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Application of high resolution NMR, FTIR, and GC–MS to a comparative study of some indigenous seed oils from Botswana

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ABSTRACT

Spectroscopic techniques utilizing FTIR, ^{1}H and ^{13}C NMR spectral fingerprints of the neat oils, as well as standard titrimetric and GC–MS analyses were all employed to determine the quality parameters and fatty acid (FA) profiles for Soxhlet-extracted seed oils of four indigenous plants from Botswana: *Tylosema esculentum* (morama), *Schinziophyton rautanenii* (mungongo), *Citrullus lanatus* (kgengwe) and *Bauhinia petersiana* (mogose). The physicochemical parameters and FA composition obtained from spectroscopic methods were found to be similar to those obtained from classical procedures indicating that oil quality parameters can reliably be obtained from spectroscopic data. The FA analysis showed the presence of 73–84% unsaturated FAs in the four seed oils. In addition, spectroscopic data clearly established the presence of the uncommon tri-unsaturated FA, α-eleostearic acid (α-ESA) in mungongo seed oil which was quantified as 25% by ^{13}C NMR. Generally, the high levels of unsaturated FAs in the oils indicate their suitability in health food supplements.

Industrial relevance: The four plants studied are highly treasured in the areas where they grow due to the fact that they have for centuries provided food security, and means of livelihood for populations living in the Kalahari Desert and other marginal regions of the southern Africa region. Currently, there is a drive to add value to such often underutilized plants to aid in poverty alleviation by processing and marketing the products as healthy food supplements or cosmetic formulations. To this end, reliable methods for characterization and comparison of the FA composition of the seed oils from different geographical locations is required. The development of rapid, non-destructive spectroscopic techniques that can be applied directly on the neat oils is therefore an important venture.

1. Introduction

Plant oils have been extracted and utilised by humans for centuries for both food and non-food uses like skin care, fuel, wood polish and for aromatherapy and other medicinal purposes. In recent years, there has been increasing recognition of the health benefits of various types of vegetable oils and the desirability of including them in the diet (FAO, 2008; Szostak-Wegierek, Kllosiewicz-Latoszek, Szostak, & Cybulska, 2014). Over the past 20 years or so, the importance of plant oils as a renewable energy resource to be exploited to benefit mankind has grown significantly. The drive to use plant oils as fuel (biodiesel) has gained substantial momentum due to rising concerns about the environmental impact of petroleum products (Issariyakul & Dalai, 2014). The suitability of an oil for food or industrial applications depends primarily on the fatty acid (FA) composition which ultimately determines its physicochemical properties. Generally, most vegetable oils are composed of just about five basic FA structures: palmitic (16:0), stearic (18:0), oleic (18:1Δ^{9}), linoleic (18:2$\Delta^{9,12}$) and α-linolenic (18:3$\Delta^{9,12,15}$) acids in varying proportions (Dyer, Stymne, Green, & Carlsson, 2008). However, some plants do biosynthesize unusual fatty acids with enhanced health or industrial benefits and thus there exits in Nature a wide variety of yet undiscovered, structurally diverse fatty acids that occur in the seed oils of wild plant species (Millar, Smith, & Kunst, 2000; Aitzetmüller, Matthaüs, & Friedrich, 2003; Hilderbrand, 2010). Currently, a huge percentage (about 80%) of the World production of plant oil comes from only about ten traditionally-utilised plant species: oil palm, sunflower, soybean, olive, canola, coconut, peanut, rapeseed, palm kernel and cotton (Dyer et al., 2008; USDA, 2014) but due to the steadily increasing World population and the inevitable increase in competition between the use of vegetable oil

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as food or as fuel, this limited supply of vegetable oil is under severe strain creating a dire necessity to search for other unconventional sources. The identification of unexplored indigenous plants with good oil-bearing potential and the profiling of the FA composition of their oils has therefore become a major focus of research globally, with an added goal of promoting rural economies through informed processing of local seed oils (Aitzetmüller et al., 2003; World Growth, 2011; Sabikhi & Sathish Kumar, 2012).

As part of an on-going screening process to identify and properly characterize indigenous plants of nutritional and economic significance in Botswana, seeds from the following wild plants (with local names given in brackets) were selected for study: *Tylosema esculentum* Fabaceae (morama), *Schinziophyton rautanenii* Euphorbiaceae (mungongo/manketti), *Citrullus lanatus* Cucurbitaceae (kgengwe variety/desert melon) and *Bauhinia petersiana* Fabaceae (mogose/wild coffee bean). These four plants thrive under drought conditions and have for ages provided food, famine relief and means of livelihood for communities dwelling in the Kalahari Desert and other arid areas of the southern Africa region (Lee, 1973; Muller, 1988; Bosch, 2006; SEPASAL, 2007; Jackson et al., 2010). Currently, although the plants are highly valued in the areas where they grow, their potential health and economic benefits are under examined and hence underutilized. Over the years, therefore, there has been a drive to add value to such useful drought-resistant plants by exploring strategies to cultivate them and process and market their products in order to alleviate poverty (Taylor, Wickens, Goodin, & Field, 1985; Zimba, Wren, & Stucki, 2005; Mabaleha, Mitei, & Yeboah, 2007; Mogotsi & Ulian, 2010; Kobue-Lekalake, Mogotsi, & Jackson, 2016). More studies have been conducted on the morama plant (*Tylosema esculentum*) than the other three plants used in this study and there have been some small scale projects on the production of morama bean products such as: milk, yoghurt, flour and oil (Mogotsi & Ulian, 2010; Mpotokwane et al., 2013). Despite these studies, the production of morama bean oil and other products is still currently on a very small scale in individual homes. Generally, there is scanty or no information about the use and potential health or economic benefits of the oil extracted from the seeds of all four plants. It was therefore considered necessary to focus our research on the seed oils.

Over the years, several oil quality parameters have been stipulated and official methods to determine them have been developed (IUPAC, 1992; FAO, 1999; AOCS, 2013). The traditional analysis of an oil sample generally involves the determination of physical properties such as refractive index, density and viscosity; determination by titrimetric means of chemical properties such as acid value (AV), iodine value (IV), saponification value (SV) and peroxide value (PV) and determination of the FA profile by GC–MS analysis of the fatty acid methyl esters (FAMES). The quality of the oil is then assessed by comparison of the physicochemical properties with internationally recognised standard values established by World bodies such as FAO/WHO (2016). In recent years, a trend to explore more rapid alternative procedures to obtain oil quality parameters and FA profile from spectroscopic techniques has evolved. Several methods utilizing ^{1}H NMR, ^{13}C NMR and FTIR fingerprints of vegetable oils have thus been developed (Vlahov, 1999; Knothe & Kenar, 2004; Salinero et al., 2012; Siddiqui & Ahmad, 2013). Furthermore, research has shown that chemical properties such as AV, SV, IV and PV may be determined solely from ^{1}H NMR spectra with results equivalent to those from titrimetric analysis (Miyake, Yokomizo, & Matsuzaki, 1998; Carneiro, Reda, & Carneiro, 2005; Satyarthi, Srinivas, & Ratnasamy, 2009; de Souza et al., 2011). Spectroscopic methods are, in fact, currently considered as highly desirable alternate approaches to edible oil characterization due to several advantages over the traditional titrimetric methods such as: being non-destructive; less time-consuming; not requiring much reagents or solvents hence reducing hazards of waste chemical disposal; providing a unique fingerprint of the oil from which several oil quality parameters can be determined; requiring only small quantities of neat oils with minimal pre-processing or sample preparation, with the capacity for running a large number of samples at a time for convenient comparison and yet yielding valuable information on all components of the oil in one spectrum. In this study we sought to apply these rapid spectroscopic techniques to the study of the seed oils of the four plants. The objective of the study was to establish, from existing literature, a working protocol for the determination of vital oil characterization parameters solely based on spectroscopic fingerprints (^{1}H NMR, ^{13}C NMR and FTIR,) of the neat oils. To this end, the selected spectroscopic methods were applied alongside the traditional methods and the results obtained were compared.

2. Materials and methods

2.1. Seed samples and chemicals

Seeds of *Tylosema esculentum* (morama), *Schinziophyton rautanenii* (mungongo), *Citrullus lanatus* (kgengwe) and *Bauhinia petersiana* (mogose) were obtained from the Food Science Department of Botswana University of Agriculture and Natural Resources (BUAN). The solvents and reagents used in this work unless otherwise stated, were all of analytical grade and were obtained from Rochelle chemicals (South Africa) or BDH (Merck Chemicals, PTY Ltd., UK).

2.2. Extraction of the oils

The seeds from each batch were deshelled and ground into powder form with a mortar and pestle. A total lipid extraction was carried out on about 30 g – samples of the powdered seeds by Soxhlet using 2-propanol - hexane (1:3) at a temperature of 60 °C. The extracts obtained were filtered before removal of solvents under reduced pressure on a Büchi Rotary evaporator (R-215) followed by blowing a stream of nitrogen through each sample. The oils were then stored in brown glass vials at ambient temperature.

2.3. Determination of physical properties of the oils

The refractive indices of the oils were measured using an ATAGO RX5000 refractometer (Tokyo, Japan) attached to a Labcon low temperature water bath (23.1 °C). Densities were measured on an Anton Paar DMA 4500 Density meter (Graz, Austria).

2.4. Determination of chemical properties of the oils

2.4.1. Chemical parameters from standard titrimetric procedures

Chemical properties of the oils were determined according to standard AOCS methods for analysis of fats and oils: Acid value (AV), Cd 3d-63; Saponification value (SV), Cd 3–25 and Peroxide value (PV), Cd 8b-90. The values were determined in triplicate for each oil and recorded as the average value ± the standard deviation. Due to unavailability of Wij's reagent during this study, the iodine values, IV, were not determined. However, values determined in our laboratory in previous years for the same plant seed oils have been recorded for comparison (Ketshajwang, Holmback, & Yeboah, 1998; Mabaleha et al., 2007).

2.4.2. Chemical parameters from ^{1}H NMR spectroscopy

^{1}H NMR spectra at 300 MHz, were recorded for triplicate samples of each oil on a Bruker Avance DPX 300 spectrometer. Typically, 20–30 mg of seed oil was dissolved in ≈ 0.7 mL deuterated chloroform, $CDCl_3$, in an NMR tube and readings were taken between 0 and 14 ppm at 298 K. The chemical shifts are reported in ppm and referenced to the residual $CDCl_3$ solvent signal at δ7.295 ppm. The integrals of each of the signals labelled **A – H**, as indicated in Fig. 1, were manually determined using Bruker TopSpin software. The integral reference number for protons was set at 3.00 for the broad peak due to terminal

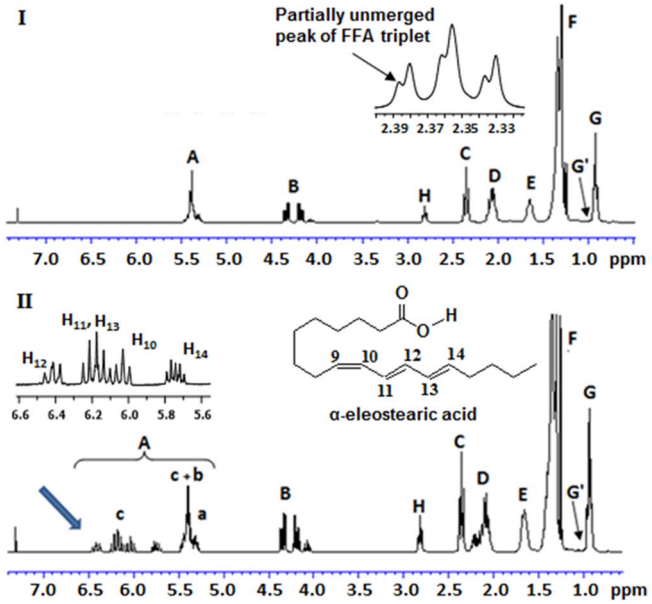


Fig. 1. Comparison of ^{1}H NMR spectra of morama (I) and mungongo (II) seed oils.

–CH_3 protons at ≈ 0.89–0.91 ppm. The average integrals for signals **A** – **H** obtained for each oil are given in Table 1. The relevant integrals were fed into the formulas described in Eqs. 1 to 5 given below and computed using Microsoft Excel 2013 software to yield the chemical parameters AV, SV and IV. The average molecular weight (M_w) of each oil was determined first from the ^{1}H NMR spectra as it was required to determine the other parameters.

The average molecular weight, $\boldsymbol{M_w}$, of each oil was determined by the method developed by Miyake et al. (1998) and was calculated from the formula, given in Eq. (1).

$$\mathbf{M_w} = 15.034\,\mathbf{G}\Big/3 + \frac{14.026(\mathbf{C}+\mathbf{D}+\mathbf{E}+\mathbf{F}+\mathbf{H})}{2} + 173.100\mathbf{B}\Big/4 + \frac{26.016(\mathbf{A}-\mathbf{B}/4)}{2} \quad (1)$$

Where the letters **A – H** represent the areas of signals as labelled in Fig. 1 (which shows the ^{1}H NMR spectra for morama and mungongo seed oils) and in Table 1, which shows the average values of the integrals obtained for each oil. [Note that Eq. (1) gives the molecular weight for one FA unit which is then multiplied by 3 to give the weight of the oil (triacylglycerol)].

Acid value (AV) was determined from ^{1}H NMR using the methodology described by Satyarthi et al. (2009). First, the percentage of free fatty acids (% FFA) was determined. Quantification of FFA is based on the fact that the triplet peaks of the α-CH_2 hydrogens of FFA appear at slightly higher δ values than those of glycerol esters in the ^{1}H NMR spectrum. When free fatty acids are present together with glycerol esters, the two triplets are invariably partially merged and appear like a triplet of doublets (td) in the region δ2.30 – δ2.40 ppm as shown in the expansion of signal **C** in Fig. 1 (I) in the ^{1}H NMR spectrum of morama oil. This allows the *unmerged portion* of the FFA triplet to be integrated and % FFA is then calculated according to Eq. (2):

$$\%\text{FFA} = \frac{4 \text{ x area of unmerged peak of } \alpha CH_2 \text{ of FFA}}{\text{total area of } \alpha CH_2 \text{ of both FFA and ester}} \quad (2)$$

AV as mg KOH/g can then be calculated from % FFA by the simple formula:

AV = 1.99 × % FFA (Kirk & Sawyer, 1989; Da Tech, 2016). This formula is however only an approximation as it considers all the fatty acids as oleic with molecular weight 282 g/mol. In this study, we therefore first derived the average molecular weight of the fatty acids (MW_{FA}) in each oil from the already determined average molecular weight of the oil (M_w) using the formula, $MW_{FA} = (M_w - 41.07)/3$. Where 41.07 is the molar mass of the glycerol backbone. % FFA was then converted to AV from the formula given in Eq. (3) where 56.11 is the molecular weight of KOH.

$$\text{AV} = \frac{56.11}{MW_{FA}/10} \text{ x\%FFA} \quad (3)$$

Saponification value (SV) was determined from the ^{1}H NMR spectra by adapting the methodology developed by Carneiro et al. (2005). The correlation between our titrimetric SVs and average molecular weight, M_w determined from ^{1}H NMR was established by linear regression on Microsoft Excel (2013). The following formula given in Eq. (4) was obtained (with $R^2 = 0.9857$ for $n = 5$) and was used to calculate SV for each oil sample:

$$\text{SV} = 343.55 - 0.1911 * M_w \quad (4)$$

Iodine value (*IV*) was determined from the ^{1}H NMR spectra using the formula given in Eq. (5) (Fig. 1 and Table 1). This method was initially developed by Miyake et al. (1998) and later adapted by Nehdi, Sbihi, Tan, and Al-Resayes (2013), and Sbihi, Nehedi, and Al-Resayes (2014).

$$\text{IV} = 253.8 \text{ x } \frac{\mathbf{A} - (\mathbf{B}/4)}{2 \mathbf{x} \mathbf{M_w} \mathbf{x} (\mathbf{B}/4)} \text{ x } 100 \quad (5)$$

Where,**A** is the signal area of the olefinic protons (5.0–6.5 ppm) + the methine protons of the glycerol group.**B** is the signal area of the 4 methylene protons (4.0–4.4) in the glycerol group.

$\mathbf{M_w}$ is the average molecular weight of the oil (determined previously from ^{1}H NMR, Eq. (1)).

2.5. Determination of fatty acid composition

2.5.1. Fatty acid composition by GC–MS

The fatty acid methyl esters (FAMEs) were prepared by refluxing the seed oils (≈ 2 g samples) in dry methanol that contained ethanoyl chloride (Christie, 1989; Mitei, 2007). Analysis of the FAMES was then carried out in an Agilent 7890A gas chromatograph coupled to an Agilent 5975C inert XL EI/CI MSD mass spectrometer with a Triple-Axis detector. Separation was carried out on a HP-5MS capillary GC column (0.25 µm × 0.25 mm × 30 m) from J & W Scientific (California, USA) consisting of (5%-Phenyl)-methylpolysiloxane phase. UHP helium was

Table 1
Average integrals (I) for the signals A - H obtained from ^{1}H NMR spectra of the oils.

	A		B		C		D		E		F		G		H	
	-CH = CH–		CH_2 -glyceride		α-CH_2		CH_2 -allylic		β-CH_2		CH_2-envelope		CH_3– terminal		CH_2-diallylic	
	δ/ppm	I	δ/ppm	I	δ/ppm	I	δ/ppm	I	δ/ppm	I	δ/ppm	I	δ/ppm	I	δ/ppm	I
Morama	5.38	2.28	4.12–4.39	1.43	2.35	1.9	2.09	2.85	1.65	2.20	1.34	20.23	0.94	3.00	2.80	0.55
Mungongo	5.20–6.50	3.78	4.13–4.40	1.32	2.35	1.92	2.15	3.29	1.66	2.14	1.36	16.62	0.92	3.00	2.8	0.72
Kengwe	5.38	3.04	4.14–4.38	1.23	2.35	2.02	2.08	3.16	1.65	2.18	1.35	17.13	0.92	3.00	2.78	1.24
Mogose	5.39	2.16	4.12–4.40	1.24	2.35	1.94	2.09	2.85	1.65	2.01	1.34	18.14	0.93	3.00	2.79	0.75

used as carrier gas at a flow rate of 1 mL/min. The initial temperature was 100 °C held for 3 min and then ramped to 200 °C at the rate of 10 degrees per minute. It was then held for 3 min before the second ramp at the rate of 8 degrees per minute to 300 °C. Solutions of the samples (100 ppm in chloroform) were injected manually at 250 °C. Injection volume was 1.0 μL in the splitless mode. Mass spectra were obtained by EI at an electron energy of 70 eV over the scan range *m/z* 50 to 800. The FA composition was determined for each oil sample from the total ion chromatograms (TICs) obtained from the GC analysis. Each fatty acid, at a given retention time, was identified from its mass spectrum by comparison with those of the NIST 05 L mass spectral library. Relative concentrations of the FA components were calculated from GC peak areas and the average molecular weight was determined by weighted average of the FA composition using Eq. (6).

$$M_{w(GC-MS)} = \left[\left[\left(\Sigma\frac{\%FA}{100}\, x\, MM\right) - 15.0\right]^{*} 3\right] + 41.07 \quad (6)$$

Where % FA is the percentage of a particular fatty acid in the mixture; MM is the molar mass of the fatty acid; 15.0 is the mass of a methyl group of the FAME and 41.07 is the mass of the glycerol backbone ($-CH_2CHCH_2-$).

2.5.2. Fatty acid composition by ^1H NMR

The relative compositions of the saturated fatty acids (SFA), monounsaturated fatty acids (MUFA, as oleic) and diunsaturated fatty acids (DUFA, as linoleic) were determined from Holmback's equations (Holmbäck, 2000; Mitei, Ngila, Yeboah, Wessjohann, & Schmidt, 2008) which are derived by comparing the relative sizes of integrals of the signals for the olefinic (ca. 5.38 ppm), allylic (ca. 2.08 ppm) and diallylic (ca. 2.80 ppm) protons and relating them to the total integrals of the signals of all methyl protons (ca. 0.93–0.98 ppm).

2.5.3. Fatty Acid composition by ^{13}C NMR.

The ^{13}C spectra were recorded for the neat oils (≈ 40 mg dissolved in 0.7 mL $CDCl_3$), as well as their FAMEs on a Bruker Avance DPX 300 spectrometer operating at 75.475 MHz, spectral width 238.3 ppm, acquisition time 1.822 s, and pulse delay 2 s. The chemical shifts are reported in ppm and referenced to the residual $CDCl_3$ at δ_C 77.00 ppm. The FA composition was determined from the ^{13}C NMR spectra by two methods:

Method 1 was adapted from a previously described method (Ng & Ng, 1983; Akintayo, Adeyeye, Akintayo, & Oyewusi, 2004). The method is based on the following general observations on ^{13}C NMR of fatty acids: Referring to the labelling shown in Fig. 2, which shows the ^{13}C NMR of morama oil (I) compared to the ^{13}C NMR of mungongo oil (II), the peak **(*a*)** at $\delta_C \approx$ 24.8–24.9 ppm belongs to C_3 of all fatty acid chains and so represents the total of all the FAs. The peak(s) **(*b*)** at $\delta_C \approx$ 25.3–25.7 belongs to a carbon that is allylic to both double bonds of a *cis-cis*-diene such as C_{11} of linoleic acid and thus represents the total number of diene chains. The peak **(*c*)** at $\delta_C \approx$ 27.2 ppm belongs to the two carbons allylic to a *cis* double bond such as C_8, C_{11} of oleic acid or C_8, C_{14} of linoleic acid and therefore represents twice the total of all mono-unsaturated (MUFA) plus diunsaturated fatty acids (DUFA). In this study, this reasoning was extended to involve peaks in the olefinic carbon region δC125.0–135.5 ppm which are observed when there are conjugated diene or triene chains in the oil (Gunstone, 2007). Thus the peak **(d)** at $\delta_C \approx$ 125.9–126.0 ppm was identified as belonging to one of the middle carbons of a conjugated tri-unsaturated fatty acid (C-TUFA) and is unique to C_{12} of the *cis-trans-trans* stereochemistry of α-ESA. Peak **(d)** therefore represents the total of α-ESA in the oil. The percentage FA composition of the oils was thus calculated from the integrals of peaks **(*a*)**, **(*b*)**, **(*c*)**, **(d)** according to Eqs. 7, 8, 9 and 10:

$$\%\text{SFA (Saturated fatty acid)} = [(\boldsymbol{a}–0.5\boldsymbol{c}–\boldsymbol{d})/\boldsymbol{a}] \times 100 \quad (7)$$

$$\%\text{MUFA (Oleic acid)} = [(0.5\boldsymbol{c}–\boldsymbol{b})/\boldsymbol{a}] \times 100 \quad (8)$$

$$\%\text{DUFA (Linoleic acid)} = (\boldsymbol{b}/\boldsymbol{a}) \times 100 \quad (9)$$

$$\%\text{CTUFA } (\alpha - \text{ESA}) = (\boldsymbol{d}/\boldsymbol{a}) \times 100 \quad (10)$$

NB. Peak (***d***) was only observed in mungongo seed oil as shown in Fig. 2 (II).

Method 2 was first described by Tulloch and Bergter (1979) for determination of the composition of seed oils containing mixtures of conjugated trienoic acids and is based on identifying carbons of the *same* fatty acid and averaging the peak heights (Mallet, Gaydou, & Archavlis, 1990). The peak at δ24.8 ± 0.2 ppm is always due to C_3 of all fatty acids present in the oil and this is used to normalize the integrals and set at 100 representing the total of all FAs in the oil (Fig. 2 (II)). The peak integrals of carbons of the *same* fatty acid are then averaged to determine relative percentage in the oil. The difficulty with this method was that the chemical shifts of palmitic, oleic, linoleic and stearic acids were generally overlapping and not easily distinguished, making it difficult to individually quantify these acids. However, signals due to α-ESA in mungongo oil (C_{10}, C_{11}, C_{12} and C_{14}) on the other hand,

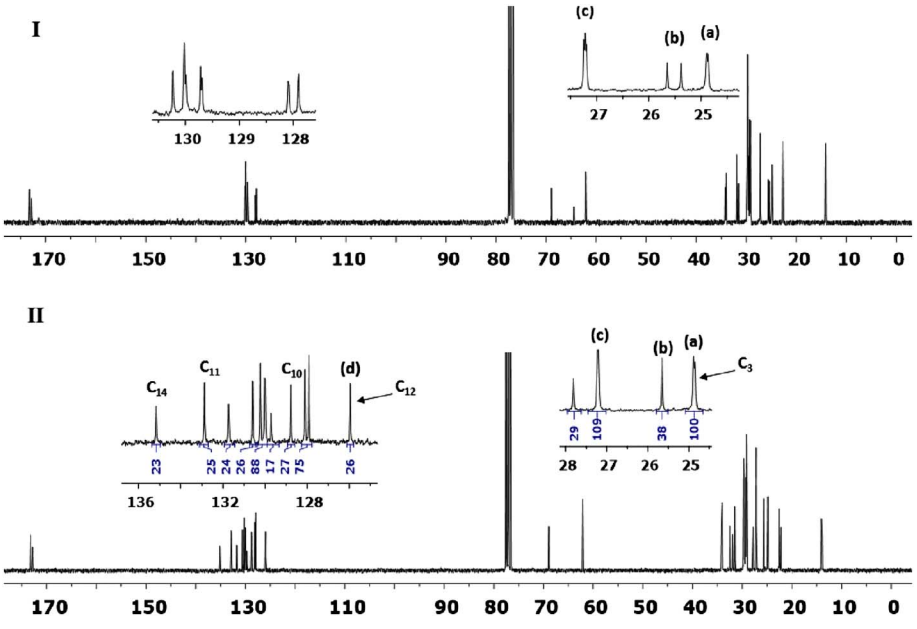


Fig. 2. Comparison of ^{13}C NMR spectra for seed oils of morama (I) and mungongo (II), showing some identified carbons of α-ESA in mungongo.

were easily identified by using the assignment of Cao et al. (2007) as shown in Fig. 2 (II), making the quantification of this trienoic acid relatively simple by this method.

2.6. FTIR spectral fingerprints

FTIR spectra of the oil samples were recorded on a Perkin-Elmer Spectrum 100 spectrophotometer (Spectrum Two) fitted with a universal attenuated total reflectance (UTAR) sampling device. A drop of each oil sample was placed directly onto the Universal diamond ATR crystal and all spectra were measured against a background spectrum of air in the wavenumber range from 4000 to 600 cm^{-1}.

2.7. Statistical analysis

All parameters were determined in triplicate for each oil sample and recorded as the average value ± the standard deviation. The data obtained from the different methods (traditional and spectroscopic) were compared and analysed using the correlation and one-way ANOVA ($\alpha = 0.05$) functions from Microsoft Excel statistical package.

3. Results and discussion

3.1. Physical characteristics

The oil yields and physical properties of the oils are given in Table 2. High oil yields were obtained for mungongo (60%) and morama (43%) seed oils, while rather lower yields were obtained from mogose (19%) and kgengwe (16%). It should however be noted that this range of oil yields is comparable to the oil yields for some leading vegetable oils on the world market such as; sunflower oil (45–55%) and soyabean oil (17–22%). Refractive index (RI) is a measure of ease of passage of light through the sample medium. For edible oils, RI increases with degree of unsaturation, conjugated unsaturation and chain length. The RI values of the four seed oils ranged from 1.467 for morama to 1.487 for mungongo suggesting a higher degree of unsaturation in mungongo seed oil which indeed, as will be shown later, contains a conjugated tri-unsaturated fatty acid. The densities ranged from 0.902 for morama seed oil to 0.929 g/mL for kgengwe. Generally, the density and RI values found in this study for the four oils were comparable to those reported (FAO, 1999) for standard edible vegetable oils like olive oil (0.911 g/mL and 1.467–1.471) and sunflower oil (0.916 g/mL and 1.461–1.471).

3.2. Chemical properties of the oils

Chemical properties are vital measurements required in the characterization of oils as they provide important quality and structural information. Over the years, several sets of standard methods of measurements of these chemical properties have been compiled and are regularly updated (AOCS, 2013). In recent years it has been shown that these chemical properties can also be obtained from spectroscopic techniques especially from ^{1}H NMR spectra. A wide range of spectroscopic techniques are now published in literature that have been shown to provide chemical properties of oils that are equivalent to those obtained from traditional titrimetric procedures (Miyake et al., 1998; Carneiro et al., 2005; Satyarthi et al., 2009; de Souza et al., 2011; Sbihi et al., 2014). In this study, we applied both traditional AOCS official methods and ^{1}H NMR methods to obtain the usual chemical parameters. In the ^{1}H NMR method, the desired parameters were obtained by substituting the relevant integrals of the signals **A – H** (Fig. 1) into the appropriate equations (Eq.(1–5)). The average integrals obtained for signals **A – H** in the ^{1}H NMR spectra are presented in Table 1. The average molecular weight (M_w) of the oil was required to determine the other parameters so it was determined first by substituting the relevant integrals from Table 1 into Eq. (1). Acid value, saponification value and iodine value were then obtained from Eq. (2–5). The results from the two methods (titrimetric and ^{1}H NMR) are compared in Table 3.

Acid values from the traditional titrimetric method ranged from 0.77 mg KOH/g for morama seed oil to 2.73 mg KOH/g for kgengwe. The values from the ^{1}H NMR method were much lower and ranged from 0.60 for mogose seed oil to 1.36 for morama oil. A rather large difference in the values from the two methods (titration/^{1}H NMR) was noted for kengwe (2.73/0.77) and mogose (2.47/0.60) seed oils. The larger titration values could possibly be due to the fact that these two oils have stronger colour (greenish and orange, respectively) than morama or mungongo oils (both pale yellow) which could have clouded the endpoint detection. Acid value (AV) is a measure of free fatty acids produced by the breakdown of the triacylglycerols in the oil and is therefore a measure of hydrolytic rancidity and gives an indication about edibility of the oil. The AVs of the four oils from both methods fall well below the limit of 6.6 mg KOH/g which is the accepted Codex standard for virgin olive oil indicating good oil quality and are thus safe for consumption (FAO, 1999).

Saponification values of the oils obtained from the traditional titrimetric method were within a narrow range from 172.8 mg KOH/g for morama oil to 185.1 mg KOH/g for mogose. Similar values were obtained from the ^{1}H NMR method; 173.6 mg KOH/g for morama oil to 187.2 mg KOH/g for mogose. Saponification value (SV) is a measure of the average molecular weight (or chain length) of all the fatty acids present. The smaller the SV, the larger the average molecular weight (or chain length) of the fatty acids present. The narrow range of SVs obtained from the two methods suggests that the four oils contain FAs of similar chain length of 16–18 carbons as they are comparable to some commercially produced oils such as mustard seed oil (168–184 mg KOH/g) and rapeseed oil (168–181 mg KOH/g). The SVs obtained in this study are good agreement with those obtained for these oils in previous studies [morama and manketti/mungongo by Mitei et al. (2008); desert melon/kgengwe by Mabaleha et al. (2007) and *B. peretsiana*/mogose by Ketshajwang et al., 1998].

The iodine value (IV) is a measure of unsaturation (number of double bonds) in the fatty acids of the oil. The higher the iodine number, the more C=C bonds are present in the oil that can react with iodine. In this study, we were unable to measure IV due to unavailability of Wij's reagent. However, IV values from previous studies on these oils are given in Table 3 for comparison with those determined from ^{1}H NMR. The IV values previously obtained from the traditional method range from 95.0 g/100 g for morama oil to 124 g/100 g for kengwe (Ketshajwang et al., 1998; Mabaleha et al., 2007; Mitei et al., 2008),suggesting that kengwe has the highest unsaturation among the four oils. The IVs from ^{1}H NMR in this study ranged from 78.9 g/100 g for morama oil to 136.6 g/100 g for kengwe and 142.2 g/100 g for mungongo suggesting that mungongo oil has the highest unsaturation closely followed by kengwe. The higher IV values for mungongo and kgengwe oils indicate that they contain polyunsaturated FAs while the lower values for morama and mogose oils suggest the presence of mainly monounsaturated FAs.

Peroxide value (PV) is a measure of the amount of hydroperoxides in the oil which arise from primary lipid oxidation. PVs are therefore a good indicator of oil quality and stability. The PVs reported in Table 3 were obtained only from the traditional titrimetric method. We were

Table 2
The physical characteristics of the oils.

Seed sample	Oil yield (%)	Refractive index (24.4 °C)	Density (g/mL) (25.0 °C)
Morama	43.1 ± 2.1	1.4666 ± 0.0002	0.9015 ± 0.0012
Mungongo	57.7 ± 0.9	1.4868 ± 0.0002	0.9081 ± 0.0000
Kgengwe	16.1 ± 2.0	1.4738 ± 0.0002	0.9292 ± 0.0001
Mogose	18.5 ± 2.0	1.4738 ± 0.0002	0.9085 ± 0.0002

Table 3
Comparison of the chemical parameters determined by titration/GC–MS with values estimated from ^{1}H NMR.

Seed sample	Method	AV (mg KOH/g)	SV (mg KOH/g)	IV (g/100 g)	PV (mEq O_2/kg)	M_w (g/mol)
Morama	Titration	1.17 ± 0.03	172.8 ± 0.6	95.0 ± 3.0[c]	7.84 ± 1.33	881.8 ± 3.2 (GC–MS)
	^{1}H NMR	1.36 ± 0.61	173.6 ± 1.1	78.9 ± 6.3	nd	889.2 ± 8.3
Mungongo	Titration	0.77 ± 0.11	173.3 ± 1.3	121.8 ± 1.2[c]	5.04 ± 0.09	870.9 ± 1.2 (GC–MS)
	^{1}H NMR	0.65 ± 0.02	177.2 ± 3.5	142.2 ± 7.8	nd	870.5 ± 6.5
Kgengwe	Titration	2.73 ± 0.30	180.3 ± 3.7	124.0 ± 0.2[b]	5.08 ± 0.08	873.0 ± 0.5 (GC–MS)
	^{1}H NMR	0.77 ± 0.14	180.6 ± 0.8	136.6 ± 10.8	nd	852.7 ± 6.8
Mogose	Titration	2.47 ± 0.26	185.1 ± 5.8	98.0 ± 3.0[a]	4.65 ± 0.47	870.4 ± 1.6 (GC–MS)
	^{1}H NMR	0.60 ± 0.01	187.2 ± 1.0	92.0 ± 6.4	nd	818.7 ± 5.5

nd, Not determined.
[a] Ketshajwang et al., 1998.
[b] Mabaleha et al., 2007.
[c] Mitei et al., 2008.

unable to determine PV from our ^{1}H NMR spectra in this study due to the fact that we did not observe the pertinent (−OOH) hydroperoxide proton signals in the δ10 – δ11 ppm region when the sample was dissolved in $CDCl_3$ (Skiera, Steliopoulos, Kuballa, Holzgrabe, & Diehl, 2012). The PVs obtained from the traditional titrimetric method ranged from 4.65–7.84 mEq. O_2/kg and were all within the standard Codex value of 20 mEq. O_2/kg for unrefined vegetables oils, indicating good oxidative stability of the oils.

All chemical properties obtained from traditional titrimetric methods in this study were comparable to those obtained in previous studies (Ketshajwang et al., 1998; Mabaleha et al., 2007; Mitei et al., 2008). Good correlation (R^2 = 0.9964) was observed between the chemical properties obtained from traditional methods and those estimated from ^{1}H NMR as shown in Fig. 3. In addition, the results obtained from one way ANOVA analysis, F (1,30) = 0.0005, $p > 0.05$, indicate that there is no statistically significant difference between the values obtained by traditional titrimetric methods and those obtained by ^{1}H NMR, thus confirming the observation that vital chemical properties of oils can be obtained solely from the ^{1}H NMR spectra (Carneiro et al., 2005; Satyarthi et al., 2009; de Souza et al., 2011).

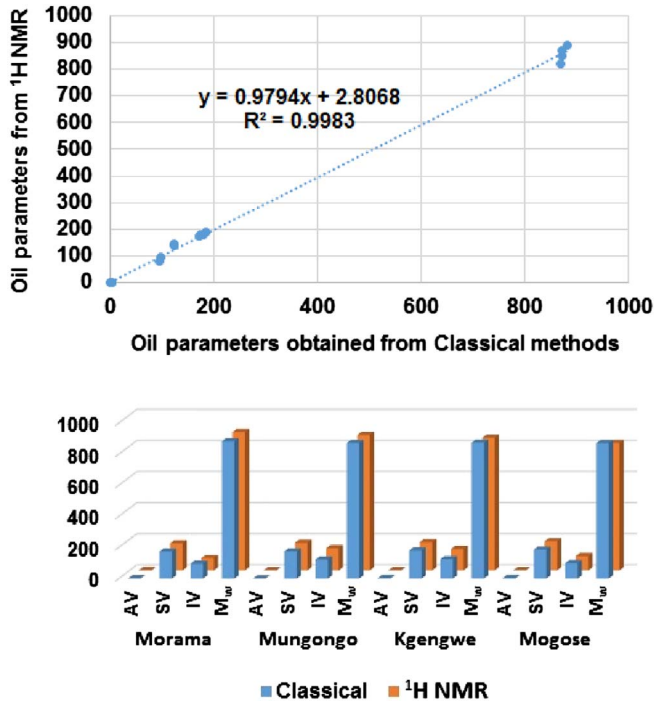


Fig. 3. Correlation between chemical parameters derived from ^{1}H NMR and those from classical methods.

3.3. Fatty acid composition

The fatty acid composition of an oil determines all the physical and chemical properties. Traditionally, FA composition is determined by GC–MS of the FAMEs of the oils. However, over the years, a number of alternative methods for determining FA composition directly from spectroscopic fingerprints have been developed. In this study we determined FA composition by the traditional GC–MS analysis as well as from ^{1}H NMR, and ^{13}C NMR spectral fingerprints. The results obtained from all the methods are compared in Table 4.

3.3.1. Fatty acid composition from GC–MS analysis

GC–MS analysis of FAMEs is now the method of choice to provide the composition of individual fatty acids in an oil. The major fatty acids detected in each of the oils by GC–MS are given in Table 4. The fatty acids found in morama oil were: palmitic (16:0, 14%), linoleic (18:2, 31%), oleic (18:1, 42%), stearic (18:0, 8%), arachidic (20:0, 3%), behinic (22:0, 0.7%) and lignoceric (24:0, 0.4%). Thus from GC–MS, morama oil has a total of 27% saturated and 73% unsaturated FAs. A similar profile (without any lignoceric acid) was previously determined by Mitei et al. (2008). The fatty acids in mungongo oil were: palmitic (16:0, 14%), linoleic (18:2, 47%), oleic (18:1, 26%), stearic (18:0, 10%) and α-eleostearic (18:3, 3%) giving mungongo oil a total of 24% saturated and 76% unsaturated (mainly di-unsaturated) FAs. In a previous study for mungongo (manketti) oil, the same fatty acids were obtained in slightly different ratios and α-eleostearic acid was not observed at all and total unsaturation was mainly monounsaturation (Mitei et al., 2008). In this study, α-eleostearic acid was detected at RT of 20.10 min as the last peak with intensity of 3%. The fatty acids observed in kengwe oil: were: palmitic (16:0, 9%), linoleic (18:2, 71%), oleic (18:1, 13%), and stearic (18:0, 7%) giving a total of 16% saturated and 84% unsaturated (mainly di-unsaturated) FAs. This profile was similar to the FA profile obtained in a previous study (Mabaleha et al., 2007). In mogose oil, the fatty acids were: palmitic (16:0, 14%), linoleic (18:2, 47%), oleic (18:1, 33%), and stearic (18:0, 6%) giving a total of 16% saturated and 83% unsaturated (roughly equal amounts of mono and di-unsaturated FAs), similar to results from a previous study (Ketshajwang et al., 1998). Linolenic acid (18:3) was not detected in any of the oils.

3.3.2. Fatty acid composition from ^{1}H NMR spectra

The ^{1}H NMR spectrum provides useful information about the fatty acid classes present in an oil. The usual fatty acids in an oil have similar functional groups which produce signals in the same region of the NMR spectrum making it difficult to assign them to individual FAs. What can be identified from the signals in the ^{1}H NMR spectrum are the total

Table 4
Percentage FA compositions determined by GC–MS, ^{1}H NMR and ^{13}C NMR.

	Morama			Mungongo			Kgengwe			Mogose		
Fatty acid	GC–MS	^{1}H NMR	^{13}C NMR	GC–MS	^{1}H NMR	^{13}C NMR	GC–MS	^{1}H NMR	^{13}C NMR	GC–MS	^{1}H NMR	^{13}C NMR
Palmitic	13.57 ± 0.66	[a]	[a]	14.04 ± 2.21	[a]	[a]	9.35 ± 0.95	[a]	[a]	14.24 ± 1.25	[a]	[a]
Linolenic (TUFA)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Linoleic (DUFA)	31.00 ± 0.80	33.18 ± 0.05	44.05 ± 0.78	47.05 ± 5.78	34.60 ± 0.05	37.76 ± 0.37	70.54 ± 2.62	61.05 ± 0.03	62.83 ± 0.10	46.50 ± 1.10	37.50 ± 0.03	49.17 ± 0.42
Oleic (MUFA)	42.15 ± 4.15	47.20 ± 0.06	38.26 ± 2.51	26.46 ± 3.62	44.80 ± 0.01	16.53 ± 0.24	13.05 ± 0.51	17.35 ± 0.05	13.27 ± 0.18	33.32 ± 2.50	33.40 ± 0.03	23.31 ± 0.98
Stearic	7.87 ± 0.49	[a]	[a]	10.30 ± 1.74	[a]	[a]	7.07 ± 1.17	[a]	[a]	5.93 ± 1.13	[a]	[a]
α-ESA (CTUFA)	ND	ND	ND	2.87 ± 1.30	[b]	25.31 ± 0.44	ND	ND	ND	ND	ND	ND
Arachidic	3.23 ± 0.55	[a]	[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Behenic	2.41 ± 0.67	[a]	[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lignoceric	0.83 ± 0.42	[a]	[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Σ Saturated	26.87 ± 3.42	19.62 ± 0.05	17.70 ± 1.73	24.35 ± 3.78	20.60 ± 0.02	20.50 ± 0.44	16.42 ± 2.11	21.6 ± 0.05	23.90 ± 0.09	20.17 ± 3.10	29.10 ± 0.01	27.51 ± 0.84
Σ Unsaturated	73.13 ± 3.42	80.38 ± 0.05	82.30 ± 1.73	75.65 ± 3.78	79.40 ± 0.02	79.50 ± 0.44	83.59 ± 2.11	78.40 ± 0.05	76.10 ± 0.09	79.83 ± 3.10	70.90 ± 0.01	72.49 ± 0.84

TUFA = Tri-unsaturated FA; DUFA = di-unsaturated FA; MUFA = monounsaturated FA; CTUFA = conjugated tri-unsaturated FA;
[a] Determined together with other saturated FAs.
[b] Determined with the other unsaturated FAs.

integrals due to the different types of unsaturation (mono-, di-, tri- and conjugated tri-unsaturation). The application of equations developed by Holmbäck (2000) to the ^{1}H NMR spectra thus yielded the relative composition of fatty acid classes in each oil as compiled in Table 4. Morama oil was found to contain 47% MUFA (as oleic), 33% DUFA (as linoleic) and 0% TUFA (as linolenic), giving a total of 80% unsaturated and 20% saturated FAs; mungongo oil contained 45% MUFA, 35% DUFA, and 0% TUFA giving a total of 79% unsaturated and 21% saturated; kengwe oil had 17% MUFA, 61% DUFA and 0% TUFA giving a total 78% unsaturated and 22% saturated and mogose oil had 33% MUFA, 38% DUFA and 0% TUFA, a total of 71% unsaturated and 29% saturated. All four oils displayed a high percentage of total unsaturated FAs ranging from 70.9% for Mogose oil to 80.38% for Morama. This was in close agreement with the overall results from GC–MS given above.

In addition to determining the FA classes, the ^{1}H NMR spectrum provides a unique fingerprint for each oil which can show up the presence of any uncommon fatty acids in the oil. A fatty acid with a functional group that is different from the groups usually present in an oil, will show signals in a new region of the spectrum making it possible to identify it. The ^{1}H NMR spectra for three of the seed oils, morama, kgengwe and mogose, looked almost identical to the naked eye while the spectrum of mungongo oil was slightly different as shown in Fig. 1. The ^{1}H NMR chemical shifts for a typical edible oil are well known and have been previously assigned (Popescu et al., 2015) as summed up in Table 5 for the four seed oils. Mungongo oil displayed extra signals in the olefinic double bond region between δ5.6 – *δ* 6.4 ppm. These signals were ascribed to the presence of the conjugated *cis-trans-trans* double bonds of the uncommon fatty acid α-eleostearic acid (α-ESA) by comparison with data from literature (Cao et al., 2007; Sbihi et al., 2014). The expansion of the olefinic peak region δ5.6 – δ6.5 ppm for mungongo oil (II) was similar to that obtained by Sbihi et al. (2014) for white mahaleb (*Prunus mahaleb* L.) seed oil which contains about 38% α-ESA. This made possible the assignment of the chemical shifts of protons H-9 to H-14 of α-ESA as shown in Table 5. Thus, the ^{1}H NMR spectrum directly identified the presence of the unusual fatty acid α-ESA in mungongo oil which was not in the other three oils.

3.3.3. Fatty acid composition from ^{13}C NMR spectra

The ^{13}C NMR spectrum, like ^{1}H NMR, provides useful information mainly about the fatty acid classes present in the oil as it is not easy to identify or differentiate signals due to individual FAs in the spectrum. Application of Eq. (7) – Eq. (1)0 developed by (Ng & Ng, 1983) to the ^{13}C NMR spectra (Fig. 2) yielded the relative composition of the FA classes as compiled in Table 4. Morama oil was found to contain 38% MUFA (as oleic), 44% DUFA (as linoleic) and 0% TUFA (as linolenic) giving 82% unsaturated and 18% saturated FAs; mungongo oil contained 17% MUFA, 38% DUFA, 0% TUFA and 25% CTUFA (conjugated tri-unsaturated FA, as eleostearic acid) giving 79% unsaturated and 21% saturated; kengwe oil had 13% MUFA, 63% DUFA and 0% TUFA (76% unsaturated and 24% saturated) and mogose oil had 23% MUFA, 49% DUFA and 0% TUFA (72% unsaturated and 28% saturated). All four oils displayed a high percentage of total unsaturated FAs ranging from 71% for Mogose oil to 82% for Morama. This was in close agreement with the overall results from GC–MS and from ^{1}H NMR given above.

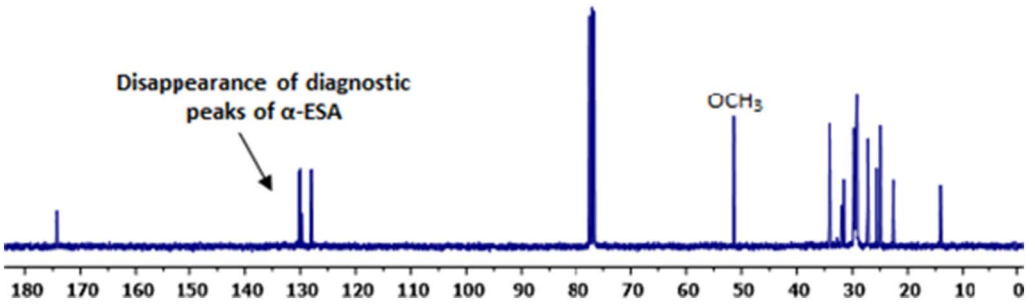
The ^{13}C NMR spectra for morama, kgengwe and mogose, looked similar to the naked eye while the spectrum of mungongo oil displayed some additional signals in the olefenic double bond region δ128– δ136 ppm (Fig. 2). These were once more assigned to the conjugated *cis-trans-trans* double bonds of α-ESA (Cao et al., 2007). The signals for carbons C_3, C_{10}, C_{11} C_{12} and C_{14} of α-ESA were easily identified and the integrals were obtained as labelled in Fig. 2 (II). This identification of signals belonging specifically to α-ESA in the spectrum, made it possible to quantify it by two methods: In Method 1, % CTUFA as α-ESA is given by [(d) / (a)]*100 (Eq.(10) and Fig. 2) which gives (26 /

Table 5
Assignment of the ^{1}H NMR (300 MHz) chemical shifts of the seed oils.

Ref.	Assignment	Compound responsible	^{1}H δ (ppm) *			
			Mungongo	Morama	Mogose	Kgengwe
G	$-CH_2-CH_2-CH_2-CH_3$	All FAs except linolenic	0.94 (m)	0.94 (m)	0.91 (m)	0.92 (m)
G´	$-CH=CH-CH_2-CH_3$	linolenic acid	ND	ND	ND	ND
F	$-(CH_2)_n-$	All acyl chains	1.24–1.44 (bs)	1.25–1.35 (bs)	1.25–1.30 (bs)	1.25–1.33 (bs)
E	$-CH_2-CH_2-COR$	All acyl chains, β-methylenes	1.66 (m)	1.66 (m)	1.65 (m)	1.65 (bs)
D	$-CH_2-CH_2=CH-$	All unsaturated fatty acids	2.04–2.25 (m)	2.02–2.12 (m)	2.03–2.10 (m)	2.04–2.12 (m)
C	$-CH_2-COOH$	All fatty acids, α-methylenes	2.35 (t)	2.36 (t)	2.35 (t)	2.35 (t)
H	$-CH=CH-CH_2-CH=CH-$	linoleic & linolenic (Bis-allylic)	2.81 (t)	2.82 (t)	2.81 (t)	2.81 (t)
B	$-CH_2-O-COR$	Glycerol α-position	4–4.4 (dd)	4–4.4 (dd)	4.26 (dd)	4.26 (dd)
Aa	$-CH-O-COR$	Glycerol β-position	5.30 (m)	5.30 (m)	5.31 (m)	5.32 (m)
Ab	$-CH=CH-$	Usat. FAs except α-ESA	5.40 (m)	5.40 (m)	5.40 (m)	5.39 (m)
Ac	H-9	α-eleostearic acid	5.40 (m)	ND	ND	ND
	H-14	(conjugated trienoic acid)	5.74 (m)	´´	´´	´´
	H-10		6.03 (m)	´´	´´	´´
	H-12		6.15 (t, J = 10.8 Hz)	´´	´´	´´
	H-11		6.20 (qui., J = 11.1 Hz)	´´	´´	´´
	H-13		6.41 (t, J = 13.5 Hz)	´´	´´	´´

Signal multiplicity: bs, broad singlet; t, triplet; qui. quintet; m, multiplet; dd, doublet of doublet; ND, not detected.

Fig. 4. ^{13}C NMR of mungongo oil FAME.



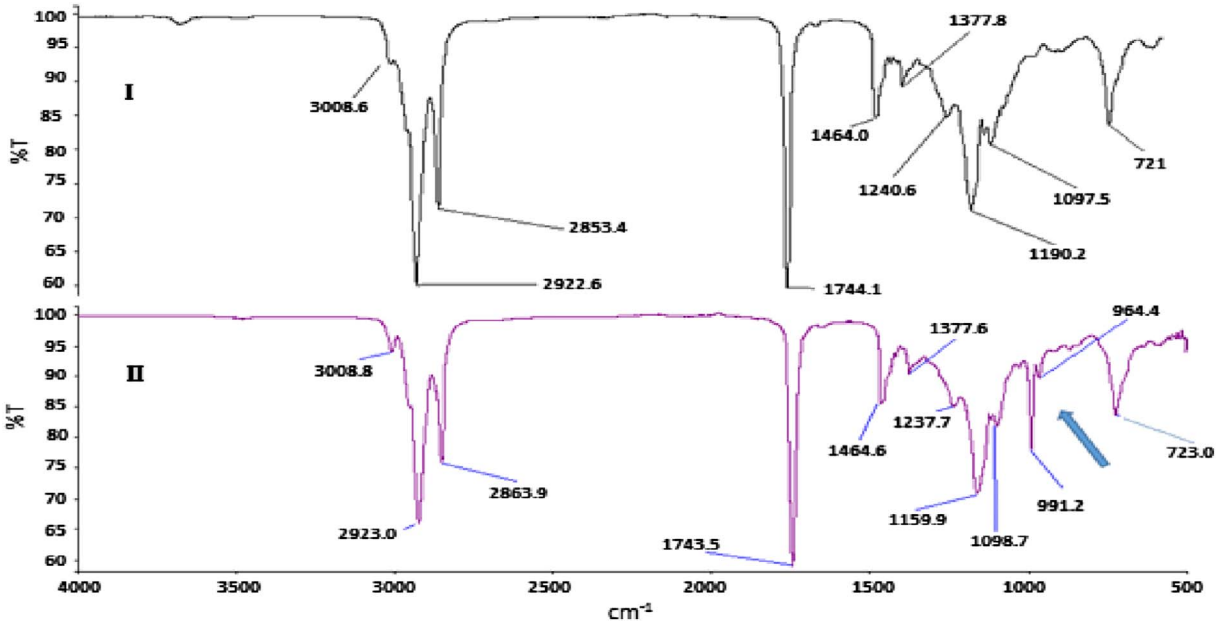


Fig. 5. Comparison of FTIR spectra of morama (I) and mungongo (II) seed oils.

100)*100 = 26% (Ng & Ng, 1983; Akintayo et al., 2004). In Method 2, the integral for C_3, which belongs to all FAs in the oil, is first set at 100 then percentage of α-ESA is given by the average of the integrals for all identified peaks that are due only to α-ESA. Thus from Fig. 2 (II), % α-ESA is given by the average of integrals for C_{14}, C_{11}, C_{10}, and C_{12} = [23 + 25 + 27 + 26]/4 = 25.25% (Tulloch & Bergter, 1979; Mallet et al., 1990). Taking the average from the two methods, % α-ESA in mungongo oil was found to be 25.6%. The fact that the GC–MS analysis showed up only 3% of α-ESA in this study and none at all in previous studies, can be explained by the fact that α-ESA was destroyed during the preparation of the FAME, suggesting that a less destructive method should be adopted in future (see Fig. 4). There are several research reports on the health benefits of α-ESA as a tumour suppressing agent and in inhibition of breast cancer (Tsuzuki, Tokuyama, Igarashi, & Miyazawa, 2004; Grossmann et al., 2009). Oils containing α-ESA are also valuable industrially because they possess unique drying

Table 6
Assignment of absorption bands in the IR spectra of the oils.

Peaks (cm^{-1})				Assignment of absorption bands
Morama	Mungongo	Mogose	Kgengwe	
3008.6	3008.8	3008.6	3009.4	C-H stretching vibrations of the *cis* double bond (=CH)
2922.6	2923.0	2923.2	2923.5	Symmetric and asymmetric stretching vibrations of methylene ($-CH_2$) groups
2853.4	2863.9	2853.6	2853.9	
1744.1	1743.5	1744.1	1743.9	Ester carbonyl (−C=O functional group of the triacylglycerols) stretch
1464.8	1464.6	1464.4	1464.5	Bending vibrations of the CH_3 and CH_2 aliphatic groups
1377.8	1377.6	1377.6	1377.8	Bending vibrations of the CH_3 groups
1240.6	1237.7	1237.7	1241.0	Stretching vibrations of the C−O ester groups
1160.2	1159.9	1160.1	1161.7	Stretching and vibration of C=O group
1097.5	1098.7	1098.0	1098.8	−CH bending and −CH deformation vibration of fatty acids
ND	991.2	ND	ND	*cis-trans-trans* conjugated double bonds of α-eleostearic acid
	964.4			
722.0	723.0	722.2	722.1	CH_2 rocking vibration and out of plane vibration of cis-disubstituted olefins

ND, Not detected.

qualities and are used for the formulation of inks, dyes, coatings and resins (Peabody, 2007). This study has shown that ^{13}C NMR can be used as a reliable non-destructive tool to quantify α-ESA in future further studies of oils like mungongo which contain this fatty acid.

Good correlation was observed when comparing the results from the three methods (^1H NMR, ^{13}C NMR and GC–MS) given in Table 4. Correlation coefficients, R^2, ranged from 0.8596–0.9956. The results of a one way ANOVA analysis, $F(2,45) = 0.0219, p > 0.05$, confirmed that there is no statistically significant difference between the results of the three methods. The ^1H NMR values for mungongo seed oil showed the greatest deviation from GC–MS values ($R^2 = 0.8596$) and from the ^{13}C NMR values ($R^2 = 0.8603$). This is due to the fact that the percentage of unsaturation due to α-ESA in mungongo oil was not identified by both ^1H NMR and GC–MS. Nevertheless, the statistical analyses all indicate that the results obtained from NMR spectra are similar to those obtained by traditional GC–MS analysis indicating that ^1H and ^{13}C NMR can be used on their own to obtain reliable data on the FA composition.

It is noted however that the GC–MS method is the most accurate of the methods for determination of FA composition as it detects all the individual fatty acids in the oil, whereas the NMR methods on the other hand, provide information about the fatty acid classes in the oil (i.e. totals of saturated, monounsaturated and polyunsaturated FAs). However, the NMR fingerprints can, at a glance, show up unusual fatty acids in the oil.

3.3.4. Information derived from FTIR spectra

As was the case with the ^1H and ^{13}C NMR, the FTIR spectra were essentially identical for morama, kengwe and mogose seed oils while the spectrum of mungongo oil was slightly different as shown in Fig. 5 where a representative spectrum for morama oil (I) is compared with the spectrum for mungongo oil (II). The spectrum for mungongo oil displayed an additional prominent peak at 991.2 cm^{-1} together with a weaker band at 964.4 cm^{-1}. IR absorptions for the four oils were assigned as given in Table 6 by comparison with literature (Lerma-García, Ramis-Ramos, Herrero-Martínez, & Simó-Alfonso, 2010). The additional peaks in mungongo oil were unequivocally ascribed to the characteristic doublet displayed by the presence of conjugated *cis-trans-trans* double bonds in α-ESA (Prashantha, Premachandra, & Amarasinghe, 2008; Sbihi et al., 2014). IR spectra revealed that three of the seed oils (morama, kengwe and mogose) contain similar fatty acids with no unusual fatty acids while mungongo oil contains α-ESA. Thus the IR spectrum provides a unique fingerprint of each oil which, can be used to detect the presence of unusual FAs.

4. Conclusions

This study has demonstrated that a single ^1H NMR spectrum of an oil can provide information both about vital chemical properties such as AV, SV, and IV and also provide information about the fatty acid classes in the oil. Furthermore, a ^{13}C NMR spectrum of the same sample can provide not only the same information on the FA classes but can also provide more detailed compositional and structural information about the fatty acids in the oil that cannot be attained from traditional methods of oil analysis. This aspect has been shown in this work by the detection and quantification of a-eleostearic acid in mungongo oil. In combination with the FTIR technique, ^1H NMR and ^{13}C NMR studies on oils can provide superior structural and compositional information about edible oils. These spectroscopic techniques are non-destructive and require very little sample quantities (one or two drops of the neat oil) with no tedious sample preparation and hence can save both time and resources. This study has therefore established a working protocol for seed oil characterization based on spectroscopic techniques.

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