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ORIGINAL PAPER

Compositional and Structural Studies of the Major and Minor Components in Three Cameroonian Seed Oils by GC–MS, ESI-FTICR-MS and HPLC

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Abstract The lipid components of three Cameroonian seed oils, ke tchock (*Aframomum arundinaceum*), njangsa (*Ricinodendron heudelotii*) and calabash nutmeg (*Monodora myristica*), have been investigated. Gas chromatography (GC)–mass spectrometry (MS) fatty acid (FA) analysis showed *M. myristica* seed oil to be dominated by linoleic (49.29%) and oleic (37.17%) acids; *R. heudelotii* was mainly linoleic (58.73%), followed by stearic (15.00%) and oleic (14.21%) acids; *A. arundinaceum* was predominantly oleic (65.76%) and palmitic (20.36%) acids. Electrospray ionization (ESI)-Fourier transform ion cyclotron resonance (FTICR)-MS analysis showed seven major triacylglycerol (TAG) classes for *M. myristica*, with C54:5, C54:4 and C54:6 dominating. *R. heudelotii* had eight major TAG classes with C54:8, C54:7 and C54:6 being most abundant. *A. arundinaceum* also had eight major TAG classes with C52:2, C54:3 and C50:2 dominating. ^{13}C nuclear magnetic resonance (NMR) analysis of the TAGs showed that both *sn*-1,3 and *sn*-2 positions were predominantly occupied by linoleoyl and oleoyl chains. High-performance liquid chromatography (HPLC) fluorescence detector (FLD) analysis showed that *M. myristica* contained only α- and β-tocopherols (195.40 and 73.95 μg/g, respectively), *R. heudelotii* contained mainly γ-tocopherol (289.40 μg/g), and *A. arundinaceum* had mainly γ- and β-tocopherols (236.78 and 124.93 μg/g, respectively). GC–MS analysis of the unsaponifiable matter showed that β-sitosterol was the most abundant phytosterol in all three seed oils. The absolute amounts of 4-desmethylsterols were 196.15, 608.71 and 362.15 μg/g for *M. myristica*, *R. heudelotii* and *A. arundinaceum* seed oils, respectively. These compositional and structural studies provide justification for the use of all three seed oils in food products.

Keywords *Aframomum arundinaceum* · *Ricinodendron heudelotii* · *Monodora myristica* · Fatty acids · Triacylglycerols · GC–MS · HPLC-FLD · ESI-FTICR-MS · Phytosterols · Tocopherols · Tocotrienols

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Introduction

Edible seeds are consumed by both animals and humans in all communities throughout the world. However, in less affluent communities, edible seeds are particularly important as major sources of nutrients such as proteins, fats and carbohydrates. In some communities, seeds are not only used for food, but also serve as sources of spices, cosmetic ingredients and as herbal medications. As part of our general objective of comprehensive characterization of seed oils in the sub-Sahara region of Africa, we report herein on the lipid profiles of the principal and minor components of three seed oils which are of considerable socio-economic importance in the West African region. In this work, seeds of calabash nutmeg (African nutmeg,

Monodora myristica), njangsa (*essessang*, *Ricinodendron heudelotii*) and ke tchock (*Aframomum arundinaceum*) were obtained from Cameroon, although the seeds are commonly grown throughout the West African region.

Calabash nutmeg, *M. myristica*, also commonly known as African nutmeg, belongs to the Annonaceae family. The plant also grows in the East African forests of Uganda and Western Kenya, where it is known as *lubushi*. The roasted seed is used for flavouring foods in West Africa. In addition to triglyceride oil, calabash nutmeg also contains a volatile, pleasant-smelling essential oil (1.3% w/w), which in West Africa is used as a spice and for medication. Indeed, the oil has been shown to have antimicrobial activity against *Bacillus subtilis*, *Candida albicans* and *Staphylococcus aureus* [1]. Ke tchock, *A. arundinaceum*, belongs to the Zingiberaceae family, which includes over 1,400 species distributed among 50 genera. The Zingiberaceae family is widespread in West and Central Africa, where five of the genera are indigenous. The genus *Aframomum* is made up of 80 species, about 25 of which are found in the Cameroon–Gabon region of West Africa. Seeds of the *Aframomum* species are used for a variety of different medical conditions such as dysmenorrhoea, broncho-pulmonary disorders, migraines, sexual asthenia etc., by different tribal groups in West Africa. Crude extracts of *Aframomum melegueta* have been shown to have potent bactericidal activities against several bacterial strains [2]. The sweet-tasting seeds of *A. arundinaceum* are roasted and eaten by the Bamileke people in Western Cameroon (personal communication). Njangsa is the name of the oily seed of a fast-growing tree, *Ricinodendron heudelotii*, which occurs throughout tropical West Africa. *Ricinodendron heudelotii* belongs to the Euphorbiaceae family. The tree is called by different names by different communities, such as *essessang* in Cameroon, *munguella* in Angola, *wama* in Ghana and *okhuen* in Nigeria [3]. Njangsa seeds have been reported to be rich in protein and oil but low in carbohydrates; the kernels are crushed into paste and used both as a thickener and as a spice in West African dishes [3, 4].

Even though the three seeds in this study are widely traded in the West African region for use as food, spices and in a limited way for medicinal purposes, their extracted oils have so far not been of much value to the people in the region. However, with the current ever-increasing world demand for vegetable oils for both food and nonfood uses, rich oil-bearing plants such as *Ricinodendron heudelotii* and *Aframomum arundinaceum* (Table 1) are potentially important natural resources on account of their seed oils, which must be studied and evaluated for their potential commercial uses. There are some literature reports about the fatty acid composition of the seed oil from njangsa *R. heudelotii* [4, 5], but detailed compositional information about the different lipid classes in all three seed oils is not available in the scientific literature. Thus, this study was undertaken to determine the composition of the saponifiable matter (the principal components), i.e. the fatty acids and triacylglycerols, and also to determine the composition of the major components of the unsaponifiable matter (UM), i.e. the phytosterols, tocopherols and the tocotrienols, in the seed oils of calabash nutmeg (*Monodora myristica*), njangsa (*Ricinodendron heudelotii*) and ke tchock (*Aframomum arundinaceum*).

Experimental Procedures

Materials

Ke tchock seeds (*A. arundinaceum*, 200 g), njangsa seeds (*R. heudelotii*, 800 g) and African nutmeg (*M. myristica*, 500 g) were obtained from an open market in the City of Dschang in Cameroon, West Africa.

Extraction: Solvents and Reagents

All solvents and reagents used in determinations of chemical parameters and other experiments, unless otherwise stated, were of analytical grade, while solvents for HPLC and high-resolution MS were of HPLC grade. The solvents were obtained from Rochelle Chemicals (SA), BDH (Merck Chemicals Pty. Ltd.), Riedel-de Haën (Sigma–Aldrich, GmbH) or JT Baker Chemical Co. (Phillipsburg, NJ, USA). The seeds and nuts were manually dehulled and, after thorough cleaning, macerated using a Warring blender (Gateshead, UK). The resulting powders were extracted with a 3:1 (v/v) solvent system of *n*-hexane and 2-propanol for about 8 h.

Physicochemical Properties

The bulk physical properties (Table 1) were determined according to standard International Union of Pure and Applied Chemistry (IUPAC) methods for analysis of oils

Table 1 Yield, refractive index and relative density of some Cameroonian seed oils

Sample	Yield (%)	Refractive index at 25 °C	Density (g/cm^3) at 20 °C
M. myristica	40.5	1.486 ± 0.001	0.926 ± 0.002
R. heudelotii	43.3	1.501 ± 0.001	0.961 ± 0.001
A. arundinaceum	10.1	1.499 ± 0.001	0.990 ± 0.001

Table 2 Percentage composition of lipid classes in the test seed oils as estimated from adsorption column chromatography

Sample	Hydrocarbons	TGAs + FFAs	Cholest esters	Cholest	DAGs	MAGs	Glylipid	Phoslipid
M. myristica	1.72	86.26	1.01	2.14	2.37	2.74	0.30	3.96
R. heudelotii	4.44	78.70	2.95	1.37	1.33	1.70	1.64	7.83
A. arundinaceum	1.96	86.50	1.05	0.94	1.46	3.43	0.98	3.68

Values are means of two determinations

FFAs free fatty acids, *DAGs* diacylglycerols, *MAGs* monoacylglycerols

and fats [6]. Unless otherwise stated, all experiments were conducted in triplicate.

Composition of Lipid Classes

Analysis of the lipid classes (Table 2) in each oil sample was carried out by adsorption column chromatography, using Florisil (7% water w/w; Saarchem Pty. Ltd., Muldersdrift, Republic of South Africa) and gradient elution using *n*-hexane, mixtures of *n*-hexane/diethyl ether, diethyl ether, methanol and acetone [7].

Separation of Acylglycerols

Triacylglycerols (TAGs), diacylglycerols with free fatty acids (DAGs + FFAs) and monoacylglycerols (MAGs) in the oil samples were further separated by gradient elution on silica gel (5% H_2O w/w; Saarchem Pty. Ltd.) using benzene (100%), benzene:diethyl ether (9:1) and diethyl ether (100%), respectively [7].

Fatty Acid Composition: Sample Preparation

For analysis of fatty acid methyl esters (FAMEs), the oil samples (2 g each) were transesterified by refluxing in anhydrous methanol that contained ethanoyl chloride to yield FAMEs, and were stored under nitrogen and later used for GC–MS and ^{1}H NMR analyses. For analysis of picolinyl esters, the method of Destaillats et al. [8] was adopted in this preparation. Oil samples (2 g) were dissolved in anhydrous dichloromethane and allowed to react with a mixture of potassium *tert*-butoxide in tetrahydrofuran and 3-hydroxymethylpyridine at room temperature for 2 min. Sodium bicarbonate solution was then added, and the organic layer was extracted, dried with anhydrous sodium sulphate and stored under nitrogen for GC–MS analysis.

Instrumentation and Separation Conditions

FAMEs and picolinyl esters in dichloromethane were analysed in a ThermoQuest Voyager GC–MS coupled to ThermoQuest Trace GC 2000 series (San Jose, CA, USA). Separation was effected on a DB-5MS capillary column (0.25 μm × 0.25 mm × 30 m; J & W Scientific, CA, USA) consisting of 5% phenyl-methylpolysiloxane stationary phase. Ultra-high-purity (UHP) helium was used as carrier gas at flow rate of 1 mL/min. Injection temperature was 220 °C, while interface temperature was 300 °C. The initial temperature of the oven was 60 °C, held for 1 min, and then ramped to 200 °C at rate of 15 °C/min. It was then held for 1 min before the second ramp at rate of 5 °C/min to 300 °C. It was then held isothermally for 25 min. These conditions were suitable for *M. myristica* and *R. heudelotii* FAMEs which did not contain 18:3 FA. However, for *A. arundinaceum* FAMEs, separation was carried out using a CP-WAX 52 CB capillary column (0.25 μm × 0.25 mm × 30 m) from Chrompak International BV (Middelburg, The Netherlands) consisting of 100% polyethylene glycol phase. Injection temperature of 200 °C and interface temperature of 240 °C were used. UHP helium gas was also used as carrier gas at the same flow rate of 1 mL/min. The initial temperature of the oven, 60 °C, was held for 1 min and then ramped to 240 °C at rate of 5 °C/min, after which it was held isothermally for 10 min.

Nuclear Magnetic Resonance Analysis

^{1}H NMR spectra of the FAMEs, dissolved in $CDCl_3$, were acquired at 300 MHz using a Bruker Avance DPX 300 spectrometer. The relative compositions of the saturated, monounsaturated, diunsaturated and linolenic FAs together with their average chain lengths were determined from the relative sizes of the integrals of the signals for the allylic, diallylic and methyl protons using Holmback's equations [2, 9].

Analysis of Triacylglycerols (TAGs)

For high-resolution mass spectrometric analysis, TAG extracts (0.25 mg) were dissolved in methanol, and the methanolic solutions were introduced continuously via a syringe pump into an electrospray ionization source

(APPOLO) on a high-resolution FTICR mass spectrometer (Bruker Daltonics Apex III) at flow rate of 2 μL/min. All data were acquired with 512 k data points and zero-filled to 2,048 k by averaging 32 scans before application of Fourier transform and magnitude calculation. After acquisition, mass spectral raw data were post-processed using Bruker XMASS acquisition and processing software (version 6.12) according to full-width at half-maximum (FWHM) criteria and taken as input data for calculations. Molecular formulas were assigned by use of the software above. Molecular formulas were limited to 200 ^{12}C, 300 ^{1}H, 30 ^{16}O and 1 ^{23}Na atoms.

^{13}C NMR Analysis of Triacylglycerols

Standard compounds (tri-palmitin, tri-olein, and tri-linolein) were obtained from Fluka and used without further purification. About 200 mg sample was dissolved in deuterated chloroform ($CDCl_3$), and the solution (700 μL) was placed in a 5-mm-diameter NMR tube. ^{13}C NMR spectra were recorded on a Varian Mercury 400 spectrometer operating at 100.6 MHz. ^{13}C NMR spectra for quantitative analyses were recorded with spectral width of 1,300 Hz, 64 k data points, pulse repetition time of 60 s, 90° flip angle and full proton decoupling. Two hundred scans were accumulated per spectrum. The free induction delays (FIDs) were zero-filled to 128 k prior to Fourier transform, resulting in digital resolution of 0.02 Hz/point. Line broadening of 0.05 Hz was used for the exponential weighting. Peaks were assigned by spiking standard compounds into the real test samples.

Analysis of Tocopherols and Tocotrienols

Saponification

Phane oil samples for analysis of tocopherols and tocotrienols were saponified according to the method reported by Panfili [10]. Oil sample (2.0 g) in an amber screw-capped bottle was flushed with nitrogen, and 10 M potassium hydroxide (2.0 mL) was added. Absolute ethanol (2.0 mL) and 0.2 M sodium chloride solution (2.0 mL) were then added, and the mixture flushed again with nitrogen. Ethanolic pyrogallol (0.5 M, 5.0 mL) was finally added as antioxidant and then flushed with nitrogen. The bottle was placed in a 70 °C water bath and mixed after every 5 min for 45 min, after which the bottle was cooled in ice and 0.2 M sodium chloride solution (15.0 mL) was added. The suspension was extracted twice with 9:1 (v/v) *n*-hexane-ethyl acetate (15.0 mL). The combined organic layer was evaporated to dryness. The dry residue was dissolved in *n*-hexane-isopropanol (99:1) and passed through a silica Chromabond™ solid-phase extraction (SPE) cartridge. The filtrate was dried and re-dissolved in *n*-hexane (10.0 mL). This was then appropriately diluted prior to HPLC-postcolumn fluorescence derivatization (FLD) analysis.

HPLC-FLD Analysis

A Merck HPLC system (Darmstadt, Germany) consisting of a Merck-Hitachi HPLC L-7100 Intelligent pump, a Rheodyne injector fitted with a 5 μL loop and a Merck L-7480 fluorescence detector was used for tocopherol and tocotrienol analysis. The analysis was achieved using normal-phase HPLC on Nucleosil 100-5 column (5 μm × 4 mm × 25 cm) from Macherey–Nagel (Düren, Germany). The mobile phase was *n*-hexane-isopropanol (99.7:0.3 v/v) at flow rate of 1.0 mL/min. Fluorometric detection of all peaks was performed at excitation wavelength of 295 nm and emission wavelength of 330 nm. Tocopherol peaks were identified and quantified against authentic tocopherols used as external standards. For each extract, quantitative analysis was performed in triplicate.

Analysis of Phytosterols

Saponification of Oil Sample

To oil sample (2.5 g) in 250-mL round-bottomed flask was added 0.5 M ethanolic potassium hydroxide solution (25.0 mL), and the mixture was refluxed for 1 h. Water (100.0 mL) was then added down the condenser, and the mixture was extracted with diethyl ether (100 mL × 3) in a 500-mL separatory funnel. The combined ether solution was washed with water (40.0 mL × 3) and then washed successively with 0.5 M aqueous potassium hydroxide (40.0 mL), distilled water (40.0 mL) and repeatedly with aqueous 0.5 M KOH (40.0 mL) before finally washing with more 40.0 mL portions of distilled water until the washings were neutral to phenolphthalein indicator. The ether solution was then dried under anhydrous magnesium sulphate, and the solvent evaporated by distillation on a water bath to release the unsaponifiable matter.

Acetylation

The whole unsaponifiable matter (UM) was acetylated according to the method described by Wilson et al. [11]. UM (2.0 mg) was dissolved in 2:1 (v/v) pyridine-acetic anhydride solution (600 μL), and the reaction was allowed to proceed at room temperature overnight. Excess reagents

were then removed by slight warming under a stream of nitrogen gas. The mixture was re-dissolved twice in dichloromethane, and the solvent removed in a stream of nitrogen. The acetylated products were transferred to a sample vial and dissolved again with dichloromethane, flushed with nitrogen and stored at 4 °C for GC–MS analysis.

Analysis by GC–MS

The acetylated lipids in dichloromethane were analysed in a ThermoQuest Voyager GC–MS coupled to ThermoQuest Trace GC 2000 series (San Jose, CA, USA). Xcalibur version 1.3 software from Thermo Fischer Scientific (San Jose, CA, USA) was used to process the data. A DB-5MS capillary GC column (0.25 μm × 0.25 mm × 30 m) from J & W Scientific (CA, USA) was used for separation, and UHP helium was used as carrier gas at flow rate of 1 mL/min. The injection temperature was 220 °C, while the interface temperature was 300 °C. The initial temperature was 60 °C, held for 1 min and then ramped to 200 °C at rate of 15 °C/min. It was then held for 1 min before the second ramp at the rate of 5 °C/min to 300 °C. This was then held isothermally for 25 min.

SPE Fractionation of Sterols

The SPE method of Azadmard-Damirchi and Dutta [12] was modified for this determination. A 500 mg, 3 mL silica solid-phase extraction (SPE) cartridge from Macherey–Nagel (Düren, Germany) was used per sample. The cartridge was attached to a Macherey–Nagel vacuum manifold (Düren, Germany). Waste tubes were positioned to collect the conditioning solvent. The cartridges were conditioned by passing *n*-hexane (5.0 mL) through them. After conditioning, the *n*-hexane was discarded and a clean test-tube was positioned to collect the phytosterol fraction. UM sample aliquots (2.0 mg) in 2 mL *n*-hexane were then loaded onto the cartridges. The UM was then washed with *n*-hexane-ethyl acetate (10.0 mL), 99:1(v/v), to remove any non-sterol compounds. Vacuum was applied at 5 mmHg for each elution. This fraction was discarded after checking by analytical thin-layer chromatography (TLC) that it did not contain compounds of interest. 4-Methylsterols were then eluted with *n*-hexane-ethyl acetate (10.0 mL), 99:1(v/v), followed by 14.0 mL 97:3 (v/v) *n*-hexane-ethyl acetate. Prior to eluting pure 4-desmethylsterols, additional *n*-hexane-ethyl acetate (2.0 mL), 97:3(v/v), was used to wash the cartridges. 4-Desmethylsterols were then eluted with *n*-hexane-ethyl acetate (25.0 mL), 97:3(v/v). The fractions were dried, spiked with 10 μg 5α-cholestane as internal standard and acetylated prior to GC-MS analysis. The components were quantified against 5α-cholestane.

Data Analysis

Experiments for determination of physicochemical parameters, HPLC-FLD for tocols and GC–MS analysis of fatty acid composition were carried out in triplicate, and results are expressed as mean values ± standard deviation (SD). NMR and high-resolution MS results are expressed as values for single runs.

Results and Discussion

The percent oil yields (w/w) from the seeds of njangsa (*R. heudelotii*) and calabash nutmeg (*M. myristica*), 43.3% and 40.5%, respectively, as shown in Table 1, compare very favourably with the reported oil yields for rapeseed (40–45%) and groundnut (45–50%). Thus, *R. heudelotii* and *M. myristica* seeds can be classified as high-oil-bearing seeds, whose oils merit study to collect detailed information on the compositional and structural properties of their lipid classes to evaluate the oils for potential food and non-food uses. Even though ke tchock seeds, *A. arundinaceum*, cannot be described as oil bearing, having oil content of 10.1%, inclusion of its oil in this study is considered worthwhile on account of its wide use in the West African region.

The refractive indices and relative densities given in Table 1 for the test seed oils are generally higher than corresponding values for typical household unsaturated vegetable oils such as soybean (1.466–1.470, 0.919–0.925), sunflower (1.467–1.470, 0.922–0.927), groundnut (1.460–1.465, 0.914–0.917) and olive (1.468–1.471, 0.910–0.916) [13], therefore indicating that the test seed oils from *A. arundinaceum*, *R. heudelotii* and *M. myristica* are highly unsaturated, since refractive indices for oils and fats usually increase with increasing degree of unsaturation.

The composition of lipid classes in the test seed oils, as estimated from adsorption column chromatography, is given in Table 2, which shows that all three seed oils were composed mainly of neutral lipids, i.e. triacylglycerols. The low content of diacylglycerols (DAG) and monoacylglycerols (MAG) indicates that lipase hydrolysis had been minimal in all three seed oils. The phospholipid content in the test oils appears significant, with *R. heudelotii* having the highest content at 7.83% and *M. myristica* and *A. arundinaceum* containing 3.96% and 3.68%, respectively. The implication is that, for commercial production, all three seed oils will have to be degummed in the refining process.

Triacylglycerols (TAGs) as a class of lipids constitute the bulk of the seed oils, as shown above. Thus, to gain in-depth understanding of the nature of the seed oils would require detailed study of the composition and structure of

the TAG molecules and their component acyl chains, the fatty acids (FAs). In this study the FAs were analysed by GC–MS as fatty acid methyl esters (FAMEs) and also as fatty acid picolinyl esters (FAPEs). The FAMEs were also analysed by ^{1}H NMR, which gave estimates of the fatty acid classes in the oils to complement the GC–MS analysis of the FA compositions. The GC–MS analysis of the FAMEs provided data on the molecular weight as well as the retention times of the respective FAs, whilst the GC–MS analysis of the FAPEs provided distinct diagnostic ions in the mass spectra that facilitated the location of the positions of the double and triple bonds in the acyl chains [14] as explained in our earlier publication [15].

The FA compositions of the test seed oils as determined from the GC–MS analysis are given in Table 3, which shows the percent total unsaturated FAs ranging from 72.94% for *R. heudelotii* through 73.12% for *A. arundinaceum* to 86.46% for *M. myristica*. These high levels of total unsaturation corroborate the rather high refractive indices and the relative densities that were determined for the seed oils as given in Table 1. The FA profile for *M. myristica*, which is dominated by linoleic acid (9c, 12c-18:2; 49.29%) and oleic acid (9c-18:1; 37.17%), is quite similar to the FA profiles for groundnut oil (oleic acid 36.4–67.1%, linoleic acid 14.0–43.0%), corn oil (oleic acid 20–42.2%, linoleic acid 39.4–65.6%) and sesame oil (oleic acid 35.5–44.1%, linoleic acid 40.3–50.8%) [16], all of which have linoleic and oleic acids as the dominant FAs.

Table 3 Percentage FA composition by GC–MS analysis of the FAMEs of the test seed oils

Fatty acid	*M. myristica*	*R. heudelotii*	*A. arundinaceum*
12:0	ND	ND	ND
14:0	ND	ND	3.63 ± 0.03
9c-16:1	ND	ND	3.50 ± 0.07
16:0	9.00 ± 0.04	12.05 ± 0.30	20.36 ± 0.12
17:0	ND	ND	ND
9c, 12c, 15c-18:3	ND	ND	0.78 ± 0.01
9c, 12c-18:2	49.29 ± 0.09	58.73 ± 0.11	3.08 ± 0.01
9c-18:1	37.17 ± 0.07	14.21 ± 0.13	65.76 ± 0.25
11c-18:1	ND	ND	ND
18:0	4.08 ± 0.10	15.00 ± 0.32	2.89 ± 0.02
20:0	0.47 ± 0.02	ND	ND
13-22:1	ND	ND	ND
22:0	ND	ND	ND
15-24:1	ND	ND	ND
24:0	ND	ND	ND
Total unsaturated	86.46	72.94	73.12
Total saturated	13.55	27.05	26.88

ND not determined

Combining its FA profile with its high oil yield of 40.5% (w/w), *M. myristica* seed oil should be considered as an alternative or additional source of vegetable oil for home consumption and also for non-food uses such as biodiesel for the West African region.

As shown in Table 3, GC–MS analysis of the FAMEs from *R. heudelotii* revealed only four major FAs, dominated by linoleic acid (58.73%), followed by stearic acid (15.00%), oleic acid (14.21%) and palmitic acid (12.05%). This FA profile for *R. heudelotii* is quite similar to that of manketti seed oil *Ricinodendron rautanenii*, which we reported in our previous paper [15]. Again, as was the case for *R. rautanenii*, we did not find α-eleostearic acid (9Z, 11E, 13e-18:3) in our analysis of *R. heudelotii* seed oil, in contrast to Kapseu et al. [17], who in 1995 found α-eleostearic acid when they analysed an oil sample from *R. heudelotii* seeds also from Cameroon. Our findings agree more with those of Manga et al. [18], who in 2000 also did not find α-eleostearic acid but found a rather large amount of linoleic acid (60%) in *R. heudelotii* seed oil, also obtained from Cameroon. The FA composition found in this work rather resembled those of corn and sunflower oils, and here again this makes the seed oil from *R. heudelotii*, with yield of 43.3%, an attractive candidate for commercial exploitation as an additional source of vegetable oil for the West African region.

The FA composition of the seed oil from *A. arundinaceum*, presented in Table 3, was dominated by oleic acid (65.76%), and rather like olive oil, it contained significant amount of palmitoleic acid (9-16:1, 3.50%). The other FAs were palmitic acid (20.36%), myristic acid (14:0, 3.63%), linoleic acid (3.08%), stearic acid (2.89%) and α-linolenic acid (9c,12c,15c-18:3, 0.78%). Even though the seeds of *A. arundinaceum* cannot be described as high oil-bearing on account of its low yield of 10.1% (w/w), the FA profile of the oil confers good nutritive quality to the seed and hence justifies its use as a relish among communities in Western Cameroon.

In our search to gain in-depth information about the major components of the seed oils, we sought to find out how the FAs were combined with glycerol to form the principal components, the triacylglycerols (TGAs), of the test seed oils. As mentioned elsewhere, whilst the composition of intact TAGs can serve as a fingerprint in confirming the authenticity of individual oils [19], the regiospecific distribution of the FAs on the glycerol backbone has been shown to impact on human health and nutrition [20]. In this work we attempted to determine the composition of intact TAGs in the seed oils and their regiochemistry by a combination of high-resolution mass spectrometry-Fourier transform ion cyclotron resonance interfaced with an electrospray ionization source, ESI-FTICR, and ^{13}C NMR spectrometry, respectively. The

essential details of the two techniques were described in our recent publication [15].

The percent composition of the TAG classes, C*x*:*n*, where *x* denotes the total number of carbon atoms and *n* stands for the number of double bonds in each TAG class, as determined by the ESI-FTICR mass spectrometric technique, is shown in Table 4. Table 5 gives the percent distribution of the saturated, oleoyl and linoleoyl/linolenyl acyl chains in the *sn*-1,3 and *sn*-2 positions in the glycerol backbone. As can be seen from Table 4, *M. myristica* seed oil had seven major TAG classes, of which the dominant classes were C54:5 (26.98%), C54:4 (19.77%) and C54:6 (19.22%), a TAG composition very much in agreement with the FA composition of the oil, given in Table 3, which shows C18 FAs as the predominant acyl chains in *M. myristica* seed oil. Table 5 shows that linoleoyl and oleoyl acyl chains predominantly occupied the *sn*-1,3 positions to a combined level of 79.5% and occupied the *sn*-2 position to a combined level of 100%, with the linoleoyl acyl chain being the chief occupant (72.51%) at this position on the glycerol backbone of the major TAG classes in *M. myristica* seed oil.

As shown in Table 4, the seed oil from *R. heudelotii* contained eight major TAG classes, of which the dominant classes were C54:8 (31.22%), C54:7 (19.16%) and C54:6 (12.03%). Even though the GC–MS analysis of the FAMEs from *R. heudelotii* seed oil did not detect the presence of 18:3 FAs, the large amounts of C54:8 and C54:7 TAG classes detected in the seed oil of *R. heudelotii* by the ESI-FTICR mass-spectrometric technique point to the presence of 18:3 FAs in the oil. We encountered a similar situation in our earlier work on seed oil from *R. rautanenii* [15] which we have already mentioned above. Once again we explain the anomaly as due to the instability of α-eleostearic acid, which is susceptible to undergo transformations during chemical processing as mentioned by Kapseu and Tchiegang [17]. The regiospecific distribution of the acyl chains on the glycerol backbone of the TAGs, according to Table 5, was linoleoyl 100% at the *sn*-2 position and 81.56% at the *sn*-1,3 positions, whilst saturated FAs occupied the remaining *sn*-1,3 positions (18.44%). It would appear that the oleoyl acyl chain, which constituted 14.21% of the fatty acid composition of *R. heudelotii* seed oil, was not detected at either *sn*-2 or the

Table 4 Assignment of major mass peaks of triacylglycerols in positive-ion ESI-FTICR mass spectra of test oil fractions

Observed mass (*m/z*)	Mass ion $[M + Na]^+$	Theoretical mass	TAG CN:DB	Relative intensity	Composition (%)
M. myristica					
877.7271	$[C_{55}H_{98}O_6Na]^+$	877.7256	C52:4	0.3434	9.277
879.7435	$[C_{55}H_{100}O_6Na]^+$	879.7412	C52:3	0.3941	10.63
881.7569	$[C_{55}H_{102}O_6Na]^+$	881.7569	C52:2	0.1917	5.17
901.7272	$[C_{57}H_{98}O_6Na]^+$	901.7256	C54:6	0.7125	19.22
903.7399	$[C_{57}H_{100}O_6Na]^+$	903.7412	C54:5	1.0000	26.98
905.7564	$[C_{57}H_{102}O_6Na]^+$	905.7569	C54:4	0.7327	19.77
907.7720	$[C_{57}H_{104}O_6Na]^+$	907.7725	C54:3	0.3323	8.96
R. heudelotii					
873.6982	$[C_{55}H_{94}O_6Na]^+$	873.6943	C52:6	0.17875	5.54
875.7144	$[C_{55}H_{96}O_6Na]^+$	875.7099	C52:5	0.3177	9.92
877.7236	$[C_{55}H_{98}O_6Na]^+$	877.7256	C52:4	0.944	2.95
879.7443	$[C_{55}H_{100}O_6Na]^+$	879.7412	C52:3	0.0327	9.93
897.695	$[C_{57}H_{94}O_6Na]^+$	897.6943	C54:8	1.0000	31.22
899.7100	$[C_{57}H_{96}O_6Na]^+$	899.7099	C54:7	0.6138	19.16
901.7258	$[C_{57}H_{96}O_6Na]^+$	901.7256	C54:6	0.3853	12.03
903.7412	$[C_{57}H_{100}O_6Na]^+$	903.7412	C54:5	0.2968	9.26
A. arundinaceum					
825.69369	$[C_{51}H_{94}O_6Na]^+$	825.6943	C48:2	0.1342	4.38
827.70872	$[C_{51}H_{96}O_6Na]^+$	827.7099	C48:1	0.1472	4.80
853.72462	$[C_{53}H_{98}O_6Na]^+$	853.7256	C50:2	0.4201	13.71
855.73937	$[C_{53}H_{100}O_6Na]^+$	855.7412	C50:1	0.3556	11.60
879.74083	$[C_{55}H_{100}O_6Na]^+$	879.7412	C52:3	0.1598	5.21
881.75559	$[C_{55}H_{102}O_6Na]^+$	881.7569	C52:2	1.0000	32.63
905.75438	$[C_{57}H_{102}O_6Na]^+$	905.7569	C54:4	0.0799	2.61
907.77165	$[C_{57}H_{104}O_6Na]^+$	907.7725	C54:3	0.7679	25.05

Table 5 Percentage positional distribution of fatty acyl chains on the glycerol backbone of triacylglycerols in test oil samples as determined by ^{13}C NMR

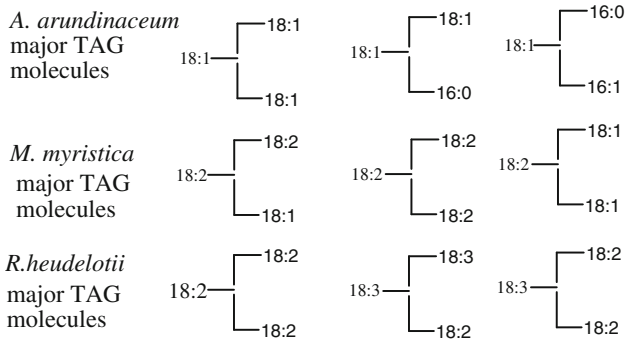
Sample	*sn*-1,3 composition			*sn*-2 composition		
	Saturated	Oleoyl	Linoleoyl	Saturated	Oleoyl	Linoleoyl
M. myristica	20.63	32.52	46.85	ND	27.51	72.49
R. heudelotii	18.44	ND	81.56	ND	ND	100
A. arundinaceum	40.50	59.50	ND	ND	100	ND

sn-1,3 positions. This anomaly could be attributed to the poor resolution of the oleoyl and linoleoyl signals in the ^{13}C NMR spectrum, a situation which was observed in other cases when one signal was much more prominent than the other [15].

The positive-ion ESI-FTICR-MS spectrum showed that *A. arundinaceum* seed oil was made up of eight major TAGs, of which the most abundant were C52:2 (32.63%), C54:3 (25.05%) and C50:2 (13.71%). This composition of the TAG classes was very consistent with the FA composition, which showed oleic acid (65.76%) and palmitic acid (20.36%) as the most abundant FAs in *A. arundinaceum* seed oil. The regiospecific distribution of the acyl chains, as shown in Table 5, was that the oleoyl chain occupied 100% of the *sn*-2 and 59.50% of the *sn*-1,3 positions, while saturated acyl chains occupied 40.50% of the *sn*-1,3 positions. Once again, because of the preponderance of the oleoyl chain, there was poor resolution between the signals of the oleoyl and the other minor acyl chains in the ^{13}C NMR spectrum, and hence the minor chains were not detected, as shown in Table 5.

Combining Tables 3, 4 and 5 we can predict the structures shown in Scheme 1 as probably some of the most abundant TAG molecules in the test seed oils.

In agreement with Table 5 all these structures have the *sn*-2 position occupied by unsaturated acyl chains, a structural feature which is consistent with oils of plant origin [21]. As far as we are aware this is the first report on the compositional and structural studies of the TAGs in seed oils of *R. heudelotii*, *M. myristica* and *A. rautanenii*.



Scheme 1 Major TAG molecules in the test seed oils

In recent times, information about the qualitative and quantitative composition of the minor components of vegetable oils, i.e. the components of the unsaponifiable matter, has become essential in the evaluation of seed oils for food uses and as nutraceuticals. The major components of the unsaponifiable matter, the phytosterols, tocopherols and tocotrienols, have become vital markers for the authenticity of individual vegetable oils. Besides, the health benefits of tocopherols and tocotrienols as antioxidants and the health benefits of certain phytosterols as cancer-preventing and total plasma cholesterol-lowering biomolecules are well documented [22, 23]. As described in the experimental section, the tocopherols and tocotrienols were determined by carefully extracting the unsaponifiable matter from each oil sample and subjecting it to normal-phase HPLC (with fluorescence detection) analysis (HPLC-FLD). Normal-phase HPLC was used in this case as it is capable of resolving α- and *β*-tocopherols and tocotrienol homologues. The tocols were eluted in order of increasing polarity, corresponding with the decreasing order of methylation on the chromanol ring of the tocols, i.e. α-, *β*-, *γ*- and *δ*-homologues [24]. The quantitative analysis was performed using calibration curves. A linearity test was carried out over the concentration range of 1 to 5 ppm, from which regression analysis of the plot of area response versus concentration for each isomer gave an excellent relationship with correlation coefficients of 0.9997 for α-tocopherol, and 1.0000 each for *β*-, *γ*- and *δ*-tocopherols.

The most abundant tocol component found in *R. heudelotii* seed oil was *γ*-tocopherol at the level of 289.40 μg/g, as given in Table 5. This was followed by *δ*-tocopherol at 5.90 and α-tocopherol at 0.46 μg/g, making a total tocol content of 295.8 μg/g, which is within the typical tocol range of 200–800 μg/g for vegetable oils. Only two tocol components were found in seed oil of *M. myristica*, i.e. α-tocopherol (195.40 μg/g) and *β*-tocopherol (73.95 μg/g), making a total of 269.0 μg/g. Seed oil of *A. arundinaceum* had three tocol components consisting of *γ*-tocopherol (236.78), *β*-tocopherol (124.93) and α-tocopherol (15.23 μg/g), making a total tocol content of 376.36 μg/g.

Table 6 Tocopherol and tocotrienol content (μg/g, mean ± SD) of selected test oils obtained by HPLC-FLD

Sample	α-T	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	Unknown	Total tocol
M. myristica	195.40 ± 15.46	73.95 ± 6.52	ND	ND	ND	ND	ND	[a]	269.0 ± 10.8
R. heudelotii	0.46 ± 0.21	ND	289.40 ± 3.16	5.90 ± 0.50	ND	ND	ND	ND	295.8 ± 3.5
A. arundinaceum	15.23 ± 2.41	124.93 ± 21.51	236.78 ± 17.23	ND	ND	ND	ND	[a]	376.9 ± 36.6

No δ-T3 was detected in all samples

[a] Some signals could not be assigned and were not quantified

Thus, even though no tocotrienol was found in any of the three seed oil samples, they all contained a fair amount of total tocopherols to protect them from oxidative attack, hence ensuring good shelf-life.

The composition of the phytosterol components of the test seed oils was determined in two separate experiments. In the first experiment the relative percent composition of all the phytosterols in the acetylated total unsaponifiable matter was determined using GC–MS. In this analysis the peaks in the region where phytosterols eluted were selected and the sterols were identified by comparing their retention times against reference spectra of authentic standards. Calculation of the relative composition of each sterol was done by expressing the peak area as a ratio to the total peak area of all identifiable sterol peaks in the sample. Of the three classes of phytosterols, i.e. 4-desmethylsterols, 4-monomethylsterols and 4,4-dimethyl sterols, Table 6 shows that, in all three test oils, 4-desmethylsterols were the predominant class of phytosterols. Indeed, the phytosterol content in *A. arundinaceum* seed oil was 100% made up of 4-desmethylsterols, while 4-desmethylsterols constituted about 90% and 70% of the phytosterol content in *R. heudelotii* and *M. myristic* seed oils, respectively. Also in agreement with literature reports, β-sitosterol was the single most abundant phytosterol in all three seed oils [25]. There was no 4-monomethylsterols detected in any of the test seed oils, however *A. arundinaceum* seed oil contained cycloartenol (2.04%) and 24-methylene-cycloartanol (3.73%), which were the only 4,4-dimethysterols found in the test seed oils (Table 7).

The second experiment for the phytosterol analysis involved determination of the absolute amount of each 4-desmethylsterol in each seed oil. The absolute amounts of 4,4-dimethylsterols and 4-monomethylsterols were not determined due to lack of authentic standards. As described in the experimental section, a modified Damirchi SPE method was employed to pre-fractionate the unsaponifiable matter prior to acetylation and GC–MS analysis. 5α-Cholestane was used as internal standard [12]. The absolute amount of each identifiable 4-desmethylsterol was calculated as micrograms per gram of the seed oil by using the relationship, 4-desmethylsterol $= (A_z \cdot m_{is})/(A_{is} \cdot m)$, where A_z = start peak area, A_{is} = internal standard peak area, m_{is} = mass (μg) of internal and m = mass of oil in g weighed for the analysis [26].

Table 7 Relative percent composition (mean ± SD) of phytosterols in the test oil samples obtained by GC–MS

Compound	*M. myristica*	*R. heudelotii*	*A. arundinaceum*
Cholesterol	ND	ND	ND
22-Dehydrocholesterol	ND	ND	ND
24-Me-cholesterol	ND	ND	4.93 ± 0.41
Campesterol	13.11 ± 1.10	7.76 ± 0.07	14.47 ± 0.57
Campestanol	ND	ND	ND
Stigmasterol	38.28 ± 1.93	ND	6.70 ± 0.36
Stigmastanol	ND	ND	ND
Sitosterol	40.31 ± 3.32	78.59 ± 0.03	37.65 ± 0.5
β-Amyrin	ND	ND	ND
Δ^5-Avenasterol	8.3 ± 0.29	9.49 ± 0.03	10.54 ± 0.42
Sitostanol/Δ^5-avenasterol	ND	ND	ND
24-Methyllophenol	ND	ND	ND
Cycloartenol	ND	0.68 ± 0.05	2.04 ± 0.03
Lupeol	ND	ND	ND
22-Dihydrospinasterol	ND	0.61 ± 0.04	2.63 ± 0.14
Δ^7-Avenasterol	ND	2.88 ± 0.01	ND
24-Me-cycloartanol	ND	ND	3.73 ± 0.13
Citrostadienol	ND	ND	ND
Others	ND	ND	3.08 ± 0.08

The results of the determination of the absolute amounts of 4-desmethylsterols in the seed oils were in complete agreement with the results of the determination of the relative composition of phytosterols in confirming the order of abundance of the 4-desmethylsterols in the seed oils. β-Sitosterol was the most abundant sterol in all three seed oils, with *R. heudelotii* seed oil containing the largest amount (527.23 μg/g), followed by *A. arundinaceum* (181.32 μg/g) and *M. myristic* (89.88 μg/g) (Table 8). The second most abundant sterol in the oil of *R. heudelotii* was Δ^5-avenasterol (44.87 μg/g), followed by stigmasterol (29.36 μg/g). Campesterol and 22-dihydrospinasterol were present in small amounts, i.e. 4.43 and 2.82 μg/g, respectively, making the total 4-desmethylsterol content in *R. heudelotii* seed oil as 608.71 μg/g. This level of phytosterol content compares quite favourably with that of

Table 8 Content of 4-desmethylsterols (μg/g of oil) in test oil samples determined by GC–MS after SPE fractionation

Compound	*M. myristica*	*R. heudelotii*	*A. arundinaceum*
Cholesterol	ND	ND	ND
22-Dehydrocholesterol	ND	ND	ND
24-Methylenecholesterol	ND	ND	30.63
Campesterol	28.44	4.43	76.53
Campestanol	ND	ND	ND
Stigmasterol	59.14	29.36	33.28
Sitosterol	89.88	527.23	181.32
Δ^5-Avenasterol	18.69	44.87	33.8
22-Dihydrospinasterol	ND	2.82	4.89
Δ^7-Avenasterol	ND	ND	1.70
Total 4-desmethylsterols	196.15	608.71	362.15

ND not detected

coconut oil (470–1140 μg/g) and makes seed oil of *R. heudelotii* a moderately good source of phytosterols. The order of abundance of 4-desmethylsterols in *A. arundinaceum* seed oil after *β*-sitosterol was campesterol (76.53), Δ^5-avenasterol (33.80), stigmasterol (33.28) and 24-methylenecholesterol (30.63), followed by small amounts of 22-dihydrospnisterol (4.89) and Δ^7-avenasterol (1,70 μg/g), making a total of 362.15 μg/g 4-desmethylsterols. *M. myristica* seed oil contained the smallest amount of 4-desmethylsterols (196.15 μg/g), made up of *β*-sitosterol (89.88), stigmasterol (59.14), campesterol (28.44) and Δ^5-avenasterol (18.44 μg/g).

Conclusions

This study has revealed that seed oils from *M. myristica*, *R. heudelotii* and *A. arundinaceum* are highly unsaturated, with unsaturation levels ranging from 72.94% for *R. heudelotii* through 73.12% for *A. arundinaceum* to 86.46% for *M. myristica*. The FA profiles for the seed oils of *M. myristica*, *R. heudelotii* and *A. arundinaceum* are very similar to those of groundnut oil, sunflower oil and olive oil, respectively. The ESI-FTICR and ^{13}C NMR study of the triacylglycerols from all the test seed oils showed that the *sn*-2 position of the glycerol backbone was predominantly occupied by unsaturated fatty acids. Thus, all three seed oils are nutritionally good unsaturated vegetable oils, as the structure of the TAGs makes the unsaturated FAs readily available for metabolism. HPLC analysis of the unsaponifiable matter has shown that the test seed oils contain fair amounts of the vitamin E compounds, the tocols, to protect them from oxidative attack. Also, GC–MS analysis of the unsaponifiable matter has shown that *R. heudelotii* and *A. arundinaceum* seed oils contain modest amounts of a variety of phytosterols, which are important bioactive molecules. Thus, the results of this study justify the use of all three seed oils in food products and further identify the three seed oils as good candidates for commercial exploitation.

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