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ORIGINAL PAPER

NMR, GC–MS and ESI-FTICR-MS Profiling of Fatty Acids and Triacylglycerols in Some Botswana Seed Oils

Y. C. Mitei · J. C. Ngila · S. O. Yeboah · L. Wessjohann · J. Schmidt

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Abstract In the search for non-traditional seed oils, physicochemical parameters, fatty acid (FA) and triacylglycerol (TAG) profiles for five Botswana seed oils, obtained by Soxhlet extraction, were determined. GC–MS and ^{1}H-NMR analyses showed the FA profiles for mkukubuyo, *Sterculia africana*, and manketti, *Ricinodendron rautanenii*, seed oils dominated by linoleic and oleic acids, 26.1, 16.7 and 51.9, 24.4%, respectively, with *S. africana* containing significant amounts of cyclic FAs (19.9%). Mokolwane, *Hyphaene petersiana*, seed oil was typically lauric; 12:0 and 14:0 acids were 25.9 and 13.4%, respectively. Morama, *Tylosema esculentum*, seed oil resembled olive oil; 18:1 (47.3%) and 18:2 (23.4%) acids dominated. Moretologa-kgomo, *Ximenia caffra*, seed oil had 45.8% of 18:1 FA, plus significant amounts of very long chain FAs: 26:1 (5.8%), 28:1 (13.9%), 30:1 (3.9%), and acetylenic acids, 9a-18:1 (1.5%) and 9a, 11t-18:2 (16.0%). TAG classes and regiochemistry were determined with ESI-FTICR-MS, and ^{13}C-NMR spectra, respectively. Morama showed seven major TAG classes with C54:4 and C54:3 dominating; mokolwane had 16 major classes with C32:0, C38:0 and C42:2 dominating; manketti had 11 major classes with C54:7, C54:6 and C54:4 dominating; mkukubuyo had 12 major classes with C52:4, C52:3 and C54:4 dominating; moretologa-kgomo had 30 major TAG classes with C64:5, C64:3 and C62:3 dominating. Saturated FAs were generally distributed over the *sn*-1(3) position for morama, manketti, and moretologa-kgomo but at the *sn*-2 position for mokolwane and mkukubuyo. These findings indicate that morama and manketti seed oils can be developed for food uses, whilst moretologa-kgomo and mkukubuyo seed oils only for nonfood uses.

Keywords NMR · GC–MS · ESI-FTICR-MS · Fatty acid and triacylglycerol profiles · Mkukubuyo · *Sterculia africana* · Manketti · *Ricinodendron rautanenni* · Mokolwane · *Hyphaene petersiana* · Morama · *Tylosema esculentum* · Moretologa-kgomo · *Ximenia caffra*

Introduction

The world's production of seed oils has been increasing steadily over the last 30 years as a result of the growing demand for both food and non-food uses. The world's actual production of seed oils in the year 2004–2005 was about 113 MMT (million metric tons), which was twice as much as was produced 30 years earlier [1]. The demand level for seed oils is set to rise even more rapidly in the foreseeable future because of the anticipated escalation in the production of oleochemicals, especially biodiesel, in the coming years. The growth in the production of biodiesel, which is principally fatty acid methyl esters (FAME), has been phenomenal in the last 10 years because of the general desire to cut down on the release of greenhouse gases into the atmosphere, and also as a result of the increasing cost of fossil fuels. Thus vegetable oil producing countries are under pressure to expand production to meet the ever increasing demand. At the same time there is also

Y. C. Mitei · S. O. Yeboah (✉)
Chemistry Department, University of Botswana, Gaborone, Botswana
e-mail: yeboahso@mopipi.ub.bw

J. C. Ngila
Chemistry Department, University of Kwa-Zulu Natal, Durban, South Africa

L. Wessjohann · J. Schmidt
Leibniz Institute of Plant Biochemistry, Halle, Germany

concern about the strain on the world's supply of vegetable oils for food uses as more vegetable oil resources are diverted towards production of biodiesel. And yet the world has a vast resource potential in as yet undeveloped oil-bearing seeds which grow in many parts of the world. When managed with scientific and economic skills, such non-traditional seed oil sources will have a tremendous impact on the world's capacity to satisfy the ever growing demand for this important renewable commodity.

It is worth noting that the world's supply of vegetable oils is currently obtained from only about 15 plant species out of nearly half a million plant species known to man. Apart from the traditional commodity oils obtained from the seed/nuts of soybean, palm nut, sunflower, rapeseed-canola, groundnut, cottonseed and a few others, there are many oil-bearing seeds distributed all over the world, especially in tropical areas of the world, whose agro-economic potential has not been evaluated by the formal economic systems in the growing countries. Most rural communities in Africa have traditional methods of extracting vegetable oils from local plant species for use in food products, in cosmetic and medicinal formulations and indeed for use as fuel to produce light in lanterns. These plant species are often grown on small family plots to provide for the needs of the family and immediate neighbours. Thus there is often little or no scientific information about the use of these locally grown plant species, and yet many of these plants are rich in oil and are grown year by year. There are therefore a large number of oil-bearing seeds in many parts of the world that are potentially good candidates for scientific study and development for commercial exploitation to augment the world's supply of vegetable oils.

We report here studies carried out to characterise fully five seed oils of some local economic importance in Botswana in order to evaluate their uses and potential for commercial exploitation. The test seed oils were: mkukubuyo, *Sterculia africana* (Sterculiaceae); manketti (also known as mongongo), *Ricinodendron rautanenni* (Euphorbiaceae); mokolwane, *Hyphaene petersiana* (Arecaceae); morama, *Tylosema esculentum* (Fabaceae) and moretologa-kgomo, *Ximenia caffra* (Olacaceae). The study started with preliminary characterisation of the seed oils by collecting data on their bulk physicochemical properties such as relative density, refractive index (RI), acid value (AV), iodine value (IV), saponification value (SV), peroxide value (PV), *p*-anisidine value (*p*-AV) and unsaponifiable matter (UM). The study continued with the profiling of the principal components, i.e. the triacylglycerols and their constituent fatty acids. Fatty acid composition was determined by GC–MS analysis of the fatty acid methyl esters and also the fatty acid picolinyl esters. This was complemented with proton NMR estimation of the fatty acid classes in the seed oils [2]. The composition of the triacylglycerol classes was determined using a high resolution mass spectrometric technique that consisted of an electrospray ionisation unit coupled to a Fourier transformed ion cyclotron resonance mass spectrometer, ESI-FTICR-MS [3]. The regio-distribution of the fatty acids on the glycerol backbone was determined by ^{13}C-NMR spectrometry [4]. As far as we are aware this is the first report on the ESI-FTICR-MS profiling of the triacylglycerol classes and the ^{13}C-NMR determination of the triacylglycerol regiochemistry in the five seed oils from Botswana.

Experimental Procedures

Materials

Manketti seeds, *R. rautanenii* (500 g), mokolwane nuts, *H. petersiana* (150 g), morama beans, *T. esculentum* (1.00 kg), mkukubuyo seeds, *S. africana* (200 g) and moretologa seeds, *X. caffra* (250 g), were purchased from Botswana Forestry Association, Kumakwane Village, Botswana.

Extraction: Solvents and Reagents

Solvents and reagents used in this work, unless otherwise stated, were all of analytical grade. Solvents used for high resolution MS were of HPLC grade. Solvents were obtained from Rochelle Chemicals (South Africa) BDH (Merck Chemicals, Pty Ltd, UK), Riedel-de Haën (Sigma Aldrich, GmbH) or JT Baker Chemicals Co. (Phillipsburg, NJ, USA). The seeds and nuts were manually dehulled and after thorough cleaning were macerated in a Waring commercial blender (Gateshead, UK). The powders were extracted with a mixture of *n*-hexane/2-propanol (3:1, v/v) in a Soxhlet apparatus for 6 h.

Physicochemical Properties

The bulk physical and chemical properties (Table 1) were determined according to standard IUPAC methods for the analysis of oils and fats [5]. All experiments, unless otherwise stated, 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 [6].

Table 1 Percentage yields, physical and chemical properties of the test oil samples

Sample	Yield (%)	RI at 25 °C	Density g/cm^3 at 20 °C	SV (mg KOH g^{-1})	IV (Wijs)	AV (mg KOH g^{-1})	PV (mequiv/kg)	*p*-AV	UM (%, w/w)
Manketti	41.5	1.481 ± 0.003	0.907 ± 0.002	185.26 ± 1.23	121.76 ± 1.23	0.36 ± 0.01	2.51 ± 0.0.3	3.85 ± 0.05	0.58 ± 0.06
Mkukubuyo	31.7	1.470 ± 0.001	0.905 ± 0.002	161.24 ± 0.48	53.07 ± 1.56	5.05 ± 0.03	5.26 ± 0.03	2.18 ± 0.04	0.84 ± 0.04
Mokolwane	5.0	1.458 ± 0.001	0.880 ± 0.003	209.00 ± 1.00	65.68 ± 0.42	2.02 ± 0.04	15.70 ± 0.25	2.37 ± 0.03	0.08 ± 0.01
Moretologa-kgomo	38.5	1.468 ± 0.002	0.906 ± 0.002	141.00 ± 0.33	82.00 ± 1.00	1.67 ± 0.02	6.45 ± 0.04	0.98 ± 0.04	0.10 ± 0.002
Morama	38.4	1.465 ± 0.002	0.903 ± 0.003	174.00 ± 2.00	95.00 ± 3.00	2.96 ± 0.03	4.01 ± 0.05	3.62 ± 0.05	0.51 ± 0.01

Values represent the average of three replicate analyses ± SD

SV saponification value, *IV* iodine value, *RI* refractive index, *AV* acid value, *PV* peroxide value, *p-AV para*-anisidine value and *UM* unsaponifiable matter

Separation of Acylglycerols

Triacylglycerols (TAG), diacylglycerols with free fatty acids (DAG + FFA) and monoacylglycerols (MAG) in the oil samples were further separated by gradient elution on silica gel (5% H_2O, Saarchem Pty Ltd) using benzene (100%), benzene:diethyl ether (9:1) and diethyl ether (100%), respectively [6].

Fatty Acid Composition: Sample Preparation

FAME

The oil samples (2 g each) were transesterified by refluxing in dry methanol that contained ethanoyl chloride to yield fatty acid methyl esters (FAME). These were stored under nitrogen and used for GC–MS and ^{1}H-NMR analyses.

Picolinyl Esters

The method of Destaillats et al. [7] was adopted in this preparation. Portions of the oil samples were dissolved in dry dichloromethane and allowed to react with a mixture of potassium *tert*-butoxide in tetrahydrofuran and 3-hydroxy methylpyridine 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

FAME and picolinyl esters in dichloromethane were analysed in a ThermoQuest Voyager GC–MS coupled to ThermoQuest Trace GC 2000 SERIES (San Jose, California, USA). Separation was effected on a DB-5MS capillary column (0.25 μm × 0.25 mm × 30 m; J&W Scientific, California, USA) consisting of 5% phenylmethylpolysiloxane stationary phase. UHP Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. 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 a rate of 15 °C per minute. It was then held for 1 min before the second ramp at a rate of 5 °C per minute to 300 °C. This was then held isothermally for 25 min.

Nuclear Magnetic Resonance Analysis

Proton NMR spectra of the FAME, dissolved in $CDCl_3$, were acquired at 300 MHz using a Bruker Avance DPX 300 spectrometer. The relative compositions of the saturated, monounsaturated and diunsaturated fatty acids together with their average chain lengths were determined

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

Sample	Hydrocarbons	Triacylglycerols + FFA	Sterol esters	Free sterols	DAG	MAG	Glycolipids	Phospholipids
Manketti	0.49	79.89	1.38	3.06	1.18	0.50	0.41	2.73
Mkukubuyo	0.67	88.34	0.87	1.98	6.71	1.15	0.64	3.36
Mokolwane	1.09	82.67	1.07	2.57	15.35	2.24	0.81	2.56
Moretologa-kgomo	0.96	86.51	0.08	0.96	16.02	6.32	0.97	3.02
Morama	1.37	82.77	1.79	1.94	3.41	5.87	1.59	1.26

Values are means of two determinations

FFA free fatty acids, *DAG* diacylglycerols, *MAG* monoacylglycerols

from the relative sizes of the integrals of the signals for the allylic, diallylic and methyl protons using Holmback's equations, Table 4 [2, 8].

Analysis of Triacylglycerols

High Resolution Mass Spectrometric Analysis

TAG extracts (0.1–0.5 mg) were dissolved in methanol and the methanolic solutions were introduced continuously via a syringe pump into an electrospray ionisation source (APPOLO) on a high resolution FTICR mass spectrometer (Bruker Daltonics Apex III) at a flow rate of 2 μL min^{-1}. All data were acquired with 512 K data points and zero-filled to 2,048 K by averaging 32 scans and applied to 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 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 of sample was dissolved in deuterated chloroform ($CDCl_3$) and the solution (700 μL) was placed in a 5 mm (diameter) NMR tube. Carbon-13 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 a spectral width of 1,300 Hz, 64 K data points, a pulse repetition time of 60 s, a 90° flip angle and full proton decoupling. Two hundred scans were accumulated per spectrum. The FIDs were zero-filled to 128 K prior to Fourier transform resulting in a digital resolution of 0.02 Hz/point. A line broadening of 0.05 Hz was used for the exponential weighting. Peaks were assigned by spiking the standard compounds into the real test samples.

Data Analysis

Experiments for the determination of the physicochemical parameters and the GC–MS analysis of fatty acid composition were carried out in triplicate and results are expressed as mean values ± SD. Statistical analysis was carried out using a one-way ANOVA with a significance level of $p < 0.05$. Software used for the statistical analysis was the SPSS for Windows (v.10.0.6; Chicago, IL, USA) statistical package. NMR and MS results are expressed as values for single runs.

Results and Discussion

Some pertinent bulk physicochemical parameters were first determined in this work in order to collect preliminary information about the general nature of the seed oils under investigation. The results of these measurements are shown in Table 1, which also shows the average yields of oils obtained from two Soxhlet extractions. Apart from mokolwane kernels, with a yield of 5% (w/w), the average oil yield of the other four seeds was about 37% (w/w), which is much higher than the oil yield from soybean (17–25% w/w), currently the leading seed oil on the world market. Indeed higher oil yields from morama (48% w/w), and moretologa-kgomo (41% w/w) have previously been reported by one of us and also elsewhere in the literature [9, 10].

The ranges of refractive index (RI 1.481–1.458) and relative density values (0.907–0.880), shown in Table 1, are quite comparable with literature values for such well known seed oils as grapeseed, soybean and palm kernel. Refractive indices for oils and fats usually increases with the degree of unsaturation, especially polyunsaturation, and chain length. In Table 1, manketti seed oil has the highest refractive index at 1.481, which is consistent with it having the highest iodine

value (IV = 121.76 Wij), indicating that manketti seed oil would have the highest degree of polyunsaturation among the test seed oils. This indeed was the case as can be observed from the GC–MS and proton NMR estimation of the fatty acid composition shown in Tables 3, 4. The rather low RI (1.468) for moretologa-kgomo seed oil can be rationalised on the basis that even though it has long fatty acid chains, as indicated by its low SV (141.00), its acyl chains are mainly monounsaturated and thus it has rather a low degree of polyunsaturation, as shown in Table 3. This structural effect again explains its moderate IV of 82.00 Wij. Mokolwane seed oil had the highest SV at 209.00 mg KOH g^{-1}, indicating that the oil would be mainly lauric acid, and so like palm kernel oil (SV = 230–254 mg KOH g^{-1}, RI = 1.448–1.452) [11], mokolwane seed oil had the lowest RI of 1.458. The physicochemical parameters of the morama bean oil: RI = 1.465, relative density = 0.903, SV 174.00 mg KOH g^{-1} and IV = 95.00 Wij are very similar to those of olive and groundnut oils. As these parameters furnish information on the chemical structure of fatty acids in oils, the morama bean oil would therefore be expected to have a fatty acid composition similar to those of olive and groundnut oils.

The seeds that were extracted for this investigation had been in storage for several months before they were purchased and therefore the stability parameters such as acid value AV, peroxide value PV, and *p*-anisidine value *p*-AV, shown in Table 1 are quite reasonable as all the values fall within the Codex recommended ranges for virgin olive oil [11]. However, mokolwane seed oil with PV of

Table 3 Percentage fatty acid compositions by capillary GC–MS analysis of the FAME from test seed oils

FA	Mkukubuyo	Manketti	Mokolwane	Moretologa	Morama
12:0	ND	ND	25.92 ± 0.65	ND	ND
14:0	ND	ND	13.13 ± 0.27	ND	ND
9-16:1	0.94 ± 0.07	ND	ND	ND	ND
16:0	19.97 ± 0.35	11.95 ± 1.36	10.39 ± 0.93	0.94 ± 0.06	12.93 ± 0.06
17:0	ND	ND	ND	ND	ND
9,12,15-18:3	ND	ND	ND	ND	ND
Undefined 18:2	0.93 ± 0.13	ND	ND	ND	ND
9,12-18:2	26.11 ± 0.28	51.93 ± 1.09	4.29 ± 0.02	0.26 ± 0.02	23.40 ± 0.42
9-18:1	16.74 ± 0.29	24.35 ± 0.72	42.37 ± 0.18	45.76 ± 0.06	47.27 ± 0.43
11-18:1	3.40 ± 0.08	ND	ND	ND	ND
18:0	5.80 ± 0.11	11.77 ± 0.29	3.90 ± 0.18	1.15 ± 0.07	8.82 ± 0.12
9a-18:1	ND	ND	ND	1.47 ± 0.13	ND
9a,11t-18:2	ND	ND	ND	15.98 ± 0.01	ND
Undefined 20:4	ND	ND	ND	ND	ND
Undefined 20:3	3.33 ± 0.14	ND	ND	ND	ND
8,11-20:2	ND	ND	ND	ND	ND
11,13-20:2	ND	ND	ND	ND	ND
9-20:1	1.84 ± 0.04	ND	ND	0.81 ± 0.02	0.61 ± 0.00
20:0	1.10 ± 0.03	ND	ND	ND	3.31 ± 0.03
13-22:1	ND	ND	ND	ND	2.63 ± 0.01
22:0	ND	ND	ND	ND	1.03 ± 0.02
15-24:1	ND	ND	ND	4.30 ± 0.03	ND
24:0	ND	ND	ND	2.09 ± 0.01	ND
17-26:1	ND	ND	ND	5.84 ± 0.05	ND
26:0	ND	ND	ND	2.11 ± 0.04	ND
19-28:1	ND	ND	ND	13.94 ± 0.23	ND
28:0	ND	ND	ND	1.39 ± 0.03	ND
21-30:1	ND	ND	ND	3.95 ± 0.14	ND
Cyclic fatty acids	19.82 ± 1.00	ND	ND	ND	ND
Total unsaturated	53.29	76.28	46.66	92.31	73.91
Total saturated	26.87	23.72	53.34	7.68	26.09

Values represent the average of three replicate analyses ± SD

ND not detected

Table 4 Composition of fatty acid classes as estimated from ^{1}H-NMR spectra of test oils

Sample	CH_2 (methylene envelope)		CH_2-diallylic		CH_2-allylic		CH_3		CH_3-ω3	
	δ/ppm	Integral	δ/ppm	Integral	δ/ppm	Integral	δ/ppm	Integral	δ/ppm	Integral
Manketti	1.30	22.03	2.79	1.00	2.06	3.81	0.89	3.88	–	–
Mokolwane	1.27	20.71	–	–	2.04	1.24	0.88	3.54	–	–
Mkukubuyo	1.27	19.68	2.77	0.41	2.04	2.11	0.88	3.38	–	–
Morama	1.27	22.24	2.77	0.34	2.04	2.94	0.88	3.30	–	–
Moretologa-kgomo	1.27	26.62	–	–	2.02	3.19	0.88	3.08	–	–

	α-Linolenic	Diunsaturated	Monounsaturated	Total unsaturated	Total saturated.	CN
Manketti	ND	40.3	35.4	75.7	24.3	17
Mokolwane	ND	ND	26.2	26.2	73.8	14
Mkukubuyo	ND	18.1	28.7	46.8	53.2	15
Morama	ND	15.6	51.1	66.7	33.3	17
Moretologa-kgomo	ND	ND	77.6	77.6	22.4	20

% Composition of FA classes

CN average carbon number

15.70 mequiv kg^{-1}should be showing signs of oxidative rancidity. The levels of *p*-AVs in Table 1 generally indicate that not much secondary oxidative products were present in the oils. Thus the stability parameters shown in Table 1 indicate that all the test oils should have good keeping capacity.

Determination of the composition of lipid classes in oils by adsorption column chromatography essentially gives general information about the relative amounts of neutral and polar lipids that are present in the respective oils. Table 2 shows that all the five test seed oils were predominantly composed of neutral lipids with triacylglycerols being the dominant lipid components. However, mkukubuyo, mokolwane, morama and moretologa-kgomo seed oils appeared to contain fair amounts of polar lipids like diacylglycerols and monoacylglycerols, which would suggest the occurrence of lipase catalysed hydrolysis in these oils. Indeed the acid values (AV) of 5.05, 2.02, 2.96 and 1.67 mg KOH g^{-1}, respectively, for these seed oils, tend to support this observation. Table 2 shows rather moderate to low levels of phospholipid and glycolipid content in the test seed oils.

The principal components of oils and fats on which all the physical and chemical properties depend are the TAG molecules. The TAGs are in turn made up of a variety of FAs esterified to the hydroxyl groups on glycerol molecules. In profiling the principal components we first determined the FA acid compositions of the seed oils using the GC–MS technique. This was complemented with proton NMR estimation of the FA classes. The GC–MS determination of the FA compositions was carried out by analysing both FA methyl esters (FAME) and FA picolinyl esters of the oils. The FAME analysis provided data on the molecular weight as well as retention times of the respective compounds, whilst analysis of the FA picolinyl esters on the other hand provided distinct diagnostic ions in the mass spectra that facilitated the location of the positions of double and triple bonds and also cyclic rings in the acyl chains [12–14]. When the FA acid picolinyl ester is ionised in the mass spectrometer, it is the nitrogen atom rather than the alkyl chain that carries the charge and hence double bond ionisation and migration are minimised. Thus in the picolinyl esters of saturated FAs radical-induced cleavage occurs evenly along the chain, leading to a regular pattern of fragments *m/z* 14 apart in the mass spectra. In the mass spectra of picolinyl esters of monounsaturated FAs the position of the double bond was indicated by a gap of *m/z* 26, representing the abstraction of allylic hydrogen atoms on each side of the double bond, and a gap of *m/z* 40, representing the fragmentation of the double bond and the adjacent methylene group on the carboxyl side [13, 14]. In the mass spectra of diunsaturated FA picolinyl esters, the gaps of *m/z* 40 on the carboxyl side were easier to identify and in addition two gaps of *m/z* 26 occurred to locate positions of the double bonds [4, 13]. The positions of triple bonds and cyclic rings were identified in a similar way. For example the mass spectrum of the picolinyl ester of stearolic acid, shown in Fig. 1, showed a gap of *m/z* 24 between *m/z* 234 and 258 and a gap of *m/z* 38 between *m/z* 220 and 258 on the proximal side that indicated the position of the acetylenic bond at Carbon-9 of the acyl chain. This finding is to the best of our knowledge the first report of the presence of stearolic acid in the oil of moretologa-kgomo, *X. Caffra*. In a similar way, the mass spectrum of

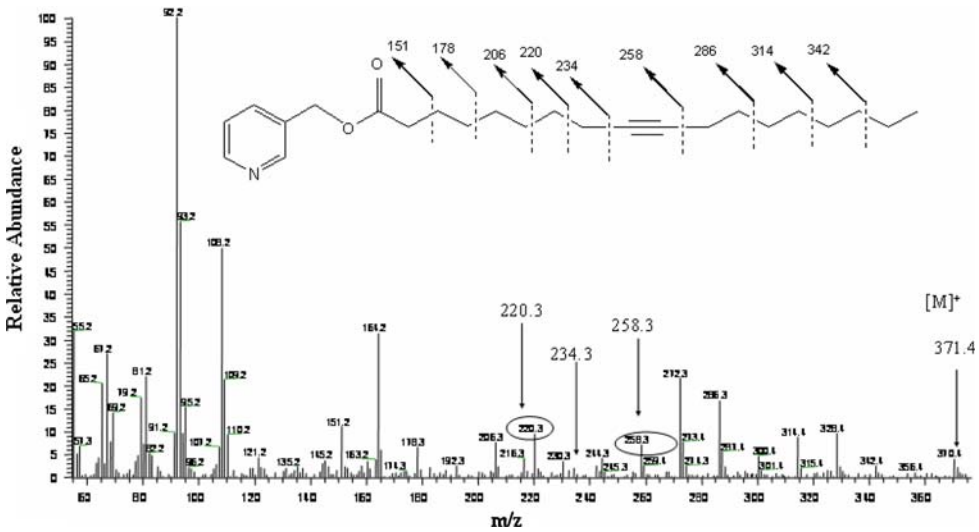

Fig. 1 EI mass spectrum of stearolic acid (9a-18:1) picolinyl ester in the full mode scan at 70 eV

the FA picolinyl ester of xymenynic acid showed a gap of *m/z* 24 between *m/z* 234 and 258 indicating the position of a triple bond, and in addition showed a gap of *m/z* 26 between *m/z* 258 and 284 indicating the position of a double bond conjugated to the triple bond. Thus the presence of xymenynic acid in the moretologa-kgomo oil was unambiguously demonstrated. Table 3 shows the detailed FA composition of the oil from moretologa-kgomo as dominated by monounsaturated FAs to the extent of 76.59%, which included stearolic acid 9a-18:1. The predominant unsaturated FA was oleic acid, 9-18:1 (45.76%), followed by xymenynic acid 9a, 11t-18:2 (15.98%). Rather unusually, the remaining monounsaturated FAs (about 30%) consisted of very long fatty acid (VLFA) chains like 15-24:1 (4.30%), 17-26:1 (5.84%) 19-28:1 (13.94%) and 21-30:1 (3.95%). The structural effect of these acyl chains on the bulk physicochemical properties of moretologa-kgomo seed oil has been mentioned earlier. The relatively high proportion of VLFA chains in this oil should make it a good candidate for use as lubricating oil in a similar way to the use of high erucic rapeseed oil in lubricating applications [15].

As shown in Table 3, the total unsaturation in morama seed oil was about 73.91%, dominated by oleic acid (45.76%) and linoleic acid, 9,12-18:2 (23.40%). The saturated FAs were palmitic acid, 16:0 (12.93%) and stearic acid 18:0 (8.82%). Overall the general FA profile for morama seed oil is quite similar to that of olive oil. In both oils, in addition to the dominance of oleic acid, they each contain minor but measurable amounts of long chain FAs such as 20:0, 22:0 and 24:0 (about 3% in total) in olive oil and 20:0, 9-20:1, 22:0, 9-22:1 and 13-22:1 (about 7% in total) in morama oil. The level of erucic acid, 13-22:1, in morama seed oil (2.63%) is comparable to that allowed by Codex for low erucic canola oil and thus morama seed oil should be safe for consumption [16]. Indeed morama seed oil is widely used for food purposes throughout the Kalagadi regions of Botswana, Namibia, and South Africa.

The mokolwane kernel oil, with an oil yield of 5% (w/w), cannot be described as an oil-bearing palm. It was however included in this study in order to confirm its nature as a lauric oil since there has not been any report on its characteristics in the literature. With FA composition of 12:0 (25.92%), 14:0 (13.13%), 16:0 (10.39%), 18:2 (4.29%), 18:1 (42. 37%) and 18:0 (3.90%), the mokolwane kernel oil resembles both coconut and palm kernel oils, hence confirming its nature as a lauric oil.

The GC–MS analysis of the FAME from manketti seed oil revealed only four major FAs, dominated by linoleic acid (51.93%) and oleic acid (24.35%); the other FAs were palmitic (11.95%) and stearic (11.77%) acids. In 1966 Chisholm [17] reported the presence of α-eleostearic acid, 9Z, 11E, 13E-18:3, in manketti seed oil. However in 1970 Engelter [18] did not identify α-eleostearic acid but reported an unidentified component FA to the extent of 21.70% in manketti seed oil. In this work we did not find α-eleostearic acid but found higher amounts of linoleic (51.93%) and oleic (24.35%) acids than previously reported. It is worth noting that Kapseu et al. [19] in 1995 found α-eleostearic acid (about 50%) in the seed oil of *R. heudelotii* from Cameroon, whilst in 2000 Manga et al. [20] found a larger amount of linoleic acid (60%) but found no α-eleostearic acid in *R. heudelotii* seed oil, also from Cameroon. The FA profile for manketti seed oil found in this work very much resembled that of maize (corn) oil *Zea mays*, thus indicating that manketti seed oil can be used in similar ways as corn oil in food products. Indeed manketti seed oil has been reported as an important ingredient in food preparations as well as in skin care formulations in some regions of southern Africa [21].

The GC–MS analysis of mkukubuyo FAME revealed a rather unusual FA profile. Table 3 shows that in addition to the common seed oil FAs: palmitic acid (19.97%), stearic acid (5.80%), oleic acid (21.14%) and linoleic acid (26.11%), mkukubuyo seed oil contained such long chain FAs as arachidic acid 20:0 (1.10%), eicosenoic acid 9-20:1 (1.84%), and an undefined 20:3 (3.33%). Furthermore this oil also contained cyclopropanoid and cyclopropenoid FAs at a total level of 19.82%. In the mass spectrum of the picolinyl ester of cyclopropenoid FA, a gap of *m/z* 66 between fragments *m/z* 220 and 286 clearly showed the presence of the cyclopropene ring between C9 and C10, hence showing the presence of sterculic acid, cp9, 10-18:1. The cyclopropanoid FA was indicated by the presence of the $[M\text{-}H]^+$ ion at *m/z* 386 which was more abundant than the expected $[M]^+$ ion at *m/z* 387, and also the presence of the unusual but very prominent odd numbered ion at *m/z* 247, representing a cleavage at the ring [14], and giving a gap of *m/z* 139 (equal to C10 H19), indicated that the cyclopropane ring was located between C9 and C10, and hence the presence of 9,10-methylene-octadecanoic acid in the mkukubuyo seed oil. This finding is consistent with the reported presence of three-membered ring FAs in seed oils of the Sterculiaceae family [22]. Further still, the GC–MS analysis showed two more components 20:3 (3.33%) and 18:2 (0.93%) in the mkukubuyo seed oil whose picolinyl ester mass spectra were too poorly resolved to allow for the positions of unsaturation to be identified. On account of its FA profile mkukubuyo seed oil may not be recommended for food uses as cyclopropenoid FAs have been reported to be toxic to higher animals and possibly including humans [23].

Table 4 shows the proton NMR estimation of the FA classes in the test seed oils. In this analysis, the composition of saturated, monounsaturated (as oleic) and diunsaturated (as linoleic) FAs were estimated by comparing integrals of the signals from the olefinic (ca. 5.2 ppm), allylic (ca. 2.02 ppm) and bis-allylic (ca. 2.7 ppm) protons and relating them to the total integrals of the proton signals from the methyl groups at 0.85 and 0.98 ppm. The n-3 (ω3) FAs (as linolenic) were estimated by comparing the methyl triplet at 0.98 ppm to the methyl groups at 0.85 ppm [2, 8]. As shown in Tables 3, 4 there was good agreement between the proton NMR estimation of the FA classes and the GC–MS determination of the FA compositions of the oils. For example, both techniques agreed that there was no detectable presence of n-3 FAs, i.e. there was no linolenic acid in any of the five seed oils. Thus the unidentified component 20:3 acid (3.33%) detected in the GC–MS analysis of mkukubuyo seed oil was probably a non-ω3 FA. There was good agreement between the proton NMR and GC–MS estimation of diunsaturated FAs in the seed oils. For example proton NMR analysis did not detect any diunsaturation in mkukubuyo and moretologa-kgomo seed oils, and indeed the more accurate technique, GC–MS, estimated only 4.29 and 0.26% diunsaturation, respectively, for these two seed oils. Again both proton NMR and GC–MS techniques showed that linoleic acid was the most abundant FA in manketti seed oil. Similarly both proton NMR and GC–MS estimations of monounsaturation in the test seed oils were in good agreement; for example, both techniques estimated that monounsaturated FAs were the most abundant FAs in both morama and moretologa seed oils. However, compared to GC–MS, the proton NMR technique slightly overestimated monounsaturation in mkukubuyo and manketti seed oils, whilst it underestimated monounsaturation in mokolwane seed oil. On the whole, the two techniques generally agreed closely on the pattern of the FA profiles for the test seed oils as evidenced in the good agreement in their estimation of the ratios of total unsaturated to total saturated FAs in all the seed oils, Tables 3, 4.

FAs in oils and fats exist almost exclusively as triesters of glycerol molecules, the triacylglycerols (TAGs). The biosynthesis of TAGs, like all other biomolecules, is enzymatically controlled to yield products with specific stereo and/or regiochemistry. There is increasing evidence to suggest that the enzymatically controlled regiospecific distribution of fatty acids on the glycerol backbone in oils and fats has important physiological effects and nutritional value on humans [24, 25]. Furthermore data on intact TAGs and their composition in oils can often serve as fingerprint in confirming the authenticity of the oils [26]. In this work the composition of intact TAGs in the test seed oils was studied using FTICR-MS attached to an electrospray ionisation (ESI) source. The combined ESI-FTICR-MS technique offers ultrahigh mass resolving power, high mass accuracy measurement and rapid analysis of complex mixtures like TAGs. Indeed it has been shown that the positive ion ESI-FTICR mass spectral relative abundances of particular homologous components are characteristic of a particular vegetable oil [3].

In this report each TAG is represented as C*x*:*n*, where *x* denotes the total number of carbon atoms and *n* denotes the total number of double bonds in the three FAs, e.g. C54:3. In processing the spectral data, application of elemental analysis software (XMASS) on the spectral data combined with high resolution and exact mass measurement of the FTICR mass spectrometer allowed assignment of sodiated adduct ions to the larger peaks from which the most suitable elemental compositions were computed. Since the ESI-FTICR-MS analysis was not preceded by separation, the elemental composition data obtained does not distinguish between regioisomers. What is presented here can safely be taken as TAG classes, C*x*:*n* [27]. Figure 2 shows

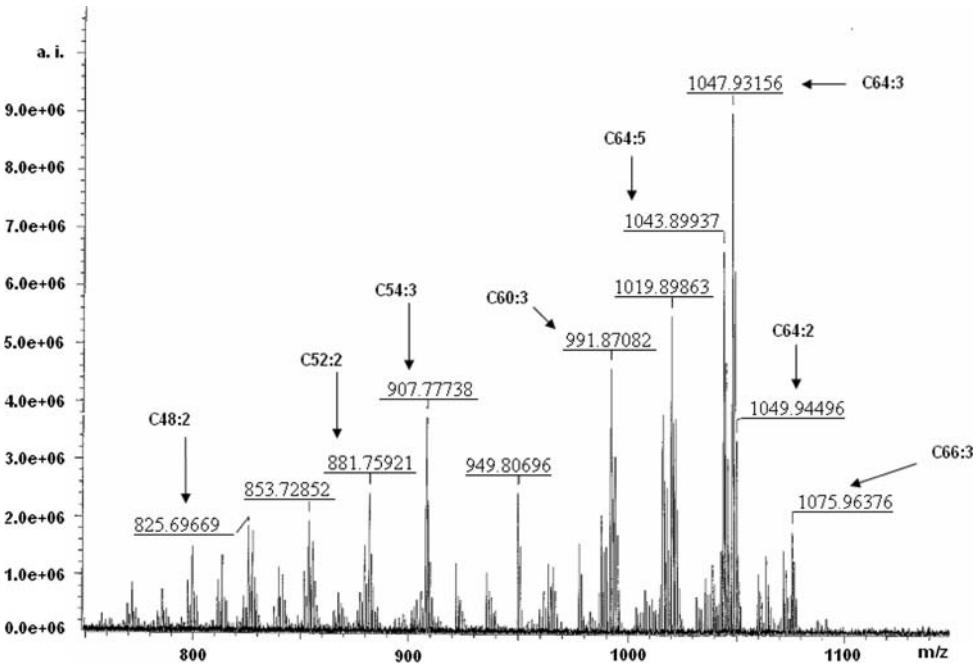

Fig. 2 Positive ion ESI-FTIRC mass spectrum for moretologa-kgomo triacylglycerols

the positive ESI-FTICR mass spectrum for moretologa-kgomo triacylglycerols.

The regiochemical distribution of the FAs on the glycerol backbone was determined using ^{13}C NMR under full NOE. Under these conditions carbonyl carbons resonated according to both degree of unsaturation of the acyl chains and the *sn*-1/3 and *sn*-2 positions on the glycerol backbone. The carbonyl carbons resonated in the frequency range of 172.5–173.5 ppm, where the signals from the carbonyl carbons of *sn*-1/3 chains were shifted about 0.42 ppm at higher frequency from those of carbonyl carbons of *sn*-2 chains [28]. Within each set of signals the saturated, oleoyl and linoleoyl chains appeared from higher to lower frequency in that order as shown in Fig. 3 for morama seed oil. The integrated carbonyl resonances of saturated, oleoyl, and linoleoyl chains in the test samples were used to calculate the acyl chain composition as well as the distribution between the *sn*-1/3 and *sn*-2-glycerol positions [2, 4].

Table 5 shows only the seven most abundant TAG classes in each of the test seed oils, whilst Table 6 shows the positional distribution of the fatty acyl chains on the glycerol backbone. It can be observed from Table 6 that independent of the total amount, saturated FAs were exclusively distributed at the *sn*-1/3 positions in the seed oils of morama, moretologa-kgomo and manketti, whilst

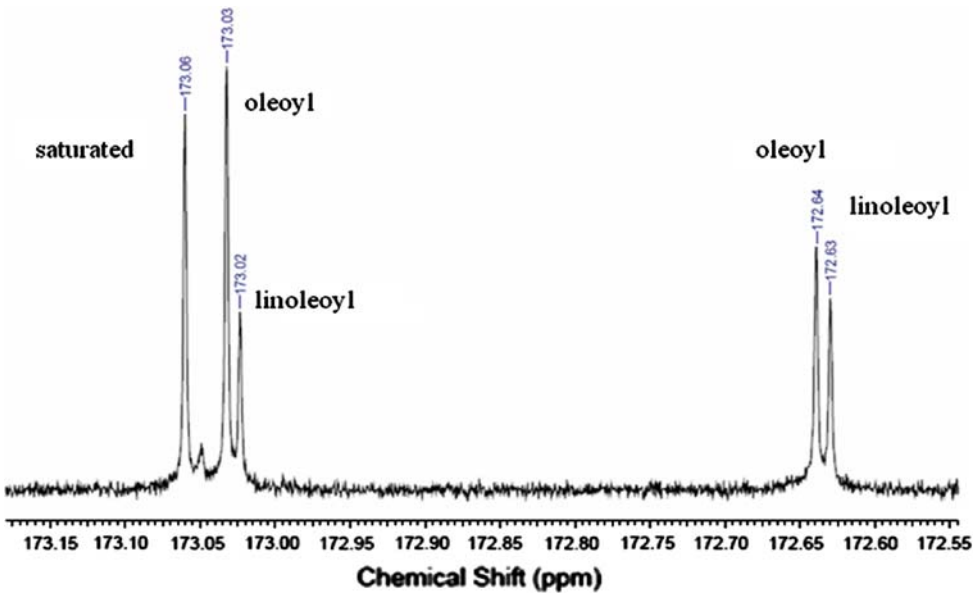

Fig. 3 Expansion of the carbonyl region of the ^{13}C-NMR spectrum of the morama triacylglycerols in $CDCl_3$ at 100.6 MHz

Table 5 Major mass peaks of triacylglycerols in the positive ion ESI-FTIRC mass spectra of test seed oil fractions

Observed mass (*m/z*)	Mass ion $[M + Na]^+$	Theoretical mass	TAG CN:DB	Relative intensity	% composition	Observed mass (*m/z*)	Mass ion $[M + Na]^+$	Theoretical mass	TAG CN:DB	Relative intensity	% composition
Morama						*Manketti*					
877.7258	$[C_{55}H_{98}O_6Na]^+$	877.7256	C52:4	0.3822	7.58	875.7107	$[C_{55}H_{96}O_6Na]^+$	875.7099	C52:5	0.2264	5.66
879.742	$[C_{55}H_{100}O_6Na]^+$	879.7412	C52:3	0.7623	15.12	877.7248	$[C_{55}H_{98}O_6Na]^+$	877.7256	C52:4	0.2272	5.68
881.7558	$[C_{55}H_{102}O_6Na]^+$	881.7569	C52:2	0.6871	13.63	879.7423	$[C_{55}H_{100}O_6Na]^+$	879.7412	C52:3	0.1482	3.70
901.7267	$[C_{57}H_{98}O_6Na]^+$	901.7256	C54:6	0.5891	11.68	897.6957	$[C_{57}H_{94}O_6Na]^+$	897.6943	C54:8	0.2091	5.22
903.7396	$[C_{57}H_{100}O_6Na]^+$	903.7412	C54:5	0.6816	13.52	899.7111	$[C_{57}H_{96}O_6Na]^+$	899.7099	C54:7	0.7867	19.65
905.7563	$[C_{57}H_{102}O_6Na]^+$	905.7569	C54:4	1.0000	19.83	905.7558	$[C_{57}H_{102}O_6Na]^+$	905.7569	C54:4	0.7757	19.38
907.7718	$[C_{57}H_{104}O_6Na]^+$	907.7725	C54:3	0.9395	18.63	907.7722	$[C_{57}H_{104}O_6Na]^+$	907.7725	C54:3	0.3839	9.59
Moretologa						*Mokolwane*					
881.7556	$[C_{55}H_{102}O_6Na]^+$	881.7569	C52:2	0.0691	3.44	605.4752	$[C_{35}H_{66}O_6Na]^+$	605.4752	C32:0	0.5720	8.70
907.7724	$[C_{57}H_{104}O_6Na]^+$	907.7725	C54:3	0.1066	5.30	633.5067	$[C_{37}H_{70}O_6Na]^+$	633.5065	C34:0	0.4125	6.28
991.8708	$[C_{63}H_{116}O_6Na]^+$	991.8664	C60:3	0.1312	6.53	661.5377	$[C_{39}H_{74}O_6Na]^+$	661.5378	C36:0	1.0000	15.22
1019.8986	$[C_{65}H_{120}O_6Na]^+$	1019.8977	C62:3	0.1573	7.83	687.5529	$[C_{41}H_{76}O_6Na]^+$	687.5534	C38:1	0.6012	9.15
1021.9122	$[C_{65}H_{122}O_6Na]^+$	1021.9134	C62:2	0.1064	5.29	689.569	$[C_{41}H_{78}O_6Na]^+$	689.5691	C38:0	0.6417	9.77
1043.8994	$[C_{67}H_{120}O_6Na]^+$	1043.8977	C64:5	0.1892	9.40	743.6164	$[C_{45}H_{84}O_6Na]^+$	743.6160	C42:1	0.8863	13.49
1047.9316	$[C_{67}H_{124}O_6Na]^+$	1047.9290	C64:3	0.2573	12.80	825.6932	$[C_{51}H_{94}O_6Na]^+$	825.6943	C48:2	0.5391	8.20
						Mkukubuyo					
						853.7259	$[C_{53}H_{98}O_6Na]^+$	853.7256	C50:2	0.3497	6.48
						877.7248	$[C_{55}H_{98}O_6Na]^+$	877.7256	C52:4	0.9209	17.06
						879.7407	$[C_{55}H_{100}O_6Na]^+$	879.7412	C52:3	0.6547	12.13
						891.7419	$[C_{56}H_{100}O_6Na]^+$	891.7412	C53:4	0.4024	7.46
						901.7253	$[C_{57}H_{98}O_6Na]^+$	901.7256	C54:6	0.5729	10.61
						903.7378	$[C_{57}H_{100}O_6Na]^+$	903.7412	C54:5	0.5850	10.84
						905.7556	$[C_{57}H_{102}O_6Na]^+$	905.7569	C54:4	0.6431	11.91

Table 6 Positional distribution of fatty acyl chains on the glycerol backbone of triacylglycerols in test oil samples determined by ^{13}C NMR

Sample	*sn*-1,3 composition					*sn*-2 composition				
	Saturated	Oleoyl	Linoleoyl	uk	uk	Saturated	Oleoyl	Linoleoyl	uk	uk
Mokolwane	81.26	18.74	ND	ND	ND	52.03	47.97	ND	ND	ND
Morama	45.04	38.37	16.59	ND	ND	ND	56.38	43.62	ND	ND
Moretologa	55.11	31.56	ND	13.33	ND	ND	95.39	ND	4.61	ND
Mkukubuyo	37.33	11.34	15.02	22.57	13.74	11.49	22.77	36.73	22.88	6.12
Manketti	26.03	ND	73.97	ND	ND	ND	ND	100	ND	ND

uk unknown, *ND* not detected

there was some distribution of saturated FAs in the *sn*-2 position, albeit to a minor extent, in the seed oils of mkukubuyo and mokolwane. Conversely unsaturated fatty acyl chains were much more abundantly distributed at the *sn*-2 position than at the *sn*-1/3 positions in all the test seed oils. This pattern of distribution of the FA classes is consistent with literature reports [29]. Table 6 also shows that moretologa-kgomo and mkukubuyo seed oils had other acyl chains other than saturated, oleoyl and linoleoyl which were labelled "uk" (unknown) because of lack of authentic standards for identification. However from the GC–MS analysis of the fatty acid composition, Table 3, these unknown acyl chains for moretologa-kgomo could be attributed to the acetylenic fatty acids, stearolic and xymenynic acids. For mkukubuyo these unknown FAs could be ascribed to the cyclic FAs.

The positive ion ESI-FTICR spectrum revealed 18 TAG classes in mokolwane, of which the most abundant class was C36:0, and here trilaurin was most likely to be the major regioisomer in accordance with the FA composition of the mokolwane seed oil. Now the presence of such prominent TAG classes as C32:0, C34:0 and C38:0 in the mokolwane seed oil, as shown in Table 5, would suggest the presence of short FA chains like 8:0 and 10:0, which however were not detected in the GC–MS analysis. One explanation could be that the separation conditions did not favour the separation of such short acyl chains. Table 5 shows the presence of TAG classes with large total carbon numbers greater than C54, such as C60:3, C62:3 and C64:3 in the moretologa seed oil. Such TAGs served as evidence of the substantial amounts of VLFA chains detected in the GC–MS analysis of the moretologa-kgomo seed oil. As shown in Table 6, saturated FAs were distributed only at the *sn*-1/3 positions whilst the *sn*-2 position was occupied principally by unsaturated fatty acids in the TAG classes contained in the moretologa-kgomo seed oil.

The positive ion ESI-FTICR spectrum showed that manketti seed oil was actually composed of 11 TAG classes, of which the C54 TAG classes constituted over 70%. This was very consistent with the GC–MS estimation of the fatty acid classes, Table 3, which shows that the C18 FAs constitute over 75% of all the other acyl chains. The presence of C54:7 and C54:8 TAG classes would suggest the presence of 18:3 FAs in the manketti seed oil. Such FAs, however, were not detected in either the GC–MS or the proton NMR analyses as mentioned earlier. Perhaps the failure to detect 18:3 FAs was due to the susceptibility of α-eleostearic acid to undergo transformations during chemical processing [19]. It was noted that in spite of oleic acid constituting about 24.35% of the manketti seed oil, no oleic acid was detected at either the *sn*-1/3 or the *sn*-2 positions in the glycerol backbone as shown in Table 6. This anomaly could be attributed to the poor resolution of the oleoyl and linoleoyl signals in the ^{13}C-NMR spectrum.

The TAG classes for mkukubuyo seed oil included odd numbered total carbon TAGs, such as C53:4, C53:3, C55:6 and C57:6. This finding was consistent with the presence of cyclic FAs in the mkukubuyo seed oil. Indeed the ^{13}C-NMR analysis of the distribution of FAs on the glycerol backbone showed that FAs other than saturated, oleoyl and linoleoyl were present at both *sn*-1/*3* and *sn*-2 positions. On the basis of the FA composition these unknown FAs could be ascribed to the cyclic FAs detected in the mkukubuyo seed oil by the GC–MS analysis. The preponderance of C54 TAG classes (up to 63.7%) in the morama seed oil was quite consistent with the GC–MS analysis of the FA composition which showed that C18 FAs were dominant to the extent of 79.49% in the morama oil. It would appear from Table 6 that the oleoyl acyl chain had a greater preference for the *sn*-2 position than the linoleoyl acyl chain in the morama oil. This unexpected regiochemistry could be attributed to the fact that the content of oleic acid was more than double that of linoleic acid in the morama seed oil.

The application of the positive ion ESI-FTICR mass spectrometry in profiling the TAGs in the test seed oils has demonstrated the mass accuracy and high resolution features of the technique. The positive ion ESI-FTICR-MS was able to show the relative abundances of TAG classes containing the same number of carbons: examples include the $[M + Na]^+$ ion with *m/z* 1043.8994 in moretologa-kgomo which was shown to be C64:5, whilst the $[M + Na]^+$ ion

with *m/z* 1047.9317 was shown to be C64:3. Furthermore the percentage composition of the TAG classes appears to be quite diagnostic for each of the test seed oil samples. Another attribute of the technique is that even though it does not identify individual TAGs, the ESI-FTICR-MS technique avoids cumbersome separation processes such that in one experiment it reveals nearly all the possible TAG molecules that are present in each seed oil.

The comprehensive profiling of the compositional and structural characteristics of the seed oils investigated in this study has provided some indications about the possible uses of the test vegetable oils. Indeed the results of the study justify the food uses of morama and manketti seed oils, which are respectively dominated by oleic and linoleic acids, rather similar to olive and sunflower oils. Moreteloga-kgomo seed oil, on account of its high content of VLFAs, could be recommended for use as a lubricant as well as for use in cosmetics and also for biodiesel. Mkukubuyo seed oil may not be safe for consumption but could be recommended for cosmetic formulations and for biodiesel. All the four seed oils mentioned above are obtained from high oil-yielding seeds which must be recognised as potential candidates for development to produce commercial quantities of vegetable oils for appropriate uses.

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