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Fatty acid composition of seed oils from selected wild plants of Kahuzi-Biega National Park and surroundings, Democratic Republic of Congo

K. Minzangi¹, A. N. Kaaya^{2*}, F. Kansiime³, J. R. S. Tabuti³, B. Samvura⁴ and O. Grahl-Nielsen⁵

¹Laboratory of Phytochemistry, Biology Department, CRSN Lwiro, Bukavu, Democratic Republic of Congo.

²Department of Food Science and Technology, Makerere University, P. O. Box 7062 Kampala, Uganda.

³Institute of Environment and Natural Resources (MUIENR), Makerere University, P.O. Box 7062 Kampala, Uganda.

⁴Department of Chemistry, Insitut Supérieur Pédagogique de Bukavu, Democratic Republic of Congo.

⁵Department of Chemistry, University of Bergen, Allegt 41, N-5007 Bergen, Norway.

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Oils were extracted from seeds of *Carapa grandiflora* Sprague (Meliaceae), *Carapa procera* DC. (Meliaceae), *Cardiospermum halicacabum* Linn (Sapindaceae), *Maesopsis eminii* Engler (Rhamnaceae), *Millettia dura* Dunn (Fabaceae), *Myrianthus arboreus* P. Beauv. (Cecropiaceae), *Myrianthus holstii* Engl. (Cecropiaceae), *Pentaclethra macrophylla* Benth (Mimosaceae), *Podocarpus usambarensis* Pilger (Podocarpaceae), *Tephrosia vogelii* Hook. (Fabaceae) and *Treculia africana* Decne (Moraceae) collected from a forest in Kahuzi-Biega National Park and the surrounding areas in Democratic Republic of Congo. Fatty acids in the oils were determined by gas chromatography to detect potential sources of various quality oils. Twenty-four fatty acids were detected in the seed oils and the most abundant were palmitic (16:0), stearic (18:0), oleic (18:1n9), linoleic (18:2n6) and α -linolenic (18:3n3) acids. Four fatty acids with *trans* double bonds and two fatty acids with non-methylene separated double bonds were detected in oils from *C. halicacabum*, *M. dura* and *T. vogelii*. There were remarkable occurrences of very long chain fatty acids, particularly lignoceric and behenic acids. *M. eminii*, *P. usambarensis*, *T. vogelii* and *M. dura* seed oils have potential for use in foods because of the contents of essential fatty acids. The oils from *C. grandiflora*, *C. procera*, *M. arboreus*, *M. holstii* and *P. usambarensis* showed a high potential for use in the cosmetic industry due to their fatty acids profile and high unsaponifiable matter content.

Key words: Seed oils, fatty acids, *trans* fatty acids, nonmethylene-interrupted fatty acids, nutritional potential.

INTRODUCTION

Plant seeds are important sources of oils of nutritional, industrial and pharmaceutical importance (Alvarez et al., 2000). The suitability of oil for a particular purpose, however, is determined by its characteristics and fatty acid composition. No oil from any single source has been

found to be suitable for all purposes as oils from different sources generally differ in their fatty acid composition (Dagne and Jonsson, 1997).

The world production of fatty acids (FAs) from the hydrolysis of natural fats and oils totals about 4 million metric tons per year. FAs are utilized in a wide variety of end-use industries that include food, medicine, rubber, plastics, detergents, and cosmetics (Gunstone, 1996). Fats and oils make up the greatest proportion of raw materials in the chemical industry (Biermann et al., 2000).

*Corresponding author. E-mail: ankaaya@agric.mak.ac.ug. Tel: 256-772440046. Fax: 256-414-531641.

Table 1. Species of wild oil plants studied from Kahuzi-Biega National Park (KBNP) and the surrounding areas.

Scientific name	Family	Local name	Locality
<i>Carapa grandiflora</i> Sprague	Meliaceae	Igwerhe	KBNP/Tshibati
<i>Carapa procera</i> DC	Meliaceae	Ewechi	Irangi
<i>Cardiospermum halicacabum</i> Linn	Sapindaceae	Mubobogo	Mugeri
<i>Maesopsis eminii</i> Engler	Rhamnaceae	Omuguruka	Lwiro
<i>Millettia dura</i> Dunn	Fabaceae	Nshunguri	KBNP/Tshibati
<i>Myrianthus arboreus</i> P. Beauv.	Moraceae	Bwamba	Irangi
<i>Myrianthus holstii</i> Engl.	Moraceae	Chamba	KBNP/Tshibati
<i>Pentaclethra macrophylla</i> Benth	Mimosaceae	Lubala	Irangi
<i>Podocarpus usambarensis</i> Pilger	Podocarpaceae	Omufu	KBNP/Tshibati
<i>Tephrosia vogelii</i> Hook.	Fabaceae	Mukulukulu	Lwiro
<i>Treulia Africana</i> Decne	Moraceae	Bushingu	Irangi

However, the sources of oils and fats are diminishing, implying that there is the growing need for new sources of oil to supplement the existing ones (Mohammed et al., 2003).

In this study, we analyzed the fatty acid composition of oils from seed of eleven wild plant species of Kahuzi-Biega National Park (KBNP) and surrounding areas in Eastern D.R. Congo in order to establish their potential for use in the food and cosmetic industry. Our earlier studies (Kazadi et al., 2011) quantified the oil content from each of these plant species and found that it ranged from 17.2 to 64.4%. This oil content is higher than that of some food crops such as soybean and olive seed indicating that these plant species have a high potential as oil sources.

MATERIALS AND METHODS

Collection of seed material and its preparation for oil extraction

Seeds were collected from the 11 plant species (Table 1) from KBNP and the surrounding areas in South-Kivu Province, Eastern D.R. Congo located at 2°30'S 28°45'E. These plants were chosen because they are the dominant oil tree species in the primary forest of KBNP and the surrounding swamp areas (Basabose, 2004) and their fruits have been utilized for several purposes but not for oil extraction. At least 500 g of mature seeds were collected for each species. Seed samples were taken to Phytochemistry Laboratory of Centre de Recherche en Sciences Naturelles de Lwiro where they were sun dried for one week before drying at 105°C till constant weight in an oven. After drying, the seeds were shelled by hand to remove the kernels which were crushed using a coffee-mill to produce fine seed flour from which oil samples were extracted.

Extraction of oil from plants seeds

Oil from the flour was extracted using the Soxhlet's procedure (Barthet et al., 2002), by repeated washing with petroleum ether (boiling point 40 to 60°C). After 8 h, the Soxhlet extraction flask containing oil and solvent mixture was removed from Soxhlet apparatus. The oil dissolved in petroleum ether was filtered using

filter paper (Whatman No. 1) and the solvent evaporated under vacuum in a rotary evaporator. The remaining solvent traces were removed by heating the flask containing oil in a water bath at 90°C. The oil obtained, was thereafter stored in hermetically closed bottles and kept in a refrigerator at 4°C till further analyses.

Analysis of fatty acids (FAs)

The FA composition of oils was determined by converting into FA methyl esters (FAMES) followed by gas chromatography (GC). Samples of 50 mg oil were accurately weighed in thick-walled 15 ml glass tubes. The tubes were prepared in advance with an accurately determined amount of the saturated FA, nonadecanoic acid, 19:0 as internal standard. This was added to the tubes by pipetting 50.0 µl of a solution of 19:0 in chloroform into the tubes, and then allowing the chloroform to evaporate. This pipetting was carried out with the handystep electronic, motorized repetitive pipette. Anhydrous methanol, 750 µl, containing hydrogen chloride in a concentration of 2 mol/L, was added, the tubes were securely closed with teflon-lined screw caps and placed in an oven at 90°C for 2 h. The lipids were methanolysed, leaving all FAs as FAMES. After cooling to room temperature, approximately half the methanol was evaporated under a stream of nitrogen after which 0.5 ml distilled water was added.

The FAMES were extracted 2 times from the methanol/water-phase with 1 ml hexane by vigorous shaking by hand for 1 min each time, followed by centrifugation at 3000 rpm. Analysis was carried out with a Hewlett-Packard 5890A gas chromatograph and Hewlett-Packard 7673A auto-sampler. A fused silica capillary column with polyethylene glycol as the stationary phase with a thickness of 0.2 µm (CP-WAX 52CB from Chrompac, 25m × 0.25mm) was used. The carrier gas was helium at a flow rate of 1.7ml/min at 40°C. 1 µl of the combined FAMES extracts was automatically injected splitless (the split was opened after 4 min), on a capillary column. The temperature program was 90°C for 4 min, 90 to 165°C with 30°C/min, 165 to 225°C with 3°C/min, 225°C for 10 min, total run time 43 min, cooling included. Injector and detector temperatures were 260 and 330°C, respectively.

Samples were chromatographed in random order with a standard solution, GLC 68D containing 20 FAMES, for each 8th sample. The quantitatively most important FAs were identified in the samples by way of the standard mixture and by using previous experience (Grahl-Nielsen et al., 2000) of relative retention times of FAMES and mass spectrometry.

The response factors for the FAMES for which there were no standards, were estimated by comparing with the standard FAMES

Table 2. Relative amounts, as percent of sum of fatty acids in seed oils from plants of Kahuzi-Biega National Park and surroundings areas in D.R. Congo. The values are means of three replicate analyses with approximately 1% relative SD. Cases where amounts of fatty acids were below the detection limit of 0.1% are indicated with-.

Division	Magnoliophyta										Pinophyta
Subclass	Rosidae						Hamamelidae				Gymnosperm
Order	Sapindales			Fabales			Rhamnales	Urticales			Pinales
Family	Maliaciae			Fabaceae			Rhamnaceae	Cecropiaceae		Moraceae	Podocarpaceae
Genus	<i>Carapa</i>	<i>Carapa</i>	<i>Cardiaspermum</i>	<i>Milletia</i>	<i>Pentactelehra</i>	<i>Tephrosia</i>	<i>Maesopsis</i>	<i>Myrianthus</i>		<i>Treculia</i>	<i>Podocarpus</i>
Species	<i>grandiflora</i>	<i>procera</i>	<i>halicacabum</i>	<i>dura</i>	<i>macrophylla</i>	<i>vogelii</i>	<i>eminii</i>	<i>arboreus</i>	<i>holstii</i>	<i>africana</i>	<i>usambarensis</i>
Fatty acids											
Myristic 14:0	0.1	-	-	-	-	0.1	-	0.1	0.1	-	-
Palmitic 16:0	23.6	1.9	10.7	4.1	4.9	14.0	8.3	3.9	4.0	18.3	4.0
Stearic 18:0	4.4	52.3	8.5	3.3	1.9	5.8	17.8	2.8	3.3	14.6	3.5
Arachidic 20:0	1.1	0.2	2.4	0.8	2.0	2.0	2.3	0.1	0.2	0.7	0.4
Behenic 22:0	0.6	-	1.5	7.3	6.3	5.8	0.6	-	0.1	0.4	0.3
Lignoceric 24:0	0.2	0.1	2.0	2.6	9.8	1.5	0.2	0.1	0.1	0.3	0.2
Total SFA	30.0	54.5	25.1	18.1	24.9	29.2	29.2	7.0	7.8	34.3	8.4
Palmitoleic 16:1n7	0.5	-	0.2	-	0.1	-	-	0.2	0.2	0.3	-
Oleic 18:1n9	40.2	42.5	36.8	31.8	30.1	19.6	36.7	9.8	8.6	29.2	39.6
cis-vaccenic18:1n7	1.0	0.3	0.7	0.5	0.5	0.3	0.3	2.5	1.2	1.6	0.3
Eicosenoic 20:1n9	0.3	0.1	0.5	2.4	2.4	0.7	2.2	0.3	0.4	0.2	2.0
Erucic 22:1n9	-	-	-	0.7	0.3	-	-	0.2	-	-	-
Nervonic 24:1n9	-	-	-	0.1	0.1	-	-	-	-	-	-
Total MUFA	42.0	42.9	38.2	35.5	33.5	20.6	39.2	13.0	10.4	31.3	41.9
Linoleic 18:2n6	26.4	1.6	35.1	20.2	40.6	40.3	26.8	77.2	80.2	30.0	29.7
α-linolenic 18:3n3	1.1	0.6	0.6	21.2	0.1	7.6	3.8	0.5	0.5	1.2	9.0
Eicosadienoic 20:2n6	-	-	-	0.2	0.2	0.1	0.1	0.3	0.2	-	2.9
Eicosatrienoic 20:3n3	-	-	-	-	-	-	0.4	0.1	0.1	-	0.1
c9, t12-18:2	0.1	0.1	0.4	0.7	0.3	0.8	0.1	0.6	0.3	1.5	0.1
t9, c12-18:2	-	0.1	0.4	0.7	0.2	0.7	0.1	0.5	0.3	1.3	0.1
c9, c12, t15-18:3	-	0.1	-	1.2	-	0.2	-	0.1	-	0.1	0.2
t9, c12, c15-18:3	-	0.1	-	1.7	-	0.3	-	-	-	0.1	0.2
c5, c11, c14-20:3	-	-	-	0.1	-	-	-	0.2	0.2	-	5.4
c5, c11, c14, c17-20:4	-	-	-	-	-	-	-	0.1	0.1	-	2.0
Total PUFA	27.6	2.6	36.5	46.0	41.4	50.0	31.3	79.6	81.9	34.2	49.7

Table 2. Contd.

ω6/ω3 ratio	24.0	2.7	58.5		1.0	408.0	5.3		6.4		129.2	134.0		25.0		3.6
% fatty acids in oils	81.2	82.2	82.0		78.8	82.0	61.3		79.7		63.8	79.4		80.9		84.4

which resembled each of those most closely in terms of chain length and number of double bonds. The relative amount of each FA in a sample was expressed as a percentage of the sum of all FAs in the sample. Three replicate analyses were carried out for all samples. Analysis of variance (ANOVA) was performed for FA composition of seeds from all plant species using GenStat Statistical Computer Package (GenStat Release 7.1, 2003) and the means were separated using LSD ($P \leq 0.05$).

RESULTS

Twenty four FAs were detected in the oils from the sampled plant species. Six were saturated FAs (SFAs), six were monounsaturated FAs (MUFAs) with 16 to 24 carbons, and 12 were polyunsaturated FAs (PUFAs). Four of the PUFAs had *trans* double bonds and two had nonmethylene-interrupted double bonds (Table 2). The relative amounts of the FAs varied largely among the plant species.

Palmitic (16:0) and stearic (18:0) acids were the most abundant among the SFAs. This acid was most abundant in *C. grandiflora* and *T. africana* with 23.6 and 18.3% respectively, while stearic acid was present in very high amounts in *C. procera* (52.3%). Three of the species, *M. dura*, *P. macrophylla* and *P. usambarensis*, also contained high levels of the very long-chain SFAs notably behenic (22:0) and lignoceric (24:0) acids.

Oleic acid (18:1n9) was the single dominating MUFA extracted, and ranged from 8.6% in *M. holstii* to 42.5% in *C. procera*.

The PUFA found were dominated by essential

fatty acids *Omega-6* ($\omega-6$) and *Omega-3* ($\omega-3$) that is, linoleic acid (18:2n6) (LA) and α -linolenic acid (18:3n3) (ALA). The linoleic acid was found with levels between 20 and 80% in all species except *C. procera* which had 1.6% (Table 2 and Figure 1).

FAs with *trans* double bonds were also detected in the seeds (Table 2). c9, t12-18:2 and t9, c12-18:2 were present in levels above 0.1% in all seeds, and the two α -linolenic acid isomers, c9, c12, t15-18:3 and t9, c12, c15-18:3, were present in six species above trace levels. Finally, two nonmethylene interrupted FAs: c5, c11, c14-20:3 and c5, c11, c14, c17-20:4 were found in trace amounts in three of the species, and in substantial levels in *P. usambarensis*.

The fractions of very long chain FAs (chain length higher than 18 atoms of carbon) in analyzed oils ranged from 0.5 to 21.3% (Figure 2). The highest was from *P. macrophylla* and the lowest from *C. procera*. *P. macrophylla*, *M. dura* and *T. vogelii* had respectively, 21.3, 14.5 and 10.2% of their total FAs constituted of these very long chain FAs.

DISCUSSION

Saturated FAs

C. procera oil contained the highest levels of SFAs among all analyzed plant species. Its FAs profile was found to be similar to those of other saturated oils such as: shea butter (*Vitellaria*

paradoxa) and cocoa butter (*Theobroma cacao*) which have stearic acid as their main component followed by oleic acid. Cocoa butter is mostly used in the food industry, while shea butter is mainly used in cosmetics, although it has also been authorized for use as an alternative to cocoa butter in chocolate products (Foubert, 2003). Therefore, all these oils, including that from *C. procera* seed, contain approximately half of SFAs and their nutritional interest lies in the neutrality of stearic and oleic acids with regard to plasma lipid composition (Dubois et al., 2007).

T. africana and *C. grandiflora* oils are also characterized by relatively high fractions (34.3 and 30% respectively) of SFAs. Foma and Abdala (1985) found in samples from northern D.R. Congo oleic acid (32.7%) followed by linoleic (25.8%) and palmitic (25.7%) acids as predominant FAs in *T. africana*. *M. eminii* oil had 29.2% of SFAs. Theagarajan et al (1986) reported stearic (26.48%), oleic (47.49%) and linoleic (14.79%) acids in *M. eminii* samples from India.

Monounsaturated and polyunsaturated FAs

C. grandiflora and *C. procera* contained respectively 40.2 and 42.5% of oleic acid and compare quite well with palm oil and palm olein commodities of high economic importance which contain respectively 39.1 and 46.0% oleic acid (Mohammed et al., 2003). With regard to *C. procera*, Kabele (1975) reported oleic (48.9%); palmitic (26.4%); linoleic (14.4%) and stearic

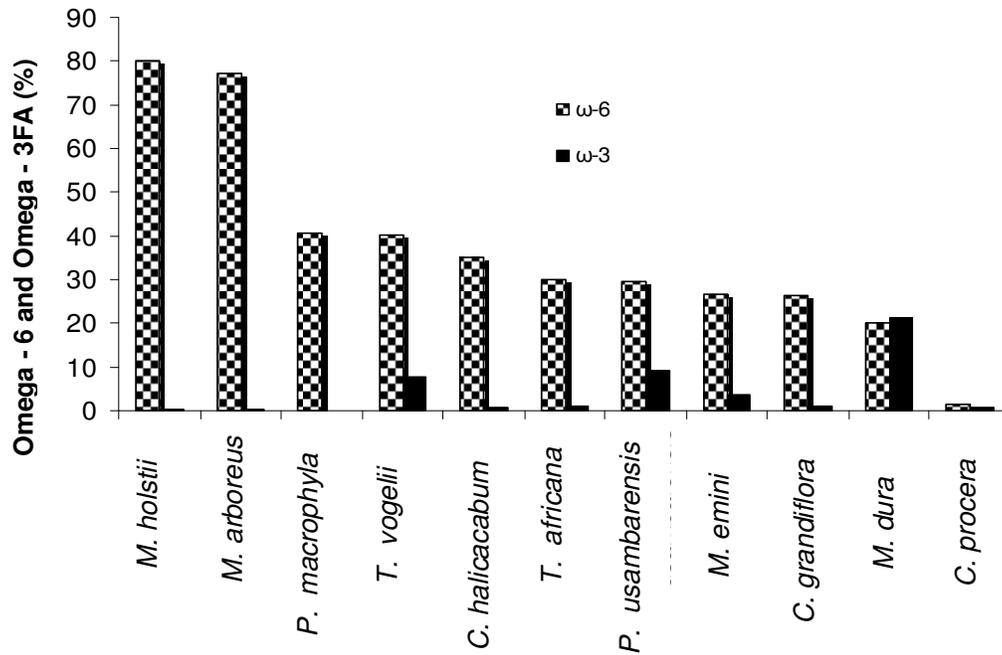


Figure 1. Omega-6 and Omega-3 FAs content in oils of plants obtained from Kahuzi-Biega National Park and surroundings areas in D.R. Congo.

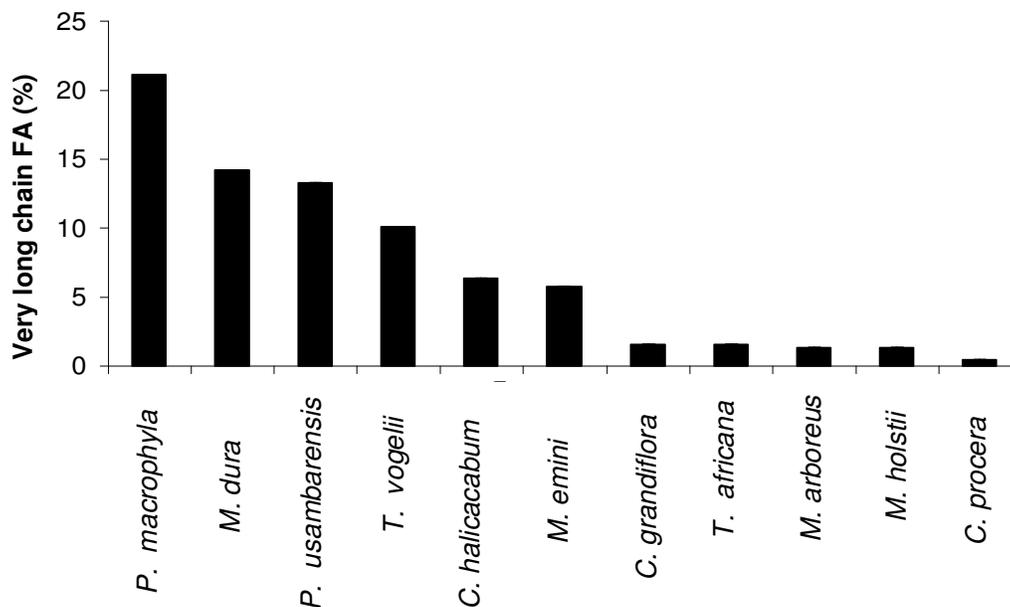


Figure 2. Very long chain FAs content in oils of plants obtained from Kahuzi-Biega National Park and surroundings areas in D.R. Congo.

(8.0%) acids in related plant samples from western D.R. Congo. Oldham et al. (1993) reported that the main FAs of the oil from *C. procera* were palmitic (26.6%), linoleic (25.2%), stearic (11.8%), linolenic (10.5%), oleic (9.9%) and arachidic (9.4%) acids. All the reported plants in the

current study except *C. procera* had more than 60% of their FA content unsaturated. Thus, these plant species may have a high potential in nutrition (Dubois et al., 2007). The α-linolenic acid (ALA) content of *M. dura* seed oil (21.2%) is about three times richer than soybean oil

(7.8%) and canola oil (7.9%) which are common crops that provide this type of acid (Zamora A, 2005).

Regarding *C. procera*, our oleic acid finding (42.5%) is a bit near to that of Kabele (1975) which was 48.9% but very different from that reported by Oldham et al. (1993) (9.9%). In addition, about SFA the reported contents (54.5%) in the current study are different from those reported by the two cited authors (34.4 and 47.8%) respectively. This could be due to the identity of the seed material. The three samples could be from different varieties of the same species if not from different species of same family. Further taxonomic and chemotaxonomic investigation is necessary.

The concentrations of linoleic acid found in *M. arboreus* and *M. holstii* (77.2 and 80.2% respectively) are higher than that found in common edible oils, such as: cottonseed oil, grape seed oil and oils from soybean, sunflower, safflower and corn (Dubois et al., 2007). These results indicate that oils extracted during this study can be used as alternatives for commercial oils for nutritional purposes.

Our results further show that *M. eminii*, *P. usambarensis*, *T. vogelii* and *M. dura* seed oils are nature's most balanced oil among the analyzed plants as dietary and health oils, because they had the most complete profile of essential FAs. They also have an equilibrated ω -6/ ω -3 ratio closer to the recommended ratio of 6 (Wijendran and Hayes, 2004) than soybean oil (Dubois et al., 2007). *T. vogelii* seed oil which has poisonous products (Lambert et al., 1993) can only be considered in nutrition after extensive refining (Orthofer and List, 2007). On the whole the unsaturated fraction in *M. arboreus* and *M. holstii* was near to 94% implying that the oils of the seeds of these two *Myrianthus* species could be used very effectively to make varnishes for timber (Giuffre et al., 1996).

FAs with double bonds in *trans* configuration

FAs with *trans* double bonds are rarely found in plant seed oils. The few cases reported are mostly uncommon FAs, like conjugated linolenic acids (Özgül-Yücel, 2005), acetylenic FAs (Aitzetmüller et al., 2003) or non-methylene interrupted FAs (Tsevegsüren et al., 2000). In the current study, we detected *trans* double bonds in the common FAs, linoleic acid and α -linolenic acid. *Trans* double bonds that is, t9, t12-18: 2 have been detected in linoleic acid in the seed oils of *Chilopsis linearis* (Chisholm and Hopkins, 1963), and c9, t12-18:2 in the seed oils of *Crepis rubra* (Morris and Marshall, 1966). In the case of α -linolenic acid, our findings of the *trans* isomers, c9, c12, t15-18:3 and t9, c12, c15-18:3 in *M. dura*, and trace amounts in *C. procera*, *T. vogelii*, *T. africana* and *P. usambarensis*, apparently seem to be the first detection of α -linolenic acid with *trans* double bonds in plant seed oils. It has been shown that *cis-trans*

isomerisation may occur during acid methanolysis (Mjøs et al., 2006). However, this was the case when prolonged reaction times, > 4 h at 100°C, were used, and when the methanolysis was performed directly on the tissue sample (herring in this case). No isomerisation took place when the methanolysis was performed on the extracted lipids, indicating that the isomerization was caused by non-extractable catalysts in the tissue (Mjøs et al., 2006). It is therefore clear that the *trans* FAs observed in the present study, with relatively mild methanolysis conditions, that is, 90°C for 2 h, performed on extracted oils, were really present in the samples and not caused by isomerisation during the work-up procedure.

Non-methylene-interrupted FAs

The presence of all *cis*- Δ 5-unsaturated polymethylene-interrupted FAs (Δ 5-UPIFA), 5,11,14-20:3 (sciadonic acid) and 5,11,14,17-20:4 (juniperonic acid) in *P. usambarensis*, were expected, since these two acids together with other polymethylene-interrupted FAs, are often present in all gymnosperms in levels around 5% of the total FAs, with the former usually being the most abundant (Mogrand et al., 2001; Wolff and Christie, 2002). So far, 5-UPIFAs have been determined in 6 out of the approximately 100 *Podocarpus* species (Mogrand et al., 2001; Wolff et al., 1999; Bagci and Karagacli, 2004).

Very long chain, chemotaxonomically relevant FA

The very long chain FAs (VLCFAs) has essential chemotaxonomic significance (Bagci and Şahin, 2004). In the current study *P. macrophylla* seed oil had the highest fraction (21.3%) of VLCFAs including lignoceric (9.8%), behenic (6.3%) and eicosenoic acids (2.4%). Jones et al. (1987) have also identified hexacosanoic (C26:0) and octacosanoic (C28:0) acids in *P. macrophylla* seed oil. In the current investigation and as reported by Kabele (1975) and Foma and Abdala (1985) the occurrence of these two very long-chain FAs was not established.

M. dura seed oil had the second highest fraction (21.3%) of VLCFAs including lignoceric acid 2.6%, eicosenoic acid 2.4%, erucic acid 0.7% and remarkably high percentage (7.3%) of behenic acid. Behenic acid was also reported by Ezeagu et al., (1998) in related species *Milletia thonningii* seed oil at content of 8.93% which is almost similar to that found from *M. dura* seed oil in the current study. Ezeagu et al. (1998) had found from *Milletia thonningii* samples from Nigeria FAs profile very alike to that of analyzed samples of *M. dura* in the current study. The behenic, lignoceric and eicosenoic acids seem to be characteristic to this *Milletia* genus. The VLCFAs fraction of *T. vogelii* seed oil (10.2 %) contained notably, behenic acid (5.8%) and lignoceric acid (1.5%).

Groundnut oil which has arachidic acid content ranging from 1.1 to 2.3% (Davis et al., 2008) is regarded as vegetable source of this FA. Among the analyzed plant species, *C. grandiflora*, *C. halicacabum*, *M. eminii*, *P. macrophylla* and *T. vogelii* had arachidic acid content similar to that of groundnut oil with respectively 1.1, 2.4, 2.3, 2.0, and 2.0%. In addition, the FA profile of *M. eminii* oil analyzed was found to resemble that of oil from peanut cultivars reported by Davis et al. (2008).

Eicosadienoic acid (20:2n6) occurred in relatively high levels in *P. usambarensis* seed oil (2.9%) while podocarpic acid (ω 6-eicosatrienoic acid: 20:3n6) known to be unique to *Podocarpus nagera* (Zamora, 2005) was not found in this *P. usambarensis* seed oil. In *C. halicacabum* seed oil, more than 6% of total FAs are constituted of VLCFA. In our study, just like Chisholm and Hopkins (1958), we did not find the 11-eicosenoic (gadoleic) acid as major FA as reported in previous works (Ahmad, 1992). These differences may be due to variety, time of harvest, source and seasonal variation (Foubert, 2003). The VLCFA identified in the seed oils can be used in the manufacture of some products such as the high grade candles (Weiss et al., 1979).

The studied plant species can be domesticated for oil production like olives and palm trees because some of them are known as fast growing plants (Binggeli and Hamilton, 1993). Thus, commercial utilization of these plants is possible through mass propagation and cultivation than harvesting them from the wild.

Conclusions

We have established that a variety of FAs can be found in the studied plant species' oils. Many of these have FAs in amounts in the range or sometimes surpassing currently used species in industry. This implies that these plant species have a potential to substitute several species currently used in industry and therefore should be recommended for domestication. In order to extend the range of use of the oil, these oils should be refined and the FA composition, taste, smell, colour and potential toxicity of the refined oils determined since these impact on consumer acceptance.

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