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Potential New Sources of Oleic Acids from Wild Plants from Kivu, D.R. Congo

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ABSTRACT

Oleic oil is one of the better known fatty acid for consumption and from a health standpoint. It is known to slow the development of heart disease and to produce antioxidants. Oils from *Carapa grandiflora* and *Carapa procera* (Meliaceae), *Cardiospermum halicacabum* (Sapindaceae), *Maesopsis eminii (*Rhamnaceae*), Millettia dura* (Fabaceae), *Pentaclethra macrophylla* (Fabaceae), *Podocarpus usambarensis* (Podocarpaceae) and *Treculia africana* (Moraceae) were analysed using gas chromatography to determine their fatty acids composition. These plants are very frequent and grow wild in Kahuzi-Biega National Park and surrounding in the Kivu region, D.R. Congo and are used by the local population mainly for nutrition and medical purposes. Twenty-four fatty acids were determined and oleic acid was the predominant fatty acid in the seed oil from six of the eight analyzed plant species, with 41.2% in *C. grandiflora*, 42.8% in *C. procera*, 39.9% in *P. usambarensis*, 37.5% in *C. halicacabum*, 37.0% in *M. eminii*, 30.8% in *T. africana* and 32.3% in *M. dura*. The oleic acid fraction in all these 8 analyzed oils ranges 30.8 - 42.8%. In comparison to palm oil and palm olein, commodities with high economic importance which comprise respectively 39.1 and 46.0% oleic acid, *C. grandiflora* and *C. procera* (41.2 and 42.8% of oleic acid, respectively) can be new cheap sources of this acid. All analyzed oils here can be used as sources of olein, because the oleic content of all 8 plant species is enough high to be enhanced by fractionation to become a good source of high-oleic oils.

Keywords: *Oilseed plants; olein; fatty acids composition; Kahuzi-Biega National Park.*

INTRODUCTION

Oleic acid (9-octadecenoic acid) is a mono-unsaturated fatty acid (FA) naturally found in many plants and in some animal products. It is an omega-nine fatty acid, and considered one of the healthier sources of fat in the diet [1]. Oils rich in monounsaturated FAs (e.g. oleic acid) are generally more stable to oxidative rancidity and stable as deep frying oils [2]. They have many applications as plant-based lubricants or as feedstock for the oleochemical industry [3]. As a fat, oleic oil is one of the better known ones for consumption and from a health standpoint. It exhibits further such benefits as total cholesterol, whereas it is known to slow the development of heart disease and to produce antioxidants [4]. The administration of oleic and erucic acids has been widely publicized as a possible cure for adrenomyeloneuropathy a cerebral disease [5]. Due to the importance placed on dietary monounsaturated FAs (MUFAs), it has been recommended that MUFAs intake be as high as half of the total recommended dietary intake of calories from fat (30%) as a means to reduce the risk of coronary artery and heart disease [6-8]. In addition, oleic acid is part of a number of products, such as soap and cosmetics in which it is a great moisturizer [1]. Oleic acid is the main component of numerous vegetable oils, including olive and rapeseed oils, and is the major dietary mono-enic acid. One of the chief sources of oleic acid in foods is olive oil. It might have a slight and controversial positive effect on LDL-cholesterol. Olive oil has long been described as healthy because of the presence of oleic acid, but it seems that other compounds might be considered as the active ones [9].

Due to ever-diminishing sources of fats and oils, there is the growing need for the search of new sources of oil as well as exploiting sources that are currently under-exploited in order to supplement the existing ones [2]. Furthermore, the price of edible oil is increasing due to the effects of turning edible oil into energy sources [10]. Similarly the biodiesel market is growing [11]. Thus, to promote plants conservation, oils from many wild plant species of Kahuzi-Biega National Park (KBNP) and surrounding in Kivu, Democratic Republic of the Congo (DRC) were analyzed [12]. These plants grow wild in this region of east of DRC where some important plant species are threatened with extinction [13].

Exploitation of non-timber forest products, particularly fruits and seeds as a source of oil can help to reduce oil costs by diversifying the sources for this commodity. This form of exploitation can be more sustainable than timber extraction, because this is often

viewed as a means of sustainable forest management affecting the structure and function of forests much less than other uses [14]. Demonstration of tangible economic values can lay the foundation for rational use and protection of plant resources, because people tend to conserve plants which they know are important for their needs. This study sought to establish the importance of some local plants from Kivu by assessing their chemical properties in order, to support rural people's needs in ways that are in harmony with environment.

In the present work, oleic acid rich oils from 8 plant species analyzed are reported in order to appoint them as potential source of oleic acid, a fatty acid of economic importance. These plants are *Carapa grandiflora* and *C. procera* (Meliaceae), *Cardiospermum halicacabum* (Sapindaceae), *Maesopsis eminii (Rhamnaceae), Millettia dura* (Fabaceae), *Pentaclethra macrophylla* (Mimosaceae), *Podocarpus usambarensis* (Podocarpaceae) and *Treculia africana* (Moraceae). These plants grow wild in Kahuzi-Biega National Park and surrounding in Kivu region, D.R. Congo and some of them are being domesticated for timber or others forestry benefits. Many of them have not been analysed as oil sources.

EXPERIMENTAL

Seed samples from the above cited plant species were collected from KBNP and surrounding in Sud–Kivu Province, Eastern DRC. Mature seed samples were collected from beneath the trees and kept in plastic bags. Only entire seeds whose kernels were protected by seed coat were collected to avoid contamination. At least 500 g of seeds were collected from 5 to 7 trees for each plant species. Voucher specimens of these plant species were identified in Herbarium of "Centre de Recherche en Sciences Naturelles de Lwiro" (CRSN/Lwiro) in Sud–Kivu, DRC and in Herbarium of Department of Botany of Makerere University to confirm the plants identification. The oil extraction and identification and quantification of FAs were determined following the American Oil Chemists Society official methods [15]. Oil samples were extracted from seed kernels by petroleum ether in Soxhlet's apparatus [16].

Identification and quantification of fatty acids was done using Gas Chromatography (GC) in the laboratory of Department of Chemistry, University of Bergen, in Norway by Dr. Otto Grahl-Nielsen. The oil samples were weighed to approximately 50 mg, and were transferred to thick-walled 15 mL glass tubes, avoiding water contamination. The tubes were prepared with an accurately determined amount of the saturated fatty acid, nonadecanoic acid (19:0; Nu Chek Prep, Elysian, Minn., USA) as internal standard. This was added to the tubes by pipetting 50.0 µL of a solution of 19:0 chloroform into the tubes, and then allowing the chloroform to evaporate. This pipetting was carried out with Handystep electronic, motorized repetitive pipette and 750 µL anhydrous methanol containing hydrogen chloride were added to the methanol as dry gas, in a concentration of 2 $mol.L^{-1}$ to allow hydrolysis of oil triglycerides. The tubes were securely closed with teflon-lined screw caps. After keeping the tubes in an oven at 90°C for two hours, the samples were then methanolysed by the replacement of glycerol in the triglyceride by methanol. So, all fatty acids were converted to fatty acid methyl esters (FAMEs). After cooling to room temperature, approximately half the methanol was evaporated by nitrogen-gas bubbling, and 0.5 mL distilled water was added.

The FAMEs were extracted from the methanol/water-phase with 2 x 1.0 mL hexane by vigorous shaking by hand for one minute each time, followed by centrifugation at 3000 rpm. The FAMEs extracted were recovered in a 4 mL vial with teflon-lined screw cap. The concentration of the FAMEs in the extracts was adjusted to obtain levels suitable for gas chromatography.

One µL of the adjusted extract was automatically injected splitless (the split was opened after 4 min), on a capillary column. Samples were analyzed in random order with a standard solution, GLC 68D from Nu Chek Prep (Elysian, Minn., USA) containing 20 FAMEs. The 20 - 40 quantitatively most important fatty acids were identified in the samples, by way of the standard mixture following previous experience of relative retention times of FAMEs and mass spectrometry. The smallest peaks, that are those with areas of less than 0.1% of the total area of all peaks, were not considered. The peaks were integrated by Chromeleon software and the resulting area values exported to Excel, where they were corrected by response factors.

These empirical response factors, relative to 18:0, were calculated from the 20 FAMEs, present in known proportions in the standard mixture. An average of 10 runs of the standard mixture was used for these calculations. The response factors for the FAMEs for which there were no standards, were estimated by comparison with the standard FAMEs which resembled each of those most closely in terms of chain length and number of double bonds. The relative amount of each fatty acid in a sample was expressed as percentage of the sum of all fatty acids in the sample. The data analysis were performed with at least 3 replicates and the mean values and standard deviation (mean ± SD) calculated and all data subjected to analyses of variance (ANOVA). The least significant differences of means (LSD) test at 5% probability level was also carried out. All analyses were done using the GenStat computer package programme, GenStat release 7.1, Copyright 2003, Lawes Agricultural Trust (Rothamsted Experimental Station), Seventh Edition.

RESULTS AND DISCUSSION

Table 1 give fatty acid composition of oil from *Carapa grandiflora* , *Catapa procera, Cardiospermum halicacabum* , *Maesopsis eminii, Millettia dura, Pentaclethra macrophylla, Podocarpus usambarensis* and *Treculia africana* from Kahuzi-Biega National Park and surrounding in Kivu region, D.R. Congo.

As it can be seen from this table twenty four FAs were determined and identified in the studied plant species. The composition levels of the FAs from the oils were significantly different ($p<0.001$). Notably the oils contained linoleic (18:2n6), oleic (18:1n9), stearic (18:0), palmitic (16:0) and α-linolenic (18:3n3) acids as well as very long chain FAs. The total FA content in oils for studied plant species ranged from 79.7% for *Maesopsis eminii* to 84.4% for *Podocarpus usambarensis*. The palmitic, stearic, oleic, linoleic, α-linolenic, arachidic, eicosenoic, eicosadienoic, eicosapentaenoic and lignoceric acids are found existing in various amounts in all studied plant species.

Among the twenty four fatty acids determined and oleic acid was the predominant fatty acid in the seed oil from six of the eight analyzed plant species.

Table 1: Fatty acid (FA) composition (wt. % of total of FA) of oil from plants of Kahuzi-Biega National Park and surrounding areas in D.R. Congo. All FAs showed significant variation among species (p<0.001)

FA & symbol	*C. grandiflora*	*C. procera*	*C. halicacabum*	*M. eminii*	*M. dura*	*P. macrophylla*	*P. usambarensis*	*T. africana*	LSD	CV %
Myristic 14:0	0.1	-	-	-	-	-	-	-	0.01729	19.0
Palmitic 16:0	23.6	1.9	10.7	8.3	4.1	4.9	4.0	18.3	0.1535	0.9
Palmitoleic 16:1n7	0.5	-	0.2	-	-	0.1	-	0.3	0.02615	10.5
Stearic 18:0	4.4	52.3	8.5	17.8	3.3	1.9	3.5	14.6	0.2304	1.1
Oleic 18:1n9	40.2	42.5	36.8	36.7	31.8	30.1	39.6	29.2	0.2382	0.4
Oleic 18:1n7	1.0	0.3	0.7	0.3	0.5	0.5	0.3	1.6	0.03344	1.8
LA 18:2n6	26.4	1.6	35.1	26.8	20.2	40.6	29.7	30.0	0.5279	0.7
ALA 18:3n3	1.1	0.6	0.6	3.8	21.2	0.1	9.0	1.2	0.05142	0.4
Arachidic 20:0	1.1	0.2	2.4	2.3	0.8	2.0	0.4	0.7	0.008787	1.2
Eicosenoic 20:1n9	0.3	0.1	0.5	2.2	2.4	2.4	2.0	0.2	0.10636	4.1
Eicosadienoic 20:2n6	-	-	-	0.1	0.2	0.2	2.9	-	0.01547	2.1
Eicosatrienoic 20:3n3	-	-	-	0.4	-	-	0.1	-	0.004386	3.5
EPA 20:5n3	0.3	0.1	0.2	0.2	0.3	0.2	0.2	0.2	0.08086	18.0
Behenic 22:0	0.6	-	1.5	0.6	7.3	6.3	0.3	0.4	0.06019	1.0
Erucic 22:1n9	-	-	-	-	0.7	0.3	-	-	0.01446	3.6
Lignoceric 24:0	0.2	0.1	2.0	0.2	2.6	9.8	0.2	0.3	0.09896	2.7
DHA 22:6n3	-	-	-	-	0.1	-	-	-	0.01382	23.0
Nervonic 24:1n9	-	-	-	-	0.1	0.1	-	-	0.013086	19.4
c9, t12	0.1	0.1	0.4	0.1	0.7	0.3	0.1	1.5	0.012148	1.1
t9, t12	-	0.1	0.4	0.1	0.7	0.2	0.1	1.3	0.01531	1.6
c9, c12, t15	-	0.1	-	-	1.2	-	0.2	0.1	0.011512	1.8
t9, c12, c15	-	0.1	-	-	1.7	-	0.2	0.1	0.008787	1.0
c5, c11, c14	-	-	-	-	0.1	-	5.4	-	0.05273	5.0
c5, c11, c14,c17	-	-	-	-	-	-	2.0	-	0.008152	2.2
ω–6/ω-3 Ratio	18.86	2.29	43.88	6.11	0.94	136	3.51	21.43		
%FA Tot.	81.2	82.2	82.0	79.7	78.8	82.0	84.4	80.9		

LA = linoleic acid, ALA = α-linolenic acid, DHA = Docosahexaenoic acid, EPA = Eicosapentaenoic acid; LSD: Least significant differences of means (5% level), CV%: coefficients of variation, (-) = was found in trace.

Among the twenty four fatty acids determined and oleic acid was the predominant fatty acid in the seed oil from six of the eight analyzed plant species.

Figure 1 give percentage of oleic acid in the plants

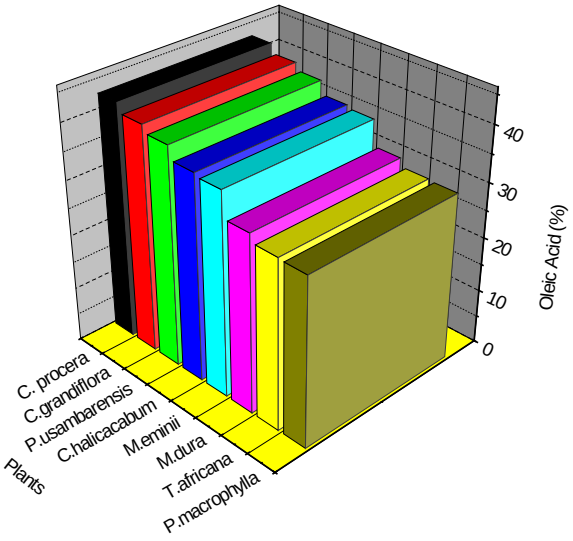

Figure 1: Oleic acid % in oils of plants from Kahuzi-Biega National Park and surroundings areas in D.R. Congo.

This figure show that te oleic acid fraction in all 8 analyzed oils is from 30.8 to 42.8%. The highest is from *Carapa procera* (42.8%) and the lowest from *Pentaclethra* macrophylla (30.6 %). This monounsaturated FA content in the extracted oils constituted more than 30% of the total FAs in each of all 8 plant species studied: *Carapa grandiflora* seed oil (41.2%), *Podocarpus usambarensis* seed oil (39.9%), *Cardiospermum halicacabum* seed oil (37.5%), *Maesopsis eminii* seed oil (37.0%), *Milletia dura* seed oil (32.3%) and *Treculia africana* seed oil (30.8%).

In comparison to palm oil and palm olein, commodities of high economic importance which comprise respectively 39.1 and 46.0% oleic acid [9], *Carapa grandiflora* and *C. procera* can be new cheap sources of oleic acid (40.2 and 42.5% respectively). There appears to be no previous reported work on FA composition of *Carapa grandiflora*, but for *Carapa procera*, Kabele [17] has reported 48.9% of oleic acid in samples from western D.R. Congo. Oldham *et al.*[18] found from *Carapa procera* 9.9% of oleic acid. A related species, *Carapa guianensis* was found oil rich source of usual FAs including oleic acid [19].

According to Susan [20], oil having oleic acid content from 30% of its FAs is suitable for olein production by oil fractionation. Thus, all 8 analyzed oils here can be used as source of olein which is useful for production of oils having high oxidative stability, more stable to oxidative rancidity and stable as deep frying oils [2]. Palm oil, a commodity of high economic importance and big source of olein has around 39% oleic acid only while olein itself has 46% [9].

Production of high-oleic oils for use in food applications has been receiving increased attention due to the health benefits attributed to oils of content high in monounsaturated/oleic fatty acid and their high stability even in demanding applications such as deep-frying [21]. Fractionation provides versatility for different food applications, with the additional advantage that this process doesn't bring apparition of *trans* fatty acids as in the case of hydrogenation process [22]. By enzymatic transesterification and fractionationthus Abdulkarim *et al.* [23] have enhanced the content of oleic acid in *M. oleifera* seed oil until 75.2%. Thus, the oleic content of our 8 plant species (31.8 to 42.8%) is enough high to be enhanced by fractionation to become good source of high-oleic oils.

CONCLUSION

This works showed that eight plants from Kahuzi-Biega National Park and surrounding in Kivu region, D.R. Congo have oils of a composition of oleic acid high enough to be enhanced by fractionation to become a good source of high-oleic oils. Other studies are ongoing in order to determine oil composition of other plants in this area.

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