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Fatty Acids and Tocopherols Content in Fractionated Oils from Five Wild Oilseed Plants Native to Kahuzi-Biega National Park, Kivu-DR Congo

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KM, ANK, BS and JNK conceived experiment and designed the experiments. Authors KM, BM and PVD performed experiments. Authors JNK, KM, PTM and BM analyzed the data. Authors DEM, KB, ANK, KM and JNK wrote the paper. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Most natural oils only have limited application in their crude forms, but fractionation can provide a variety of food applications. The objective was to fractionate crude oils from five selected plants growing wild in Kahuzi-Biega National Park (KBNP) in DR Congo, to analyze fatty acids and tocopherols contents in their respective resulting olein and stearin fractions, and to evaluate whether fractionation improves food quality.
Methods: Solvent fractionation of oil, Gas Chromatography (GC) for FAs analysis and High Performance Liquid Chromatography (HPLC) for Tocopherols analysis were used. Studied plants include *Carapa grandiflora* (Meliaceae)*, Cardiospermum halicacabum* (Sapindaceae), *Maesopsis eminii (Rhamnaceae), Millettia dura* (Fabaceae) and *Pentaclethra macrophylla* (Fabaceae).
Results: Fractionation gave the highest olein value yield (79.3%) from *M. eminii* oil and the highest stearin value yield (53.4%) from *C. grandiflora* oil. Eighteen FAs were detected, from plamitic acid (16:0) to very long chain FA cerotic acid (26:0). Differences were found between the profiles of fractions obtained and their corresponding crude oils. The profile of *C. halicacabum* olein is dominated by MUFAs (77%) and close to the known profile of olive oil (77%). In all olein and stearin fractions, unsaturated FAs dominated SFAs, contrary to the palm oil. *M. dura* and *P. macrophyla* oils are richest in active vitamin E compounds (tocopherols, tocotrienol and plastochromanol) with the highest content 9.6 mg/100 g in *M. dura* stearin and olein fractions.
Conclusions: The solvent fractionation used is relatively efficient to modify the proportions of SFA-MUFA-PUFA content in olein and stearin fractions. By its profile, *C. halicacabum* olein has similarities with olive oil, and would thus be used as a substitute for this expensive oil after some adaptations. Fractionation allows adding nutritional value to crude oils by increasing essential unsaturated fatty acids like nevronic acid while reducing the levels of unwanted fatty acids such as behemic acid. The high content of tocopherols in *M. dura* stearines also adds nutritional value to future derivate food products. It would be possible to adjust the final cooling temperature of the crude oils in order to modify processing yield and generate fractions of different qualities.

Keywords: Olein; stearin; fractionation; wild oilseed plants; fatty acids; tocopherols.

1. INTRODUCTION

Majority of natural plant oils only have limited applications in their crude forms, yet most of them have good structural chemical profiles [1]. To make them more suitable, hydrogenation as a method of oil modification has been used for a long while, but now is in decline due to nutritional concerns about trans-fatty acids (t-FAs) formed in the process and environmental concerns about disposal of nickel catalysts used during hydrogenation [2]. Trans-FAs negatively impact on human plasma lipoprotein profiles and surely have untoward implications for atherogenesis [3].

Alternatively, oil fractionation at controlled temperatures has become the more preferred process for oil quality improvement as it allows versatile transformation of oils for different food applications with the additional advantage that this process does not generate t-FAs as in the case of the above-mentioned hydrogenation process [4].

Direct oil crystallization from solvent is used mainly to purify individual saturated FAs or to make a crude separation of saturated and unsaturated FAs. For instance, preparation of a single FA like oleic acid from *Olea europea* oil [5] or from *Moringa oleifera* oil [6] was easily made by low temperature crystallization.

Previous studies, performed among the rich flora of Kahuzi-Biega National Park (KBNP) in DRC, helped identify a number of oilseed plants of potential interest for nutrition and medical uses [7], among which *Carapa grandiflora* (Meliaceae)*, Cardiospermum halicacabum* (Sapindaceae), *Maesopsis eminii (Rhamnaceae), Millettia dura* (Fabaceae), and Pentaclethra macrophylla (Fabaceae), locally named Igwerhe, Mubobogo, Omuguruka, Nshunguri*,* and Lubala respectively.

The oil from *C. grandiflora* (Cg) seeds is used as a substitute for Vaseline, while oils from some *Millettia dura* (Md) and related species are reported to be effective in preventing skin infection. *C. halicacabum* (Ch), an introduced weed, is used in several applications and has been found to have anti-arthritic effect. *M. eminii* (Me) and *Pentaclethra macrophylla* (Pm) are

large African forest trees introduced to many parts of the tropics and grown in monoculture plantations as fast-growing timber trees [7-9].

However, further studies were deemed necessary to determine their physicochemical characteristics and chemical composition after fractionation as the latter would make them more suitable as edible and/or medicinal oils.

The present work was carried out to fractionate crude oils from the above five mentioned plants in olein and stearin components, identify fatty acids and active vitamin E compounds in resulting fractions, and evaluate whether fractionation would improve their food quality.

2. MATERIALS AND METHODS

2.1 Reagents and Apparatus

All reagents used were of extra pure GC or HPLC quality (Merck, Darmstadt, Germany), except distillate water, petroleum, and acetone used to clean seeds and extract oils. We used as apparatus, electronic oven (model Boekel, Arthur H. Thomas Co. Philadelphia, USA) for drying seeds; coffee-mill (model Corona 01, Landers & CIA, SA) for crushing seeds; Soxhlet system and rotary evaporator (model Eyala of Tokyo Rikakikai Co. Ltd) for extraction; Varian 5890 gas chromatograph (GC) with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 µm) for FAs quantification; High Performance Liquid Chromatography (HPLC) Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump (Merck-Hitachi, Darmstadt, Germany), a Merck-Hitachi F-1000 (Darmstadt, Germany) fluorescence spectrophotometer with detector wavelengths for excitation 295 nm, for emission 330 nm), a Merck-Hitachi 655-A40 autosampler, and a ChemStation integration system (Agilent Technologies Deutschland GmbH, Böblingen, Germany) for vitamin E compounds analysis.

2.2 Seeds Collection and Oils Extraction

The general procedures for seed collection and oil extraction have been described elsewhere [7-9]. The process consisted of the following steps: seeds harvest from wild plants, cleaning, 3 days sun-drying, 1 hour 105ºC oven heating, hand-shelling to expel the respective kernels, and then crushing to produce fine seed flour. Approximately 500g aliquots of fine flours were submitted to Soxhlet's extraction for 8 hours using petroleum fraction at 40-60ºC, and then evaporated under vacuum in water bath at 50ºC for one hour. Oils thus obtained were stored in hermetically closed bottles and kept refrigerated till fractionation.

2.3 Oil Solvent Fractionation Procedure

Fractionation was done following the cooling procedure described elsewhere [1,10-12]. The principle consists of dissolving a 100g oil sample in 400 ml acetone and keeping the mixture at 4ºC for 24 hours. At this temperature, stearin solidifies while olein remains liquid. The two fractions are then separated by filtration using filter paper (Whatman no. 1). The obtained solid stearin is then washed with fresh cold acetone to remove any trapped liquid/olein. A typical acetone:oil ratio is 4:1 with 2 x 1:1 for washing. The solid dry stearin fraction is then weighed (Ps), stored hermetically in closed bottles and kept refrigerated till further analysis. Acetone fractions containing olein are gathered and the solvent removed under vacuum at 50ºC, using a rotary evaporator. The olein residue fraction is then weighed (Po) and treated in the same manner as the solid stearin fraction. Fractionation was done for each plant in three replicates. Yield of each fraction is expressed as percentage Ps% and Po% through (100*Ps/Pr) and (100*Po/Pr), where Pr is the weight of the oil sample. Values are presented as mean±standard deviation for 3 replicates.

2.4 Fatty Acids GC Analysis

Test conditions have been described elsewhere [9-14]. FAs composition from each plant oil fractions was determined by GC following ISO standard 5509:2000 procedure [15]. In brief, one oil drop dissolved in 1 ml n-heptane is methylated with 50 µl sodium methylate. Then 100 µl of pure water is added, the mixture centrifuged at 4500 g for 10 min and the lower aqueous phase removed. Then 50 µl of HCl (1 mol with methylorange) is added, the solution shortly mixed, and the lower aqueous phase rejected. About 20 mg of sodium hydrogen sulphate (monohydrate, extra pure) is added, and after centrifugation at 4500 g for 10 min and the top n-heptane phase transferred to a vial and injected in GLC under the following operating conditions: temperature program from 155ºC to 220ºC (1.5ºC/min); 10 min isotherm; injector 250ºC; carrier gas 36 cm/s hydrogen; split ratio 1:50; detector 250ºC; gas 30 ml/min hydrogen, 300 ml/min air and 30 ml/min nitrogen; manual injection volume less than 1 µl. Peak areas were

computed by integrated software, and percentages of fatty acid methyl esters (FAMEs) were obtained by direct internal normalization.

2.5 Tocopherols HPLC Analysis

For the determination of tocopherols, DGF F-II 4a method was used [16,17]. Generally, from a solution of 250 mg oil in 25 ml n-heptane, 20 µl volume is automatically sampled and injected onto a Diol phase HPLC column 25 cm × 4.6 mm ID. The mobile phase used was n-heptane/tert-butyl methyl ether (99+1, v/v) at flow rate of 1.3 ml/min. Mean values are given as mg/100 g without the standard deviation, because this value would represent only the deviation of the method and not the variation of the respective samples.

3. RESULTS AND DISCUSSION

Table 1 presents the olein and stearin fraction yields corresponding to each plant.

Table 2 gives the magnitude of individual fatty acids detected for each fraction.

Table 3 gives tocopherols content for each fraction.

Fig. 1 shows the proportional composition of all studied plant oils in saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) comparing to Palm, Olive, and other commonly marketed oils.

3.1 Olein and Stearin Fractions Yields

(Table 1) the solvent fractionation procedure used here allowed separating oleines and stearines at different ratios (olein/stearin) depending on the plant. The olein fraction yield dominated the stearin fraction for *Me-oil* (79/17) and *Md-oil* (65/26); but inversely stearin fraction dominated olein fraction for *Cg-oil* (53/39), *Pm-oil* (50/43) and *Ch-oil* (49/43).

In a previous study [7], it was found that the melting point (MP) of the analyzed crude oils ranged from -12 to 32°C. Cross-linking the stearin yields to MP showed positive correlation, meaning the higher MP the higher relative stearin yield.

Fractionation is currently being used to process a broad range of fats and oils including palm oil, palm kernel oil, butterfat, fish oil and lard, thus broadening the scope of food industry applications [14,18]. The value of fractionation has been demonstrated particularly for Palm oil. Both crude as well as refined palm oil are fractionated in a multistage process leading to oil palm olein and stearin products of several applications [1,14]. Many of those products find uses in ice cream, dry and canned soup mixes, with little or no further modifications [4]. Oil palm stearin is a very useful source of fully natural, hard fat which is a component for products such as shortening, pastry and bakery margarines. Like whole palm oil, palm olein is also widely used as frying oil; much of its popularity is due to its good resistance to oxidation and formation of breakdown products at frying temperatures, and long shelf life of its finished products [18,19].

3.2 FAs Detected in Fractionated Oils

(Table 2) after cooling fractionation, some FAs remained about fifty-fifty distributed in the two fractions olein and stearin, while for others one fraction was found enriched in particular FAs.

Globally eighteen FAs were detected (%>0.05) of which seven are dominant: lineloic, oleic, alpha-linolenic, vaccenic, eicosenoic, palmitic and stearic, *Cg*-oil has the highest content of oleic 18:1 D9 (43% of olein fraction), Vaccenic 18:1 D11 (42% of olein) and palmitic 16:0 (22% of stearin) acids.

Ch-oil is richest in eicosenoic 20:1 11(25% of olein), arachidic 20:0 (6% of olein), palmitoleic 16:1D9 (1.8% of olein), cerotic 26:0 (3.3% of stearin) and eicosadienoic (0.8% of olein).

Table 1. Olein and stearin yields in crude oils of studied plants from KBNP, Kivu, DRC, expressed as a percentage (mean % ± SD, n=3) of total oil

Plants family: Local name (Abbreviation)	Olein%	Stearin%
Maesopsis eminii Rhamnaceae*:* Omuguruka (Me-oil)	79.3±2.9	17.3±1.9
Millettia dura Fabaceae*:* Nshinguri (Md-oil)	65.4±2.8	26.5±2.7
Cardiospermum halicacabum Sapindaceae*:* Mubobogo (Ch-oil)	43.3±2.4	49.3±2.8
Pentaclethra macrophyla Fabaceae*:* Lubala (Pm-oil)	43.2±2.9	50.3±2.3
Carapa grandiflora Meliaceae*:* Igwerhe (Cg-oil)	39.5±2.1	53.4±2.7

Table 2. Fatty acid compositions of olein and stearin fractions of studied plants oils from KBNP, Kivu/DRC (expressed as a percentage of total fatty acids)

Fatty acids	Fraction	*C. grandiflora*	*C. halicacabum*	*M. dura*	*M. eminii*	*P. macrophylla*
Palmitic (16:0)	Olein	21.36	2.83	4.10	7.42	5.23
	Stearin	22.11	4.13	4.82	7.43	5.34
Palmitoleic (16:1D9)	Olein	0.86	1.78	ND	ND	ND
	Stearin	0.87	1.25	ND	ND	ND
Stearic (18:0)	Olein	5.06	3.03	3.2	17.2	2.3
	Stearin	4.96	4.01	3.39	17.96	2.29
Oleic (18:1)	Olein	ND	ND	ND	ND	ND
	Stearin	ND	0.26	ND	ND	ND
Oleic (18:1 D9)	Olein	42.54	20.91	29.22	38.96	30.62
	Stearin	1.24	22.10	29.6	38.61	29.72
Vaccenic	Olein	1.27	12.27	0.3	0.25	0.42
(18:1 D11)	Stearin	41.32	9.13	ND	0.24	0.42
Lineloic (18:2)	Olein	25.88	5.60	14.85	25.84	49.6
	Stearin	25.11	12.37	14.84	25.5	50.95
Alpha-Linolenic (18:3)	Olein	1.14	1.53	30.09	4.07	ND
	Stearin	1.21	1.11	27.78	3.98	ND
Arachidic (20:0)	Olein	1.14	6.27	0.9	2.39	2.08
	Stearin	1.23	5.05	0.98	2.47	2.28
Eicosenoic (20:1)	Olein	ND	16.4	0.09	2.51	ND
	Stearin	0.47	14.0	ND	ND	2.51
Eicosenoic	Olein	ND	25.49	2.7	2.46	2.45
(20:1 11)	Stearin	ND	20.89	2.76	ND	ND
Eicosadienoic (20:2 11,14)	Olein	ND	0.77	0.19	0.13	0.43
	Stearin	ND	0.66	0.15	0.11	0.32
Eicosatrienoic (20:3 5,11,14)	Olein	ND	ND	0.1	ND	ND
	Stearin	ND	ND	0.52	ND	ND
Eicosatetraenoic (20:4 5, 11, 14, 17)	Olein	ND	ND	0.12	ND	0.51
	Stearin	ND	ND	ND	ND	0.22
Behenic (22:0)	Olein	ND	ND	9.2	1.1	ND
	Stearin	0.67	0.27	ND	ND	5.79
Eicosatetraenoic (20:4)	Olein	0.63	ND	ND	ND	4.96
	Stearin	ND	0.54	7.87	1.01	ND
Nervonic (24:1)	Olein	ND	ND	ND	ND	ND
	Stearin	ND	ND	2.99	ND	ND
Cerotic (26:0)	Olein	ND	ND	ND	ND	ND
	Stearin	ND	3.33	ND	ND	ND

ND=Non-detected

Md-oil is richest in ALA (30% of olein), behemic acid (9% of olein) and eicosatetraenoic 20:4 5,11,14,17 (8% of stearin), nervonic acid (3% of stearin) and eicosatrienoic acid (0.5% of stearin).

Ch-oil and *Md-oil* are the richest sources of very long fatty acids (VLCFAs).

Me-oil is the richest for stearic acid (18% of stearin).

Pm-oil is richest in LA (51% of stearin).

Nervonic acid that was found at 0.1% in crude *Md*-oil increased to 3% in the stearin fraction and was cleared from the olein fraction. This fatty acid is essential to help maintain brain health. There is growing interest in using nervonic acid for the treatment of neurological diseases associated with dementia, especially Alzheimer's disease, multiple sclerosis and adrenal eukodystrophy [18-21]. Epidemiological studies

have shown that in areas where mustard, known to be rich in nervonic acid, is much used, there are less cardiovascular diseases. Thus, *Md*-stearin would be a good candidate to be considered for medicinal use. Furthermore, fractionation also improved nutritional values of both *Md*-stearin and *Pm*-olein fractions by removing behenic acid which was present in the crude oils of these plants at 7.3% and 6.3% levels respectively. Behenic acid is a fatty acid that raises blood cholesterol in humans and is therefore not rather desired component in food industry [8].

3.3 Percentage of SFA, MUFA, PUFA

Classification of FAs in saturated FAs, monounsaturated (MUFAs) and polyunsaturated FAs (PUFAs) gave the profiles presented in Fig. 1. Fractionation also slightly modified the profile of SFA-MUFA-PUFA proportions. For some oils, the distribution of FAs in olein and stearin was almost equivalent; for others fractionation enriched one fraction in particular FAs.

As example, oleic acid (18:1 D9) is more concentrated in olein than stearin for *Cg*-oil (16.82% vs 0.67%), *Md*-oil (19.9% vs 8.2%), and *Me*-oil (30.19% vs 6.86%). Another example among PUFAs is LA which is more concentrated in *Me*-olein (20.02%vs4.53%). In general, fractionation increased MUFAs content and decreased SFAs content in olein fractions. Comparison has been made with common marketed fats and oils [22] to find similarities.

In the case of Ch-olein, the profile is dominated by MUFAs (77%) and close to the known profile of olive oil (77%) that was incorporated in the histogram for comparison.

For all oils but Ch-oil, all olein fractions have high content of unsaturated fatty acids and poor content of SFAs in comparison to Palm oil that also was incorporated in the histogram for comparison. Md-olein profile is close to cottonseed oil. Production of MUFAs for food applications has been receiving increased attention due to the health benefits attributed to oleic fatty acids and their high stability even in demanding applications such as deep-frying oils [6]. This adds value to their nutritional applications.

3.4 Active Vitamin-E Compounds Content

(Table 3) eight active vitamin E compounds were globally detected in the five studied plant oils. They are relatively better presented in three plants, i.e. *M. dura* (9.6 mg/100 g), *P. macrophylla* (6.7mg/100g). and *M. eminii* (3.5 mg/100 g). The majority comprised of β-Tocopherol and γ-Tocopherol besides α-Tocopherol, α-Tocotrienol, Plastochromanol-8, γ-Tocotrienol and δ-Tocotrienol that are present in relatively small quantity (0.1 to 1.4 mg/100 g).

Similar contents were found in other investigated plants [9] like *S. sesban* seed oil (10.7 mg/ 100 g), *E. abyssinica* seed oil (7.9 mg/ 100 g) and *M. kilimandscharica* seed oil (4.9 mg/ 100 g). Most food plants contain low to moderate levels of vitamin E activity. They display antioxidant activity which protects the body tissues against the damaging effects, caused by the free radicals resulting from many normal metabolic functions [23,24]. What is interesting is that fractionation also can modify their distribution in the two fractions for some oils. Example is Ch-oil where all the content was accumulated in stearin leaving olein free.

Table 3. Levels of tocopherols and tocotrienol in olein and stearin fractions of studied plants oils from KBNP, Kivu/DRC (expressed as mg/100 g of oil)

Vitamin E compounds	*C. grandiflora*		*C. halicacabum*		*M. dura*		*M. eminii*		*P. macrophyla*	
	Po	Ps	Po	Ps	Po	Ps	Po	Ps	Po	Ps
α-Tocopherol	ND	ND	ND	ND	ND	ND	0.1	ND	0.2	0.1
β-Tocopherol	0.6	ND	ND	1.2	3.6	3.5	2.7	2.7	3.9	3.8
γ-Tocopherol	ND	0.1	ND	0.6	3.3	3.3	ND	ND	0.8	0.9
δ-Tocopherol	ND	0.1	ND	0.1	0.3	0.3	ND	ND	0.1	0.1
α-Tocotrienol	0.1	ND	ND	0.1	0.3	0.9	0.1	0.1	0.2	0.2
Plastochromanol-8	ND	ND	ND	ND	0.1	0.1	ND	0.1	0.1	0.1
γ-Tocotrienol	0.2	0.3	ND	0.3	1.4	1.4	0.8	0.9	1.3	1.3
δ-Tocotrienol	ND	0.1	ND	ND	0.2	0.2	ND	ND	0.2	0.1
Total Vit E mg/100 g	0.9	0.6	ND	2.3	9.2	9.7	3.7	3.8	6.8	6.6

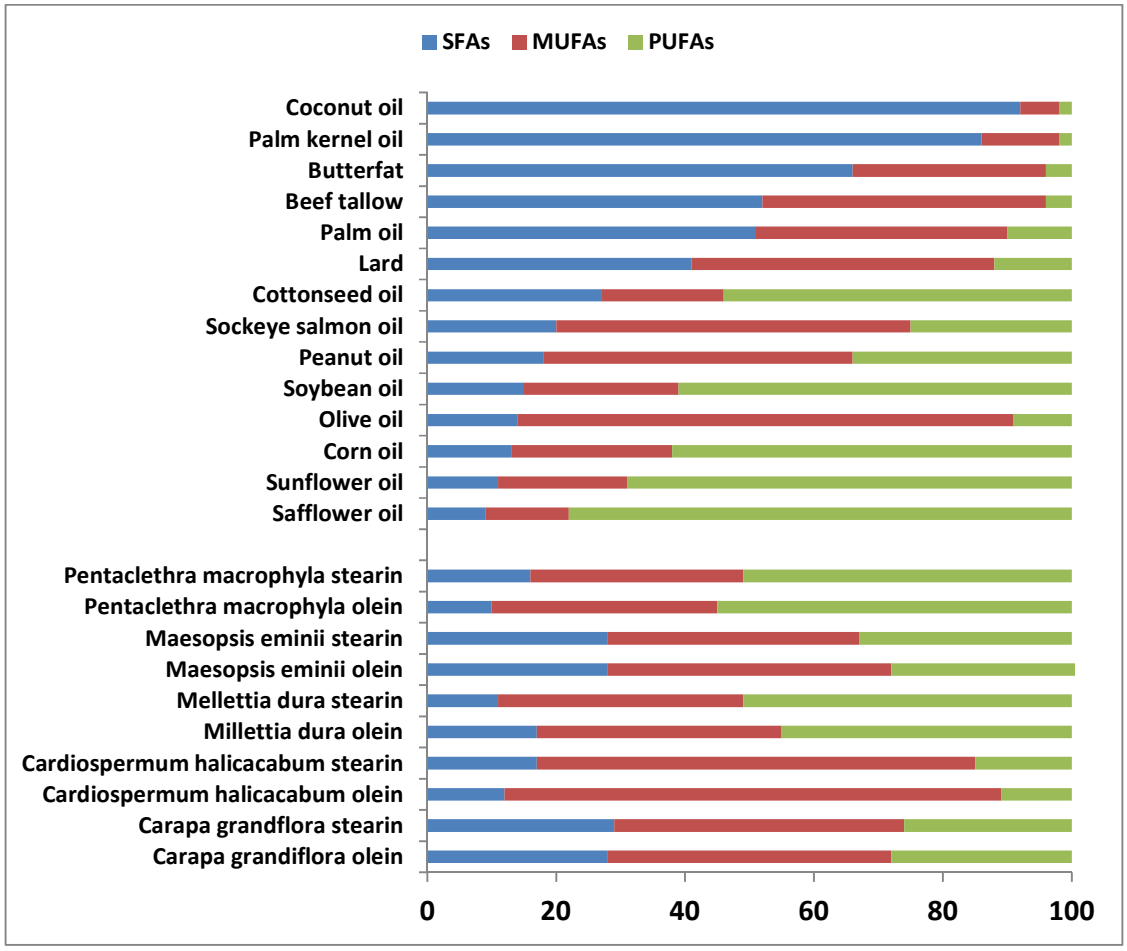

Fig. 1. SFA, MUFA and PUFA composition of studied plant fractionated oils from KBNP, DRC compared with common market oils [24]. Percentage of total fatty acids

4. CONCLUSION

The solvent fractionation used is relatively efficient to separate olein and stearin fractions and modify the proportions of saturated to unsaturated fatty acids.

Fractionation allows adding nutritional value to crude oils by increasing essential unsaturated fatty acids like nevronic acid while reducing the levels of unwanted fatty acids such as behemic acid.

By its profile, *C. halicacabum* olein has similarities with olive oil, and would thus be used as a substitute for this expensive oil after some adaptations.

High content of active vitamin E compounds in *M.dura* stearin and olein fractions greatly contributes to the nutritional value of these oil products.

It would be possible to adjust the final cooling temperature of the crude oils in order to modify processing yield and generate fractions of different qualities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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