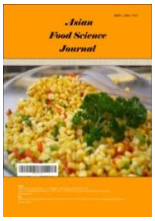

Asian Food Science Journal

19(1): 1-9, 2020; Article no.AFSJ.62510
ISSN: 2581-7752

Composition of Fatty Acids, Total Sterols and Total Polyphenol Content of the Oils of Six Oilseeds in Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Author YNJC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AAA and NBF managed the analyses of the study. Author AAF managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2020/v19i130227
Editor(s):
(1) Dr. Surapong Pinitglang, University of the Thai Chamber of Commerce, Thailand.
Reviewers:
(1) Uday Deokate, Government College of Pharmacy, India.
(2) Ramya Kundayi Ravi, RAK College of Nursing, RAK Medical & Health Sciences University, UAE.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62510

Original Research Article

Received 27 August 2020
Accepted 01 November 2020
Published 12 November 2020

ABSTRACT

Aims: This study aims to determine the fatty acid, sterol and total polyphenol composition of *Ricinodendron heudelotii, Terminalia catappa, Moringa oleifera, Cyperus esculentus, Sesamum indicum* and *Coula edulis* oils.
Study Design: Plant material consisting of almonds of *Ricinodendron*. *heudelotii*, *Terminalia catappa*, *Moringa oleifera*, rhizomes of, *Cyperus esculentus*, seeds of *Sesamum indicum* and hazelnuts of *Coula edulis* were collected in different producing areas of Côte d'Ivoire in 2017.
Place and Duration of Study: This study was conducted between November 2017 and June 2018 at the Laboratory of Industrial Processes, Synthesis, Environment and New Energies, National Polytechnic Institute Félix Houphouët-Boigny, Côte d'Ivoire.
Methodology: The extraction of oil from the oil-bearing organs of different plants was carried out by maceration of the crushed parts of these organs in hexane at a crushed/solvent ratio of 1:10 (grams/volume) for 6 hours at cold temperature. These oils were analyzed by gas chromatography

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for the determination of fatty acid profile, then by gas chromatography coupled with a mass spectrometer for the determination of sterols and Ultraviolet-visible spectrophotometer for the quantification of polyphenols.
Results: The results show a fatty acid composition of the different oils dominated by four fatty acids: oleic (18.89 - 93.93%), linoleic (1.03 - 43.2%), palmitic (2.65 - 35.6%) and stearic (1.09 - 11.66%). We also note average unsaponifiable matter (0.5 - 1.71%) and sterols dominated by sitosterol, stigmasterol and campesterol. Concerning polyphenols, the values obtained range from 52.09 to 863.67 µg gallic acid equivalent/g oil.
Conclusion: The presence of sterols, phenolic compounds and unsaturated fatty acids in interesting proportions show that these oils are important sources of edible oils beneficial to human health.

Keywords: Oil; fatty acids; sterols; polyphenols.

1. INTRODUCTION

Epidemiological evidence suggests that populations consuming a high percentage of fat calories have a higher incidence of cardiovascular disease than populations with low fat consumption [1]. The higher the saturated fat intake, the higher the incidence of cardiovascular disease. This would be one of the reasons for the contradictions related to the consumption of palm oil, which is qualified as a potential cause of cardiovascular disease due to its high saturated fatty acid content (50%). Indeed, palm oil is the main dietary oil in most sub-Saharan countries.

However, in the rural areas of these countries, some spontaneous food plants are potential sources of lipid intake for the populations, but the latter are often unaware of the nutritional values of these nutrients.

Thus, following the observation of the dietary habits of some populations in Côte d'Ivoire, we were interested in almonds *Ricinodendron heudelotii*, *Terminalia catappa, Moringa oleifera*, rhizomes of *Cyperus esculentus*, grains of *Sesamum indicum* and hazelnuts of *Coula edulis*. These oleiferous organs, with a relatively high oil content, although consumed irregularly, may not only be sources of significant lipid intake, but may also contain biologically active molecules beneficial to health.

Much work has been done on the glyceride fraction of these oils [2, 3, 4, 5, 6, 7], but little information on the composition of the unsaponifiable fractions is available. The nutritional quality of some of these oils is no longer to be demonstrated, but the revealed presence of biologically active molecules such as phytosterols and polyphenols within these oils could not only lead to their use as edible oils for mass consumption but also allow their incorporation into pharmaceutical or cosmetic products and revive interest in them, hence the need for this study.

Plant sterols or phytosterols constitute an important part of the unsaponifiable fraction of oils. They have the capacity to lower blood cholesterol in humans [8].

As for polyphenols, they are attracting increasing interest because of their importance for the body, both nutritionally and for health [9]. These constituents are natural substances with antioxidant properties.

The objective of this study is to determine the fatty acid composition, total sterols and total polyphenol contents of *Ricinodendron heudelotii, Terminalia catappa, Moringa oleifera, Cyperus esculentus, Sesamum indicum* and *Coula edulis* oils in order to encourage their large-scale consumption in sub-Saharan countries.

2. MATERIALS AND METHODS

2.1 Plant Material

Plant material consisting of almonds of *Ricinodendron heudelotii, Terminalia catappa, Moringa oleifera* were purchased at the market of Yamoussoukro (city located in the center of Côte d'Ivoire). The rhizomes of *Cyperus esculentus*, the seeds of *Sesamum indicum* come from Touba (city located in the north-west of Ivory Coast). The hazelnuts of *Coula edulis* were bought at the market of Grand Bereby (city located in the south-west of Ivory Coast).

2.2 Oil Extraction

Extraction was performed according to the method described by Rombaut [10]. The oil

contained in the almonds, rhizomes, seeds and hazelnuts is extracted by maceration in hexane at a solid/liquid ratio of 1:10 (g/v) for 6 hours in the cold. After vacuum filtration using a Buchner-type device, the filtrate obtained is evaporated under reduced pressure in a rotary evaporator (Rotavapor, Buchi- Switzeland) at 40°C. The fat obtained is dried on anhydrous magnesium sulphate to remove traces of water and then steamed at 70°C for 12 hours to remove traces of hexane. The oil obtained from each sample was kept in a dark bottle in the refrigerator at 4°C until it was used for the various analyses.

2.3 Determination of Fatty Acid Composition of Oils

Fatty acid methyl esters were obtained by methylation of fatty acids in oils by the method described by [11] prior to GC-MS analysis. Three drops of oil were added to 3 mL of sodium methoxide solution and the mixture was heated at 60°C for 10 min. After this step, 3 mL acetyl chloride and 10 mL methanol (50 mL/625 mL) were added to the previously heated mixture. The new mixture was heated for 10 min and then cooled to room temperature. Then 10 mL distilled water and 15 mL hexane were added successively. The mixture was stirred vigorously and left to stand until two phases were obtained. The upper hexane phase containing the fatty acid methyl esters was transferred to a vial for GC-MS analysis. The fatty acid methyl ester analysis was performed by gas chromatography coupled to a GC-MS (GC-MS) labelled Shimadzu QP2012-SE with a Zebron ZB-5MS apolar capillary column (20 m x 0.18 mm x 0.18 µm). The flow rate of the carrier gas (helium) is set at 2 mL/min. The furnace programming is set at 100°C for 5 min, to reach 230°C at a rate of 10°C/min. The volume of fatty acid methyl esters injected is 1 µL. The temperatures of the injector and the detector are 250 and 260°C respectively. The peak areas were treated with a Merck D-200 type integrator. Fatty acids were identified by comparison of their retention time of palm oil fatty acids and by comparison with values in the literature.

2.4 Extraction and Dosage of Total Phenols

The total phenolic contents were evaluated according to the colorimetric method of Folin-Ciocalteu to [12] with some modifications: 1 to 2 g of oil are dissolved in 5 mL of hexane in an Erlenmeyer flask, then 5 mL of methanolic solution (CH_3OH/H_2O; 60/40, v/v) are added, then the methanolic phase is recovered using a separating funnel. A total of three identical extractions are performed and the methanolic phases are collected and concentrated in the rotary evaporator. To 50 µL of the concentrated fraction (phenolic extract) are added successively 1.95 mL distilled water and 0.5 mL Folin-Ciocalteu reagent (0.5 N). This mixture is incubated for 3 minutes and then 0.4 mL Na2CO3 (62.5 g/L) is added. The phenol determination is performed spectro-photometrically by measuring the absorbance of the phenolic solutions at 760 nm using a calibration line with gallic acid at different concentrations (20 µg/mL). The content of total polyphenols (Q), expressed in microgram gallic acid equivalent per gram of oil (µg G.A Eq/g oil) is calculated according to formula:

$$Q= (V \times C \times d)/m$$

V: final volume of the extract (mL)
C: concentration of the extract obtained from the calibration curve (g/mL)
d: dilution
m: mass of oil (g)

2.5 Unsaponifiable Matter Content

The method used for the extraction of unsaponifiables was that of the AFNOR NF [13]. Five (5) grs of oil sample were heated under reflux in 50 mL of ethanolic KOH at 1 N for 1 hour. After addition of 50 mL distilled water, the cold solution was extracted 3 times with 50 mL hexane. The hexane phases were then collected and washed with distilled water until the wash solution was neutral. The hexane phase was dried on anhydrous magnesium sulfate and then filtered and the solvent evaporated in the rotary evaporator. The residue obtained was dried in an oven at 103°C and then cooled down in a desiccator. This residue constitutes the unsaponifiable fraction. The tests were carried out in triplicate. The content of unsaponifiable matter was calculated according to expression:

$$Ins\ (\%) = (m_1/m_2)\times 100$$

m_1: mass of unsaponifiables (g)
m_2: mass of the test sample (g).

2.6 Dosage of Sterols

Sterol compounds were determined according to the method described by Melchert HU et al. [14]

after saponification with potassium hydroxide in ethanolic solution. The unsaponifiable matter was then extracted with hexane. The sterol fraction was separated from the unsaponifiable fraction by thin layer chromatography. The sterols recovered from the plate were converted to trimethylsilyl ethers (TMS) and analyzed by gas chromatography coupled to a Shimadzu QP2012-SE mass spectrometer (GC-MS) with a Zebron ZB-5MS apolar capillary column (20 m x 0.18 mm x 0.18 µm). The flow rate of the carrier gas (helium) is set at 0.9 mL/s. The furnace temperature program is 80-280°C for 42 min (4.75 C/min), 280-310°C for 18 min (1.66°C/min). The injection was carried out in split 30 mode. The temperature of the injector was set to 250°C and that of the detector to 280°C. The parameters of the mass spectrometer for the electronic impact mode are: ionization source temperature (230°C), electron energy (70 eV), scan speed (50 scans/s) and acquisition speed (10.000 u.m.a/s). Peak identification is based on comparison of the mass spectra with those of Wiley's library (HPCHEM, Wiley, 275, 6th ed.) or by comparison of their retention time with those of TMS derivatives prepared from standard compounds and in some cases by comparison with those in the literature. The amounts of the different sterols expressed in milligrams per 100 grams of oil were calculated as follows:

$$\text{Stérol X (mg/100 g d'huile)} = \frac{\text{Ax.ms}}{\text{As.m}} \text{ x } 100$$

Ax: sterol "X" peak area,
As: area of the peak of 5α-cholestane,
ms: mass of 5α-cholestane added (mg),
m: mass of the oil sample taken (g).

3. RESULTS AND DISCUSSION

3.1 Fatty acid Composition of Oils

Fatty acid contents of *Ricinodendron heudelotii, Terminalia catappa, Moringa oleifera, Cyperus esculentus, Sesamum indicum* and *Coula edulis* oils are presented in Table 1.

Four fatty acids commonly found in oils were identified in all the oils studied. These are palmitic acid (16:0), stearic acid (C18:0), oleic acid (18:1n-9) and linoleic acid (18:2n-6). Analysis of the table indicates that these four main fatty acids alone account for approximately 95% of the total fatty acids contained in each of these oils. However, their respective contents differ greatly when we switch from one oil to another. Thus, the palmitic acid content is 8.7; 35.6; 7.04; 17.26; 9.4 and 2.65% respectively for *Ricinodendron heudelotii, Terminalia catappa, Moringa oleifera, Cyperus esculentus, Sesamum indicum*, *Coula edulis*. Palmitic acid is a saturated fatty acid whose responsibility is sometimes implicated in hypercholesterolemia and cardiovascular diseases. Given its relatively high content in *Terminalia catappa* oil, this oil could constitute a potential risk for cardiovascular disease, as some authors have pointed out Keys A, Dubois V et al. [15, 16].

As regards oleic acid, its content is higher in *Coula edulis* (93.93%), *Moringa oleifera*, (72.5%), *Cyperus esculentus* (67.76%), intermediate in *Sesamum indicum* (44.99) and *Terminalia catappa* (30.95%) and low in *Ricinodendron heudelotii* (18.89%).

Oleic acid is the majority fatty acid in olive oil with contents ranging from 55 to 83% [17]. The beneficial health effect attributed to olive oil is partly due to its high oleic acid content, which is attributed a cholesterol-lowering effect [17]. The oleic acid contents of *Coula edulis*, *Moringa oleifera Cyperus esculentus* oil are within a range of the oleic acid contents of olive oil. With regard to these oleic acid contents, these three oils could have the same properties as olive oil, i.e. the cholesterol-lowering effect. Large scale production and consumption of these oils could be beneficial for populations.

We also note the presence of stearic acid in the different oils studied at levels ranging from 1.09 to 11.66%. According to the literature, stearic acid has no effect on serum lipid parameters [18, 19] and therefore does not constitute a danger or a benefit following its consumption.

As for linoleic acid, its contents are respectively 43.2; 28.02; 1.03; 9.67; 39.61 and 1.6% for *Ricinodendron heudelotii*, *Terminalia catappa, Moringa oleifera, Cyperus esculentus, Sesamum indicum*, *Coula edulis*. Linoleic acid is essential fatty acid because it cannot be synthesized by humans. Moreover, it has been shown in the literature that this fatty acid has a positive effect on blood cholesterol, i.e. a cholesterol-lowering effect [20]. Its presence in these oils at variable levels is a source of essential fatty acids.

Following these fatty acids frequently found in vegetable oils, we note the presence of

elaeostearic acid (C18:3n-5) found in *Ricinodendron heudelotii* oil (17.55%), heptadecanoic acid (C17:0), eicosenoic acid (C20:1n-9) and docosanoic acid (C22:0) in M. oleifera oil with respective contents of 1.7%, 2.15% and 6.61%.

3.2 Unsaponifiable Matter Content

The unsaponifiable matter contents of the oils studied are presented in Table 2. The unsaponifiable matter contents determined are relatively common to those for palm oil (between 0.5 and 1.2%) [21] and those found in the literature for *Terminalia catappa* oil (0.47%) [3], sesame oil (1.65%) [6].

Unsaponifiables are a group of compounds consisting of sterols, vitamin E (tocopherols and tocotrienols), higher aliphatic alcohols, carotenoids, and natural hydrocarbons. Some of these compounds (vitamin E and carotenoids) have antioxidant and vitamin activities.

Unsaponifiables give fats certain pharmacological and cosmetological properties. Thus, they are used in the composition of cleansing milks and nutritive creams against aging. These unsaponifiable compounds are recommended in food [22], as well as in medicine for their anticancer [23], and/or anti-inflammatory [24].

The presence of unsaponifiable matter indicates that these oils are dietary sources of compounds that are beneficial to health.

3.3 Sterols Composition of Oils

Table 3 shows the total sterol contents of crude oils and palm olein. From the analysis of this table we observe that the total sterol contents of these oils range from 128.91 to 873.34 mg/100 g oil. These values are in the same order of magnitude as some conventional oils such as sunflower (352 mg/100 g), soybean (297 mg/100 g), olive (150 mg/100 g), rapeseed (668 mg/100 g), peanut (171 mg/100 g), cotton (300 mg/100 g). According to Bouic PJD et al. [25], sterols help fight certain cardiovascular diseases because they help reduce intestinal adsorption of dietary cholesterol. Sterols have been shown to act as an antioxidant and anti-polymerizing agent in frying oils. To this end, the actual presence of sterols in these crude oils would therefore constitute important sources of phytosterols, which are attributed to their ability to lower blood cholesterol in humans. This hypocholesterolemic role of sterols is largely attributed to sitosterols [26] which were found in all the oils studied. Sitosterols are therefore the major sterol in these oils, particularly in S. indicum oil.

With regard to stigmasterol, the highest value was found in *Moringa oleifera* oil (110.68 mg/100 g). The stigmasterol contents of all the oils studied are higher than those of campesterol except in *Sesamum indicum* oil.

Campesterol is the third most common sterol in the oils studied. Its content varies from 9.61 mg/100 g) (*Terminalia catappa*) to 158.8 mg/100 g) (*Sesamum indicum*).

3.4 Total Polyphenol Content of Oils

Phenolic compounds are the subject of numerous studies today, especially for their potential for human health prevention [27]. These natural compounds play a very important role in the characterization of oils and for their nutritional interest [28]. According to Fedeli E [29], simple and complex phenolic compounds found in olive oil increase its stability, confer antioxidant properties and modulate its flavor. In this work, we determined the content of these natural antioxidants in the six oils studied. The result shown in Table 4 showed that *Coula edulis* oil recorded the highest value (863.67±0.62 µg Gallic Acid Eq/g). This oil as well as that of *Cyperus esculentus* (170.62±0.73) have higher values than that of olive oil (167.29±2.71 µg gallic acid eq/g) reported by Merouane A et al. [30]. This family of compounds are powerful antioxidants that protect oils against oxidation processes and give them better stability during storage Boskou D [31]. In view of these interesting results, in terms of total polyphenols, we can say that oils are potential sources of polyphenol intake for food and therefore sources of natural antioxidants.

Table 1. Fatty acid composition (%) of oils

Oils	Palmitic acid C16:0	Heptadecanoic acid C17:0	Stearic acid C18:0	Oleic acid C18:1	Linoleic acid C18:2	Elaeostearic acid C18:3	Eicosenoic acid C20:1	Docosanoic acid C22:0
R. heudelotii	8.7	-	11.66	18.89	43.2	17.55	-	-
T. catappa	35.6	-	5.37	30.95	28.02	-	-	-
M. oleifera	7.04	1.7	6.22	72.5	1.03	-	2.15	6.61
C. esculentus	17.26	-	5.31	67.76	9.67	-	-	-
S. indicum	9.4	-	5.97	44.99	39.61	-	-	-
C. edulis	2.65	-	1.09	93.93	1.6	-	-	-

Table 2. Unsaponifiable matter content of oils (%)

Oils	*R. heudelotii*	*T. catappa*	*M. oleifera*	*C. esculentus*	*S. indicum*	*C. edulis*
Values	0.75±0.09	0.57±0.07	0.5±0.02	0.62±0.03	1.71±0.11	0.63±0.14

Table 3. Sterol composition of oils (mg/100 g)

	Oils					
	R. heudelotii	*T. catappa*	*M. oleifera*	*C. esculentus*	*S. indicum*	*C. edulis*
Stigmastérol	-	13.24	17.75	43.45	87.29	30.97
Sitostérol	93.36	70.47	347.61	147.72	487.24	90.57
Campestérol	-	3.8	92.94	29.42	158.8	-
Clionastérol acetate	-	-	-	-	-	185.11
Stigmasta-5,22-dien-3-ol	-	-	-	-	-	86.46
Other sterols	6.63	12.47	72.25	18.61	145	239.95
Total Sterols	128.91	252.76	623.48	239.2	878.34	633.06

Table 4. Total polyphenol content of oils (µg gallic acid eq/g oil)

Oils	*R. heudelotii*	*T. catappa*	*M. oleifera*	*C. esculentus*	*S. indicum*	*C. edulis*
Values	69.95±0.4	105.3±0.67	52.09±0.23	170.62±0.73	137.82±1.15	863.67±0.62

4. CONCLUSION

Vegetable oils are some food source rich in nutrient compounds. The objective of this study was to evaluate the composition of oil plant oils from the biodiversity of Côte d'Ivoire. From the point of view of fatty acid composition, it appears that the oils studied are mainly composed of oleic acid which could give them the same nutritional properties as that of olive oil. In terms of sterol composition, it is mainly sitosterol, stigmasterol and campesterol that are present in almost all the oils studied. As regards total phenols, the preponderance of these compounds in *Coula edulis, Cyperus esculentus* and *Sesamum indicum* oils is noteworthy. These relatively high levels of fatty acids, sterols and total polyphenols open the way to many applications, especially in the food, medical and even cosmetic fields.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62510