

Bioavailability of Epoxidized Triglycerides in Seed Oil of *Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg. from Congo-Brazzaville

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Abstract

This study aims at the physico-chemical characterization of *Alchornea cordifolia* oil isolated from the seed of this plant, to identify the bioavailability of epoxidized triglycerides. The ripe fruits of *Alchornea cordifolia* were harvested in southern Congo-Brazzaville in the localities of Goma-tsétsé and Dolisie. The oil is extracted from the seeds by three different methods: cold method (Folch), Soxhlet method, and water method. The fat contents are of the order of 18 to 28% by the water method, 30 to 37% by the Folch method and 45 to 56% by the Soxhlet method. All these methods exhibit similar fatty acid profiles. They are characterized by the presence of palmitic acids (C16: 0) 8 to 15%, stearic (C18: 0) 1 to 2%, oleic (C18: 1) 8 to 12%, linoleic (C18: 2) 8 to 12%, epoxidized fatty acid 48 to 63%, selacholeic (C24: 1) 2 to 3%. The epoxidized fatty acid contents are of the order of 50 to 63% by the water method, 50 to 61% by the Folch method and 48 to 58% by the Soxhlet method. The oil extracted with water has slightly higher epoxidized fatty acid contents than that extracted by other methods. The fatty acid composition of *Alchornea cordifolia* seed oil from Congo is characterized by high levels of epoxidized fatty acids in the order of 48-63%. This multi-functional acid has considerable commercial potential.

Keywords: *Alchornea cordifolia*, bioavailability of epoxidized fatty acid, fatty acid composition, oil extraction methods

INTRODUCTION

Starting from the destruction of the Amazonian Forest, the "Rio Summit" accelerated awareness of the challenges of tropical forests in general, those of the Congo Basin in particular (Loumouamou, 2011). The

management of forest ecosystems recognizes the ecological and socio-economic importance of Non-Ligneous Forest Products (NLFPs) (Nikiema *et al.*, 2019).

NLFPs are the food, medicinal, cultural, and socio-economic basis of forest populations. Long considered

in the Congo as having no influence on the sustainable management of forests and not intervening in economic roles, their considering, although still timid, is a recent situation.

Non- Ligneous Forest products constitute a range of varied products including plant species or parts thereof not concerned by logging, and all animal species. Among these products, we also find unconventional oilseeds.

Several studies which contributing to the valuation of unconventional oilseeds in Congo have been carried out. We can cite studies on safou (Silou, 2012), *Irvingiagabonensis* and *Irvingiawombulu* (Silou *et al.*, 2004, 2011), *Irvingiasmithii* (Loumouamou *et al.*, 2013), *Allanblackia floribunda* (Loumouamou *et al.*, 2014), *Raphia sese* and *Raphia laurenthii* (Goteni *et al.*, 2011; Silou *et al.*, 2012), the cucurbits (Silou *et al.*, 1999; MvoulaTsiéri *et al.*, 2005).

Nkuinkeu (2003) took stock of the situation and prospects for the valuation of oilseeds in the Congo Basin. Indeed, he notes that some unconventional oilseeds such as *Ricinodendronheudelotii*, *Raphia sese*, *Raphia laurenthii*, the Cucurbits ... are used locally as cosmetics and food, others on the other hand such as *Irvingiagabonensis*, *Irvingiawombulu*, *Dacryodes edulis* ... are marketed locally, regionally, and internationally. Unfortunately, and overall, this work has remained too ad hoc and of unequal importance in the different countries.

The studies carried out by Loumouamou (2011) on oilseeds from the Congo Basin made it possible to select a species of the genus *Alchornea cordifolia* often encountered in Congo regarding its production potential in vegetable oils.

Alchornea cordifolia is a plant whose species is part of the Euphorbiaceae family, also called the djeman tree. It is widely present in many African countries and used in traditional African medicine from Senegal to Uganda. Many medicinal virtues that this plant could have been very varied. Therefore, especially sought after, this plant has been the subject of several subjects of study and research for its anti-inflammatory properties, to treat diseases such as dermatitis, asthma, hepatitis, colitis and many more. The most recent studies have identified the active compounds that provide these properties (lipophilic compounds, flavonoids, and alkaloids) (Manga *et al.*, 2004).

The parts of the plant used are leaves, roots, stem bark, fruits, and seeds. Depending on the chemical compositions of the different organs, a repertoire of applications may be established. In the leaves, roots, and bark of the stem, we find terpenoids, steroidal heterosides, flavonoids (2-3%), tannins (10%), saponins, carbohydrates, and alkaloids (especially di-

isopentenylguanidine) (Okoye *et al.*, 2011). The leaves alone are composed of hydroxybenzoic acids: gallic acid and its ethyl ester, gentisic acid, anthranilic acid (vitamin L1), protocatechuic acid, and ellagic acid (alizarin yellow) (Manga *et al.*, 2008). The seeds produce oil whose triglycerides carry epoxy functional groups. Thus, the seed oil is composed of epoxidized triglycerides including a C20 homolog called alchornoic acid ((+) cis-14,15-epoxy -cis- 11 - eicosenoic acid), of vernolic acid (cis-12, 13-epoxyoleic acid). The latter has 18 carbons and epoxy groups on C9, C12 and C15 (Kleiman *et al.*, 1977). 3-Acetoxy-7, 8-epoxylanostan-1-ol was found in the hexane-extracted fraction of the leaves (C₃₂H₅₄O₄) (Manga *et al.*, 2004).

However, studies to characterize the oils from seeds of *Alchornea cordifolia*, native to the Central African region, are non-existent. This study presents the chemical (oil content, fatty acid profiles, minor compounds) and physicochemical (peroxide value, acid value, oxirane content) properties of oils from the seeds of the Congo Brazzaville species obtained by three methods of extraction.

MATERIALS AND METHODS

Materials: *Alchornea cordifolia* is a plant known by the vernacular names of Mbunzila in southern Congo in the department of pool, Mbunzi in southern Congo in the department of niari and Abunzi in northern Congo. It is a shrub reaching 4 to 5 m in height. The fruits (Fig. 1) of *Alchornea cordifolia* are capsular, two-lobed about 1.5×1.5 cm, lobes slightly compressed, smooth, briefly hairy, green to red, two-seeded. The seeds (Fig. 1) are ovoid-ellipsoid, about 6 mm long, smooth, bright red. In Congo-Brazzaville, the fruits of *Alchornea cordifolia* ripen from December to February and anglers use its red seeds as bait to trap birds.

The ripe fruits of *Alchornea cordifolia*, were harvested in the south of Congo-Brazzaville in the localities of Goma-tsésé (4°13'44" South and 15°7'52" East) and Dolisie (4°11'54" South and 12°39'59" East). The fruits are harvested manually, during the period from January to March.

The fruits are shelled manually. The shelled seeds are dried in an oven at 70°C for 2 days and are crushed using a manual mechanical grinder.

Sampling is carried out on 2 localities. It is performed on 8 trees, i.e., 4 trees from Goma-tsésé (noted G) and 4 trees from Dolisie (noted D).

Methods:

Oil extraction methods: We used three different methods to extract the lipid material: The Folch method (F), the Soxhlet method (S), and the water method (E).



Tree and fruits



Seeds

Fig. 1: *Alchornea cordifolia* (tree and fruits, seeds)

The different lipid extractions made with the three methods are repeated at least three times.

The Folch method (modified Folch) (Folch *et al.*, 1957): Twenty-five gram of seeds crushed with a mechanical grinder is mixed with 100 mL of the chloroform-methanol mixture (2: 1, v/v) in a 300 mL beaker. The mixture is stirred for 10 min using a magnetic stirrer. To facilitate contact extraction, the mixture is allowed to stand for 30 min. Then vacuum filtered. The operation on the residue is repeated a second time and the two extracts are combined in a 500 mL separating funnel. After adding 50 mL of a 20% sodium chloride solution, the resulting mixture is shaken vigorously and then left to stand for 1 h. After settling of the solution, the organic phase (fat) is recovered and filtered using a filtration system consisting of a glass funnel fitted with a paper filter in which a few grams of anhydrous sodium sulfate have been deposited. The oil is recovered after evaporating the mixture of solvents on a rotary evaporator at 40°C under vacuum at 1.5 mbar. The oil obtained is dried in an oven for 24 h at 70°C. The extraction yield is then determined relative to the mass of ground material.

The Soxhlet method: Dried *Alchornea cordifolia* seeds were ground using a mechanical grinder. For solvent extraction by Soxhlet method, we have placed 30 g of ground seeds into the extraction cartridge. Using 200 mL of hexane contained in a 250 mL flask, the fat contained in the ground material is extracted by heating the solvent at reflux (about 80°C) for 6 h. The extract is cooled and then dried with anhydrous sodium sulfate. The oil is obtained after evaporation of the solvent on a rotary evaporator at 40°C under vacuum at 1.5 mbar.

The yield of oil extraction is then determined based on the mass of ground material.

The water method: The powder of 100 g of seeds dried in an oven for 24 h at 100°C is mixed with 500 mL of water in an aluminum pan. The mixture is heated for 1 h 30 min at a temperature of 150°C. After boiling, two phases are obtained. The oil floats in the upper phase and the rest in the lower phase. We take the upper phase and put it in a separating funnel for 24 h and the oily phase is separated with the aqueous phase. The oil is dried using anhydrous sodium sulfate. The yield of oil extraction is then determined based on the mass of ground material.

Determination of the physico-chemical characteristics of oils extracted: The physicochemical parameters were determined using standard analytical methods described by the French Standards Association (AFNOR, 1998).

Determination of the Fatty Acid composition (FA): The methyl esters of fatty acids are obtained by basic transesterification: 0.2 mL of methanolic sodium hydroxide solution (2 N) to 60 µL of oil in 1 mL of hexane is added; after stirring, 0.4 mL of hydrochloric acid (1 N) and 1 mL of cyclohexane are added. The organic phase is recovered for analysis.

The analyzes were carried out on a chromatograph of type HP 5890 equipped with an apolar column (HP 5M, 30 m long, 0.25 mm internal diameter and 0.2 µm thick) and a Flame Ionization Detector (FID). The following experimental conditions were used: carrier gas, helium at constant flux: 1 mL/mm; the oven

Table 1: Physico-chemical characteristics of oils of *Alchornea cordifolia* seeds

Parameters	SG2	EG2	FG2	ED2	FD2	SD2
Acid number (mg KOH/g oil)	64.06±0.05	63.53±0.08	54.11±0.06	63.35±0.06	48.25±0.07	63.98±0.05
Peroxide number (mg O ₂ /g of oil)	1.88±0.04	1.90±0.06	1.80±0.07	1.88±0.07	1.53±0.06	1.88±0.05
Hydroxyl number (mg KOH/g of oil)	44.96	nd	nd	nd	nd	nd
Density (28°C)	0.96	0.96	0.97	0.96	0.96	0.97

S: Soxhlet extraction; E: Water extraction; F: Extraction by the Folch method; G2: Sample 2 from Goma Tsé-tsé; D2: Sample 2 from Dolisie; nd: not determined

Table 2: Comparison of the oxiran content (epoxy number) of *Alchornea cordifolia* seed oil with those of certain epoxidized vegetable oils

Parameter	<i>Alchornea cordifolia</i> seed oil (this study)	<i>Vernonia anthelmintica</i> seed oil (Perdue <i>et al.</i> , 1986)	<i>Vernonia galamensis</i> seed oil (Carlson and Chang, 1985)
Oxiran content (%)	3.23	3.71	4.1

temperature is programmed from 50 to 280°C with a gradient of 5°C/min; injector temperature: 250°C; detector temperature: 280°C. The quantity injected is 1 µL.

A mixture of known fatty acids in defined proportions is injected under the same conditions as the oil to be studied. The retention time and area of each control fatty acid are determined. The fatty acids of the oil studied are identified by comparison of retention times and assayed by their areas, referring to the area of an internal standard of known concentration (heptadecanoic acid: C17: 0).

Determination of the content and composition of unsaponifiable matter: The extraction of unsaponifiables was carried out according to the NF T 60-205-2 standard (Wolff, 1968).

The unsaponifiable fraction of the oil was analyzed with a gas chromatograph of Agilent type and model 7890, coupled with a mass spectrometry apparatus of the Agilent 5979C type. The operating conditions were as follows: DB5 MS column: 20 m × 0.18 mm, thickness 0.18 µm; carrier gas, helium: 1 mL/min; ionization energy: 70 eV; injector temperature: 320°C; detector temperature: 280°C; ion source temperature: 230°C; oven programming: from 50°C (3.2 min) to 320°C at 8°C/min, 3 min; split mode injector 1: 150.

Mass spectra were acquired in the m/z range 33-450 scanning mode. The compounds were identified by comparison of the spectra with those available in databases.

Statistical processing: Statistical processing (calculation of means and standard deviations, Principal Component Analysis (PCA), Ascending Hierarchical Classification (AHC)) were carried out with the XLSTAT 2006.2 software that is a macro command of Excel 10.1 from Microsoft.

RESULTS AND DISCUSSION

The physico-chemical characterization of extracted oils: The physico-chemical analysis of the seed oil of

Alchornea cordifolia gave the results shown in Table 1. The chemical properties of the oil are very important parameters that determine the quality of the oil.

Table 1 shows that the acid number values are high regardless of the extraction method used. This index varies from 48.25 mg of KOH/g (Folch method) to 64.06 mg of KOH/g (Soxhlet extraction). The oils obtained by the water and Soxhlet methods have somewhat higher acid numbers. For the two studied samples (G and D), the oil extracted with the Folch method shows the lowest acid number values.

The peroxide value ranges from 1.53 mg O₂/g oil (Folch's method) to 1.90 mg O₂/g oil (water extraction). This index is relatively low. However, the lowest value is given by Folch's method. The water method gives oils with slightly higher values.

Table 2 compares the epoxy value of the oil from *Alchornea cordifolia* seeds to that of naturally epoxidized vegetable oils found in the literature.

According to Table 2, the epoxy value of *Alchornea cordifolia* seed oil is 3.23%. Perdue *et al.* (1986) and Carlson and Chang (1985) obtained comparable results, respectively with values of 3.71 and 4.1% on the oils of *Vernonia anthelmintica* and *Vernonia galamensis*.

Oil content and fatty acid composition: The oils extracted from samples of seeds distributed in the two localities (Goma-tsétsé (G) and Dolisie (D)) were studied. The oils studied were obtained by 3 different methods Soxhlet (S), Folch (F) and water Extraction (E). The oil contents and the fatty acid compositions are reported in Table 3.

The very low values of the standard deviations of the oil content (Table 3) indicate a reproducible extraction on each sample therefore the sample appears to be homogeneous. This content varies from 45 to 56%, from 30 to 37% and from 18 to 28% respectively for the Soxhlet, Folch and water extractions. However, for each sample, the Soxhlet method extracts more oil than the other two methods. The Soxhlet extraction method is therefore better from a quantitative point of view. The oil contents obtained by the Soxhlet method are close to or even higher than the data in the literature

Table 3: Variation in the FA content and composition of *Alchornea cordifolia* oils depending on the extraction methods

Samples	Oil content (%)	C16: 0	C16: 1	C18: 0	C18: 1	C*18: 1	C18: 2	C18: 3	Epoxidized fatty acid	C24: 1
Soxhlet method										
SG1	46.66±0.37	11.57	0.29	1.96	10.71	1.16	9.74	0.59	55.90	3.48
SG2	46.68±0.39	11.50	0.43	2.14	9.93	1.21	10.00	0.70	54.40	3.56
SG3	53.55±0.35	13.73	0.38	1.89	8.39	1.30	10.37	0.67	53.91	3.49
SG4	51.67±0.25	11.02	0.34	2.11	12.45	1.25	10.27	0.80	52.38	3.26
SD1	42.69±0.43	10.48	0.33	2.19	8.76	1.17	8.95	0.62	57.70	3.61
SD2	53.47±0.38	15.23	0.44	1.94	12.09	1.28	10.71	0.67	47.70	3.61
SD3	45.20±0.33	11.60	0.39	2.29	8.44	1.08	9.89	0.73	55.50	3.61
SD4	56.09±0.35	13.26	0.49	1.95	11.30	1.43	10.61	0.78	49.70	3.42
Folch method										
FG1	35.05±0.21	9.89	0.26	1.97	9.48	1.05	8.98	0.59	61.14	2.82
FG2	34.93±0.32	11.20	0.42	2.21	9.99	1.15	10.30	0.71	56.90	2.69
FG3	37.31±0.29	13.90	0.36	1.84	9.04	1.35	11.00	0.72	54.10	2.78
FG4	37.31±0.28	11.33	0.29	1.99	10.70	1.16	9.41	0.71	56.90	2.94
FD1	30.66±0.36	11.14	0.36	2.20	9.07	1.14	9.66	0.68	57.94	3.17
FD2	37.34±0.20	13.98	0.41	2.07	11.45	1.27	10.44	0.64	51.11	2.86
FD3	31.22±0.35	14.60	0.43	2.22	9.21	1.20	11.20	0.90	53.30	3.16
FD4	36.47±0.32	14.60	0.43	1.87	11.90	1.33	11.10	0.86	49.90	2.84
Water extraction method										
EG1	20.79±0.24	8.42	0.24	1.90	8.81	1.07	8.30	0.52	62.63	3.43
EG2	21.22±0.39	8.88	0.36	2.25	9.26	1.20	9.55	0.58	59.10	3.72
EG3	20.81±0.38	13.27	0.38	1.86	9.00	1.40	11.24	0.73	53.82	3.24
EG4	28.57±0.36	10.14	0.30	1.99	10.38	1.12	8.65	0.59	58.37	3.59
ED1	21.07±0.24	10.28	0.34	2.14	8.71	1.16	9.09	0.58	59.50	3.58
ED2	20.15±0.24	12.63	0.41	1.94	10.98	1.24	9.91	0.58	54.28	3.15
ED3	18.48±0.25	14.00	0.53	2.42	10.20	1.29	12.10	0.95	50.20	3.18
ED4	20.33±0.38	11.50	0.39	1.96	10.80	1.38	9.84	0.67	54.30	3.52

that indicate 42% according to the work of Kabélé-Ngiéfu *et al.* (1976) and 49% according to the work of Loumouamou (2011).

The water extraction method is appealing. It shows that water is not such a bad solvent for extracting this oil. This yield is still satisfactory in relation to the solvent power of water by its solubility parameters $\delta_h = 34.2$, $\delta_p = 31.3$, $\delta_d = 12.3$ (Barton, 1975). Its polar interactions through bonds expressed by high values of δ_h and δ_p do not contribute to the quantitative extraction of the oil. Rather, its dispersal interactions favor high extraction of epoxidized fatty acid. As for the Soxhlet extraction method, hexane acts exclusively through its solubility parameter δ_d ($\delta_d = 14.9$, $\delta_p = 0$, $\delta_h = 0$). This is linked to dispersal interactions. Folch's method uses a MeOH/CHCl₃ mixture. These are two complementary solvents. An effect of association or breaking of hydrogen bonds between methanol ($\delta_d = 15.1$, $\delta_p = 12.3$, $\delta_h = 22.3$) and chloroform ($\delta_d = 18$, $\delta_p = 3.7$, $\delta_h = 3.7$) results in the formation of monomers, dimers or oligomers (Ibbitson and Moore, 1967) enhances the solvent power of these entities in favor of the oil of *Alchornea cordifolia*. The extraction of triglycerides rich in epoxidized acid is favored. The performances are close to those of the cold and aqueous route (Mouloungui *et al.*, 2006). The water extraction method gives a higher content of epoxidized fatty acid compared to the Soxhlet method using hexane.

However, the acid numbers are very high by hydrolysis of triglycerides (Table 1).

Knowing that the oils studied consist of a large part of free fatty acids, the similarities, and differences in the composition of fatty acid between the oils extracted by different methods are visualized using the "radar plot" representation. This last representation allows an easier comparison than a histogram when we have samples with a large number of characteristic variables (Silou *et al.*, 2004).

Thus, starting from the fatty acid composition of the oils studied, a figure is constructed on which the constituents are distributed on graduated axes, uniformly distributed on a circle (360°). The content of each component on the corresponding axis defines the representative point of the component. These points connected by straight segments define a characteristic geometric figure of the oil (radar-plot).

Figure 2a and 2b show the great resemblance of the oils extracted by three methods from the samples of Goma tsé-tsé and Dolisie. These two figures illustrate, more generally, a great stability of the qualitative composition given by the identity of shape of the radar-plots representative of the oils and the small variation in size of the radar-plots reflecting a moderate variability of the quantitative composition.

Whatever the extraction method used, the epoxidized fatty acid presents a clear predominance and

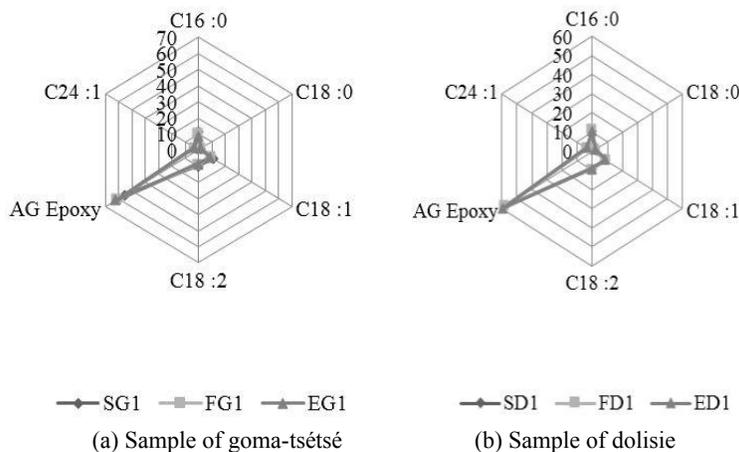


Fig. 2: “Radar plots” representation of the oils from the seeds of *Alchornea cordifolia* studied

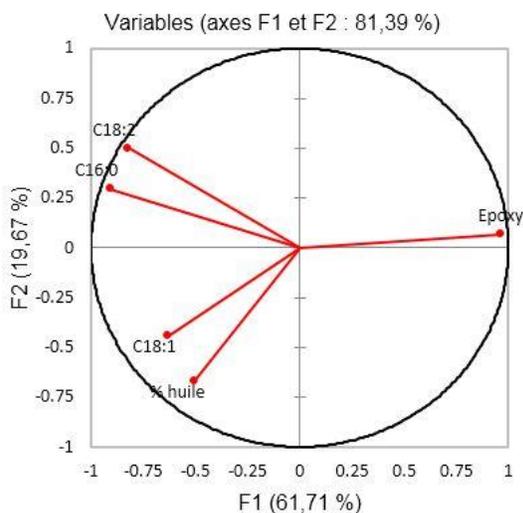


Fig. 3: Correlation circles in principal component analysis of the fatty acid composition of *Alchornea cordifolia* seeds oils

remains the major constituent of the oil of *Alchornea cordifolia* followed by palmitic (C16: 0), oleic and linoleic acids with levels of 1 order of ten. Selacholeic (C24: 1) and stearic (18: 0) acids are in very low amounts. This should lead to a fatty acid profile at cumulative contents of nearly 90% following:

Epoxidized fatty acid >> C16: 0> C18: 1> C18: 2> C24: 1> C18: 0

In sample SG4, we note the following profile:

Epoxidized fatty acid >> C18: 1> C16: 0> C18: 2> C24: 1> C18: 0

The fatty acid profile of SG4 shows a quantitative inversion between C16:0 and C18: 1.

Principal Component Analysis makes it possible to visualize the link between variables and the similarity between individuals. The circles of correlations on the F1F2 plane, representing 81% information on the total variability indicate a positive correlation between C18: 2 and C16: 0, and between C18: 1 and the oil content, both of which are anti correlated with epoxidized fatty acid (Fig. 3).

The oils are distributed on the F1F2 plane according to their composition (Fig. 4).

In relation to this occupation of space, the oils are distributed regardless of the method of extraction and the origin of the seeds. This shows that the fatty acid composition is not influenced by the method of extraction and the origin of the seeds. We therefore have oils that are more homogeneous.

These results of the fatty acid composition of seeds of *Alchornea cordifolia* from Congo, with a remarkable predominance of epoxidized fatty acid agree with those found by Kleiman *et al.* (1977), who worked on the oil of seeds of *Alchornea cordifolia* from Ghana extracted with petroleum ether. However, there are small differences in the composition of these two oils. The "radar-plots" representation brings out the similarities and the differences (Fig. 5).

Figure 5 shows two non-superimposable geometric figures. This figure gives a better representation of the oils of the seeds of *Alchornea cordifolia* from Ghana and Congo which are essentially differentiated by the presence in significant quantity or not of selacholeic acid (C24: 1) and vernolic acid (C18 epoxidized). Ultimately, there is a total absence of selacholeic acid (C24: 1) in oil from Ghana and a total absence of

Table 4: Content of unsaponifiable matter

Sample	SD2	FD2	ED2	SG3	FG3	EG3	SG4	FG4	EG4
Content (%)	0.58	0.80	0.62	0.72	1.26	0.62	0.79	1.23	0.60

Table 5: Composition of the unsaponifiable matter of the oil of *A. cordifolia*

Compounds	Squalène	Bêta-sitostérol	Stigma-stérol	Alpha-tocophérol	Delta.5 ergostenol	Fucos-terol	Cyclo-artenol	Friedelin	A	B
Content (%)	3.13	30.84	3.73	4.16	7.81	7.10	2.02	10.45	1.51	2.70

A: bis (p-octylphenyl) amine; B: Phénol, 2,2'-methylenebis [6-(1,1-dimethylethyl)-4-méthyl

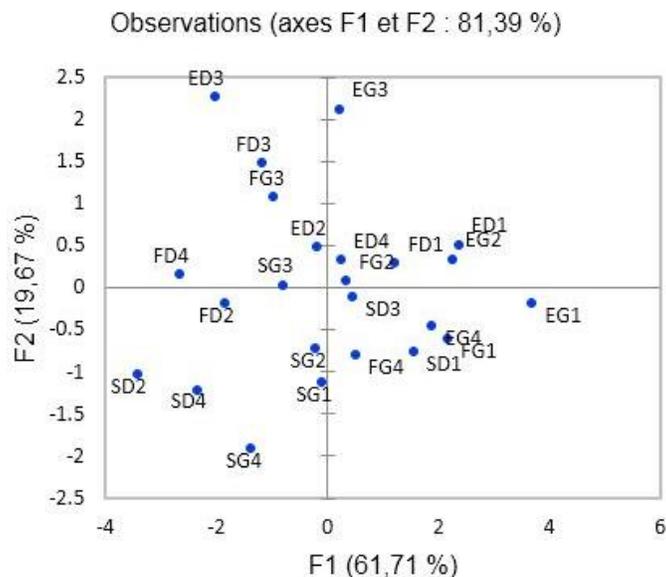


Fig. 4: Distribution of individuals in principal component analysis of the fatty acid composition of *Alchornea cordifolia* seeds oils

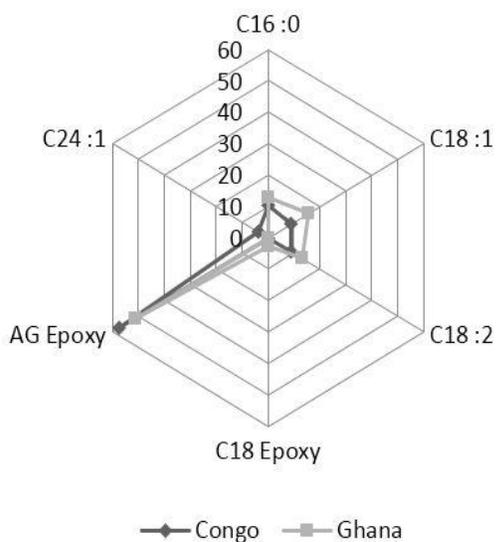


Fig. 5: “Radar plots” representation of oils from Ghana and Congo

epoxidized C18 in oil from Congo (Fig. 5). The seed oil of *Alchornea cordifolia* from Congo-Brazzaville contains triglycerides carrying epoxy groups like that from Ghana.

Content and composition of the unsaponifiable matter: The composition of the unsaponifiable fraction of *Alchornea cordifolia* is given in Table 4.

The oils of *Alchornea cordifolia* studied have unsaponifiable matter contents, which vary from 0.58 to 2.45% (Table 4). The contents obtained with the oils extracted by the Folch method are slightly higher than those of other methods regardless of the sample and the origin.

These contents are less than 2%, the maximum content of unsaponifiable matter in commonly encountered vegetable oils (Loumouamou *et al.*, 2013).

A sample of oil extracted by the Soxhlet method was subjected to a fine analysis for the study of the unsaponifiable composition. The results are given in Table 5.

According to Table 5, the composition of the unsaponifiable matter of the oil of *Alchornea cordifolia* shows the presence of hydrocarbons (3.13%) represented mainly by squalene. Beta-sitosterol constitutes the major compound of unsaponifiables with a content of 30.84% followed by fiedelin (10.45%), delta.5 ergosterol (7.81%), fucosterol (7.1%), alpha-tocopherol (4.16%), stigmasterol (3.73%) and other constituents in trace amounts.

CONCLUSION

This study focused on the characterization of oils obtained from seeds of *Alchornea cordifolia* according to the Soxhlet, water and Folch methods. In terms of analysis and characterization, we find that the Soxhlet method extracts more oil than the other two methods. The fatty acid composition is not influenced by the method of extraction and the origin of the seeds. We therefore have oils that are more homogeneous. However, the water extraction method gives a higher content of epoxidized fatty acids compared to the Soxhlet method using hexane and the Folch method. However, the acid numbers are very high by hydrolysis of triglycerides. The physico-chemical characterization of the oils reveals high acid number values regardless of the extraction method used. This index varies from 48.25 mg of KOH/g (Folch method) to 64.06 mg of KOH/g (Soxhlet extraction). By its fatty acid composition and its epoxy content, it is thus shown that the oil from the seeds of *Alchornea cordifolia* from Congo-Brazzaville contains triglycerides carrying an epoxy group. It should be classified among naturally epoxidized oils in the same way as *Vernonia galamensis* cultivated on 200,000 ha in Kenya and 20,000-30,000 ha in Zimbabwe (Gunstone, 1997). Thanks to the commercial interest aroused by the epoxidized fatty acid containing these oils (Carlson and Chang, 1985), we must continue our investigations by studying the determination of the structures of the epoxidized fatty acids contained in the triglycerides of the oil of *Alchornea cordifolia*.

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CONFLICT OF INTEREST

We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

REFERENCES

- AFNOR (Association Française de Normalisation), 1998. Corps gras, graines oléagineuses, produits dérivés. 4e édition, Afnor, Paris.
- Barton, A.F.M., 1975. Solubility parameters. Chem. Rev., 75(6): 731-753.
- Carlson, K.D. and S.P. Chang, 1985. Chemical epoxidation of a natural unsaturated epoxy seed oil from *Vernonia galamensis* and a look at epoxy oil markets. J. Am. Oil Chem. Soc., 62(5): 934-939.
- Folch, J., M. Less and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226(1): 1497-1509.
- Goteni, S., D. Mampouya and T. Silou, 2011. Fatty acids, triacylglycerols, carotenes, retinols, tocopherols and phytosterols in oils of *Raphia sese* and *R.laurentii* from Congo-Brazzaville. J. Food Technol., 9(2): 61-65.
- Gunstone, F.D., 1997. Epoxidized Oils. In: Gunstone, F.G. and F.B. Padley (Eds.), Lipid Technologies and Applications. Marcel Dekker Inc., Chapter No. 29, pp: 759-769, and references cited: Editor, Natural plant material, Oils Fats Int. 1990, (2): 9.
- Ibbitson, D.A. and L.F. Moore, 1967. Association of n-alcohols in non-polar solvents. Part I. The dielectric polarisations, apparent dipole moments, and near-infrared spectra of n-alcohols in carbon tetrachloride and cyclohexane. J. Chem. Soc., B: 76-79.
- Kabélé-Ngiéfu, C., C. Paquot and A. Vieux, 1976. Plantes à huiles du Zaïre II. Familles botaniques fournissant des huiles d'insaturation moyenne. Oléagineux, 31(12): 545-547.
- Kleiman, R., R.D. Plattner and G.F. Spencer, 1977. *Alchorneacordifolia* seedoil: A rich source of a new C20 Epoxide, (+) cis-14,15-epoxy-cis-11-eicosenoic acid. Lipids, 12(7).
- Loumouamou, B.W., 2011. Contribution à la valorisation des oléagineux du Bassin du Congo. Composition chimique et potentialités technologiques des amandes du Genre Irvingia. Ph.D. Thèse, Université Marien Ngouabi, pp: 142.
- Loumouamou, B.W., J.P.M. Gomoufatan, T. Silou, J.M. Nzikou, G.V. MbayaGindo, G. Figueredo and J.P. Chalard, 2013. Extraction and chemical composition of seed kernel oil from *Irvingiasmithii* of Congo Basin. Adv. J. Food Sci. Technol., 5(5): 506-513.
- Loumouamou, B.W., A.F. Binaki and T. Silou, 2014. Oleaginous character and profiles in fatty acids and in triacylglycerols of the seeds of *Allanblackia floribunda* Oliv. of Congo. Adv. J. Food Sci. Technol., 6(3): 308-315.

- Manga, H.M., D. Brkic, D.E.P. Marie and J. Quetin-Leclercq, 2004. *In vivo* anti-inflammatory activity of *Alchornea cordifolia* (Schumach. &Thonn.) Müll. Arg. (Euphorbiaceae). J. Ethnopharmacol., 92(2-3): 209-214.
- Manga, H.M., M.Haddad,L.Pieters,C. Baccelli,A. Penge and J. Quetin-Leclercq, 2008. Anti-inflammatory compounds from leaves and root bark of *Alchorneacordifolia* (Schumach. &Thonn.) Müll. Arg. J. Ethnopharmacol., 115(1): 25-29.
- Mouloungui, Z., S. Pelet, V. Eychenne and S. Mateo, 2006. La lipochimie. In: Colonna, P. (Ed.), Chapter 12, Chimie Verte. TEC & DOC, Lavoisier, Londres-Paris-New-York, pp: 305-356.
- MvoulaTsiéri, M., T. Silou and A. Tremolieres, 2005. Nature et composition des classes de lipides de quatre espèces de curcurbitacées alimentaires du Congo Brazzaville, Rivis. Ital. Sostanse Grasse, 82: 140-144.
- Nikiema, D., Z. Mouloungui, K. Oi Koua, M. Cerny, E. Lacroux, R. Valentin and A. Ané, 2019. Effect of dehulling method on the chemical composition of the lipid constituents of the kernels and oils of *Ricinodendronheudelotii* seeds. Ind. Crop. Prod., 140: 111614.
- Nkuinkeu, R., 2003. Perspectives sur la promotion soutenue des plantes oléagineuses dans le Bassin du Congo avec référence spécial au Cameroun. Actes séminaires safou 2003, Brazzaville, Congo.
- Okoye, F.B.C., P.O. Osadebe, C.S. Nworu, N.N. Okoye, E.O. Omeje and C.O. Esimone, 2011. Topical anti-inflammatory constituents of lipophilic leaf fractions of *Alchorneafloribunda* and *Alchorneacordifolia*. Nat. Prod. Res., 25(20): 1941-1949.
- Perdue, R.E., K.D. Carlson and M.G. Gilbert, 1986. *Vernonia galamensis*, potential new crop source of epoxy acid. Econ. Bot., 40(1): 54-68.
- Silou, T., D. Mampouya, W.D. Loka Lonyange and M. Saadou, 1999. Composition globale et caractéristiques des huiles extraites de 5 espèces des cucurbitacées du Niger. Rivist. Ital. Sostanze Grasse, 76: 141-144.
- Silou, T., S. Biyoko, S. Heron, A. Tchaplà and M.G. Maloumbi, 2004. Caractéristiques physico-chimiques et potentialités technologiques des amandes de *Irvingiagabonensis*. Riv. Ital. Sostanze Gr., 81: 49-57.
- Silou, T., B.W. Loumouamou, S. Nsikabaka, T. Kinkela, M. Nzikou, P. Chalard and G. Figueredo, 2011. Contribution to the varietal delimitation of *Irvingiagabonensis* and *Irvingiawombulu* chemical composition variability of fats extracted from kernels. J. Food Technol., 9(1): 36-42.
- Silou, T., 2012. Oil and Fats for the Future. Case Study Safou: *Dacryodes edulis* from Congo Basin Countries. Nava Edn., Publisher NY, USA, pp: 120.
- Silou, T., S. Goteni, C. Makondzo Mondako, A. Malumba Mukaya and D. Mampouya, 2012. Oils from *Raphia* species from Congo basin: Composition, biodiversity, and quality. Rec. Prog. Med. Plant, 34: 237-268.
- Wolff, J.P., 1968. Manuel d'analyse des corps gras. Azoulay éditeur, Paris France, pp: 519.