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Chemical characterization of *Lophira lanceolata* and *Carapa procera* seed oils: Analysis of Fatty Acids, Sterols, Tocopherols and Tocotrienols

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Abstract

The chemical profiles of two non conventional seed oils from Carapa procera (Cp) and Lophira lanceolata (Ll) have been characterized. Both oils contain ~31% of saturated fatty acids, mainly palmitic acid. Cp oil is highly monounsaturated (58.08%) whereas Ll oil has a high content in polyunsaturated fatty acids (52.46%). Ll oil is rich in tocopherols (3.61 mg/100g) while the Cp oil exhibits higher tocotrienol contents (5.63 mg/100g). A higher total sterol content was found for Ll (100,13 ± 0,04 mg/g) than for Cp (29,43 ± 0,01 mg/100g). A high content of lanosterol was observed for Cp (28.03 % w/w). Both studied vegetable oils showed very different chemical profiles. Ll oil exhibited interesting potential nutritional value. The high contents in polyunsaturated essentials fatty acids, tocopherols and phystosterols, could properly respond to nutritional deficiencies. However, Cp oil may serve as cosmetic additive given to its content in tocotrienol and lanosterol.

Keywords: Fatty acids, Tocopherols, tocotrienols, *Lophira lanceolata*, *Carapa procera.*

Introduction

The majority of Sub-Saharan Africa's population relies on forest products for subsistence uses. Exploitation of non timber African forest products, particularly seeds, can be a sustainable and more economically rewarding use of forests than timber extraction[1,2]. In African folk medicine, numerous seed oils from non-conventional sources recovered from savannah plants have been locally used for centuries for food, pharmaceutical and cosmetic applications[3]. From the livelihoods perspective, seed oils commercialization, defined as increasing the value of oils in trade, is expected to increase income and employment opportunities, especially for poor and otherwise disadvantaged people[4]. From the conservation side, oil commercialization could provide opportunities for forest utilization and even create incentives for conservation of individually valuable species and the environment in which they grow. However, in most of the cases, very little information has been reported on the chemical profiles of these seed oils. Mainly composed of triglycerides, vegetable unconventional oil contains various bioactive components, which include various triterpenoids, carotenoids, tocopherols, tocotrienols and phytosterols[5,6]. It is known since a while that ingestion of phytosterols prevents intestinal absorption of cholesterol in humans, resulting in a lowering of serum cholesterol[7]. In addition to beta-sitosterol, campesterol and stigmasterol, vegetable oils can contain other phytosterol compounds that give them high vitaminic value. More recently, a positive correlation between the presence of lanosterol and maturation of oocytes of prepubertal sheep was demonstrated and the anticancer properties of lupeol on gastric cells have also been shown[8,9]. Tocotrienols and tocopherols have also been linked to the ability to inhibit the proliferation of breast cancer cells and that to lower serum cholesterol levels when administered in the diet of chickens, swine, rats and hypercholesterolemic humans[10]. Several preparations composed of vegetable unconventional oils enriched with additional substances like vitamins or compounds showing an antioxidant activity are commonly available on the market. For this reason, the identification and the quantification of fat-soluble vitamins as well as their precursors or derivatives has been recently gaining interest[11].

Lophira lanceolata (Ll) and *Carapa procera* (Cp) are two wild promising oil seeds plants from savannah regions. *Lophira lanceolata* grows to about 12 m with twisted short branches. Its fruits develop between February and April in which tough reddish elongated seeds are found. Ll seeds are mainly used to extract edible oils. The oil also has cosmetic and medicinal uses and is suitable for making soap. In traditional medicine the oil is used to treat dermatosis, toothache and muscular tiredness. Rubbing the skin with the oil prevents dryness. *Carapa procera* is a tree of economic importance distributed throughout tropical forests in Africa and America. The oil extracted from seeds is used for non edible utilizations, traditionally as repellent and for massage, as well as in the fabrication of candles and various cosmetic products such as soap, shampoos, and other personal care products.

However, there is a lack of information on the characteristics of Ll and Cp oils; the chemical components of these oils have not been investigated in detail. Few data on Cp oil are available and for Ll the available data are incomplete and concern only the physicochemical properties of the oil[12].

Therefore, the main objective of this study was to evaluate the physicochemical properties of oils extracted from *Lophira lanceolata* and *Carapa procera*. The tocopherol, tocotrienol and sterol profiles of these plant oils were investigated to determine the functional compounds in these plant seeds and improve the economic utility of these seeds as a source of edible/non-edible lipids.

Material and Methods

Samples: Fresh *Lophira lanceolata* Tiegh. ex Keay and *Carapa procera* DC. fruits were collected respectively from Boribansinfa, Departement of Atacora in the North-West and from Sakete, Plateau's Department (Benin). They were respectively identified at the National Herbarium of Abomey Calavi University (Benin) in the number AA 6486/HNB and AA6485/HNB. The seeds were separated manually, cleaned for any adhering flesh and dried at 50°C for 48 h. The dried seeds (1.5 kg) were ground with RETSCH GRINDOMIX apparatus in 2min and 5x1000rpm were used as parameters then the grounded seeds were extracted with hexane in Soxhlet apparatus. Oils extracted were conserved in dry place in 4°C for analysis.

Reagents: All solvents and reagents of analytical grade (or HPLC) were obtained from Sigma Chemicals Company Co. (St. Louis, USA) as lupeol (25MG, 98%), cholesterol (500MG, 99%), lanosterol (1MG, 93%), campesterol (1MG, 65%), 7-dehydrocholesterol (5G, 98%). Reference standards for tocols were obtained from Chroma Dex (Santa Ana, CA, USA) and included α,γ,δ and β-tocopherols and tocotrienols with high purity. Stock solutions containing 2.5g.L^{-1} of tocols were prepared in HPLC-grade methanol and stored in the dark at 4°C for at least 2 months.

Chemical properties of studied oils: Acid, peroxides, iodine and saponification values were determined according to International Organization for Standardization (ISO)[13-16].

Fatty acid methyl esters (FAMEs) determination: Crude oils were analyzed as methyl esters (FAMEs) to determine the fatty acids composition. FAMEs were obtained by using Ackman methods and then analyzed by capillary column gas chromatography (GC) (Shimadzu GC-201O Plus) equipped with a flame ionization detector (FID), as described by Belhaj *et al.* (2010)[17,18].

Determination of tocols (tocopherols and tocotrienols): Quantification of tocopherols (TCP), Tocotrienols (TCT) (or tocols) and phytosterols was performed using an HPLC-MS equipment (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an ion trap LTQ (Linear Trap Quadripod) as mass analyzer. The data were processed using the Xcalibur (version 2.1) software. Elution of the compounds was carried out on a reverse phase column Alltima C18 (150 * 2.1 mm, porosity of 5 microns - Grace / Alltech, Darmstadt, Germany) equipped with an Alltima C18 pre-column (7.5 * 2.1 mm, 5 µm for porosity - Grace / Alltech, Darmstadt, Germany) at 25°C. These compounds were eluted by an isocratic method with a flow rate of 0.2 mL.min^{-1}, using MeOH / H_2O / HCOOH mixture (97/3/0.1) as the mobile phase for tocols and methanol at 0,1% formic acid for the phytosterols.

The APCI (Atmospheric-Pressure Chemical Ionization) interface mass spectrometry was used in the positive mode for the determination and quantification of tocols and phytosterols. Spectrometric conditions were optimized to achieve high sensitivity by direct injection of the standard solutions (1mg.L^{-1}) in methanol. These conditions are summarized in table-1 below.

Table-1
Conditions of LC-MS spectrometric analysis

Category	Parametry	
Voltages	Corona	5µA
	Capillary	23V
	Lens	75V
	Bi-lens	-36V
	frontal lens	-6,25V
Tempature	Vaporisator	400°C
	Capillary	175°C
Gaz	sheating	40 min^{-1}
	auxiliary	10 min^{-1}
	scanning	10 min^{-1}

Detecting with high sensitivity of the specific compounds of tocols was performed following the sons ions derived from MS2 mass fragmentation of pseudo-molecular parental ions [M+H]+. These ions, as well as equations of calibration curves are presented in table-2. However, because of MS2 fragmentation patterns similar (qualitative and semi-quantitative), it was not possible to distinguish the beta and gamma isomers (TCP / TCT). They have therefore been quantified together.

For the detection of phytosterols, they shall also be made by mass spectrometry from the son ions obtained from the fragmentation of parent pseudo-molecular ions [M+H-H2O]+; except for lanosterol whose parental form was [M]·+. It was in MS2: 367,5m/z for 7-dehydrocholesterol; 369,5m/z for cholesterol; 383,5m/z for campesterol; 395.5 m/z for stigmasterol; 397,5m/z for beta-sitosterol and 409,5m/z for lupeol and lanosterol. The calibration curves were obtained by MS2 from standard mixtures of these compounds of concentration ranging between 0.5 and 100 ppm. These calibration curves are shown in each case, not only a very good linear correlation (R^2> 0.99) but also a good stability in MS response.

Table-2
Characteristics of ion fragments of mass spectra of tocols in APCI mode and regression quantified compounds

		Tr (mn)	Some characteristic ions (m/z)	Regression (ppm)
TP	α	21,10	431,5 ; 165	$y = 137770x;\quad R^2 = 0{,}9995$
	(β+γ)	17,64	417,5 ; 151	$y = 644591x;\quad R^2 = 0{,}9993$
	δ	14,34	403,5 ; 137+177	$y = 261804x;\ R^2 = 0{,}9972$
TCT	α	10,8	425,5 ; 165 + 205 + 273	$y = 710847x - 386449{,}1;\quad R^2 = 0{,}9988$
	(β+γ)	9,2	411,5 ; 151.00 + 163.00 + 177.00 + 191.00 + 205.00 + 219.00 + 247.00 + 259.00 + 273.00 + 287.00	$y = 448801x;\ R^2 = 0{,}9974$
	δ	7,7	397,5+137.00 + 163.00 + 177.00 + 191.00 + 205.00 + 247.00 + 259.00 + 273.00 + 287.00	$y = 562034x;\quad R^2 = 0{,}9986$

Statistical analysis: Data from three independent replicate trials were subjected to statistical analysis using Statistica version 6.0. Differences between means were tested using Z-test.

Results and Discussion

Chemical properties: The chemical characteristics of *Lophira lanceolata* and *Carapa procera* oils, obtained by Soxhlet apparatus with hexane are presented in table-3[19]. The oil yield (42.32%) from Ll seeds is in good agreement with previously reported results and is closed to the oil contents of rapeseed and sunflower[20]. For Cp, the value of 74.76% is higher than the results reported in the literature (47.91-61.5%) and obtained by the same way as reported by Vieux *et al.* (1970)[21]. A high free oleic acid value (A) for Cp oil was observed (18.74 %). This is in agreement with previous results since high levels of free fatty acids in Cp oil were already reported by Djenontin *et al.*[22]. The saponification values (SV) of the Ll oil is comparable with the values for common oils i.e., palm oil (196-205 $mg.g^{-1}$), groundnut oil (188-196 $mg.g^{-1}$) and corn oil (187-196 $mg.g^{-1}$) and justify the use of this oils by population to prepare soap[23]. The iodine values for both seed oils suggest that Ll (76.59±1.18E-4 g/100g w/w) is more unsaturated than Cp [60-63.77±2.89 g /100g w/w]. Nevertheless, Cp oil's shows better texture with a brighter light (L *, Carapa: 92.65 and Lophira: 35.85), more extreme in the green hue (a *, Carapa: -3.49 and Lophira: 6.08), and yellow (b *, Carapa: Lophira and 56.27: 41.10), than the oil of Lophira; indicating the presence of compounds such as vitaminic tocols.

Fatty acids: Methyl esters fatty acid obtained from vegetable oils are shown in table-4 below. There are high values of polyunsaturated fatty acids (PUFA) in Ll (> 50%). These are followed by the values of saturated fatty acids (SFA) which are double of that obtained for monounsaturated fatty acids (MUFA ≈15%). Specifically, α-linolenic acid (an essential ω–6 type fatty acid which proportion is > 31%), palmitic acid (≈30%), arachidonic acid and oleic acid (> 13%) are the most predominantly obtained for this oil. Ismail *et al.* (2008) recommended vegetable oils rich simultaneously in oleic and linoleic acids in nutrition to reduce cardiovascular disease[24]. Then, these acids are recognized as GRAS (Generally Recognize as safe) in the increasing of the immune defense[25]. The fatty acids of Cp differ from those of Ll by their content. Better composition is observed in MUFA (58.2%) with oleic acid as major (57.75%). The palmitic acid (20.39%), α-linoleic acid (9,80%) and α-linolenic acid (1.18%) were lesser quantified in Ll oil's. But, better content of stearic acid (10.09%) is noted in Cp oil. Such specificities in oleic, palmitic and stearic acids is closed to the fatty acids compositions of *Vitalaria paradoxa* vegetable oils and *Theobroma cacao*, commonly used for chocolates in industry[26].

Table-3
Extraction yields, colors and chemical properties of *Lophira l.* and *C. procera* oils

	Lophira lanceolata	*Carapa procera*
Yield (%)	$42.32±0.41^b$	$74.76±1.27^a$
Acidity (%)	$0.18±0.01^b$	$18.74±0.05^a$
IV (g/100g)	$76.59±1.18E\text{-}4^a$	$63.77±2.89^b$
IP ($mleqO_2/kg$)	$21.84±1.05^a$	ND
IS (mgKOH/g)	$201.57±5.07^a$	ND
Colors (L, a, b)	$35.85±0.27^b$	$92.65±0.07^a$
	$6.08±0.08^a$	$-3.49±0.02^b$
	$41.10±0.12^b$	$56.27±0.13^a$

IP: Peroxid value; IV: Iodine value et IS: Saponification value; ND: not detected; *Data in the column followed by different letters are significantly different (p < 0.05). The values are means of three repetitions ± standard deviation*

Tocols (tocopherols and tocotrienols): The tocols proportions of both oils are showed in table-5. As predisposed by her best texture, Cp has more tocols contents than Ll. Alpha-tocopherol is the most tocochromanol type showed in Ll oil whereas Cp has a higher content in (β+γ) tocotrienol (4,64mg/100g representing more than 70% by all tocols). These tocotrienols contents give to Cp, very positive connotations in cosmetics where their anti-tumor activity was proven by Husain *et al.*[27]. However, beta and gamma isomers of each of these compounds could not be differentiated due to their identical molecular weight. Similar observations were made by Evans *et al.* and Surai, although other authors have been able to separate these compounds[28,29].

Furthermore**,** tocols levels quantified here are lower than those of usual oils such as peanut and sunflower, but larger than some of non-conventional oils. For example, Fanali *et al.* (2011) by using an HPLC method were unable to quantify 0.4 mg/100g of alpha tocopherol (ten times less than that of Ll oil), in Inca peanut (*Plukentia volubilis* L.) vegetable oils[30]. This would justify the preferential food uses of this oil in rural areas of Benin.

Sterols: Like tocols, six phytosterols and lupeol were quantified by LC-APCI-MS method (table- 6). *Lophira lanceolata* oil, has three times more triterpene compounds (100,13mg/100g) than Cp. This finding is contrary to that observed for tocols where *Carapa procera* oil was the richest. As shown by many studies, the β-sitosterol is the most abundant phytosterol in vegetable oils (> 60% and 47.04% respectively for *L. lanceolata* and *Carapa p.*). It is followed by campesterol (LI: 63,82mg/100g and Cp: 13,85mg/100g) and stigmasterol (LI: 13.83 mg/100g and Cp: 3,19mg/100); which are four to five times richer in *Lophira lanceolata* oil. However, lanosterol was not quantified in this oil; while 28.03% of this compound has been identified in Cp oil. Similarly, 1.49% of lupeol were quantified in Ll oil while this substance was absent in Cp oil. If the analgesic and anti-inflammatory properties and the destructive action of lupeol on cancer cells have been respectively proven by Oliveira *et al.* and Wu *et al.*, those of lanosterol were also highlighted[9,31]. Cholesterol and its peer have only been poorly quantified in both oils compared to the usual vegetable oils. This is a good finding because a high concentration of these types of sterols can cause cardiovascular diseases. However, the values recorded for phytosterols in Carapa oil are lower than those found by Djenontin *et al.*[24].

Table-4
Fatty acids profils of *Lophira lanceolata* and *Carapa procera* oils

Sub-class	Squelettons	Acids	*L. lanceolata*	*C. procera*
SFA	C16	Palmitic	30.03±0.08[a]	20.39±0.05[b]
	C18	Stearic	2.03±0.05[b]	10.09±0.18[a]
Total SFA			32.06	30.48
MUFA	C16:1n7c	Palmitoleic	0.14±0.00[a]	0.28±0.04[a]
	C18 1n9c	Oleic	14.21±0.20[b]	57.75±0.69[a]
	C20:1n9	Gadoleic	0.37±0.10[a]	0.17±0.01[a]
	C22:1n9c	Erucic	0.74±0.05[a]	ND[a]
Total MUFA			15.46	58.2
PUFA	C18 2n6c	α-linoleic	31.81±0.52[a]	9.80±0.05[b]
	C18:3n3	α-linolenic	0.21±0,00[a]	ND[a]
	C18:3n6	γ-linolenic	2.19±0.08[a]	1.18±0.02[b]
	C18:4n3	stearidonic	2.02±0.09[a]	0.18±0.01[b]
	C20:4n6	Arachidonic	16.23±0.26[a]	ND[b]
		unknown	ND	0.16
Total PUFA			52.46	11.32
		Total	99.98	100

SFA: Saturated Fatty acids, MUFA: Monounsaturated, PUFA: Polyunsaturated fatty acids; *Data in the column followed by different letters are significantly different (p < 0.05). The values are means of three repetitions ± standard deviation*

Table-5
Tocols composition of oils of *Lophira lanceolata* obtained by differents methods

	L. lanceolata	*C. procera*
α-TCP	1,71±0,00[a]	0,92±0,00[a]
(β+γ)-TCP	1,85±0,23[a]	0,10±0,01[b]
δ-TCP	0,23±0,10[a]	0,01±0,00[a]
α–TCT	0,12±0,04[b]	0,91±0,11[a]
(β+γ)-TCT	0,55±0,03[b]	4,64±0,60[a]
δ-TCT	0,18±0,11[a]	0,07±0,07[a]
Total TCT	0,84±0,03[b]	5,63±0,23[a]
Total tocols	4,45±0,06[b]	6,58±0,18[a]

TCP: Tocopherols, TCT: Tocotrienols

Table-6
Phytosterols of studied Lophira l, seed oils

	L.lanceolata		*C.procera*	
	(mg/100g)	(%)	(mg/100g)	(%)
7-dehydrocholesterol	0,013±0,00[a]	0,01	0,018±0,00[a]	0,06
Lupeol	1,49±0,10[a]	1,49	nd[a]	0,00
Lanostérol	nd[b]	nd	8,25±0,06[a]	28,03
Cholestérol	0,05±0,00[a]	0,05	0,04±0,00[a]	0,13
Stigmastérol	13,83±0,13[a]	13,81	3,19±0,01[b]	10,83
Campestérol	20,92±0,02[a]	20,89	4,09±0,003[b]	13,91
β-sitostérols	63,82±0,07[a]	63,74	13,85±0,03[b]	47,04
Total	100,13±0,04[a]		29,44±0,01[b]	

Data in the column followed by different letters are significantly different ($p < 0.05$). The values are means of three repetitions ± standard deviation

Conclusion

The chemical characterizations of *Lophira lanceolata* and *Carapa procera* oils, two non-conventional seed oils from Benin, were described. The two oils showed very different chemical and nutritional qualities and seem to be of high economic value in different ways. *Lophira lanceolata* oil exhibited interesting potential nutritional value (good organoleptic properties, high polyunsaturated fatty acids and phytosterols contents). Concerning *Carapa procera*, the oil yield and the tocotrienols content were particularly high. Furthermore, the presence of a relatively high content in lanosterol, whose anticanceric effects have been proven, was observed. These properties could explain and justify the endogenous uses of *Lophira lanceolata* and *Carapa procera* oils in sub-Saharan Africa's population.

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