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Composition of fatty acids triacylglycerols and unsaponifiable matter in *Calophyllum calaba* L. oil from Guadeloupe

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Abstract

The composition of the kernel oils of two *Calophyllum* species (*Calophyllum calaba* L. and *Calophyllum inophyllum* L.) was investigated. The physico-chemical properties and fatty acid composition of the kernel oils were examined. In two species, oleic acid C18:1 (39.1–50%) is the dominating fatty acid followed by linoleic acid C18:2 (21.7–31.1%) as the second major fatty acid. Stearic C18:0 (13.4–14.3%) and palmitic C16:0 (11–13.7%) acids are the major saturates. The oils contains an appreciable amount of unsaturated fatty acids (70.8–73.10%). Most of the fatty acids are present as triacylglycerol (76.7–84%), twenty one triacylglycerols are detected with predominantly unsaturated triacylglycerols. The total unsaponifiable content, its general composition and the identity of the components of the sterol and tocopherol fractions are presented. In both species, analysis of the unsaponifiable fractions revealed the preponderance of phytosterols, mainly stigmasterol (35.8–45.1%) and β-sitosterol (41.1–43.1%). Among the eight tocopherols and tocotrienols present in two species, variations exist; α-tocopherol (183 mg/kg) is the main tocopherol in *Calophyllum calaba* L. and Δ-tocotrienol (236 mg/kg) is the dominant tocotrienol in *Calophyllum inophyllum* L.

Keywords: *Calophyllum calaba*; *Calophyllum inophyllum*; Guttiferae; Gas and Liquid chromatography; Kernel oil; Fatty acid; Triacylglycerol; Sterol; Tocopherol; Tocotrienol

1. Introduction

The genus *Calophyllun* of the Guttiferae family is a large group of tropical trees consisting of approximately 180–200 different species. Although a handful of species has been identified in the New World, the genus is primarily found in the Indo-Pacific region, particularly Malaysia (Morel et al., 2000). A number of medicinal and therapeutic properties have been described to various parts of *Calophyllum* multipurpose tree, including the treatment of rheumatism, varicose veins, hemorrhoids and chronic ulcers (Cottiglia et al., 2004). Plants from *Calophyllum* are known to be rich in coumarins (Kashman et al., 1992; Spino et al., 1998; Cao et al., 1998; Guilet et al., 2001; Ito et al., 2003; Ma et al., 2004) and xanthones (Kumar et al., 1982; Goh and Jantan, 1991; Linuma et al., 1996, 1997; Sartori et al., 1999; Kijjoa et al., 2000; Ito et al., 2003). Many species have been studied and biflavonoids (Ito et al., 1999), chalcone, benzofurans and triterpenoids (Ito et al., 2002) have been identified as constituents. Previous work has focused on the characterisation of xanthones and

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coumarins especially since the isolation of the calanolides, prototypes of a unique subclass within the nonucleodide HIV-1 reverse transcriptase inhibitors, have been reported (Dharmaratne et al., 1999; McKee et al., 1998; Itoigawa et al., 2001). *Calophyllum inophyllum* L. is the most widespread and has superior timber qualities. Is a tree commonly found in the Coastal regions of South India and Madagascar. The kernel oil is used in folk medicine for its healing properties in Asia (Adeyeye, 1991), nowadays, with those antiaging (protection against UV) properties, its oil can be used for industrial means (Boucher et al., 2000). *Calophyllum calaba* L. is one of species distributed in the islands of Caribbean. The tree is a 25 m high; the fruits are green or greenish yellow, drupaceous, 2.8 cm diameter and ripen between July and September, it is frequently used for reforestation. (Fournet, 2002). The latex from the trunk has been employed medicinally, and lamp oil is extracted from the kernels (Little et al., 1964). The kernel contained high lipid content. Neither the physicochemical properties, triacylglycerol and fatty acid composition, nor the unsaponifiable composition of the kernel oil of the Caribbean species of *C. calaba* L. has been reported previously. Earlier analysis of the kernel oil of *C. inophyllum* L. have been made (Adeyeye, 1991; Hemavathy and Prabhakar, 1990). The aim of the present investigation is to determine composition of *C. calaba* L. kernel oil with comparative experiments with *C. inophyllum* L. kernel oil.

2. Results and discussion

2.1. Physicochemical properties

The kernel of *C. calaba* represented 65% of the dry fruit. The total lipids in *C. calaba* kernel are presented in Table 1. The percentages of lipid content showed high kernel oil contents (67.2%). In the literature, the value of *C. inophyllum* L. was about 60.1% (Hemavathy and Prabhakar, 1990). Some of the chemical and physical properties of the crude oils extracted from *C. calaba* and *C. inophyllum* kernels are shown in Table 2. Density and refractive indices were similar to the two species tested, and the values compared well with the 1.4701 value reported for *C. inophyllum* kernel oil (Hemavathy and Prabhakar, 1990). Acid values were similar and the stability of the extracted oils is conveyed by low peroxide value. Oxidation stability value of *C. inophyllum* L. kernel oil (21.9 ± 3.3 h) was lower than the value of *C. calaba* L. kernel oil (>75 h). Saponification values (178–194) were similar to those reported in the literature for cotton kernel oil (189–198) (Hemavathy and Prabhakar, 1990). Both oils show high saponification values suggesting their use in production of liquid soap and shampoos. The two oils samples had relatively high iodine values, thus reflecting a high degree of unsaturation, and place them in the semi-drying group oil. These iodine values were confirmed by the fatty acid composition of the oils presented in Table 3. *C. calaba* kernel oil has less unsaponifiable matter than *C. inophyllum* kernel oil. The phosphorus content is higher for *C. calaba* L. than it is in *C. inophyllum* L.

Table 1
Fruit characteristics and kernel oil extraction results[a]

	Calophyllum calaba
Oil/kernel (%)	67.2 ± 1.4
Kernel/dry fruit (%)	65.1 ± 0.5
Oil/dry fruit (%)	43.7 ± 1.9

[a] Values are mean ± standard deviation of duplicate determinations.

2.2. Fatty acid composition

The results of the analysis of the fatty acid fraction of the kernel oils are summarised in Table 3. There were wide variations in the contents of palmitic, stearic, oleic and linoleic acids among the oils studied, leading to differences in saturated and unsaturated fatty acids. The major saturated fatty acids in crude oils of both *C. calaba* L. and *C. inophyllum* L. kernels, were stearic C18:0 (13.4–14.3%) and palmitic C16:0 (11–13.7%) acids. Saturated fatty acid content of *C. inophyllum* L. kernel oil was higher (29.1%) than *C. calaba* L. kernel oil (26.70%). Both oil samples had high amounts of unsaturated fatty acids, representing 73.10% for *C. calaba* L. kernel oil and 70.8% for *C. inophyllum* L. kernel oil which consisted mainly of oleic (39.1%) followed by linoleic (31.1%) and stearic (14.3%) acids. *C. calaba* L. had the highest content of oleic acid (50%) and the lowest amount of linoleic acid (21.7%) of the kernel oil.

2.3. Lipid classes

The quantitative data of lipid fractions of crude oils of both species are shown in Table 4. Triacylglycerol (TAG) were the predominant lipid class in all the samples, representing 84%, 76.7% for crude oils of *C. calaba* and *C. inophyllum* kernels, respectively; followed by diacylglycerol (DAG), then free fatty acid (FFA). FFA and DAG contents of *C. inophyllum* kernel oil were higher (5.1%, 7.0%, respectively) than *C. calaba* kernel oil (0.8%, 2.2%, respectively). The presence of monoacylglycerol (MAG) and FFA in oil samples may be due to the partial enzymatic hydrolysis of TAG during storage of the kernels.

2.4. Triacylglycerol composition

Identification of molecular species of TAG and percentage of the oil samples have been presented in Table 5.

Table 2
Physicochemical characteristics of the extracted kernel oils[a]

	Species		AFNOR methods
	Calophyllum calaba	*Calophyllum inophyllum*	
Density	0.99	0.99	NF T 60-214
Refractive index (25 °C)	1.475	1.479	NF T 60-212
Acid value (mg KOH/g)[a]	22 ± 1	22.4 ± 0.6	NF T 60-204
Peroxide value (μg/g)	3.84 ± 0.1	1.45 ± 0.1	NF T 60-220
Oxidation stability (98 °C, 20 l/h, 3 g)	>75 h	21.9 ± 3.3 h	NF ISO 6886
Saponification value (mg KOH/g)[a]	178 ± 5	194.4 ± 6	NF T 60-206
Iodine value (mg iodine/g)	119 ± 5	90.05 ± 5	NF T 60-203
Unsaponifiable matter (%)	0.79 ± 0.3	1.98 ± 0.2	NF ISO 3596-1
Phosphorus content (mg/kg)	32 ± 7	9 ± 2	NF ISO 10540-1
Phospholipids content (mg/kg)	800 ± 6	225 ± 5	–
Carotene content (mg/kg)	0.2 ± 0.09	<0.1	NF ISO 12823-2
Lycopene content (mg/kg)	<0.1	<0.1	NF ISO 12823-2

[a] Values are mean ± standard deviation of duplicate determinations.

Table 3
FA composition of the kernel oils[a]

FA (%)	Species	
	Calophyllum calaba	*Calophyllum inophyllum*
Myristic acid	<0.1	<0.1
Palmitic acid	11 ± 0.8	13.7 ± 0.8
Palmitoleic acid	0.10	0.20
Stearic acid	13.4 ± 0.8	14.3 ± 0.8
Oleic acid	50 ± 1.7	39.1 ± 1.4
Linoleic acid	21.7 ± 1	31.1 ± 1.4
Linolenic acid	0.5 ± 0.2	0.3 ± 0.1
Arachidic acid	1.1 ± 0.4	0.6 ± 0.3
Gondoic acid	0.4 ± 0.1	0.10
Behenic acid	0.4 ± 0.1	0.20
Erucic acid	0.10	<0.1
Lignoceric acid	0.20	0.20
Nervonic acid	0.20	<0.1
Saturated fatty acids	26.70	29.1
Unsaturated fatty acids	73.10	70.8

[a] Values are mean ± standard deviation of duplicate determinations.

Table 4
Lipid classes of kernel oil[a]

Glyceride (%)	Species	
	Calophyllum calaba	*Calophyllum inophyllum*
FFA	0.8	5.10
MAG	<0.1	<0.1
DAG	2.2	7.00
TAG	84	76.70
Not identified	13	11.2

[a] *Abbreviations*. FFA, free fatty acid; MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol. Data are means of duplicate results.

It can be estimated that there are altogether 21 identifiable forms of TAG found in the oil samples. Considering the average values it can be indicated that the mains forms of TAGs present in *C. calaba* L. kernel oil were: OOO (14%), and OOL (13.4%), followed by StOO (13.1%), in the case of POO, POL, StOL and OOLn, their contents vary between 10% and 7.5%. It can be observed that percentages of forms of TAGs for *C. inophyllum* L. kernel oil were: OLL (10.8%), and POL (10.7%), followed by OOL (10.4%), between 9% and 7.2%, we had StOL, StOO, POO and OOO. *C. calaba* L. and *C. inophyllum* L. contains a lower amount of monounsaturated forms of TAGs (e.g., PPO, StStO, PStO, etc.) and a higher amount in di and tri-unsaturated forms of TAGs (POO, StOO, OOO, etc.).

Table 5
TAG[a] composition of kernel oil by partition number[b]

TAG (%)	Partition number	Species	
		Calophyllum calaba	*Calophyllum inophyllum*
OLnLn	40	–	3.9 ± 1.0
PLnLn		–	0.2 ± 1.0
LLL	42	1.3 ± 1.0	–
OLLn		0.2 ± 1.0	–
OOLn	44	7.5 ± 1.1	–
PLL		2 ± 1.0	2.04
OLL		–	10.70
OOL	46	13.4 ± 1.2	10.4 ± 1.2
StLL		2 ± 1.0	4.8 ± 1.1
POL		8.4 ± 1.1	10.7 ± 1.2
PPL		1.4 ± 1.0	2.5 ± 1.0
POLn		0.4 ± 1.0	–
OOO	48	14 ± 1.3	7.2 ± 1.1
StOL		7.8 ± 1.1	9 ± 1.2
POO		10 ± 1.2	8.4 ± 1.1
PStL		2 ± 1.0	4 ± 1.1
PPO		2.2 ± 1.0	2.4 ± 1.0
StOO	50	13.1 ± 1.2	8.5 ± 1.1
StStL		1.4 ± 1.0	2.4 ± 1.0
PStO		4.1 ± 1.1	4.7 ± 1.1
PPSt		1.6 ± 1.0	0.7 ± 1.0
StStO	52	3.5 ± 1.0	2.8 ± 1.0

[a] *Abbreviations*. St, stearic acid; P, palmitic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid; Po, palmitoleic acid.
[b] Values are mean ± standard deviation of duplicate determinations.

2.5. Sterol composition

In almost all vegetable fats and oils, sterols are the most quantitatively important unsaponifiable component. They are also characteristic of the genuineness of vegetable oils. The results of the analysis of the sterol fraction of the oils were given in Table 6. Thirteen sterols were identified in the fraction. Cholesterol, was detected in both species in small amounts (0.5–1.2%). The main components for *C. calaba* were stigmasterol (45.1%), β-sitosterol (41.1%), campesterol (9.6%); and the minor components were Δ5-avenasterol (0.8%), Δ7-stigmaterol (0.8%), Δ5,23-stigmatadienol + clerosterol (0,8%), campestanol (0.4%), stigmastanol (0.4%), Δ7-avenasterol (0.3%) and Δ5,24 stigmatadienol (0.2%). We have observed for *C. inophyllum*L. the presence of β-sitosterol (43.1%) as the

Table 6
Sterol composition of the extracted kernel oils[a]

Sterol (%)	Species	
	Calophyllum calaba	*Calophyllum inophyllum*
Cholesterol	0.5	1.2
Brassicasterol	<0.1	<0.1
Campesterol	9.6	14.4
Campestanol	0.4	0.6
Stigmasterol	45.1	35.8
Δ7-Campesterol	<0.1	<0.1
Δ5,23-Stigmatadienol + Clerosterol	0.8	0.8
β-Sitosterol	41.1	43.1
Stigmastanol	0.4	<0.1
Δ5-Avenasterol	0.8	1.3
Δ5,24-Stigmatadienol	0.2	1.7
Δ7-Stigmasterol	0.8	1.1
Δ7-Avenasterol	0.3	<0.1
Not identified	0.1	<0.1
Sterols content (mg/100 g)	148.0	131.0

[a] Data are means of duplicate results.

Table 7
Tocopherol and tocotrienol contents of the extracted kernel oils[a]

Tocopherol/tocotrienol (mg/kg)	Species	
	Calophyllum calaba	*Calophyllum inophyllum*
α-Tocopherol	183.0	15.0
β-Tocopherol	5.0	3.0
γ-Tocopherol	102.0	36.0
Δ-Tocopherol	10.0	16.0
α-Tocotrienol	29.0	2.0
α-Tocotrienol	<2	<2
γ-Tocotrienol	130.0	64.0
Δ-Tocotrienol	103.0	236.0
Tocopherol/tocotrienol content	562.0	370.0
Vitamin (α-tocopherol equivalent/100 g)	20.6	2.1

[a] Data are means of duplicate results.

major component, stigmasterol (35.8%) and campesterol (14.4%). The minor components were Δ5,24 stigmatadienol (1.7%), Δ5-avenasterol (1.3%), Δ7-stigmaterol (1.1%), Δ5,23 stigmatadienol + clerosterol (0.8%) and campestanol (0.6%).

The sterol pattern in species of the same family is sometimes very different and can be used for chemotaxonomical investigations. In the case of the *Calophyllum* genus, sterol composition is different from one species to another. Stigmasterol (45.1%) was the main component in the case of *C. calaba* L., while the main component for *C. inophyllum* L. was β-sitosterol (43.1%).

2.6. Tocopherol and tocotrienol composition

Tocopherol and tocotrienol are natural antioxidant compounds which give stability to oils. The tocopherol and tocotrienol composition of *Calophyllum* kernel oils presents significant variations from one species to another as shown in Table 7.

α-Tocopherol is the main product (183.0 mg/kg) in *C. calaba*, and in *C. inophyllum* the major component is Δ-tocotrienol (236.0 mg/kg). γ-Tocotrienol was the second principle component found at higher concentration in both species (64–130 mg/kg).

3. Conclusion

The results of our investigation provide useful information on the *C. calaba* L. kernel oil composition: FA, TAGs, sterols and tocopherols; its oil can be used for cosmetic preparations.

4. Experimental

4.1. Plant material

Fruits of *C. calaba* L. were harvested during the ripening season 2002–2003 in Capesterre (Guadeloupe). They were stored at ambient temperature before extraction of kernels. The crude oil of *C. inophyllum* L. was purchased in a Madagascar market. Authentic oils and standard components were purchased from Merck, Fluka and Sigma.

4.2. Kernel oil extraction

Fruits after storage at ambient temperature are slightly crushed to safely extract the kernels which are then sundried. Once dried, the oils are extracted from the kernel by cold-pressing system with a single conveying screw and then filtrated.

4.3. Physicochemical parameters

Several physicochemical indices of the extracted oils were determined. The following were evaluated according to the methods listed in the "Association Francaise de Normalisation" (AFNOR, 1984): (i) density (AFNOR NF T 60-214); (ii) refractive index (AFNOR NF T 60-212); (iii) acid value (AFNOR NF T 60-204); (iv) saponification value (AFNOR NF T 60-206); (v) peroxide value (AFNOR NF T 60-220); (vi) iodine value (AFNOR NF T 60-203); (vii) unsaponifiable value (AFNOR NF EN ISO 3596-1); (viii) oxidation stability (98 °C, 20 l/h, 3 g) (AFNOR NF EN ISO 6886); (ix) carotene and lycopene content (AFNOR NF EN 12823-2); (x) phosphorus content (AFNOR NF EN ISO 10540-1).

4.4. FA analysis by gas chromatography

The crude oils were methylated to determine their FA composition. FAs were esterified by boron trifluoride/methanol followed by potash/methanol treatment, and analysed by capillary GC with FID detection, as described by AFNOR method (AFNOR NF EN ISO 5509 and NF EN ISO 5508). Methyl ester solution (1 μl) was injected onto a TRACE 2000 THERMO-ELECTRONICS gas chromatograph equipped with a flame ionisation detector (FID), capillary column was

a 30 m BP20/SGE with 0.22 mm i.d., 0.25 μm film thickness. Column temperatures were programmed from 200 °C for 10 min, with a rise of 5 °C/min to 240 °C for 10 min. The detector temperature was set at 250 °C, and the injector was maintained at 240 °C. The carrier gas was hydrogen at 1.2 ml/min, and analyses were performed in duplicate. FAME were identified by comparison of retention times with authentic standards, and quantification was performed by internal normalisation method.

4.5. Lipid classes by column chromatography

Mono-, di- and triacylglycerol were determined by column chromatography. Crude oil (1 g) was dissolved in 15 ml of chloroform. The extract was submitted to preparative column chromatography on silica gel (Merck 0.10–0.15 mm). This lead to three fractions, triacylglycerol fraction was eluated with benzene; diacylglycerol and free fatty acid fraction with a mixture of diethyl ether/benzene: 10:90; and monoacylglycerol fraction with diethyl ether. The purity of the three fractions have been checked by thin-layer chromatography, using silica gel ascending development with a hexane/diethyl ether 80:20 and visualisation with chromic-sulphuric acid at 180 °C. Each fraction was analysed by gas chromatography with a FISON GC 8000 gas chromatograph equipped with an FID detector. The capillary column was a 15 m DB-1 fused silica capillary column (15 m * 0.32 mm i.d., 0,1 μm film thickness). A programmed oven temperature was used at 80 °C and then raised to 380 °C at a rate of 10 °C/min. The detector temperature was set at 380 °C. The carrier gas was hydrogen, and analyses were performed in duplicate. Samples (1 μl) were injected using an on column injector. Peaks were recorded and identified with authentic standards by comparison of their retention times. Peak areas were computed using internal normalisation.

4.6. Triglycerides analysis by HPLC

The analytical separation of triacylglycerols was performed according to the official method of IUPAC 2.324. Crude oil (0.5 g), exactly weighed, was dissolved in 10 ml of acetone. Aliquots of 10 μl of this solution were injected for HPLC analysis. The apparatus consisted of a VARIAN liquid chromatograph coupled with a refractive index detector and a software interface for the processing of the acquired data. Injection was by means of a rheodyne injection valve with 20 μl fixed loop. The chromatographic separation was performed in a 250 mm in length and of internal diameter 4 mm Superspher100 RP-18 Merck, 4 μm column. The temperature of the column was held at 23 °C. Isocratic elution was carried out at a flow rate of 1 ml/min with a mixture of acetone/acetonitrile (65/35, v/v) as mobile phase. Quantification was performed by internal normalisation method and identification by comparison of retention times with authentic standards.

4.7. Sterols analysis by gas chromatography

Unsaponifiable content obtained by AFNOR Method NF EN ISO 3596-1, was derivitised with the addition of pyridine (0.5 ml), hexamethyldisilazane (0.1 ml) and trimethylchlorosilane (0.04 ml). The upper layer obtained after centrifugation was analysed by gas chromatography with a FISON GC 8000 gas chromatograph equipped with an FID detector (AFNOR NF ISO 6799). The capillary column was a 25 m DB-5 fused silica capillary column (25 m × 0.25 mm i.d., 0.25 μm film thickness). A programmed oven temperature was used at 80 °C and then raised to 260 °C at a rate of 10 °C/min and then held at this temperature for 20 min before finally being raised to 300 °C at 5 °C/min and kept at this temperature for an additional 5 min. The detector temperature was set at 320 °C. The carrier gas was hydrogen, and analyses were performed in duplicate. Samples (1 μl) were injected using an on column injector. Peaks were recorded and peak areas computed using an integrator. Sterols were identified by comparing their retention times with those of standards under the same operating conditions and quantified by internal normalisation method and internal standard.

4.8. Tocopherols and tocotrienols analysis by HPLC

Tocopherols and tocotrienols in the kernel oils were extracted and the content determined with the aid of High Performance Liquid Chromatography analysis, described in (AFNOR NF ISO 9936). The apparatus consisted in a TSP AS 3000 liquid chromatograph coupled with a FP 1520 JASCO fluorescence detector set at an excitation wavelength at 290 nm and an emission wavelength of 330 nm and integrator. 10 mg of the crude oil was dissolved in 10 ml hexane. Aliquot of this solution were injected for HPLC analysis, onto a column of silice (25 cm, 4 mm).Injection was by means of a rheodyne injection valve with 20 μl fixed loop. The mobile phase was hexane/isopropanol (99.4/0.6 v/v) at a flow rate of 1.1 ml/min. The contents of tocopherols/tocotrienols in the sample were obtained from external standard, by comparison of retention times with authentic standards.

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