J Am Oil Chem Soc (2011) 88:1297–1303
DOI 10.1007/s11746-011-1796-x

ORIGINAL PAPER

Fatty Acids, Tocopherols and Sterols of *Cephalocroton cordofanus* in Comparison with Sesame, Cotton, and Groundnut Oils

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Received: 10 August 2010 / Revised: 18 October 2010 / Accepted: 4 November 2010 / Published online: 23 March 2011

Abstract *Cephalocroton cordofanus*, a perennial much-branched shrub, is dominant in the eastern and western states of Sudan. The seeds of *C. cordofanus* sesame, groundnut, and cotton were compared for their oil and protein content as well as for fatty acids, tocopherols, and sterols. Fatty acids and sterols were analyzed by GC while tocopherols were analyzed by HPLC. The oil of *C. cordofanus* showed low levels of saturated fatty acids in comparison with the other three oils. The other reported fatty acids of *C. cordofanus* were 8.60 % oleic, 17.2% linoleic, 64.2% vernolic, and 2.0% coronaric acids. Neutral lipids, glycolipids, and phospholipids of *C. cordofanus* oil accounted for 77.5, 14.4, and 8.1% of the total lipid fraction, respectively. The oil of *C. cordofanus* showed higher levels of tocopherols (113.53 mg/100 g) in comparison to sesame, groundnut, and cottonseed oils, with 64.74, 27.96, and 77.83 mg/100 g, respectively. The primary tocopherol of *C. cordofanus* was γ-tocopherol (106.21 mg/100 g), which amounted to 93.8% of the total tocopherols. β- and δ-tocopherol were present at levels below 5.0 mg/100 g. In comparison to sesame, groundnut, and cottonseed oils, *C. cordofanus* oil contains more (304.4 mg/100 g) total sterols than ground nut (294.0 mg/100 g), but less than sesame (774.9 mg/100 g) and cotton seed (492.4) oils. Due to its high level of epoxy fatty acids, *C. cordofanus* oil is used for industrial rather than edible applications.

Keywords *Cephalocroton cordofanus* · Cottonseed · Epoxy acids · Groundnut · Phytosterols · Sesame · Tocopherols

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Introduction

Although the oil and fat business is based almost entirely on a limited number of commodity oils differing in fatty acid composition, there are many other plant oils with fatty acid composition not too dissimilar from the commodity oils. These could be used as food lipids but unless they were sufficiently differentiated, it would be difficult for them to compete with the commodity oils. There are also plants that produce uncommon fatty acids such as epoxy acids, acids with conjugated unsaturation, or oils with very high levels (>80%) of a single fatty acid [1].

Identification of new oil sources from a large number of commonly grown vegetables, fruits, and wild plants in Sudan has been of recent research interest [2–10]. One of these wild plants is *Cephalocroton cordofanus,* a perennial much-branched shrub 0.3–3 m high with simple alternating leaves. The fruit is a hairy, deeply 3-lobed, 3-seeded capsule 12 mm in diameter. Seeds are ovoid to nearly globose, 7.5 × 6 mm, smooth, evenly grayish or

dark brown flecked and mottled, and somewhat shiny. The seeds, locally called dingili (eastern Sudan) and umgutni (western Sudan), are eaten and the highly unsaturated oil, is occasionally extracted and used in cooking. The plant occurs naturally in northern Nigeria, eastern Sudan to Ethiopia and Eritrea, and south to north-eastern Tanzania [11].

The seeds contain 42% oil, the kernel about 56%. The oil has a pleasant odor and taste. It consists chiefly of *cis*-12:13-epoxyoleic acid (62%) along with saturated acids (7%), oleic acid (10%), linoleic acid (17%), and 12:13-dihydroxyoleic acid (4%) [12].

The fatty acid (FA) composition determines the physical properties, the stability, and the nutritional value of the oil. All fatty acids in glycerides of natural origin consist of saturated, monoenoic, and polyunsaturated fatty acids (PUFA) in various proportions and differ in detailed fatty acid composition. The fatty acid distribution of triacylglycerols as well as of phospholipids affect the physical properties, lipolytic and oxidative stability, and nutritional availability of the oil. In plants, monoenoic fatty acids and PUFA are dominant at the *sn*-2 position [13].

The tocochromanols comprise a group of chemically related tocopherols and tocotrienols, which are similar in molecular structure and occur in plants, plant oils, nuts, grains, fruits, and vegetables. All compounds including α, β, λ, and δ homologues, exhibit antioxidative and vitamin E activity. Acting as chain-breaking antioxidants, tocopherols react with lipid radicals to convert them into more stable products [14].

Phytosterols, or plant sterols, are minor components of vegetable origin. They function as structural components in membrane lipids and as precursors to steroid hormones [15]. There is increasing interest in isolating these biologically active components for nutraceutical applications and as ingredients for functional foods [16] (Holser et al. 2004). Phytosterols exhibit some beneficial properties such as anti-inflammatory and antitumor activity. The principal sterols in edible oils (olive, peanut, sesame, and hazelnut) are β-sitosterol with campesterol, stigmasterol, and $\Delta(5)$-avenasterol as minor sterol compounds [17].

Many authors have reported on sterols determination in different conventional edible oils [15–17], while Mariod et al. [3] identified phytosterols in three unconventional Sudanese oils.

This study aims to investigate the compositional properties including fatty acids, tocopherols, and sterols of *C. cordofanus* in comparison with groundnut, sesame, and cottonseed oils, and to shed light on the potential of *C. cordofanus* as an epoxy fatty acid source for industrial uses rather than edible applications.

Materials and Methods

Materials

All solvents used were of analytical grade: *n*-hexane, *n*-heptane, diethyl ether, ethanol, and methanol were acquired from Merck, Darmstadt, Germany.

Crude oils of groundnut, sesame, and refined cotton oil were collected randomly from Khartoum north central market, Sudan, while seeds of *C. cordofanus* were collected from Gadarif, eastern Sudan.

Methods

Oil Extraction

The stored oil seeds were crushed and ground with a grinding mill (Petra electric, Burga, Germany). The oil was extracted from the ground material with *n*-hexane at 50–60 °C in a Soxhlet apparatus for 6 h following the AOCS method [18]. The oil content was determined as a percentage of the extracted oil to the sample weight (w/w). The samples were analyzed in triplicate, and the mean and standard deviation was calculated. The oil obtained was stored at 4 °C for further investigation.

Crude Protein

Crude protein analyses of the different samples was determined and nitrogen content was determined by the semi-micro-Kjeldahl digestion, distillation, and titration method, as described by the official methods of Association of Official Analytical Chemists (AOAC) [19].

Fatty Acid Composition

The fatty acid compositions of *C. cordofanus*, groundnut, sesame, and cottonseed oils were determined following the International Organization of Standards (ISO) draft standard [20]. In brief, one drop of the oil was dissolved in 1 mL of *n*-heptane, 50 μL 2 M sodium methanolate in methanol was added, and the closed tube was agitated vigorously for 1 min. After addition of 100 μL of water, the tube was centrifuged at 4,500×*g* for 10 min. and the lower aqueous phase was removed. Fifty (50) μL 1 M HCl was added to the heptane phase, the two phases were briefly mixed and the lower aqueous phase was discarded. About 20 mg of sodium hydrogen sulphate (monohydrate, extra pure, Merck, Darmstadt, Germany) was added, and after centrifugation at 4,500×*g* for 10 min, the top *n*-heptane phase was transferred into a vial and injected into a Varian 5890 gas chromatograph with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 μm). The

temperature program was: from 155 °C heated to 220 °C (1.5 °C/min.), 10 min isotherm; injector 250 °C, detector 250 °C; carrier gas 1.07 mL/min hydrogen; split ratio 1:50; detector gas 30 mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen; manual injection volume less than 1 μL. The integration software computed the peak areas and percentages of fatty acid methyl esters (FAME) were obtained as weight percent by direct internal normalization.

Lipid Fractionation

Separation of the lipids into the individual classes, namely neutral lipids (NL), glycolipids (GL), and phospholipids (PL) was achieved by silica gel column chromatography. Crude lipids obtained from 1 g of *C. cordofanus*, groundnut, sesame, and cottonseed oils were fractionated on a column (2 × 18 cm) of activated silica gel (15 g silica gel 60, 60–120 mesh; Merck). The individual classes were eluted with chloroform (200 mL), acetone (400 mL), and methanol (200 mL) [21]. For elution of glycolipids, mixtures of varying proportions of chloroform: acetone to pure acetone were used. The elutes were monitored for various types of lipids on TLC silica gel plates (Merck).

Tocopherol Determination

Solutions of 250 mg of *C. cordofanus*, ground nut, sesame, and cottonseed oils in 25 mL *n*-heptane were prepared for HPLC analysis of tocopherols. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump, a Merck-Hitachi F-1000 Fluorescence Spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm), and a D-2500 integration system. Twenty microliters of each sample were injected by a Merck 655-A40 Autosampler onto a Diol phase HPLC column 25 cm × 4.6 mm ID (Merck, Darmstadt, Germany) using a flow rate of 1.3 mL/min. The mobile phase was *n*-heptane/tert, butyl methyl ether (99 + 1, v/v) [22].

Sterol Determination

The sterol compositions of *C. cordofanus*, groundnut, sesame, and cottonseed oils were determined by silylation with *N*-methyl-*N*-trimethyl-silyl-heptafluorobutyramide, and the assignments were made by using the retention times (RTs) of the individual sterols and calculation of the relative RTs in relation to betulin as an internal standard following the ISO/FIDS [23] draft standard. In brief, 250 mg of oil was saponified with a solution of ethanolic potassium hydroxide by boiling under reflux. The unsaponifiable matter was isolated by solid-phase extraction on an aluminium oxide column (Merck) on which fatty acid anions were retained and sterols passed through. The sterol fraction from the unsaponifiable matter was separated by thin-layer chromatography (TLC) on 20 × 20 cm silica gel plates of 0.25 μm layer thickness using hexane/diethyl ether (1/1 [v/v]) as the developing solvent (Merck). After re-extraction from the TLC material, the compositions of the sterol fractions were determined by gas liquid chromatography using betulin as an internal standard (gas chromatography mass spectrometry is used only in unclear cases). The compounds were separated on an SE 54 CB (Macherey–Nagel, Düren, Germany) (50-m long, 0.32-mm i.d., 0.25-mm film thickness). Further parameters were hydrogen as carrier gas; split ratio, 1:20; injection and detection temperature adjusted to 32 °C; temperature program, 245–260 °C at 5 °C/min.

Statistical Analysis

The analyses were performed with three replicates. The mean values and standard deviation (means ± SD) were calculated and tested using the Student *t* test ($P < 0.05$). Statistical analysis of variance (ANOVA) was performed on all values using the statistical program Statgrafics® Statistical Graphics System version 4.0 (Statgraphics® 1985–1989) [24].

Results and Discussion

Oil and Protein Content

The four samples, *C. cordofanus*, sesame, groundnut, and cottonseed, showed significant differences ($P < 0.05$) in oil and protein content (Table 1). Lipid levels were 43.0, 50.0, 42.5, and 16.8.0 g/100 g, for *C. cordofanus*, sesame,

Table 1 Oil, protein content, and lipid classes of *Cephalocroton cordofanus*, ground nut, sesame, and cotton seeds

Seed	Oil content (%)	Protein content (%)	NL (%)	GL (%)	PL (%)
CC	43.0 ± 0.2	29.4 ± 0.6	77.5 ± 0.2	14.4 ± 0.2	8.1 ± 0.1
SE	50.0 ± 05	25.1 ± 0.3	77.9 ± 0.3	14.3 ± 0.2	7.8 ± 0.1
GN	42.5 ± 04	26.7 ± 0.5	79.8 ± 0.3	13.1 ± 0.2	7.1 ± 0.1
CS	16.8 ± 1.0	24.3 ± 0.4	78.7 ± 0.3	13.6 ± 0.2	7.7 ± 0.1

All determinations were carried out in triplicate and mean values ± standard deviations (SD) are reported

CC Cephalocroton cordofanus, *SE* sesame, *GN* groundnut, *CS* cottonseed, *NL* neutral lipid, *GL* glycolipids, *PL* phospholipids

groundnut, and cotton seed, respectively. The oil content of *C. cordofanus* seems to be very high relative to cottonseed and groundnut but less than sesame seed. This result for *C. cordofanus* is in good agreement with that of Oyen, [11] who reported 42.0% oil. Protein analyses showed that *C. cordofanus* seed has a higher level of protein (29.4 g/100 g) than sesame, groundnut, and cottonseed (25.1, 26.7, and 24.3 g/100 g), respectively. Many authors reported the same oil and protein results for sesame [25], cottonseed, and groundnut [8].

Fatty Acid Composition

The fatty acid compositions of *C. cordofanus* (CCO), groundnut (GNO), sesame (SEO), and cottonseed (CSO) oils are given in Table 2. *C. cordofanus* was lower in saturated fatty acids (14:0, 16:0, 17:0, 18:0, and 20:0) relative to groundnut, sesame, and cottonseed oils. Other fatty acids and their levels reported in *C. cordofanus* were 8.60 % oleic acid, 17.2% linoleic acid, 64.2%, and the epoxy fatty acids vernolic acid and 2.0% coronaric acid. These results were to some extend different from that of Bharucha, and Gunstone [12] who earlier reported *cis*-12:13-epoxyoleic acid (62%) along with linoleic (17%), oleic (10%), *threo*-12:13-dihydroxyoleic acid (4%), and saturated acids (7%). The first natural epoxy acid discovered, vernolic (*cis-12,13-epoxyoleic*) acid, was discovered by Gunstone [26] in 1954 from *Vernonia anthelmintica* seed oil. The second was coronaric acid (*cis-9,10-epoxy-cis-12-octadecenoic),* an isomer of vernolic acid, discovered by Smith et al. [27] from *Chrysanthemum coronarium* seed oil. The remarkable differences in the fatty acid composition of *C. cordofanus* relative to groundnut, sesame, and cottonseed oils usually used in Sudan (Table 2) was its high level of vernolic and coronaric acids, which are unusual for commonly used edible oils. This high content is comparable to that of *Vernonia galamensis* (or ironweed), a plant native to eastern Africa. The seeds of this plant contain about 40–42% oil of which 73–80% is vernolic acid [28]. The occurrence of vernolic acid in the oil of *Hibiscus esculentus* (okra) and *Hibiscus cannabinus* (kenaf) was also reported [27]. Many products can be made from epoxy oils include epoxies for manufacturing adhesives, varnishes, paints, and industrial coatings. Their low viscosity suggests their use as nonvolatile solvents in oil-based and low VOC paints since they will become incorporated into the dry paint rather than evaporating into the air [28]. From Table 2, the fatty acid profile of *C. cordofanus* generally indicates 17.8% polyunsaturated fatty acids, 7.0% saturated fatty acids, and 93.0% total unsaturated fatty acids which were significantly ($P < 0.05$) different from that of groundnut, sesame, and cottonseed oils. Also *C. cordofanus* showed a higher ratio of unsaturated/

Table 2 Fatty acid composition (% of total) and summary of the important fatty acid parameters (g/100 g) of *Cephalocroton cordofanus*, ground nut, sesame, and cotton seed oils

Oil	14:0	16:0	16:1	17:0	18:0	18:1**Δ9**	18:1**Δ11**	18:1[a]	18:1[b]	18:2	18:3	20:0	20:1	22:0	PUFA	TSFA	TUSFA	Ratio unsaturated/saturated
CCO	0.1	3.90	n.d	n.d	3.20	8.60	0.70	64.00	2.00	17.2	0.6	n.d	0.1	n.d	17.8	7.00	93.0	13.28
	±0.1	±0.1			±0.2	±0.3	±0.1	±0.5	±0.1	±0.2	±0.1		±0.1		±0.3	±0.2		
SES	0.02	9.76	0.17	0.10	6.17	39.83	0.97	n.d	n.d	41.43	0.34	0.68	0.17	0.19	41.7	17.04	82.93	4.87
	±0.1	±0.3	±0.1	±0.1	±0.3	±0.4	±0.1	n.d	n.d	0.3	±0.2	±0.1	±0.1	±0.1	±0.4	±0.2		
GNO	0.03	10.95	0.10	0.10	3.55	45.83	0.64	n.d	n.d	29.85	0.10	1.61	1.20	3.43	29.8	21.88	78.09	3.56
	±0.1	±0.3	±0.1	±0.1	±0.3	±0.4	±0.2	n.d	n.d	±0.3	±0.1	±0.2	±0.2	±0.2	±0.3	±0.3		
CSO	0.92	23.63	0.61	0.20	2.61	17.46	0.92	n.d	n.d	48.82	0.16	0.31	1.34	0.22	48.9	27.97	69.42	2.48
	±0.2	±0.3	±0.2	±0.1	±0.2	±0.3	0.2	n.d	n.d	±0.4	±0.1	±0.1	±0.2	±0.1	±0.4	±0.3		

Data are means of triplicate results

n.d not identified, *CCO Cephalocroton cordofanus* oil, *SEO* sesame oil, *GNO* groundnut oil, *CSO* cottonseed oil are codes based on oil names, *PUFA* polyunsaturated fatty acids, *TSFA* total saturated fatty acids, *TUSFA* total unsaturated fatty acids

[a] cis-12:13-epoxyoleic acid

[b] 12:13-dihydroxyoleic acid

Table 3 Tocopherol composition (mg/100 g) of *Cephalocroton cordofanus*, ground nut, sesame, and cotton seed oils

Oil	α-T	β-T	γ-T	P8	δ-T	Total
CCO	3.03 ± 0.1	0.00 ± 0.0	106.21 ± 0.5	0.00 ± 0.0	4.30 ± 0.2	113.53
SEO	0.60 ± 0.1	0.00 ± 0.0	63.32 ± 0.4	0.23 ± 0.1	0.59 ± 0.1	64.74
GNO	12.66 ± 0.2	0.56 ± 0.1	12.99 ± 0.2	1.02 ± 0.1	0.72 ± 0.2	27.96
CSO	28.62 ± 0.2	0.35 ± 0.1	45.88 ± 0.3	2.61 ± 0.1	0.33 ± 0.1	77.83

All determinations were carried out in triplicate and mean values ± standard deviations (SD) are reported

T tocopherol, *P8* plastochromanol, CCO *Cephalocroton cordofanus* oil, *SEO* sesame oil, *GNO* groundnut oil, *CSO* cottonseed oil are codes based on oil names

Table 4 Phytosterol composition (mg/100 g oil) of *Cephalocroton cordofanus*, ground nut, sesame, and cotton seed oils

Sterols	CCO	SEO	GNT	CSO
Cholesterol	1.4 ± 0.1	0.0 ± 0.0	1.6 ± 0.1	3.2 ± 0.1
Campesterol	27.7 ± 0.2	13.0 ± 0.2	42.9 ± 0.3	43.9 ± 0.3
Stigmasterol	72.8 ± 0.3	48.1 ± 0.3	24.3 ± 0.3	5.3 ± 0.1
β-sitosterol	160.4 ± 0.3	467.7 ± 0.5	183.9 ± 0.4	403.3 ± 0.5
Δ5-avenasterol	20.4 ± 0.2	70.6 ± 0.3	28.7 ± 0.2	15.5 ± 0.3
Δ7-avenasterol	5.7 ± 0.1	8.4 ± 0.2	2.9 ± 0.1	2.1 ± 0.1
Δ7-stigmasterol	5.9 ± 0.1	6.4 ± 0.1	0.7 ± 0.1	3.4 ± 0.1
[a]Others	10.1 ± 0.2	170.7 ± 0.4	9.0 ± 0.1	15.7 ± 0.3
Total	304.4	774.9	294.0	492.4

All determinations were carried out in triplicate and mean values ± standard deviations (SD) are reported

CCO Cephalocroton cordofanus oil, *SEO* sesame oil, *GNO* groundnut oil, *CSO* cottonseed oil are codes based on oil names

[a] Others include 24-methylcholesterol, campestanol, clerosterol, sitostanol, 5,24-stigmastadienol

saturated fatty acids than the edible oils conventionally used in Sudan.

Lipid Fractionation

Silica gel column chromatography was used to fractionate lipids into their individual classes based on polarity. Neutral lipids, glycolipids, and phospholipids of *C. cordofanus* accounted for 77.5, 14.4, and 8.1% of the total lipid fraction (Table 1), which are lower than GNO, SEO, and CSO lipid fractions. The lipid fractions of CCO were significantly different ($P < 0.05$) than that of GNO, SEO, and CSO. The neutral lipid fraction was found to contain non-polar compounds such as mono and diacylglyceols, and hydrocarbons. The neutral lipid fractions also contained the chlorophyll and carotenoid pigments, which were later separated by preparative TLC.

Tocopherols in *C. cordofanus*, Groundnut, Sesame, and Cottonseed Oils

The tocopherol contents of *C. cordofanus,* groundnut, sesame, and cottonseed oils are given in Table 3. α- and γ-Tocopherols are the main tocopherols found in vegetable oils and fats [29]. The main function of tocopherols is as a radical-chain breaking antioxidant in membranes and lipoproteins, as well as in foods [30]. From Table 3 the oil of CCO showed higher amount of tocopherols (113.53 mg/100 g) in comparison to sesame, groundnut, and cottonseed oils, in which levels of 64.74, 27.96, and 77.83 mg/100 g respectively, could be found. The main tocopherol of CCO was γ-tocopherol (106.21), which amounted to about 93.8% of the total tocopherols, wherein β- and δ-tocopherol were present in minor amounts or traces below 5.0 mg/100 g.

Phytosterols in *C. cordofanus*, Groundnut, Sesame, and Cottonseed Oils

Phytosterols concentrations for *C. cordofanus,* groundnut, sesame, and cottonseed oils are listed in Table 4. Vegetable oils are generally considered to be important sources of phytosterols since they generally contain relatively higher levels than vegetables and fruits [31]. The main sterol found in the four oils was β-sitosterol. In comparison to other edible oils usually used in human nutrition in Sudan, *C. cordofanus* oil showed lower amounts of total sterols than SEO and CSO. Sesame oil had the highest levels of total sterol (774.9 mg/100 g oil) and groundnut oil the

lowest (294.0 mg/100 g oil). The mean values of total sterols were 492.4, and 304.4 mg/100 g oil for cottonseed oil and *C. cordofanus* oil samples, respectively.

In the four oils studied, a remarkable high amount of Δ5-avenasterol, known to act as an antioxidant and as an antipolymerization agent in frying oils, was found [32, 33]. These authors pointed out that those sterols with an ethylidene group in the side chain are most effective as antioxidants and suggested that a synergistic effect of the sterols with other antioxidants may occur.

Conclusions

This study suggests that *C. cordofanus* oil, with its high level of epoxy fatty acid, can be used for industrial applications such as the manufacture of adhesives, varnishes, paints, and coatings rather than as an edible oil. The oil content of *C. cordofanus* is very high in comparison with cottonseed and groundnut but less than sesame and from an economical point of view, the production of oil from this source could be interesting. The oil of *C. cordofanus* proved to be a good source of sterols and tocopherols, containing 4.0 times greater concentration of total tocopherol than groundnut oil, the most commonly used oil in Sudan.

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