A Study on Cashew Nut Oil Composition

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Eight samples of cashew nut oil were assayed, and the component triacylglycerols, fatty acids and several unsaponifiable compounds were analyzed by gas chromatography (GC) and high-performance liquid chromatography (HPLC). Total lipid amount, unsaponifiable percentage, fatty acids, sterols, triterpene alcohols and tocopherols are reported here. The combination of GC and HPLC enhanced the resolution of compound classes.

KEY WORDS: Cashew nut oil, fatty acids, gas chromatography, liquid chromatography, oil, oil minor components, sterols, tocopherols, triacylglycerols, unsaponifiable.

The cashew (*Anacardium occidentale* L.), an evergreen arboreal species native to tropical America, can attain a 10–12-m height. The species belongs to the class of the dicotyledons, order terebintals, family Anacardiaceae (1). The cashew's botany, cultivation, pest and disease resistance, products and their uses were surveyed by Agnoloni and Giuliani (2).

The growing interest in cashew can be ascribed to the purported dual role of the kernel: It can be used as a substitute for peanut and almond in the confectionery industry and as an important source of lipids and proteins. Kernel composition (protein and oil) has been investigated and determined in numerous studies (3–7). However, it may be necessary to reappraise kernel composition, because most of these studies are dated. Nagaraja (8), for example, reports surprising differences in proportions of triacylglycerol and sterol fractions, noting that the triacylglycerol fraction in oil varied from 3.7 to 71% and the sterol fraction from 84 to 23%.

In the present study, the lipid fractions of eight cashew samples were extracted from the kernel to determine total lipids, triacylglycerols (never before analyzed), fatty acids and unsaponifiable matter. The latter was used for analyses of sterols, methylsterols, triterpene alcohols and tocopherols.

EXPERIMENTAL PROCEDURES

Benzene, hexamethyldisilazane, trimethylchlorosilane, potassium hydroxide and *n*-hexane were supplied by Carlo Erba (Rodano, Milan, Italy). Ethyl ether, chloroform, anhydrous sodium sulfate, bidistilled water, acetone, methanol and ethyl acetate were supplied by Baker Analyzer (Deventer, Holland). Anhydrous pyridine and 2,7-dichlorofluorescein were supplied by Merck (Darmstadt, Germany). The trilinolein, trilinolenin, triolein, tripalmitin, dipalmitoylolein, dioleoylpalmitine and distearoylolein, triglyceride standards were supplied by Sigma Chemical Co. (St. Louis, MO). The tocopherol standards were purchased from Merck. The high-performance liquid chromatography (HPLC) solvents were filtered through a 0.45 μm Alltech filter (Alltech Europe, Eke, Belgium) and degassed *via* an ERC-3312 Degasser (Erma Inc., Tokyo, Japan). The silica thin-layer chromatography (TLC) plates were Carlo Erba Stratocrom (Milan, Italy), 20 × 20 cm, with a 0.25 mm thickness.

Gas chromatography (GC) was performed with a Carlo Erba 4160, equipped with a flame-ionization detector and interfaced with a Spectra Physics SP 4270 integrator-calculator (San Jose, CA). The columns were a fused-silica capillary (25 m × 0.25 mm i.d., 0.1 μm film thickness) with a 50% phenyl-/50% methyl-polysiloxane stationary phase (TAP, Chrompack, Middelburg, The Netherlands); a fused-silica capillary coated with SE52 (Mega, Milan, Italy; 25 m × 0.32 mm i.d., 0.1–0.15 μm film thickness); and a fused-silica Supelco SP 2340 (Bellefonte, PA; 30 m × 0.32 mm i.d., 0.2 μm film thickness).

The HPLC analyzer included a Rheodyne 7125 injector (Cotati, CA), a Waters Associates M-6000A pump (Milford, MA), a Knauer UV spectrophotometer (Berlin, Germany) and an ACS 750/14 mass detector (Applied Chromatographic System, Macclesfield, United Kingdom) interfaced with a Spectra Physics SP 4270 integrator-calculator. The column was a S5 Spherisorb ODS2 Phase Sep (Deeside, United Kingdom; 15 cm × 4.6 mm, 5-μm spherical particles).

The eight cashew samples were supplied by Oltremare S.p.a. (Bologna, Italy). Three originated from Indonesia (samples 1, 2 and 3), two from India (samples 4 and 5), two from Brazil (samples 6 and 7) and one from Thailand (sample 8). These samples were ground, the resulting meals were extracted with *n*-hexane for 6 h by a Soxhlet apparatus, and the total lipids were collected and weighed after filtering and solvent removal. Fatty acid and glyceride compositions, tocopherols and unsaponifiable matter were determined for all eight samples.

Fatty acids. A 0.5 g sample of total lipids was dissolved in 5 mL *n*-hexane, 0.25 mL of metanolic KOH 2N (9) was added, and the solution was stirred for 20 s, then centrifuged at 4000 rpm, passed through a dehydrating column (Na_2SO_4) and analyzed by GC. GC conditions involved the SP 2340 capillary column, GC oven temperature was programmed from 180 to 200°C at 4°C per min, and injector and detector temperatures were 200°C.

Triacylglycerols. Solutions of 2% in *n*-hexane were analyzed by GC (TAP column) by the method of Frega *et al.* (10).

Tocopherols. Solutions (10%) of total lipids in ethyl acetate were analyzed by HPLC by the method of Tonolo *et al.* (11).

Unsaponifiable matter. Total lipids were saponified according to the Norme Grassi e Derivati (NGD) C-12 method (12), and the unsaponifiable fraction was treated with diazomethane (CH_2N_2) (13) to convert acid groups to methyl esters. The material was then analyzed by preparative TLC on silica gel plates (Stratocrom, Carlo Erba) with an elution mixture of *n*-hexane/ethyl ether (60:40, vol/vol). Plates were sprayed with 0.2% ethanolic solution of 2,7-dichlorofluorescein (sodium salt). After separation and subsequent extraction of the bands, sterols, 4-methyl sterols and 4,4′-dimethyl sterols

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(triterpene alcohols) were silylated (14) and then analyzed separately by GC with the TAP column (200–320°C temperature programmed at a rate of 5°C/min) and the SE52 column (isothermally at 265°C, with 290°C injector and detector temperatures). Peak identification was carried out by comparing relative retention times with those reported in the literature (15–21) and with retention times of standards (supplied by Sigma Chemical Co., and Extrasynthase, Genay, France). Cholesterol identification was confirmed by means of combined GC/mass spectrometry (MS). The analysis was run on an MFC 500 GC with a WCOT fused silica 25 m × 0.24 mm coating CP-SIL 8CB (Chrompack, Middelburg, The Netherlands) and a QMD 1000 MS (Carlo Erba). The temperature for on-column injection analysis of cholesterol (as trimethylsilyl ether) was programmed from 60 to 260°C at 50°C/min and then to 310°C at 1°C/min, where it was held for 10 min.

RESULTS AND DISCUSSION

Total triacylglycerol and unsaponifiable contents for all eight oil samples are given in Table 1, and agree with previously reported findings (4). Our results contrast with the anomalous lipidic compositions reported by Nagaraja (8). Total lipids ranged from 43 to 50% on the whole seed (avg. 46.5%), which showed that the cashew kernel has a high fat content. Unsaponifiable matter, which also fell within the normal unsaponifiable range for edible oils, varied from 0.9 to 1.9% (avg. 1.4%).

GC analysis of total lipids was performed to determine triacylglycerol composition, which has not been reported before for cashew-nut oil. Established statistical methodology (10) showed that eight repetitions of the same sample had standard deviations of 0.59 for triacylglycerols with a percentage of 41.7, and 0.19 for triacylglycerols with a percentage of 9.2.

Figure 1 shows the trace recorded for one sample. Data for all eight samples are reported in Table 2. Note that dioleoylstearin, triolein and dioleoyllinolenin account for up to 74.1% of the triacylglycerols that predominantly contain oleic acid, which has been re-evaluated for its energy properties and ready assimilation (22).

Table 3 shows the fatty acid composition of the eight samples of cashew nut oil. The average content of unsaturated fatty acids is 78.9% of total fatty acids which approaches the 85% composition for an ideal fat (23). This is of particular interest, considering that 99.6% of the unsaturated fatty acids can be attributed to oleic and linoleic acids, both of which are important from a nutritional point of view. The former is of interest because it is one of the most readily metabolized fatty acids (24), and the latter because it is a precursor of prostaglandin and, as such, an essential fatty acid (25). The stability to oxidative alterations, deriving from the higher levels of oleic and lower ones of linoleic acid, makes cashew oil more suitable than peanut oil (26) for cooking and frying (27).

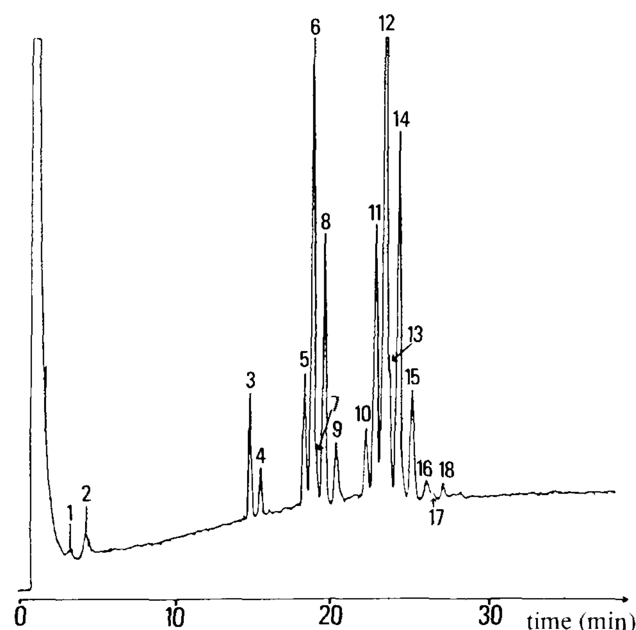

FIG. 1. Gas chromatographic separation of triacylglycerols. Peak identification: 1, Diacylglycerols (D34); 2, Diacylglycerols (D36); 3, POP; 4, PLP + POPo; 5, POS; 6, POO; 7, Not identified; 8, POL; 9, PLL; 10, SOS; 11, SOO; 12, OOO; 13, SOL; 14, OOL; 15, OLL; 16 and 17, Not identified; 18, LLL. Abbreviations: P, palmitic acid; Po, palmitoleic acid; S, stearic acid; O, oleic acid; L, linoleic acid.

TABLE 1

Amounts of Total Lipid and Unsaponifiable Fraction

Sample	Total lipids (g/100 g of seeds)	Unsaponifiable (g/100 g of oil)
1	45	1.3
2	46	1.9
3	50	1.3
4	45	1.8
5	46	0.9
6	43	1.6
7	50	1.2
8	47	1.2
Mean	46.5	1.40

TABLE 2

Triacylglycerol Composition (%)[a] of Cashew Kernel Lipids Determined by GC (TAP column)

	Sample							
TG[b]	1	2	3	4	5	6	7	8
POP	4.4	4.6	4.2	3.2	3.3	2.4	2.2	3.0
PLP + POPo	1.9	2.2	1.9	1.3	1.5	1.0	1.0	1.2
POS	5.5	5.9	5.9	4.7	3.5	3.2	3.1	4.5
POO	18.3	18.6	17.5	16.8	16.5	16.3	15.2	16.8
POL	10.1	10.6	10.0	8.6	9.2	8.3	7.9	8.7
PLL	2.3	2.6	2.5	1.7	2.3	2.1	2.0	2.3
SOS	2.6	2.5	3.2	2.7	2.2	2.0	2.2	2.2
OOS	11.3	11.5	11.8	12.1	11.3	10.6	10.9	10.7
OOO	19.5	19.1	19.0	24.8	26.4	28.5	29.4	24.9
SOL	4.8	4.9	4.8	4.1	3.9	3.5	3.8	4.1
OOL	12.4	11.8	12.3	13.5	15.1	16.0	16.7	14.5
OLL	3.7	3.6	3.7	3.7	4.6	5.0	5.1	4.6
LLL	0.5	0.5	0.5	0.5	trace	0.4	trace	0.5
Other	2.7	1.6	2.7	2.3	0.2	0.7	0.5	2.0

[a]Calculated on the basis of gas chromatographic (GC) areas.
[b]TG, triacylglycerols; P, palmitic acid; Po, palmitoleic acid; S, stearic acid; O, oleic acid; L, linoleic acid.

TABLE 3

Fatty Acid Composition (%) (methyl esters)[a] of Cashew Kernel Lipids Determined by GC (SP 2340 column)

	Fatty acids							
Sample	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C20:0	C18:3
1	14.1	0.4	0.1	10.2	57.3	17.2	0.5	0.2
2	14.2	0.3	trace	11.2	58.0	15.6	0.7	trace
3	13.4	0.3	0.2	11.6	57.6	16.1	0.8	trace
4	11.3	0.4	0.1	8.7	62.6	16.3	0.6	trace
5	10.4	0.3	0.2	8.7	62.9	17.1	0.4	trace
6	9.0	0.3	0.1	6.8	65.1	18.3	0.4	trace
7	9.2	0.3	0.1	6.3	65.1	18.6	0.4	trace
8	11.1	0.3	trace	7.6	63.2	17.5	0.3	trace

[a]Calculated on the basis of gas chromatographic (GC) areas.

Special emphasis was also placed on determining the unsaponifiable components, which often prove to be a true "fingerprint" of the oil's botanical origin (28). Sterol, 4,4′-dimethyl sterol and 4-methyl sterol compositions were checked with a TAP polar column and compared to those assayed by a SE52 nonpolar column (data not reported), which is traditionally employed in the analysis of the total unsaponifiable fraction.

Table 4 shows the composition of sterol fractions from the eight cashew nut oils analyzed by GC with a polar (TAP, Chrompack) column. The results obtained for the two columns were fairly similar, but only the polar column yields complete separation between β-sitosterol and fucosterol. Note that β-sitosterol predominates (Table 4), ranging from 76.2 to 82.7% (avg. 80%). Lower amounts of campesterol, 24-methylencholesterol (tentative), cholesterol, stigmasterol, fucosterol and Δ^5-avenasterol were identified, and trace amounts of as yet unidentified sterols were also detected. The identification of cholesterol, which has been detected in low amounts in cashew germ oil (18), as well as in other plant oils (16,17,19), was confirmed by GC/MS analysis. Unidentified compounds averaged 3.7% of the total sterol fraction, the maximum being 7.4% in sample 2 (Indonesia) and the minimum 2.1% in sample 3 (Indonesia). Triterpene alcohol (4,4′-dimethyl sterol) analysis confirmed that this fraction is a true "fingerprint" of the oil's botanical origin (Table 5).

The predominant components in all samples were cycloartenol and 24-methylencycloartanol, the latter being particularly low (16.3%) only in sample 7 (Brazil). β-Amyrin eluted in both GC systems (TAP and SE52 columns) together with an unidentified component. This has also been confirmed by various studies on other lipid fractions (17,18). It would be worthwhile to establish the nature of the unidentified component, which is quantitatively comparable to β-amyrin. Also, in the case of triterpene alcohols, several hitherto undetermined components, were identified. They account for an average 6.5% of the triterpene alcohol fraction.

TABLE 4

Sterol Fraction Composition (%) (TMS ethers)[a] Determined by GC (TAP column)

Sample	Cholesterol (0.84)[b]	Campesterol 24-Methylencholest[c] (0.87 + 0.94)	Stigmasterol (0.95)	β-Sitosterol (1.00)	Fucosterol (1.01)	Δ^5-Avenasterol (1.03)	Other
1	0.7	6.4	0.3	79.4	0.8	9.4	2.9
2	0.7	6.2	0.3	76.2	0.8	8.4	7.4
3	0.9	6.8	trace	78.8	0.8	10.6	2.1
4	1.3	6.6	trace	79.6	0.9	6.7	4.9
5	0.6	6.0	0.2	81.6	0.9	7.2	3.5
6	0.6	6.6	0.2	81.9	0.7	7.4	2.6
7	0.3	6.4 + 0.3	0.2	82.7	0.6	6.4	3.1
8	0.4	6.0 + 0.3	0.1	80.2	0.6	9.3	3.1

[a]Calculated on the basis of gas chromatographic (GC) areas. TMS, trimethylsilyl.
[b]Numbers in parentheses are the relative retention time, which was calculated by assuming that the retention time of β-sitosterol equalled 1.000.
[c]Tentative identification.

TABLE 5

Composition (%)[a] of the 4,4′-Dimethyl Sterol Fraction (TMS ethers) Determined by GC (TAP column)

Sample	Cycloartanol (0.97)[b]	β-Amyrin* (0.98 + 0.99*)	Cycloartenol (1.06)	Cyclolaudenol[c] (1.06 + 1.07[c])	24-Methylencycloartanol (1.09)	Other
1	3.4	6.4 + 3.8	27.0	3.7 + 4.3	44.5	6.9
2	2.7	6.6	30.5	5.7 + 5.3	43.0	6.2
3	2.9	5.4	29.1	6.1 + 4.5	45.2	6.8
4	2.5	8.8	40.2	4.2 + 3.2	36.9	4.2
5	3.1	8.6	37.6	6.1 + 5.1	35.5	4.0
6	6.7	5.5	34.0	13.1 + 5.9	26.1	8.7
7	12.1	5.3 + 4.1	31.2	17.2 + 6.9	16.3	6.9
8	4.4	6.4	32.5	9.8 + 6.9	31.8	8.2

[a]Calculated on the basis of GC areas. Abbreviations as in Table 4.
[b]Numbers in parentheses are the relative retention time, calculated considering retention time of β-sitosterol = 1.000.
[c]Tentative identification.

TABLE 6

Tocopherol Composition (mg/100 g of oil) of Cashew Kernel Lipids Determined by High-Performance Liquid Chromatography

Sample	δ-Tocopherol	γ-Tocopherol	α-Tocopherol
1	3.6	51.6	3.8
2	3.4	45.3	3.6
3	5.1	51.6	3.7
4	5.8	70.3	8.2
5	5.9	83.5	5.8
6	3.3	58.6	7.5
7	3.4	61.1	2.8
8	2.0	72.5	5.0

The GC analysis with a SE52 column of the 4-methyl sterols fraction shows that the principal compounds are obtusifoliol, dihydroobtusifoliol, cyclolaudenol, gramisterol and citrostadienol, findings that agree with previous data reported in the literature for the unsaponifiable composition of cashew germ (18). The identification of these components with the TAP polar column is in progress.

Table 6 lists the results for the tocopherol fraction. It is a known fact that the vitamin activity decreases from α- to δ-tocopherol, whereas the antioxidant activity increases in the same direction. Note that cashew oil is particularly rich in γ-tocopherol, which is very active among tocopherols as an antioxidant. This, along with high oleic acid, confirms that cashew nut oil is particularly suited to cooking and frying of foods (27).

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