Camelina Oil and Its Unusual Cholesterol Content

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ABSTRACT: The oil in *Camelina sativa* L. Crantz has a combined linolenic and linoleic acid content that is greater than 50% and a relatively low saturated FA content (~10%). Although the FA composition has been reported, no information is available on the sterol composition of camelina oil. The derivatized plant sterols were separated and quantified with capillary GC and their identity confirmed with GC–MS. The refined camelina oil sample contained approximately 0.54 wt% unsaponifiables, and over 80% of the unsaponifiables were desmethylsterols. Perhaps the most unusual characteristic of camelina oil is its relatively high content of cholesterol, particularly for a vegetable oil, since it contains several times the cholesterol found in other "high-cholesterol" vegetable oils. Camelina oil also contains relatively large amounts of another unusual sterol, brassicasterol. The major sterols identified in the camelina oil included cholesterol (188 ppm), brassicasterol (133 ppm), campesterol (893 ppm), stigmasterol (103 ppm), sitosterol (1,884 ppm), and Δ^5-avenasterol (393 ppm).

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KEY WORDS: Brassicasterol, camelina oil, cholesterol, desmethylsterols, α-linolenic acid, sterols, unsaponifiables, unsaturated fatty acids.

Camelina is known primarily in the United States as a weed, although it is probably best described as an underutilized oilseed. The plant is under renewed interest owing to its excellent potential as an oilseed crop and because of the recent search for new sources of EFA, particularly the n-3 FA. Camelina does have a long history of cultivation. *Camelina sativa* ssp. seeds have been found in archaeological sites from the Bronze Age in Scandinavia and other parts of Western Europe (1). *Camelina sativa* L. Crantz is a member of the Crucifereae (Brassicaceae) family, which includes mustard, rapeseed, turnip, broccoli, cabbage, rutabaga, cauliflower, brussels sprouts, and several others that are less well known. The common names used in the North American botanical literature include gold-of-pleasure, dutch flax, and false flax. It is considered a low-input crop, since the nitrogen demand is moderate to low and chemical plant protection is generally not needed. It grows well in cool and semi-arid regions that have a relatively short growing season, e.g., it matures 21 d earlier than flaxseed. Camelina competes well with most other plants, is fairly tolerant of insects and weeds, and grows well on relatively poor and saline soils in northerly climates. It also can survive frost and freeze–thaw cycles after emergence. It has been tested for various agronomic factors in North America, Russia, and Europe, including the Scandinavian countries (2–4).

Camelina seeds contain >40% oil on a dry weight basis. The FA composition of camelina oil has been published (2,3), as has research using genetic modification to alter the FA composition of the oil (5). Sterol fractions from numerous vegetable oils, although not camelina oil, have been analyzed using GC (6–9). Plant oils generally contain significant amounts of plant sterols, particularly campesterol, stigmasterol, and/or sitosterol, usually in the range of 0.2 to 2% (w/w) of total sterols in the oil. The sterol concentrations can be altered depending on the method of oil purification. Kochhar (9) wrote an excellent review outlining the effects of processing on the sterol content of oils. Vegetable oils are thought to contain very little cholesterol, although trace amounts (a few ppm) of cholesterol have been found in many vegetable oils (6,9,10). A few of the tropical fats and oils (palm, coconut, and cocoa) and a few of the field crop oils (peanut, cottonseed, linseed, and rapeseed) have modest amounts (up to ~60 ppm) of cholesterol, although the concentration varies depending on the source of the oil (6,9,10). The objective of this research was to confirm the FA composition, and to identify and quantify the unusual sterol pattern that can occur in camelina oil. However, characterization of the range in FA composition and sterol content of camelina oil from various sources and locations was beyond the scope of this work.

EXPERIMENTAL PROCEDURES

Commercial *Camelina sativa* L. Crantz oil samples were used for both the FA and sterol analysis. The oil was cold-pressed in France from a spring variety of camelina, Celine. Duplicate analyses were done on duplicate samples from a single lot for both the FA and sterol analysis. The means and SEM were determined and reported.

FA analysis. The FA composition analysis was based primarily on IUPAC procedures 2.301 and 2.303 (11). Each oil sample was saponified with KOH in methanol (MeOH), then esterified with BF_3 in MeOH. FAME in hexane were separated with a CP Sil 88 column (Chrompak, Varian Instruments, Walnut Creek, CA; 50 m × 0.25 mm × 0.2 μm film thickness), with an initial column temperature of 100°C for 2 min, then 30 to 150°C, followed by a 5°C/min ramp to 225°C, and then it was held at 225°C for 16 min. The GC had an initial injector

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temperature of 50°C (with a rapid temperature ramp to 250°C in 12 s) and an FID temperature of 250°C. The GC was a PerkinElmer autosystem XL. The injection volume was 0.2 µL with a 1:100 split. The mobile phase was helium at a head pressure of 124 kPa. The FA were identified based on standards purchased from Nu-Chek-Prep (Elysian, MN).

Cholesterol analysis, GC. To quantify the amount of cholesterol in the oil sample, 40 µg of 5α-cholestane (Sigma-Aldrich Sweden AB, Tyresö, Sweden) was added as an internal standard to the lipid sample. After addition of 2 mL of 2 M KOH in 95% ethanol to a 40 mg lipid sample, the mixture was vortexed for approximately 10 s in ground-glass stoppered tubes. The tubes were shaken for 45 min at 60°C in a glycerol bath. After saponification, each sample was cooled to room temperature under cold running water, followed by addition of 1.0 mL of distilled water, 2.0 mL of hexane, and 0.2 mL of absolute ethanol to each tube. The tubes were shaken vigorously, centrifuged at 3000 × *g* for 3 min, and the upper hexane layer containing the unsaponifiable material was transferred into clean glass tubes. The extract was dried with a stream of nitrogen gas. To confirm complete saponification, TLC can be used with the following solvent system, hexane/diethyl ether/acetic acid (85:15:1) on silica gel plates [detection: phosphomolybdic acid, followed by heating for 10 min at 120°C (12)].

After solvent removal, the samples were derivatized with 100 µL of Tri-Sil reagent (Pierce, Rockford, IL) in glass tubes covered with a ground-glass stopper, mixed well (ultrasonic bath for 1 min, followed by brief vortexing), and incubated at 60°C for 45 min. The solvent was removed under a stream of nitrogen, the derivatized sterols were dissolved in hexane (0.1 mL), and the tubes were sonicated for 2 min, vortexed, and centrifuged for 3 min at 3000 × *g*. The hexane layer was transferred into another tube and analyzed by GC–FID and GC–MS, as described below. After derivatization, the samples can be stored for as long as 3 d (at −20°C) for subsequent analysis (in closed tubes to prevent air contact).

For sterol quantification using GC–FID and identification using GC–MS, sample components were separated with a DB-5MS (J&W Scientific, Folsom, CA, 30 m × 0.25 mm × 0.50 µm film thickness) capillary column (11). For quantification using GC–FID, the column was connected to a Varian Star 3400 CX gas chromatograph. The injector temperature was 250°C, and the sample was injected in a split mode with a ratio of 20:1. The detector temperature was 320°C. Helium was used as a carrier gas at an inlet pressure of 83 kPa, and nitrogen was used as makeup gas for the FID at 30 mL/min. The concentration of sterols was based on the GC results and the percentage of unsaponifiables in the oil. A temperature program starting at 285°C for 15 min, then 1°C/min to 300°C, followed by 1 min at 300°C was used. Five standard samples of sterols (cholesterol, brassicasterol, campesterol, stigmasterol, and sitosterol) were purchased from Larodan Fine Chemicals (Malmö, Sweden). All the sterols were quantified by GC–FID. GC identification was done by comparing retention times of the standards with the sample chromatogram. Confirmation of cholesterol, brassicasterol, campesterol, stigmasterol, and sitosterol was done by GC–MS analysis by comparing mass spectra of standard samples and sample sterols. The two other sterols, Δ^5-avenasterol and cycloartenol, are not commercially available, but they were identified by their retention times in GC and by GC–MS analysis by comparing MS data (12,15).

Cholesterol identification, GC–MS. For GC–MS analysis, a GC 8000 Top Series GC (ThermoQuest Italia S.p.A., Rodano, Italy) coupled to a Voyager mass spectrometer with a MassLab data system version V1.4 (Finnigan, Manchester, England) was used. The TMS sterol derivatives were separated on a DB-5MS column (30 m × 0.25 mm × 0.50 µm); helium was used as the carrier gas at an inlet pressure of 79 kPa. The injector temperature was 250°C, and the samples were injected in a splitless mode with a purge delay time of 0.6 min. A programmed oven temperature starting at 60°C for 1 min, then 25°C/min to 290°C, then 20 min at 290°C, followed by 1°C/min to 300°C, and then finally 25 min at 300°C (12).

RESULTS AND DISCUSSION

FA profiles. The camelina oil FA profile is shown in Table 1, which agrees with published results (2,3). The FA composition of camelina oil (also referred to as false flax) is similar to flaxseed with high concentrations of linolenic, linoleic, and oleic acids, with the major difference being that there are substantial amounts of the longer-chain FA, particularly C20:1 (>10%), in camelina oil (13). Like many of the Cruciferae, camelina does contain significant amounts of erucic acid (14), although the content (2.5%) is approximately equal to that found in low-erucic acid rapeseed oil (~2%). Upon comparison of the polyunsaturated to monounsaturated FA ratios, camelina oil has a ratio similar to soybean oil, a higher ratio than canola oil, and a ratio lower than flaxseed and the traditional sunflower oils. Budin *et al.* (3) suggested that this ratio is probably a better indication of oil susceptibility to autoxidation than the saturated to unsaturated FA ratio, since the monounsaturated FA are much less susceptible to oxidation than the PUFA.

Sterol composition. The unsaponifiables totaled 0.54% (w/w) and were calculated based on the GC chromatogram. The major desmethylsterols were separated and identified (Fig. 1). Their concentrations in the camelina oil sample are shown in Table 2. The six identified desmethylsterols constituted 0.36%, while the remaining 0.18% may have been a mixture of other desmethylsterols, 4-monomethylsterols, 4,4-

TABLE 1
FA Profile of Refined, Bleached, and Deodorized Camelina Oil

FA	Mean ± SEM (%)	FA	Mean ± SEM (%)
C16:0 (palmitic)	5.3 ± 0.1	C20:0 (arachidic)	1.4 ± 0.1
C18:0 (stearic)	3.0 ± 0.0	C20:1 (eicosenoic)	11.6 ± 0.1
C18:1 (oleic)	18.7 ± 0.1	C22:1 (erucic)	2.5 ± 0.1
C18:2 (linoleic)	16.0 ± 0.1	Other FA	3.4 ± 0.1
C18:3 (linolenic)	38.1 ± 0.1		

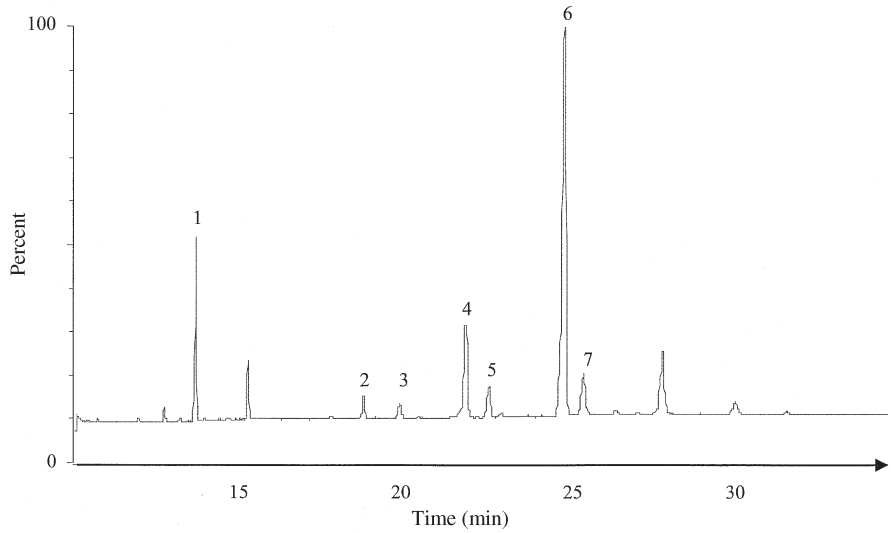

FIG. 1. GC–MS total ion chromatogram of sterols from camelina oil. (1) 5α-Cholestane, (2) cholesterol, (3) brassicasterol, (4) campesterol, (5) stigmasterol, (6) sitosterol, and (7) Δ^5-avenasterol.

dimethylsterols, and possibly some tocopherols (15). There were three components present in substantial amounts (>50 ppm) whose identity could not be confirmed. However, based on published data (15), two of the components were identified as cycloartenol (515 ppm) and 24-methylene cycloartenol (124 ppm). There were several (>10) minor components that were not present in sufficient quantities to identify without additional concentration steps.

Some tropical oils have relatively large amounts of cholesterol, e.g., cocoa butter—59 ppm, coconut oil—23 ppm, linseed oil—42 ppm, palm oil—26 ppm, and palm kernel oil—39.6 ppm (9), although less than that found in camelina oil (188 ppm). Vegetable oils from the Brassicaceae, e.g., rapeseed and mustard, can also have modest amounts of cholesterol. Oils from the Brassicaceae can also contain substantial amounts of brassicasterol (in one case >600 ppm), although the amount varies widely with the source and growing conditions (6,9,10,16). Brassicasterol is either absent or present only in trace amounts in most other oils. Brassicasterol was present in the camelina oil samples at 133 ppm. Campesterol and stigmasterol are present in vegetable oils over a very large concentration range (over 100-fold), up to very high concentrations (>1,000 ppm), depending on the oil source. Camelina contains relatively modest amounts of stigmasterol but greater amounts of campesterol. Sitosterol is present at very large concentrations in the large majority of vegetable oils (from 924 to 17,336 ppm). It was also present in camelina oil in substantial amounts, 1,884 ppm. Finally, the other major desmethylsterol reported, Δ^5-avenasterol, was present within the range reported for most other vegetable oils (10). The unusual sterols present include brassicasterol (unusual because it is present in few other commercial oil sources) and cholesterol (camelina has the greatest concentration reported for a vegetable oil, 188 ppm) (Figs. 2A,B). Most oils contain 1 to 5 g sterols per kg of oil, and the commercial camelina oil samples reported here are within this range (6,9).

TABLE 2
The Concentration of the Major Desmethylsterols in Camelina Oil

Sample	Mean ± SEM (μg/g of oil)	Sample	Mean ± SEM (μg/g of oil)
Cholesterol	188 ± 8	Sitosterol	1884 ± 144
Brassicasterol	133 ± 9	Δ^5-Avenasterol	393 ± 41
Campesterol	893 ± 74	Total of all six sterols	3604 ± 288
Stigmasterol	103 ± 18	Total unsaponafiables	5404 ± 407

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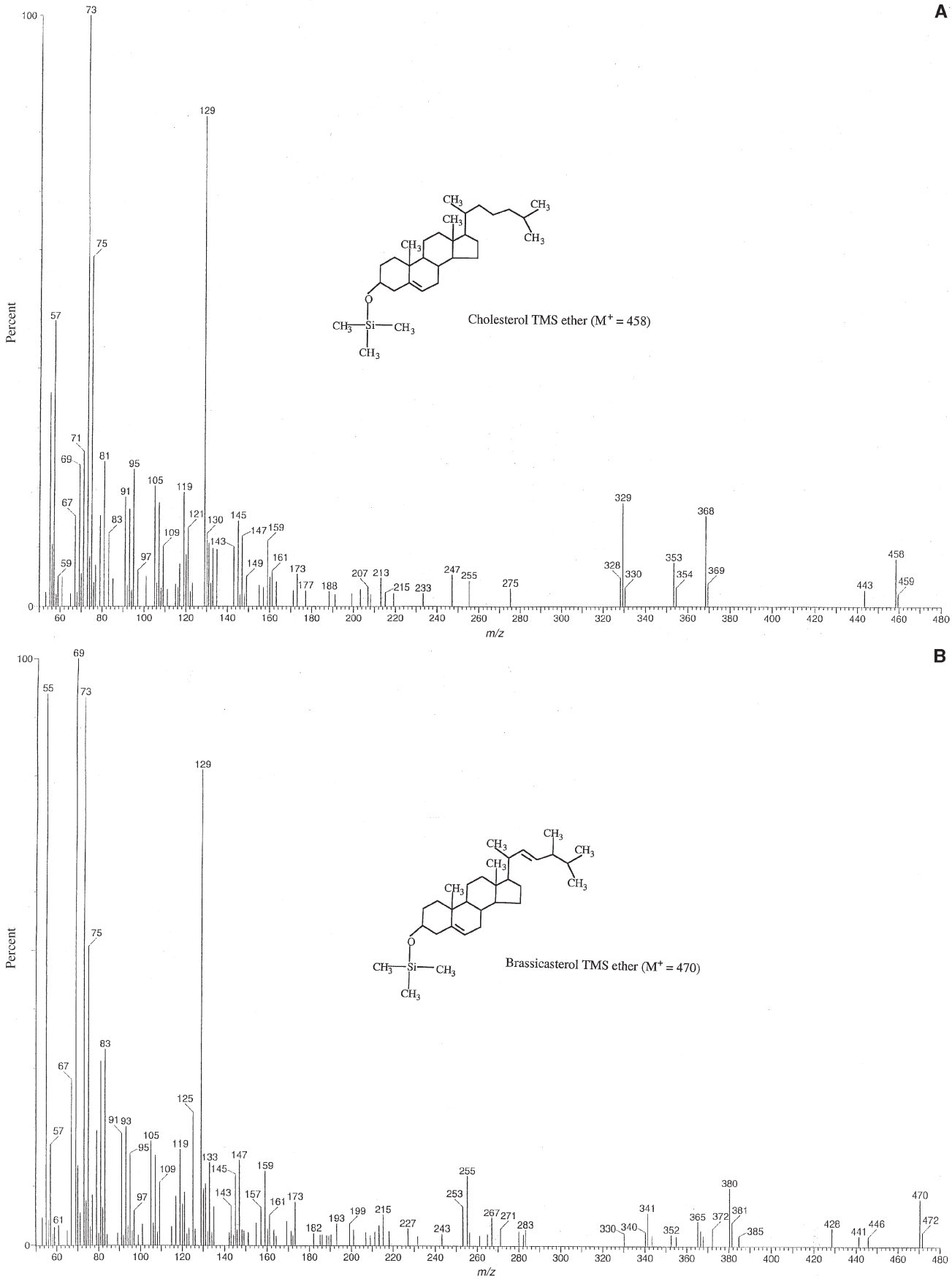

FIG. 2. Full-scan mass spectrum of the trimethylsilyl (TMS) ether derivative of (A) cholesterol with the molecular ion (M^+ = 458) and other typical ion fragments, with an insert of the structure of the TMS ether of cholesterol, and (B) brassicasterol with the molecular ion (M^+ = 470) and other typical ion fragments, with an insert of the structure of the TMS ether of brassicasterol.

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