Characterization of Corn Oil, Soybean Oil and Sunflowerseed Oil Nonpolar Material

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Normal phase preparative and semi-preparative liquid chromatography were used to isolate fractions of varying polarity from corn, soybean and sunflowerseed oils. Reported here is the composition of one fraction, less polar than triglycerides, determined by isolating the individual "peaks" of a semi-preparative separation using as starting material the mix of compounds obtained from a large scale separation. These peaks were then analyzed by high performance liquid chromatography (LC), gas chromatography (GC), mass-spectrometry (MS) with and without GC, in both electron impact (EI) and chemical ionization (CI) modes, and carbon-13 nuclear magnetic resonance (NMR) spectroscopy. Semi-quantitative data were obtained for many of the components found in these semi-preparative isolates including hydrocarbons, steryl esters, triterpenyl esters, phytyl esters and geranylgeranyl esters. The weight percent and composition of the preparative fraction differed substantially among the three oils. Corn oil had the greatest amount, at 1.25% of the starting oil, and was composed mostly of steryl and triterpenyl esters. Sunflowerseed oil, at 0.7%, and soybean oil, at 0.3%, showed greater variety in that branched chain esters were included with the steryl/triterpenyl distributions.

Vegetable oils are natural products and, as such, contain many substantially different classes of compounds. These compounds have been studied in many ways and from several perspectives. For example, quality assessments are often made based on nontriglyceride composition (1). Most of the analytical methods used to monitor product quality measure the level of selected minor components; examples include free fatty acid, phosphorus, tocopherol, chlorophyll and trace metal analyses (2). As a rule, the relative weight percent of nonglycerides decreases as an oil is processed, because edible oil processing itself is a systematic means of separating and isolating nontriglyceride species.

Studies of triglycerides are well documented (3), but isolation and identification of minor components are more difficult and less explored. Among the many approaches to the isolation of minor components [such as molecular distillation (4), size exclusion or saponification with extraction (5)], chromatographic separations are now considered the best way to obtain enriched fractions of unmodified minor components. It is known that the heat necessary to separate materials using molecular distillation can lead to chemical modification of the desired product. Chemical modification of the triglycerides is the main goal of typical saponification methods. The resulting unsaponifiables can also be altered by this method, and information concerning nonglyceride ester distributions will be lost. As an example, steryl esters are an important class of compounds in plant metabolism and are known to exist in vegetable oils. The saponification process may produce at least two unwanted effects. First, the reaction may not go to completion (that is, to a free sterol and a metallic salt of a fatty acid). If so, when the reaction mixture is extracted with a hydrocarbon solvent, unreacted steryl ester will be extracted as an unsaponifiable. This leads to an inaccurate estimate of total sterols and possibly a modified compositional estimate. Second, modification of the unsaturation in both the fatty acid and in the sterol is possible. Kochhar (6) reports that silylated sterol profiles can be altered after only several days of storage at 4 C. This may well explain some of the widely varying sterol distributions reported in early literature for a given oil or product. It is our opinion that the concept of "unsaponifiables" cannot accurately represent the unmodified minor component composition of an oil.

Our focus is on chromatographic methods as a means to obtain minor oil components. Modern preparative liquid chromatography allows routine separations at resolutions, loadings and throughput levels which are significantly greater than older gravity methods. Using normal phase preparative HPLC, we are able to fractionate oil at the 100 g level and isolate concentrated nontriglyceride materials. Then, using a step by step fractionation scheme by preparative and semi-preparative LC, simplified mixtures for analysis and structural elucidation are obtained.

Because adsorption chromatography effects a separation through the general mechanism of polarity, we organize our classification of oil minor components according to their general elution order on silica using hydrocarbon solvents, as opposed to a classification scheme based on structure or size. One of the major nontriglyceride fractions we isolate from finished vegetable oil is the fraction which is less polar than triglycerides. This fraction elutes from our preparative system at the solvent front and continues until the onset of the elution of the triglycerides. This paper reports the significant species found in this nonpolar material (NPM) and estimates the amounts for each oil studied. Specifically, we report the distributions of hydrocarbons, steryl esters, triterpenyl esters, phytyl esters, geranylgeranyl esters and selected carbon-13 nuclear magnetic resonance chemical shifts for previously unreported compounds.

EXPERIMENTAL PROCEDURES

Preparative high pressure liquid chromatography. To separate the NPM from the triglycerides, 100 g of corn

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oil was dissolved in 75 ml n-hexane and injected, through the pump, onto a Waters Prep 500A high pressure liquid chromatograph at 100 ml/min. The chromatograph was equipped with four compression chambers, connected in series, each containing one Prep Pak 500 silica cartridge (57 mm × 30 cm, 37-55 μm), resulting in a single column with a void volume of 2000 ml. The cartridges were unused and were conditioned by a preliminary wash of one column volume (2000 ml) hexane/ethyl acetate (96/4, v/v), followed by one column volume of hexane/ethyl acetate (98/2, v/v) mobile phase, with all solvents sent to waste. This preliminary wash was to ensure that the mobile phase would not be depleted of polar modifier by the virgin silica as well as to remove any silica "fines." After this conditioning, the columns were brought into equilibrium with the mobile phase (ca. 20,000 ml) by recirculation at 500 ml/min for ca. 90 min. After injection, the NPM was eluted from the column at 200 ml/min in a volume of ca. 1500 ml. Fraction collection started several hundred milliliters before the expected solvent front, and continued until a capacity factor (k′) of about 0.7 had been reached. The major peak for triglycerides had a k′ = 1.2 under these conditions. Solvent was removed with gentle heat (ca. 50 C) under a stream of dry UHP nitrogen; 1.22 g of triglyceride-free NPM was recovered.

Preliminary experiments indicated that sunflowerseed oil and soybean oil had significantly lower levels of NPM than did corn. Thus, to collect enough NPM for subsequent fractionation the preparative chromatographic

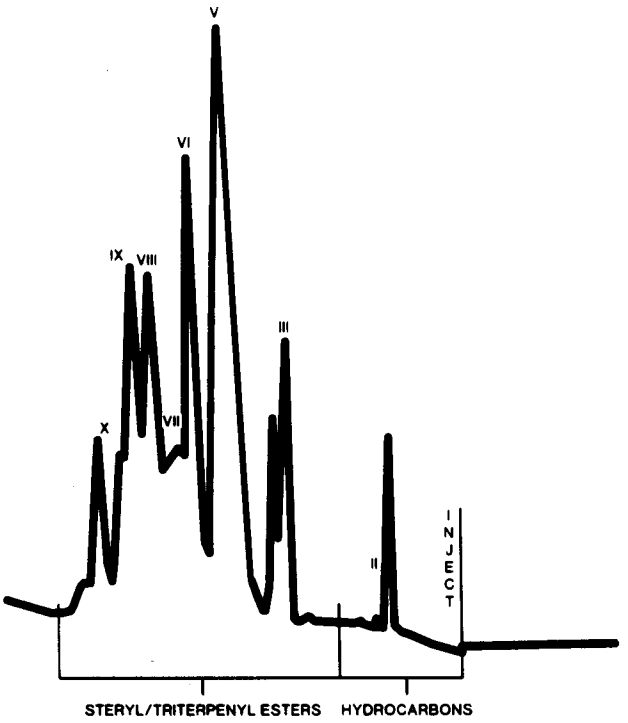

FIG. 2. Soybean oil nonpolar material analytical scale, HPLC refractive index response. Column, Waters μ porasil; mobile phase, 99.5 n heptane and 0.45 butyl acetate; flow rate, 0.6 ml/min; sample size, 250 μg.

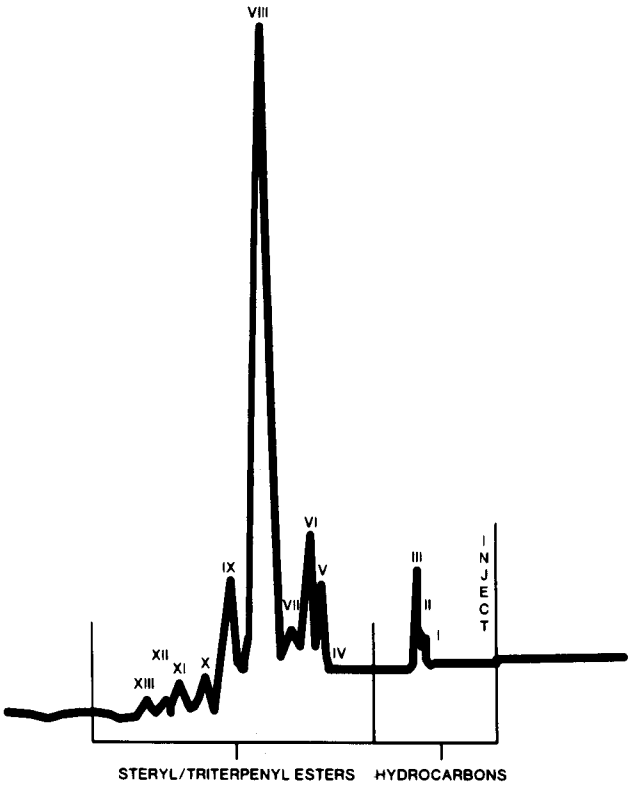

FIG. 1. Corn oil nonpolar material analytical scale, HPLC refractive index response. Column, Waters μ porasil; mobile phase, 99.55 n heptane and 0.45 butyl acetate; flow rate, 0.6 ml/min; sample size, 250 μg.

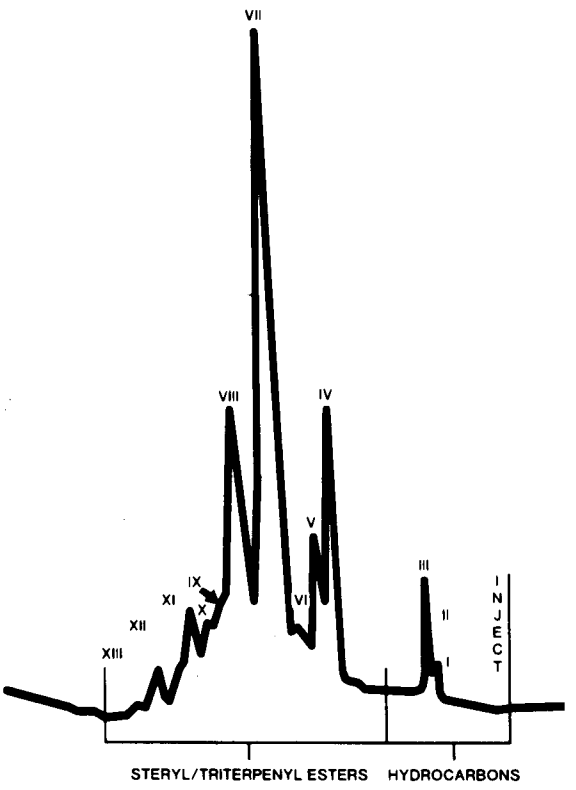

FIG. 3. Sunflowerseed oil non polar material analytical scale, HPLC refractive index response. Column, Water μ porasil; mobile phase, 99.5 n heptane and 0.45 butyl acetate; flow rate, 0.6 ml/min; sample size, 250 μg.

procedure was modified. In particular, 5-100 g runs were made on one set of four Prep Pak 500 silica columns each day for two days, resulting in 10-100 g runs on two sets of four columns for each oil. It was known that NPM, isolated after the first run, would include residual triglycerides from the previous separation, because triglycerides tail at this loading under these chromatographic conditions. To ensure triglyceride-free NPM, a final preparative separation was done using the pooled NPM fractions for each oil from the 10-100 g runs under conditions stated for corn oil; 2.79 g of NPM was obtained from soybean oil and 6.40 g from sunflowerseed oil. All of the starting oils were commercial consumer products.

Semi-preparative high pressure liquid chromatography. The eluent corresponding to individual peaks of the NPM was isolated using a Beckman 101A pump, modified Waters U6K injector, a Whatman Partisil 10-Magnum 20 column (22 mm i.d. × 50 cm, 10 μ) having a void volume of 120 ml, a Waters 403 RI detector, and a Beckman 165 UV/VIS detector. A strip chart recorder was used to monitor detector outputs. It was found that, at typical flows (20 ml/min), excessive back pressure was generated when the injector was in the load position. Reducing the length of the restriction loop eliminated this situation. More troublesome, however, was the instability of the refractive index baseline at 20 ml/min. This instability was found to be flow dependent and was eliminated by including a flow splitter (6:1) in the system. This splitter consisted of a stainless steel tee with short sections of stainless steel tubing of differing inner diameters generating the necessary stream splitting and reducing the flow through the detector to about 3.4 ml/min. All eluent was collected. The usual mobile phase for NPM peak isolation was hexane/ethyl acetate (99.70/0.30, v/v), and 100 mg NPM was injected for each run in 200 μl total injection volume; the injection solvent was mobile phase. To ensure that sufficient amounts of peak isolates would be obtained for further characterization, multiple runs were required. The solvent was removed with gentle heat under a stream of dry nitrogen.

Analytical scale high pressure liquid chromatography. The NPM, and its subfractions, were characterized by normal phase LC. The equipment used was a Beckman/Altex 100A pump, a Waters U6K injector, a Waters μ-Porasil column (3.9 mm i.d. × 30 cm, 10 μm), and a Beckman/Altex 156 refractive index (RI) detector. The mobile phase most commonly used was heptane/butyl-acetate (99.55/0.45, v/v) at 0.6 ml/min. Resolution was sample load dependent. A reasonable balance between resolution and signal to noise was found at a loading of ca. 250 μg in a 10-μl injection for the intact fraction and at 50-150 μg on the column for individual subfractions, depending on the retention time. The detector outputs went to a combination of strip chart recorders and integrators, including a Spectra Physics SP4100 and a Varian Vista 402.

Gas chromatography. Several GC methods were used to characterize the corn NPM. The main instrument was a Varian 6000 series chromatograph with dual flame ionization detectors, interfaced with a Varian 402 data system capable of storing raw data on floppy disks. All injections were on column in both packed and capillary column modes.

Intact steryl esters were chromatographed using a 60 cm × 2 mm i.d. glass column packed with 3% Dexsil 300 on Supelcoport 100/120 (Supelco, Inc., Bellefonte, Pennsylvania). The instrumental conditions were as follows: injector, 350 C; detector, 400 C; helium flow rate, 30 ml/min; initial column temperature, 200 C for

TABLE 1

Capillary GC of Corn, Soybean and Sunflower Hydrocarbons[a]

ECL[b]	Corn	Soybean	Sunflower
27.01	1.7	-	6.3
28.00	-	-	1.1
28.27 (squalene)	57.0	67.6	19.3
28.78	1.2		
28.92	-	1.7	-
29.02	1.6	-	30.1
29.10	1.5		
29.17	-	1.7	-
30.01	5.6	1.0	2.2
30.44	3.9	4.7	-
30.76	3.2	4.1	-
30.93	-	5.7	-
31.01	2.7	1.2	27.1
31.13	1.3	1.0	1.3
31.38	13.6	-	3.8
31.50	1.0		
32.84	-	0.9	1.9
Peak % of NPM	3.3	2.3	5.0
Wt of % of oil	0.04	0.006	0.015

[a]% Total hydrocarbons.
[b]Equivalent chain length.

TABLE 2

^{13}C NMR Chemical Shifts[a] at 90.5 MHz Δ^5 steryl ester

Carbon Δ^5 steryl moiety[b]	PPM Downfield from internal TMS
1	37.11
2	27.92
3	73.74
4	38.26
5	139.84
6	122.58
7	31.98
8	31.98
9	50.19
10	36.70
Fatty acid moiety[c]	
1	173.12
2	34.77
3	25.11

[a]PPM downfield from TMS in $CDCl_3$.
[b]Sitosteryl, campesteryl/dihydrobrassicasteryl, stigmasteryl and isofucosteryl.
[c]Palmitate, oleate, linoleate and linolenate.

two min; temperature program, 200 to 400 C at 10 C/min. About one μl of one μg/μl was a typical injection.

Capillary column GC data were also obtained on a Carlo-Erba Fractovap series 4160 equipped with a flame ionization detector and on column injector. Conditions used were as follows: injection onto a 120 C column, hold two min; temperature program, 7.5 C/min to 350 C. The column was a 15 meter × 0.25 mm fused silica column coated with a DB-1 methyl silicone.

Selected peak isolates were transmethylated according to Luddy's (7) method. Methyl esters were analyzed as follows: injector, 275 C; column, 182 cm × 2 mm i.d. glass packed with 10% SP2340 on 100/120 Supelcoport (Supelco, Inc., Bellefonte, Pennsylvania); temperature program 100 C, hold for three min after injection; 25 C/min to 162 C, hold 10 min; 10 C/min to 224 C, hold 10 min; detector, 300 C; helium flow 35 ml/min. All typical methyl esters from C8:0 to C24:0 were well resolved.

The sterols and other alcoholic products of the transmethylation method were characterized using the following conditions: column, 182 cm × 2 mm i.d. glass packed with 3% OV-17 on 100/120 Supelcoport (Supelco, Inc., Bellefonte, Pennsylvania); injector, 374 C; temperature program, 150 C, hold to one min; 10 C/min to 230 C, hold 10 min; 5 C/min to 315 C, hold five min; detector, 375 C; helium flow, 35 ml/min. These conditions allowed nearly baseline resolution between underivatized stigmasterol and campesterol.

Gas chromatography/chemical ionization mass spectroscopy. In order to obtain molecular weights and structural information on selected semi-preparative peak isolates, mass spectroscopic methods were used. Preliminary work was done in the electron impact (EI) mode at 12eV using both direct insertion and packed column gas chromatographic techniques. These did not usually yield a molecular ion. Wakeham and Frew (8) reported a procedure, which we adapted, for characterizing various esters using chemical ionization mass spectroscopy (CIMS) with capillary GC. This method had at least two potential advantages. First, a molecular ion was obtained. Second, selective fragmentation occurred, primarily at the ester bond. This gave information concerning both the fatty acid and the alcohol distributions within each peak.

The instrumentation used was a Hewlett-Packard 5985B quadrupole mass spectrometer operating in the CI mode using methane as the reagent gas. Methane was introduced to the source through the interface using a separate gas line; methane used as the carrier gas resulted in poor chromatography. The chromatographic conditions were as follows: instrument, H/P 5840 with capillary inlet option; column, 7.5 m × 0.25 mm i.d. SGE BP-1 (SGE); helium flow rate, 37.9 cm/sec at 0.45 kg/cm^2; injector temperature, 375 C; column temperature, 240 C; hold two min then 7.5 C/min to 350 C; interface temperature, 370 C. Mass spectroscopic conditions were as follows: ion source temperature, 200 C, ion source pressure, 0.5 to 0.8 Torr; ion source voltage, greater than 15eV; electron multiplier, 2400 to 3000 volts; scanning, 80 to 800 amu in 3.6 seconds.

Nuclear magnetic resonance. Three spectrometers were used to obtain spectral data on the semi-preparative peak isolates. Initially, continuous wave (CW) ^{1}H spectra were obtained on a Varian EM-390 (90 MHz). Samples (1 to 100 mg) were dissolved in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. Sweep width was 10 ppm; sweep times varied from 5–20 min. Natural abundance Fourier Transform (FT) ^{13}C NMR spectra were obtained at 36 C using a Varian FT80 spectrometer (20.1 MHz) with complete proton decoupling. A spectral width of 4000 Hz was acquired in 16K data points. A 90 degree flip angle was used with no pulse delay; the cycle time was two seconds. Samples (10 to 100 mg) were dissolved in ca. 500 μl of $CDCl_3$ and placed in 5-mm tubes which were then plugged; a 5-mm probe insert was used. The number of transients needed for usable signal to noise varied from 3,000 to 85,000 depending on sample size; TMS was used as an internal reference.

Subsequent work was done on a Bruker WM-360. Broadband decoupled FT ^{13}C spectra (90.5 MHz) were obtained on peak isolates by dissolving 4 to 100 mg in ca. 500 μl of $CDCl_3$ in a 5mm tube. The typical sweep width was 20 KHz; 32 K data points were used to acquire the free induction decay. No pulse delay was used; the flip angle was 70 degrees and cycle time was 0.8 sec. The number of scans varied from 3000 to 100,000 depending on the sample size. All chemical shifts are expressed as ppm downfield from TMS. Although more

TABLE 3

^{13}C NMR Chemical Shifts[a] at 90.5 MHz 4 Monomethy Steryl Moieties in Corn NPM

Carbon common name	Peak XI Citrostadienyl	Peak XII 24 Methylenelophenyl
1	36.74	36.75
2	27.26	27.23
3	78.28	78.28
4	37.14	37.15
5	46.85	46.87
6	26.73	26.73
7	117.32	117.33
8	139.23	139.16
9	46.90	49.61
10	34.84	34.81
11	21.47	21.46
12	39.60	39.52
13	43.46	43.46
14	55.01	55.00
15	22.97	22.94
16	27.99	27.93
17	56.14	56.17
18	11.89	11.87
19	14.05	14.01
20	36.61	36.21
21	18.97	18.87
22	36.07	34.81
23	28.22	31.21
24	145.91	156.83
25	28.71	33.92
26	21.03	21.89
27	21.11	22.01
28	116.61	106.06
29	12.76	-
30	15.18	15.14

[a]PPM downfield from TMS in $CDCl_3$.

than 100 mg of several peaks were isolated, it was found that exceeding ca. 100 mg/500 μl $CDCl_3$ introduced concentration effects that caused changes in chemical shifts and peak intensities. The most pronounced changes were observed for the carbonyl carbon and the olefinic carbon in the fatty acid moiety.

A relatively new NMR experiment, the Distortionless Enhancement by Polarization Transfer (DEPT), was used on selected samples. This experiment uses a pulse sequence that, after appropriate spectral editing, allows the discrimination of carbons by the number of attached protons. Thus, it is possible to determine the number of methyl, methylene and methine carbons on an unknown, as well as their chemical shifts, without tedious and insensitive off-resonance or undecoupled experiments. In practice, four separate acquisitions are obtained, including a normal broadband decoupled acquisition. As a result, the experiment needed either a relatively large amount of sample (ca. 50–100 mg) or a long run time (24 hr) to obtain the signal to noise suitable for our work.

RESULTS AND DISCUSSION

Hydrocarbons. Figures 1–3 show typical analytical LC RI responses for corn, soybean and sunflowerseed oil NPM, respectively. The NPM recovered represents 1.25% of the starting corn oil, 0.28% of the starting soybean oil and 0.64% of the starting sunflowerseed oil. At this solvent strength, two distinct regions can be seen for NPM of all three oils. The first is near the solvent front, and consists of multiple, closely spaced peaks. NMR and capillary GC data show this region to consist of hydrocarbons. Both ^{13}C and ^{1}H NMR spectra show evidence for a mixture of straight and branched chain, saturated hydrocarbons. Because there are few peaks in spectral regions associated with aromatic structures and no evidence for oxygenated compounds such as ethers or esters, we conclude that hydrocarbons are the major components of this region. This is confirmed in Table 1 which quantitates the responses for this region using the GC method described above and identifies the components by equivalent chain length (ECL) based on the retention times of straight chain hydrocarbons. Squalene is confirmed in all oils by the obtained equivalent retention times of an authentic sample when analyzed both by GC and LC methods as well as in spectral data. These data compare well with Worthington's studies (9) on the hydrocarbons of several vegetable oils. NMR data show evidence of branching and unsaturation explaining the fractional ECL values. Both analytical and semi-preparative LC, using heptane:butyl acetate and hexane:ethyl acetate, respectively, show no peaks between the hydrocarbon and ester region, eliminating the possibility of an oil component having a refractive index identical to a mobile phase.

Based on the analytical scale HPLC, in most of the isolates the target peak (as identified by NMR/MS) is by far the major component. The major contaminants are the preceding and succeeding peaks. This does not mean, however, that each peak is a single species. Coelution of species with equivalent polarity was detected through GC (Tables 6–8) and NMR experiments.

Unlike the hydrocarbon region, the later eluting compounds are much more complicated in structure and distribution. To simplify the discussion and structure determinations each oil is discussed separately for this region.

Corn oil NPM. Peak isolates from the putative steryl ester region (Fig. 1) have 42 to 50 carbon atoms based on high temperature GC. Using cholesterol and choles-

TABLE 4

^{13}C NMR Chemical Shifts[a] at 90.5 MHz Phytyl Ester Soybean Oil NPM Peak IV

Phytyl Carbon	Chemical Shift
1	61.24
2	118.28
3	142.47
4	39.88
5	25.06
6	36.67
7	32.71
8	37.47[b]
9	24.48
10	37.41[b]
11	32.83
12	37.34[b]
13	24.80
14	39.42
15	27.98
16	23.63[c]
17	22.72[c]
18	18.75
19	19.71
20	16.36

[a]PPM downfield from TMS in $CDCl_3$.
[b]May be interchanged.
[c]May be reversed.

TABLE 5

^{13}C NMR Chemical Shifts[a] at 90.5 MHz Geranylgeranyl ester

Geranylgeranyl carbon	Chemical shift
1	61.14
2	118.59
3	141.97
4	39.51[b]
5	26.57[c]
6	124.32
7	134.84
8	39.76[b]
9	26.72[c]
10	124.32
11	135.24
12	39.76
13	26.83
14	124.48
15	131.14
16	25.47
17	17.63
18	16.02
19	16.02
20	15.96

[a]PPM downfield from TMS in $CDCl_3$.
[b]May be interchanged.
[c]May be reversed.

TABLE 6

Corn Oil NPM - Transmethylation Analyses

Relative fatty acid composition		Peak V	VI	VII	VIII	IX	X	Intact NPM
(%)								
C16:0		81.1	9.4	8.4	1.0	1.4	2.1	5.0
C18:0		6.7	0.7	0.6	-	0.3	-	0.3
C18:1		0.9	88.9	38.2	3.3	6.7	8.4	14.4
C18:2		-	1.0	53.5	95.7	78.9	85.2	78.8
C20:0		2.7	-	-	-	0.6	-	-
C18:3		1.8	-	-	-	12.0	3.8	1.5
Relative sterol composition								
RRT[a]	Sterol	(%)						
0.66		-	0.4	0.2	0.4	0.2		0.3
0.71		-	0.2	0.6	0.4	2.4	0.4	-
0.82		-	0.2	0.2	0.2	0.2	0.6	0.6
9.95	Campesterol/ dihydrobrassicasterol	18.2	19.6	12.8	18.5	17.3	-	17.4
0.97	Stigmasterol	8.9	6.6	2.3	8.5	6.2	-	7.9
0.99		-		-	-	-	3.4	-
1.00	Sitosterol	72.7	73.4	83.8	72.0	15.8	10.0	70.7
1.03	Isofucosterol	-	-	-	-	55.8	36.9	8.1
1.06		-	-	-	-	2.0	7.2	1.6
1.09		-	-	-	-	-	41.5	1.3

[a]Retention time relative to sitosterol.

teryl esters as model compounds, as well as current literature (10-21) for ^{13}C NMR chemical shift assignments, the regions from peaks IV through IX in Figure 1 are primarily steryl esters. Selected ^{13}C NMR data are presented in Table 2 reporting the Cl to C10 chemical shift for the 5 steryl group esterified to a long chain fatty acid.

Transmethylation methodology was developed using a model system consisting of cholesteryl oleate, cholesteryl palmitate, behenyl behenate and C35 hydrocarbon, as well as unfractionated NPM. This transmethylation procedure is necessary because typical saponification methods applied to our model system and several selected peak isolates had poor recoveries and irreproducible fatty acid/alcohol profiles. At least 15 mg are required for transmethylation to ensure reasonable recoveries. This is several orders of magnitude greater than the amounts needed for the actual GC methods used. As a result, transmethylations were done only where sufficient sample existed; the rest require the development of suitable microtechniques. Peaks V through X are a distribution of steryl and triterpenyl esters chromatographically resolved by a combination of unsaturation and chain length. Peaks V through VIII, for the most part, are separated by LC according to the unsaturation in the fatty acid moiety. Overlap between peaks prevents complete quantitation, but the essential compositions are: Peak V, sitosteryl, campesteryl/dihydrobrassicasteryl and stigmasteryl palmitate; Peak VI, sitosteryl, campesteryl/dihydrobrassicasteryl and stigmasteryl oleate; Peak VII, mixture; Peak VIII, sitosteryl, campesteryl/dihydrobrassicasteryl and stigmasteryl linoleate; Peak IX, isofucosteryl linoleate; Peak X, 24 methylene cycloartanyl linoleate.

Peaks XI through XIII account for about 5.4% of the total fraction weight. The ^{13}C NMR spectra show that each of these peaks are complicated mixtures of esters including several 4-monomethyl sterols. As Kornfeldt and Croon have shown (22), the 4-monomethyl and 4,4-dimethyl sterol distributions are more complicated than the desmethyl distribution in corn oil. Because the peaks were collected sequentially, the appearance and disappearance of responses aid in determining the related carbons as much as do the relative resonance intensities. No relaxation time or nuclear Overhauser effect measurements were made due to the long run times needed for such limited sample size. The chemical shifts for the 4-monomethyl steryl groups and the peak in which others predominate are found in Table 3. Assignments were made through comparison with model compounds. Linoleate is the major fatty acid moiety found by NMR, perhaps indicating that these metabolic intermediates may be specifically esterified to linoleic acid. These species are not unexpected, as Nes (23) has clearly outlined. It is somewhat unusual that, as yet, no trace of cycloartanyl esters has been found in this fraction.

For these latter peaks, some effects of chemical structure on elution order can be seen. Δ^7 avenasteryl linoleate elutes at $k' = 4.0$, whereas Δ^5 avenasteryl linoleate elutes at $k' = 2.8$. A 4-methyl steryl ester elutes before the equivalent desmethyl ester. A 24-methylene steryl ester is retained longer than the equivalent 24-ethylidene ester.

Soybean oil NPM. From LC retention data obtained on corn oil NPM, we expected Peak IV in Figure 2 to

TABLE 7

Soybean Oil NPM - Transmethylation Analyses

Relative fatty acid Composition (%)		Peak V	VI	VII	VIII	X	XI	XIII	Intact NPM
16:0		37.9	12.5	1.8	2.7	2.2	-	-	2.4
18:0		14.2	2.6	-	3.2	-	-	-	2.1
18:1		11.6	66.9	3.0	4.2	19.6	7.6	-	8.5
18:2		0.6	0.9	86.1	35.6	67.5	85.6	82.3	46.0
18:3[a]		12.1	0.9	-	40.7	8.3	-	17.3	4.5
20:0		4.	1.1	-	3.3	-	-	-	2.7
22:0		10.2	4.1	2.4	5.0	-	-	-	18.9
23:0		2.6	1.7	1.3	1.1	-	-	-	5.3
24:0		5.1	4.0	5.5	3.6	2.4	-	-	9.5
RRT[b]	Relative sterol composition Sterol	(%)							
0.66		-	-	-	-	-	-	-	1.0
0.72		-	-	0.4	-	-	-	-	0.6
0.86		-	-	-	3.1	-	-	-	0.8
0.95	Campesterol/ dihydrobrassica-sterol	15.5	20.4	15.4	13.8	-	-	-	13.3
0.97	Stigmasterol	5.8	7.6	7.5	61.8	-	-	-	7.2
0.99		-	-	-	-	8.1	-	-	-
1.00	Sitosterol	78.7	72.1	76.7	-	4.5	6.8	-	70.9
1.02		-	-	-	21.3	5.9	8.9	-	-
1.03	Isofucosterol	-	-	-	-	81.5	6.8	-	4.1
1.04		-	-	-	-	-	8.9	16.8	1.7
1.06		-	-	-	-	-	-	80.3	-
1.09		-	-	-	-	-	77.2	2.9	-

[a]Methyl esters of C18:3, C21:0 co-elute with phytol; total response reported.
[b]Retention time relative to sitosterol (RT ca. 23 min).

contain the typical Δ^5 steryl esters with saturated fatty acid moiety. Instead, we find a distribution of saturated phytyl esters. Table 4 lists the proposed ^{13}C NMR chemical shifts for the phytyl moiety of the esters. The spectra show no unsaturation typical of fatty acids; the carbonyl carbon is at 173.80 ppm, compared with 173.12 for Δ^5 steryl ester and 173.96 for a straight chain wax ester (behenyl arachidate) analyzed as a model compound. Transmethylation analyses, as well as high temperature GC methods, allow an estimate of the intact ester distribution which is discussed later.

From qualitative studies using high temperature GC, it is clear that most of the steryl/triterpenyl esters are typically of a C18 fatty acid, whereas a group of compounds with retention times similar to the phytyl esters are found in Peaks VII through X in Figure 2. Because the separations by normal phase LC system are based partially on unsaturation and because the fatty acids are clearly saturated, we propose that the unsaturation is in the alcohol portion of the ester. The ^{13}C NMR data suggest a polyisoprenyl structure for at least a portion of the above peak isolates. One structure consistent with the GC/LC/NMR data is a long chain fatty acid esterified with geranyl geranol. The proposed chemical shifts for the geranylgeranyl moiety are found in Table 5. Furthermore, capillary GC/CIMS shows responses ($M^{\cdot}+1$) corresponding to the exact masses of C20:0, C21:0, C22:0, C23:0 and C24:0 geranylgeranyl esters in equivalent peak isolates from this NPM fraction.

As with the corn oil NPM, the common esterified phytosterols are found in the soybean oil fraction. The general composition for each labeled peak in Figure 2 is as follows: V, phytyl esters plus sitosteryl, campesteryl/ihydrobrassicasteryl and stigmasteryl palmitates; VI, sitosteryl, campesteryl/dihydrobrassicasteryl and stigmasteryl oleates plus phytyl oleate; VII, sitosteryl, campesteryl/dihydrobrassicasteryl, stigmasteryl linoleates plus an unidentified 4,4 dimethyl species; VIII, geranylgeranyl esters of saturated fatty acids, sitosteryl linolenate, campesteryl/dihydrobrassicasteryl and stigmasteryl linolenate; IX, 24 methylene cycloartanyl linoleate, geranylgeranyl esters, unidentified 4,4 dimethyl species; X, 22 dihydrospinasteryl linoleate, geranylgeranyl esters; XI, citrostadienyl linoleate; XII, 24 methylenelophenyl (gramisteryl) linoleate; XIII, Δ^7 avenasteryl linoleate. The ^{13}C NMR data show a distribution of 4,4 dimethyl species eluting between Peaks VI and XI of which only one, 24-methylene cycloartanyl linoleate, has all carbon resonances completed assigned.

Sunflowerseed oil NPM. Sunflowerseed oil NPM is remarkably similar to soybean oil NPM. Using Figure

TABLE 8

Sunflowerseed Oil NPM - Transmethylation Analyses

Relative fatty acid composition		Peak IV	V	VI	VIII	IX	XI	Intact NPM
16:0		7.3	3.8	3.2	0.6	1.5	0.4	3.1
18:0		5.8	1.3	7.5	0.6	0.2	0.4	2.9
18:1		41.5	18.9	25.4	22.4	11.2	1.4	13.8
18:2		2.8	41.1	23.9	75.2	85.9	97.3	49.4
18:3[a]		20.1	2.7	0.4	-	1.0	0.5	5.0
20:0		1.9	0.7	5.5	-	-	-	2.5
22:0		9.8	13.4	27.0	-	-	-	12.2
23:0		2.7	2.6	1.7	-	-	-	6.3
24:0		4.7	12.5	5.5	-	-	-	4.5
Other		3.4	3.0	-	1.2	-	-	-
Relative sterol composition								
RRT[b]	Sterol	(%)						
0.84		-	1.3	-	-	-	-	-
0.90		0.5	-	-	-	1.2	-	-
0.92		0.4	-	-	-	-	-	-
0.95	Campesterol/ dihydrobrassica-sterol	11.0	9.1	-	-	2.7	-	5.0
0.97	Stigmasterol	3.8	2.5	4.5	0.8	0.7	-	3.0
1.00	Sitosterol	82.3	71.7	30.7	14.6	18.4	4.3	46.1
1.03	Isofucosterol	1.9	0.8	21.5	1.8	-	-	22.0
1.05		-	2.4	16.1	65.9	21.4	4.5	12.6
1.08		-	-	2.0	9.8	4.9	85.7	11.0
1.10		-	-	4.6	6.7	44.8	-	-
1.22		-	3.8	4.3	-	-	-	-

[a]Methyl esters of C18:3, C21:0 co-elute with phytol; total response reported.
[b]Retention time relative to sitosterol (RT ca. 23 min).

TABLE 9

^{13}C NMR Chemical Shifts[a] Integrals at 90.5 MHz Total NPM Fraction

Chemical shift	Carbon	Integral[b] Corn	Soybean	Sunflowerseed
61.15	Cl-Phytyl/ geranylgeranyl	0.027	0.531	0.748
73.14	C3-Δ^7 Sterol	0.032	0.118	0.510
73.67	C3-Δ^5 Sterol	1.000	1.000	1.000
78.16	C3-4 Methyl sterol	0.038	0.179	0.457
78.44	C3-4 Methyl sterol	0.006	0.050	0.326
80.32	C3-4,4 Dimethyl sterol	0.055	0.054	0.133
80.54	C3-4,4 Dimethyl sterol[c]	-	0.087	0.061
80.79	C3-4,4 Dimethyl sterol[c]	-	0.096	-

[a]PPM downfield from TMS in $CDCl_3$.
[b]Normalized relative to C3 —Δ^5 sterol.
[c]Tentative assignment.

3 as a reference, the general composition of sunflowerseed oil NPM is as follows: Peak III, saturated phytyl esters; Peak IV, sitosteryl, campesteryl/dihydrobrassicasteryl and stigmasteryl oleate, phytyl esters; Peak V, sitosteryl, campesteryl/dihydrobrassicasteryl and stigmasteryl linoleate plus phytyl/eranylgeranyl esters; Peak VI, geranylgeranyl esters of saturated fatty acids, 24 methylenelophenyl oleate, isofucosteryl linoleate; Peak VII, 24 methylene cycloartanyl linoleate; Peak VIII, 22 dihydrospinasteryl linoleate; Peak IX, citrostandienyl linoleate; Peak X, Δ^7 avenasteryl linoleate.

Comparisons. Although the similarities outnumber the differences for all three oils, it is clear that soybean

oil NPM and sunflowerseed oil NPM are more like each other than the equivalent corn oil fraction. In particular, corn oil NPM has a much simpler overall composition than the other two oils. Corn oil NPM is predominantly Δ^5 steryl esters, whereas sunflowerseed oil has a more varied distribution when relative weights are considered, especially in the 4 methyl Δ^7 distributions. Although some workers have reported antioxidant effects for various sterols, further investigation is required to assess the impact of steryl esters on overall oil stability, especially the 24-methylene isomers (24-25). Similarly, the level of linolenic acid esterified in the soybean oil NPM is substantially higher on a relative basis than that found in corn. It would seem from our current work that this fraction may be unique for each oil and may have value as an indicator of suspected contamination or misidentification.

Transmethylation and sterol analysis by GC probably is not an efficient means for detecting oil adulteration, because information is lost with regard to distribution and the lack of appropriate standards. NMR analysis of the intact nonpolar fraction might be more informative because the spectral region from 85 to 60 ppm contains diagnostic resonances most of which relate to oxygen bearing compounds. Table 9 shows a comparison of selected chemical shifts and integrals for that region. Distributions can be estimated if we assume equivalent relaxation times and nuclear Overhauser effects for the carbons of interest. Note that steryl C_3 carbons are methine, whereas the branched chain alcohol C_1 carbons are methylene. In addition to showing relative amounts of each class of compounds within an oil fraction, these data allow direct comparisons for the sterol species between oils for this particular sterol pool. Like the results from saponification reported by others, sunflowerseed oil NPM is relatively rich in several 4-methylsteryl esters while soybean oil NPM is relatively rich in the 4,4 dimethyl species and corn oil is by far the richest in Δ^5 species.

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