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ORIGINAL ARTICLE

Free and Esterified Sterol Composition of Edible Oils and Fats

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The free and esterified sterol concentrations in 31 edible oils and fats were determined, including individual values for sitosterol, campesterol, stigmasterol, brassicasterol, Δ^5-avenasterol, sitostanol, campestanol, and cholesterol. Free and esterified sterols were separated by solid-phase extraction (SPE), saponified, and quantified as trimethylsilyl ether derivatives using capillary gas–liquid chromatography. Considerable variability in the proportion of free and esterified sterols was observed among different oils and fats, with free sterols ranging from 32 to 94% of total sterols. Refining or hydrogenation tended to decrease total sterols and increase esterified sterols. Differences in total phytosterol content and the proportion of free and esterified sterols also were evident for different samples of some oils. Among four soybean oils, total phytosterols ranged from 205 to 287 mg/100 g, and free sterols constituted 68–81% of total sterols; two different shortenings differed 62% in total sterol content (185–301 mg/100 g). Such variability in phytosterol composition likely reflects differences in processing, growing season, or variety of a particular plant source.

Key Words: phytosterols; solid-phase extraction; lipids; food composition; hydrogenation; oil; refining; sterol esters; sterols; stanols; dihydrobrassicasterol.

INTRODUCTION

Phytosterols have been widely studied for their hypocholesterolemic, anticarcinogenic, and other health effects (Grundy and Mok, 1976; Pollack and Kritchevsky, 1981; Raicht *et al.*, 1980; Rao and Janezic, 1992; Pelletier *et al.*, 1995; Awad *et al.*, 2000). Sterols in plants exist as free alcohols, esters with fatty acids, glycosides, and acylated glycosides (Wojciechowski, 1991). In edible oils, sterols are primarily in the free and esterified forms. Free and esterified sterols may have different physiological effects, and the physical properties of free and esterified sterols also vary (Miettinen and Gylling, 1999). For example, steryl esters but not free sterols are readily soluble

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in oil–water mixtures (Jandacek *et al*., 1977). This characteristic of steryl esters has been capitalized upon in the preparation of sterol- and stanol-ester fortified margarines (Miettinen and Gylling, 1999). The esterified sterol content and minor sterol composition of commercial oils has also been used to detect adulteration (Miller and Gordon, 1995; Gordon and Griffith, 1992; Mandl *et al*., 1999).

Whereas data on the sterol content of many edible oils have been published (Itoh *et al*., 1973; Jeong *et al*., 1974; Weihrauch and Garndner, 1978), information about the free and esterified sterol composition is lacking. Typical analytical procedures for phytosterols involve saponification prior to gas-chromatographic analysis of individual sterols (Thompson and Merola, 1993; Reina *et al*., 1997; Toivo *et al*., 1998), and sterols that were esterified in the original material are not distinguished. Some of the available data on the free and esterified sterol composition of edible oils are for crude oilseed extracts, including sunflower kernel, poppy seed, rapeseed (Johansson and Appelqvist, 1978; Johansson, 1979a), soybean (Hirota *et al*., 1974), and peanut (Worthington and Hitchcock, 1984). These data may not apply to commercially available refined oils due to the effects of processing on sterol content and composition. For example, refining has been reported to decrease the total sterol content of crude oils and to increase the proportion of esterified sterols (Kochhar, 1983). Data on the free and esterified sterol content of refined and/or commercially distributed oils have been presented for soybean (Milkova *et al*., 1977a; Johansson, 1979b), corn (Worthington and Hitchcock, 1984; Milkova *et al*., 1977b), olive (Dimitrios and Ioanna, 1986; Grob *et al*., 1990), and sunflower (Milkova *et al*., 1977b) oils. Kochhar (1983) compiled data for several crude and refined oils, but many reports are dated and sterol values were from colorimetric, not chromatographic, analysis. Differences in analytical methods might also confound inter-study comparison of values for different oils. Many older studies involved thin-layer chromatographic separation and/or colorimetric quantification of sterols and lacked validation of quantitative analyte recovery. More recent work of Grob *et al*. (1990) and Plank and Lorbeer (1994) employed coupled LC-GC, and the equipment for this analysis might not be available in all laboratories.

The goal of the present study was to develop a simple, validated, method of separating free and esterified sterols and to apply that method to survey the free and esterified sterol composition of a variety of commercially available edible oils and fats.

MATERIALS AND METHODS

Samples

Samples were purchased from local retail outlets in Blacksburg, VA, except for the following. Five rapeseed oil samples were obtained from retail outlets in Finland. One olive oil sample was a composite of individual samples purchased from 20 retail outlets nationwide in the U.S.A., and one corn oil sample was a composite of individual samples from 24 retail outlets nationwide (Pehrsson *et al*., 2000). Crude and refined evening primrose and borage, palm, cottonseed, hydrogenated palm, hydrogenated cottonseed, and partially hydrogenated soybean oils were received from commercial suppliers, as indicated in Table 1. Multiple samples of some oil types, as well as some refined, unrefined, and hydrogenated oils and fats were assayed to evaluate potential variability in the sterol distribution. The crude and refined borage and evening primrose oils from the same source were taken from the same batches of raw material and so reflect changes in sterol composition due to the

TABLE 1

Description of samples

Oil sample	Brand/supplier	Description
Canola 1[1,2]	Smartbeat® (GFA Brands, Inc., Cresskill, NJ)	100% pure Canadian canola oil
Canola 2	Crisco (Procter & Gamble, Cincinnati, OH)	Canola oil (*Brassica napus*)
Rapeseed 1	Raisio Group, Finland	Kultasula rypsioljy (*Brassica rapa*)
Rapeseed 2	Van Den Bergh Foods, Finland	Rypsioljy (*Brassica rapa*)
Rapeseed 3	TukoSpar Oy, Finland	Eldorado rapsioljy (origin: Belgium) (*Brassica napus*)
Rapeseed 4	Suomen Spar Oyj, Finland	Spar rapsioljy (origin: Belgium) (*Brassica napus*)
Rapeseed, cold-pressed	Kankaisten Oljykasvit Oy, Finland	Virgino-rapsioljy (cold-pressed rapeseed oil) (*Brassica rapa*)
Soybean 1	Crisco (Procter & Gamble, Cincinnati, OH)	Soybean oil
Soybean 2	Wesson (Hunt-Wesson, Inc., Fullerton, CA)	Soybean oil
Soybean 3	C&T Refinery (Charlotte, NC)	"Soybean salad oil"
Soybean 4	Kroger (Cincinnati, OH)	Soybean oil
Soybean, partially hydrogenated	C&T Refinery (Charlotte, NC)	"Master Chef 727" partially hydrogenated soybean oil
Soybean oil margarine	Imperial® (Van Den Bergh Foods Co., Lisle, IL)	(70% fat). Vegetable oil blend (partially hydrogenated soybean oil and liquid soybean oil), whey, water, salt, vegetable mono- and diglycerides, soy lecithin, potassium sorbate, citric acid, artificial flavor, beta-carotene vitamin A palmitate
Peanut	Planter's (Nabisco, Inc., East Hanover, NJ)	Peanut oil
Peanut, cold-pressed	Spectrum Naturals (Petaluma, CA)	100% pure cold-pressed unrefined peanut oil
Corn 1	Mazola® (Best Foods Division, CPC International, Inc., Englewood Cliffs, NJ); single sample, locally purchased	Corn oil
Corn 2	Mazola®; nationwide composite from 24 retail outlets (Pehrsson *et al*., 2000)	Corn oil
Corn oil margarine	Kroger (Cincinnati, OH)	80% fat. Liquid and partially hydrogenated corn oil, water, salt, whey, soy lecithin, vegetable mono- and diglycerides, sodium benzoate, citric acid, artificial flavor, beta-carotene vitamin A palmitate
Olive 1	Bertolli Classico (Bertolli U.S.A., Inc., Secaucus, NJ); single sample, locally purchased	Cold-pressed olive oil, cotton-filtered
Olive 2	Bertolli Classico; nationwide composite of samples from 20 retail outlets (Pehrsson *et al*., 2000)	Cold-pressed olive oil, cotton-filtered
Olive, extra virgin	Bertolli extra virgin	Cold-pressed extra virgin olive oil, cotton-filtered
Coconut 1	Spectrum Naturals (Petaluma, CA)	100% refined coconut oil; no preservatives or additives
Coconut 2	C&T Refinery (Charlotte, NC)	"Ultimate 76 degree coconut oil" (non-hydrogenated)

TABLE 1 (Continued)

Oil sample	Brand/supplier	Description
Coconut, hydrogenated	C&T Refinery (Charlotte, NC)	"Ultimate 110 degree coconut oil"; fully hydrogenated coconut oil, with 2% hydrogenated soybean oil
Avocado	Tree of Life (St. Augustine, FL)	100% expeller-pressed avocado oil
Palm, hydrogenated	C&T Refinery (Charlotte, NC)	Partially hydrogenated palm oil
Palm	Famar N.A. (Needham, MA)	Palm oil
Sesame, extra-virgin	Loriva Supreme Foods, Inc. (Ronkonkoma, NY)	Extra virgin sesame oil
Sesame, toasted	Tree of Life (St. Augustine, FL)	Sesame oil
Safflower, unrefined	Spectrum Naturals (Petaluma, CA)	100% pure pressed unrefined high oleic variety safflower oil
Cottonseed	C&T Refinery (Charlotte, NC)	"Master Chef" cottonseed
Cottonseed, hydrogenated	C&T Refinery (Charlotte, NC)	"Master Chef 946"; partially hydrogenated cottonseed oil
Lard	Fischer's Packing Co. (Louisville, KY)	Lard, BHT & BHA added
Shortening 1	Kroger (Cincinnati, OH)	Partially hydrogenated soybean and cottonseed oils, mono- and diglycerides
Shortening 2	Crisco (Procter & Gamble, Cincinnati, OH)	Partially hydrogenated soybean and cottonseed oils, mono- and diglycerides
Evening primrose, crude 1	Albolier (Nieuwe-Tonge, Holland)	Expeller-pressed oil
Evening primrose, crude 2	VSP (Nieuwe-Tonge, Holland)	Expeller-pressed oil
Evening primrose, refined 1	Albolier (Nieuwe-Tonge, Holland)	Refined oil[3]
Evening primrose, refined 2	VSP (Nieuwe-Tonge, Holland)	Refined oil[3]
Borage, crude 1	Albolier (Nieuwe-Tonge, Holland)	Food-grade hexane extract
Borage, crude 2	VSP (Nieuwe-Tonge, Holland)	Food-grade hexane extract
Borage, refined 1	Albolier (Nieuwe-Tonge, Holland)	Refined oil[3]
Borage, refined 2	VSP (Nieuwe-Tonge, Holland)	Refined oil[3]
Sunflower	Kroger (Cincinnati, OH)	Sunflower oil
Sunflower, expeller-pressed	Hain Food Group, Inc. (Uniondale, NY)	Expeller-pressed sunflower oil, with mixed tocopherols added

[1]The names "canola" and "rapeseed" are retained to differentiate the European and North American samples. Both canola and rapeseed oils are from *Brassica rapa* or *B. napus*. All commercial rapeseed oils contain $<5\%$ erucic acid. By definition, "canola" oil contains $<2\%$ erucic acid and $<30\,\mu$mol glucosinolates per gram (*INFORM*, 1999). All of the canola and rapeseed samples assayed are low-erucic acid oils.

[2]Control sample, analyzed in each assay batch.

[3]Degummed with phosphoric acid; neutralized with caustic soda; bleached using earth and active carbon; deodorized under vacuum with steam at 185°C.

refining process. Since the goal of this study was to apply newly developed solid-phase extraction/gas-chromatographic method to survey a wide range of edible oils and fats, multiple samples of every oil type were not analyzed. Analysis of multiple samples and composites of selected oils was included to evaluate the potential variability within a given product and directions for further research.

Chemicals

Campesterol, stigmasterol, β-sitosterol, sitostanol, 5α-cholestane, dihydrocholesterol, pyrogallol, pyridine, and dimethyldichlorosilane (used to silanize test tubes) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Brassicasterol and epicholesterol were obtained from Stearoloids (Newport, RI, U.S.A.). Campestanol was from Research Plus (Bayonne, NJ, U.S.A.). Absolute ethanol was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KY, U.S.A.). Potassium hydroxide (ACS grade), and hexane (GC/HPLC/residue analysis grade) were obtained from Fisher Scientific (Fairlawn, NJ). *bis*(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane was from Alltech Associates (Deerfield, IL, U.S.A.). Cyclohexane and anhydrous diethyl ether were purchased from Aldrich (Milwaukee, WI, U.S.A.).

Sample Preparation

Silanized glass test tubes (25 mm × 150 mm) were pre-loaded with 50 μg each dihydrocholesterol and 5α-cholestane (0.5 mL each of 0.1 mg/mL ethanol solutions, evaporated to dryness in a 60°C water bath under a stream of nitrogen). The oil sample (100–200 mg) was weighed into each tube. For oils with β-sitosterol > 300 mg/100 g (determined by gas chromatography after direct saponification), a 100 mg aliquot was assayed; if β-sitosterol was < 300 mg/100 g, a 200 mg aliquot was assayed (in animal fat, cholesterol rather than sitosterol concentration was used to determine sample size). Solid fats were completely liquefied before aliquotting by heating in a capped container in a 50–60°C water bath for 15–20 min with occasional agitation. Extra care was taken to maintain homogeneity of these materials while taking the analytical aliquots. One milliliter of diethyl ether/hexane (DEE/HEX) 20:80 (v/v) was added to each sample and thoroughly mixed.

Solid-phase Extraction Cartridge Conditioning, Sample Loading, and Elution of Esterified Sterols

Neutral alumina solid-phase extraction (SPE) cartridges (Sep-Pak® Alumina N, 6 mL/1 g; Waters Chromatography, Milford, MA, U.S.A.), one per sample, were attached to a vacuum manifold (Visaprep DL™; Supelco, Bellefonte, PA, U.S.A.) fitted with a small vacuum pump. Waste tubes were positioned to collect the conditioning solvent. Cartridges were conditioned with 2 × 5 mL hexane, which was eluted just into the frit above the adsorbent, and were not allowed to run dry during the procedure. After conditioning, the hexane was discarded and a clean test tube was positioned to collect the esterified sterol fraction. The oil sample solutions were quantitatively transferred to the SPE cartridges by first pipetting the sample into the cartridge, eluting the solvent just into the frit above the adsorbent, and then following with 2 × 2.5 mL DEE/HEX rinses. These rinses were eluted one at a time from the SPE cartridge. The remaining esterified sterols were eluted with 2 × 5 mL DEE/HEX. The second 5 mL DEE/HEX was eluted to just above the frit on top of

the adsorbent. Vacuum was applied to slow-eluting samples as necessary to maintain dropwise throughput.

Elution of Free Sterols

A clean test tube was positioned to collect the free sterol fraction, and the solvent remaining in the cartridge was eluted just into the frit above the adsorbent. The free sterols were eluted with 3×5 mL 50:25:25 ethanol/hexane/diethyl ether (v/v/v) (ET/HEX/DEE). After elution of the third 5 mL ET/HEX/DEE, the remaining solvent was drawn through the SPE cartridge using maximum vacuum.

Saponification, Derivatization, and Gas Chromatography

Each fraction was quantitatively transferred into a 25 mm × 150 mm test tube containing 50 μg epicholesterol (internal standard) using two 1 mL hexane rinses. Samples were placed in a 60°C water bath, and the solvent was evaporated completely under a stream of nitrogen. Saponification (1.34 M potassium hydroxide/ 85 ± 4°C/20 min), derivatization to trimethylsilyl ethers, and gas chromatographic analysis were done as previously described (Phillips *et al.*, 1999), except in the present study the injector temperature was 280°C and the column flow rate was 0.75 mL/min (linear velocity = 25.6 cm/s) for GC analysis. Sitosterol, campesterol, stigmasterol, brassicasterol, Δ^5-avenasterol, sitostanol, campestanol, and cholesterol concentrations were measured.

When gas chromatography–mass spectrometry (GC–MS) was used to confirm peak identity, a Hewlett Packard 5790 GC and VG 7070EHF mass spectrometer [Micromass (formerly VG Analytical)] with electron impact ionization was employed under the following conditions: column: 30 m × 0.32 mm i.d. (5% phenyl/95% methylpolysiloxane) (HP5); helium carrier gas at 0.83 bar; injector: temperature 200°C, splitless; oven temperature: 75–280°C at 10°C/min.; ion source temperature: 200°C; electron energy: 70 eV; scan range: 50–600 amu at 1.5 s/decade.

Method Validation and Quality Control

Prior to routine use, the SPE separation of free and esterified sterols was validated by checking the recovery of standards added to oils at a concentration similar to that of analytes expected in the samples. Recovery experiments were performed with dihydrocholesterol, 5α-cholestane, cholesteryl palmitate and cholesteryl acetate as follows. Canola oil (50 mg) was weighed into separate test tubes each previously spiked with 50 μg of dihydrocholesterol, 5α-cholestane, and either cholesteryl palmitate or cholesteryl acetate. In addition, peanut oil was studied since it contained < 0.3 mg/100 g cholesterol based on analysis after direct saponification. Peanut oil (200 mg) was weighed into separate test tubes each previously spiked with 50 μg dihydrocholesterol, 5α-cholestane, and cholesteryl palmitate. All samples were prepared in duplicate and fractionated by SPE, saponified, derivatized, and analyzed by gas chromotography as described. Recovery of cholesterol from samples spiked with cholesteryl palmitate and cholesteryl acetate was adjusted by subtracting the contribution from indigenous cholesterol determined by analysis of unspiked samples.

In routine analyses, the following quality control measures were implemented. The SPE separation of each sample was validated by evaluating the recovery of the dihydrocholesterol and 5α-cholestane standards added prior to SPE (i.e. confirming that dihydrocholesterol eluted with the free sterol fraction and 5α-cholestane eluted

with the esterified sterol fraction). Each sample was analyzed in duplicate, usually in a separate assay batch. A quality control material (''Canola oil 1'', Table 1) was included with each group of samples to monitor run-to-run precision. For each oil, recovery of total free plus esterified sterols after SPE was compared to total sterols determined after direct saponification.

Each oil sample was assayed in duplicate, usually in a separate analytical batch. An estimate of the standard error of each mean was calculated using the data for duplicate measurements in all of the samples, as follows. For each component (e.g. free sitosterol, esterified sitosterol, free campesterol, esterified campesterol), an analysis of variance (PROC ANOVA) (SAS version 8.01; SAS Institute, Cary, NC) was performed using the model, Component = Oil, with class = Oil. An estimate of the standard error for duplicate measurement of the component in an individual oil was then calculated as the square root of (MSE/2), where MSE is the mean square error from the ANOVA.

RESULTS AND DISCUSSION

Free, Esterified, and Total Sterol Composition

Table 2 summarizes the concentration of free and esterified individual sterols and the total sterol content of each oil sample. The average composition of each oil and fat is illustrated in Figure 1. Crude evening primrose oil had the highest concentration of total sterols (1098 mg/100 g), and the hydrogenated palm oil sample had the lowest sterol content (58 mg/100 g) (Fig. 1(a)). As expected, cholesterol constituted 99% of total sterols in lard (Table 1). The proportion of free and esterified sterols varied widely. Figure 1(b) illustrates the sterol composition of the oils in order of increasing proportion of free sterols. In soybean, sesame, olive, cottonseed, coconut, palm, borage, safflower, expeller-pressed sunflower, shortening, and cold-pressed peanut oils, free sterols constituted 54–85% of total sterols. In lard, 94% of total cholesterol was free. On the other hand, in the canola, rapeseed, corn, refined peanut, avocado, evening primrose, and refined sunflower oils esterified sterols predominated, with free sterols representing only 32–44% of total sterols.

The data for soybean, corn, peanut, and olive oil were compared to values reported in the literature (Table 3). The average values of 76% of total free sterols and 24% esterified in refined soybean oils agree between the present study and that of Johansson (1979b). The average results for olive oil in our analysis agreed remarkably well with the average proportion of free and esterified sterols in eight Italian olive oils as determined by Grob *et al.* (1990) using LC-GC (Table 3). There was some difference in the sterol distribution in corn, sunflower, and olive oils found in the present study and by other researchers. Dimitrios and Ioanna (1986) found a much higher average level of free sterols in a variety of Greek olive oil total sterols (86%) compared to our results and those of Grob *et al.* (1990) (63%). There was also a discrepancy in the free sterol concentration of corn oil sterols in this study compared to that of Worthington and Hitchcock (1984) (Table 3). Interestingly, one corn oil sample assayed by the latter was the same brand (Mazola®) as the corn oil we analyzed. Values for free and esterified sterols (as percent total sterols) were 40 and 60% in the present work versus 21 and 79% (Worthington and Hitchcock, 1984), respectively. These inter-study discrepancies might be due to differences in plant variety, growing conditions, storage, processing, or assay methods. A lack of certified reference materials precludes the absolute determination of analytical accuracy and inter-laboratory bias, but the present study

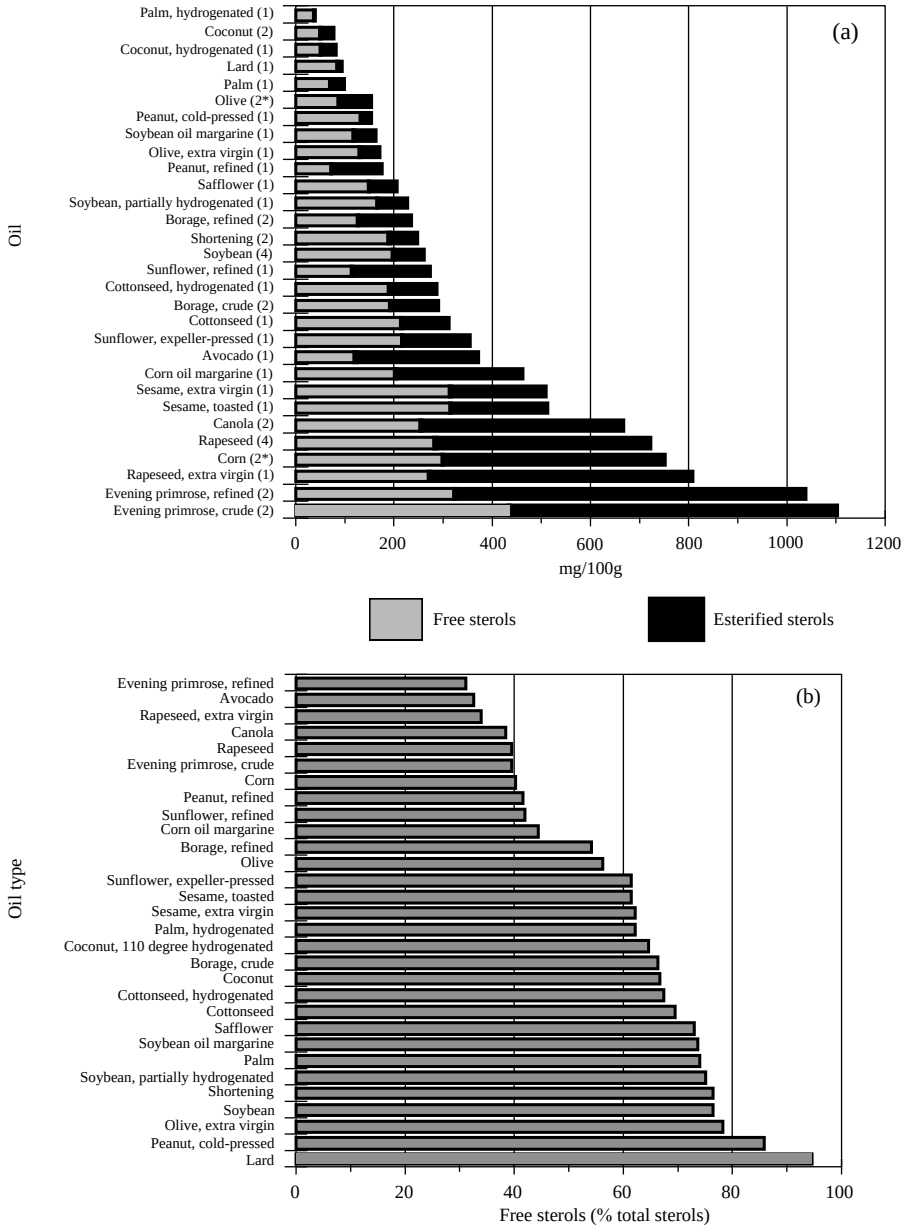

FIGURE 1. Sterol composition of edible oils and fats. (a) Total sterols in mg/100 g. (b) Total free sterols as percent of total sterols. The number of samples analyzed for each oil is shown in parentheses in (a); "*" indicates that one sample was a composite of 20–24 individual samples (see sample descriptions in Table 1).

as well as other work (Worthington and Hitchcock, 1984; Kochhar, 1983; Johansson, 1979b; Grob *et al.*, 1990; Dimitrios and Ioanna, 1986) indicates that variation does occur in the proportion of free and esterified sterols within individual samples of a particular oil type.

TABLE 2

Free and esterified sterol concentrations in edible oils and fats (mg/100 g)

Oil sample[1]		Sitosterol		Campesterol[2]		Stigmasterol		Brassicasterol		Δ5-Avenasterol		Sitostanol		Campestanol[3]		Cholesterol		Total sterols[4]
		Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	
Canola 1	Free	145.5	(3.41)	56.2	(1.70)	7.6	(0.33)	28.2	(0.84)	3.6	(0.20)	2.3	(0.10)	3.7	(0.77)	1.0	(0.08)	639
	Esterified	235.9	(5.10)	108.2	(3.38)	8.8	(0.26)	22.7	(0.65)	14.1	(0.72)	3.9	(0.19)	2.3	(0.43)	0.9	(0.07)	(*659*)
Canola 2	Free	133.3	(1.18)	63.2	(0.59)	2.6	(0.33)	44.4	(0.17)	1.3	(0.28)	0.7	(0.09)	2.5	(0.28)	1.4	(0.25)	658
	Esterified	224.4	(2.23)	136.4	(1.16)	nd	(0.11)	35.2	(0.20)	12.5	(1.16)	0.7	(0.13)	nd	(0.18)	1.5	(0.08)	(*668*)
Rapeseed 1	Free	117.4	(1.18)	63.2	(0.59)	2.0	(0.33)	35.7	(0.17)	6.3	(0.28)	0.5	(0.09)	nd	(0.28)	1.2	(0.25)	716
	Esterified	246.4	(2.23)	173.5	(1.16)	0.5	(0.11)	36.3	(0.20)	29.7	(1.16)	1.8	(0.13)	0.6	(0.18)	1.6	(0.08)	(713)
Rapeseed 2	Free	141.5	(1.18)	82.8	(0.59)	2.1	(0.33)	48.7	(0.17)	9.6	(0.28)	0.5	(0.09)	nd	(0.28)	1.4	(0.25)	736
	Esterified	231.8	(2.23)	154.8	(1.16)	<0.5	(0.11)	38.8	(0.20)	20.4	(1.16)	1.5	(0.13)	nd	(0.18)	1.6	(0.08)	(*730*)
Rapeseed 3	Free	162.2	(1.18)	74.8	(0.59)	3.4	(0.33)	56.3	(0.17)	4.8	(0.28)	0.7	(0.09)	nd	(0.28)	1.8	(0.25)	722
	Esterified	232.3	(2.23)	132.5	(1.16)	nd	(0.11)	35.5	(0.20)	15.2	(1.16)	1.4	(0.13)	0.6	(0.18)	1.2	(0.08)	(*721*)
Rapeseed 4	Free	167.8	(1.18)	77.0	(0.59)	3.5	(0.33)	57.6	(0.17)	3.8	(0.28)	0.7	(0.09)	nd	(0.28)	1.6	(0.25)	715
	Esterified	227.2	(2.23)	125.6	(1.16)	nd	(0.11)	33.9	(0.20)	13.3	(1.16)	1.4	(0.13)	0.6	(0.18)	1.5	(0.08)	(*709*)
Rapeseed, extra virgin	Free	139.3	(1.18)	75.0	(0.59)	1.8	(0.33)	37.4	(0.17)	6.6	(0.28)	2.6	(0.09)	nd	(0.28)	1.3	(0.25)	785
	Esterified	253.4	(2.23)	200.2	(1.16)	0.7	(0.11)	31.6	(0.20)	29.3	(1.16)	4.6	(0.13)	1.0	(0.18)	1.3	(0.08)	(*803*)
Corn 1	Free	172.2	(1.18)	53.2	(0.59)	18.2	(0.33)	nd	(0.17)	10.1	(0.28)	4.5	(0.09)	4.1*	(0.28)	0.7	(0.25)	699
	Esterified	290.8	(2.23)	70.9	(1.16)	34.1	(0.11)	nd	(0.20)	25.0	(1.16)	9.3	(0.13)	4.7*	(0.18)	0.8	(0.08)	(*732*)
Corn 2	Free	210.6	(1.18)	65.9	(0.59)	22.6	(0.33)	nd	(0.17)	13.1	(0.28)	5.9	(0.09)	5.5	(0.28)	0.9	(0.25)	766
	Esterified	298.8	(2.23)	73.0	(1.16)	35.9	(0.11)	nd	(0.20)	28.2	(1.16)	10.0	(0.13)	5.1	(0.18)	1.0	(0.08)	(*766*)
Corn oil margarine	Free	131.8	(1.18)	37.4	(0.59)	13.8	(0.33)	nd	(0.17)	4.7	(0.28)	5.1	(0.09)	4.2	(0.28)	0.9	(0.25)	442
	Esterified	174.8	(2.23)	37.3	(1.16)	18.3	(0.11)	nd	(0.20)	10.0	(1.16)	7.4	(0.13)	3.4	(0.18)	0.5	(0.08)	(*457*)
Soybean 1	Free	117.9	(1.18)	48.7	(0.59)	56.2	(0.33)	0.7	(0.17)	2.4	(0.28)	4.1	(0.09)	2.4	(0.28)	0.9	(0.25)	285
	Esterified	40.3	(2.23)	6.4	(1.16)	4.0	(0.11)	nd	(0.20)	2.4	(1.16)	0.7	(0.13)	nd	(0.18)	nd	(0.08)	(*297*)
Soybean 2	Free	76.4	(1.18)	26.2	(0.59)	30.7	(0.33)	nd	(0.17)	1.4	(0.28)	2.9	(0.09)	1.6	(0.28)	0.6	(0.25)	203
	Esterified	47.5	(2.23)	8.0	(1.16)	6.2	(0.11)	nd	(0.20)	2.8	(1.16)	1.0	(0.13)	nd	(0.18)	nd	(0.08)	*(210)*
Soybean 3	Free	95.9	(1.18)	35.0	(0.59)	45.0	(0.33)	nd	(0.17)	3.2	(0.28)	3.5	(0.09)	2.1	(0.28)	0.7	(0.25)	239
	Esterified	39.6	(2.23)	5.5	(1.16)	4.3	(0.11)	nd	(0.20)	5.1	(1.16)	1.1	(0.13)	nd	(0.18)	<0.5	(0.08)	(*250*)

TABLE 2 (Continued)

Oil sample[1]		Sitosterol		Campesterol[2]		Stigmasterol		Brassicasterol		Δ5-Avenasterol		Sitostanol		Campestanol[3]		Cholesterol		Total sterols[4]
		Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	
Soybean 4	Free	101.1	(1.18)	46.4	(0.59)	45.8	(0.33)	nd	(0.17)	3.2	(0.28)	3.6	(0.09)	2.8	(0.28)	1.1	(0.25)	257
	Esterified	43.6	(2.23)	5.9	(1.16)	4.3	(0.11)	nd	(0.20)	2.6	(1.16)	nd	(0.13)	nd	(0.18)	nd	(0.08)	(*274*)
Soybean oil margarine	Free	62.5	(1.18)	23.8	(0.59)	28.4	(0.33)	nd	(0.17)	2.5	(0.28)	2.7	(0.09)	1.4	(0.28)	0.8	(0.25)	164
	Esterified	32.4	(2.23)	4.3	(1.16)	3.4	(0.11)	nd	(0.20)	2.9	(1.16)	0.7	(0.13)	0.6	(0.18)	nd	(0.08)	(*158*)
Soybean, partially hydrogenated	Free	81.7	(1.18)	30.4	(0.59)	38.6	(0.33)	nd	(0.17)	3.2	(0.28)	3.3	(0.09)	1.8	(0.28)	0.6	(0.25)	212
	Esterified	38.4	(2.23)	5.3	(1.16)	4.5	(0.11)	nd	(0.20)	5.0	(1.16)	1.0	(0.13)	nd	(0.18)	<0.5	(0.08)	(*221*)
Shortening 1	Free	67.6	(1.18)	28.8	(0.59)	27.3	(0.33)	0.5	(0.17)	2.3	(0.28)	2.4	(0.09)	1.6	(0.28)	2.1	(0.25)	183
	Esterified	36.6	(2.23)	7.4	(1.16)	3.7	(0.11)	nd	(0.20)	2.8	(1.16)	0.9	(0.13)	0.7	(0.18)	0.6	(0.08)	(*198*)
Shortening 2	Free	135.3	(1.18)	45.5	(0.59)	50.6	(0.33)	<0.5	(0.17)	3.0	(0.28)	4.5	(0.09)	2.4	(0.28)	0.9	(0.25)	298
	Esterified	43.2	(2.23)	5.9	(1.16)	3.8	(0.11)	nd	(0.20)	3.7	(1.16)	1.0	(0.13)	0.6	(0.18)	<0.5	(0.08)	(*294*)
Safflower	Free	86.4	(1.18)	18.4	(0.59)	12.7	(0.33)	nd	(0.17)	14.7	(0.28)	6.9	(0.09)	1.7	(0.28)	nd	(0.25)	192
	Esterified	34.3	(2.23)	9.8	(1.16)	2.1	(0.11)	nd	(0.20)	3.6	(1.16)	2.7	(0.13)	0.9	(0.18)	nd	(0.08)	(*204*)
Peanut	Free	47.2	(1.18)	8.3	(0.59)	8.1	(0.33)	0.6	(0.17)	3.0	(0.28)	1.6	(0.09)	0.9	(0.28)	<0.5	(0.25)	167
	Esterified	67.8	(2.23)	15.4	(1.16)	3.9	(0.11)	0.6	(0.20)	9.9	(1.16)	0.9	(0.13)	<0.5	(0.18)	<0.5	(0.08)	(*171*)
Peanut, cold-pressed	Free	83.9	(1.18)	11.9	(0.59)	13.4	(0.33)	nd	(0.17)	13.9	(0.28)	2.0	(0.09)	1.2	(0.28)	nd	(0.25)	147
	Esterified	15.1	(2.23)	3.4	(1.16)	nd	(0.11)	nd	(0.20)	3.1	(1.16)	nd	(0.13)	nd	(0.18)	nd	(0.08)	(*153*)
Avocado	Free	101.4	(1.18)	7.5	(0.59)	2.5	(0.33)	nd	(0.17)	0.5	(0.28)	1.8	(0.09)	0.7	(0.28)	nd	(0.25)	353
	Esterified	218.7	(2.23)	10.5	(1.16)	0.5	(0.11)	nd	(0.20)	7.7	(1.16)	1.9	(0.13)	nd	(0.18)	nd	(0.08)	(*367*)
Lard	Free	nd	(1.18)	0.5	(0.59)	nd	(0.33)	<0.5	(0.17)	<0.5	(0.28)	nd	(0.09)	0.5	(0.28)	85.7	(0.25)	91
	Esterified	nd	(2.23)	nd	(1.16)	nd	(0.11)	nd	(0.20)	<0.5	(1.16)	nd	(0.13)	nd	(0.18)	5.3	(0.08)	(*89*)
Sesame, extra virgin	Free	217.3	(1.18)	36.3	(0.59)	24.1	(0.33)	nd	(0.17)	24.5	(0.28)	1.1	(0.09)	1.6	(0.28)	nd	(0.25)	492
	Esterified	113.9	(2.23)	38.2	(1.16)	8.9	(0.11)	nd	(0.20)	26.9	(1.16)	0.7	(0.13)	nd	(0.18)	nd	(0.08)	(*506*)
Sesame, toasted	Free	221.0	(1.18)	36.3	(0.59)	26.9	(0.33)	nd	(0.17)	22.8	(0.28)	1.1	(0.09)	1.1	(0.28)	nd	(0.25)	504
	Esterified	114.4	(2.23)	40.1	(1.16)	9.8	(0.11)	nd	(0.20)	30.6	(1.16)	0.9	(0.13)	nd	(0.18)	nd	(0.08)	(*511*)
Olive 1	Free	70.3	(1.18)	2.2	(0.59)	1.6	(0.33)	nd	(0.17)	6.7	(0.28)	1.8	(0.09)	0.7	(0.28)	<0.5	(0.25)	
	Esterified	52.0	(2.23)	2.1	(1.16)	1.1	(0.11)	nd	(0.20)	9.4	(1.16)	1.6	(0.13)	nd	(0.18)	<0.5	(0.08)	(150)
Olive 2	Free	74.0	(1.18)	2.3	(0.59)	1.4	(0.33)	nd	(0.17)	7.7	(0.28)	1.5	(0.09)	0.7	(0.28)	<0.5	(0.25)	156
	Esterified	55.2	(2.23)	2.0	(1.16)	0.9	(0.11)	nd	(0.20)	9.5	(1.16)	1.3	(0.13)	nd	(0.18)	<0.5	(0.08)	(*155*)

Olive, extra virgin	Free	105.5	(1.18)	3.4	(0.59)	0.9	(0.33)	nd	(0.17)	15.2	(0.28)	0.9	(0.09)	0.7	(0.28)	<0.5	(0.25)	162
	Esterified	27.1	(2.23)	1.1	(1.16)	nd	(0.11)	nd	(0.20)	6.6	(1.16)	0.9	(0.13)	nd	(0.18)	<0.5	(0.08)	(*166*)
Cottonseed	Free	180.7	(1.18)	11.7	(0.59)	4.3	(0.33)	nd	(0.17)	3.1	(0.28)	1.7	(0.09)	0.9	(0.28)	0.7	(0.25)	292
	Esterified	75.4	(2.23)	8.5	(1.16)	0.7	(0.11)	nd	(0.20)	4.4	(1.16)	1.2	(0.13)	nd	(0.18)	nd	(0.08)	(*308*)
Cottonseed, hydrogenated	Free	151.4	(1.18)	12.4	(0.59)	7.3	(0.33)	nd	(0.17)	2.6	(0.28)	1.7	(0.09)	1.0	(0.28)	0.6	(0.25)	263
	Esterified	72.6	(2.23)	8.1	(1.16)	1.1	(0.11)	nd	(0.20)	4.3	(1.16)	1.2	(0.13)	nd	(0.18)	nd	(0.08)	(*280*)
Coconut 1	Free	30.0	(1.18)	3.7	(0.59)	8.3	(0.33)	nd	(0.17)	6.2	(0.28)	0.5	(0.09)	1.5	(0.28)	<0.5	(0.25)	70
	Esterified	12.2	(2.23)	2.0	(1.16)	2.8	(0.11)	nd	(0.20)	4.7	(1.16)	<0.5	(0.13)	nd	(0.18)	<0.5	(0.08)	(*71*)
Coconut 2	Free	24.1	(1.18)	3.2	(0.59)	7.1	(0.33)	nd	(0.17)	8.5	(0.28)	nd	(0.09)	1.4	(0.28)	<0.5	(0.25)	69
	Esterified	13.8	(2.23)	2.2	(1.16)	3.5	(0.11)	nd	(0.20)	6.2	(1.16)	nd	(0.13)	nd	(0.18)	<0.5	(0.08)	(*74*)
Coconut, hydrogenated	Free	24.4	(1.18)	4.6	(0.59)	5.0	(0.33)	nd	(0.17)	9.6	(0.28)	2.9	(0.09)	1.2	(0.28)	<0.5	(0.25)	73
	Esterified	15.4	(2.23)	2.7	(1.16)	3.0	(0.11)	nd	(0.20)	4.6	(1.16)	0.6	(0.13)	nd	(0.18)	<0.5	(0.08)	(*77*)
Palm	Free	28.7	(1.18)	10.7	(0.59)	6.5	(0.33)	nd	(0.17)	1.0	(0.28)	<0.5	(0.09)	nd		1.0	(0.25)	66
	Esterified	10.8	(2.23)	4.1	(1.16)	2.2	(0.11)	nd	(0.20)	0.6	(1.16)	<0.5	(0.13)	nd		0.5	(0.08)	(*93*)
Palm, hydrogenated	Free	17.7	(1.18)	5.6	(0.59)	4.0	(0.33)	nd	(0.17)	<0.5	(0.28)	nd	(0.09)	3.3	(0.28)	1.0	(0.25)	48
	Esterified	12.0	(2.23)	4.0	(1.16)	2.5	(0.11)	nd	(0.20)	0.5	(1.16)	nd	(0.13)	<0.5	(0.18)	0.5	(0.08)	(*58*)
Evening primrose, crude 1	Free	368.9	(1.18)	23.4	(0.59)	4.4	(0.33)	nd	(0.17)	12.2	(0.28)	9.1	(0.09)	6.3*	(0.28)	1.3	(0.25)	1087
	Esterified	493.2	(2.23)	47.4	(1.16)	nd	(0.11)	nd	(0.20)	108.6	(1.16)	7.4	(0.13)	4.6*	(0.18)	nd	(0.08)	(*1107*)
Evening primrose, crude 2	Free	377.7	(1.18)	23.9	(0.59)	4.9	(0.33)	nd	(0.17)	13.3	(0.28)	9.0	(0.09)	6.1	(0.28)	nd	(0.25)	1087
	Esterified	499.0	(2.23)	47.1	(1.16)	nd	(0.11)	nd	(0.20)	105.0	(1.16)	7.1	(0.13)	4.8	(0.18)	nd	(0.08)	(*1094*)
Evening primrose, refined 1	Free	260.6	(1.18)	15.9	(0.59)	3.4	(0.33)	nd	(0.17)	6.8	(0.28)	7.2	(0.09)	4.4	(0.28)	0.8	(0.25)	998
	Esterified	526.9	(2.23)	53.1	(1.16)	nd	(0.11)	nd	(0.20)	116.3	(1.16)	7.4	(0.13)	5.0	(0.18)	nd	(0.08)	(*1037*)
Evening primrose, refined 2	Free	292.6	(1.18)	18.8	(0.59)	3.6	(0.33)	nd	(0.17)	7.2	(0.28)	7.6	(0.09)	4.6	(0.28)	nd	(0.25)	1026
	Esterified	521.8	(2.23)	51.6	(1.16)	nd	(0.11)	nd	(0.20)	115.5	(1.16)	6.8	(0.13)	5.0	(0.18)	nd	(0.08)	(*1034*)
Borage, crude 1	Free	76.4	(1.18)	48.8	(0.59)	7.5	(0.33)	nd	(0.17)	50.8	(0.28)	2.6	(0.09)	1.9	(0.28)	1.3	(0.25)	282
	Esterified	32.0	(2.23)	34.8	(1.16)	1.3	(0.11)	nd	(0.20)	25.1	(1.16)	1.2	(0.13)	0.7	(0.18)	<0.5	(0.08)	(*292*)
Borage, crude 2	Free	75.6	(1.18)	48.3	(0.59)	7.5	(0.33)	nd	(0.17)	50.2	(0.28)	2.2	(0.09)	1.4	(0.28)	1.2	(0.25)	281
	Esterified	32.2	(2.23)	35.5	(1.16)	1.4	(0.11)	nd	(0.20)	26.0	(1.16)	1.4	(0.13)	0.7	(0.18)	<0.5	(0.08)	(*287*)
Borage, refined 1	Free	59.3	(1.18)	33.5	(0.59)	5.3	(0.33)	nd	(0.17)	20.1	(0.28)	1.6	(0.09)	1.7	(0.28)	0.9	(0.25)	226
	Esterified	40.5	(2.23)	36.4	(1.16)	1.3	(0.11)	nd	(0.20)	25.6	(1.16)	1.3	(0.13)	nd	(0.18)	<0.5	(0.08)	(*234*)

TABLE 2 (Continued)

Oil sample[1]		Sitosterol		Campesterol[2]		Stigmasterol		Brassicasterol		Δ5-Avenasterol		Sitostanol		Campestanol[3]		Cholesterol		Total sterols[4]
		Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	Mean[5]	S.E.[6]	
Borage, refined 2	Free	59.3	(1.18)	33.4	(0.59)	5.3	(0.33)	nd	(0.17)	20.1	(0.28)	1.6	(0.09)	1.7	(0.28)	0.9	(0.25)	225
	Esterified	39.8	(2.23)	36.2	(1.16)	1.3	(0.11)	nd	(0.20)	25.7	(1.16)	1.4	(0.13)	nd	(0.18)	<0.5	(0.08)	(*232*)
Sunflower	Free	79.34	(1.18)	9.61	(0.59)	12.99	(0.33)	0.6	(0.17)	4.7	(0.28)	1.0	(0.09)	1.2	(0.28)	<0.5	(0.25)	263
	Esterified	114.84	(2.23)	17.48	(1.16)	4.7	(0.11)	1.5	(0.20)	14.0	(1.16)	1.9	(0.13)	nd	(0.18)	<0.5	(0.08)	(*270*)
Sunflower, expeller-pressed	Free	145.10	(1.18)	21.08	(0.59)	28.27	(0.33)	nd	(0.17)	11.2	(0.28)	2.0	(0.09)	nd	(0.28)	<0.5	(0.25)	340
	Esterified	100.26	(2.23)	12.42	(1.16)	3.5	(0.11)	nd	(0.20)	14.0	(1.16)	1.9	(0.13)	nd	(0.18)	<0.5	(0.08)	(*352*)

[1]See Table 1 for sample descriptions.

[2]24-methylcholesterol; palm oil (only) was assayed by HPLC-^{1}HNMR (Giner *et al.*, 2001) and the "campesterol" peak was a mixture of campesterol (67%) and dihydroliassicasterol (33%).

[3]Identity for values marked with * was confirmed by GC–MS (see text).

[4]Total free plus total esterified sterols, with total sterols after direct saponification shown in parentheses. Total sterols = sitosterol + campesterol + stigmasterol + sitostanol + campestanol + brassicasterol + delta-5-avenasterol + cholesterol.

[5]nd = not detected.

[6]Standard error, except for Canola 1, for which the value in parentheses is the standard deviation ($n = 22$).

TABLE 3

Comparison of free and esterified sterol composition determined in the present study to literature data

	Present study (% total sterols)[1]		*Literature* (% total sterols)[1]		
Oil	Free	Esterified	Free	Esterified	References
Corn	40	60 (25)	23	77 (2)	Worthington and Hitchcock (1984)
Peanut	63	37 (2)	70	30 (2)	Worthington and Hitchcock (1984)
Soybean	76	24 (4)	76	24 (2)	Johansson (1979b)
Olive	63	37 (21)	86	14 (6)	Dimitrios and Ioanna (1986)
			63	37 (8)	Grob *et al.* (1990)
Sunflower (unrefined)	61	39 (1)[2]	55	45 (1)[3]	Johansson (1979a)

[1]Each value is the average across the number of samples indicated in parentheses.
[2]Expeller-pressed (commercially prepared).
[3]Hexane/ethanol extract (prepared in the laboratory).

Individual Sterols

As expected, the predominant phytosterols in all vegetable oils and fats were sitosterol and campesterol, and only rapeseed/canola oils contained more than a trace of brassicasterol. Only a trace of cholesterol (<3 mg/100 g) was found in all oils and fats, except of course in lard (91 mg/100 g). Δ^5-avenasterol was present in all samples in small amounts, but borage, sesame, and evening primrose oils contained substantially more than other oils (46–76, 51–53, and 118–123 mg/100 g, respectively). Low concentrations (<5 mg/100 g) of sitostanol and campestanol were present in all of the samples but a relatively higher concentration of sitostanol occurred in corn and crude evening primrose oils (14–16 mg/100 g). Since palm, corn and evening primrose oils appeared to have a comparatively large campestanol content by GC-FID, one sample of each of these oil types was also assayed by GC–MS. In corn and evening primrose oils, GC–MS verified the compound to be campestanol, but in palm oil, the component was not a sterol.

The distribution of sitosterol between the free and esterified sterol fractions essentially parallels the pattern for total sterols. This result is not surprising since sitosterol represents 38–91% of total sterols in the vegetable oils and fats. Similar results were seen for campesterol, except in refined borage oil and the sesame oils. In these cases, campesterol was predominately esterified while the majority of total sterols were free. Interestingly, in all samples but corn oil, corn oil margarine, peanut oil, and one canola oil sample, most of the stigmasterol was free regardless of the overall proportion of free and esterified sterols. This finding for soybean oil is inconsistent with that of Naudet *et al.* in 1973 (Naudet *et al.*, 1983), who found stigmasterol to be primarily esterified in soybean oils, regardless of processing conditions. In the rapeseed/canola oils, brassicasterol was predominately free, while overall most of the sterols were esterified. In lard, 94% of the cholesterol was unesterified. In all oils except crude borage, unrefined safflower, extra virgin olive, coconut (hydrogenated and non-hydrogenated), and cold-pressed peanut oils, most of the Δ^5-avenasterol was esterified, despite the overall distribution of free and esterified sterols.

An increase in the concentration of the saturated sterols (sitostanol and campestanol) might be expected in hydrogenated versus non-hydrogenated samples of the same oil. However, the present results show similar sitostanol and campestanol levels in the hydrogenated and non-hydrogenated soybean and

cottonseed oils which were from the same source (C&T Refinery). Though small amounts (< 5 mg/100 g) of the saturated sterols were found in the hydrogenated oils, shortening, and margarine samples, the highest concentrations occurred in some of the non-hydrogenated oils (corn, crude evening primrose, and palm; Table 2). The saturation of sterols during vegetable oil hydrogenation might depend on the specific procedure used, including the type of catalyst (Parodi, 1975).

Comparison of Crude and Refined Oils

Two evening primrose oil samples and two borage oil samples were received from the supplier in both crude and refined form (Table 1). In each case, refining reduced the total sterol and free sterol contents, while the concentration of esterified sterols increased. A similar trend was evident when comparing the composition of retail oils of the same type but from different sources. Figure 2 illustrates the free, esterified, and total sterol content of all oils and fats for which crude and refined samples were assayed. The mean reduction in total and free sterols for the six oil types was 9 and 31%, respectively. These findings are consistent with reports that refining (degumming, neutralization, bleaching, deodorization, and steam distillation) removes sterols from crude oils, and that interesterification of free sterols and preferential removal of free sterols during distillation also occur (Kochhar, 1983). Interestingly, the refined peanut oil in the present study had an only slightly higher total sterol content than the cold-pressed oil (168 versus 148 mg/100 g), though the latter still contained substantially more free sterols (126 versus 70 mg/100 g). Another exception was extra virgin rapeseed oil, which had a higher total sterol content than the average for the four refined rapeseed oil samples (786 versus 723 mg/100 g) but a lower free sterol content (264 versus 282 mg/100 g). These deviations from the overall

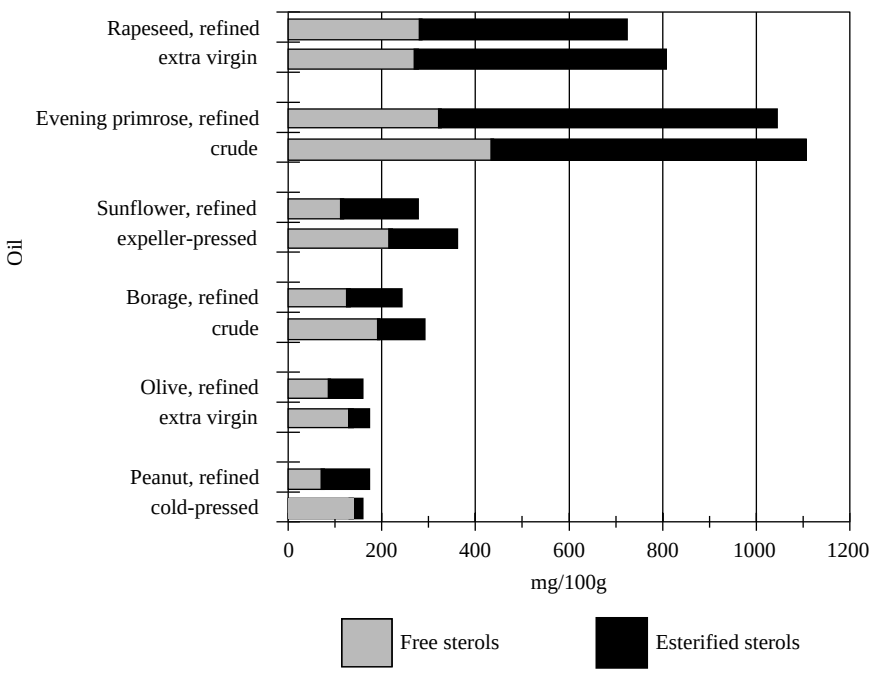

FIGURE 2. Sterols in crude and refined oils.

trend of less free sterols and less total sterols in refined versus crude oils might be due to other causes of variability, since the crude and refined samples of each oil were not from the same source.

Hydrogenated Oils

Hydrogenated and non-hydrogenated samples of cottonseed, palm, soybean, coconut, and corn oils were analyzed (see Table 1). The cottonseed and soybean oils in hydrogenated and non-hydrogenated form were obtained at the same time from the same source (C&T Refinery). Figure 3 shows results for the total sterol and free sterol content of these oils and fats. In both cases, hydrogenation decreased the total sterol and free sterol concentrations. In the cottonseed and soybean oils, total sterols in the hydrogenated samples were reduced by 9 and 12%, respectively, and free sterols decreased by 12 and 14%. An analysis of variance comparing mean free sterols in the hydrogenated and non-hydrogenated samples, the mean esterified sterols in the hydrogenated and non-hydrogenated samples, and the mean total (free plus esterified sterols) in the hydrogenated and non-hydrogenated samples for each of soybean and cottonseed oils confirmed the difference in the means to be significant ($P < 0.03$; $\alpha = 0.05$) for all but the esterified sterols in the soybean oils ($P = 0.33$ at $\alpha = 0.05$).

Differences Among Samples of the Same Oil Type

Two to four samples each of canola, rapeseed, corn, soybean, olive, crude and refined evening primrose, crude and refined borage oils and shortening were assayed,

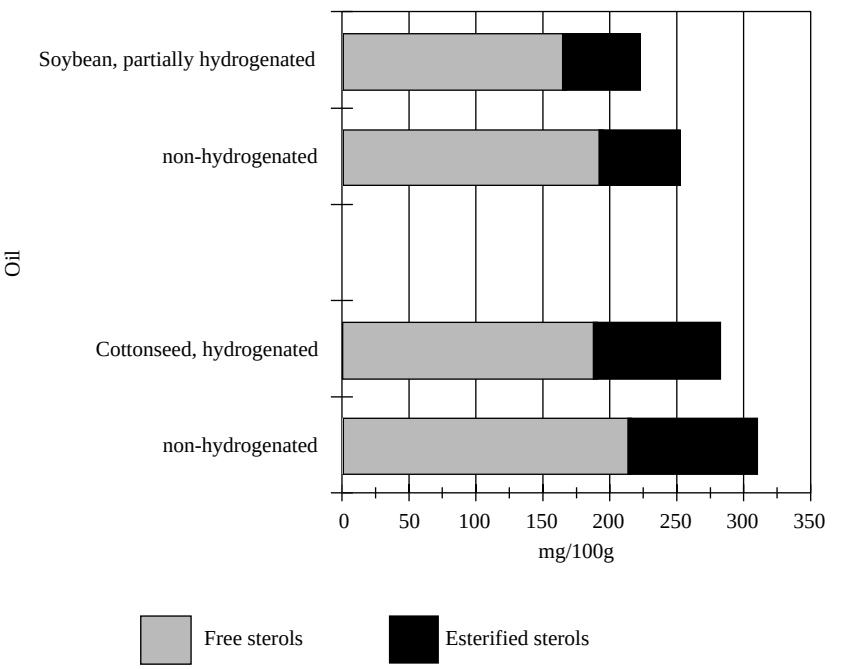

FIGURE 3. Sterol composition of hydrogenated and non-hydrogenated oils from the same source.

and the results are illustrated in Figure 4. In most cases, the samples were from different sources (see Table 1). For corn and olive oils, however, multiple samples of the same brand/type were assayed: one locally procured sample and one nationwide composite of samples from 20 to 24 retail outlets (Table 1). In the olive, sesame, canola, borage, and evening primrose oils, the total, free, and esterified sterol contents were similar among all samples of the same oil. Differences were observed, however, among the other oils. The two corn oil samples (same brand) differed, 77 mg/100 g in total sterols and 62 mg/100 g in free sterols. All four rapeseed oil samples had similar total sterol concentrations (716–736 mg/100 g), but one sample (Rapeseed 1, Fig. 3) had a notably lower level of free sterols than the other three (226 versus 287–312 mg/100 g). The two shortenings had markedly different sterol contents: 301 versus 185 mg/100 g total sterols and 242 versus 133 mg/100 g free sterols. The variability in sterol content and composition among different samples of the same type of oil is likely due to differences in processing, storage, growing season, or variety of a particular plant source.

We also compared the values for individual sterols in two of the rapeseed oil samples [Raisio Group, Finland and Van Den Bergh Foods, Finland (Table 1)] to concentrations determined on separate samples of the same brands in an independent study in 1996 that used a direct saponification/gas chromatography method validated by analysis of BCR 162 Soya-Maize Oil Reference Material (Toivo *et al*., 1998). The average total sterol concentrations (mg/100 g) for these two oils from the present study and Toivo *et al*. (1998), respectively, were: sitosterol, 368 and 373; campesterol, 237 and 272; stigmasterol, 2.5 and <2; brassicasterol, 80 and 54; Δ^5-avenasterol, 33 and 23; cholesterol, 3 and 5.

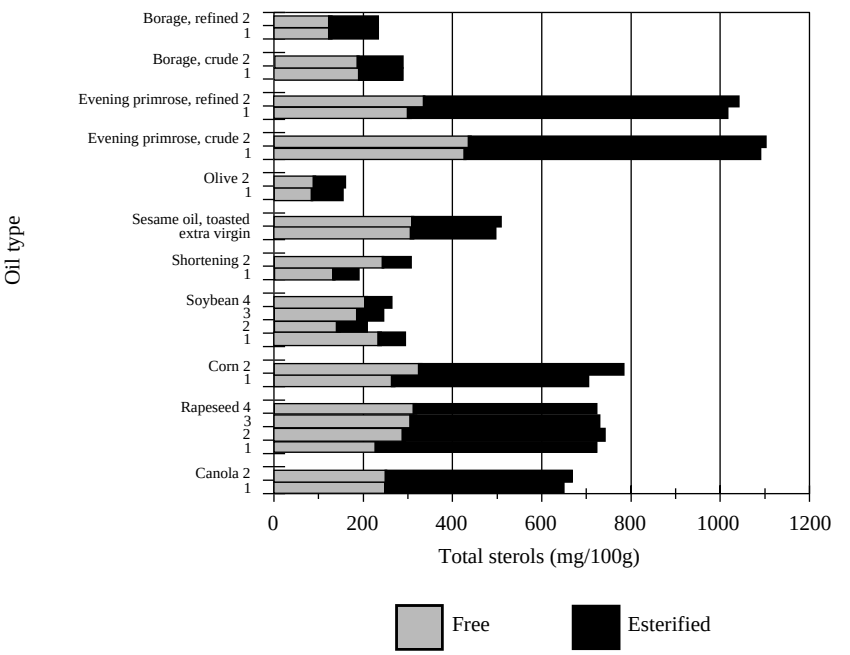

FIGURE 4. Total free and esterified sterol composition of multiple samples of the same oil type.

Quality Control Results

Table 4 summarizes the results from preliminary method validation studies on the recovery of standards spiked into canola and peanut oils. The results for dihydrocholesterol and 5α-cholestane suggest complete fractionation of polar and non-polar sterols into the ET/HEX/DEE (free sterol) and DEE/HEX (esterified sterol) fractions. The data for cholesterol recovered from added cholesteryl palmitate and cholesteryl acetate indicate essentially complete recovery of these components after SPE. The values for peanut oil might be more reliable since it contained virtually no endogenous cholesterol (<0.3 mg/100 g) compared to 1.6 mg/100 g in the canola oil.

The separation and recovery of added 5α-cholestane and dihydrocholesterol was used to validate the separation of free and esterified sterols. If $>1\%$ of the added dihydrocholesterol was found in the esterified sterol fraction or $>1\%$ of added 5α-cholestane was recovered with the free sterols (and no dihydrocholesterol or 5α-cholestane was evident in the corresponding directly saponified oil), data for that sample were rejected. None of the added 5α-cholestane was found in any of the free sterol fractions, and on an average 100% was recovered with the esterified sterols (range 82–111%; S.D., 4%). The only cases in which data were rejected for incomplete separation of dihydrocholesterol occurred early in the study when the SPE cartridges were suspected to have been exposed to ambient conditions for an extended time period. Probably water uptake interfered with the activity of the adsorbent. After precautions were taken to protect the cartridges from ambient moisture (e.g. opening a new packet immediately before each assay), fractionation was complete in all samples. On an average, 105% of the dihydrocholesterol was recovered with the free sterols (range 97–118%; S.D., 3.6%). Different factors could compromise the solid phase extraction, such as variability in adsorbent activity or elution solvent concentration. Since the SPE step solely differentiates free and esterified sterols in this method, it is critical to include the recovery surrogates, especially dihydrocholesterol.

Another quality control measure, applied to each sample, was comparison of total free plus esterified sterols determined after SPE to total sterols after direct saponification. For 110 samples, recovery of total free and esterified sterols averaged 98% of total sterols after direct saponification (S.D., 3%; range 89–105%). The slightly lower values after SPE could be attributed to the decreased sample size resulting after SPE fractionation of the oil, and consequent decreased overall

TABLE 4

Results for recovery of standards spiked into oils assayed by the proposed method. ET = ethanol; HEX = hexane; DEE = diethyl ether. Percent recovery[1]

Standard	Canola oil—free sterol fraction (ET/HEX/DEE)	Canola oil—esterified sterol fraction (DEE/HEX)	Peanut oil—free sterol fraction (ET/HEX/DEE)	Peanut oil—esterified sterol fraction (DEE/HEX)
Cholesteryl palmitate	1.6 (0.42)	95 (1.4)	0.05 (0.02)	92 (0.0)
Cholesteryl acetate	1.3 (0.42)	97 (0.0)	n/a[2]	n/a[2]
5α-Cholestane	nd[3]	102	nd[3]	105
Dihydrocholesterol	98 (0.8)	nd[3]	101 (0.0)	nd[3]

[1]Mean ($n=2$); S.D. in parentheses.
[2]Not determined.
[3]Not detected.

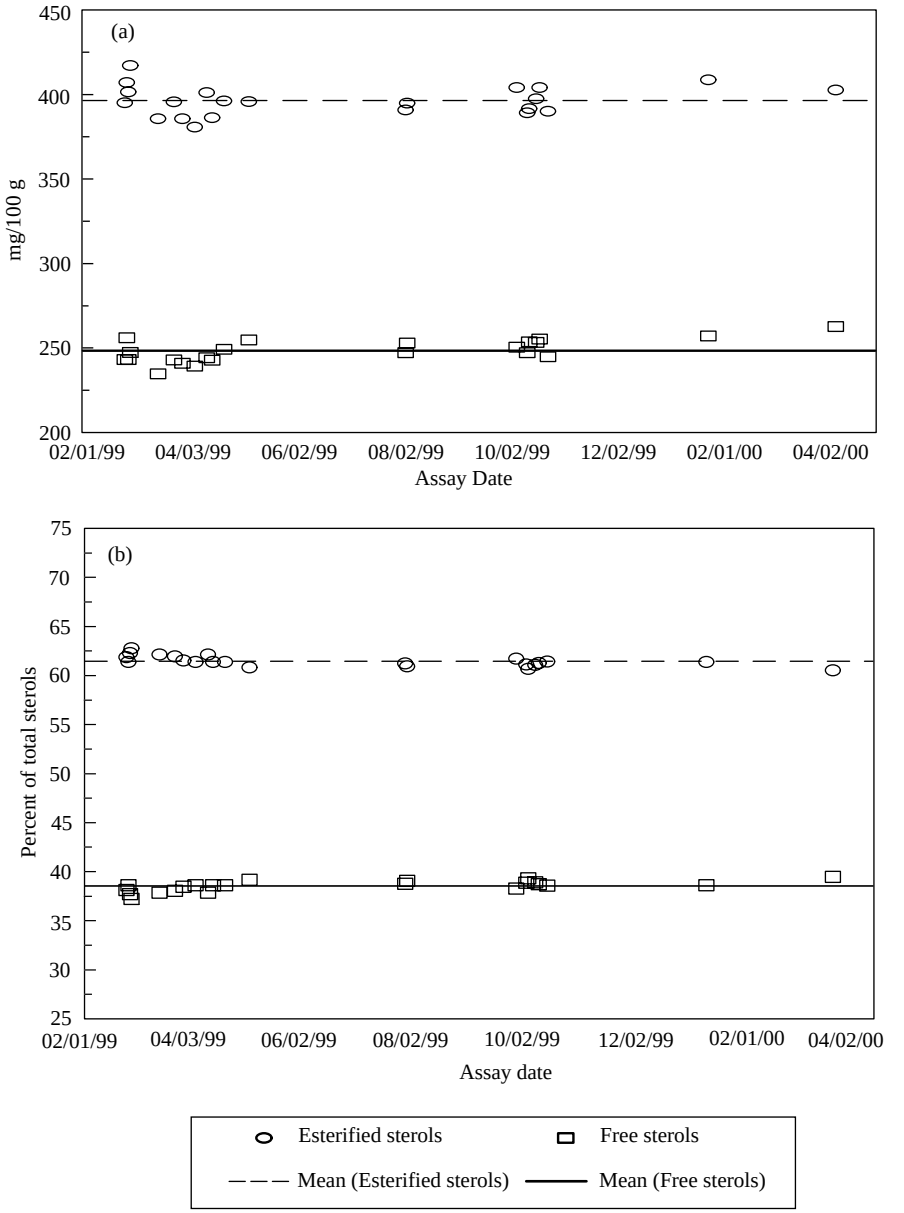

FIGURE 5. Quality control results for canola oil sample over 19 assays, 13 months, and 3 analysts. (a) Values in mg/100 g. Standard deviation = 6.8 and 8.8 mg/100 g for free and esterified sterols, respectively. (b) Values as percent of total sterols. Standard deviation = 0.6% for both free and esterified sterols.

sensitivity for low-level components such as sitostanol, campestanol, and cholesterol in most of the oils and fats. The maximum sample amount is limited by the capacity of the SPE cartridge. It was not possible to add the epicholesterol internal standard prior to SPE; it would elute only with the free sterols. Due to potential differences in interaction with the stationary phase, it could also not be relied upon that

epicholesterol would exactly mimic the behavior of all analytes during SPE. While using dihydrocholesterol and 5α-cholestane as internal standards was considered, this approach would preclude their function as recovery surrogates, and we also could not be certain that all oils and fats would be free of these components or others that interfered with their quantitation in the gas chromatographic analysis.

Results for 19 separate assays of the canola oil quality control material over 13 months and three analysts are shown in Figure 5. For total free sterols and total esterified sterols, respectively, the relative standard deviations were 2.8 and 2.2% in mg/100 g, and 1.4 and 0.9% as percent of total sterols. These data suggest excellent analytical precision over time.

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