Sterol Content of Some Plant Oils; Further Observations on Fast-Reacting Sterols

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Abstract

Total and free sterols were measured by a modified Sperry-Webb procedure in raw and refined corn, cottonseed, safflower seed, linseed, soybean and wheat germ oils. Wheat germ and safflower seed oil sterols were relatively rich in fast-reacting sterols, which predominated in the sterol ester fraction. Photometric constants following color reaction were obtained for cholesterol and plant sterols and applied to a procedure for analysis of cholesterol and plant sterols in mixtures thereof.

Introduction

In a recent study by Norcia et al. (9) it was observed that sterol digitonides from lipids of pea plant shoots were "fast-reacting" (6,7) to the Liebermann-Burchard reagent. This same characteristic was demonstrated for sterol digitonides from a number of common plant oils which were surveyed for fast-reacting sterols and also for content of esterified and free sterols. An adaptation of the Sperry and Webb (13) method for cholesterol was used for the latter measurements. The quantitative measurements and the fast-reacting characteristics of the plant oil sterols are reported here.

Materials and Methods

Plant Sterols

Stigmasterol (I), recrystallized (Southeastern Biochemicals, Inc., Morristown, Tenn.); stigmasterol (II), as received (Calbiochem, Los Angeles, Calif.); sitosteryl acetate, recrystallized (Southeastern Biochemicals, Inc.); β-sitosterol, as received (Calbiochem); phytosterol, recrystallized, plant source unknown (Southeastern Biochemicals, Inc.); soybean sterols, recrystallized (Mann Research Laboratories, Inc., New York, N. Y.).

For determining the sterol content of the plant oils a modification of the Sperry-Webb (13) procedure for cholesterol was used. Measurements were made against a cholesterol standard and an empirical factor was used to convert the result to a plant sterol basis.

The empirical factor was found by assaying standard solutions of the plant sterols by the Sperry-Webb procedure (both free and total sterol) against a cholesterol standard. The data are presented in Table I. The percent recovery values for stigmasterol (I) and sitosteryl acetate were averaged and the mean was again averaged with those values for phytosterol and soybean sterols, thus producing a new mean value of 65.5% recovery. The plant oils were assayed for sterol content against a cholesterol standard using a recovery factor of 100/65.5. It may be seen that the molecular weight correction and the chromogenicity correction are both applied in the recovery factor.

The modification of the Sperry-Webb procedure was in the saponification step. The sample in a 3 ml aliquot of an acetone-alcohol solution was placed in a 15 ml conical-tipped centrifuge tube with 7 drops of KOH solution instead of 3 drops. After the saponification and neutralization with acetic acid solution, the tube contents were made to 6 ml volume with acetone-alcohol and then the sterols were precipitated by adding 3 ml of the digitonin solution. Following color development on the digitonides the absorbance was measured at 625 mμ in a Beckman DB spectrophotometer with a 1 cm light path cuvet and against a cholesterol standard. If an instrument equipped with 5 cm light path microcuvets is available the modification of the saponification step can be obviated since much smaller samples of lipid may be used.

Precipitation of the digitonides of the plant sterols was shown to be quantitatively complete by comparing color development on aliquots of plant sterol solutions (see Table I for solutions studied) from which the solvent had been evaporated with color development on aliquots from which the sterols were precipitated as digitonides by the procedure described here.

The absorption spectra following Liebermann-Burchard color development on the sterols, not as digitonides (Sperry-Webb conditions) were determined for cholesterol, stigmasterol (I), β-sitosterol, and soybean sterols. The spectra are presented in Figure 1. Two further analytical considerations were apparent from the spectral data. First, a wavelength setting of 630 mμ would be a more optimal setting than 625 mμ for assay of plant sterols against a cholesterol standard. From the spectral data a correction factor of 100/67.7 was obtained for use at 630 mμ during analysis of plant sterols. Second, differences in the absorption spectra at 630 and 420 mμ could be used to analyze mixtures of cholesterol and plant sterols in a manner similar to the approach used by Avigan et al. (1) for mixtures of cholesterol and desmosterol.

For analysis of mixtures of cholesterol and plant sterols, simultaneous linear equations were developed as follows:

Let

(1) All color development be done at 6 ml total volume per tube.

(2) Color development at 630 mμ, 30 min, 25C be done on digitonides or free sterols; and color development at 420 mμ, 90 min, 25C be done on free sterols only (cf Avigan et al. (1). Letter *a* indicates milligrams of cholesterol per tube; *b*, milligrams of plant sterols per tube; *m*, absorbance at 630 mμ of cholesterol per millogram per tube per 1 cm light-path = 0.720; *n*, absorbance at 630 mμ of plant sterols per milligram per tube per 1 cm light-path = 0.488; *o*, absorbance at 420 mμ of cholesterol per milligram per tube per 1 cm light-path = 0.793; *p*, absorbance at 420 mμ of plant sterols per milligram per tube per 1 cm light-path = 0.810.

Then

(3) $a\,m + b\,n = A_{630}$

(4) $a\,o + b\,p = A_{420}$

TABLE I

Assay of Plant Sterol Solutions Against a Cholesterol Standard by the Sperry-Webb Procedure [a]

Sterol	Mass of plant sterol present, mg	Sterol found calculated as mg $C_{27}H_{46}O$	Mass recovery of plant sterol %
Stigmasterol (I)	65.17	40.25	61.8
Stigmasterol (II)	105.55	63.9	60.6
Sitosteryl acetate	48.10 [b]	34.0	70.6
β-Sitosterol	120.17	86.3	71.7
Phytosterol	59.52	38.6	64.9
Soybean sterols	54.77	35.8	65.4

[a] Absorbance read at 625 mμ, 30 min, 25C.
[b] Mass as free sterol.

Since A_{630} and A_{420} are measured values of the absorbance of the unknown tubes and m, n, o, and p are known constants; equations (3) and (4) may be solved simultaneously for a and b.

If unequal aliquots are used for color development at 630 and 420 mμ then either equation (3) or (4) must have the a and b terms multiplied by an appropriate fraction. As an example, assume that color development at 420 mμ is done on an aliquot of the sterols that is 0.9 the aliquot used for color development at 630 mμ; then equations (3) and (4) become

$$a\,m + b\,n = A_{630}$$
$$0.9\,a\,o + 0.9\,b\,p = A_{420}$$

and a and b represent the milligrams of cholesterol and plant sterol, respectively, in the 630 mμ tube; and $0.9a$ and $0.9b$ represent the milligrams of cholesterol and plant sterol, respectively, in the 420 mμ tube.

In order to check the goodness of fit of the equations and constants to the amounts of cholesterol and plant sterols in the tubes and the absorbance of the colored reaction products at the two wavelengths, standard solutions of cholesterol and soybean sterols were used to prepare known mixtures. Aliquots of the standard solutions were carefully pipetted into color development tubes. Color was developed after evaporation of solvent. Results obtained in this way are presented in Table II.

Fast-reacting characteristics to the Liebermann-Burchard reagent of the digitonides from the total and free sterols (Sperry-Webb method) of the plant oils were scored negative (−), slight (+), medium (++), intense (+++), and very intense (++++). These were scored upon visual observation immediately after color reagent addition. For purposes of making this observation, an aliquot was taken such that the absorbance at 625 mμ after 30 min would be between 0.200 and 0.300 for a 1 cm light-path.

TABLE II

Analyses of Standard Mixtures of Cholesterol and Soybean Sterols Spectrophotometrically Following Liebermann-Burchard Color Development

	Cholesterol, mg	Soybean sterols, mg	Total sterols, mg
Mixture 1			
Present	0.040	0.394	0.434
Found	0.029	0.406	0.435
Mixture 2			
Present	0.200	0.197	0.397
Found	0.201	0.198	0.399
Mixture 3			
Present	0.400	0.0315	0.432
Found	0.415	0.020	0.435

Results

The sterol content of the various oils studied and the fast-reacting characteristics are presented in Table III. Total sterols range from 0.4 to 5.7% of the oil depending on the plant source. Considerable variation is encountered in the ester/free ratios. These ratios for the two wheat germ oils are quite different from one another. The VioBin material was prepared for veterinary use in animals while the Lilly material was prepared for use in humans.

The fast-reacting characteristic was striking for the wheat germ oils but was also present in corn and safflower oils. This characteristic predominates in the ester sterol fraction of these oils but also appears in the free sterol fractions. No fast-reacting characteristics were shown by any of the crystalline plant sterol materials we studied, including soybean sterols and phytosterol. However, in studying soybean oil itself (Table III) the presence of these materials was questionable. Fast-reacting scores were quite similar for refined and unrefined oils. The data suggest that processing and refining may affect the ester/free sterol ratio; however, the design of the experiment and the number of oils studied do not permit conclusions to be drawn in this regard.

Discussion

Fast-reacting characteristics were shown to be particularly prominent for the sterols from wheat germ oil. Wheat germ oil sterols are known to be a relatively rich source of the "α-sitosterols" (2,14). Bernstein and Wallis (2) showed that α-sitosterol was really a mixture of compounds and that $\Delta^{5,8}$ compounds were present among them (2,3). The $\Delta^{5,8}$ structure is known to impart fast-reacting characteristics to sterols; therefore, it is quite probable that this characteristic of sterols from phanerogam plant

TABLE III

The Sterol Content of Plant Oils and Fast-Reacting Characteristics [a]

Oil [b]	Total sterol mg/100 mg oil	Free sterol mg/100 mg oil	Ratio ester/free	Fast-reacting score [c]	
				Total sterol	Free sterol
Corn oil, Mazola	1.097	0.423	1.59	++	−
Cottonseed oil, Wesson	0.397	0.278	0.43	−	−
Cottonseed oil (bleachable, prime, summer yellow)	0.574	0.459	0.25	− to +	+
Soybean oil crude (solvent oil, not degummed)	0.488	0.356	0.37	− to +	−
Soybean oil, once refined (an alkali refined oil)	0.481	0.333	0.44	− to +	−
Soybean oil, alkali refined	0.446	0.287	0.55	− to +	−
Linseed oil, raw (conventional raw linseed oil)	0.478	0.209	1.29	− to +	−
Linseed oil, alkali refined	0.393	0.170	1.31	− to +	−
Safflower seed oil, raw (crude, solvent oil, not degummed)	0.626	0.425	0.47	+++	+++
Safflower seed oil (alkali refined)	0.563	0.298	0.89	++	++
Wheat germ oil (solvent extracted with ethylene dichloride not refined or degummed), Viobin Corp.	5.403	0.345	14.7	++++	− to +
Wheat germ oil (cold pressed, refined), Eli Lilly Co.	5.667	1.407	3.03	++++	+

[a] Analyses against cholesterol standard, 625 mμ, correction factor of 100/65.5. See methods.
[b] The description of the oils is with terms commonly used in commerce.
Complete detail on the processing of some of the particular oils studied is not available.
[c] Negative (−), slight (+), medium (++), intense (+++), and very intense (++++).

lipids is due to the presence of $\Delta^{5,8}$ α-sitosterols. Cryptogam plant lipids would contain ergosterol which is also a fast-reacting sterol.

It is to be noted that no correction in Table III was made for fast-reacting sterols when measurements of total and free sterols of plant oil sterols were made. It is recognized that this can be a source of error (6). It is believed that for most of the oils studied such errors are not large. The measured sterol contents of safflower seed and wheat germ oils which had high fast-reacting scores are in agreement with known unsaponifiable matter content for these oils: safflower seed oil below 1.5%, and wheat germ oil 2–5% (Table I 1–46 Official and Tentative Methods of the American Oil Chemists' Society). Approaches to making such corrections have been described (4-7). We attempted to approximate the amounts of fast-reacting sterols in wheat germ oil and safflower oil by making photometric measurement after 1½ min as described by Moore and Baumann (7) and using extinction coefficients they have published for some fast-reacting sterols. Our approximations showed that for the Lilly wheat germ oil total sterols, slow-reacting sterols comprise probably not less than 4.1 mg/100 mg oil and fast-reacting sterols probably not more than 0.8 mg/100 mg oil. For the total sterols of the raw safflower oil slow-reacting sterols comprise probably not less than 0.40 mg/100 mg oil and fast-reacting sterols probably not more than 0.11 mg/100 mg oil.

For analysis of mixtures of cholesterol and plant sterols, e.g., margarines compounded of animal and vegetable fat and fat from mixed diets, the simultaneous equations presented in the *Methods* section may be used with confidence provided two conditions are met. First, color development at 420 mμ must be on the sterols and not on the digitonides, since digitonin contributes to color development at this wavelength (1). Secondly, since an internal standard is not used, the reagents and conditions for color development must be such that the known extinction coefficient [1] for cholesterol color development (8,12) can be accurately reproduced. Feasibility of standardization in this way has been demonstrated by the work of Schoenheimer and Sperry (12) and Norcia (8). Thoroughgoing studies concerning the purification of cholesterol and the use of cholesterol as a standard substance have been reported more recently (10,11,15).

Haust and Beveridge (5) have reported a differential photometric method for the estimation of cholesterol and sitosterol in the presence of certain other 3β-hydroxysterols; Avigan et al. (1) have reported measuring cholesterol and desmosterol in mixtures by a photometric procedure.

[1] Specific extinction-coefficient $k = (-\log_{10} T \text{ soln.}/T \text{ solv.})\ b\ c$, b in centimeters, and c in grams cholesterol per liter. $k = 4.35$, Norcia (8).

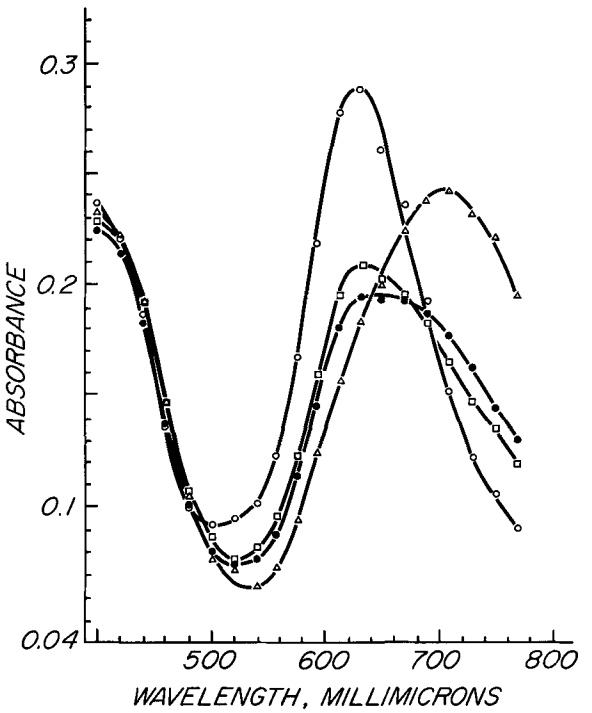

FIG. 1. Absorption spectra of sterols: △-stigmasterol, ●-soybean sterols, □-β-sitosterol, ○-cholesterol.

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