Polar Lipids of Sunflower Meal and Isolates

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ABSTRACT

The polar lipids present in the 86% ethanol extract obtained from sunflower meal and isolates were studied. The isolates were obtained in 2 ways: (a) by precipitation at the isoelectric point of the alkaline extract of the meal and further washing of the curd with water, and (b) by precipitation at the isoelectric point of the extract obtained with a 0.25% sodium sulfite solution and washing the curd with water, ethanol and acetone, successively. The polar lipids eluted from a Florisil column with chloroform/acetone (1:1) and with methanol, glycolipids and phospholipids, respectively, were studied both qualitatively and quantitatively. The components found were glycolipids, including sterol glycosides and esterified sterol glycosides, and phospholipids, including phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingomyelin.

INTRODUCTION

In an earlier paper (1) the combined neutral lipids of sunflower meal and the protein isolates obtained with 86% ethanol were studied. Besides neutral lipids, we found polar lipids, e.g., glycolipids and phospholipids, which were the objective of the present paper.

As we pointed out (1), no previous studies have been done on the nature of the combined lipids of protein isolate apart from some work on soybean isolates (2-4). However, the role played by these lipids, especially the unsaturated ones, on the stability of foods and feed during their processing and storage is important. Foods containing proteins and polyunsaturated fatty acids turn brown on storage, and as has been shown (5,6) the browning reactions are preceded by autoxidative reactions in the lipids.

Little is known about the nature of the interactions of sunflower lipids with proteins and this will be the subject of further investigation once we have obtained as much information as possible on the main lipid components of the protein isolates.

EXPERIMENTAL PROCEDURES

Materials

Meals from prepress hexane-extracted partially dehulled sunflower seeds were supplied by industry (original meal O). Two types of isolates were obtained: (a) by precipitating at the isoelectric point, pH 4.3, after extracting the original meal O with 0.2% sodium hydroxide and then washing the curd with water (isolate A); (b) the same procedure except extracting the meal with a 0.25% sodium sulfite solution at pH 11.0 and washing the curd successively with water, ethanol and acetone (isolate B).

A mixture of fatty acid methyl esters was prepared by transesterification of an equilibrated blend of coconut oil and linseed oil. Sterol glycosides (SG) and esterified sterol glycosides (ESG) were obtained from potato tubers by the method of Lepage (7). Phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) were from Sigma Chemical Co., St. Louis, MO, and sphingomyelin (SP) was from Fluka AG, West Germany. A standard mixture of sterols containing cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol and Δ^7-stigmasterol was obtained by extracting the unsaponifiable matter of a mixture of equal amounts of rapeseed oil and cocoa butter. From the extract, the sterols were recovered by preparative thin layer chromatography (TLC). To this mixture, 1 mg cholesterol was added. The sterols of the mixture obtained were identified by comparison with the retention times of pure standards.

Standards of D-glucitol, galactitol, xylitol and inositol were donated by Dr. Gil, University of Seville. Alditol acetates were prepared by treating the alditols with acetic anhydride in dry pyridine, and purified by crystallization.

General Methods

Extraction and separation of lipids. Free and combined lipids were obtained from the original meal and isolates (1). The preliminary separation into types of components was carried out with a Florisil column (8) and the elution of different components monitored by TLC. Glycolipids were eluted with chloroform/acetone (1:1) and phospholipids with methanol. The eluent for glycolipids on TLC was chloroform/methanol/7N ammonia (100:15:2) and the detection reagent was either iodine vapors or spraying with sulfuric acid and charring. The glycolipids appear as violet spots with the second reagent. For phospholipids, a mixture of n-butanol/acetic acid/water (40:7:32) was used as eluent and the Vaskosvky reagent (9) for detection. Blue spots on a white background were obtained.

Quantitation. Preparative TLC was used to quantitatively determine the different compounds. SG and ESG were quantified by the colorimetric method of Huang et al. (10). As the phospholipids contain the ester group in their molecule, the hydroxamic acid method (11) was used for their quantitation. Once the different compounds were separated on TLC plates with the proper eluents, the bands were scraped off the plates and extracted with chloroform. The residue was analyzed by the above methods.

Gas chromatography. A Hewlett-Packard Model 5711A was used under the following conditions: for fatty acid methyl esters, a 200 mm × 0.25 mm column of butanediol succinate (BDS), 2.5% on Supercoport, 80-100 mesh; oven temperature 180 C; detector and injector, 250 C; for sterols, a 200 mm × 0.25 mm column of OV-17, 2.5% on Supelcoport, 80-100 mesh; oven temperature, 270 C, detector and injector, 300 C. For alditol acetates, a Perkin-Elmer F-7 chromatograph was used; column packing was of 3% ECNSS-M(ethylenesuccinate-cyanoethylsilicone copolymer) on Gas-Chrom Q, 100-200 mesh, with a 200 mm × 0.25 mm column. The column was operated at 200 C, with injection port at 250 C and detector at 300 C.

Hydrolysis. The sample was taken into a 10 mL Pyrex tube and hydrolyzed with one mL of a 5% HCl solution in methanol at 100 C for 4 hr. After cooling, one mL water and 2 mL chroform were added. The mixture was shaken and the organic layer, containing free sterols if the sample was SG and free sterols and fatty acid methyl esters in the case of ESG, separated. The aqueous phase contained the hydrolyzed sugars.

The organic phase was chromatographed on preparative TLC of Silica gel G using hexane/diethyl ether (80:20) as eluent and cholesterol and fatty acid methyl esters as standards. The bands were visualized with iodine vapor, scraped off the plates, extracted in small chromatographic columns with chloroform and analyzed by gas liquid chromatography (GLC).

The aqueous phase, containing the sugars, was concen-

trated and processed as follows: an excess of freshly prepared 0.3 M sodium borohydride solution was added, and after 3 hours the excess reagent was destroyed with acetic acid. Sodium ions were removed by passing the solution through an Amberlite IR-120(H^+) column, and 5-bed column. After evaporation to dryness under diminished pressure, boric acid was removed by several distillations with dry methanol (5 mL × 20 mL). The dry residue, containing the alditols, was treated with acetic anhydride and pyridine for 1 hr at 95 C. The resulting solution was chromatographed on a Silica gel G plate with isopropyl alcohol/acetic acid/water (60:20:20) as eluent and reference standard. The band with the same Rf as the standard was scraped off the plate, extracted and a sample analyzed by GLC.

Phospholipids. To establish the identity of the different phospholipids after their detection and tentative identification by TLC, they were hydrolyzed as follows: the extracted samples from the plates were transferred to 10 mL Pyrex tubes. After the addition of 5 mL of HCl/methanol (1.7 N) the tubes were heated for 4 hr at 100 C. The mixture was taken to dryness and the residue extracted with 1 mL of 0.1 N HCl. The fatty acid methyl esters and bases were separated by TLC. Two methods were used for identification of the bases: (a) by TLC with methanol/water/6N ammonia (60:30:10) as eluent and reference standards, and (b) by liquid-liquid chromatography as described below.

Determination of the long-chain base (LCB) of the sphingomyelin. Once the sphingolipid had been isolated, the analysis of the LCB was performed as follows: the extracted material was taken with a few mL of a solution containing 8.6 mL concentrated HCl, 9.6 mL water and 81.8 mL methanol. The mixture was heated for 18 hr at 70 C in a 10 mL Pyrex tube. The fatty acid methyl esters were recovered by extracting with hexane (3 mL × 1 mL). The fatty acid composition was determined by GLC.

For the LCB identification, the residual methanolic phase was taken to a small volume with a stream of nitrogen; the solution was made strongly alkaline with 7 N sodium hydroxide and the bases extracted with diethyl ether (3 mL × 1 mL). After drying the extract, the base was identified by TLC following the procedure of Sambasivarao and McCluer (12). The solution of the bases was chromatographed on silica gel plates using chloroform/methanol/2N ammonia (40:10:1) as eluent and comparing the Rf with the reported values.

Liquid chromatography. A modular apparatus was used with 2 pumps and a 1 cm × 50 cm column filled with Aminex A-9 (Bio-Rad), a strongly acidic ion exchanger resin, particle size 11.5 ± 0.5 micra. A fluorescent detector, model LD 1309 (Jobling) with a dual flow cell, 13 μL each; excitation, 360 nm standard; emission, 400-700 nm standard was used. The following parameters and conditions were used: for choline and ethanolamine, sodium citrate buffer, pH 6.5; pumping rates 40 mL/hr for buffer and 90 mL/hr for the o-phtalaldehyde reagent. The temperature was 60 C. Under these conditions the retention times for choline and ethanolamine were 7.5 min and 76 min. For serine, the buffer used was sodium citrate, pH 4.25, the temperature was 50 C and the pumping rates were the same as before. Under these conditions the retention time for serine was 54 min.

RESULTS AND DISCUSSIONS

From prepress and solvent-extracted sunflower meal, as well as from the 2 types of isolates, A and B, referred to above, the free and combined lipids were extracted with hexane and 87% ethanol, respectively. The percentages and general chemical characteristics of these lipids were presented in an earlier paper (1).

Fractionation of the combined lipids in a Florisil column yielded glycolipids and phospholipids, besides the neutral lipids. Table I summarizes the percentages of the different types of compounds in these 3 samples.

Glycolipids. SG and ESG were identified after TLC separation with standards. Figure 1 shows the chromatogram of the 3 samples, i.e., the combined lipids of the original meal O and isolates A and B, together with standards SG and ESG from potato tubers. Further confirmation of the nature of the 2 compounds was obtained by acidic hydrolysis. Free sterols and sugars were obtained after hydrolysis of the more polar compound (SG) and free sterols, sugars and fatty acid methyl esters were obtained from the less polar component (ESG).

TABLE I

Weight Distribution of Glycolipids and Phospholipids of Meal and Protein Isolates (Percentage in the Lipids)

Sample	Glycolipid			Phospholipids		
	SG	ESG	PE	PS	PC	SP
Meal O	15.6	1.6	8.4	12.7	23.2	2.5
Isolate A	11.6	2.9	6.9	9.5	12.0	1.4
Isolate B	9.0	4.2	9.1	20.4	12.9	6.2

SG = sterol glycoside, ESG = esterified sterol glycoside, PE = phosphatidylethanolamine, PS = phosphatidylserine, PC = phosphatidylcholine, SP = sphingomyelin.

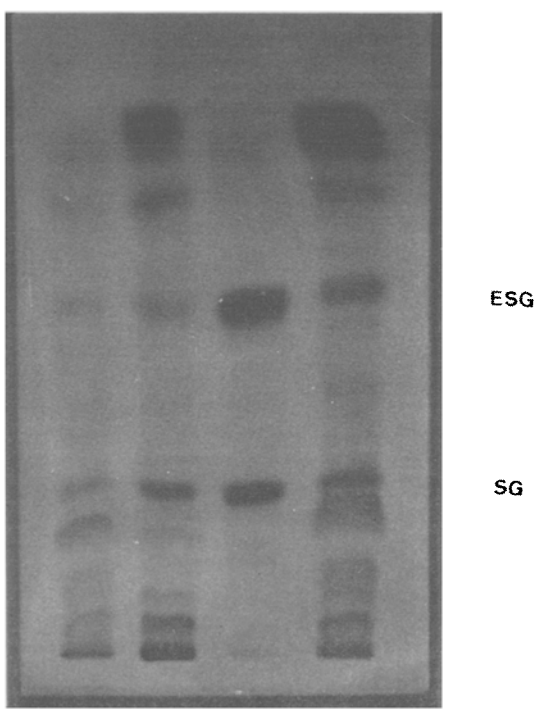

FIG. 1. TLC on Silica gel G of glycolipids: (a) isolate A; (b) original meal O; (c) glycolipids from potato tubers; (d) isolate B. Eluent: isopropyl alcohol/acetic acid/water (60:20:20). Detection: sulfuric acid and charring.

TABLE II

Percentage of Constituent Sterols of SG[a] and ESG[a] of Meal and Protein Isolates

Sample		Cholesterol	Brassicasterol	Campesterol	Stigmasterol	β-Sitosterol	Δ^7-Avenasterol
Meal O	SG	6.0	3.4	–	8.6	82.6	–
	ESG	18.3	–	–	3.6	78.1	–
Isolate A	SG	3.5	3.0	6.8	7.6	78.8	–
	ESG	6.0	–	1.1	–	53.8	39.1
Isolate B	SG[b]	19.4	–	2.1	–	73.1	–
	ESG	–	–	–	34.8	28.3	36.8

[a]SG = sterol glycoside, ESG = esterified sterol glycoside.
[b]SG of isolate B also contain 5.4% Δ^7-stigmasterol.

TABLE III

Percentage of Constituent Sugars of SG and ESG of Protein Isolates

Sample		Xylose	Galactose	Glucose	Inositol
Isolate A	SG	0.1	6.8	38.7	54.6
	ESG	0.4	15.6	47.3	37.0
Isolate B	SG	6.1	30.1	12.4	51.5
	ESG	15.9	18.9	37.4	27.8

SG = sterol glycoside, ESG = esterified sterol glycoside.

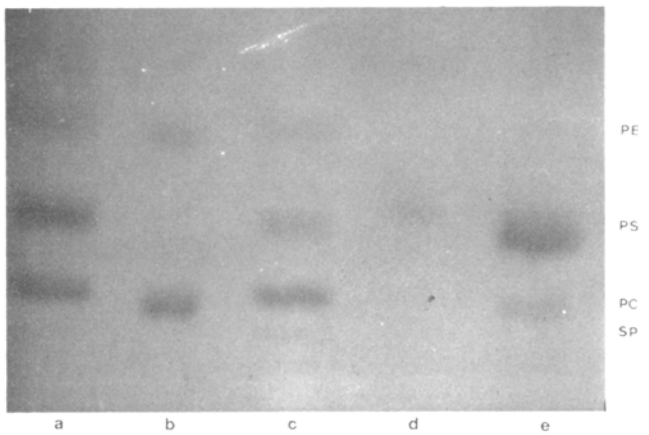

FIG. 2. TLC on Silica gel G of phospholipids: (a) original meal O; (b) standards, PE and PC; (c) isolate A; (d) standard, PS; (e) isolate B. Eluent: n-butanol/acetic acid/water (40:7:32). Detection: reagent of Vaskovsky et al. (9).

TABLE IV

Percentage of Constituent Fatty Acids of ESG of Meal and Protein Isolates

Sample	14:0	14:1	15:0	16:0	18:0	18:1	18:2
Meal O	4.4	1.8	–	40.5	10.0	42.1	–
Isolate A	1.8	1.2	1.1	31.8	18.1	27.8	14.9
Isolate B	2.5	0.5	1.7	85.2	7.2	2.2	–

ESG = esterified sterol glycoside.

Table II shows the GLC analyses of the sterols. Note the high proportion of Δ^7-avenasterol in the ESG of the lipids of both isolates A and B, as well as the considerable amount of stigmasterol in the ESG of the lipids of isolate B. In all cases, as should be expected, the main component appears to be β-sitosterol.

The sugars were quantitatively determined as their alditols by GLC. The results obtained from the SG and ESG of isolates A and B are included in Table III. The qualitative study of sugars of SG and ESG of lipids of original meal O showed they were the same as the sugars of isolates A and B. Xylose, galactose, glucose and inositol were constituents of the 3 samples.

The fatty acids of the ESG of the 3 samples were analyzed by GLC and the results are shown in Table IV. They were rather saturated, particularly those from isolate B, with up to 85.2% of palmitic acid as the main component. This is unusual as most plant tissues contain a relatively high proportion of unsaturated acids (13).

Phospholipids. PC, PS, PE and SP were identified by TLC in the 3 samples. Figure 2 shows a chromatogram of the phospholipids of the samples with standards. Final confirmation of these species was achieved through acid hydrolysis. After hydrolysis, the fatty acid methyl esters were extracted with hexane and analyzed by GLC. The fatty acid composition of the 4 phospholipids of the 3 samples is presented in Table V. The compositions resemble each other; however, linolenic acid is absent in the PE of the 3 samples.

Note that the unsaturation of the phospholipids of the original sample and those of the isolates were practically the same, with the exception of the component PC of the isolate B, which had a calculated iodine value distinctly higher than those of the original sample and isolate A. This is true for the other 3 components, PE, PS and SP, but was not the case when we considered the unsaturation of triglycerides, free fatty acids, diglycerides or fatty acid components of waxes, methyl esters or ESG. In these cases, the calculated iodine values were much lower in the samples from isolate B than the other two (see Table VI).

The bases, choline, ethanolamine and serine, were identified by TLC as well as by liquid-liquid chromatography by the methods described before. Peaks with retention times of 7.5 min, 76.0 min and 54.0 min were obtained under the indicated conditions, agreeing with those of the standards.

From the hydrolysis products of the sphingomyelin, a

TABLE V

Percentage of Constituent Fatty Acids of Phospholipids of Meal and Protein Isolates

Sample	14:0	15:0	16:0	16:1	18:0	18:1	18:2	18:3
				Phosphatidylethanolamine				
Meal O	5.5	–	31.2	5.3	4.7	18.2	34.9	–
Isolate A	1.5	0.8	28.5	4.6	7.0	27.0	29.8	–
Isolate B	2.1	1.3	25.1	4.7	5.8	18.5	38.1	–
				Phosphatidylserine				
Meal O	0.9	–	45.7	2.0	5.0	9.7	32.4	2.8
Isolate A	2.9	–	23.6	8.3	9.1	17.8	30.5	1.6
Isolate B	1.3	–	31.1	2.9	6.5	14.2	37.8	4.2
				Phosphatidylcholine				
Meal O	1.2	–	23.3	2.6	5.3	22.0	38.8	5.5
Isolate A	3.5	–	24.5	9.6	7.2	19.5	24.6	3.5
Isolate B	2.8	–	30.0	6.2	6.9	25.1	16.8	7.6
				Sphingomyelin				
Meal O	2.1	–	26.6	4.7	6.4	25.8	22.3	8.3
Isolate A	3.4	–	28.6	7.2	7.8	25.1	14.1	6.0
Isolate B	2.3	–	29.6	4.9	7.0	27.5	15.7	8.4

TABLE VI

Calculated Iodine Value of the Constituent Fatty Acids of the Different Types of Lipids

Type of compound	Sample		
	Meal	Isolate A	Isolate B
Triglycerides[a]	111.5	122.5	41.9
Free fatty acids[a]	125.8	129.4	20.1
Waxes[a]	57.2	72.3	12.2
Methyl esters[a]	121.4	128.6	86.8
Diglycerides[a]			
1,3-	103.5	107.8	0.5
1,2-	90.7	27.4	32.2
ESG	29.9	53.1	2.6
Phospholipids:			
PE	82.5	90.0	84.5
PS	83.5	95.0	76.5
PC	81.0	89.5	107.1
SP	72.3	92.5	91.3

[a]Data taken from reference 1.
ESG = esterified sterol glycoside, PE = phosphatidylethanolamine, PS = phosphatidylserine, PC = phosphatidylcholine, SP = sphingomyelin.

base was liberated with similar properties to those of threo-sphingosine. The Rf was 0.66, identical to the one assigned to this compound by Sambasivarao and McCluer (12) under the same chromatographic conditions. As the basic component in the sphingosine was serine, the compound present in all our lipid samples was serine sphingomyelin with threo-sphingosine as the base.

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