Analysis of Soybean Lecithin by Thin Layer and Analytical Liquid Chromatography

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ABSTRACT

The application of thin layer and analytical liquid chromatography to the analysis of two samples of commercial soybean lecithins is described. A combination of column chromatography and quantitative thin layer chromatography showed that these products consisted of ca. 82% mixture of the major phospholipids of soybeans, phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl inositol. The remainder of these products contained essentially the entire spectrum of lipid classes found in soybean oil—some 24 known and unknown glycolipids and phospholipids, in addition to the neutral lipids. Applications of analytical liquid chromatography to these lecithins gave a composition profile of the lipid classes comparable to two-dimensional thin layer chromatography. The potential of this method for the complete analysis of complex lipids, such as soybean lecithins, is indicated.

INTRODUCTION

Commercial lecithins generally consist of a complex mixture of phosphatides, inasmuch as the specifications for this product are defined only in terms of acetone and benzene soluble and insoluble material, moisture, color, and acid value (1). Total phosphorus content also may be determined and the ca. phosphatide content estimated (1). By application of a combination of silicic acid column and thin layer chromatograph (TLC), Szuhaj, et al., (2) identified some 17 different lipid classes in commercial soybean lecithins. In addition to the common phosphatides, these investigators detected sterol glucosides, esterified steryl glucosides, and cerebrosides. Described here is the application of thin layer and analytical liquid chromatographic analysis to two commercial soybean lecithin products.

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EXPERIMENTAL PROCEDURES

Materials

Azolectin was purchased from Associated Concentrates, Woodside Long Island, N.Y., and lecithin from Central Soya Company, Inc., Chemurgy, Chicago, Ill. Lipid class standards were purchased from the Lipids Preparation Laboratory, The Hormel Institute, Austin, Minn.

Methods

Two-dimensional and one-dimensional TLC were carried out by procedures and with solvent systems that have more or less become standard for the separation of the lipid classes (3-6), except for slight modifications that were indicated because of the particular conditions existing in our laboratory. The specific conditions used for the two-dimensional TLC are included in the legend for the analysis of these compounds. In addition to the use of Rf values of pure standards for comparison, identification of compounds also was made by color reactions on the chromatoplates. These included tests for phospholipids (7),

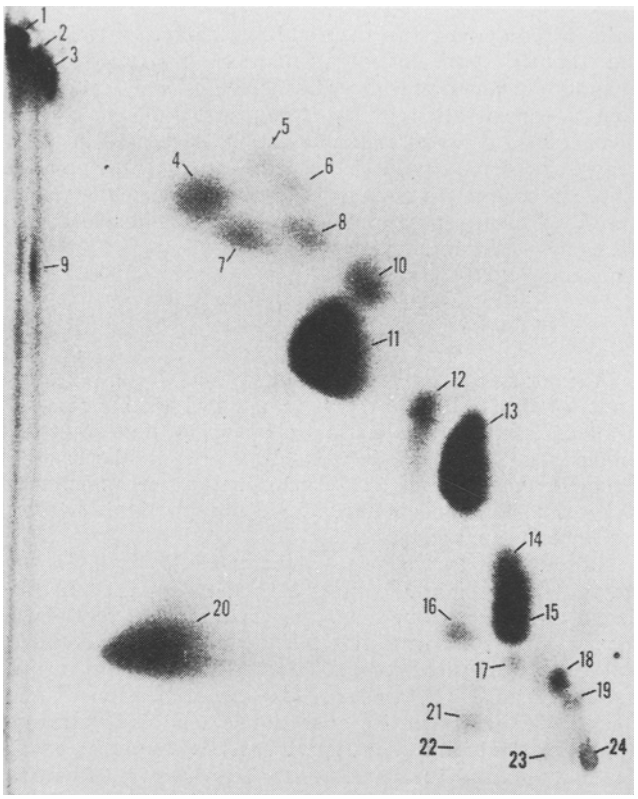

FIG. 1. Thin layer chromatography of commercial lecithin on Silica Gel G (Merck A.G., Darmstadt, Germany). First dimension: chloroform:methanol:7N ammonium hydroxide (65:30:4); second dimension: chloroform:methanol:acetic acid:water (170:25:25:6). 1=neutral lipid, 2 = ESG, 3=APE, 4=SG, 5=X_1, 6=X_2, 7=X_3, 8=Ce, 9=FFA, 10=PG, 11=PE, 12=DGDG, 13=PC, 14=LPE, 15=PI, 16=PS, 17=X_4, 18=LPC, 19=X_5, 20=PA, 21=X_6, 22=X_7, 23=X_8, 24=origin, X=unidentified components. (See Table I for abbreviations.)

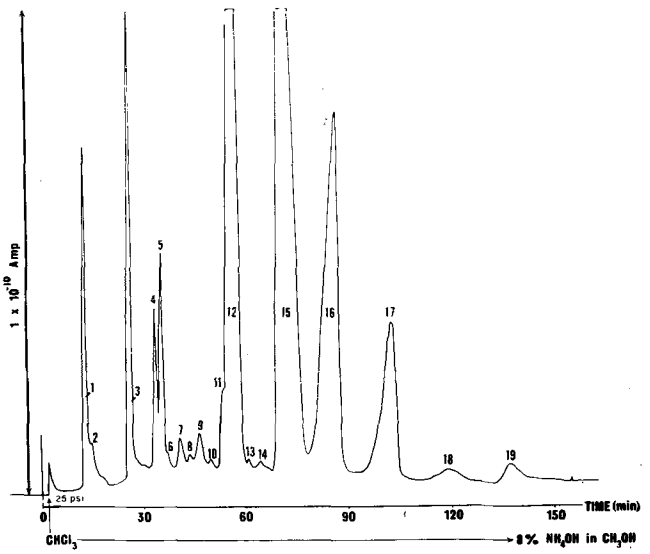

FIG. 2. Liquid chromatography of commercial lecithin with Corasil II (mesh 35-50 μ diameter, Waters Assoc., Inc., Framingham, Mass.) treated with ammonium hydroxide (column 2.8 mm x 1 m); flow rate = 0.1 ml/min, sample size ca. 900 μg. 1=TG and other neutral lipids, 2=FA, 3=ESG, 4=APE, 5=SG, 6=X_1, 7 and 8=CE and minor glycolipids, 9=PG, 10=X_2, 11=DGDG, 12=PE, 13=X_3, 14=X_4, 15=PC, 16=PI, 17=PA, 18=LPC, 19=very polar lipids, X=unidentified components. (See Table I for abbreviations.)

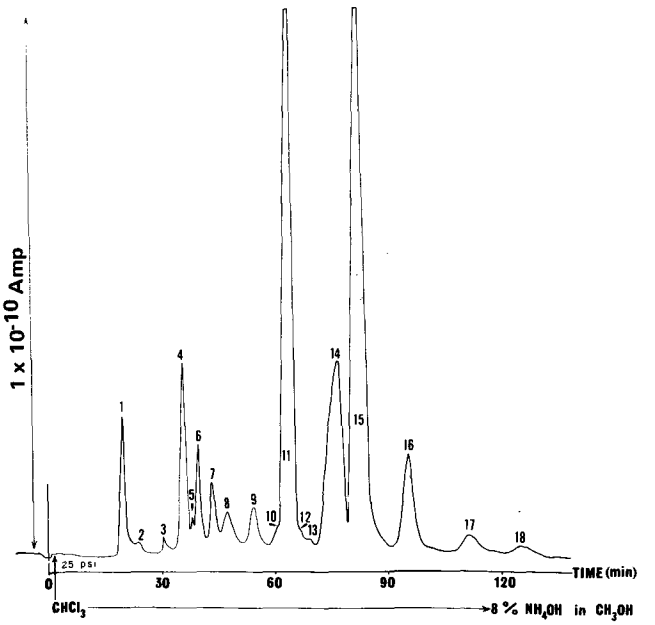

FIG. 3. Liquid chromatography of commercial lecithin with silicic acid (Bio-Rad, minus 325 mesh) (column 2.8 mm x 1 m), flow rate = 0.1 ml/min, sample size ca. 900 μg. 1=TG and other neutral lipids, 2=FA, 3=X_1 4=ESG, 5=X_2, 6=APE, 7=SG, 8=CE, 9=PG, 10=DGDG, 11=PE, 12=X_3, 13=X_4 14=PI, 15=PC, 16=PA, 17=LPC, 18=very polar lipids, X=unidentified components. (See Table I for abbreviations.)

glycolipids, sterols and derivatives, and amine group containing compounds by the ninhydrin test (8).

Column chromatographic separation of the major lipid groups, neutral lipid, glycolipids, and phospholipid fractions was carried out by silicic acid chromatography on ca. 50 mg samples with a silicic acid column (50 cm x 1.25 cm) (5). Neutral lipids were eluted with a mixture of chloroform:carbon tetrachloride (2:1), glycolipids with acetone, and phospholipids with methanol. The percentage of each of these fractions was determined by gravimetric analysis. Components within each fraction were determined quantitatively by TLC using the charring-densitometry techniques (5,9). Analysis of known compounds was made by comparing standard curves prepared from authentic standards. Unknown components were determined collectively by the difference between the amount of sample applied to the plate and the sum of the analyzed compounds.

Analytical liquid chromatography was carried out with the apparatus and by the procedure described by Stolyhwo and Privett (10) using a flame ionization detector of new design (11).

RESULTS

Azolectin and Central Soya lecithin were similar in composition varying only quantitatively, as might be expected, from batch to batch. Therefore, only the results on Azolectin are reported in detail. The general composition of this product is illustrated in Figure 1 by two-dimensional TLC. To simplify the mixture, the sample was separated into the major lipid groups, namely neutral lipids, glycolipids, and phospholipids, by column chromatography and analyzed quantitatively by TLC (Table I). These products contained over 80% phospholipid consisting primarily of phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl inositol. However, examination of Figure 1 showed that they contained essentially the entire spectrum of lipid classes found in soybean oil (12). Some 24 components were detected by TLC of the polar lipids, a number of which were unknown.

The complexity of these products also was demonstrated by analytical liquid chromatography (Figs. 2 and 3). The

TABLE I

Thin Layer Chromatographic Analysis of Commercial Lecithin (% by wt.)[a]

Neutral Lipid				
TG	FFA	ST	UN[c]	Total
2.0[b] ±0.3	0.12 ±0.01	0.23 ±0.07	0.15 ±0.02	2.5
UN contained DG, MG, STE, pigments and other unknowns				

Glycolipids				
ESG	SG + CE	DGDG	UN	Total
6.2 ±0.3	3.7 ±0.3	1.7 ±0.3	3.4 ±0.7	15.0
UN components not identified				

Phospholipids					
PE	PC	PI	PA	UN	Total
23.5 ±0.7	29.0 ±2.1	15.1 ±0.8	7.0 ±0.8	7.9 ±1.2	82.5
UN contained APE, DPG, LPE, LPC and other unknowns					

[a]TG=triglyceride, FFA=free fatty acids, ST=sterols, DG=diglycerides, MG=monoglycerides, STE=sterol esters, ESG=esterified steryl glucosides, SG=steryl glucosides, CE=cerebroside, DGDG=digalactosyl diglyceride, PE=phosphatidyl ethanolamine, PC=phosphatidyl choline, PI=phosphatidyl inositol, PA=phosphatidic acid, APE=acylphosphatidyl ethanolamine, DPG=diphosphatidyl glycerol, LPE=lysophosphatidyl ethanolamine, and LPC=lysophosphatidyl choline.

[b]M±SD.

[c]Unanalyzed components determined by the difference between amount of total sample and analyzed components.

peaks of components in these chromatograms were identified on the basis of the retention times of known compounds, simultaneous TLC analysis, and, generally, from a consideration of the composition of the eluting solvent. Two types of column packings were used in these analyses to demonstrate that elution order of the components can be changed to assist in identification and to effect a more efficient separation of some components. Just as in the analysis of two-dimensional TLC, a number of components could not be identified.

DISCUSSION

In accordance with the report by Szuhaj, et al., (2), the results of this study show that commercial lecithin consists of a large mixture of compounds. The major portion of the sample was phospholipid (82.5%) which, in the sample analyzed, consisted of 23.5% phosphatidyl ethanolamine, 29.0% phosphatidyl choline, 15.1% phosphatidyl inositol, and 7.0% phosphatidic acid.

Although at present it is necessary to use TLC in conjunction with liquid chromatography to assist in the identification of compounds, especially those for which there are no standards, this technique gives a qualitative profile of the lipid composition that approximates a two-dimensional TLC analysis. Like two-dimensional TLC, it cannot be employed for quantitative analysis at present. However, eventually quantitative analysis should be possible, inasmuch as the detector has been shown to have a wide linear dynamic range for the lipid classes (11). Likewise, improvement in resolution can be expected with the use of more efficient columns. An indication of the versatility of the method is illustrated by the difference in the pattern of elution of the components by treating the packing with ammonium hydroxide. For example, by changing the order of elution of phosphatidyl inositol and phosphatidyl choline, the separation of the former from phosphatidyl serine, which is generally difficult, should be

accomplished readily. Insofar as identification is concerned, much can be learned by the composition of the eluting solvent and by a cursory knowledge of the composition of the sample, because easily identified compounds within each group can serve as markers. Hence, in general, liquid chromatography, as demonstrated in these preliminary studies, should be useful for the analysis of complex mixtures of lipid classes, as represented by commercial lecithins.

ACKNOWLEDGMENT

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