The HPLC Separation and Quantitation of Lecithin in Chocolate

W.J. HURST and **R.A. MARTIN, JR.**, Technical Center, Hershey Foods Corporation, Hershey, PA 17033

ABSTRACT

A method is described for the analysis of phosphatidylcholine (chemical lecithin) in chocolate products. The chocolate is extracted with a modified Folch reagent and interfering compounds eliminated with a Sep-pak™. The extract is chromatographed using normal phase chromatography with a solvent system of CH_3CN/CH_3OH/H_2O and detection at 210 nm. The method shows good recovery from soy lecithin and chocolate-type matrices. It also shows good precision with % Cv ranging from 1-8% depending on the matrix. Studies of the effects of lecithin on the rheological behavior of chocolate during processing can now be easily performed, as well as the monitoring of lecithin ingredient levels in chocolate products.

INTRODUCTION

Lecithin is used in chocolate as an emulsifier and surface active agent. It is extracted from soybeans by leaching with solvent. Technically, chocolate is a dispersion of fine solid particles in a fat matrix. In milk chocolate, milk is added to the other solid components. Lecithin's major effect is that of a viscosity reducer with other minor effects and the ability to employ higher processing temperatures without viscosity changes (1).

Lecithin analysis is an important need in the chocolate industry both as an ingredient check and as a means to monitor rheological phenomena throughout processing.

This soy lecithin (1) consists of 3 major phosphatides: 19.7% phosphatidylcholine (PC) (chemical lecithin), 19.7% phosphatidylethanolamine (PE) (cephalin) and 21.0% phosphatidylinositol (PI). In this study we chose to analyze lecithin for chemical lecithin rather than for all of the phospholipids.

Lecithin analysis traditionally has been performed by an extraction of acetone insolubles with phosphorus determination on the resulting fraction (2). This technique is time-consuming and results are difficult to reproduce.

Phospholipid analysis has also been made by liquid chromatography, using various supports such as silica gel, TEAE-cellulose and DEAE-cellulose with TLC on the resulting fraction (3-9). High performance liquid chromatography (HPLC) for the analysis of phospholipids has not

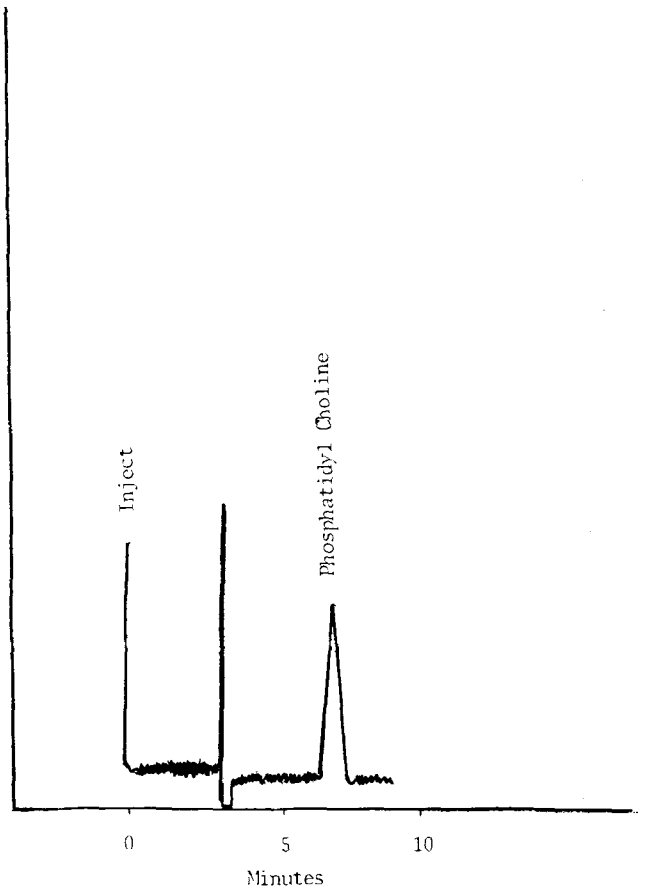

FIG. 1. Phosphatidylcholine standard (20 μg). Column: μPorasil (Waters Assoc.); mobile phase: 65:21:14 CH_3CN/MeOH/H_2O; flow rate: 2 ml/min; detector: Waters Assoc. Model 401 Refractive Index 8x.

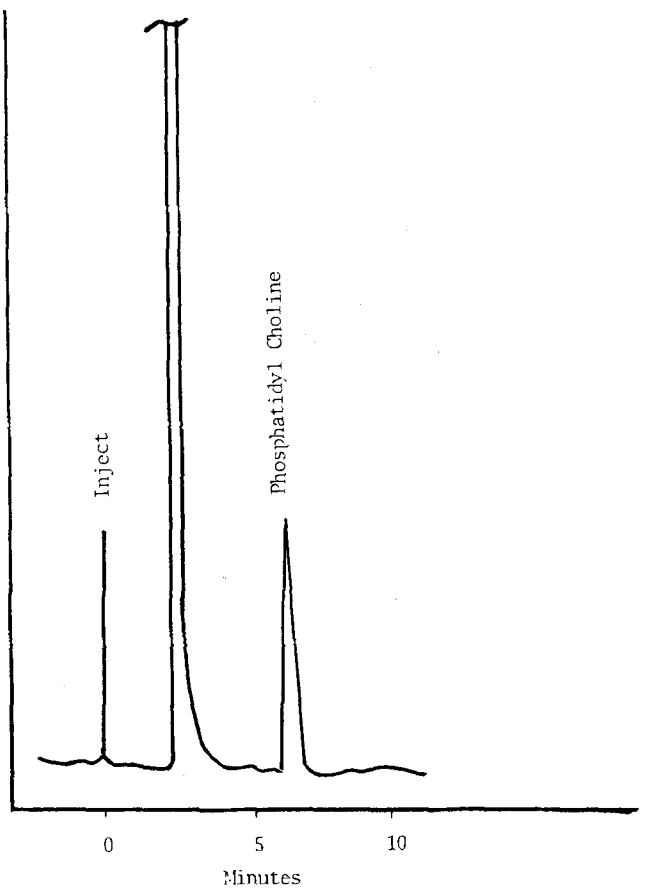

FIG. 2. Phosphatidylcholine standard (2 μg). Column: μPorasil (Waters Assoc.); mobile phase: 65:21:14 CH_3CN/MeOH/H_2O; flow rate: 2 ml/min; detector: Waters Model 450 @ 210 nm (.01 AUFS).

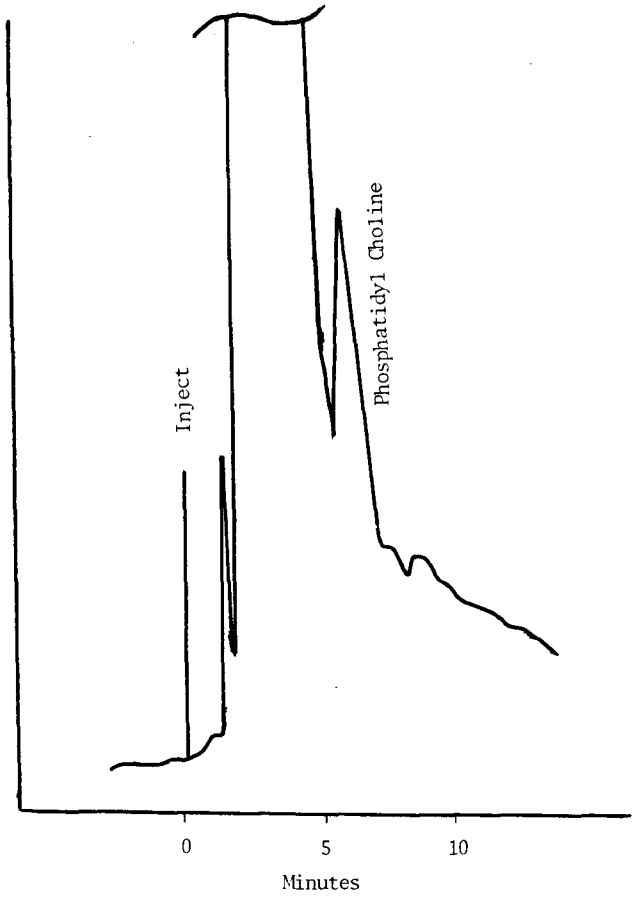

FIG. 3. Chocolate extract without Sep-pak™ clean up. Column: μPorasil (Waters Assoc.); mobile phase: 65:21:14 CH_3CN/MeOH/H_2O; flow rate: 2 ml/min; detector Waters Model 450 @ 210 nm (0.01 AUFS).

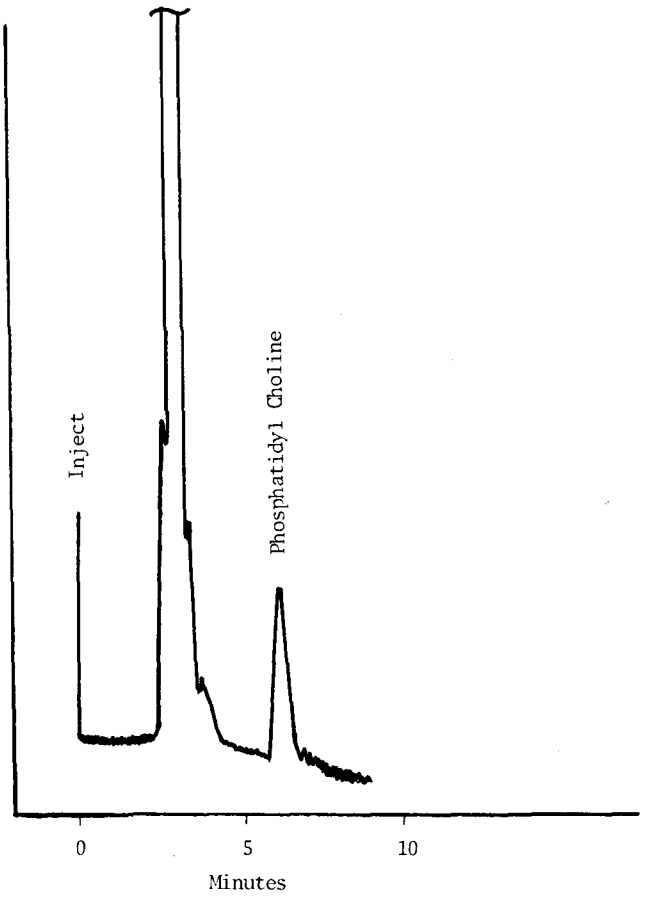

FIG. 4. Chocolate extract with Sep-pak™ clean up. Column: μPorasil (Waters Assoc.); mobile phase: 65:21:14 CH_3CN/MeOH/H_2O; flow rate: 2 ml/min; detector: Waters Assoc. Model 401 Refractive Index (4x).

been very successful since both refractive index (RI) and flame-ionization detection methods are insensitive. The phospholipids do not have strong absorption peaks but do have unsaturated centers and functional groups which exhibit absorption in the 203-214 nm region (10). It has been reported that this absorption primarily results from isolated double bonds with detector response proportional to the degree of unsaturation (11).

In this paper, a method is described for the analysis of chemical lecithin, PC, in chocolate using normal phase HPLC with detection at 210 nm. Samples are extracted and interferents eliminated using a commercially available Sep-pak™. The method is accurate, precise and is time-conservative; analysis time is less than 30 min.

EXPERIMENTAL PROCEDURES

HPLC

The HPLC apparatus consisted of an M45 or M6000A Solvent Delivery System (Waters Associates), a Porasil column (4.8 nm id x 30 cm) (Waters Associates), 2 detectors, a data unit and miscellaneous ancillary equipment. The 2 detectors used were an M401 Refractive Index Detector (Waters Associates) and a Model 450 Variable Wavelength Detector at 210 nm (Waters Associates). Data acquisition and evaluation were performed by the Shimadzu E-1A Data Unit. The HPLC mobile phase was CH_3CN/CH_3OH/H_2O (65:21:14, v/v/v) at a flow rate of 2.0 ml/min. All solvents were LC grade.

Samples and Standards

Soy lecithin and chocolate samples were obtained at the Hershey Chocolate Company, Hershey, PA. Different lots of soy lecithin were obtained compliments of A.E. Staley Mfg. Co. All other samples were made at Hershey Research Laboratories, Hershey, PA. The standard used was L-α-lecithin from soybeans. (Sigma Chemical Co.)

Extraction of Samples

All samples were extracted using a modified Folch reagent (12) consisting of $CHCl_3$:CH_3OH (2:1) plus BHT added at the 0.005% level to prevent oxidative degradation of unsaturated lipids (8). Soy lecithin samples were extracted and injected without further purification. Chocolate samples are extracted and the extract further purified using the silica gel Sep-pak™. Ten g of ground chocolate were extracted with 2 100-ml portions of modified Folch. The resulting solution was centrifuged and the resulting $CHCl_3$/CH_3OH poured into a round-bottomed flask. This solution was evaporated under vacuum at 35-40 C until only the fat remained. The fat was dissolved in 25 ml of $CHCl_3$ and 10 ml of this solution passed through a silica gel Sep-pak™. The Sep-pak™ was washed with 10 ml of 7% petroleum ether in ethyl ether. The PC was eluted with 30 ml of CH_3OH and subjected to analysis.

Analysis

Samples and standards were injected onto the column in duplicate. Calculation of results was obtained by comparing

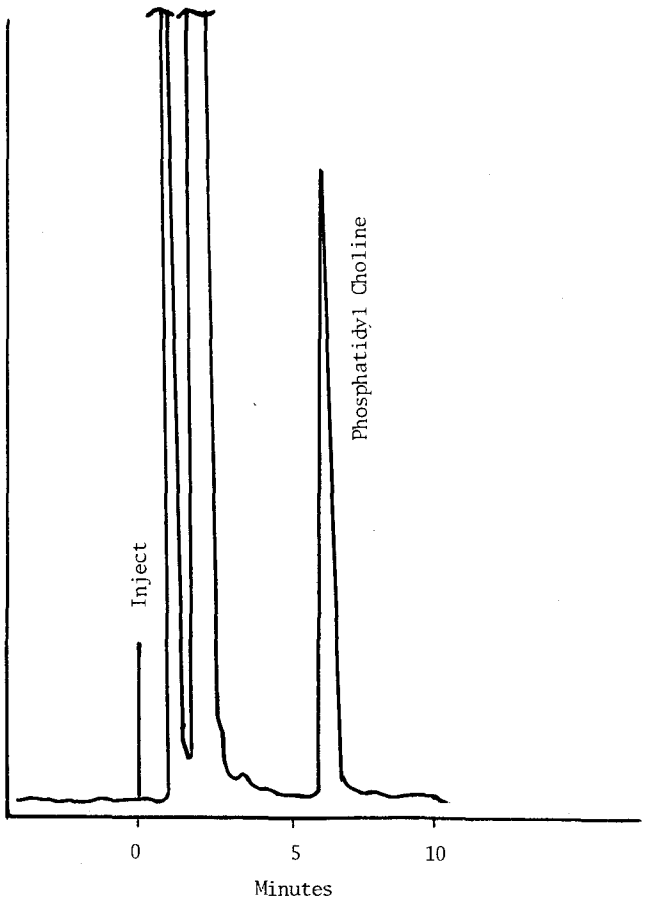

FIG. 5. Chocolate extract with Sep-pak™ clean up. Column: μPorasil (Waters Assoc.); mobile phase: 65:21:14 CH_3CN/MeOH/H_2O; flow rate: 2 ml/min; detector: Waters Model 340 @ 210 nm (0.01 AUFS).

peak areas obtained by injection of samples and standards. Figures 1 and 2 show sample chromatograms of lecithin standards using UV and RI detectors whereas Figure 3 shows a chromatogram of a chocolate extract without the Sep-pak™ clean up. Figures 4 and 5 show chocolate extract chromatograms with Sep-pak™ clean up with UV and RI detection.

Thin Layer Chromatography (TLC)

Ten μl of Folch extracts were spotted on silica gel TLC plates (Si-60; E. Merck). The plates were developed with $CH_3Cl_3/CH_3OH/HOAC/H_2O$ (25:15:4:2) (10). The phospholipids were visualized using either 10% ethanolic phosphomolybdic acid followed by exposure to heat of 110 C or exposure to I_2 vapors.

RESULTS

Six different manufacturers' lots of the same type of soy lecithin were analyzed for PC. The results are reported in Table I; the lot-to-lot coefficient of variation was less than 7%.

Table II shows the precision studies for analyses of different matrices with % Cv ranging from ca. 1 to 8%.

Duplicate recovery studies were conducted on 2 matrices, commercial lecithin and matrix consisting of 50% cocoa butter, 40% sugar and 10% cocoa. Table III shows good recovery from commercial lecithin and Table IV shows good recovery from the second matrix.

Cocoa liquor has indigenous PC; it was therefore necessary to elucidate the percentage of PC in various liquors used in chocolate products.

With the information on the choline content of soy lecithin used in the product and information on indigenous PC levels in chocolate liquor, it is therefore possible to calculate the the amount of lecithin in the finished product. Lower limits of detection are ca. 150 ng within the matrix and data are linear from 150 ng to over 4 μg.

TABLE I

Phosphatidylcholine in Lecithin Lots

Phosphatidylcholine (%)
27.31
26.43
28.64
23.91
24.70
27.88
$\bar{x}$ = 26.49
% Cv = 6.98

TABLE II

Precision Studies

Sample	*n*	% Cv
Phosphatidylcholine standard (soy)	10	4.34
Commercial soy lecithin	9	1.08
High liquor milk chocolate extract	10	1.99
Liquor extract	9	7.79
Low liquor milk chocolate extract	5	5.48

TABLE III

Recovery Study of Phosphatidylcholine Standard Added to Commercial Lecithin (n = 2)

Amount added (%)	Amount recovered (%)	Recovery (%)
+ 2	1.93	96.6
+ 5	4.83	96.5
+10	10.26	102.6
		Average 98.6

TABLE IV

Soy Lecithin Added to Cocoa Butter, Sugar and Cocoa Matrix

Amount added (g)	Amount recovered (g)	Recovery (%)
0.04571	0.04534	99.19
0.10260	0.09238	90.32
0.41350	0.50530	98.41
1.00240	0.92380	92.16
1.50690	1.36670	90.64
		Average 94.14

DISCUSSION

The data generated show the utility of this technique as an analytical tool. It allows the monitoring of ingredients and observations of various rheological phenomena to which lecithin is related. Additionally, without the use of the Sep-pak™ this would not be possible, since the chocolate extract is not sufficiently clean to permit analysis. It is

extremely important that the variable wavelength UV be used since the RI detector, while a universal detector, is only ca. 10% as sensitive as the UV. Finally, it would be possible to analyze for PE and other phospholipids, although they do elute extremely close to the solvent front using this mobile phase.

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REFERENCES

1. Minifie, B.W., Chocolate, Cocoa and Confectionery: Science and Technology, Avi Publishing Co., 1970.
2. Official Methods of Analysis, 12th Edition, edited by W. Horwitz, 1975, p.218.
3. Rouser, G., G. Kritchevsky, A. Yamamoto, G. Simon, C. Galli and A.J. Bowman, Methods Enzymol. 14:272 (1969).
4. Lipid Chromatographic Analysis, 2nd Edition, Vol. 3, edited by G. Rouser, G. Kritchevsky and A. Yamamoto, Marcel Dekker, New York, 1976, p.713.
5. Weenick, R.O., and A.P. Tullock, JAOCS 43:327 (1965).
6. Blood Lipids and Lipoproteins, edited by G.J. Nelson, Wiley-Interscience, New York, 1972, pp. 3-24.
7. Shipski, V.P., and M. Barclay, Methods Enzymol. 14:530 (1969).
8. Aitzetmuller, K., J. Chromatogr. 113:231 (1975).
9. Dieffenbacher, A., and U. Bracco, JAOCS 55:642 (1978).
10. McCluer, R.H., and F.B. Jungalwala, Adv. Exp. Med. Bio. 76: 533 (1976).
11. McCluer, R.H., and F.B. Jungalwala, Chromatogr. Sci.79:10 (1979).
12. Folch, J., M. Lees and G.H.S. Stanley, J. Biol. Chem. 226: 497 (1957).

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The Properties of *Cucurbita foetidissima* Seed Oil

J.A. VASCONCELLOS, J.W. BERRY and **C.W. WEBER,** Department of Nutrition and Food Science, University of Arizona[1], Tucson, AZ, and **W.P. BEMIS,** and **J.C. SCHEERENS,** Department of Plant Science, University of Arizona, Tucson, AZ 85721

ABSTRACT

Oils from the seeds of 15 different selections of the buffalo gourd, *Cucurbita foetidissima*, were characterized in terms of their physical and chemical properties, which indicate that this oil is similar to other common edible oils. Xanthophylls were the predominant carotenoid pigments present in the crude oil, ranging from 51-232 mg/kg oil. Linoleic acid, the predominant fatty acid, ranged from 39-77% with an average level of 61%. Although conjugated unsaturated acids are a significant component in some other xerophytic cucurbit oils, the levels of conjugated dienoic and trienoic fatty acids in this species are only 2.3 and 0.03%, respectively.

INTRODUCTION

The buffalo gourd, *Cucurbita foetidissima* HBK, a feral xerophytic gourd, has been the subject of a number of recent studies (1-5) because of its potential as a source of oil, protein and starch. The domestication of this plant as a crop adapted to arid land agriculture is currently under study (1). Previous investigation of the crude oil from the seed suggested that it could be processed to yield an edible oil (6). Its highly unsaturated nature should make it very attractive for food purposes.

The purpose of this work was to determine the physical and chemical properties of the crude oil from the seeds of this xerophytic plant.

EXPERIMENTAL PROCEDURES

Open-pollinated seed lots from 15 genetically different selections of *C. foetidissima* grown at the University of Arizona Experiment Station were studied. The seeds were obtained by submerging the fruits in water until fermentation disintegrated the placental tissue. They were then washed, air-dried and kept at room environmental conditions. Seed aliquots were taken as needed for different analyses, thus assuring that the determinations were performed on freshly extracted oil.

Seed and Oil Analyses

Seed aliquots were ground in a laboratory Wiley mill to 10-mesh size through a nickel-plated delivery tube. Moisture content was obtained by drying in a vacuum oven for 16 hr at 60 C. Crude oil content was determined with 5 g samples by Soxhlet extraction with hexane; crude protein content of defatted meal was measured by the micro-Kjeldahl method using a conversion factor of 6.25.

Oil for physical and chemical analyses was extracted as already described. The hexane solution was filtered and the oil recovered in a rotary evaporator. Residual hexane was removed by heating the sample to 60 C while flushing the oil with a stream of N_2. Characterization of the oil samples was made using AOCS methods (7).

Total Carotenoids: Visible Spectrophotometry

Carotenoids were determined using matched silica cells of 1-cm path length read in a Perkin-Elmer 202 spectrophotometer. Oil samples were dissolved in cyclohexane (2.5% w/v) and the spectra recorded in the range 350-550 mμ. For

TABLE I

Composition of *Cucurbita foetidissima* Seed

Properties	Range	Mean ± SD
Moisture (%)	4.1 - 8.4	6.2 ± 0.2
Oil (%)	31.8 - 39.4	36.0 ± 2.0
Protein content[a], defatted meal (%)	49.0 - 66.0	54.6 ± 4.6

[a]Crude protein = % N X 6.25.

[1] Arizona Agricultural Experiment Station No. 3081.