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# Analysis of Phosphatidylcholine in Soy Lecithins by HPLC

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## ABSTRACT

A simple and rapid high pressure liquid chromatographic method with RI detector was developed to determine the content of phosphatidylcholine in soy lecithins.

## INTRODUCTION

During the course of our recent study on soy lecithin production by solvent extraction (1), we needed a rapid method of analysis for phosphatidylcholine (PC). Analysis of phospholipids using high pressure liquid chromatography (HPLC) has been extensively studied by several researchers (2-6). However, these methods were based on either UV detector in the range of 203-214 nm (2-4) or FID (5,6). Recently, Nasner and Kraus (7) reported a very sensitive HPLC method for determination of PC in soy lecithins by using a UV detector at 206 nm.

## EXPERIMENTAL

### Materials

Crude soy lecithin was obtained from a local company (Cheil Sugar Co. Seoul). Acetone-insoluble fraction and 2-propanol-soluble fraction were made according to the methods of Weenink and Tulloch (8) and Liebing (9), respectively. PC, phosphatidylethanolamine (PE), phosphatidylinositol (PI), sphingomyelin (Sph), triolein and cardiolipin were purchased from Sigma (St. Louis, MO). Solvents of chromatographic grade were purchased from Burdick & Jackson (Muskegon, MI). All other reagents and chemicals were of technical grade.

### HPLC Equipment and Conditions

HPLC was performed with a Waters Associates' ALC/GPC-244 (Milford, MA) equipped with R401 RI detector and the column used was a $\mu$-Porasil column (Waters Associates). The operating conditions were: flow rate, 2 mL/min; solvent, chloroform/methanol/acetate/water (14/14/1/1, by vol); sample size, 6 mg; attenuation, 32X; chart speed, 0.5 cm/min. Integration of peak area was done by multiplying the height of the peak by the width at half-height (10).

## RESULTS AND DISCUSSION

Retention times of triolein, PE, PI, cardiolipin, PC, and Sph were 1.6, 2.0, 2.2, 2.3, 6.4 and 10.3 min, respectively.

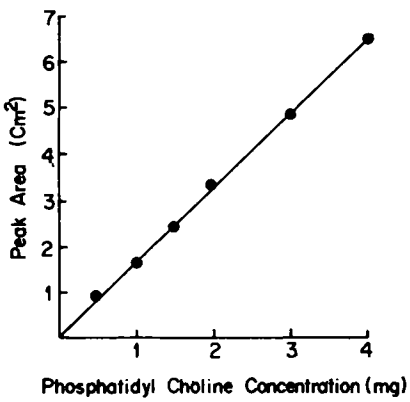


FIG. 1. Quantitative analysis of phosphatidylcholine standard by HPLC.

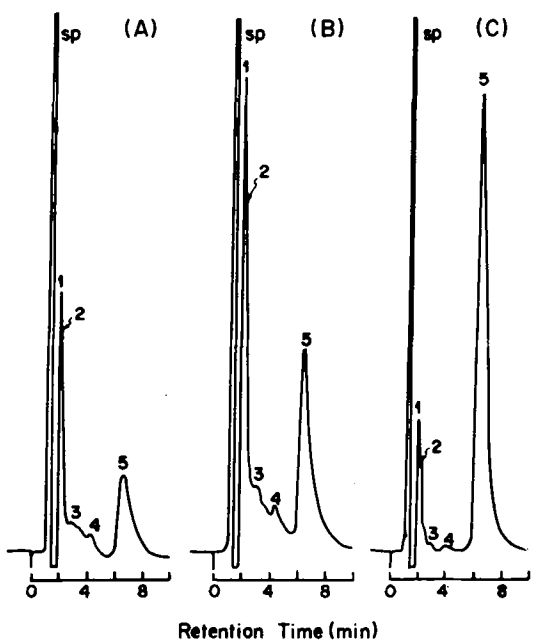


FIG. 2. (A) HPLC of crude lecithin, (B) acetone-insoluble fraction, and (C) 2-propanol-soluble fraction. SP, solvent peak; 1, phosphatidylethanolamine; 2, phosphatidylinositol; 3 and 4, unknowns; 5, phosphatidylcholine. Operating conditions: flow rate, 2 mL/min; solvent, chloroform/methanol/acetate/water (14/14/1/1, by vol); sample size, 6 mg; attenuation, 32X; chart speed, 0.5 cm/min.

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TABLE I

**Phosphatidylcholine Content Determined by HPLC of Soy Lecithins**

| Sample | Content (%)[a] |
|---|---|
| Crude lecithin | 18.4 ± 1.3[b] |
| Acetone-insoluble fraction | 27.3 ± 1.0 |
| 2-Propanol-soluble fraction | 57.6 ± 1.2 |

[a]Content (%) = (phosphatidylcholine [mg] /lecithins [mg] ) X 100.
[b]Mean ± SD based on 3 samples.

Therefore, we could separate PC but we could not separate another 2 major components of the lecithin, PE and PI. Furthermore, PC was found to be completely separated from Sph. Guerts van Kessel et al. (3) and Hax and Guerts van Kessel (4) noted the difficulty of separating PC from SpH with UV detector in the range of 203-214 nm and with *n*-hexane/2-propanol/water mixture as eluting solvent, whereas Jungalwala et al. (2) reported that PC and Sph could be separated with acetonitrile/methanol/water mixture as eluting solvent using gradient system.

In order to quantify the amount of PC, aliquots of standard solutions ranging from 0.5 to 4.0 mg PC were chromatographed and the plot of peak area against PC concentration of the samples was found to be linear as shown in Figure 1. Based on this calibration curve, PC contents of crude lecithin, acetone-insoluble fraction and 2-propanol-soluble fraction were obtained from HPLC as shown in Figure 2 and the results are shown in Table I. The results indicate that PC contents of crude lecithin, acetone-insoluble fraction and 2-propanol-soluble fraction were 18.4 ± 1.3, 27.3 ± 1.0, 57.6 ± 1.2%, respectively, and these values were very close to the data obtained by Erdahl et al. (6) and Sullivan and Szuhaj (11). The HPLC method described in this paper is a rapid and simple analytical tool for the determination of PC in soy lecithins.

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# ⁂Cyclopropenoid Fatty Acid Content and Iodine Value of Crude Oils from Indian Cottonseed

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## ABSTRACT

Cyclopropenoid fatty acid (CPFA) and iodine value (IV) of oils extracted from 30 varieties of cottonseed belonging to 4 botanical species, *Gossypium arboreum*, *G. herbaceum*, *G. hirsutum* and *G. barbadense*, have been reported. The CPFA determined by HBr titration method ranged from 0.66 to 1.51%. The IV of the oils ranged from 93.3 to 111.2. CPFA contents were significantly different among the species and nonsignificant within the species. The mean values of CPFA for different species were 0.67 (*G. barbadense*), 0.83 (*G. hirsutum*), 1.14 (*G. herbaceum*) and 1.40% (*G. arboreum*). There was no correlation between IV and CPFA content.


## INTRODUCTION

Cottonseed oil contains cyclopropenoid fatty acid (CPFA) as malvalic and sterculic acids in its triglycerides. Although the concnetration of CPFA in cottonseed oil is small (1-7), the incorporation of low levels of cottonseed oil containing CPFA that give a positive Halphen test into the diet of laying hens results in unusual biological effects (2,8-11). The dietary CPFA can cause pink-white, pasty yolks in the eggs, defects in the reproductive capacity of the bird, growth inhibition, altered fatty acid distribution and fat accumulation (12-14). Cottonseed oil is widely used in food for human consumption and the possibility exists that the CPFA may produce deleterious effects in man. Thus, data on CPFA content in oils of different varieties of cottonseed will provide useful information. Data on CPFA content in cottonseed oil of American varieties have been reported whereas little is known about those of Indian cottonseed. The object of this study was to provide basic information on CPFA content of oils of different varieties of Indian cottonseed.

In this investigation, 30 samples of crude cottonseed oil belonging to 4 cultivated botanical species were analyzed for CPFA content, expressed as percentages of malvalic acid using the HBr-dilution technique (15) and iodine value (IV). Significant variation in CPFA content in cottonseed oil among different species was observed.

## EXPERIMENTAL

### Materials

Thirty cottonseed samples included in this study belonged to 4 botanical species; 11 from *G. arboreum*, 5 from *G. herbaceum*, 12 from *G. hirsutum* and 2 from *G. barbadense*. The cottonseed samples were obtained from cotton