Enrichment of Phospholipids from Neutral Lipids in Peanut Oil by High-Performance Liquid Chromatography[1]

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Phospholipids from crude peanut oil were enriched on a 2-cm silica column and subsequently separated from neutral lipids within the chromatographic system without prior concentration. Hexane effectively removed the bulk neutral lipids, leaving the adsorbed phospholipids on the silica precolumn. Individual phospholipids were separated from the remaining neutral lipids and from each other by using two mixed solvents and a gradient program. This method separates the phospholipids in approximately 27 min after the desired enrichment level has been reached.

KEY WORDS: HPLC, oil, peanuts, phospholipids.

Naturally occurring oils are complex mixtures of triglycerides, diglycerides, monoglycerides, phospholipids and other minor components. These components of natural oils are important to the food industry because they impart mouthfeel, texture and flavor, as well as aid in the solubility and stabilization of solids (1). Therefore, the analysis of lipids and development of lipid methodology becomes important to monitor quality of raw natural products as well as processed food products. Measurement of phospholipid components can be used to evaluate the quality of crude oil from oilseeds that have been damaged due to environmental conditions in the field, high moisture content and abuse during storage or length of storage (2). In recent years, high-performance liquid chromatography (HPLC) has become an effective tool in the separation and detection of lipids (2–6). To fractionate phospholipids, they must first be separated from crude oil and then the phospholipid fraction may be separated into various components (2). Previously, many of the separations were accomplished by thin-layer chromatography, which usually included a multistep procedure (7–10). HPLC appears to be a significantly improved alternative method of separation and detection.

Because the concentration of the phospholipid fraction is low in crude peanut oil (1.04–1.65%), some method of preconcentration is necessary before HPLC analysis to give useful profiles for evaluation (2,6). Previously, phospholipids were separated from the bulk neutral lipids by low-pressure gravity columns or by Sep-Pak columns (11, 12). After the neutral lipids were separated from the phospholipids, the sample had to be concentrated prior to analysis by HPLC. This report describes a method developed to effectively enrich the phospholipid concentration from crude peanut oil, achieve class separation and separate the individual phospholipids within the chromatographic system without any modifications to existing HPLC systems. This method also reduces the amount of solvent that is used when compared to procedures previously used.

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EXPERIMENTAL PROCEDURES

Materials. HPLC solvents, methanol, chloroform, hexane and water were obtained from Fisher Scientific (Fair Lawn, NJ). Phosphatidic acid, phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylcholine used for standards were obtained from Sigma Chemical Company (St. Louis, MO).

Lipid extraction. A 50-g peanut sample was blended with 300 mL of chloroform/methanol (2:1) in an Omni blender (Sorvall-Omni, Norwalk, CT) for 1 min. The blended material was filtered and the peanut meal cake resuspended in an additional 300 mL of solvent and reground for 1 min. This procedure was carried out three times, resulting in approximately 900 mL of solvent containing the crude lipid. Water was added to the chloroform/methanol lipid mixture in a separatory funnel to remove water-soluble nonlipid components. The layers were allowed to separate, the chloroform fraction was removed, placed in a solvent repurification apparatus (Kontes, Vineland, NJ), and chloroform was evaporated under vacuum at 40°C.

Phospholipid enrichment and separation. A 500-μL loop was used to inject approximately 4 mg of crude oil onto the precolumn (40 μ silica). Repeated injections were made every 12 s until the sample was sufficiently enriched to give a usable profile. The number of injections required to inject 4 mg had been predetermined. Hexane was used as the eluting solvent during the enrichment process. Neutral lipids were eluted to the waste flask by hexane, while the phospholipids were retained on the precolumn. The HPLC system was then flushed with the starting solvent (solvent A, 4:3 isopropanol/hexane). The precolumn was attached to the analytical column. Solvent was then diverted through the precolumn, thereby eluting the adsorbed phospholipid fraction onto the analytical column (250 mm × 4.6 mm 5-μm silica column, Supelco Inc., Bellefonte, PA). Phospholipids were separated with two mixed solvents and a gradient program (Table 1). The gradient HPLC program was optimized by using known phospholipid standards.

TABLE 1

High-Performance Liquid Chromatography Gradient Program for the Separation of Phospholipids

Time (min)	%A[a]	%B[b]	Event
0	100	0	3
.1	100	0	4
.5	100	0	3
20	0	100	
40	0	100	
40.1	100	0	

[a]Solvent A, isopropanol/hexane (4:3).

[b]Solvent B, isopropanol/hexane/water (8:6:1.5).

RESULTS AND DISCUSSION

Phospholipids are important to the food industry because of their unique properties. These substances add smoothness and texture to processed food products and act as antioxidants as well as emulsifiers. The phospholipid profile in seed oils can reflect both pre- and postharvest changes that might have occurred due to environmental stress or to postharvest conditions. In peanuts, the phospholipid fraction constitutes approximately 1–1.65% of the total lipid fraction. Therefore, the development of a method to separate the bulk neutral lipids from the phospholipids and to separate the individual phospholipids at the same time by HPLC was the main objective of this study. Figure 1 shows the HPLC separation of peanut phospholipids from the neutral lipid fraction and separation from each other. Retention times for standard phospholipids and those enriched from crude peanut oil are included in Table 2, showing excellent agreement between standards and sample phospholipids. This HPLC method provides enrichment of peanut phospholipids from the bulk neutral fractions within the chromatographic system. It can be used to monitor the quality of crude oil from peanuts, which can be affected by environmental stress and postharvest conditions. For example, PE and PI increased in concentration when peanuts were exposed to −10°C (data not shown).

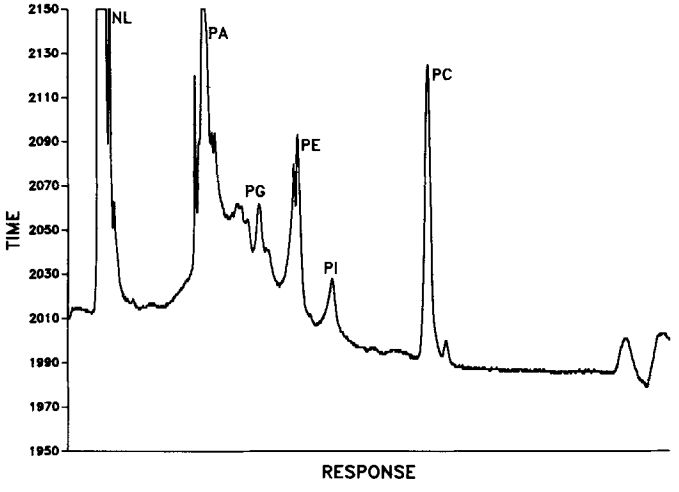

FIG. 1. High-performance liquid chromatography separation of phospholipids from neutral lipids in crude peanut oil. NL, neutral lipids; PA, phosphatidic acid; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PC, phosphatidylcholine. Column: 4.6 mm × 250 mm silica.

TABLE 2

Retention Time for Phospholipid Standards and Phospholipids Extracted from Peanut Seed

Phospholipid	Retention time of phospholipid standards (min)	Retention time of phospholipids from peanut seed oil (min)
Phosphatidic acid	11.21	11.28
Phosphatidylglycerol	16.35	16.37
Phosphatidylethanolamine	19.18	19.02
Phosphatidylinositol	21.92	22
Phosphatidylchlorine	29.39	29.94

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