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Analyses of Phospholipids in Soybean Oils by HPLC

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HPLC를 이용한 대두유 인지질의 분석

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Abstract

The qualitative and quantitative analyses of phospholipids in chloroform-methanol extracted crude and hexane extracted deodorized soybean oils were carried out by High-Performance Liquid Chromatography (HPLC). The phosphorus contents in hexane extracted crude, degummed, refiend, bleached and deodorized soybean oil were 510, 120, 5, 1.4 and 1 ppm, respectively. The chloroform-methanol extracted crude soybean oil had 800 ppm phosphorus. The phospholipids found in crude oil were phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and phosphatidic acid. Only phosphatidylchonine and phosphatidylethanolamine were detected in the deodorized soybean oil. The HPLC method described in this report can separate and detect individual phospholipids in soybean oil at 0.1 ppm level in 30 min.

Introduction

Phospholipids in soybean oil have long been recognized as an important factor influencing the keeping-quality of soybean oil.[1] Soybeans undergo several processing steps to produce a deodorized oil with good flavor quality. These processing steps produce crude, degummed, refiend, bleached, and deodorized soybean oils, which have different levels of phospholipids. The reproted phospholipids in crude oil and degummed oil are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), lysophosphatidylcholine (LPC), and lysophosphatidylethanolamine (LPE).[2,3] The only information available on the phospholipids of deodorized soybean oil is the phosphorus content determined by AOCS method.[4] Qualitative and quantitative information on phospholipids in refiend, bleached, and deodorized soybean oils are not available due to the lack of resolution and sensitivity of conventional analytical methods used in the analysis of phospholipids in soybean oil. It is, however, important to fully understand the effects of different processing steps on the quantitative and qualitative information of phospholipids in soybean oils.

The purposes of this investigation were (1) to determine the effects of processing steps on the phosphorus content in crude, degummed, refiend, bleached, and deodorized soybean oil, (2) to develop a rapid and sensitive HPLC method which can analyze phospholipids in different soybean oils qualitatively, and (3) to study the effects of extracting solvents on the phospholipids in crude soybean oil.

Materials and Methods

Samples

Soybeans (AmCor-4603) used for chloroform-methanol extracted crude oil preparation were obtained from the Department of Agronomy, The Ohio State University. Hexane extracted crude, degummed, refined, bleached, and deodorized soybean oils were obtained from Capital City Products Co. (Columbus, OH.). Standard phospholipids were obtained from Sigma Chemical Co. (St. Louis, MO.), and Supelco (Bellefonte, PA.).

Sample Preparation

Chloroform-methanol extracted crude oil was prepared from mature soybeans by the method of Folch, et al.[5] after they were ground.

Phosphorus Determination

Phosphorus content in soybean oils and individual phospholipids were determined by the AOCS method[4] and by the method of Rouser, et al.,[6] respectively.

Separation of Phospholipids

The HPLC instrument consisted of 2 solvent pumping systems (Model 100, Altex Scientific Co.), injection system (Model 500, Altex Scientific Co.), and ultraviolet detector at 205 *nm* (LC-75, Perkin-Elmer, Norwalk, CT.). The column was a 4.6 *mm* × 25 *cm* Zorbax Sil column (DuPont Instruments). Phospholipids were separated by a moidification[7] of the method of Chan, et al.[8] The mobile phases were hexane-isopropanol (3:2, *v/v*) for Solvent A and hexane-isopropanol-water (56.7:37.8:5.5, *v/v/v*) for Solvent B. For the gradient elution, the mixture of Solvents A and B (1:1) was run for 10.5 min at 1.5 *ml/min* and then the proportion of Solvent B increased to 100% at 10% increase/*min* durig the next 5 min. Solvent B was maintained for 6 min and was decreased to 50% during the next 3 *min*, and then the mixture of Solvents A and B (1:1, *v/v*) was run until the analysis was completed.

The injection volume for methanol-chloroform extracted crude soybean oil analysis was 25 *μl* choloroform solution ocntaining 2.5 *mg* of crude oil. The volume of deodorized soybean oil was 25 *μl* chloroform containing 4.5 *mg* deodorized oil.

Identification

The unknown compounds in the chromatograms were identified by comparing the retention times with those of authentic phospholipids.

Results and Discussion

The results of degumming, refining, bleaching, and deodorization effects on the phosphorus content in soybean oil are given in Table 1. Phosphorus contents in crude and deodorized oils were 510*μg* and 1 *μg* phosphorus/*g* oil, respectively. Degumming removed 77% of phosphorus-containing compounds in the crude oil, and the combined degumming and refining processes removed 99% of phosphorus compounds in the crude oil. The deodorized oil, which is the final product, contained only 0.2% of the phosphorus compound in the crude soybean oil.

The crude soybean oil extracted with the mixture of chloroform and methanol (2:1, *v/v*) contained more phospholipids (800*μg* phosphorus/g) than the hexane extracted crude oil (510 *μg* phosphorus/*g*), as was expected. The mixture of chloroform and methanol, being more polar than hexane, is a better solvent for extracting phsophorus compounds.

Table 1. The effects of processing steps on the phosphorus content in soybean oil

Processing Step	Phosphorus[a] (*μg* P/l *g* oil)
Crude oil extracted with Hexane	510
Degummed oil	120
Refined oil	5.0
Bleached oil	1.4
Deodorized oil	1.0
Crude oil extracted with $CHCl_3$-CH_3OH	800

[a] Average of duplicate determinations.

Separation of Phospholipids

The chromatograms of crude and deodorized soybean oils are shown in Fig. 1. The chromatogram of crude soybean oil extracted with chloroform and methanol mixture shows 5 phospholipids with relatively good resolution. This chromatogram indicates that 5 major phospholipids in soybean oil can be qualitatively analyzed without any specific sample preparation.

However, the chromatographic conditions still need to be imporved to have better separation of PI from PA. The chromatogram of deodorized oil showed only PC and PE.

The phosphorus contents of major phospholipids in crude and deodorized soybean oils are listed in Table 2. The phosphorus contents in PC and PE in crude oil were 500.8 *μg* and 213.6 *μg/g* oil, respectively, and represent 62 and 27% of total phosphrus in crude oil. The phosphorus contents in PC and PE in deodorized oil were 0.86 *μg* and 0.11 *μg/g* oil respectively, and represent 86 and 11% of total phosphorus in deodorized oil.

The HPLC method discussed here can separate and detect a phospholipid at a concentration of phosphorus 0.1 *μg/g* of oil.

The peaks eluted before the retention time of 10 min were not identified positively, but they are not

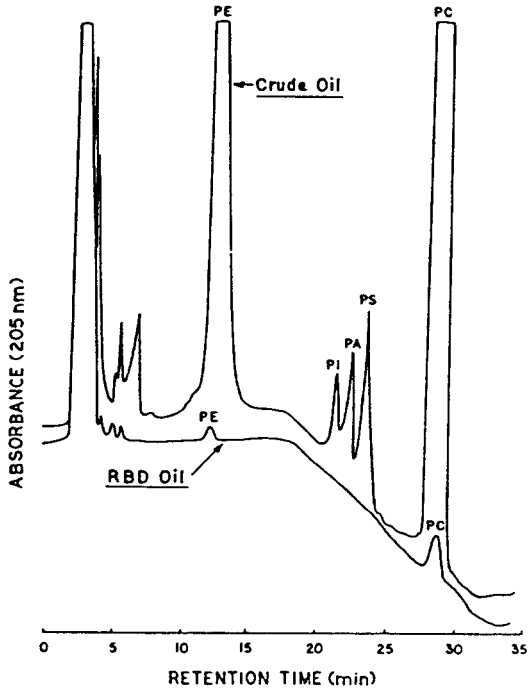

Fig. 1. HPLC chromatogram of phospholipids in crude and deodorized soybean (RBD) oil

Table 2. Phosphorus content in phospholipids in crude and deodorized soybean oils

Phospholipid	Crude Oil (μg P/g)	Deodorized Oil (μg P/g)
Phosphatidylcholine	500.8	0.86
Phosphatidylethanolamine	213.6	0.12

phosphorus-containing compounds according to the phosphorus assay.

This study provides a rapid, sensitive, and simple HPLC method for determining major phospholipids in different soybean oils. Work is still needed to improve the resolution of PI and PA.

요 약

헥산으로 추출한 대두유의 탈취유와 클로로포름-메타놀로 추출한 대두원유내에 존재하는 인지질의 정성정량분석을 고성능 액체크로마토그라피를 사용하여 행하였다. 헥산으로 추출한 대두원유, 탈검유, 정제유, 탈색유 및 탈취유의 인함량은 각각 510, 120, 5, 1. 4, 1 ppm이었으며 클로로포름-메타놀로 추출한 대두원유의 인함량은 800 ppm이었다. 원유내에서 확인된 인지질은 포스파티딜콜린, 포스파티딜에타놀아민, 포스파티딜이노시톨, 포스파티딜세린, 포스파티딜산이었으며, 탈취유에서는 포스파티딜콜린과 포스파티딜에타놀아민만이 검출되었다. 이 분석방법을 사용하면 대두유내의 모든 인지질을 30분이내에 개별적으로 분리하고, 0. 1ppm수준까지 정량분석할 수 있다.

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