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Quantitative Analysis of Lysophosphatidyl Choline (LPC) in Wheat Starch Lipids by High Performance Liquid Chromatography

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고속액체크로마토그래피에 의한 밀전분 지방질에 함유된 리소레시친의 정량

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ABSTRACT. The content of lysophosphatidyl choline (LPC) in wheat starch lipids from six cultivar representing three classes of wheat was determined by a high performance liquid chromatography using UV-detection methodology (HPLC-UV). The HPLC-UV assay had a sensitivity of LPC concentrations above 5 μg/50 μl and required 80 minutes per chromatogram.

요 약. HPLC-UV방법을 이용하여 밀전분 지방질의 주성분인 LPC를 정량하기 위해 HRW 밀전분 지방질로부터 순수한 LPC를 분리하여 LPC의 표준곡선을 작성하였다. 밀 품종별 LPC에 대한 HPLC peak responses는 시료간 큰 차이를 보여주지 않아 HRW 밀전분 지방질로부터 만든 LPC의 HPLC-UV 표준곡선은 밀전분 지방질에 함유되어 있는 LPC의 함량을 신속하고 정확하게 정량하는 데 이용할 수 있음을 보여 주었다.

Key Words: HPLC, lysophosphatidyl choline, wheat starch, quantitative analysis

1. INTRODUCTION

The extraction and determination of the composition and distribution of lipids associated with wheat starch have been the focus of several studies[1~5]. Most wheat starch lipid occur inside the granule and are composed of about 90% lysophospholipids, and the surface of wheat starch also contains not only

phospholipid but also non-polar and glycolipid[1,6].

Silicic acid column chromatography has been used to quantitate starch lipids[12]. This column chromatographic procedure is time consuming and requires large amounts of lipids. A more detailed analysis of the full range of lipid classes in wheat starches has been achieved using three or four of one-dimensional separations on thin-layer chromatograms followed by quantifying phospholipids from phosphorus content and other lipids by gas chromatography as fatty acid methyl esters[6,7]. This gas chromatographic procedure is also slow and tedious. A high performance liquid chromatography theoretically offers a rapid methodology for lipid analysis[8,13]. Nevertheless, a major limitation has been the lack of a suitable chromophore in lipids and the lack of a sensitive universal detector[8].

The purpose of our study was to investigate the use of HPLC-UV to quantitate LPC in wheat starch lipid from three classes of wheat.

2. EXPERIMENTAL

2.1 Lipid Reference Standard

Qualitative identification of lipid in wheat starch was based on the following reference standard purchased from Sigma Chemical Co. (St. Louis, MO 63178, U.S.A.) ; linoleic acid, triolein, monogalactosyl diglyceride (MGDG), digalactosyl diglyceride (DGDG), phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PG), phosphatidyl inositol (PI), lysophosphatidyl ethanolamine (LPE), lysophosphatidyl glycerol (LPG), phosphatidyl serine (PS), lysophosphatidyl inositol (LPI), lysophosphatidyl serine (LPS), phosphatidyl choline (PC), and lysophosphatidyl choline (LPC). Quantitative analysis of LPC by HPLC was using standard LPC isolated from wheat starch, which is described in "2.5 Isolation of Reference Standard for quantitation of Wheat Starch LPC" of the Experimental.

2.2 Wheat Starch

The hard red winter (HRW) wheats Victory and Mustang were provided by the U.S. Grain Marketing Research Laboratory, Manhattan, KS, while the Durum wheat Vic was obtained from North Dakota State University, Fargo, ND. Three samples of soft red winter (SRW) wheats were obtained from the Soft Wheat Quality Laboratory, Wooster, Ohio ; Caldwell, a soft wheat with relatively strong gluten ; Cardinal with medium gluten ; and Titan with weak gluten.

Each wheat sample was milled on a Buhle Laboratory mill, give flour of 72% extraction. Starch was isolated from flour-water doughs according to established procedures. After repeated washing and centrifugal separations, the starches were air-dried[9]. The recovery yields of the six starches were about 50% based on flour weight and their protein contents ranged from 0.3 to 0.5% (*Table* 1).

Table 1. Composition of wheat starches from six cultivar

Sample	Wheat class	Moisture content (%)	Protein (%)	Ash (%)
Victory	HRW	10.8	0.3	0.10
Mustang	HRW	7.6	0.4	0.15
Vic	Durum	9.6	0.3	0.12
Caldwell	SRW	8.8	0.4	0.11
Cardinal	SRW	9.2	0.4	0.06
Titan	SRW	9.0	0.5	0.13

2.3 Extraction of Starch Lipid

To the 1 *l* of round-bottom flask fitted with a condenser was added starch (40 g) and 640 m *l* of n-propanol/water (3 : 1, v/v). The mixture was stirred for 4 hours at 20°C to remove surface lipids from the starch. The mixture was filtered and the collected starch was resuspended in n-propanol/

water (3 : 1, v/v, 16 m*l* /g starch). After heating the mixture in a boiling water bath for 12 hours, cooling and filtering, the filtrate was concentrated to dryness under vacuum[1,5,6,10]. Crude starch lipids were purified by applying aliquot (20 mg) of the extract to a column of a 50g portion of sephadex G -25, and eluting the lipids with a mixture of water -saturated chloroform/methanol, 19 : 1 (v/v)[11].

2.4 High Performance Liquid Chromatography (HPLC) of Starch Lipid

HPLC-UV of starch lipids was carried out using the solvent elution system of Geurts Van Kessel et al.[8]. The HPLC-UV system in our laboratory was a Hewlett Packard Model HP 1090 liquid chromatography, a loop injector (50 μ*l*), UV-detector (210 nm), and an HP Model 3390A recorder.

Analyses were performed at 25°C and a flow rate 1 m*l* /min on a silicic acid (10 μm) column (200 × 4.6 mm, I.D., Bio-Rad). The column was developed isocratically 5 min with n-hexane/2 -propanol/water (41/54/5, v/v/v), then with a linear gradient from 41/54/5 to 39/52/9 (v/v/v) from 5 to 20 min, and finally isocratically from 20 to 80 min. Peaks were identified from the retention time of commercial reference standard. LPC was quantitated using a standard curve derived from wheat starch LPC which was isolated as described in "2.5 Isolation of Reference Standards for Quantitation of Wheat Starch LPC".

2.5 Isolation of Reference Standards for Quantitation of Wheat Starch LPC

A column (250 × 25 mm, I.D.) containing 30g of silicic acid activated for two hours at 130°C, was prepared in chloroform[12]. Wheat starch lipids (100 to 250mg) were applied, and developed with chloroform (500 m*l*), acetone (500 m*l*), 50% methanol in chloroform (100 m*l*), 67% methanol in chloroform (250 m*l*), 75% methanol in chloroform (250 m*l*) and methanol (250 m*l*). Each fraction was determined by a high performance liquid chromatography (HPLC).

3. RESULTS AND DISCUSSION

A typical HPLC chromatogram of wheat starch lipid is shown in *Figure* 1. By the comparison of retention time in chromatogram of reference standards (data not shown), we found that non-polar and glycolipids were eluted within 10 minutes, while phospholipids were eluted in 20-60 min. LPC, the major component of wheat starch lipids, was readily resolved from other phospholipids, which included LPG, LPE, LPI, LPS and PC.

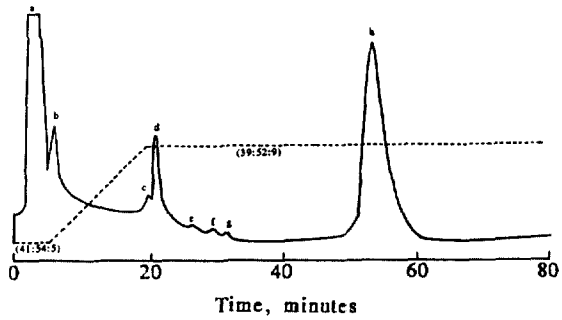

Figure 1. HPLC-UV chromatogram of starch lipid. Chromatogram (——) and eluting gradient (·····) of n-hexane/2-propanol/water (v/v/v).
a : solvent peak, non-polar and glycolipid, b : digalactosyl diglyceride (DGDG), c : lysophosphatidyl glycerol (LPG), d : lysophosphatidyl ethanolamine (LPE), e : lysophosphatidyl inositol (LPI), f : lysophosphatidyl serine (LPS), g : phosphatidyl choline (PC), h : lysophosphatidyl choline (LPC).

Geurts Van Kessel et al.[8] have shown that phospholipids containing unsaturated fatty acids give,

much greater UV absorption at 203nm than phospholipids with saturated fatty acids, and for the complex absorption behavior of phospholipid, an accurate quantitation can be obtained only from the collect reference phospholipid. The purchased LPC was not satisfactory for HPLC determination of wheat starch LPC because the purcased LPC had differing fatty acid composition, and thus different peak responded from wheat starch LPC.

To obtain wheat starch reference standard for LPC, six fractions were collected from the silicic acid column chromatographic separation of starch lipids present in HRW wheat starch. HPLC indicated that the fraction eluted with ethanol gave one peak with the same retention time as commercial standard LPC (*Figure* 2).

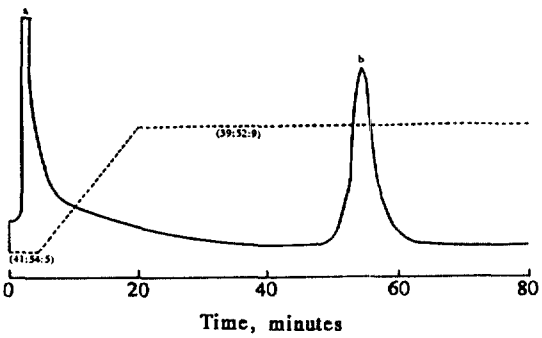

Figure 2. HPLC-UV chromatogram of lysophosphatidyl choline isolated by preparative silicic acid column chromatography of HRW wheat starch lipids.
a : solvent peak, b : lysophosphatidyl choline (LPC), eluting gradient (- - -).

HPLC-UV standard curve for LPC from HRW wheat starch lipid was linear (r=0.98) up to 150 μg, while LPC concentrations above 5μg/50μl was required for detection. The equation of standard curves was as follows ; Peak responce for LPC was $(0.1975C + 0.1710) \times 10$ where into C is lysophospholipid amount. To apply the standard curves for LPC from HRW wheat starch to those phospholipids from other classes of wheat starch, we determined the HPLC peak responses for LPC isolated from six different wheat starch lipids by silicic acid column chromatography. *Table* 2 shows there was no significant difference between responses for the six wheat starch lipids. Based on the similarity of peak responses for LPC in the different wheat starch lipids, it was concluded that the HPLC -UV procedure could be used to analyze LPC in any wheat starch lipids. A series of wheat starch lipids were analyzed for LPC contents (*Table* 3).

Table 2. HPLC peak response[a] for LPC isolated from six wheat starch lipids

Sample	Peak response (arbitrary unit × 100)
Victory	19.3
Mustang	19.0
Vic	20.2
Caldwell	19.9
Cardinal	19.2
Titan	19.1
Mean ± S.D.	19.5 ± 0.5

[a] Average of two determinations. Each injection (50μl) contained 100μg of LPC.

Table 3. Level[a] of LPC in wheat starch lipids determined by HPLC-UV

Sample	Content (%)
Victory	61.3 ± 1.2
Mustang	62.2 ± 0.9
Vic	64.4 ± 1.3
Caldwell	66.7 ± 1.6
Cardinal	68.1 ± 1.5
Titan	67.9 ± 1.0

[a] Mean ± S.D. based on three determinations.

LPC content in the six samples ranged from 61.3± 1.2 to 68.1 ± 1.5% of starch lipids.

In conclusion, the HPLC-UV method for quantitating LPC in wheat starch lipids is relatively rapid, sensitive and accurate.

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