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PHOSPHOLIPIDS OF SOYBEANS*

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The phospholipids (PLs) of ripe soybean seeds of the variety Uzbekskaya-2 have been investigated, and 10 classes of PLs have been detected, the main ones being phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositols. It has been found that this variety contains a large set of minor components. The compositions of the fatty acids (FAs) of homogeneous classes of PLs have been studied.

The seeds of Leguminosae, especially soybeans, differ from those of other plants by a high content of membrane lipids. The soybean — *Glycine max* (L.) Merr (Fabaceae) — forms an important technical and fodder crop traditional for the Far East. The polar lipids of soybeans have been widely studied, and 15 classes of PLs have been isolated from them [1-11], while a phosphoaminolipid — sphingomyelin — has been detected in soybean phospholipids [6]. However, the PLs of local varieties of soybean have scarcely been investigated [11].

We have studied the fractional and FA compositions of the PLs of ripe soybeans of the variety Uzbekskaya-2. The total PLs were extracted from the hexane-defatted beans with chloroform–methanol (2:1). According to TLC, the extract contained, in addition to the PLs, neutral lipids, carbohydrates, sterols, amino acids, etc.

The total PLs, freed from the above-mentioned classes of impurities and amounting to 1.9% of the weight of the seeds, were analyzed by two-dimensional TLC in solvent systems 1 (first direction) and 2 (second direction). Ten components were detected, with the following R_f values in the first and second (shown in parentheses) directions: 0.1 (0.1) — lysophosphatidylcholines (lyso-PCs); 0.15 (0.4) phosphatidylinositols (PIs); 0.35 (0.3) — phosphatidylcholines (PCs); 0.5 (0.6) — phosphatidylethanolamines (PEs); 0.8 (0.95) — N-acyl-PEs; 0.6 (0.85) — N-acyl-lyso-PEs; 0.1 (0.75) — phosphatidic acids (PAs); 0.4 (0.7) — phosphatidylglycerols (PGs); and 0.5 (0.9) — X_1-PLs — and 0.05 (0.5) — X_2-PLs.

From their chromatographic mobilities and qualitative reactions it may be assumed that the X_1-PLs were di-PGs (DPGs), and the X_2-PL lyso-PAs, which have been detected previously in other soybean varieties [4, 5]. In the quantitative respect, these classes of PLs were distributed in the following way (% on the total PLs): PCs — 35.8; PIs — 20.1; PEs — 19.9; lyso-PCs — 2.3; N-acyl-PEs — 5.5; N-acyl-lyso-PEs — 3.8; PAs — 3.6; DPGs — 2.8; lyso-PAs — 1.8; PGs — 4.4. It must be mentioned that this soybean variety is distinguished from varieties studied previously by a lower total PL content [10] and a larger set of minor components.

By separating the total PLs into fractions with the aid of CC on silica gel and repeated PTLC we isolated all ten components in the homogeneous form. The FA compositions of the total PLs and their individual classes were analyzed after mild alkaline hydrolysis, methylation, and GLC analysis (Table 1). With respect to increasing degree of saturation, the molecules of the homogenous classes formed the following sequence: PCs, lyso-PCs, N-acyl-PEs, lyso-PAs, N-acyl-lyso-PEs, PEs, PIs, PAs, DPGs, PGs. A total of 12 acids were detected, while in all cases palmitic predominated among the saturated acids, and linoleic among the unsaturated acids. The lowest level of the 16:0 acid was observed in the PCs (10%), and the highest in the PAs (32.2%).

The position distributions of the FAs in the main classes of PLs (PCs, PEs, PIs) were established by enzymolysis with phospholipase A_2 in Tris buffer. The lyso- compounds obtained were subjected to alkaline hydrolysis, during which the FAs

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TABLE 1. FA Composition of the Total PLs of Soybeans and of Individual Classes of Them

FAs	Total PLs	PCs			PEs			PIs			N-Acyl-PEs			N-Acyl-lyso-PEs			Lyso-PCs	PGs	Di-PGs	PAs	Lyso-PAs
		tot.	sn-1	sn-2	tot.	sn-1	sn-2	tot.	sn-1	sn-2	tot.	N-acyls	O-acyls	tot.	N-acyls	O-acyls					
10:0	0.7	0.2	Tr.	0.5	Tr.	Tr.	Tr.	1.9	2.3	0.9	1.0	2.4	0.4	0.7	–	0.3	1.5	3.1	4.7	Tr.	
12:0	0.8	Tr.	Tr.	Tr.	3.2	5.0	2.2	3.0	4.0	1.1	0.8	1.9	0.6	1.4	–	0.2	0.7	7.5	8.5	3.3	1.8
14:0	0.6	0.5	0.4	Tr.	1.9	3.0	Tr.	1.3	2.4	Tr.	0.7	2.6	2.3	2.5	–	0.4	0.7	3.6	4.4	2.6	1.0
15:0	0.3	–	–	–	2.2	3.0	Tr.	1.3	1.4	Tr.	0.6	1.2	1.3	2.6	2.0	0.4	–	4.5	5.1	3.2	1.0
16:0	18.1	10.0	20.0	3.3	23.1	35.2	11.1	32.5	52.5	13.1	22.2	27.0	12.1	17.3	18.1	18.3	11.6	34.3	28.2	32.2	22.8
16:1	0.9	0.4	Tr.	Tr.	3.1	Tr.	3.5	Tr.	–	0.4	0.7	3.0	3.2	3.0	7.5	0.9	Tr.	Tr.	Tr.	Tr.	Tr.
17:0	0.6	Tr.	Tr.	Tr.	4.0	6.8	Tr.	2.3	4.0	Cn.	1.1	3.9	2.0	3.4	2.7	0.7	Tr.	4.7	5.5	4.6	1.7
17:1	0.5	–	–	–	Tr.	Tr.	Tr.	Tr.	Tr.	0.3	0.7	1.9	1.7	3.4	Tr.	0.8	–	2.9	4.1	Tr.	1.0
18:0	7.5	5.7	12.0	1.2	8.0	15.0	1.4	11.0	17.8	2.0	6.2	10.5	5.7	8.8	8.9	5.5	6.3	11.3	12.0	13.6	6.2
18:1	11.3	8.2	7.9	8.2	11.5	4.3	15.8	9.0	2.7	12.5	9.9	9.7	9.3	13.5	6.9	10.5	7.8	9.5	11.0	9.9	12.5
18:2	51.3	68.2	55.5	77.7	39.3	24.8	60.4	34.2	10.9	65.3	51.5	31.1	53.7	38.3	49.7	60.7	63.2	15.5	16.5	22.4	47.0
18:3	7.4	6.8	4.2	9.1	3.7	2.9	5.6	3.5	2.0	4.4	4.6	4.8	7.7	5.1	4.2	1.3	8.2	3.1	–	8.2	5.0
ΣS	28.8	16.4	32.4	5.0	42.4	68.0	14.7	53.3	84.4	17.1	32.6	49.5	24.4	36.7	31.7	25.8	20.8	69.0	68.4	59.5	34.5
ΣU	71.2	83.6	67.6	95.0	57.6	32.0	85.3	46.7	15.6	82.9	67.4	50.5	75.6	63.3	68.3	74.2	79.2	31.0	31.6	40.5	65.5

of the sn-1 positions were split out (see Table 1). The amounts of unsaturated FAs in the sn-2 positions were: in the PCs — 95.0%; in the PEs — 85.3%; in the PIs — 82.9%. A particularly high specificity of the distribution of the acids was characteristic for the PCs.

There is no information in the literature on the distribution of FAs between the O and N atoms in the molecules of N-acyl-PEs and their lyso- analogs from ripe soybeans. To study this question, the N-acyl-PEs and N-acyl-lyso-PEs were subjected to mild deacylation [12], in which the acids of the glycerol moiety of the molecule (O-acyls) were split out. The FAs of the glyceryl-phosphoryl-N-acylethanolamines (N-acyls) were isolated by cold saponification. GLC analysis of the FAs showed that the N-acyls in the N-acyl-PEs and their lyso- analogs were more saturated than the O-acyls, which is typical for N-acylated plant PLs.

EXPERIMENTAL

For TLC we used silica gel and the following solvent systems: 1) $CHCl_3-CH_3OH-NH_4OH$ (65:35:5), and 2) $CHCl_3-CH_3OH-$acetone$-CH_3COOH-H_2O$ (10:5:4:2:1).

Elimination of Amino Acid Impurities from the Total PLs [13]. A suspension of the total PLs in a small volume of water was extracted with chloroform, and the chloroform layer was separated off and evaporated. The amino acids remained in the water (TLC, ninhydrin).

The quantitative levels of the individual classes of PLs in the total were determined from an analysis of the phosphorus contents of the spots according to [14]. Gas-liquid chromatography was conducted on a Chrom-4 chromatograph with a flame-ionization detector, using a stainless steel column filled with Chromaton N-AW bearing 15% of Reoplex-400, at 210°C and a rate of flow of carrier gas (helium) of 115 ml/min.

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