COMPOSITION OF THE PHOSPHOLIPIDS OF THE SEEDS OF Sapium sebiferum

M. A. Bitadze, Ts. M. Dalakishvili,
É. P. Kemertelidze, and Kh. S. Mukhamedova

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The fractional composition of the polar lipids and also the fatty-acid composition of the main components of the phospholipids of the Chinese tallow tree have been studied. It has been established that in the seeds of the Chinese tallow tree there are 11 phospholipids, two glycolipids, and two phosphonolipids. The main fatty acids of the phospholipids are palmitic and linoleic.

The Chinese tallow tree Sapium sebiferum, family Euphorbiaceae, which is common in southern China, has been introduced into cultivation on the Black Sea littoral of Georgia [1, 2]. The seeds of this plant are a valuable raw material for the production of a technical oil [2, 3].

We have studied the composition of the phospholipids (PLs) of the Chinese tallow tree growing in the Gagra mountains.

The comminuted raw material was defatted with n-hexane, and the fraction of polar lipids was extracted by Folch's method [4]. The total phospholipids, freed from impurities [5], amounted to 0.7% of the weight of the air-dry raw material (systems 1 and 2).

With the aid of TLC in system 3, the presence of monogalactosyldiacylglycerols (R_f 0.91) and of digalactosyldiacylglycerols (R_f 0.70) in the glycolipids was established. By two-dimensional TLC in a layer of silica gel and systems 3 and 4 [6, 7], 11 phosphorus-containing spots in the total PLs, the amounts of them being determined spectrophotometrically [6] (%): PCs – 45.3; PIs – 16.7; PEs – 12.9; PSs – 7.7; N-acyl-PEs – 6.2; PAs – 5.7; and lyso-PCs – 5.3 were determined. PGs, lyso-PCs, and N-acyl-lyso-PEs and an unidentified PL were detected in trace amounts. As we see, in the total PLs of the seeds of the Chinese tallow tree the main components are phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), and phosphatidylinositols (PIs).

TABLE 1. Composition and Positional Distribution of the Fatty Acids in the PCs, PIs, and PEs of the Seeds of the Chinese Tallow Tree

Fatty acid	PCs			PEs			PIs		
	total	sn-1	sn-2	total	sn-1	sn-2	total	sn-1	sn-2
12:0	2,5	4,0	—	0,1	Tr.	Tr.	—	—	—
14:0	1,7	5,1	0,9	4.5	6,2	2,1	4 4	4,6	4,4
14:1	1,1	—	2,0	0,6	Tr.	Tr.	—	—	—
15:0	1,4	2,7	0,6	3,0	4.5	1,3	2,0	1,6	2,6
16:0	43,5	68,5	18,9	32,6	46,3	18,2	38.0	51,5	24.2
16:1	1,9	1,5	2,4	6,1	10,9	2,9	4,5	6,4	3,5
17:0	0,6	—	—	2.1	3,0	—	3,8	6,0	—
18:0	8,7	11,4	8,3	7,8	8,6	7,1	10,3	14,9	7.4
18:1	12,8	4,4	20,1	17,5	15,6	20.8	11,3	9,8	12,2
18:2	17,0	1.0	30,7	17,6	2.8	30,6	15,9	5.2	26,2
18:3	3,0	1,4	3,5	2,9	2,1	6,9	1,7	—	3,0
20:4	5,3	—	12,6	5,2	—	10.1	8,1	—	16.5
Σ_s	58.4	91,7	28,7	50,1	70,7	28.7	58,5	78.6	38,6
Σ_u	41,6	8,3	71,3	49,9	29,3	71,3	41,5	21,4	61,4

I. G. Kutateladze Institute of Pharmatochemistry, Georgian Academy of Sciences, Tbilisi. Institute of Chemistry of Plant Substances, Uzbek Academy of Sciences, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 619-621, September-October, 1991. Original article submitted January 2, 1991.

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To isolate homogeneous phospholipids, the total material was chromatographed on a column of silica gel and the crude phospholipid fractions obtained were separated by preparative TLC in systems 3 and 5. The PCs, PEs, and PIs were isolated in the homogeneous state, and they were identified from their IR spectra [8-10] and also from the products of their acid hydrolysis. The fatty acids split out were analyzed by GLC (Table 1). The sets of fatty acids in the PCs and PEs included 12 components, and that of the PEs 10. The largest amount of arachidonic acids was observed in the PIs (8.1%). In all cases, palmitic acid predominated among the saturated acids, and linoleic acids among the unsaturated acids. It must be mentioned that in the PEs the levels of the 18:1 and 18:2 acids were the same.

The position-specificity of the distribution of the fatty acids in the molecules of the fatty acids investigated was determined by studying the products of their enzymatic hydrolysis.

The figures given in Table 1 show that in the PLs studied there was specificity in the distribution of the acids: 71.3% of unsaturated acids was esterified in the sn-2 positions of the PCs and PEs, and 61.4% in the PIs. Attention is attracted by the fact that arachidonic acid was localized totally in the sn-2 position.

By using the method of quantitatively determining the phosphono analogs of the PLs and also the technique of TLC separation of phospho- and phosphonolipids [5, 11], in the total PLs we also detected phosphono analogs of the PCs (R_f 0.47) and PEs (R_f 0.79).

EXPERIMENTAL

The total polar lipids were extracted from the defatted seeds with a mixture of chloroform and methanol (2:1). The total material was freed from carbohydrate impurities by gel filtration through Sephadex G-25 in chloroform–methanol–water (90:10:1), and from neutral lipids, pigments, and glycolipids by the TLC method on silica gel in solvent systems 1 and 2 [5].

For chromatography we used Chemapol silica gel (Czechoslovakia).

The acid hydrolysis of the PLs was carried out in sealed tubes in 3 N HCl for 48 h in the case of the PIs and for 24 h for the other PLs.

The enzymatic hydrolysis of the homogeneous TLCs was carried out with the aid of kufi venom phospholipase A_2 in borate buffer at pH 7.4. The products of enzymatic hydrolysis were separated by PTLC in system 3, and the FAs were methylated with diazomethane and were analyzed by GLC.

Lyso compounds were subjected to acid methanolysis, and the FAMEs were studied by the GLC method.

The following solvent systems were used: 1) hexane–ether (7:3); 2) acetone; 3) chloroform–methanol–25% ammonia (65:30:4); 4) chloroform–methanol–acetone–acetic acid–water (5:2.5:2:1:0.5); and 5) chloroform–methanol–water (65:35:5). GLC was conducted on a Chrom-41 chromatograph using a stainless steel column (2.5 × 4 mm [sic]) filled with 17% of PEGS on Chrom W (60-80 mesh). Temperature 198-200°C.

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ENZYMATIC-CHEMICAL ISOLATION OF LIPIDS, PROTEINS, AND GOSSYPOL FROM COTTON SEEDS AND MEAL. I.

S. D. Gusakova, I. P. Nazarova, A. I. Glushenkova, D. A. Khashimov, Kh. G. Alimov, and P. Kh. Yuldashev

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An enzymatic-chemical method of isolating the complex of lipids, gossypol pigments, and water-soluble proteins from cotton seeds and meal has been developed which permits: a) the achievement of the maximum yield of all types of lipids without their degradation; b) a rise in the qualitative and food value of the oil and protein products isolated from the seeds, with an increase in the solubility of the latter; c) the extraction from cotton seeds of more than 75% and from cottonseed meal of 42% of the free gossypol. The method, in combination with Folch's procedure, is suitable for the quantiative estimation of the lipids, strongly bound to the protein of the nucleus.

The seeds of oil crops (cotton, sunflower, rape, etc.) are sources not only of vegetable oils but also of biologically active phospholipids, glycolipids, polyphenols, and a valuable vegetable protein with unique organoleptic and functional properties [1, 2].

In the oils and fats industry, the technology of a two-stage treatment of the seeds first by prepressing and then by extracting the solid residue with a hydrocarbon solvent (gasoline) is used to extract vegetable oils. It presupposes a severe treatment of the raw material, which, while facilitating the extraction of the desired product, simultaneously causes a change and partial breakdown of other valuable substances, the denaturation of the proteins and a deterioration in their nutritional value, the oxidation of the polyphenols, and the binding of part of the phenols and lipids with the protein. For this reason, the industrial oil cake and meal that are treated by the traditional technology as by-products have poor fodder properties. Of the components of the oil seeds that are of interest as independent products, at the present time phosphate concentrates are obtained, together with oil from soybeans and sunflower seeds.

A promising direction in the oils and fats industry is considered to be the development of methods permitting the production, for example, from cotton seeds, of a high-quality food oil, a gossypol-free fodder meal, and gossypol as an independent product for medicinal and technical purposes [3].

The simultaneous isolation of the lipid complex and of protein with no change in their quality is possible by treating an aqueous suspension of the oil-containing raw material with enzymes of microbiological nature (pectolytic enzymes, cellulases, proteinases) breaking down the complexes of polysaccharides, proteins, and lipids [4]. Lipids are isolated from the hydrolysate by extraction with organic solvents, and then the water-soluble and insoluble protein components are obtained from the residue. Under the action of a proteinase, the high-molecular-mass proteins are broken down to low-molecular-mass water-soluble proteins and peptides.

In comparison with the traditional method, the biotechnological method requires a lower consumption of energy, is distinguished by mild conditions of treating the material, and permits an increase in the solubility of the protein components as the result of the cleav-

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