# POLAR LIPIDS OF COTTON SEEDS AND THE PRODUCTS OF THEIR PROCESSING

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The compositions and amounts of polar lipids in cotton seeds and the products of their processing have been investigated. Six glycolipids have been identified, the main ones being monogalactosyldiglyceride (MGDGs), digalactosyldiglycerides (DGDGs), and sterol glycosides. The fatty acid compositions of the neutral lipids and of the phospho- and glycolipids have been determined. In the glycolipids, saturated fatty acids – palmitic and stearic – predominated.

Polar lipids (PLs), consisting mainly of phospholipids (PhLs) and glycolipids (GLS), are the main components of cell membranes and fulfill both metabolic and structural functions. The GLS of some oil [1], cereal ]2] and fruit [3] crops have been investigated. Shcherbakov et al. [1] have shown that the GLs of the sunflower are capable of taking part in reaction with the amino groups of proteins, forming dark-colored compounds difficult to remove in the purification of the oil. Glycolipids possess surface-active properties and, together with phospholipids, they interfere with filtration and refining processes, causing losses of oil.

The phospholipids of cotton seeds have been studied fairly fully [4, 5]. There is extremely little information on the glycolipids [6], apparently because of the presence of gossypol and other phenolic compounds which interfere with the separation and identification of the components. Continuing our investigations [7], we have studied the polar lipids of first-quality seeds and the products of their processing taken in the Tashkent oils and fats combine. The total lipids were isolated from the samples with a mixture of chloroform and methanol (2:1, v/v), and the extracts were freed from nonlipid impurities by washing with a 1% aqueous solution of NaCl [1]. The characteristics of the samples are given in Table 1.

It can be seen from Table 1 that at the prepressing stage about 60% of the phospholipids present in the pulp was extracted and at the husk extraction stage almost 26%, while 8.6% of the amount in the pulp remained in the oilseed meal. The high acid number of the lipids of the flakes can be explained by their enzyme-catalyzed hydrolysis. As compared with the lipids of the other samples, the lipids of the oilseed meal contained 31.25% of free fatty acids. The action of moisture and heat promoted the binding of the gossypol with accompanying substances, which led to a fall in its amount in the products of the subsequent processing of the seeds.

The preliminary separation of the PLs and the neutral lipids (NLs) was carried out by countercurrent separation in the hexane–87% ethanol system [8]. It was mainly the NLs that passed into the hexane fraction, the PLs being present in trace amounts. The PLs were freed

TABLE 1. Characteristics of Samples of Cotton Seed Processing Products

| Sample | Moisture content of sample, % | Free gossypol, % | Total lipids content, % | Acid No., mg KOH |
|---|---|---|---|---|
| 1. Seeds | 7,1 | 0,69 | 21,1 | 7,2 |
| 2. Flakes | 8,1 | 0,57 | 29,5 | 16,6 |
| 3. Pulp | 3,8 | 0,25 | 31,4 | 6,4 |
| 4. Hulls | 6,0 | 0,30 | 10,8 | 14,4 |
| 5. Oilseed meal | 8,2 | 0,10 | 2,7 | 62,5 |
| 6. Prepressing oil | — | 0,15 | — | 3,8 |
| 7. Extraction oil | — | 0,11 | — | 4,5 |
| 8. Soapstock | 42,0 | — | 48,6 | — |

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TABLE 2. Composition of the PLs of Cottonseed Processing Products (by weight)

| Sample | Total lipids | | | PLs | |
|---|---|---|---|---|---|
| | PLs + pigments | PhLs + pigments | GLs + pigments | PhLs + pigments | GLs + pigments |
| 1. Seeds | 6,3 | 3,8 | 3,0 | 57,2 | 42,8 |
| 2. Flakes | 6,9 | 3,7 | 3,2 | 56,3 | 43,7 |
| 3. Pulp | 6,7 | 3,7 | 3,0 | 56,5 | 43,5 |
| 4. Hulls | 9,2 | 5,4 | 3,8 | 58,1 | 41,9 |
| 5. Oilseed meal | 25,6 | 13,9 | 11,7 | 55,4 | 44,6 |
| 6. Prepressing oil | 1,1 | 0,7 | 0,4 | 68,1 | 31,9 |
| 7. Extraction oil | 1,0 | 0,6 | 0,4 | 64,5 | 35,5 |
| 8. Refined oil | 0,15 | 0,02 | 0,13 | 14,5 | 85,5 |
| 9. Soapstock | 26,7 | 7,6 | 19,1 | 28,4 | 71,6 |

from the NLs by preparative TLC on silica gel in system 1, in which the PLs remained at the start while the NLs that had been found in the PL fraction (20-25%) consisted of free fatty acids (FFAs), free sterols, and fatty acid methyl esters; triacylglycerols (TAGs) were present in trace amounts.

The separation of the PLs into PhLs and GLs was achieved by PTLC on silica gel in system 2, in which the PhLs remained at the start and the GLs were separated into their individual components.

The PhL and GL fractions from the seeds and the flakes had light brown colorations and the others, apart from the refined oil, were darker. The coloration of the lipids was due to the fact that the initial samples that had not been subjected to moist heat treatment contained mainly native gossypol while the others contained a dark-colored modification. In system 3, all the polar lipids revealed free gossypol ($R_f$ 0.60), two of its modified forms ($R_f$ 0.41 and 0.45), and anthocyans ($R_f$ 0.38), which were revealed with 50% $H_2SO_4$ in the form of a pink spot. After the extraction from the soapstock of the substances soluble in a mixture of chloroform and methanol, the insoluble residue (10.4%) was saponified with 10% alcoholic KOH and decomposed with 20% $H_2SO_4$. In an ethereal extract (36.6% of the residue) we detected FFAs, free sterols, and GLs. The unsaponified part contained 57.5% of protein.

Table 2 gives the compositions of the PLs together with the pigments accompanying them. When the PLs were separated into PhLs and GLs, the pigments were distributed over both these fractions. As can be seen from Table 2, the amounts of PLs in the seeds, flakes, and pulp were practically the same. The prepressing and extraction oils contained only 1% of PLs together with pigments, while in the lipids of the oilseed meal and soapstock they amounted to more than 25%. Thus, in the processing of cotton seeds, the bulk of the PLs remained in the oilseed meal.

The amount of phospholipids in the PLs of the samples that had not been subjected to treatment with alkali was greater than that of the GLs; in the refinery oil and the soapstock their amount was considerably less, which can be explained by the partial decomposition of the PLs in the process of alkaline refining.

On the acid hydrolysis of the GLs and PLs, the pigments were isolated in the form of a black insoluble precipitate. The amounts of the precipitate after the hydrolysis of the GLs of the flakes and the oilseed meal and the PLs of the oil were 33.1, 47.3, and 51.4%, respectively. When the amount of pigments after acid hydrolysis is taken into account it is possible to calculate the amounts of GLs and PLs in the individual samples. Furthermore, these results show that a considerable proportion of the modified pigments of the gossypol group are capable of forming relatively stable complexes with the polar lipids that are not decomposed either on their treatment with the solvents usually employed or on their separation by TLC in neutral and alkaline systems.

Under the conditions of two-dimensional TLC (system 4) the phospholipids of the cotton seeds were separated into five spots, the main ones of which were phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol; lysophosphatidylcholine and an unknown PL were present in minor amounts. In the PLs of the oilseed meal and soapstock that had been subjected to various thermochemical actions, two and three, respectively, unknown components giving a positive reaction for PLs were detected.

In all the samples seven GLs were detected, the main ones being sterol glycosides (SGs),

TABLE 3. Fatty Acid Compositions of the Products of the Processing of Cotton Seeds, % by GLC

| Sample | Fatty acid, mole % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | Σ unsat | Σsat |
| Flakes | | | | | | | | |
| Neutral lipids | 0,2 | 23,7 | 1,1 | 3,4 | 21,7 | 49,9 | 72,7 | 27,3 |
| Phospholipids | 1,7 | 25,8 | 1,2 | 3,3 | 19,8 | 48,2 | 69,2 | 30,8 |
| Glycolipids | 4,3 | 35,1 | 3,0 | 10,4 | 29,8 | 17,7 | 50,5 | 49,5 |
| Residual lipids | 0,3 | 24,3 | 0,4 | 0,5 | 19,0 | 55,5 | 74,9 | 25,1 |
| Halls | | | | | | | | |
| Neutral lipids | 0,6 | 23,2 | 1,3 | 2,8 | 20,7 | 51,4 | 73,4 | 26,6 |
| phospholipids | 2,0 | 29,5 | 1,5 | 2,5 | 21,9 | 44,4 | 67,8 | 32,2 |
| Glycolipids | 3,2 | 34,7 | 1,9 | 15,0 | 25,7 | 19,5 | 47,1 | 52 9 |
| Residual lipids | 0,8 | 29,2 | 1,1 | 0,4 | 20,4 | 48,1 | 69,6 | 30,4 |
| Oilseed meal | | | | | | | | |
| Neutral lipids | 0,6 | 24,6 | 1,9 | 2,8 | 19,0 | 51,2 | 72,1 | 27,9 |
| Phospholipids | 0,4 | 26,7 | 0,3 | 1,7 | 19,6 | 51,3 | 71,2 | 28,8 |
| Glycolipids | 1,1 | 38,2 | 2,1 | 11,1 | 23,9 | 23,6 | 49,6 | 50,4 |
| Residual lipids | 2,3 | 26,4 | 1,2 | 0,3 | 20,5 | 49,3 | 71,0 | 29,0 |
| Prepressing oil | | | | | | | | |
| Neutral lipids | 0,2 | 24,0 | 0,6 | 0,9 | 19,4 | 54,9 | 74,9 | 25,1 |
| Phospholipids | 0,8 | 28,2 | 1,1 | 2,0 | 20,8 | 47,1 | 69,0 | 31,0 |
| Glycolipids | 1,9 | 36,8 | 3,4 | 15,5 | 28,5 | 13,9 | 45,8 | 54,2 |

MDGDs, and DGDGs. Sulfur lipids, cerebrosides, and esterified sterol glycosides (ESGs) were present in insignificant amounts. By TLC in system 5 and two-dimensional TLC in system 4, a glycolipid similar to a SG was detected ($R_f$ 0.53 and 0.57; and 0.63 and 0.66, respectively). The unidentified GL gave a qualitative reaction with $I_2$ for unsaturated bonds and a reaction with α-naphthol for sugars. FFAs, sugars, and an unknown substance were detected in the products of the hydrolysis of this GL.

In the refined oil, the bulk of the GLs consisted of sterol glycosides which, of all the GLs, are the fractions most difficult to refine.

It was found by the PC method that the main carbohydrate component of the GLs of the cotton seeds was glucose (68.3%), while galactose made up 31.7%. In the SGs, the main GLs of the seeds, only glucose was detected. A similar carbohydrate composition is characteristic for the GLs of the grape [3].

To investigate the fatty-acid compositions of the NLs, PLs, and GLs of the samples being studied, the total lipids were separated by CC on silica gel [9], the NLs being eluted with chloroform, the GLs with acetone, and the PhLs with methanol.

The residual lipids were eluted from the column with 1 N HCl in methanol, the amounts for the different samples ranging between 0.96 and 1.04% of the weight of the sample deposited on the column. The residual lipids were shown by TLC in system 6 to contain sterol esters, fatty acid methyl esters, FFAs, TAGs, and gossypol pigments.

On TLC in system 3, the pigments were separated into three dark spots with $R_f$ 0.35, 0.41, and 0.45. The amount of gossypol pigments in the residual lipids of the oilseed meal was 62.5% of their weight. The elution of the residual lipids only by an acidic eluent can be explained by strong sorption of mainly the gossypol pigments, part of the NLs being retained simultaneously.

Table 3 gives the fatty acid compositions of the products of cottonseed processing. With respect to their qualitative set of acids, all the samples were similar, but their quantitative compositions differed. The GLs were most enriched with the saturated fatty acids, palmitic and stearic, and contained not more than 23.6% of linoleic acid. The NLs were distinguished by their highest content of unsaturated fatty acids, the level of which was approximately the same for all the samples. The PhLs of the oilseed meal were more unsaturated through their comparatively high content of linoleic acid.

## EXPERIMENTAL

The composition of the fatty acid methyl esters was determined by GLC on a Chrom-4 instrument using a column filled with Chromaton NAW-DMC bearing 15% of Reoplex 400. CC on silica gel 100/160 and TLC on silica gel L 5/40 (Chemapol) was conducted as described in

[10]. The individual groups of PhLs and GLs were identified by comparing their migrational characteristics and also with the aid of specific color reactions. Pure MGDGs and DGDGs isolated from wheat flour were used as markers [11].

For TLC we used the following solvent systems: 1) petroleum ether–diethyl ether (60:40); 2) acetone–toluene–acetic acid–water (60:60:2:1); 3) benzene–methanol (4:1); 4) chloroform–methanol–7 N ammonia (60:30: 4) and chloroform–methanol–acetic acid–water (170:25:25:6); 5) chloroform–acetone–methanol–acetic acid–water (65:20:10:3); 6) diethyl ether–hexane (3:7); and 7) pyridine–n-butanol–water (3:2:1). The PC of the carbohydrate components of the GLs was conducted by the descending method in system 7. Quantitative determination was carried out by a colorimetric method using the aniline phthalate reagent [12].

The acid hydrolysis of the GLs was conducted with 2 N $H_2SO_4$ in the boiling water bath for 4 h. After the solution had cooled the fat-soluble products were extracted with petroleum ether. The insoluble pigments were eliminated by filtering the acidic aqueous solution through a paper filter.

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# LIPIDS OF THE LEAF VEGETABLES Spinacea oleracea, Latuca sativa, AND Rumex acetosa


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The class compositions of the lipids of leaf vegetables – Spinacea oleracea (variety Ispolinskii), Latuca sativa (variety Kucheryavets Odesskii), and Rumex acetosa (variety Odesskii-17) have been studied. The compositions of their NLs (17 groups of individual compounds were identified), GLs (nine groups of compounds were found), and PhLs (seven groups of compounds have been found) have been determined by physicochemical methods of analysis. The maximum amount of the total lipids and also of the PhLs was possessed by the spinach. Among the FAs of the leaf vegetables, USFAs predominated.


Leaf vegetables are rich in a number of biologically active substances (folic acid, choline, phylloquinone, ascorbic acid, vitamins of the B group, potassium, iodine, iron, etc.) which are responsible for a complex of curative properties in a number of blood diseases, tuberculosis, diseases of the gastrointestinal tract, nervous breakdown, etc. In


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