PROTEIN-LIPID COMPLEXES OF COTTON KERNELS AND THE PRODUCTS OF THEIR PROCESSING

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The composition and lipid content of the lipoprotein complexes of cotton kernels (I), meal (II), and isolate (III) have been investigated. It has been shown that with the aid of an acidic alcohol-containing extractant it is possible to isolate up to 99% of the strongly bound lipids (SBLs) of their total mass. In the composition of the strongly bound lipids (I-III) have been found neutral lipids comparable with the free lipids of the kernel; of polar lipids, glycolipids were present. It was found that an acid extractant extracts polyphenolic compounds together with the SBLs.

Lipids and proteins are present in the plant cell in the form of basic complexes differing in the structure and degree of stability [1]. The least stable complexes are formed by neutral (free) lipids (FLs) bound by weak electrostatic forces and consisting mainly of carbohydrates, triacylglycerols (TAGs), free fatty acids (FFAs), and other weakly polar components. These lipids are extracted from the tissues by hydrophobic solvents.

More stable, as the result of hydrogen bonds, are complexes with polar lipids — phospho- and glycolipids, the so-called bound lipids (BLs). Such lipoproteins contain not only polar but also weakly polar lipids [2]. The BLs are extracted, in the main, by mixtures of chloroform and methanol.

In the third type of complexes the lipids are most strongly bound with protein (SBLs) by covalent bonds and it is not always possible to isolate them with the retention of their native state, so that information on their composition in the primary structure is sparse.

It must be mentioned that in the course of the industrial processing of oil seeds part of the FLs bind to the denatured protein, forming secondary lipoprotein complexes [3]. The composition and structure of the lipids present in complexes with protein largely determine the food properties, color, odor, emulsifying properties, and storage times of the protein products of the oils and fats industry (meal, protein hydrolysates, isolates).

We have investigated the FLs, BLs, and SBLs of industrial samples of cotton kernels (I), meal (II), and a protein isolate from cotton meal (III). The FLs were exhaustively extracted from the samples with hexane, and then the BLs with chloroform—methanol (2:1, v/v). The crude extracts of the FLs and BLs were freed from nonlipid impurities with a 0.04% solution of calcium chloride [4]. To isolate the SBLs from the complexes with protein we used as extractant chloroform—methanol—concentrated HCl (2:1:0.03, v/v/v) in the ratio recommended by authors [5] who studied the peanut. Hydrochloric acid, disrupting the covalent bonds between the lipids and the protein, leads to the complete hydrolysis of the lipoproteins and promotes the passage of the liberated components into the organic phase. In contrast to [5], we carried out an additional treatment of the extract obtained and studied in detail the composition of the components isolated individually.

In our investigations, we used literature information on the lipoprotein complexes isolated from chloroform—methanolic extracts of wheat [6]. We added a 2.5% aqueous solution of $Zn(CH_3COO)_2$ to the extract to precipitate the protein [7] and observed a considerable amount of protein flocs in the SBL extracts. Protein was also present in trace amounts in the BL extract (II).

In order to determine the true amount of lipids in the protein fraction (II), a sample after the elimination of the SBLs was dried in the air and was freed from husks, the mass proportion of which amounted to 51.8%.

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TABLE 1. Composition of Extracts of Cotton Kernels, Meal, and Isolate

Extractant, extractive substances	Yield, % by mass on the absolutely dry matter	
	kernels	meal
Hexane	35,2	0 6 (1,1)*
Free lipids	35,1	0,5 (0,9)
Nonlipid substances	0,1	0,1 (0,2)
Chloroform–methanol	3,9	2,7 (5,1)
Bound lipids	2 9	1.2 (2,3)
Nonlipid substances	1,0	1,5 (2,8)
Chloroform–methanol–HCl	2,4	1,3 (2.4)
Strongly bound lipids	0.5	0,24(0,4)
a) weakly polar	0,1	0,04(0,08)
b) polar	0,4	0,2 (0.3)
Substances soluble in the alcohol phase	0.8	0,4 (0.8)
Lipoproteins	1,2	0.7 (1,3)
Residual lipids:	0,02	0.02
a) weakly polar		0 003
b) polar		0.017
Sum of the lipids	38.52	1.94(3,7)
Sum of the nonlipid substances	1,9	2,0 (3, 7)

*The figures in parentheses show the yields of extractive substances recalculated to the protein fraction.

Table 1 gives the amounts of FLs, BLs, SBLs, and nonlipid components in (I) and (II). It follows from Table 1 that the amount of BLs and SBLs and the ratio of polar and weakly polar lipids in the SBLs of the protein fraction (II) differ in practice from those for (I). The amount of nonlipid impurities extracted from the meal together with the BLs was considerably higher than from the kernels. The nonlipid components amounted to half the extractive substances of the meal.

The bulk of the SBLs (I) and (II) consisted of polar components, the proportion of weakly polar components amounting to 20%. We extracted the FLs and BLs from the cotton isolate obtained from (II), and their amounts were 0.1 and 0.2%, respectively.

The composition of the fatty acids was determined with the aid of GLC (Table 2). It can be seen from Table 2 that the fatty acid composition of the weakly polar fraction of the SBLs (I) was close to that for the FLs (I) while the polar fraction of the same lipids was enriched with saturated fatty acids (16:0 and 18:0).

With an increase in the binding of the lipids to the protein, the amount of saturated fatty acids increased, possibly through the polar lipids. An analogous tendency has been reported previously [8]. The BLs (II) had 7-8% more of the 16:0 acid than the FLs (II), while the closeness of the fatty acid composition of the BLs and of the weakly polar SBLs of sample (II) was apparently due to the fact that, during the processing of the cotton kernels, part of the BLs was converted into SBLs. In contrast to the SBLs (I), the polar SBLs (II) had a higher level of the 18:2 than of the 18:1 acid, which also shows a possible redistribution of the polar lipids in the lipid-protein complexes during industrial processing. It had been shown previously [8] that at the stages of storage and prepressing an enrichment of the BLs with linoleic and linolenic acids takes place.

The residual lipids were distinguished by the most highly differentiated fatty acid composition; in them the proportion of saturated acids reached 47% of which almost 10% consisted of the 12:0 and 14:0 acids. It must be mentioned that, according to the results of mass-spectrometric analysis, the fatty acids of (II) contained the 21:0-26:0 acids, these also being revealed in trace amounts of GLC. Furthermore, the presence of stigmasterol (M^+ 412), β-sitosterol (M^+ 414) and a 4,4-dimethylsterol (M^+ 426) was revealed in the residual lipids (II).

In the isolate, with an increase in the degree of binding of the lipids with the protein, the degree of saturation of the lipids increased more sharply than in the kernels, reaching 71% in the SBLs.

TABLE 2. Fatty Acid Compositions of Lipids (I), (II), and (III)

Lipids	Fatty acid, % by mass									
	14:0	16:0	16:1	17:0	18:0	18:1	18:2	20:0	H	П
Kernels										
Free	0,6	24,9	0,8	0,2	1,2	19,2	53,1	Tr.	73,1	26,9
Bound	0,2	27,5	0,6	Tr.	0,3	33,1	38,3	Tr.	72,0	28,0
Strongly bound:	0,4	29,4	0,4	0.5	2,6	20,6	46,1	Tr.	67.1	32,9
a) weakly polar	0,4	28,0	0,2	0,3	0,8	17,5	52,8	Tr.	70,5	29,5
b) polar	0,3	32,4	0.4	0,4	2,0	19 0	45,5	Tr.	64,9	35,1
Meal										
Free*	1,0	24,8	1,7	Tr.	2,4	21,8	48.3	Tr.	71.8	28 2
Bound	1,3	22 3	1,5	Tr.	3,2	11,3	50,4	Tr.	63,2	33,8
Strongly bound:	0,3	30,5	1,8	0,8	1,9	14,4	49.7	0,6	65,9	34,1
a) weakly polar	0,3	31,0	2,0	0,7	2 8	16.3	45,6	1,3	63,9	36.1
b) polar	0,2	30,2	1,7	0,6	1,6	13,9	51.5	0,3	67,1	32,9
Residual lipids** of the meal	6,1	31,4	2 0	4,1	0,1	12,6	38,1	1,7	52,7	47.3
Isolate										
Free	0,4	31,0	0.6	Tr.	2,1	23,4	42,5	Tr.	66,2	33,8
Bound	0,2	43.5	3 0	Tr.	6,6	22,1	24,6	Tr.	49,7	50,
Strongly bound	0,5	70,3	0,2	Tr.	1,0	13,1	15,0	Tr.	29,1	70,9

*Traces of an 18:3 acid (M^+ 292) were also present in the FLs (II).
†3.9% of the 12:0 acid was detected in the residual lipids (II).

The separation of the lipids into classes was carried out in systems 1-8. The lipid components were identified by comparison with authentic samples, and from qualitative reactions and literature information. All the extracts except the hexane extracts and the groups of substances isolated from them had either a brown or a red-brown coloration due predominantly to the presence of polyphenolic pigments of flavonoid [9], anthocyan [10], and gossypol [11] natures. These pigments are characteristic for cotton seeds and have a complex composition; many of them are lipophilic or can acquire lipophilicity in the course of the industrial operations [12].

The BLs of the samples investigated, (I), (II), and (III), had acid numbers of 33.2, 55.5, and 138.5 mg of KOH, respectively, which shows the lighter binding of the free fatty acids of the protein in the course of industrial processing of cotton kernels.

Of neutral lipids, in the FLs (II) and (III) the same components were detected qualitatively by TLC as in (I) [2]. In the BLs of (II) and (III) neutral lipids were identified in system 1: fatty acid methyl esters (R_f 0.080), triacylglycerols (R_f 0.75), free fatty acids (R_f 0.43), 1,2- and 1,3-diacylglycerols (R_f 0.23 and 0.13), and free sterols (R_f 0.19) and their esters (R_f 0.9).

Of polar lipids, six classes of glycolipids were detected in solvent systems 3-5 with a predominance of sterol glycosides, mono- and digalactosyldiacylglycerols, cerebrosides, and sulfatides; and nine classes of phospholipids, of which the main ones were phosphatidylcholine and phosphatidylinositol. The other phospholipids were characterized by qualitative reactions and, from their chromatographic mobilities (system 6), were assigned to the products of the hydrolysis and/or oxidation of phosphatidylcholine and phosphatidylinositol. The results showed that the protein isolate obtained from cotton meal contained an appreciable amount of protein-bound fatty acids, which affect the chemical properties of the protein.

In the weakly polar fractions of the SBLs (I) and (II), sterol esters, fatty acid methyl esters, triacylglycerols, free fatty acids (main component) and free sterols were detected by TLC.

Of the polar components of the SBLs (I) and (II), TLC in systems 3 and 4 revealed only three classes of glycolipids, which were shown up only feebly by α-naphthol because of the dark pigment bands extending over the whole chromatogram. Phospholipids were absent. Thus, the composition of the acids of the polar fraction of the SBLs (I) and (II) (Table 2) re-

flected the acids of the glycolipids. Allen et al. [5], having isolated the SBLs from peanuts with chloroform—methanol—HCl (2:1:0.03, v/v/v), detected phospho- and glycolipids in them with the aid of specific reagents, but the chromatographic mobilities of the components of these lipids did not correspond to those of main compounds. Since there is information [13] showing the possible hydrolysis of phospholipids during extraction by acidic alcohol-containing extractants, the results obtained can be explained by precisely this circumstance. Esterification and/or transesterification reactions of the lipids during such extraction are not excluded, either. A confirmation of this is provided by the results of the isolation of the SBLs (I) by an extractant with an increased activity [chloroform—methanol—HCl (2:1:0.3)]. In this case, no glycolipids were detected in the SBLs (TLC) but a considerable increase in the size of the spot of the fatty acid methyl esters was observed. From the results of TLC in system 7 and qualitative reactions, in the SBL (I) three spots (R_f 0.38, 0.40, and 0.42), and in the SBL (II) four spots (R_f 0.38, 0.40, 0.42, and 0.63), corresponding to the products of modified gossypol [11] were detected by qualitative reactions.

The polyphenolic compounds isolated from the methanol phase of SBLs (I) and (II) gave a positive reaction with $FeCl_3$ [14].

On TLC in system 8 for separating the flavonoids, the pigments of the kernels and meal (I) and (II) were separated into five spots, one of which (R_f 0.25) was colored bright pink when the plate was kept for a short time in the air; its color deepened on treatment with 50% H_2SO_4 to pink-crimson, which is characteristic for anthocyans [15]; when a parallel plate was treated with vanillin in 50% H_2SO_4, two spots (R_f 0.50 and 0.75) acquired a yellow color and two (R_f 0.70 and 0.81) a blue color.

Preparative TLC in system 8 permitted the isolation of the substance with R_f 0.25, and it was investigated by UV and IR spectroscopies and mass spectrometry.

The IR spectrum exhibited bands at (cm^{-1}) 690 (m), 770 (m), 1090 (s), 1240 (w), 1420 (s), 1745 (s), and 3000-3600 (s), and in the UV spectrum there were bands with $\lambda_{max}^{CH_3OH}$ 280 and 408 nm. Since the mass spectrum of the substance showed no characteristic molecular ions, it was subjected to acid hydrolysis. The aglycon isolated differed with respect to its mobility on TLC in system 6 (R_f 0.73) from the initial substance (R_f 0.46).

On the basis of the results obtained, the substance was assigned to a glycosidated form of an anthocyan. The sum of the substances revealed by vanillin had in its IR spectrum bands at (cm^{-1}) 950 (w), 1080 (m), 1130 (m), 1390 (s), 1475 (s), 1510 (m), 1740 (m), and 2940-2970 (s), and in the UV spectrum $\lambda_{max}^{C_2H_5OH}$ 224, 265, 355 nm. The properties of the component detected were similar to those of certain flavonoids and phenols found in various organs of the cotton plant.

Consequently, on the isolation of the SBLs by acidic alcohol-containing mixtures, polyphenolic compounds are extracted as well, and where these are lipophilic they pass into the lipids and complicate their separation and identification. The freeing of the lipids from polyphenols presents certain difficulties. With an increase in the acidity of the extractant the brown coloration of all the fractions of SBLs (I) and (II), of the aqueous methanol phase, and of the lipoproteins intensified as a result of the more complete extraction of the polyphenols.

The lipoproteins — i.e., the neutral protein phases (I) and (II) — isolated from the acid extracts had a yellow-gray coloration; when they were dried at 80°C, dry residue with a brown color was obtained.

The dry residue from the meal contained 7.98% of nitrogen, or 49.8% of protein. The FFAs were isolated from the protein precipitate by hydrolysis, and their main component was found (GLC) to be the 16:0 acid. When an extract with a higher (10-fold) HCl content was used; the protein precipitate had a darker coloration; it dissolved completely in the acid extractant. The TLC of the extract showed spots corresponding in qualitative reactions [15] to phenolic compounds.

EXPERIMENTAL

Samples of the kernels and meal were obtained from the Tashkent oils and fats combine. An isolate from cotton meal was kindly provided by workers of the experimental technological laboratory. The extraction of the FLs and BLs was performed in a Soxhlet apparatus for 24 and 18 h, respectively.

The SBLs were isolated by steeping the samples in chloroform—methanol—HCl (2:1:0.3, v/v/v) for two days five times. The acidic extract was transferred to a separatory funnel and distilled water was added until a chloroform (lower) and an aqueous methanolic (upper) phase had separated. Then the acid was neutralized with 10% aqueous ammonia solution in the presence of Methyl Orange, as a result of which an intermediate protein layer formed at the boundary of separation of the phases.

Each phase was isolated and investigated separately. Chloroform was distilled off in a rotary evaporator, giving the SBL, and then the weakly polar lipids were separated from the polar ones by PTLC on silica gel G in system 1.

The protein fration was transferred to a weighed paper filter, and was washed three times with chloroform—methanol (2:1), and, after drying to constant weight, the yield of dry residue was determined.

The methanol was distilled off from the aqueous methanolic phase, and the residue was treated three times with n-butanol. The alcoholic extract had a yellow coloration, and, after the solvent had been distilled off, the substances mainly of phenolic nature were obtained.

The residual lipids were extracted by alkaline hydrolysis according to [16].

The composition of the fatty acids was determined by the GLC of their methyl esters on a Chrom-4 instrument using a column filled with Chromaton N-AW DMCS with 15% of Reoplex 400. Mass spectrum (70-80°C, 50 eV, 0.5 mA) of the FFAMEs of residual lipids (II): 326, 340, 354, 368, 382, 396, 410 (M^+); 295, 309, 323, 337, 351, 365, 379 ($M^+ - 31$); 283, 297, 311, 325, 339, 353, 367 ($M^+ - 43$).

For TLC we used Silufol (Czechoslovakia), silica gel L 5/40 (Chemapol) with 13% of $CaSO_4$, and the following solvent systems: 1) hexane—ether (7:3); 2) hexane—ether (8:2); 3) chloroform—methanol—water (65:25:4); 4) chloroform—acetone—ethanol—acetic acid—water (65:20:10:10:3); 5) chloroform—methanol—acetic acid—water (85:15:10:4); 6) chloroform—methanol—25% NH_4OH (65:35:5); 7) benzene—methanol (4:1); and 8) ethyl acetate—ethanol (3:1).

Thus, with the aid of an acidic alcohol-containing extractant, up to 99% of the lipids present in a strong bond with protein was isolated from the cotton raw material and meal. The polyphenolic pigments and the methanol-soluble lipoproteins were extracted incidentally. It was established that in the course of such extraction a degradation of the polar lipid components, particularly the phospholipids took place. The SBLs of the cotton kernels were practically identical with those of the meal. They included not only the polar lipids but also weakly polar neutral lipids which, with respect to their set of classes and fatty acid composition, were comparable with the free lipids of the kernels.

In the composition of the fatty acids, with an increase in the strength of binding of the lipids with the protein the proportion of saturated acids, particualrly the 16:0 acid and, of unsaturated acids, the 18:1 acid rose and the level of the 18:2 acid fell.

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INTRASPECIES CHEMICAL VARIABILITY OF THE ESSENTIAL OIL OF Ledum palustre

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The compositions of the essential oils of marsh tea, Ledum palustre L., from the southern regions of Tomsk, Transbaikalia and Amur province, and also from the southern part of the island of Sakhalin have been analyzed in detail. The results obtained indicate the sharply pronounced intraspecies chemical variability of this plant. In addition to the chemotype of march tea with myrcene as a macrocomponent which is widely distributed in the European part of the USSR, another three chemotypes with the macrocomponents limonene, sabinen, and p-cymene have been revealed.

The flora of Siberia and the Far East is distinguished by the greatest variability of representatives of the genus Ledum L. [1]. Marsh tea, L. palustre, which is widely distributed here, is represented by five varieties [2, 3]. However, hitherto the opinions of botanists in the field of the systematics of the genus have been extremely contradictory [4]. In view of this, to answer the question of the status of the species and of the intraspecies taxons, in addition to the classical morphological and microscopic features, and also information on the karyology, ecology, and geography of the plants, the results of chemical analysis and, in particular, the composition of the essential oils [5] must be taken into account.

Many plants are represented by a number of chemotypes [6] characterized by a predominating content of terpenoids of different biogenetic origins. Since marsh tea is an officinal medicinal plant [7] the expectorant and antitussive effect of which is due to its content of essential oil and ledol [8, 9], information on its composition is of great practical importance. At the same time, the few publications on the study of the essential oil of marsh tea growing in the territory of Siberia and the Far East [10-12] are far from complete.

We have studied samples of the essential oil from leafy shoots of L. palustre collected in the Tomsk province, Transbaikalia, the Amur province, and on the island of Sakalin, and, in these, 71 compounds have been identified by various chromatographic and spectral methods.

The main components of the essential oil of marsh tea growing in the environs of Tomsk were identified by Klokova et al. [10]. For the isolation and identification of the minor components we carried out the fractionation of combined samples of the essential oil of plants from the environs of Tomsk and the village of Suiga, Molchanovo region of Tomsk province, which are close in composition. In the samples investigated we detected 65 compounds,

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