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LIPIDS FROM FIVE COTTON VARIETIES

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Seed characteristics and the lipid and fatty-acid compositions were determined for the new cotton varieties ASh-25, Omad, 9771-I, Termez-43, and Bukhara-6.

Key words: free and bound lipids, phospholipids, glycolipids, fatty acids.

Cotton is the principal cultured source of oil in Uzbekistan. Therefore, varieties that produce large harvests with high-quality fiber and are resistant to drought are targeted for selection.

We studied for the first time lipids and accompanying biologically active substances (BAS) from the new cotton varieties ASh-25, Omad, 9771-I, Termez-43, and Bukhara-6, which were developed in the Zaitsev Institute of Selection and Seed-Growing of the Republic of Uzbekistan. These varieties were grown in Tashkent district and southern regions of Uzbekistan.

Data on lipids of other cotton varieties have been published [1-3]. However, the tocopherols, sterols, carotinoids, and other BAS of the seeds are insufficiently characterized.

Table 1 lists the properties of seeds and content of free (FL) and bound lipids (BL) from the studied cotton varieties. It can be seen that the mass of 1000 seeds and oil content were highest for variety 9771-I. The BL contents in seeds of this variety and Omad were 1.8 and 1.7%, which is lower than that in the three other varieties.

The BL were separated by column chromatography over silica gel in order to determine the fractions of the individual classes. Neutral lipids (NL) were eluted by $CHCl_3$; glycolipids (GL), by acetone; phospholipids (PL), by methanol.

Table 2 shows that the GL dominate the BL, making up more than 50% of their mass. The fraction of PL is from 21 to 39%. The mass of NL does not exceed 20%.

The qualitative compositions of the FL and each class of BL were established using TLC over silica gel. Solvent systems 1 and 2 were used to separate the FL and NL from the BL; system 3, the GL. The PL composition was determined using two-dimensional TLC and solvent systems 4 and 5. Compounds were identified using specific reactions and comparison of their TLC mobilities with those of model compounds.

The FL and NL of the BL from all samples contained hydrocarbons, sterol esters of fatty acids, triacylglycerides, free fatty acids, and sterols. Triacylglycerides dominated the components of the analyzed lipids. The GL contained mono- and digalactosyldiglycerides, sterolglycoside esters, sterolglycosides, and cerebrosides. The principal GL components of all samples were sterolglycosides. Phosphatidylcholines, phosphatidylethanolamines, phosphatidylinosites, *lyso*-phosphatidylcholines, and *lyso*-phosphatidylethanolamines were identified among the PL. Phosphatidylcholines dominated these PL in all varieties.

The total content and composition of BAS in FL and the compositions of fatty acids of FL, GL, and PL were then determined. The total BAS isolated from FL of seeds of each cotton variety were separated into individual classes by TLC over silica gel using solvent system 2. The total contents of BAS in the FL and individual classes were found gravimetrically.

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TABLE 1. Seed and Lipid Characteristics of Five Cotton Varieties

Property	ASh-25	Omad	9771-I	Termez-43	Bukhara-6
Mass of 1000 seeds, g	93.5	105.8	117.7	107.6	115.0
Seed moisture, %	7.1	6.8	6.6	7.0	6.5
Free lipids (oil content), % of seed mass	24.2	24.8	25.7	24.0	24.4
Bound lipids, % of seed mass	2.4	1.7	1.8	2.2	2.4
Acid number, mg KOH	6.7	5.6	8.0	5.7	4.4

TABLE 2. Composition of Bound Lipids, % of Seed Mass

Lipid class	ASh-25	Omad	9771-I	Termez-43	Bukhara-6
Neutral	0.5	0.3	0.2	0.4	0.4
Glycolipids	1.4	0.9	0.9	1.1	1.3
Phospholipids	0.5	0.5	0.7	0.7	0.7

TABLE 3. Composition and Content (mass %) of Biologically Active Substances in Free Lipids from Seeds of Five Cotton Varieties

BAS	ASh-25	Omad	9771-I	Termez-43	Bukhara-6
Total content	1.70	1.30	1.20	1.70	1.80
Sterols	0.87	0.76	0.66	0.82	0.94
Hydrocarbons	0.30	0.20	0.20	0.35	0.40
Aliphatic and triterpenic alcohols + tocopherols	0.50	0.32	0.32	0.50	0.42
Carotinoids, mg % in BAS mass	31.6	22.61	21.0	29.5	37.8

Table 3 shows that sterols dominate the BAS, making up over half their mass. This was confirmed by a colorimetric method [4, a]. The contents of hydrocarbons and free alcohols in the FL were less than 0.4 and 0.5%, respectively. Tocopherols in the FL were determined by analytical TLC in $CHCl_3$ by isolating them from FL using reagents that prevented their oxidation [4, b]. The analytical results have shown that the FL from all cotton varieties contain both α- and β-tocopherols that, judging from the intensity of the color, are present in equal amounts. Soy-oil tocopherols isolated by this same method were used as models.

The carotinoid content in the BAS was 21-37.8 mg%. Fatty acids obtained by hydrolysis of FL, GL, and PL were analyzed by GC. Table 4 lists their compositions. It can be seen that the FL, GL, and PL have the same qualitative set of principal fatty acids that are typical of ripe cotton seeds. Also, the FL and PL of all varieties have very similar contents of inidividual fatty acids. The 18:2 acid dominates in these lipid classes. However, the content of saturated acids is elevated in the fatty acids of PL from the studied seeds. This has been noted previously [5-7]. A feature that distinguishes the GL fatty-acid composition from that of the FL and PL is the predominance of the 18:1 acid (Table 4).

A comparison of the fatty-acid compositions of FL from seeds of the studied cotton varieties and those previously published reveals a tendency toward a certain reduction in the content of the essential 18:2 acid [1, 5-8].

TABLE 4. Fatty-Acid Composition of FL, GL, and PL from Five Cotton Varieties, GC, mass %

Fatty acids	ASh-25	Omad	9771-I	Termez-4	Bukhara-6
			Fatty-acid composition of FL		
12:0	0.5	Tr.	0.9	0.2	0.5
14:0	1.2	3.1	0.9	0.8	1.0
16:0	24.3	26.2	20.6	24.8	24.3
18:0	1.8	3.5	2.2	2.6	1.5
16:1	0.5	2.2	0.3	0.2	0.8
18:1	19.3	20.0	20.7	20.6	20.8
18:2	49.8	45.0	51.6	49.5	49.9
Σ_{unid}	2.6	Tr.	2.8	1.3	1.2
			Fatty-acid composition of GL		
12:0	0.3	1.2	7.4	4.2	2.5
14:0	0.7	1.3	4.5	3.5	3.2
16:0	24.8	31.8	20.6	28.0	25.1
18:0	2.8	3.1	5.3	4.8	4.5
16:1	1.5	Tr.	1.7	1.4	1.2
18:1	47.4	48.5	39.6	41.2	39.3
18:2	21.3	12.4	18.0	16.2	22.8
Σ_{unid}	1.2	1.7	2.9	0.7	1.4
			Fatty-acid composition of PL		
12:0	2.5	1.9	2.1	1.4	2.0
14:0	1.2	1.9	1.4	1.0	1.5
16:0	23.1	30.8	26.9	24.8	25.8
18:0	3.0	5.1	4.4	5.3	4.1
16:1	1.4	3.5	1.1	0.9	2.1
18:1	27.4	12.3	15.9	18.0	19.5
18:2	41.4	43.0	46.2	47.2	43.5
Σ_{unid}	-	1.5	2.0	1.4	1.5

EXPERIMENTAL

GC of fatty-acid methyl esters was performed on a Chrom-5 instrument using a steel column 2.5 m in length packed with Reoplex 400 (5%) on Inerton N-AW (0.16-0.20 mm) at column temperature 190°C and N_2 flow rate 30 mL/min. TLC of lipids and BAS was performed on L 5/40 silica gel with 10% gypsum.

The solvent systems were: hexane:diethylether (4:1, 1; 1:1, 2), $CHCl_3$:$(CH_3)_2CO$:CH_3OH:CH_3CO_2H:H_2O (65:20:10:10:2, 3), $CHCl_3$:CH_3OH:NH_4OH (25%) (13:5:1, 4), $CHCl_3$:CH_3OH:CH_3CO_2H:H_2O (14:5:1:1, 5). Bands of compounds were developed by spraying with H_2SO_4 (50% aqueous, lipids and BAS), H_2SO_4 (50%) and α-naphthol (GL), Vaskovsky reagent (PL), Dragendorff's solution (phosphatidylcholines), ninhydrin solution (phosphatidylethanolamines) [9], Emmerie—Engel reagent (tocopherols) [4, b], and phosphomolybdic acid solution (sterols) [4, a].

The contents of FL, seed moisture, and acid number were determined by the literature methods [10]. BL were extracted from the pulp remaining after isolation of FL by $CHCl_3$:CH_3OH (2:1 by vol) in a Soxhlet extractor. The resulting extract was washed with aqueous $CaCl_2$ (0.05%) to remove nonlipid components. The carotinoid content was established as before [11].

Total BAS were obtained using the method for isolating unsaponifiable substances [4, c]. Hydrolysis of FL, GL, and PL and the isolation of fatty acids and their methylation have been described [12].

REFERENCES

1. T. V. Chernenko, I. Tolibaev, M. Talipova, A. I. Glushenkova, A. Kh. Ataullaev, and D. M. Sultanbekova, *Collection of Scientific Works "Complete Reprocessing of Cotton Seeds"* [in Russian], Kokand (1991), p. 79.
2. A. Kh. Ataullaev, D. M. Sultanbekova, and M. Kim, *Maslo-Zhir. Promst.*, 10 (1982).
3. M. G. Siradze and A. B. Belova, *Maslo-Zhir. Promst.*, 7 (1987).
4. *Handbook of Research Methods, Chemical Engineering Control and Accounting in the Oil-Fat Industry* [in Russian], VNIIZh, Leningrad (1974), Vol. 6, Book 2, a) 35, b) 24, c) (1967), Vol. 1, Book 2, 815.
5. L. A. Shustanova, A. Sh. Isamukhamedov, and S. T. Akramov, *Khim. Prir. Soedin.*, 449 (1975).
6. Kh. S. Mukhamedova and S. T. Akramov, *Khim. Prir. Soedin.*, 128 (1975).
7. F. Yu. Gazizov, L. A. Shustanova, and S. T. Akramov, *Khim. Prir. Soedin.*, 198 (1977).
8. K. Kadyrov, T. V. Chernenko, and A. U. Umarov, *Khim. Prir. Soedin.*, 211 (1974).
9. M. Kates, *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*, Elsevier, New York (1973).
10. *Technical Control and Accounting of Production in the Oil-Production and Fat-Reprocessing Industry* [in Russian], Pishchepromizdat, Moscow (1959), Vol. 2, pp. 18, 30, 53.
11. *Addition to the USSR State Pharmacopoeia*, X Press, Moscow (1986), Vol. 3, 321.
12. N. T. Ul′chenko, N. P. Bekker, and A. I. Glushenkova, *Khim. Prir. Soedin.*, 456 (2000).