The neutral lipids of the red muscles are characterized by a high content of palmitic and oleic acids and a low concentration of linoleic, octadecatetraenoic, eicosatrienoic, and particularly eicosatetraenoic acids. On the whole, in the neutral lipids of the red muscles the amount of saturated and monoenoic acids has risen and the amount of polyenoic acids has fallen considerably.

EXPERIMENTAL

The characteristics of the material, the method of taking the samples, the extraction of the lipids, transesterification, and the purification of the esters, the isolation of the neutral lipids, and the conditions of chromatography and identification have been described previously.

SUMMARY

1. Differences have been detected in the concentrations of individual components of the fatty acids in the lipids localized in different sites of the whitefish.

2. It has been established that the highest degree of unsaturation is possessed by the lipids of the internal fatty tissues, while the brain lipids are the most saturated.

3. In the neutral lipids of all parts the amounts of monoenoic acids rises and the amount of polyenoic acids falls.

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STEREOSPECIFIC ANALYSIS OF THE TRIACYLGLYCEROLS OF COTTONSEED OIL

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A stereospecific analysis has been made of the triacylglycerols of cottonseed oil, as a result of which it has been established that the saturated and 16:1 acids mainly occupy the sn-1 position, while 18:1 and 18:2 acids esterify the sn-2 and sn-3 positions of the triacylglycerides to equal extents.

The physiological action and nutritional value of an oil and also its stability in the processes of treatment and storage depend not only on the type and amount of the constituent fatty acids but also on the positions of the acids in the molecules of the triacylglycerols (TAGs).

The study of the structure of TAGs by lipase hydrolysis has permitted definite laws in the distribution of fatty acids in position 2 of the glycerol residue to be established [1]. The calculation of all the types of TAGs by this method assumes the equivalence of the positions of 1 and 3 with respect to the fatty acid composition, which is not always in harmony with the results obtained by other methods. Thus, the stereospecific analysis of some plant oils has shown that the distribution of the acids between the positions 1 and 3 in the TAG molecule does not bear a random nature in many cases and takes place in such a

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TABLE 1. Fatty-Acid Compositions of the Products of Two Hydrolyses (mole %)

Sample	14:0	16:0	16:1	18:0	18:1	18:2
Initial TAGs	1,0	22.5	1,7	2,2	16,7	55,9
Uncleaved TAGs	0,6	23,4	0,9	1 3	15,8	58 0
DAGs	'0.6	18,8	Tr.	0,9	16 8	62,9
D-Phosphatidylphenols	—	14,8	—	Tr.	17,1	68,1
FFAs*	—	4,5	1,3	1,1	20.0	73,1

*Obtained on lipolysis of the L-phosphatidylphenols.

TABLE 2. Fatty-Acid Compositions of the sn-1, sn-2, and sn-3 Positions of the Triacylglycerols of Cottonseed Oil (mole %)

Acid	Position in the triacylglycerols			
	1	2	3†	3‡
14:0	2,8(93,3)*	—	0,2(6,7)	Tr.
16:0	39,9(59,1)	3,3(4,9)	24,3(36,0)	26,3
16:1	4,0(78,4)	Tr.	1,1(21,6)	Tr.
18:0	6,4(97,0)	Tr.	0,2(3,0)	Tr.
18:1	17,1(34,1)	21,3(42,5)	11,7(23 4)	12 9
18:2	29,8(17,8)	75,4(15,0)	62,5(37,2)	60,8

*Proportion of the acid in a certain position as a percentage with respect to its total amount in the triacylglycerols.
†Calculated by the first method.
‡Calculated by the second method.

way that position 1 is enriched with saturated acids and acids with chain lengths of more than 18 carbon atoms, both saturated and unsaturated, are concentrated in positions 3 [2].

The aim of our work was a stereospecific analysis of TAGs of cottonseed oil by Brockerhoff's method [3]. The initial TAGs were hydrolyzed with the aid of pancreatic lipase, as a result of which monoacylglycerols with the fatty acids in the sn-2 position (sn-2-MAGs), the sum of the sn-1,2- and sn-2,3-diacylglycerols (DAGs), the free fatty acids (FFAs) and the uncleaved TAGs were obtained. The hydrolysis products, after isolation by preparative TLC in system 1 were analyzed for their fatty acid compositions (Tables 1 and 2).

The set of acids in the second position of the TAGs was judged from the fatty acid composition of the sn-2-MAGs. A similarity of the compositions of the acids of the uncleaved and the initial TAGs served as an index of the absence of isomerization in the course of lipolysis. The acid composition of the DAGs isolated corresponded to the calculated composition and, consequently, reflected the distribution of the acids in the initial TAGs [4].

The sum of the isomeric DAGs was phosphorylated with phenyl phosphorodichloridate as a result of which a mixture of L- and D-phosphatidylphenols (PPs) was obtained which was separated by column chromatography from the substances that had not reacted. The purified PPs were then hydrolyzed with phospholipase A_2. The completeness of phospholipolysis was checked by TLC in system 2 and the process was stopped when the time of hydrolysis no longer affected the yield of FFAs and lysophosphatides. According to TLC (system 2) the hydrolysate consisted of uncleaved D-PPs (R_f 0.6), the free acids split off from the sn-2 position of the L-PPs (R_f 0.4), and L-lysophosphatides (R_f 0.05). After the products of phospholipolysis had been isolated, their fatty acid compositions were determined (Table 1).

Under the GLC condition used, the free phenol formed by the decomposition of the phenyl phosphorodichloridate and also in the course of the phosphorylation of the DAGs and the isolation of the fatty acids from the lysophosphatides had a retention time on the chromatogram

TABLE 3. Stereospecific Composition of the Acylglycerides of Cottonseed Oil

Species	Mole, %	Species	Mole, %
PPP	0.32	POP	2.33
OOO	0.43	OLO	1.8
LLL	14.04	POO	0.99
PLL	23.1	OOP	0.83
OLL	9.93	PPL	0.82
PLP	8.97	LOO	0.74
LLP	5.46	POL	6.12
LPL	4.52	PLO	4.40
LOL	3.97	OLP	3.83
OOL	2.78	LOP	1.84
LLO	2.63	OPL	0.35

coinciding with that of methyl palmitate. Consequently, in all cases when the presence of phenol in the reaction products was expected, they were subjected to additional purification.

The composition of the FFAs obtained in the cleavage of the L-PPs was similar to that of the sn-2-MAGs, which confirms the absence of isomerization in the process of isolating the DAGs and, consequently, their representative nature.

The fatty acids isolated from the L-lysophosphatides characterize the distribution of the acyl radicals in the sn-1 position of the TAGs.

Thus, as a result of the analysis the fatty acid compositions of the sn-1 and sn-2 positions of the TAGs were obtained. The compositions of the acids in the sn-3 position was calculated by two methods, starting from the composition of the TAGs and the lysophosphatides and also those of the D-PPs and of the sn-2-MAGs. The distribution of the acids in each of the three positions is shown in Table 2. It can be seen from Table 2 that the saturated and 16:1 acids occupy mainly the sn-1 position in the TAGs of cottonseed oil, while the unsaturated acids esterify all three positions equally, but the 18:1 acid is bound in larger amount in the sn-2 and sn-1 positions and the 18:2 acid in the sn-2 and sn-3 positions of the TAGs.

The results obtained on the composition of the acids in each of the three positions were used to calculate the stereospecific composition of the TAGs. Since in the use of the method described the percentage error in the determination of the minor components may reach 60-100% [5], the 14:0 and 18:0 acids were combined into one group with the 16:0 acid, and the 16:1 acid was included with the 18:1 acid. As a result of the calculation, 22 individual TAGs were obtained (Table 3). It can be seen from Table 3 that in cottonseed oil more than 2/3 of the total TAG composition is represented by the species SUU (monosaturated-disaturated) (34.6%) and UUU (36.4%), the remainder being due to SUS (11.3%), UUS (11.67%), and USU (4.9%). The species USS is absent.

Thus, in the distribution of the fatty acids over the sn-1,3 positions of the TAGs of cottonseed oil a definite law is observed according to which the saturated and 18:1 acids predominantly occupy the sn-1 and the 18:2 acid the sn-3 position.

EXPERIMENTAL

GLC was carried out as described previously [6]. TLC was performed on type L 5/40 silica gel in systems 1 [petroleum ether–diethyl ether (6:4)] and 2 [chloroform–methanol–25% ammonia (8:2:0.2)], the spots being revealed on the plates with 50% H_2SO_4 followed by heating to 120°C.

The investigation was performed on seeds of the cotton plant of variety Tashkent-1. The extraction of the oil from the seeds and the isolation of the TAGs were carried out as described previously [6].

Hydrolysis with pancreatic lipase was carried out as described by Markman et al. [7], using acetone-defatted type B lipase (Olaine chemical reagents factory), the time of hydrolysis being 30 min at 40°C. The hydrolysis products were separated by preparative TLC in system 1 and were identified by comparison with markers.

Phenyl phosphorodichloridate was obtained as described by Gefter [8]. The product had bp 94-96°C (1 mm), n^{31} – 1.5174.

The DAGs were phosphorylated by Brockerhoff's method [3], except that the duration of the reaction was increased to 16-18 h. A chloroform solution of the reaction products was shaken with 2 volumes of 20% Na_2CO_3 solution, the organic layer was separated off, and the solvent was evaporated in a rotary evaporator at a temperature not exceeding 50°C.

The purification of the phosphatidylphenols (50 ml) was carried out on a column (0.5 × 12 cm) of silica gel L 100/160 mesh with a small layer of Na_2CO_3 deposited on the silica gel. Chloroform eluted the unchanged DAGs, and chloroform–methanol (9:1) the L- and D-phosphatidylphenols. The purified PPs gave a positive qualitative reaction for phosphorus.

Lipolysis of the Phosphatidylphenols by Phospholipase A_2. A solution of 50 mg of the PPs in 10 ml of purified diethyl ether was treated with a solution 20 mg of kufi venom in 1 ml of Tris buffer (pH 8) and 0.2 ml of a 0.1 M solution of $CaCl_2$. The resulting mixture was left at room temperature for 16-18 h, the completeness of hydrolysis being monitored by TLC in system 2. After the end of the reaction, the ether was evaporated off, and, to eliminate the last traces of water, the reaction mixture was distilled with benzene several times. The residue was dissolved in 1 ml of chloroform and was separated by TLC in system 2. The silica gel containing the D-phosphatidylphenols and lysophosphatides was removed from the plate and was boiled under reflux with 1 ml of benzene and 3 ml of 5% methanolic HCl for 2 h. After the end of the reaction, the methanolysis products were desorbed from the silica gel with diethyl ether, and the ethereal extracts were washed with 10% Na_2CO_3 solution, dried, remethylated with diazomethane, and analyzed by GLC.

SUMMARY

The stereospecific analysis of the triacylglycerols of cottonseed oil has been performed for the first time.

It has been established that the saturated acids are distributed mainly in the sn-1 position, the oleic acid in the sn-2 and sn-1 positions, and the linoleic acids in the sn-2 and sn-3 positions of the triacylglycerol molecules.

On the basis of a calculation of the stereospecific composition of the triacylglycerols it has been found that the oil contains none of the USS species, and more 2/3 of it is represented by the SUU and UUU species.

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