4. Yu. L. Zherebin, A. A. Kolesnik, and A. V. Bogatskii, Prikl. Biokhim. Mikrobiol., 17, No. 4, 614 (1981).
5. M. Kates, Techniques of Lipidology, American Elsevier, New York (1972) [Russian translation (1975), p. 157]; J. G. Kirchner, Thin Layer Chromatography (2nd edn.), Wiley-Interscience, New York (1978) [Russian translation, Moscow, Vol. 2 (1981), p. 257].
6. Yu. L. Zherebin and A. A. Kolesnik, Khim, Prir. Soedin., 165 (1984); Yu. L. Zherebin and A. A. Kolesnik, Khim. Prir. Soedin., 196 (1984).
7. A. V. Bogatskii, Yu. L. Zherebin, and A. A. Kolesnik, Vinod. Vinograd., SSSR, No. 6, 21 (1980).
8. Methods of Biochemical Investigations [in Russian], Leningrad (1982), p. 85.
9. B. P. Pleshkov, Practical Handbook of Plant Biochemistry [in Russian], Moscow (1985), p. 187.
10. G. G. Alkhazashvili, V. G. Leont'eva, D. N. Kalatozishvili, T. I. Mayakova, L. A. Mudzhiri, A. A. Semenov, and V. Yu. Vitkovskii, Khim. Prir. Soedin., No. 4, 536 (1983).
11. G. N. Zaitsev and T. P. Afanas'eva, Biokhimiya, 22, 1035 (1957).
12. K. N. Vekirchik, Plant Physiology (Practical Handbook) [in Russian], Kiev (1984), p. 58.
13. Pigments of the Plastids of Green Plants and Methods for Their Investigation [in Russian], Moscow (1964), p. 77.
14. Yu. L. Zherebin and A. A. Kolesnik, Khim. Prir. Soedin., 663 (1983).
15. H. H. Strain and W. A. Svec, The Chlorophylls, Interscience, New York (1966), p. 63.
16. Yu. L. Zherebin and A. A. Kolesnik, Prikl. Biokhim. Mikrobiol., 20, No. 3, 407 (1984).

STEREOSPECIFIC ANALYSIS OF THE TRIACYLGLYCEROLS OF A COTTONSEED OIL HYDROGENATE AND ITS FRACTIONS

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A cottonseed oil hydrogenate has been fractionated into three fractions: high-melting, medium-melting, and liquid. It has been established that the fat of the second fraction, because of its physicochemical indices, consistency, and the distribution of the saturated fatty acyl radicals in the extreme positions of the TAG molecules, can be regarded as a cocoa butter substitute.

The hydrogenation and fractionation of oils and fats is discussed in a number of publications [1, 2], but the structure of the triacylglycerols (TAGs) in the products obtained has been studied inadequately.

Since the quality and digestibility of a fat depends on the distribution of the acyl radicals in the TAGs, we have studied the distribution of the fatty acid radicals in the TAG molecules of individual fractions of a fractionated cottonseed oil hydrogenate obtained from the Tashkent oils and fats combine. Fractionation was performed with the aim of obtaining an additional raw material for the confectionery industry. The hydrogenates were fractionated by a method described previously [3].

The characteristics of the hydrogenate and of the fractions obtained are given in Table 1. The fat of fraction I was characterized by a high hardness, a high melting point, and a considerable content of trans-acids. Fraction II, with a high hardness, had a lower melting point. Fraction III was liquid, with a considerably smaller amount of trans-acids and

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TABLE 1. Physicochemical Indices of the Hydrogenate and its Fractions

Index	Hydrogenate	Fraction I	Fraction II	Fraction III
Kaminskii hardness g/cm	280	800	660	—
Melting point, °C	32	41	37	—
trans-Acids, %	43,18	59,04	33,03	12,70
Conjugted dienes, %	4,43	Tr.	Tr.	7,34

TABLE 2. Position Distribution of the Fatty Acids in the TAGs of the Hydrogenate and its Fractions

Object of investigation	Acid	Content of acyl residues, TAGs	sn-1	sn-2	sn-3
Hydrogenate	12:0	0,3	0,5	0,3	0,1
	14:0	0,4	0,1	—	0,1
	16:0	29,0	44,8	5,0	37,2
	16:1	2,0	3,7	1,2	1,1
	18:0	0,9	2,6	—	0,1
	18:1	56,2	40,2	80,8	47,6
	18:2	11,2	7,1	12,7	13,8
	Σsat	30,6	49,0	5,3	37,5
	Σunsat	69,4	51,0	94,7	62,5
Fraction I	12:0	0,6	0,8	0,9	0,1
	14:0	0,6	0,8	0,9	0,1
	16:0	37,0	53,5	8,7	48,8
	16:1	0,2	—	0,3	0,3
	18:0	4,3	0,1	2,1	1,2
	18:1	49,8	30,6	75,0	43,8
	18:2	7,5	4,7	12,1	5,7
	Σsat	42,5	64,7	12,6	50,2
	Σunsat	57,5	35,3	87,4	49,8
Fraction II	12:0	1,0	2,0	0,5	0,5
	14:0	1,1	2,3	0,6	0,4
	16:0	28,2	38,0	7,4	39,2
	16:1	0,9	—	1,8	0,9
	18:0	2,3	5,7	0,7	0,5
	18:1	53,2	45,7	72,5	41,4
	18:2	13,3	6,3	16,5	17,1
	Σsat	32,6	48,0	9,2	40,6
	Σunsat	67,4	52,0	90,8	55,4
Fraction III	12:0	0,9	2,2	0,5	—
	14:0	0,9	2,0	0,5	0,2
	16:0	21,5	31,9	3,8	23,8
	16:1	1,6	3,6	0,8	0,4
	18:0	1,3	3,8	—	0,1
	18:1	51,0	39,5	62,6	51,2
	18:2	22,7	17,0	31,8	19,3
	Σsat	24,6	39,9	4,8	29,1
	Σunsat	75,3	60,1	95,2	70,9

TABLE 3. Stereospecific Composition of the Triacylglycerols Present in an Amount of More Than 1.0%

Triacylglycerol	Hydrogenate	Fraction I	Fraction II	Fraction III
PPP	1,0	2,2	1,1	—
PPO	1,1	2,1	1,2	—
OPO	1,0	1,1	1,5	—
POP	13,5	19,7	10,9	6,5
POO	17,3	17,6	11,5	10,6
POL	5,0	2,3	4,7	4,0
P_0OP	1,1	—	—	—
P_0OO	1,4	—	—	1,1
SOP	1,0	3,5	1,6	—
SOO	1,0	3,3	1,7	—
OOP	12,1	11,3	13,0	7,3
OOO	15,5	10,0	13,6	11,8
OOL	4,5	1,3	5,6	4,5
LOP	2,1	1,7	1,7	3,1
LOL	1,0		1,0	1,9
LOO	2,7	1,6	1,9	5,0
PLP	2,1	3,2	2,5	3,3
PLO	2,7	2,8	2,6	5,5
PLL	1,0	—	1,2	2,0
OLP	1,9	1,8	2,9	3,7
OLO	2,4	1,6	3,3	6,1
OPP	—	1,3	1,4	—
OLL	—	—	1,3	2,3
LLP	—	—	—	1,6
LLO	—	—	—	2,6
LLL	—	—	—	1,0

P, acyls of palmitic acid; P_0, of palmitoleic acid; S, of stearic acid; O, of oleic acid; L, of linoleic acid.

a higher amound of acids with a conjugated system of double bonds as compared with fraction (I) and (II).

Consequently, from its physicochemical indices fraction II corresponds best to the demands placed upon a cocoa butter substitute [4].

The TAGs from the materials investigated were isolated by column chromatography. The compositions of the fatty acids (FAs) of the TAGs of the hydrogenate and its fractions, calculated from GLC results, are given in Table 2. The amount of unsaturated FAs in the TAGs rose from the first fraction to the third, and the amount of saturated FAs fell correspondingly.

TABLE 4. Position-Type Compositions of the Triacylglycerols, mole %

Triacyl-glycerol	S_3	S_2U			SU_2			U_3
		sn-SSU	sn-SU	sn-USS	sn-SUU	sn-USU	sn-UUS	
Hydrogenate	1,0	1,1	16,6	—	27,0	1,0	17,2	27,5
Fraction I	2,2	2,1	26,4	1,3	26,0	1,1	14,8	14,5
Fraction II	1,1	1,2	15,0	1,4	21,7	1,5	17,6	26,7
Fraction III	—	—	9,8	—	22,1	—	15,7	36,3

The position distribution of the fatty acyls in the TAG molecules was established by stereochemical analysis using Brockerhoff's method with some modifications. The results of the analysis are also given in Table 2. The TAGs of fractions I and III have a certain similarity with respect to the distribution of the individual acids. Thus, the 16:0 acid, esterifying mainly the extreme positions of the TAG molecules, predominated somewhat in the sn-1 position in fractions I and III, while in fraction II it was uniformly distributed over the extreme positions.

In the TAGs of fractions I and III, of the extreme positions, the 18:1 acid most enriched the sn-3 position, while in the TAGs of fraction II it was uniformly distributed in the Sn-1 and sn-3 positions.

The stereospecific composition of the TAGs calculated from the results of analysis (Table 3) included the following main types of TAGs: POP, POO, OOO, and OOP.

The position-type compositions of the TAGs of the hydrogenate and its individual fractions are given in Table 4. The hydrogenate was characterized by a high content of monosaturated diunsaturated (SU_2) and triunsaturated (U_3) triacylglycerols, which are responsible for the nutritional value of fats.

The amount of TAGs with saturated acyls in the sn-1 position (SUU) was greater than those with such acyls in the sn-3 position (UUS). Monounsaturated-disaturated TAGs were represented mainly by the SUS type, i.e., with a symmetrical structure [4]. Trisaturated TAGs amount to only 1%.

As the result of the fractionation of the hydrogenate, the following redistribution of the TAGs took place: the largest amount of TAGs of the SUS type – 26.4% – was concentrated into fraction I, and the smallest amount of this type – 9.8% – in fraction III.

It is known that TAGs with a symmetrical structure are responsible for a high hardness of fats [4]. Trisaturated TAGs were detected in fractions I and II in amounts of 2.2 and 1.1%, respectively. In their turn, the TAGs of the U_3 type were concentrated in fraction III – 36.3% – the amounts of them in the other two fractions being considerably less. The amount of TAGs of the SU_2 type in all the fractions remained practically at the same level.

Thus, the main difference in the compositions of the TAGs of the fractions obtained consists in the quantitative ratio of the TAGs of the U_3 and S_2U types. Among the latter, those in which the saturated acyls occupy the extreme positions in the molecules, as in the position distribution of the saturated acyls in the TAGs of cocoa butter [5] considerably predominated. It is just with respect to these types of TAGs that fraction II occupies an intermediate position between the high-melting fraction I and the liquid III.

EXPERIMENTAL

The hardness of the fats was determined by Kaminskii's method [6]. The amounts of the trans-acids were calculated from IR-spectral characteristics [7] with the measurement of the intensity of the absorption band at ν 970 cm^{-1} and a comparison of it with the absorption band of methyl elaidate.

The amount of conjugated dienes was determined by UV spectroscopy from the absorption band at λ 234 nm and was calculated from a formula [8] including the specific absorption coefficient of a pure isomer of linolenic acid containing 93.5% of conjugated double-bond system.

The gas–liquid chromatography of the fatty acid methyl esters was performed on a Chrom-4 instrument with a flame-ionization detector in the isothermal regime using a 4 mm × 2.5 m column filled with 17% of ethylene glycol succinate on Chromaton N-AW at a column temperature of 192°C with a rate of flow of carrier gas (He) of 0.8 kgf/cm^2 [sic].

The triacylglycerols were isolated from the products under investigation by column chromatography on silica gel L 100-160 μ with elution by petroleum ether (40-60°C) and diethyl ether in a ratio of 9:1.

Stereospecific Analysis of the TAGs. Pancreatic lipolysis was carried out by the method described in [9]. As a supplement to this procedure, we used 5-7 ml of hexane to dissolve 1 g of sample.

The products obtained were separated by TLC on silica gel with 5% of boric acid in the solvent system hexane—ether (6:4), which led to the isolation of the sn-2-MAGs (R_f 0.1), the sn-1,2- and sn-2,3-DAGs (R_f 0.4), the FAs (R_f 0.6), and the uncleaved TAGs (R_f 0.8).

The acylglycerols were hydrolyzed with a 10% methanolic solution of KOH. The fatty acids, in the form of their methyl esters, were analyzed by GLC. The identity of the FA compositions of the initial and the uncleaved TAGs served as proof of the fact that no isomerization took place in the course of lipase hydrolysis.

The phosphorylation of the isomeric DAGs was performed directly by Brockerhoff's phenyl phosphorodichloridate method [10], but the solution of DAGs was added slowly from a dropping funnel to a cooled solution of phenyl phosphorodichloridate. The phenyl phosphorodichloridate ($C_6H_5OPOCl_2$) was synthesized by a known procedure [11].

The L- and D-phosphatidylphenols obtained were freed from DAG residues and from the by-products of phosphorylation by CC on silica gel, with Na_2CO_3 scattered on the top layer of adsorbent [12].

Hydrolysis of the sn-1,2-Diacylphosphatidylphenols. The L- and D-phosphatidylphenols (50-100 mg) were dissolved in 15 ml of diethyl ether (previously freed from peroxides), and 20 mg of snake (kufi) venom dissolved in 2 ml of tris-HCl buffer, pH 9.0, and 0.2 ml of a 0.1 M solution of $CaCl_2$ were added.

The reaction mixture was stirred with a magnetic stirrer at 30-32°C for 1 h and was left for 12 h at room temperature, after which 1 ml of methanol was added and the solvent was evaporated off in a rotary evaporator.

In contrast to Brockerhoff's method [10], the reaction products were treated with diazomethane and were then separated by TLC on silica gel using a single solvent system — chloroform—methanol—ammonia (85:15:2). Under these conditions, the fatty acids split off from the second position of the L-phosphatidylglycerols in the form of MEs migrated with the solvent first, and their composition was identical with that of the acids of the sn-2-MAGs, which confirmed the absence of isomerization in the process of isolating the sn-1,2(2,3)-DAGs. The D-phosphatidylphenols (R_f 0.45) and the lysophosphatidylphenols (R_f 0.1) were hydrolyzed with 10% methanolic KOH solution and the fatty acids were analyzed by the GLC method. The composition of the acids in the sn-1 positions of the TAGs corresponded to the acids isolated from the lysophosphatidylphenols. The acids in the sn-3 position were calculated by two methods [13]: 1) sn-3-position = 3 × (initial TAGs) − (lyso-PPs) − (sn-2-MAGs); and 2) sn-3-position = 2 × (D-PPs) − (sn-2-MAGs).

CONCLUSION

A cottonseed oil hydrogenate has been fractionated into three fractions: high-melting, medium-melting, and liquid.

It has been shown that, in its physicochemical indices and consistency, fraction II corresponds to the demands placed upon a cocoa butter substitute.

It has been established by stereospecific analysis that the main difference in the compositions of the TAGs of the fractions obtained consists in the quantitative ratio of triunsaturated and disaturated-monounsaturated TAGs. Among the latter, TAGs with a symmetrical structure, which are responsible in some degree for the hardness of fats, predominated. It was found that with respect to these types of TAGs fraction II occupied an intermediate position between the high-melting fraction I and the liquid III.

LITERATURE CITED

1. N. V. Vengerova, Trudy VNIIZh (Proc. All-Union Scientific-Research Institute of Fats), Leningrad, No. 17, 15 (1957).
2. I. V. Pavlova and M. G. Pashkevich, Maslo-zhir. Prom-st'., No. 4, 35 (1986).

3. G. U. Tillaeva, Uzbek. Khim. Zh., No. 2, 47 (1985).
4. N. F. Shumateva and F. B. Bizhanov, The Production of Confectionary Fats and of Cocoa Butter Analogues and Substitutes [in Russian], Alma-Ata (1986).
5. H. Brockerhoff and M. Yurlowski, J. Lipid Res., 7, No. 1, 62 (1966).
6. Handbook on Methods of Investigating, Technical and Chemical Control, and the Accounting of Production in the Oils and Fats Industry [in Russian], Leningrad, Vol. III (1969), p. 243.
7. A. N. Mironova, Trudy VNIIZh (Proc. All-Union Scientific-Research Institute of Fats), Leningrad, No. 17, 63 (1957).
8. V. L. Sterlin and A. N. Mironova, Trudy VNIIZh (Proc. All-Union Scientific-Research Institute of Fats), Leningrad, No. 27, 276 (1967).
9. A. L. Markman, T. V. Chernenko, and A. U. Umarov, Prikl. Biokhim. Mikrobiol., No. 5, 5 (1965).
10. H. Brockerhoff, J. Lipid Res., 6, No. 1, 10 (1965).
11. T. Mukaiyama, T. Hata, and O. Mitsunoby, J. Org. Chem., 27, No. 5, 1815 (1962).
12. S. G. Yunusova, S. D. Gusakova, A. I. Glushenkova, and V. G. Shcherbakov, Pishch. Tekhnol., No. 2, 17 (1985).
13. A. Kuksis, Handbook of Lipid Research. 1. Fatty Acids and Glycerides, Plenum, New York (1978), p. 197.

AMOUNTS OF ARACHIDONIC ACID IN THE BUDS OF Populus balzamifera IN THE COURSE OF THE ANNUAL CYCLE

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The paper gives information on the amount of arachidonic acid in the buds of Populus balzamifera. The presence of arachidonic acid in the buds was confirmed by TLC, GLC, coulometric titration, and an iodine number calculation. The dynamics of the amount of arachidonic acid in the buds during the annual cycle are given.

It is known that arachidonic acid, the main precursor of the prostaglandins, is a structural component of the phospholipids of cell membranes, of triacylglycerides, and of esterified cholesterols in animal organisms. There is information on the presence of arachidonic acid in all mammals [1] and also in the lipids of marine organisms [2, 3]. Until very recently, it was considered that vegetable oils did not contain unsaturated acids with five and six double bonds [4]. The presence of arachidonic acid* in some species of lower plants [5] and in clover and buckwheat pollen [6] was later established.

L. Rubchevskaya [7] showed the presence of arachidonic acid in the cambial zone of Larix sibirica. É. Levin, Sh. Alaudinov, and V. Cherepanova were the first to establish the presence of prostaglandins in the living tissues of higher plants [8, 9]. This, in its turn, posed the problem of studying their precursors in these species of plants. Our aim was to establish the presence of arachidonic acid in the buds of Populus balzamifera L., and also to estimate its amount quantitatively in the course of the annual cycle.

The results of determinations of the amounts of arachidonic acid are given in Table 1. The amounts of total lipids differ in the course of the annual cycle. The maximum amount of total lipids is found in December (21.33%) and the minimum in September (13.34%). The amount of neutral lipids ranges from 6 to 48% of the total lipids, depending on the pheno-

*Arachidonic acid contains four double bonds – Publisher.

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