The fatty-acid compositions were determined by the GLC method in the Fats Research Laboratory of the Moscow branch of the All-Union Institute of Fats on a Hitachi model K-53 chromatograph with a flame-ionization detector.

The thermal treatment of the fats was performed at 180-190°C in communal feeding enterprises at a ratio of fat and product of 4:1 and a replaceability of the fat of 0.6. The time of use of the fat was 30 h. The time of cooking the articles was 2-3 min.

SUMMARY

The fatty-acid composition of a mixture of cottonseed oil and mutton fat in a ratio of 1:1 before and after thermal treatment has been studied. It has been established that the mixture obtained is more resistant to the action of heat than cottonseed oil.

LITERATURE CITED

1. T. T. Mirkhalikov, A. Abdurakhimov, and P. A. Artamonov, Khim. Prirodn. Soedin., 13 (1973).
2. A. I. Zdobnov and L. N. Zaushitsina, "A study of mixtures of cottonseed oil and mutton fat used for frying," Respublikanskii Mezhvedomstvennyi Nauchno-Tekhnicheskii Sbornik, Kiev, 9, 150 (1973).
3. M. N. Ismailov, Voprosy Pitaniya, 6, 62 (1964).
4. A. G. Vereshchagin, S. V. Skvortsova, N. I. Ishkakov, Biokhimiya, 28, No. 5, 868 (1963).
5. Handbook of Methods of Investigating Fats [in Russian], Vol. 1 (1967), p. 433.
6. N. R. Artman and D. E. Smith, J. Am. Oil Chemists' Soc., 49, No. 5, 318 (1972).

PHOSPHOLIPIDS OF THE SEEDS OF TWO SPECIES OF *Erysimum*

Yu. A. Tadzhibaev, Kh. S. Mukhamedova, and S. T. Akramov

UDC 547.953:665.37

Continuing an investigation of the phospholipids (PL's) of the seeds of plants of family Cruciferae [1-3], we have studied the PL's of the seeds of *Erysimum diffusum* Ehrh. (collected in the environs of the village of Tobolino, Chimkent oblast) and of *Erysimum sylvestris* (L.) Bess. (collected in the environs of Burchmulla, Bostanlykskii region, Tashkent oblast).

The combined PL's from the seeds were obtained and freed from accompanying carbohydrates by the methods usually used [1, 3].

The yield of total PL's freed from carbohydrates was 0.5% from the seeds of *E. diffusum* and 1% from *E. sylvestris*. The amount of phosphorus in the combined material [4] was 3.3% in both cases. The qualitative and quantitative compositions of the total PL's were established by two-dimensional TLC in systems 1 and 2 followed by the determination of the phosphorus in the spots [5]. In each case, six phosphorus-containing spots were detected: three main ones – phosphatidylcholines (PC's), phosphatidylinositols (PI's), and phosphatidylethanolamines (PE's) – and three minor ones – N-acylphosphatidylethanolamines (N-acyl-PE's), N-acyllysophosphatidylethanolamines (N-acyllyso-PE's], and lysophosphatidylcholines (lyso-PC's). The quantitative distributions of these components in the combined materials from the plants investigated are given below (%).

Phospholipid Fraction	*E. diffusum*	*E. sylvestris*
N-Acyl-PE's	3,2	7,0
N-Acyllyso-PE's	2,1	5,2
PE's	19,3	19,2
PC's	49,1	45,2
PI's	21,3	16,4
Lyso-PC's	5,0	7,0

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.

Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 623-627, September-October, 1977. Original article submitted May 24, 1977.

0009-3130/77/1305-0517$07.50

TABLE 1. Results of the GLC Analysis of the Fatty Acids in the Triglycerides, in the Total Phospholipids, and in Their Fractions from the Seeds of Two Species of Erysimum

Fraction	Fatty acid															
	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:1	22:0	22:1	24:0	24:1	ΣП	ΣН
Erysimum diffusum																
Triglycerides of the oil	1,6	1,4	—	4,5	1	1,6	9,0	25,1	29,3	5,4	—	21,1	—	—	9,1	90,9
Phospholipids	2,0	1,6	—	13,7	1,8	2,2	18,5	28,0	9,8	5,4	—	17,0	—	—	19,5	80,5
Phosphatidylcholines																
total	—	2,5	—	11,0	2,1	2,0	20,3	35,8	15,6	6,0	—	4,7	—	—	15,5	84,5
position 1	—	4,2	—	18,8	2,4	2,4	17,6	23,8	12,6	8,1	—	10,1	—	—	25,4	74,6
position 2	—	—	—	2,5	3,1	1,1	21,6	47,6	18,1	4,0	—	2,0	—	—	3,6	96,4
Phosphatidylethanolamine																
total	2,8	2,0	—	11,8	3,6	2 0	12,7	32,4	10,7	4,0	—	18.0	—	—	18,6	71,4
position 1	3,3	2,0	—	18,0	—	3,3	11,7	23,2	6,1	7	—	25,4	—	—	26,6	73,4
position 2	—	1,4	—	6,2	1,3	1,4	12,2	44,5	17.4	3,1	—	12.5	—	—	9,0	91,0
Phosphatidylinositols																
total	3,0	3,2	—	20,3	2,5	3,0	9,8	29,1	16,1	—	—	13,0	—	—	29,5	70,5
position 1	4,0	4,5	—	32,0	—	5,2	9,7	16,3	6,3	—	—	22.0	—	—	45,7	54 3
position 2	—	—	—	5,2	3,3	1,3	9.8	43,3	27,0	—	—	10,1	—	—	6,5	93.5
Lysophosphatidylcholines	1,6	1,7	—	10,8	1,7	2,2	14,6	32,8	16,5	6,6	—	11,5	—	—	16,3	83,7
N-Acylphosphatidyl-ethanolamines																
total	5,1	3,9	—	13,0	4,0	3,7	10,9	21,1	14,5	4,3	—	19,5	—	—	25,7	74,3
O-acyl	—	1,6	—	17,0	3,6	4,7	11,6	26,8	16,9	1,8	—	16,0	—	—	23,3	76,7
N-acyl	12,3	10,7	—	8,8	5,2	3,0	10,8	14,0	10,0	—	—	25,2	—	—	34,8	65,2
N-Acyllysophosphatidyl-ethanolamine	25,0	16,0	—	6,5	2,5	—	6,0	26,5	9,0	—	—	8,5	—	—	47,5	52,5
Erysimum sylvestris																
Triglycerides of the oil	—	—	1,6	5,4	2,7	2,5	12,2	12,1	30,0	2,4	5,0	26,1	—	—	14,5	85,5
Phospholipids	—	—	0,5	10,3	1,0	0,6	14,7	22,1	32,0	7,4	3,0	6,0	1,1	1,3	15,5	84,5
Phosphatidylcholines																
total	—	—	1,0	8,3	1,1	—	31,3	29,7	22,8	2,6	—	3,2	—	—	9,3	90,7
position 1	—	—	0,6	16,9	—	—	34,4	24,1	16,2	3,8	—	4,0	—	—	17,5	82,5
position 2	—	—	—	1,1	1,8	—	27,3	37,5	29,4	0,8	—	2,1	—	—	1,1	98,9
Phosphatidylethanolamines																
total	—	—	1.0	8,4	0,9	0,5	11,6	29,7	23,4	13,8	2,0	3,2	2,3	3,2	14,2	85,8
position 1	—	—	0,6	14,1	0,9	0,9	9,2	20,4	12,8	28,3	2,3	6,6	3.9	—	21,8	78,2
position 2	—	—	0,4	3,0	0,6	—	13,7	37,0	33,9	—	2,1	2,3	—	7,0	5,5	94,5
Phosphatidylinositols																
total	—	—	—	27,4	0,7	—	10,4	19,0	18,3	10,3	—	6,1	—	7,8	27,4	72,6
position 1	—	—	—	51.1	—	—	7,6	7,5	3,7	11,1	—	3,0	—	16,0	51,1	48,9
position 2	—	—	—	3,8	2,2	—	12,7	30,3	31,9	8,9	—	10,2	—	—	3,8	96,2
Lisophosphatidylcholines	—	—	2,8	16,0	—	—	28,2	29,1	21,9	2,0	—	—	—	—	18,8	81,2
N-Acylphosphatidyl-ethanolamines	—	—	—	9,3	3,7	5,0	10,0	23,1	18,8	7,9	11,9	10,3	—	—	26,2	73,8
O-acyl	—	—	—	7,8	1,8	2,6	11,5	30,0	28,4	1,2	11,3	5,4	—	—	21,7	78,3
N-acyl	—	—	—	10,7	3,7	7,8	8,8	16,4	11,8	14,2	10,6	16,0	—	—	28,9	71,1
N-Acyllysophosphatidyl-ethanolamine	—	—	0,9	6,6	2,4	2,5	8,5	18,5	27,3	18,0	5,3	3,3	—	6,7	15,3	84,7

The two combined materials contained almost the same amounts of PE's, but in E. diffusum the amount of PE's was less than that of PI's, which is normal for the seeds of the majority of plants [1, 3], while in E. sylvestris, conversely, there were more PI's than PE's.

Homogeneous PL's were obtained by column chromatography of the combined material on silica gel followed by purification by preparative TLC in systems 1 and 2, and on the basis of certain physical and chemical characteristics [6, 7] they were assigned to the group of glycerophospholipids. The fatty acids of the trigycerides of the oil, the combined PL's and the individual components were split by milk alkaline hydrolysis [8] and were analyzed by GLC in the form of their methyl esters (Table 1).

The phospholipids and triglycerides of E. diffusum contain the low-molecular-weight acids $C_{10:0}$ and $C_{12:0}$ and no high-molecular-weight minor acids $C_{22:0}$, $C_{24:0}$, and $C_{24:1}$, which are present in the triglycerides of the majority of plants of the family Cruciferae [9-14]. Conversely, in the phospholipids of E. sylvestris there are no low-molecular-weight acids and the high-molecular weight $C_{22:0}$, $C_{24:0}$, and $C_{24:1}$ acids are present, being distributed nonuniformly between the individual components of the PL's: all three are absent from the PC's and their lyso analogs. According to their degree of increasing saturation, the individual PL's in E. diffusum form the following sequence: PC's → PE's → lyso-PC's → PI's → N-acyl-PE's → N-acyllyso-PE's. In E. sylvestris the sequence is: PC's → PE's → N-acyllyso-PE's → lyso-PC's → N-acyl-PE's → PI's.

Erucic acid, $C_{22:1}$, which is characteristic for the oils and phospholipids of the overwhelming number of the plants of this family [2, 3, 9-15], is present in larger amount in the triglycerides than in the phospholipids; the bulk of this acid in E. diffusum is concentrated in the PE and N-acyl-PE fractions, while in E. sylvestris it is concentrated in the N-acyl-PE fraction.

The position distributions of the acyl radicals in the phospholipids present in greatest amount were determined by enzymatic hydrolysis with phospholipase A_2. The time of enzymatic hydrolysis increased in the sequence PC's → PE's → PI's, which is due to the fatty-acid composition of the phospholipids investigated (the PC's are the most unsaturated fraction) and, possibly, to conformational difficulties of enzymatic hydrolysis. The fatty acids from positions 1 and 2, after methylation with diazomethane, were analyzed by GLC (see Table 1). The acyl radicals esterified in positions 1 and 2 of the molecules of the phospholipids of the seeds of the species under investigation differed from one another both qualitatively and quantitatively, which depends on the total fatty acids of the initial component. However, in all samples a certain law is observed: the unsaturated acids predominantly occupy position 2. On the basis of the position distribution of the fatty-acid radicals in the PC, PE, and PI molecules we calculated their molecular compositions, which can be distributed according to types in the following way (I – E. diffusum; II – E. sylvestris):

Type	PC's		PE's		PI's	
	I	II	I	II	I	II
Disaturated	2,2	0,2	4,2	0,7	2.7	1.9
Saturated–unsaturated	30,1	16,8	30,1	18,7	44.0	49.0
Unsaturated–saturated	4,7	0,9	7,0	3,7	3,3	1,7
Diunsaturated	63,0	82,1	58,7	76,9	50,0	47,4
Number of types, M	61	40	65	52	52	50

The figures given show that the molecular compositions of the phospholipids of E. diffusum differ substantially from those of E. sylvestris: the PL's of E. diffusum are characterized by a larger number of molecular types in the main fractions, which depends on the initial fatty-acid composition and degree of selectivity of the pairing of the fatty acids in these molecules. In the PC's of E. sylvestris there are less disaturated and more diunsaturated types than in the other fractions, which is due to the comparatively high state of unsaturation of the total molecule. In all cases diunsaturated forms predominate.

In both cases, N-acylphosphatidylethanolamines were studied by the procedure described previously [16]. Analysis of the fatty acids included in the amide groups (N-acyls) and those included in ester groups (O-acyls) in the glyceride part of the molecule showed that in the N-acyl-PE's the N-acyls are more highly saturated than the O-acyls.

EXPERIMENTAL

For TLC and column chromatography we used type KSK silica gel. For the two-dimensional chromatography and preparative separation of the phospholipids we used the following solvent systems: 1) chloroform–methanol–water (65 : 35 : 5); 2) chloroform–methanol–ammonia (65 : 35 : 5). The alkaline hydrolysis of the fractions was performed in 10% methanolic KOH solution at room temperature. The methyl esters of the

saturated fatty acids were investigated on a UKh-2 chromatograph [1-3]. The enzymatic hydrolysis of the main components was performed in Tris buffer, pH 9.0, at 37°C. Kufi venom was used as the source of phospholipase A.

SUMMARY

The phospholipid complexes of the seeds of Erysimum diffusum and E. sylvestris have been investigated. It has been found that the amount of phospholipids in the seeds of E. diffusum is lower than in those of E. sylvestris, but their qualitative compositions are similar. The fatty-acid compositions of the triglycerides and of the total phospholipids of the two plants investigated have been studied. It has been established that the total phospholipids of the species of Erysimum under consideration and individual fractions of them differ by the degree of saturation and the qualitative and quantitative composition of the acids that they contain and, consequently, the phospholipids present in greatest amount differ in their molecular compositions.

LITERATURE CITED

1. Yu. A. Tadzhibaev, Kh. S. Mukhamedova, and S. T. Akramov, Khim. Prirodn. Soedin., 435 (1976).
2. Yu. A. Tadzhinaev, Kh. S. Mukhamedova, and S. T. Akramov, Khim. Prirodn. Soedin., 109 (1977).
3. Yu. A. Tadzhibaev, Kh. S. Mukhamedova, and S. T. Akramov, Khim. Prirodn. Soedin., 195 (1977).
4. D. Tevekelov, Izvestiya na Instituta po Khranene, Bolg. Akad. Nauk No. 7, 21 (1968).
5. É. V. Dyatlovitskaya, T. I. Torkhovskaya, and A. D. Bergel'son, Biokhimiya, 34, 177 (1969).
6. Handbook on Methods of Investigation and Technical and Chemical Control and the Accounting of Production in the Oils and Fats Industry [in Russian], I, Book 1, Leningrad (1967).
7. G. Y. Nelson, Lipids, 3, 104 (1968).
8. E. Stahl, Thin Layer Chromatography, Springer, New York (1969), [Russian translation of 1st German Edition (1965), p. 147].
9. A. U. Umarov, Khim. Prirodn. Soedin., 12 (1975).
10. V. S. Dolya, E. N. Shkurupii, N. A. Kaminskii, and E. D. Magerya, Khim. Prirodn. Soedin., 18 (1977).
11. K. L. Mikolajczak, T. K. Miva, F. R. Barle, I. A. Wolff, and Q. Jones, J. Am. Oil Chemists' Soc., 38, 678 (1961).
12. I. W. Hageman, K. L. Mikolajczak, and I. A. Wolff, J. Am. Oil. Chemists' Soc., 30, 196 (1962).
13. R. W. Miller, F. R. Earle, I. A. Wolff, and Q. Jones, J. Am. Oil Chemists' Soc., 42, 817 (1975).
14. F. R. Earle, J. E. Paters, J. A. Wolff, and G. A. White, J. Am. Oil Chemists' Soc., 43, 330 (1966).
15. R. O. Weenink and A. P. Tulloch, J. Am. Oil Chemists' Soc., 43, 327 (1966).
16. Kh. S. Mukhamedova and S. T. Akramov, Khim. Prirodn. Soedin., 580 (1976).