POSITION-SPECIES COMPOSITION OF THE TRIACYLGLYCEROLS OF YEAST MICROORGANISMS

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UDC 547.915.663.14

The molecular-species and olefinic composition of the triacylglycerols (TAGs) of five strains of yeast have been investigated. The predominance of a particular molecular species of TAGs in the triglyceride compositions of the strains investigated depends not only on the genus but also on the species of each particular strain. The olefinic composition of the yeast TAGs is represented mainly by TAGs with 1, 2, 3, and 4 double bonds in the molecule.

One of the urgent tasks with which the microbiological industry is faced is the creation of a biotechnology of substitutes for plant oils based on the cultivation of microorganisms actively producing lipids. To answer the question of the possibility of replacing a particular plant oil by lipids of microbial origin, it is necessary to have the full characteristics of the chemical compositions of the latter: the fatty-acid and group compositions of the total lipids and the structures and molecular compositions of the triacylglycerols (TAGs). In the study of the chemical composition of microbial lipids it is most frequently the fatty-acid and group compositions of the total lipids that are studied and only more

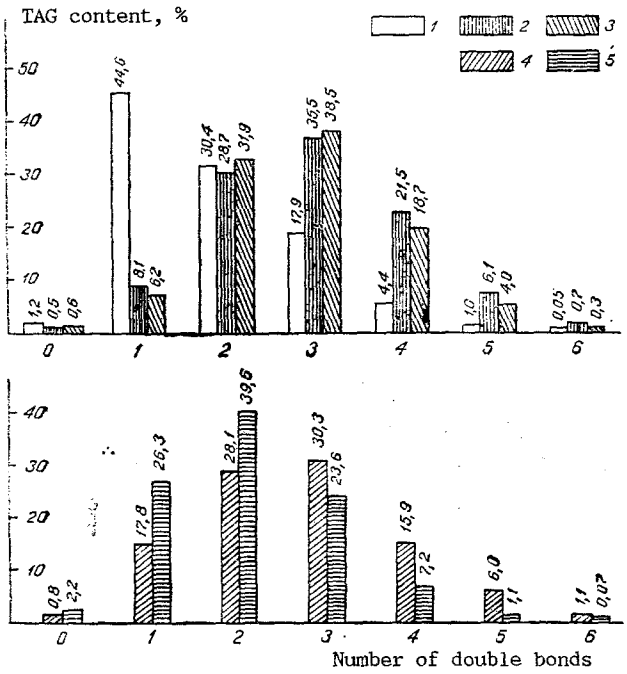

Fig. 1. Distribution of triacylglycerols according to the number of double bonds: 1) Rhodosporidium sphaerocarpum VKM u-1567; 2) Rhodosporidium sphaerocarpum VKM u-1568; 3) Cryptococcus albidus var. albidus VKM u-1955; 4) Rhodotorula gracilis VKM u-329; 5) Rhodotorula gracilis VKM u-335.

Moscow Technological Institute of the Food Industry. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 28-32, January-February, 1986. Original article submitted February 25, 1985.

0009-3130/86/2201-0025$12.50

TABLE 1. Distribution of the Yeast TAGs in Relation to the Numbers of Carbon Atoms

Total carbon atoms in the acyls of the TAGs	Rhodotorula gracilis VKM u-335 (1)		Rhodotorula gracilis VKM u-329 (2)		Rhodosporidium sphaerocarpum VKM u-1567 (3)		Rhodospordium sphaerocarpum VKM u-1568 (4)		Cryptococcus albidus var. albidus VKM u-1955 (5)	
	exp.	stat.	exp.	stat.	exp.	stat.	exp.	stat.	exp.	stat.
C_{48}	2,3	2.0	1.5	1,2	0,1	2,4	0,4	0,3	0,2	0,3
C_{50}	20,1	16,3	14,9	11,9	15,9	17,7	4,5	5,6	2,8	5,7
C_{52}	51 0	43,5	53,8	40,7	54,3	43,8	33,9	32,5	43,7	32,3
C_{54}	26,6	38,2	29,8	46,2	29,7	36,1	61,2	61,6	53,3	61,7

TABLE 2. Compositions and Position Distributions of the Fatty Acids in Yeast TAGs (% of the total amount of acids)

Fatty acid	Rhodotorula gracilis VKM u-335 (1)		Rhodotorula gracilis. VKM u-329 (2)		Rhodospordium shaerocarpum VKM u-1567 (3)		Rhodospordium sphaerocarpum VKM u-1568 (4)		Cryptococcus albidus var. albidus VKM - 1955 (5)	
	total TAGs	pos.2	total TAGs	pos.2	total TAGs	pos.2	total TAGs	pos.2	total TAGs	pos.2
14:0	1,2	0,1	1,1	Tr.	0,6	Tr.	0,4	Tr.	0,1	Tr.
15:0	0,3	0,2	Tr.	Tr.	0 1	Tr.	Tr.	Tr.	Tr.	Tr.
16:0	25,5	1,0	21,2	0,4	27,2	1,2	14,2	0,6	14,8	0,5
16:1	0 3	0,6	0,4	Tr.	Tr.	Tr.	Tr.	—	Tr.	Tr.
17:0	0,2	—	Tr.	—	0,9		0,4	—	0,4	0,1
17:1	Tr.	Tr.	—	—	Tr.	0.4	Tr.	Tr.	Tr.	0.1
18:0	9,5	0,3	7,4	—	17,5	1,2	7,1	0.4	4,7	0,2
18:1	46.9	74,7	45,2	74,5	41,2	90,5	56,8	76,4	63,5	81,5
18:2	11,2	18,9	17,0	21,3	8.0	5,6	13.8	19,8	14,9	15,7
18:3	4,3	3,8	7,1	3,8	2.2	Tr.	4,3	2,8	1,6	1,5
ΣC_{20}	0,6	0,4	0.6	—	2,3	1,1	3,0	Tr.	Tr.	0,4

rarely the fatty-acid compositions of the individual groups of lipids. There is practically no information on the molecular composition of the TAGs of lipids of microbial origin in the literature [1]. Since the molecular composition of the TAGs has a great influence on the physicochemical and technological characteristics of fats and oils, their study is essential.

The aim of the present investigation was to establish the molecular compositions of the triacylglycerol fractions of the lipids of yeast microorganisms belonging to the genera Rhodotorula, Rhodosporidium, and Cryptococcus that had previously been selected as active producers of lipids.

The yeast cultures were obtained from the All-Union Collection of Nonpathogenic Microorganisms at the Institute of the Biophysics of Microorganisms of the Academy of Sciences of the USSR [2]. The total lipids were extracted from the yeast biomass by the method of Bligh and Dyer [3]. The TAGs were isolated from the total lipids by the countercurrent separation of the neutral and polar lipids [4].

The isolated TAGs were analyzed by high-temperature gas-liquid chromatography in order to determine their compositions in relation to the total number of carbon atoms in the acyl residues. Table 1 gives the results of this analysis and the statistical figures calculated on the basis of the theory of the equiprobable sn-1, 2, 3 distribution of the acyl residues of the fatty acids in a TAG molecule. As can be seen from Table 1, the values calculated from the statistical distribution theory do not in many cases reliably reflect the true amounts of TAGs with different numbers of carbon atoms.

The fatty acid compositions of the TAGs were determined by gas-liquid chromatography (Table 2). The distribution of the acyl radicals of the fatty acids in the sn-2 positions of the TAG molecules was investigated by the method of lipase hydrolysis (Table 2) [5].

The results obtained (Tables 1 and 2) were used to calculate the compositions of the TAGs [6]. The calculation was performed on the assumption that the TAGs under investigation were derived from the four acids palmitic (P), stearic (S), oleic (O), and linoleic (L). To the amount of palmitic acid was added the amount of the other saturated acids with the exception of stearic, and to the amount of linoleic acid was added the amount of linolenic acid.

TABLE 3. Main Molecular Species of TAGs of Yeast Lipids (% on the total amount of molecular species of TAGs)

TAG	Rhodotorula gracilis VKM u-335	Rhodotorula gracilis VKM u-329	Rhodospordium shaerocarpum VKM u-1567	Rhodospordium sphaerocarpum VKM u-1568	Cryptococcus albidus var. albidus VKM u-1955
	1	2	3	4	5
SOS	—	—	6,20	—	—
POP	14,20	10,00	14,00	—	—
SOP	10,20	6,98	23,50	—	—
POO	23,40	18,10	17,70	16,70	25,50
SOO	7,22	3,93	9,32	8,34	5,97
OOO	8,52	5,05	—	10,40	23,40
POL	4,14	14,60	8,02	5,61	6,29
PLO	7,42	6,18	—	5,02	5,59
OOL	2,03	8,21		13,10	11,60
OLO	—	—	—	5,85	5,15

The main molecular species of TAGs calculated by the given method, the amounts of which exceeded 2% of the total of all the species, are given in Table 3.

An analysis of the figures in Table 3 shows that the predominance of a particular molecular species of TAG in the lipids of the strains investigated is specific not merely for the genus but also for the type of yeast. In the case of the strain Rhodotorula gracilis VKM u-335, the dominating molecular species of the PAGs were POO (24.3%), POP (14.2%), and SOP (10.2%), while for another strain of the same species, Rh. gracilis VMK u-329, in addition to POO (18.1%) and POP (10.0%), there were considerable amounts of POL (14.6%).

For the strain Rhodosporidium sphaerocarpum VKM u-1567, the predominating molecular species of TAGs were SOP (23.5%), POO (17.7%), and POP (14.0%), while for another strain of the same species, Rh. sphaerocarpum VKM u-1568, in addition to POO (16.7%), such species of TAGs as POL (19.4%) and OOL (13.1%) also predominated.

In the case of the strain Cryptococcus albidus var. albidus VKM u-1955, about 50% of all the calculated molecular species of TAGs was represented by POO and OOO, present in approximately equal amounts (25.5 and 23.4%, respectively).

It must also be mentioned that in all the strains studied the main molecular species of TAGs included POO, SOO, and POL.

For each strain the molecular species of TAGs were grouped according to the number of double bonds in the molecule. Diagrams of the distribution of TAGs with respect to the number of double bonds are given in Fig. 1. Each of the TAGs isolated from the various strains of yeast had a unique olefinic composition.

In the lipids of the strain Rhodosporidium sphaerocarpum VKM u-1567, about 45% of TAGs with one double bond in each molecule were found. The remainder was represented mainly by TAGs with two and three double bonds in the molecule, the amount of which decreased practically in arithmetical progression (30.4 and 17.9%, respectively).

In the lipids ofthe strains Rhodosporidium sphaerocarpum VKM u-1568 and Cryptococcus albidus var. albidus VKM u-1955 the bulk consisted of TAGs with two, three, and four double bonds in the molecule. In spite of the fact that these strains were representatives of different genera, the olefinic compositions of the TAGs produced by them were fairly similar.

In the lipids of the strain Rhodotorula gracilis VKM u-335, as in the strain Rhodosporidium sphaerocarpum VKM u-1567, the bulk consisted of TAGs with one, two, and three double bonds in the molecule. However, in contrast to the latter, in this strain TAGs with two double bonds in the molecule predominated (39.6%), and the amounts of the other two species of TAGs were approximately equal (26.3 and 23.6%, respectively).

The greatest diversity in olefinic composition was found in the TAGs of the lipids produced by the strain Rhodotorula gracilis VKM u-329. The lipids of this strain were represented by TAGs with one, two, three, and four double bonds in the molecule. The amounts of TAGs with one and four double bonds in the molecule were practically equal (17.8 and 15.9%, respectively) and not much more than half the amounts of TAGs with two and three double bonds in the molecule, which were 28.1 and 30.3%, respectively.

The lipids of all the strains investigated contained practically no saturated TAGs (of the type of tripalmitin and tristearin) nor TAGs with six and more double bonds in the molecule (of the type of trilinolein).

EXPERIMENTAL

The lipids were extracted from the yeast biomass that had first been frozen to −36°C and had then been disrupted mechanically. The extractant used was a 1:2 mixture of chloroform and methanol. Separation from nonlipid impurities was performed by adding potassium chloride to the solution.

The TAGs from the total lipids were isolated by the countercurrent separation of the neutral and polar lipids in the hexane–87% ethanol (1:1) system recommended by Kates for samples with a high level of TAGs in the total lipids [4].

Analysis of the TAGs according to the number of carbon atoms was performed by the GLC method on a Tsvet-100 chromatograph with a flame-ionization detector using packed Pyrex glass columns 60 cm long with an internal diameter of 2.5 mm. The support was Chromaton N-Super (0.125-0.16 mm) impregnated with the liquid phase OV-1 (3%). The temperature of the detector of the evaporator was 325°C. The temperature program of the column thermostat was 250-10-350°C. The rate of flow of the carrier gas, helium, was 60 ml/min and the ratio of helium to hydrogen to air, 1:1:10.

The TAGs isolated were hydrolyzed as described previously [5], using type A pancreatic lipase (Olaine). The hydrolysis products were separated by preparative TLC in the hexane–diethyl ether–formic acid (50:45:1) system.

The fatty-acid compositions of the total TAGs and of the 2-monoacylglycerols were determined by analyzing the corresponding methyl esters by GLC on a LKhM-8MD chromatograph with a flame-ionization detector using a packed stainless-steel column 2 m long with an internal diameter of 4 mm. The support was Inerton AW-HMDS and the stationary phase was diethyleneglycol succinate (20%). The temperature of the detector was 200°C, that of the column thermostat 170-190°C, and that of the evaporator 225°C. The rate of flow of the carrier gas, helium, was 30-40 cm^3/min, that of hydrogen 40 cm^3/min, and that of air 400 cm^3/min.

SUMMARY

1. A study of the composition of yeast TAGs has shown that in all the strains investigated the main molecular species of TAGs include POO, SOO, and POL; depending on the particular strain, the dominating molecular species of the TAGs were POP, SOP, POO, OOO, POL, and OOL.

2. An investigation of the olefinic compositions of the TAGs isolated from yeasts has shown that they consist mainly of TAGs with one, two, three, and four double bonds in the molecule.

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