COMPOSITION AND STRUCTURE OF THE TRIACYLGLYCEROLS OF *Hippophaë rhamnoides* SEEDS

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The composition and structure of the triacylglycerols (TAGs) of the seeds of ripe fruit of the sea buckthorn of the Dar Katuni, Maslichnaya, and Shcherbinka-1 varieties have been investigated with the aid of lipase hydrolysis. The amounts of fatty acid (FA) residues in the 2-positions of the TAGs, the concentrations of the FAs in the 1,3-positions, the position-type and position-species compositions of the TAGs, and also the values of the enrichment factors (EFs) and inclusion-selectivity factors (SFs) of the unsaturated FAs in the positions of the TAGs were determined. As in the seeds of the majority of other plants, in the sea buckthorn it is mainly the unsaturated FAs that are concentrated in the 2-positions of the TAGs (mol. %): linoleic (L, 47-49.6); linolenic (Le, 32.4-36.9), and oleic (O, 12.8-18.5). The EF and SF values for the O, L, and Le acids in the TAGs of the Dar Katuni variety were, respectively, 1.11, 1.21, and 0.97, and 1.00, 1.10, and 0.88. In contrast to the TAGs of the flesh of sea buckthorn fruit, palmitoleic acid is a minor component of the TAGs of the seeds (0.6-0.9 mol.%). Among the types of TAGs, trisaturated (72.4-76.2) and monosaturated-diunsaturated (22.1-25.3) predominate. According to calculation, the seeds may contain from 57 (Maslichnaya) to 98 (Shcherbinika-1) position-species of TAGs with concentrations $\geq$0.01 mol.%. The predominating species among them are LLLe (11.5-14.1), LLeLe (7.8-10.5), LeLLe (5.5-7.2), and LLL (6.0-6.9). The composition and structure of the TAGs of the seeds have also been reliably established with the aid of a mathematical method based on Litchfield's empirical formulas for determining the amounts of FAs in the 2-positions of TAGs.

Many publications have been devoted to the study of the triacylglycerols (TAGs) of the flesh of the fruit of *Hippophaë rhamnoides* L. (common sea buckthorn) [1-5]. However, the structure of the TAGs of the seeds of this valuable medicinal plant have scarcely been studied and there is only a single publication on this question [4].

We studied the composition and structure of the TAGs of ripe sea buckthorn seeds. Plants of three varieties grown in the Gor'kii province were investigated. The fatty acid (FA) composition of these TAGs ($[A]_{1,2,3}$) and their absolute amounts in the seeds have been determined previously [5].

In the first stage of the work, the TAGs were subjected to the action of pancreatic lipase, and then the FA composition of the 2-monoacylglycerols ($[A]_2$) isolated from the products of the hydrolysis of the TAGs were determined [6]. The results of the investigation of the positional distribution of the FA residues in the TAGs are given in Table 1. It can be seen that the 2-positions of the TAGs were esterified almost exclusively with unsaturated FAs — linoleic (L), linolenic (Le), and oleic (O). The linoleic acid that was the predominating residue in the initial TAGs was also predominating in the 2-positions, while oleic and linolenic acids were distributed between the 2- and 1,3-positions of the TAGs almost equally. The only exception was the TAGs of the Dar Katuni variety, which were distinguished by a somewhat higher content of the $C_{18:1}$ acid: in this case, the concentration of oleic acid residues in the 2-positions was higher than in the end-positions. Saturated acids — palmitic (P) and stearic (S) — were present in the TAGs of the seeds in small amounts concentrated almost totally in the 1,3-positions of the TAGs.

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TABLE 1. Fatty Acid Compositions of the 1,2,3-, the 2-, and 1,3-positions of the Seed TAGs*

Variety of sea buckthorn	Position of the FA in the TAG	Amounts of the acids, mole %						
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	[S]	[U]
Dar Katuni	1, 2, 3	7,1±0,5	2,7±0,6	16,6±1,1	39,5±1,2	33,5±0,9	9,8	90,2
	2	0,9±1,1	0,0	18,5±2,6	47,7±0,9	32,4±1,2	0,9	99,1
	1,3	10,2±1,2	4,1±0,6	15,7±2,8	35,4±1,5	34,0±1,5	14,3	85,7
	2 calc.**	0,5	0,0	18,2	48,1	32,6	0,5	99,5
Maslichnaya	1, 2, 3	5,8±0,6	2,7±0,4	12,5±1,0	41,3±1,0	37,7±1,5	8,5	91,5
	2	0,7±0,6	0,0	12,8±0,6	49,6±1,3	36,9±1,5	0,7	99,3
	1,3	8,4±0,8	4,0±0,4	12,3±1,2	37,2±1,6	38,1±2,1	12,4	87,6
	2 calc.	0,4	0,0	13,6	50,0	36,0	0,4	99,6
Shcherbinka-1	1, 2, 3	7,2±0,4	2,5±1,0	15,1±0,2	39,5±1,5	34,6±0,3	9,9	90,1
	2	0,9±0,4	0,0	15,7±1,0	48,4±1,2	34,3±1,2	0,9	99,1
	1,3	10,4±0,6	3,8±1,0	14,8±1,0	35,1±1,9	34,7±1,2	14,5	85,5
	2 calc.	0,4	0,0	16,5	48,5	33,7	0,4	99,6

*The values of $\bar{X} \pm E$ are given; [S] and [U] are the total amounts of saturated and unsaturated FAs, respectively. The TAGs of the Shcherbinka-1 variety also contained myristic acid ($C_{14:0}$, 0.2%) and palmitoleic acid ($C_{16:1}$, 0.9%), and the TAGs of the Dar Katuni variety the $C_{16:1}$ acid (0.6%).

†2 calc. — the amount of the FA in the 2-position of a TAG calculated on the FA composition of the initial TAGs $[A]_{1,2,3}$ (see text).

The results that we obtained (Table 1) do not agree with those published previously by other authors [4]. The wild sea buckthorn from the flood plains of the R. Zeravshan (Uzbekistan) that they investigated accumulated in the seeds TAGs close in FA composition to the TAGs of the Dar Katuni variety. However, in the 2-positions of these TAGs, according to the authors' statement, the amount of residues of the main unsaturated FAs — linoleic and linolenic — was lower than in the 1,3-positions, and the palmitic acid residues were concentrated completely in the 2-position. Such a nature of the distribution of the FAs contradicts the overwhelming majority of modern results on the structure of the reserve TAGs of plants [7-11].

We have found no other studies of the structure of the TAGs of sea buckthorn seeds.

To characterize the affinity of each type of unsaturated FA for the 2-position of the TAGs of any oil, use is made of the enrichment factor (EF), which is equal to the ratio of the concentrations of a given species of acid in the 2- and the 1,2,3-positions of the TAGs, and, for a comparative determination of this affinity in TAGs with different degrees of unsaturation, from different vegetable oils, the selectivity factor SF = $EF/([U]_2/[U]_{1,2,3})$ [8, 11]. The EF and SF values for oleic, linoleic, and linolenic acids are given in Table 2. It can be seen that the highest values of these factors characteristic for linoleic acid (L > O > Le). In the Maslichnaya and Shcherbinka-1 varieties the enrichment factors for oleic and linolenic acids were close to unity, which reflects the distribution of these FAs more fully between the 2 and the 1,3 positions of the TAGs. It must also be noted that, with respect to their SF values for the $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ acids, these two varieties were identical but they differed from the Dar Katuni variety.

It is interesting to compare the SF values obtained with corresponding literature figures for other species of plants. For all the plant oils studied, isolated both from seeds and from fruit flesh and containing oleic and linoleic acids in the TAGs, the value of this factor for the $C_{18:1}$ acid amounts to 0.8-1.0, regardless of its concentration in the TAGs, while for linolenic acid in practically all cases SF $\geq$ 1.0 [8, 11]. In TAGs containing not only oleic and linoleic acids but also linolenic acid, the value of the SF for the $C_{18:3}$ acid, amounting to 0.7-1.1, is usually lower than those for the $C_{18:2}$ and $C_{18:1}$ acids [8]. Thus, the values of the selectivity factor given in Table 2 agree with those found previously for the TAGs of the seeds of other oil plants.

On the basis of the values of $[A]_2$ and $[A]_{1,2}$ (Table 1) we calculated the position-type (PTC) and position-species (PSC) compositions of the TAGs [11]. The results of these calculations are given in Tables 3 and 4. It can be seen that the seeds contained no S_3 and the concentration of S_2U in the TAGs did not exceed 2.3 mole % (Table 3). The main TAGs of the seeds

TABLE 2. Enrichment Factors (EF) and Selectivity Factors (SF) of the Inclusion of Unsaturated FAs in the 2-Positions of the TAGs of the Seeds

FA species	EF			SF		
	Dar Katuni	Maslichnaya	Shcherbinka	Dar Katuni	Maslichnaya	Shcherbinka
$C_{18:1}$	1,11	1,02	1,01	1,00	0,94	0,94
$C_{18:2}$	1,21	1,20	1,22	1,10	1,11	1,11
$C_{18:3}$	0,97	0,98	0,99	0,88	0,90	0,90

TABLE 3. Position-Type Composition of the TAGs of Seeds (mole %)*

Position types of TAGs	Dar Katuni		Maslichnaya		Shcherbinka-1	
	1	2	1	2	1	2
SSS	0,0	0,0	0,0	0 0	0,0	0,0
S_2U	2,2	2,2	1,7	1,7	2,3	2,2
SSU	0,2	0,1	0,2	0,1	0,2	0,1
SUS	2,0	2,1	1,5	1,6	2,1	2,1
SU_2	25,0	24,9	22,1	22,2	25,3	25,2
SUU	24,3	24,5	21,6	21,9	24,6	24,8
USU	0,7	0,4	0,5	0,3	0,7	0,4
UUU	72,8	72,9	76,2	76,1	72,4	72,6

*Methods of calculation: 1) from the figures for $[S]_2$, $[U]_2$, $[S]_{1,3}$, and $[U]_{1,3}$ from Table 1; 2) from the figures for $[S]_{2\,calc}$ and $[U]_{2\,calc}$ from Table 1.

were U_3 and SUU. The Maslichnaya variety, characterized by a higher concentration of unsaturated FAs in the TAGs, differed from the other varieties by a higher level of U_3 and a smaller amount of SU_2 in the seeds.

In the calculation of the PSCs of the TAGs it was found that the sea buckthorn seeds of the Maslichnaya, Dar Katuni. and Shcherbinka-1 varieties can accumulate 57, 84, and 98, respectively, species of TAGs having levels of $\geq$0.01 mole %, and of them 43, 52, and 56 species with concentrations $\geq$0.1 mole %. The compositions of the main TAGs (26 species with levels in the Dar Katuni variety of $\geq$1.0 mole %) are given in Table 4. It can be seen that among the TAGs di- and monoacid species including linoleic and linolenic acids — LLLe, LLeLe, LeLLe, L_3, Le_3, and LLeL — predominated. In the Maslichnaya seeds, the position species of TAGs that have been mentioned accumulated in larger amounts than in the Dar Katuni and Shcherbinka-1 varieties, which differed little from one another with respect to the amounts of their main TAGs.

TAGs with concentrations of from 0.1 to 1.0 mole % are represented mainly by position species including stearic and oleic acid residues.

At the present time, it is known that practically all TAGs of plant origin are constructed according to the same rule, which consists in the fact that their 2-positions are esterified almost exclusively by unsaturated FAs [7-11]. Making use of this rule, Litchfield derived empirical formulas permitting the calculation of the amounts of individual species of FAs in the 2-positions of TAGs ($[A]_{2\,calc}$) solely from the FA compositions of the TAGs ($[A]_{1,2,3}$) without the performance of lipase hydrolysis [9, 10]. The figures given above (see Tables 1-4) show that the nature of the structure of the reserve TAGs of sea buckthorn seeds is no different from that of other smaller plants. We therefore decided to determine whether Litchfield's formulas were applicable for determining the composition and structure of the TAGs of the seeds of this plant by calculation from the $[A]_{1,2,3}$ figures in Table 1.

The results of a preliminary calculation showed that the values of $[P]_{2\,calc}$, $[O]_{2\,calc}$, and $[Le]_{2\,calc}$, were close to the corresponding values for $[A]_2$ found with the aid of lipase hydrolysis. Stearic acid (S) and palmitoleic acid (Po) are minor components of the TAGs of the majority of plant oils [7-11], and therefore Litchfield gives no formulas for them. In the present work we started from the assumption that for these minor FAs $[S]_{2\,calc}$ is equal to zero and $[Po]_{2\,calc} = [Po]_{1,2,3}$, since such an assumption has practically no effect on the results of calculations. Finally, for the main unsaturated acid of the TAGs — linoleic acid —

TABLE 4. Calculated Position-species Compositions of the TAGs of the Seeds (mol. %)*

Position species of TAGs	Variety of sea buckthorn					
	Dar Katuni		Maslichnaya		Shcherbinka-1	
	1	2	1	2	1	2
L—L—Le	11.5	11,4	14.1	14,2	11,8	11,9
L—Le—Le	7,8	7,7	10.5	10,3	8.4	8,3
L—L—L	6,0	5,9	6,9	6,8	6.0	5,9
Le—L—Le	5,5	5,6	7,2	7,4	5,8	5,9
O—L—L	5,3	5,3	4.5	4,4	5.0	4.9
O—L—Le	5.1	5,2	4.7	4,6	5,0	4.9
L—O—Le	4,5	4,3	3,6	3,9	3,8	4,0
L—Le—L	4.1	4,0	5,1	4.9	4,2	4,1
Le—Le—Le	3,7	3,8	5.4	5.3	4.1	4,1
O—Le—L	3.6	3.6	3,4	3,[illegible]	3,6	3,4
O—Le—Le	3.5	3.5	3.5	3,3	3.5	3,4
P—L—L	3,4	3,5	3,1	3,1	3,5	3,6
P—L—Le	3,3	3,4	3,2	3.3	3.5	3.6
P—Le—L	2,3	2,4	2.3	2.3	2.5	2,5
L—O—L	2,3	2,2	1,8	1,9	1,9	2,0
P—Le—Le	2,3	2,3	2,4	2.4	2.5	2,5
Le—O—Le	2,1	2,1	1.9	2,0	1,9	2,0
O—O—L	2,1	2,0	1,2	1,2	1,6	1.7
O—O—Le	2.0	2,0	1,2	1,3	1.6	1,7
P—L—O	1,5	1,6	1,0	1,0	1,5	1,5
S—L—L	1,4	1,4	1.5	1.5	1.3	1.3
P—O—L	1,3	1,3	0,8	0,9	1.2	1.2
S—L—Le	1,3	1,3	1,5	1.5	1.3	1,3
P—O—Le	1,3	1,3	0.8	0.9	1,1	1,2
O—L—O	1.2	1,2	0,8	0,7	1.1	1,0
P—Le—O	1,0	1,1	0.8	0,7	1,0	1,0
Σ of the main TAGs	89,4	89,4	93,2	93,0	88,7	88,9
Σ of the minor TAGs†	10,6	10.6	6,8	7,0	11.3	11,1

*Methods of calculation: 1) from the figures for $[A]_2$ and $[A]_{1,3}$ from Table 1; 2) from the figures for $[A]_{2\,calc}$ from Table 1.
†Minor TAGs are the position-species of TAGs present in the mixture of TAGs from the seeds of the Dar Katuni variety in amounts of 0.01-1.00 mole %.

results of calculations from Litchfield's formula were high, and therefore we determined $[L]_{2\,calc}$ from the difference: $[L]_{2\,calc} = 100 - ([P]_{2\,calc} + [Po]_{2\,calc} + [O]_{2\,calc} + [Le]_{2\,calc})$.

All the values of $[A]_{2\,calc}$ obtained by calculation are also given in Table 1. It can be seen that they are close to the corresponding values found by the enzymatic method. We therefore used them for calculating the PTCs and PSCs of the sea buckthorn TAGs. The results of these calculations are given in Tables 3 and 4. It can be seen that they correspond satisfactorily to those found from $[A]_2$.

Thus, to characterize the composition and structure of the TAGs of seeds it is sufficient to have available only information on their FA compositions. As is well known, these facts are readily susceptible to treatment on a computer [12, 13]. Therefore the mathematical method used in the present work opens up broad possibilities for the rapid performance of large numbers of analyses of the TAG compositions of the seeds of high-quality sea buckthorn varieties.

EXPERIMENTAL

The object of investigation consisted of ripe seeds of the Dar Katuni, Maslichnaya, and Shcherbinka-1 varieties of the common sea buckthorn grown at the Novinka experimental base of the Gor'kii Agricultural Institute in 1984. The fixation of the fruit, the isolation of the seeds, and the extraction of the total lipids from them were carried out as described previously [14].

Isolation of the TAGs and Their Hydrolysis. The TAGs were isolated from the extract by preparative TLC in a 0.5 × 200 × 200 mm layer of silica gel LSL_{254} 5/40 μm. On each plate was deposited 30 mg of lipids, and the mobile phase was hexane—diethyl ether (7:3, by volume). The edge of the layer was sprayed with a 0.01% aqueous solution of Rhodamine 6G, and the zone of the TAGs (R_f 0.7) was detected in UV light. The TAGs were eluted from the adsorbent with chloroform, and their FA compositions mole % were determined by the GLC method [15].

The TAGs (10 mg) were treated with pancreatic lipase (type B, Olaine chemical reagents factory, 4 mg in 2 ml of 0.01 M L-histidine solution) in 1 M Tris-HCl buffer (7 ml, pH 8.2) with the addition of a 1 N solution of NaCl (10 ml) and a 0.11 M solution of $CaCl_2$ (2 ml) at 40°C with vigorous stirring for 10 min. The monoacylglycerols (MAGs) were isolated from the TAG hydrolysis products and the quantitative composition of their FAs was determined.

Calculation of the Composition of the TAGs. Making use of the experimentally obtained FA compositions of the TAGs and MAGs and known equations, we calculated the composition of the FAs in the 1,3-positions of the TAGs, the values of the enrichment and selectivity factors, and also the PTCs and TSCs of the TAGs [8, 11].

To determine the composition and structure of the TAGs solely from the FA compositions (mathematical method), the amounts of the FA residues in the 2-positions of the TAGs were calculated from Litchfield's formulas [9, 10], and the PTCs and PSCs of the TAGs were calculated from these figures (see above).

The experiment was carried out in triplicate. The statistical treatment of the results (calculation of $\bar{X}$ — the arithmetic mean of three determinations — and of E — the confidence interval) was carried out by handbook methods [16].

SUMMARY

The 2-positions of the TAGs of the seeds of ripe fruit of the common sea buckthorn, Dar Katuni, Maslichnaya, and Shcherbinka-1 varieties, contain almost exclusively residues of unsaturated FAs: (mole %) linoleic (47.7-49.6), linolenic (32.4-36.9), and oleic (12.8-18.5). With respect to the nature of the distribution of the unsaturated and saturated FA residues between the 2- and the 1,3-positions of the TAGs, the sea buckthorn is similar to other oil plants. According to calculations, the seeds may contain from 57 to 98 position species of TAGs, among which LLLe (11.5-14.1), LLeLe (7.8-10.5), LeLLe (5.5-7.2), and LLL (6.0-6.9) predominate. The compositions and structures of the TAGs of sea buckthorn of the varieties investigated can be determined reliably by a mathematical method based only on their FA compositions.

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