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TRIGLYCERIDE COMPOSITION OF SEED OILS FROM CERTAIN PLANTS

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The type compositions of triglycerides (TAG) from seed oils of various plants were presented. The possibility of using a statistical model to estimate the ratio of TAG types formed by oleic, linoleic, and α-linolenic acids was discussed. The greatest deviations from the statistical model were observed in oils for which the TAG contained γ-linolenic, α-eleostearic, and ranunculeic acids.

Key words: HPLC, triglycerides, seed oils, type composition, statistical model for distribution of fatty-acid residues.

Much information has been accumulated on the type and positional composition of triglycerides (TAG). Enzymatic hydrolysis of total TAG [1] can determine the average degree of substitution of the β-position in TAG by various fatty acids. It was concluded [2] that petroselic acid tends not to occupy the middle position in TAG from Umbelliferae seed oils. It was noted [3] that the selectivity factor for acylating the secondary hydroxyl decreases for most plants in the order L > O > Le (L, O, and Le are linoleic, oleic, and linolenic acids, respectively) whereas this order changes for seed oils from various Labiatae plants. Certain assumptions of a theoretical nature are needed for a subsequent recalculation of the type composition. Even attempts to use the 1-, 2-, 3-random model do not always give satisfactory results according to stereospecific analysis [4]. Therefore, reliable parameters can be obtained only by preparative isolation of pure TAG types in the first step with subsequent study of the saponification products of each specimen [5].

Results from investigations of the type composition of TAG formed by oleic, linoleic, and octadecatrienoic acids are presented and discussed in this article.

The positional-type composition of TAG from *Hippophae rhamnoides* L. (seabuckthorn) seed oil has been published [6]. The sum of the fractions of positional isomers of a single type composition is equal to the fraction of TAG types that was determined assuming an equally probable statistical distribution of acids composing this oil (Table 1). According to our calculations, the same is valid for Labiatae seed oils [3].

An investigation of seed oil from seabuckthorn (mixture of varieties) grown in Belgorod region found that the TAG composition determined by HPLC does not fully correspond to the data from statistical calculations (Table 1).

Figure 1 shows the chromatogram of the acetone extract of *Daucus sativus* (Hoffm.) Roehl carrot seeds. The incremental approach [7] enables the TAG type composition to be determined. The increment Δ(O→Pe) is just over half of Δ(O→P) (Pe and P are petroselic and palmitic acids, respectively). This complicates the separation of all components. A gradual increase of TAG content in the order $O_3 < O_2Pe < (O_2P + OPe_2) < (POeP + Pe_3)$ is evident in the TAG group with an equivalent C number [8] of 48. Unfortunately, strict quantitative relationships in this instance could not be found. However, a calculation can be made if the palmitic-containing components are ignored.

The relationships below should be observed for a statistical distribution of petroselic and oleic acids within this TAG group:

$$\frac{S(O_2Pe)}{S(OPe_2)} = \frac{3p^3(O)}{3p^2(O)p(Pe)}; \quad \frac{S(OPe_2)}{S(Pe_2)} = \frac{3p(O)p^2(Pe)}{p^3(Pe)}; \quad \frac{S(O_2Pe)S(Pe_3)}{S(OPe_2)S(Pe_2)} = \frac{1}{3},$$

where p(i) is the mole fraction of acid i and S(j) is the corrected peak area of the j-th TAG.

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TABLE 1. Experimental and Calculated Compositions (α, mol %) of Principal Triglycerides from *Hippophae rhamnoides* L. Seed Oil

Triglyceride types	Chuisk		Liter. data [6], seabuckthorn variety					
			Oil		Shcherbinka-1		Gift of Katuna	
	α (stat.)	α (exp.)	α (stat.)	Σ	α (stat.)	Σ	α (stat.)	Σ
Le_3	2.2±0.2	2.9	5.4	5.4	4.1	4.2	3.8	3.7
Le_2L	10.1±0.3	8.7	17.6	17.7	14.2	14.0	13.3	13.3
LeL_2	15.1±0.1	12.6	19.3	19.2	16.2	16.0	15.7	15.6
Le_2O	5.0±0.2	3.1	5.3	5.4	5.4	5.4	5.6	5.6
Le_2P	1.6±0.1	1.4	2.5	2.4	2.6	2.5	2.4	2.3
L_3	7.5±0.2	6.9	7.0	6.9	6.2	6.0	6.2	6.0
LeLO	15.0±0.1	13.2	11.7	11.7	12.4	12.0	13.2	13.2
LeLP	4.8±0.2	5.4	5.4	5.4	5.9	6.0	5.6	5.6
Le_2O	0.5±0.1	0	1.2	1.5	1.3	1.3	1.1	1.4
L_2O	11.5±0.5	12.3	6.4	6.3	7.1	6.9	7.8	7.6
LO_2	5.6±0.2	8.2	1.9	2.0	2.7	1.6	3.3	3.3

Σ of fractions of positional isomers.

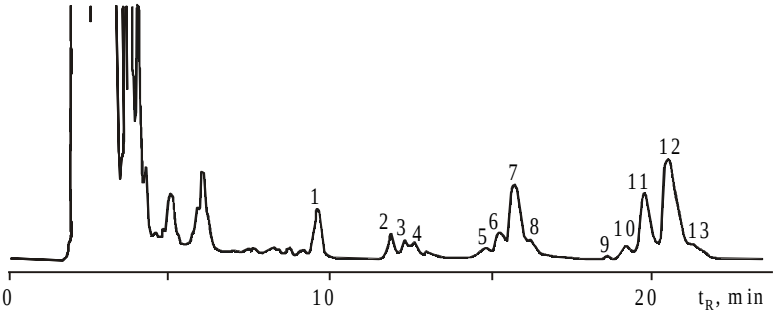

Fig. 1. Chromatogram of acetone extract of *Daucus sativus* (Hoffm.) Roehl. seeds. Triglyceride peaks: L_3 (1), L_2O (2), L_2Pe (3), L_2P (4), LO_2 (5), LOPe (6), LPe_2 + LOP (7), LPeP (8), O_3 (9), O_2Pe (10), OPe_2 + O_2P (11), Pe_3 + OPeP (12), Pe_2P (13). Eluent: acetone:acetonitrile (1:3), 1 mL/min.

The experimentally determined ratios are 0.33, 0.34, and 0.32 for seed oils of *Daucus sativus*, *Carium carvi* L., and *Coriandrum sativum* L., respectively. If the tendency (probability) of petroselic acid to acylate the secondary hydroxyl were much lower than that of oleic acid and this affected the TAG type composition, then this ratio would be less than 1/3.

In the TAG group with an equivalent C number of 46, the increase in TAG content in the order LO_2 < LOPe < (LOP + LPe_2) is even more significant. For analogous assumptions:

$$\frac{S(LO_2)}{S(LOPe)} = \frac{3p(L)p^2(O)}{6p(L)p(O)p(Pe)}; \quad \frac{S(LOPe)}{S(LPe_2)} = \frac{6p(L)p(O)p(Pe)}{3p(L)p^2(Pe)}; \quad \frac{S(LO_2)S(LPe_2)}{S((LOPe)S(LPe)} = 0.25$$

However, the experimental values were even greater than unity (1.1 for carrot seed oil and 1.6 for coriander). The first TAG pair (LO_2 and LOPe) makes almost the whole contribution to the increase because the probability relationship below is observed for the two TAG pairs (OPe_2 and Pe_3, and LOPe and LPe_2):

$$\frac{S(OPe_2)}{S(Pe_3)} = \frac{3p(O)p^2(Pe)}{p^3(Pe)}; \quad \frac{S(LOPe)}{S(LPe_2)} = \frac{6p(L)p(O)p(Pe)}{3p(L)p^2(Pe)}; \quad \frac{S(OPe_2)S(LPe_2)}{S(Pe_3)S(LOPe)} = \frac{3}{2};$$

with an experimental value of 1.7 for carrot seed oil.

TABLE 2. Triglyceride Types Formed by O and L Acids and Q Factor for Plant Oils

Specimen	Experimental mole fraction (ω, %) and Q factor of triglyceride type								p (L)
	L_3		L_2O		LO_2		O_3		
	ω	Q	ω	Q	ω	Q	ω	Q	
Capsicum annum L.	69.8	1.04	23.7	0.83	5.90	1.45	0.63	3.27	0.875
Adonis aestivalis L.	71.7	1.08	19.4	0.66	7.43	1.72	1.46	6.84	0.871
Taraxacum officinale L.	60.9	1.03	30.5	0.89	7.84	1.19	0.79	1.87	0.838
Solana melongena L.	57.0	1.06	30.5	0.82	12.2	1.44	0.35	0.54	0.814
Ambrosia trifida L.	45.9	1.00	40.7	1.00	12.4	1.02	1.08	0.90	0.771
Lycopersicom esculentum Mill.	46.4	1.08	36.7	0.87	13.6	0.99	3.33	2.23	0.754
Echinacea purpurea L.	45.2	1.08	36.8	0.87	15.3	1.07	2.74	1.71	0.748
Pumpkin oil	35.6	1.01	42.5	0.97	19.8	1.08	2.06	0.81	0.706
Corn oil	32.7	1.15	40.0	0.90	19.0	0.82	8.18	2.04	0.658
Cucurbita pepo L. *var giraumons* Duch.	26.4	1.14	38.9	0.89	27.2	0.99	7.51	1.31	0.614
Pyrus communis L.	26.1	1.36	35.5	0.84	23.8	0.77	14.5	1.93	0.578
Beta vulgaris L.	13.1	0.97	41.6	1.08	31.2	0.85	14.1	1.22	0.512
Sesamum indicum L.	17.3	1.34	33.9	0.89	32.1	0.87	16.7	1.38	0.506
Viburnum opulus L.	14.9	1.32	32.4	0.90	35.4	0.92	17.3	1.25	0.483
Prunus tomentosa (Thunb.) Wall.	5.36	0.90	32.0	1.14	37.2	0.86	25.4	1.13	0.391
Pistacia L.sp.	7.89	1.87	22.3	0.95	36.2	0.82	33.6	1.21	0.348
Amygdalus L. sp.	4.68	1.31	24.4	1.12	36.1	0.81	34.8	1.16	0.330
Prunus domestica L.	8.05	2.72	17.6	0.89	33.5	0.76	40.9	1.24	0.309
Corylus L. sp.	1.88	9.61	5.82	1.42	20.2	0.70	72.1	1.08	0.125
O = sum of 18:1 (*cis*-6 and *cis*-9)									
Carum carvi L.	11.7	1.84	21.0	0.73	42.7	0.99	24.6	1.14	0.399
Anthriscus cerefolium (L.) Hoffm.	0.67	0.22	9.64	0.46	75.1	1.69	14.6	0.47	0.321
Daucus sativus (Hoffm.) Roehl.	7.84	4.02	14.5	0.91	28.2	0.65	49.4	1.27	0.270
Anisum vulgare Gaertn.	1.83	1.20	14.8	1.07	39.2	0.93	44.1	1.04	0.248
Pastinaka sativa L.	1.82	1.88	9.53	0.89	39.4	1.00	49.3	1.01	0.213

The approach given below can be conveniently used to evaluate using HPLC the adequacy of the statistical calculation of the type composition of TAG formed by a large number of fatty acids owing to the presence of problematic TAG pairs.

We selected the group of TAG from the specimen that are formed by two types of acids (A and B): A_3, A_2B, AB_2, and B_3 with mole fractions $\omega(A_3)$, $\omega(A_2B)$, $\omega(AB_2)$, $\omega(B_3)$ (normalized to 100%). We calculated for this group the relative fraction of component acids:

$$p_A = [3\omega(A_3)+2\omega(A_2B)+\omega(AB_2)]/\Sigma; \quad p_B = [3\omega(B_3)+2\omega(AB_2)+\omega(A_2B)]/\Sigma; \quad \Sigma = 3[\omega(A_3)+\omega(A_2B)+\omega(AB_2)+\omega(B_3)].$$

Finally, we found the relationships:

$$Q(A_3) = \omega(A_3)/p_A^3; \quad Q(A_2B) = \omega(A_2B)/3p_A^2p_B; \quad Q(AB_2) = \omega(AB_2)/3p_Ap_B^2; \quad Q(B3) = \omega(B_3)/p_B^3.$$

It is easy to convince oneself that this approach is rigid (for a valid statistical model). In this instance an experimental value less than unity (up to a value slightly less than 0.66) will correspond to a lower (compared with statistical) probability of forming the sum of positional isomers of the corresponding TAG type composition (without a tendency, for example, of acid A to occupy the 2-position).

TABLE 3. Triglyceride Types Formed by L and Le Acids and Q Factor for Plant Oils

Specimen	Experimental mole fraction (ω, %) and Q factor of triglyceride type								p (Le)
	Le_3		Le_2L		LeL_2		L_3		
	ω	Q	ω	Q	ω	Q	ω	Q	
Le =9,12,15-octadecatrienoic acid									
Soy oil	<0.05	-	5.11	1.40	25.1	0.91	69.8	1.02	0.118
Osmium basilicum L.	46.7	1.15	34.1	0.80	13.8	0.92	5.39	3.08	0.740
Hippophae rhamnoides L.	10.1	1.30	29.8	0.95	37.9	0.90	22.2	1.17	0.426
Linum usitatissimum L.	55.0	1.04	32.6	0.87	12.1	1.36	0.24	0.34	0.808
Rosa canina L.	3.99	1.87	15.6	0.93	40.2	0.92	40.2	1.07	0.278
Hissopus officinalis L.	51.9	1.10	32.0	0.80	14.1	1.23	2.01	1.87	0.779
Phaseoluns vulgaris L.	34.1	1.08	40.8	0.92	20.1	0.97	4.92	1.51	0.681
Le = 6,9,12-octadecatrienoic acid									
Borago officinais L.	1.4	0.34	28.6	1.23	41.8	0.94	28.1	1	0.344
Le = 5,9,12-octadecatrienoic acid									
Aquilegia vulgaris L.	6.86	0.32	67.9	1.57	23.4	0.81	1.82	0.28	0.599
Pinus sibirica Du Tour	0.33	0.22	3.34	0.24	66.4	1.58	30.0	0.70	0.247
Le = 9,11,13-octadecatrienoic acid									
Cerasus vulgaris Mill.	0.64	0.29	11.1	0.66	59.9	1.38	28.3	0.76	0.280
Prunus padus L.	0.54	0.34	7.15	0.50	59.5	1.41	32.8	0.78	0.252

Table 2 lists the experimental data for the L—O pair. The equally probable distribution does not correspond to any of the studied oils. However, the amount of homotriglycerides (L_3 and O_3) in most instances is noticeably greater than for the equally probable distribution. The situation is more complicated for mixed TAG: if $\omega(A_2B) > \omega(AB_2)$, then the content of the first TAG is less than for the equally probably distribution. For the opposite case, the content of the second TAG is lower. A decrease of the fraction relative to the equally probable distribution is typical for both TAG if their contents are about equal.

The situation for the Le—L pair (Le = α-linolenic acid in this instance) is analogous to that examined above. However, a substantially different distribution is obtained for the other octadecatrienoic acids (Table 3). For γ-linolenic (in *Borago officinalis* L. seed oil) and ranunculeic (in *Aquilegia vulgaris* L.) acids, the Le_2L TAG type is most preferred. For trienoic acids of *Pinus sibirica* Du Tour and *Cerasus vulgaris* Mill. seed oils, LeL_2 type TAG are especially abundant.

The results at first glance contradict the statistical model. However, the fatty-acid composition of the oils is not constant with time. For example, petroselic components appear in dill oil (and begin to dominate the composition) as the seeds ripen [9]. This leads to the situation that can be modeled as follows. If at the initial time point the plant oil consists of acid A (70%) and B (30%), then the TAG positional composition for a statistically equally probable distribution is A_3, 34.3%, A_2B 44.1%, AB_2 18.9%, and B_3 2.7%. Let the second half of the TAG during ripening be formed from these same acids but in a different ratio: A (30%) and B (70%). For the new TAG portion with a statistical distribution, we obtain A_3 2.7%, A_2B 18.9%, AB_2 44.1%, and B_3 34.3%. As a result, the type composition of the oil will be 34.3, 44.1, 18.9, and 2.7%, respectively, whereas the Q ratios are 1.48, 0.84, 0.84, and 1.48. The Q ratios are 1.64, 0.86, 0.86, and 1.27, respectively, for a relative fraction of 30% for the first TAG type.

Thus, the variation of the data from the statistical distribution may be a result of the changing fatty-acid composition of the oil during ripening with a fully statistical distribution at each timepoint of TAG synthesis.

The nonstatistical TAG distribution is evident in several aforementioned instances and may be interesting for studies using lipase. Even for seed oil from Umbelliferae plants, a nonstatistical distribution can be expected to increase significantly the fraction of TAG with an equivalent number of 46 C atoms if the content of linoleic acid is increased (if the 2-position is preferentially occupied by linoleic acid). In fact, the fraction of such TAG for *Anthriscus cerefolium* (L.) Hoffm. is unusually high (Table 2).

EXPERIMENTAL

Seeds of plants grown in Belgorod region and acquired at retail outlets (including commercial soy, corn, pumpkin, and pine oils) were used in the experiments.

The triglyceride composition of the oils obtained by acetone extraction was studied as before [10]. A correction factor that was inversely proportional to the difference of the calculated values of the indices of refraction of the TAG types (calculated using the ACD Labs program) and the mobile phase was applied in the calculations.

The data indicate that the positional composition of the acids (oleic, linoleic, and α-linolenic) is not absolutely accurate. In several instances the TAG type composition may be statistical or close to it. Deviations of the type composition from statistical may be due to changes in the fatty-acid composition as the seeds ripen. If certain positional isomers form in mixed TAG, then these ratios are a consequence of the specifics of the synthesis sites and may not relate to the TAG type composition.

It was shown that the deviation of the type composition from the statistical one is large for several oils. Therefore, the data should be recalculated after enzymatic hydrolysis taking into account at least the type composition determined by an independent method. This can give different ratios of positional isomers. For example, one certain positional structure and not ratios in a mixture of positional isomers, which could not be separated by the method we used, was found for several TAG isolated by preparative HPLC [5].

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