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Changes in Triacylglycerol Composition during Ripening of Sea Buckthorn (*Hippophaë rhamnoides* L.) Seeds

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Changes in the quantitative composition of triacylglycerols (TAGs) in maturing sea buckthorn (*Hippophaë rhamnoides* L.) seeds were determined by lipase hydrolysis. As a whole, the rate of synthesis of separate TAG classes increased in proportion to both their unsaturation and relative content (weight percent) in total TAGs. Up to the 80th day of maturation, the formation of triunsaturated TAGs was predominant. Subsequently, at the terminal stage of seed ripening, the absolute content (in nanomoles per seed) of a major group of these TAGs containing linolenic and linoleic acyls decreased by ~7%, and the increase in the total TAG content was mainly due to the synthesis of TAG molecules including stearic and palmitic acyls in the *rac*-1,3 positions, as well as those containing oleate in the *sn*-2 position. At each maturation stage, the composition of the TAGs formed was controlled both by the composition of fatty acids available for TAG synthesis and by the rate of incorporation of a particular fatty acid into the *sn*-2 position of the TAGs.

KEYWORDS: Sea buckthorn; *Hippophaë rhamnoides* L.; seed maturation; fatty acids; triacylglycerols; lipase hydrolysis; *sn*-2 acylation of glycerol; positional-type composition; positional-species composition; kinetic constant of relative rate of biosynthesis

INTRODUCTION

Triacylglycerols (TAGs) differ from other major reserve substances of seeds in the fact that, in the course of fruit ripening, their quantitative, and frequently also their qualitative, composition changes considerably. Therefore, to solve one of the most important problems of modern lipidology, viz., the production of vegetable oils with a predetermined composition and the increase in their content in the seeds, it is necessary first to elucidate the pattern of these changes as a basis for the mechanism of reserve TAG biosynthesis (*1*). Nevertheless, in most cases, the TAGs of maturing seeds have been investigated only at the level of their fatty acid (FA) composition, and the changes in the molecular species composition of the TAGs themselves remain little explored (*2*).

As the object for determining these changes, sea buckthorn (*Hippophaë rhamnoides* L.) fruits are of special interest because they are characterized by two distinct types of oil accumulation localized in mesocarp and seeds and because, in many respects, they differ from the reserve organs of other plants as regards the patterns of TAG biosynthesis and metabolism (*3*).

Previously, changes in the quantitative TAG species composition in the course of sea buckthorn fruit maturation were determined solely in their mesocarp (*4*), and investigations of seed TAGs involved only the determination of their composition and structure in mature fruits (*5*), changes in their FA composition during growth (*3*), and the pattern of this composition in the ecotypes of sea buckthorn originating from different geographic locations (*6*). These investigations showed that seed TAGs are, in many respects, quite different from those formed in mesocarp (*3*, *5*−*7*).

These differences involve a higher rate of seed TAG biosynthesis and a shorter period of their accumulation (*3*). Moreover, these TAGs are characterized by a prevalence of C_{18} FA residues, and in this regard, they are different from the mesocarp TAGs, where C_{16} FA residues predominate (*3*, *5*−*7*). Finally, various ecotypes of sea buckthorn are similar to each other in the FA composition of seed TAGs, but can be considerably different as regards the mesocarp TAG composition and the mechanism of their biosynthesis (*6*). These differences between the seeds and mesocarp can be assumed to be caused by genetic factors, because, in all cases, seed embryos include equal portions of genes of both parents, whereas sea buckthorn mesocarp is a somatic organ with a solely maternal genotype (*3*).

Thus, a better understanding of direct causes of the differences observed and a more detailed outline of TAG biosynthesis in sea buckthorn fruits would be of considerable interest. To this end, it is necessary to determine the changes in the principal categories of the quantitative composition of TAGs not only in mesocarp (*8*) but also in the maturing seeds of these fruits. The objective of this work was to solve this problem. Changes in these indices in the developing embryos of sea buckthorn, as also in other plant species with oil-bearing mesocarps, were not investigated earlier (*3*).

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MATERIALS AND METHODS

As in our previous studies, plants of the cultivar Dar Katuni were used. Seed samples were collected on the *i*th day after pollination (DAP), where $i = 29$, 53, 80, and 107. The qualitative and quantitative TAG composition in each sample was determined essentially as described earlier (*3*−*6*), using a somewhat modified standard technique of lipase hydrolysis (*9*). Briefly, total TAGs (10 mg) isolated from the hexane extract of seeds by preparative TLC were treated with 4 mg of pancreatic lipase in 1 M Tris-HCl buffer (7 mL, pH 8.2) containing 10 mL of 1 N NaCl and 2 mL of 0.11 M $CaCl_2$ for 10 min at 40 °C with intense mixing. The FA content and composition of the resulting *sn*-2 monoacylglycerols and the initial TAGs were established by GLC with an internal standard. Data from these analyses were used for determining two categories of TAG composition by means of standard calculation methods (*9*).

The first of these categories is the quantitative composition of TAG positional types (weight percent of total TAGs), which includes possible triple combinations of total saturated FAs (S, considered from this viewpoint as a single FA) and total unsaturated FAs (U), that is, UUU, as well as positional isomers of the S_2U molecular type (SUS and SSU) and the SU_2 molecular type (SUU and USU). In this notation, the middle letter in a designation of a positional type indicates the FA residue located in the *sn*-2 position. Moreover, to determine the pattern of TAG positional-type formation in more detail, we calculated the contents of classes I and II of these types, which represent, respectively, the sums of the types containing S and U in the *sn*-2 position of TAGs.

The second category is the positional-species composition of TAGs (weight percent of total TAGs), which is analogous to the positional-type composition (see above), but instead of FA *types*, S and U, it involves individual FA molecular *species*, that is, palmitic (P, 16:0), stearic (St, 18:0), hexadecenoic (H, 16:1), oleic (O, 18:1), linoleic (L, 18:2), and linolenic (Le, 18:3) acids. This approach takes into account all possible isomers (positional species) within a particular TAG molecular species, for example, OLL and LOL within the OL_2 molecular species; PLLe, LPLe, and PLeL within the (PLLe) molecular species, etc. (*9*). In addition, for a more detailed investigation of the formation of TAG positional species, we calculated the contents of several *groups* of these species that differ from each other either in the qualitative FA composition or, in the case of identical FA compositions, in the nature of FA species located in the *sn*-2 position of the TAGs.

The dynamics of accumulation of TAG types and species were determined as the changes in both their concentration (weight percent of total TAGs) and absolute content (*P*, nanomoles of esterified FAs per seed) by the *i*th DAP; the *P* values were plotted on a logarithmic scale, because, during seed development, the differences between them could be as high as several orders of magnitude (*3*). Moreover, the concentrations of TAG types and species (weight percent of total TAGs) formed solely at a particular stage of seed development, viz., at 29−53 DAP (stage 1), 53−80 DAP (stage 2), and 80−107 DAP (stage 3), were also estimated (*8*).

The kinetics of TAG biosynthesis were determined as the average intensity of formation of TAG types, classes, species, and groups in the course of seed development. This parameter was assessed by the kinetic constant of the relative rate (k, day^{-1}) of this process (*3*, *4*) calculated by the equation

$$k = (k_{i,1} + k_{i,2} + ... + k_{i,n})(n - 2)^{-1} \quad (1)$$

where

$$k_i = \{\ln[(P - P_0)P_0^{-1}] - \ln[(P - P_i)P_i^{-1}]\}T_i^{-1} \quad (2)$$

is the rate constant at the *i*th DAP (see above); n is the number of maturation stages studied here, i.e., the number of seed samples of various ages; P, P_0, and P_i (nanomoles of esterified FAs per seed) are TAG absolute content values in mature seeds (107 DAP), green-fruit seeds (29 DAP), and seeds at the *i*th DAP, respectively; and T_i (days) is the number of days between the 0th (29 DAP) and *i*th DAP (*10*). Earlier, eq 2 was mainly used in chemical kinetics to characterize an autocatalytic monomolecular reaction, but at the same time, it was employed for a quantitative description of growth processes and reserve substance accumulation in plants (*11*, *12*).

Table 1. Dynamics and Kinetics of TAG Positional-Type Composition in Maturing Sea Buckthorn Seeds

positional types and classes of TAGs	composition of TAGs formed (wt %)							$k \times 10^3$ (day^{-1})
	by a given DAP				at a particular stage of maturation (DAP)			
	29	53	80	107	29–53	53–80	80–107	
SSU	0.7	0.4	0.1	0.2	0.4	0.1	1.2	156
USU	1.6	0.6	0.2	0.7	0.6	0.2	5.5	168
class I	**2.3**	**1.0**	**0.3**	**0.9**	**1.0**	**0.3**	**6.7**	**162**
SUS	3.3	6.7	1.3	2.0	6.7	1.1	8.7	222
SUU	29.5	38.0	20.5	24.3	38.0	20.0	60.8	222
UUU	64.9	54.3	77.9	72.8	54.3	78.6	23.8	239
class II	**97.7**	**99.0**	**99.7**	**99.1**	**99.0**	**99.7**	**93.3**	**228**

Earlier, it was found that, in sea buckthorn mesocarp, the dynamics and kinetics of TAG formation were markedly affected by the affinity of unsaturated FA species to the *sn*-2 position of TAGs during oil accumulation (*8*). Therefore, we have determined changes in the factor of selectivity of the incorporation of various FAs into this position in the course of seed maturation. This factor was calculated by the equation $([U]_{1,2,3} \times [U_j]_2)/([U_j]_{1,2,3} \times [U]_2)$, where U is the sum of unsaturated FAs (O + L + Le); U_j is the *j*th unsaturated FA (O, L, or Le); [U] and $[U_j]$ are the concentrations (weight percent) of the total unsaturated FAs and the *j*th FA, respectively; 1, 2, and 3 designate total TAGs; and 2 designates the *sn*-2 position of the TAGs (*4*, *5*, *8*).

The values in the tables and figures presented here represent means from experiments performed in three replications; in all cases, relative standard deviations did not exceed 7% of the mean.

RESULTS

Formation of TAG Positional Types. Only about 7% of the total TAGs of the whole sea buckthorn fruit reside in its seeds, but in TAG concentration (percentage of fresh weight), the seeds exceed the mesocarp more than 5-fold (*3*). Seed TAG formation achieved its peak at the 68th−71st DAP, and it was similar in k value to the formation of total FAs of these TAGs (*3*). Class II TAGs always predominated, with class I TAGs usually comprising only about 1−2% of total TAGs (**Table 1**).

The k values of various TAG types increased with the rise in their concentration in the order class I ≪ SUS = SUU < UUU (**Table 1**); these differences are consistent with the greater steepness of curves 5−7 for class II TAGs compared to curves 1−3 for class I TAGs in **Figure 1** (*10*). In contrast, in mesocarp, class I TAGs exceeded class II TAGs by 1.14-fold in the k value (*8*). A drastic decrease in the rate of UUU formation at stage 3 was accompanied by a rise in the synthesis of class I, SUS, and SUU TAGs, which together comprised 76.2% of total TAGs formed at this stage (**Table 1**); this rise was caused by an increased formation of S acids at stage 1 (see Table 3 in ref *3*).

Formation of TAG Positional Species of SUU Type. Developmental changes in the composition of TAG positional species were determined solely in SUU and UUU, which are the principal TAG types, amounting together to more than 97% of total TAGs of mature seeds, and only for the major species comprising 0.3% of total TAGs or more. The total contents of these species ranged from 77 to 95% (**Tables 2** and **3**).

Formation of SUU species was most intense up to the 80th DAP; however, at stage 3, their absolute content also rose (by ∼25%), and in mature-seed TAGs, their concentration reached 20.6%. In terms of the dynamics of the composition of SUU species, they could be divided into several groups (**Table 2**; **Figures 2** and **3**).

Group 1 TAGs included only P, L, and Le residues and differed from other SUU groups in the highest concentration,

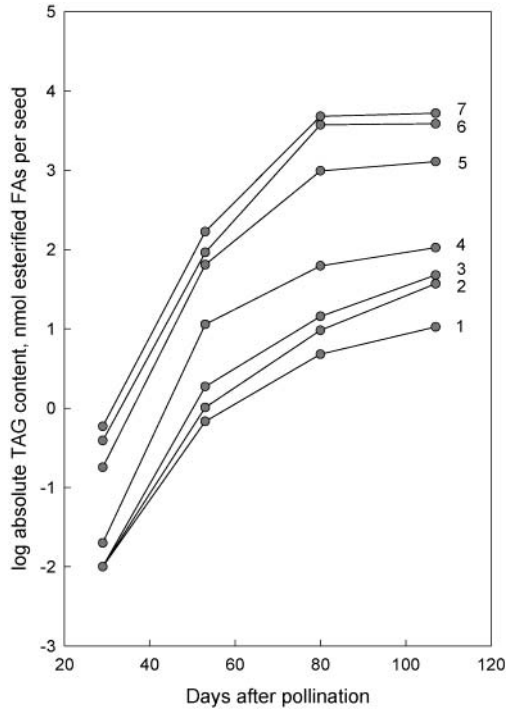

Figure 1. Dependence of the absolute content of separate triacylglycerol positional types and their classes on the number of days after pollination: 1, SSU; 2, USU; 3, class I; 4, SUS; 5, SUU; 6, UUU; 7, class II.

Table 2. Dynamics and Kinetics of SUU Triacylglycerol Positional-Species Composition in Maturing Sea Buckthorn Seeds

major SUU positional species and their groups	composition of TAGs formed (wt %)							$k \times 10^3$ (day^{-1})
	by a given DAP				at a particular stage of maturation (DAP)			
	29	53	80	107	29–53	53–80	80–107	
PLeLe	3.4	5.8	2.2	2.3	5.9	1.8	3.2	235
PLLe	4.3	4.3	3.3	3.3	4.4	2.9	3.2	228
PLeL	3.9	3.6	2.4	2.3	3.7	2.1	1.4	229
PLL	5.1	2.7	3.7	3.4	2.7	3.3	0.4	235
group 1	**16.7**	**16.4**	**11.6**	**11.3**	**16.7**	**10.1**	**8.2**	**229**
POLe	0.8	2.5	0.9	1.3	2.6	0.7	5.1	241
POL	0.9	1.5	1.0	1.3	1.5	0.9	4.1	232
POO	0.3	1.3	0.4	0.6	1.3	0.3	2.6	246
subgroup 2a	*2.0*	*5.3*	*2.3*	*3.2*	*5.4*	*1.9*	*11.8*	*240*
PLeO	1.2	3.0	0.9	1.0	3.1	0.7	2.0	242
PLO	1.5	2.2	1.4	1.5	2.2	1.2	2.4	233
subgroup 2b	*2.7*	*5.2*	*2.3*	*2.5*	*5.3*	*1.9*	*4.4*	*238*
group 2	**4.7**	**10.5**	**4.6**	**5.7**	**10.7**	**3.8**	**16.2**	**239**
StLeLe	0.3	1.1	0.5	0.9	1.1	0.4	4.7	243
StLLe	0.3	0.8	0.8	1.3	0.8	0.7	6.1	242
StLL	0.4	0.5	0.8	1.4	0.5	0.7	7.1	229
group 3	**1.0**	**2.4**	**2.1**	**3.6**	**2.4**	**1.8**	**17.9**	**238**
total SUU	**22.4**	**29.3**	**18.3**	**20.6**	**29.8**	**15.7**	**42.3**	**237**

the lowest *k*, and the least intense formation at stage 3, because of a decline in the rate of L and Le synthesis during the same period (see Table 3 in ref *3*). Group 2 differed from group 1 by a lower content, the presence of oleate, and a higher *k*. At stage 3, the total group 2 TAG content increased by more than 33% as a result of the resumption of P formation; however, the level of *sn*-2-O species (subgroup 2a) rose in this case by more than 50%, whereas the levels of *sn*-2-L + *sn*-2-Le species (subgroup 2b) increased by only 20%. This distinction was caused by the enhanced inclusion of oleate into the *sn*-2 position of TAGs at stage 3 (**Figure 4**). Finally, group 3 TAGs were marked by the presence of only St, L, and Le residues, by lower concentrations and similar *k* values as compared to the group 2 TAGs, and by doubling of their absolute content at stage 3. This dramatic

Table 3. Dynamics and Kinetics of UUU Triacylglycerol Positional-Species Composition in Maturing Sea Buckthorn Seeds

major UUU positional species and their groups	composition of TAGs formed (wt %)							$k \times 10^3$ (day^{-1})
	by a given DAP				at a particular stage of maturation (DAP)			
	29	53	80	107	29–53	53–80	80–107	
group 4[a]	**7.9**	**5.0**	**0.7**	**0.7**	**5.0**	**0.7**	**0.7**	**202**
LLeLe	7.3	5.5	9.0	7.8	5.6	8.1	0	224
LeLLe	4.0	3.3	6.1	5.5	3.4	5.5	0	228
LLeL	4.3	1.7	5.1	4.1	1.7	4.6	0	200
LLLe	9.4	4.1	13.8	11.5	4.2	12.6	0	202
LLL	5.5	1.3	7.8	6.0	1.3	7.1	0	175
OLL	3.4	2.1	5.9	5.3	2.2	5.4	0	217
group 5	**33.9**	**18.0**	**47.7**	**40.2**	**18.4**	**43.3**	**0**	**208**
LeOLe	0.7	1.9	1.7	2.1	1.9	1.5	5.7	251
LOLe	1.7	2.3	3.7	4.5	2.0	3.3	12.0	234
OOLe	0.5	1.9	1.4	2.0	1.9	1.2	7.7	254
LOL	1.0	0.7	2.1	2.3	0.7	1.9	4.1	223
OOL	0.6	1.2	1.6	2.1	1.2	1.4	6.7	239
group 6	**4.5**	**8.0**	**10.5**	**13.0**	**7.7**	**9.3**	**36.2**	**240**
LeLeLe	3.1	4.4	4.0	3.7	4.5	3.5	0.8	255
OLeLe	2.2	4.5	3.4	3.5	4.6	3.0	4.3	247
OLeL	2.6	2.8	3.8	3.6	2.9	3.4	1.8	241
OLLe	2.9	3.4	5.2	5.1	3.5	4.7	3.9	242
OLeO	0.4	1.2	0.7	0.8	1.2	0.6	1.8	255
OLO	0.5	0.9	1.1	1.2	0.9	1.0	2.2	243
group 7	**11.7**	**17.2**	**18.2**	**17.9**	**17.6**	**16.2**	**14.8**	**247**
total UUU	**58.0**	**48.2**	**77.1**	**71.8**	**48.7**	**69.5**	**51.7**	**241**

[a] Group 4 consisted of about equal amounts of five TAG positional species including hexadecenoic acid (H) residues, viz., HLeLe, LeHL, HLeL, HLLe, and HLL; the $k \times 10^3$ values of these species were 217, 202, 198, 204, and 187 day^{-1}, respectively.

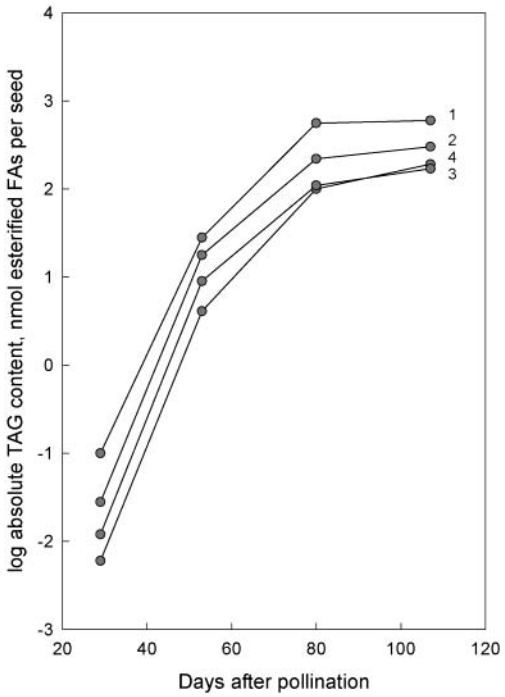

Figure 2. Dependence of the absolute content of separate groups of SUU triacylglycerol positional species on the number of days after pollination: 1, group 1; 2, group 2; 3, subgroup 2a; 4, group 3 (see **Table 2**).

increase was due to a sharp resumption of St formation during the same period (see Table 3 in ref *3*).

Formation of TAG Positional Species of UUU Type. These species exceeded SUU TAGs in *k*, and during growth, their concentration ranged from 48 to 77% (**Table 3**). The minor group 4 of UUU TAGs was marked by the lowest content and *k* value and by the presence of H in each of its species. In contrast, group 5 differed from the rest of the UUU TAGs in

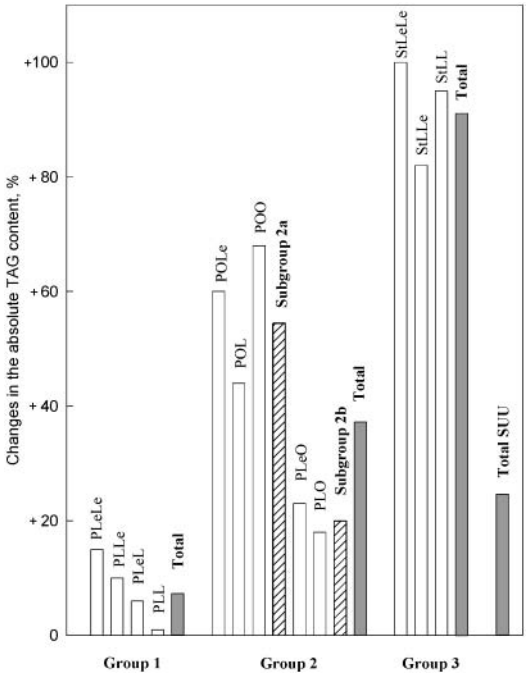

Figure 3. Percent changes in the absolute content of SUU triacylglycerol positional species, their separate groups (1–3), and total SUU triacylglycerols at stage 3 of sea buckthorn seed maturation.

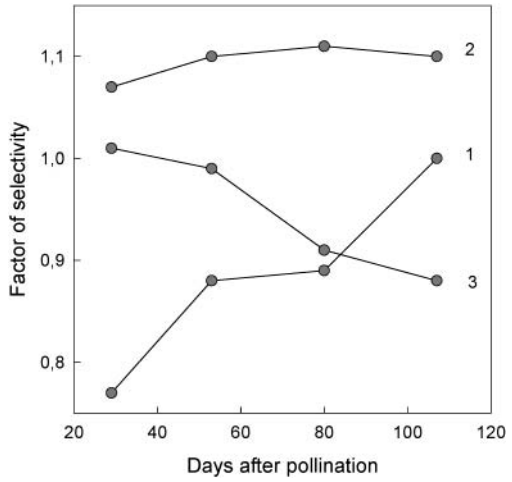

Figure 4. Dependence of the factor of selectivity of incorporation of different UFA species in the *sn*-2 position of TAGs on the number of days after pollination: 1, oleic acid; 2, linoleic acid; 3, linolenic acid.

having the highest concentration. Its TAGs, except OLL, included only L and Le, and their synthesis was most active at stage 2 because of the preferential production of L and Le (*3*). At stage 3, the absolute content of group 5 TAGs *decreased* by 7% (**Figures 5** and **6**); such a decrease, although minimal, involved also the OLL species, and that is why OLL was included in group 5. The direct cause of the observed decrease is unknown, but it seems to be partly explained by the termination of L synthesis at this stage (see Table 3 in ref *3*).

UUU TAGs outside groups 4 and 5 marked by the occurrence of O residues were divided into groups 6 and 7 according to the presence and absence, respectively, of oleate in their *sn*-2 position. In their *k* values, they exceeded all other TAG groups. The formation of group 6 TAGs, as also TAGs of subgroup 2a, was most active at stage 3 (**Figures 5** and **6**) because of an increase in the affinity of oleate to the *sn*-2 position at this stage (**Figure 4**). Finally, all group 7 TAGs were marked by the

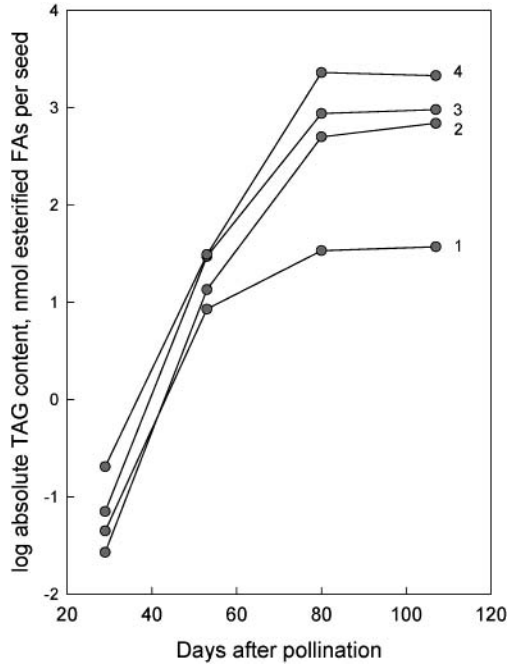

Figure 5. Dependence of the absolute content of separate groups of UUU triacylglycerol positional species on the number of days after pollination: 1, group 4; 2, group 6; 3, group 7; 4, group 5 (see **Table 3**).

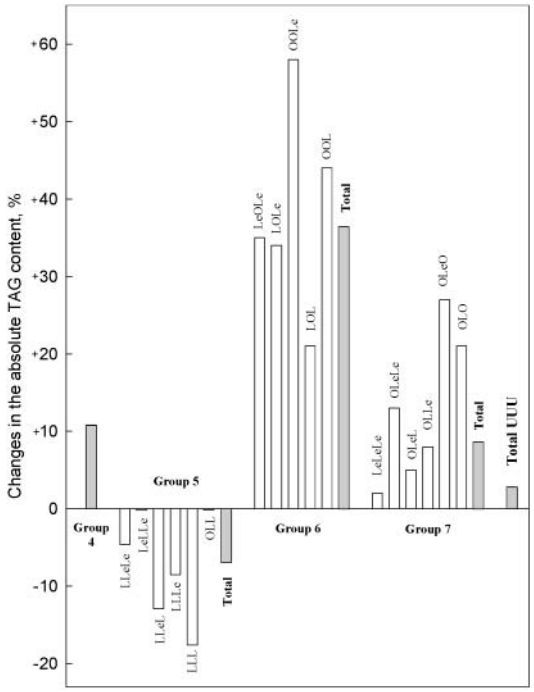

Figure 6. Percent changes in the absolute content of UUU triacylglycerol positional species, their separate groups (4–7), and total UUU triacylglycerols at stage 3 of sea buckthorn seed maturation.

presence of oleate in their *rac*-1,3 positions; LeLeLe was included in this group rather than in group 5 only because, at stage 3, its content increased in contrast to that of the group 5 TAGs. Group 7 synthesis, as also that of group 5, was most active at stage 2, and at stage 3, its content rose only by less than 9% because of a decrease in the affinity of Le to the *sn*-2 position (**Figure 4**) and a termination of L synthesis at this stage (see above).

It is concluded that there was a prevalent formation of UUU species at stages 1 and 2, TAG species with P and H acyls at

Table 4. TAG Molecular-Species Composition in Mature Sea Buckthorn Seeds Determined by Chromatography and by Lipase Hydrolysis

TAG composition determined by chromatography (*7*)			TAG quantitative composition (wt %) determined by lipase hydrolysis[c]
peak no.[a]	qualitative composition[a]	quantitative composition (wt %)[b]	
1–3	mixture of S_2U TAGs	8.2	2.2
4	PO_2	3.0	0.6
5	(POL)	3.2	2.8
6	PL_2 + (POLe)	8.6	5.7
7	(PLLe) + HL_2 + O_2L + StL_2	12.2	10.5
8	PLe_2 + (HLLe) + OL_2 + O_2Le + StLLe	13.8	14.4
9	(OLLe) + L_3	18.9	19.2
10	L_2Le + OLe_2	16.4	21.2
11	LLe_2	12.6	13.3
12	Le_3	3.1	3.7

[a] See Table 3 in ref *7*. Triacid TAG molecular species shown in parentheses include all TAG positional isomers of a given FA composition in **Tables 2** and **3**. [b] Calculated from the chromatogram shown in Figure 3B of ref *7*. [c] Calculated from the data of **Tables 1**–**3**.

stage 1, species rich in Le and L residues at stage 2, and species containing St, *sn*-2-O, and P acyls at stage 3. As distinct from TAG positional types (**Table 1**), there was no relationship between the concentrations of groups 1−7 of TAG positional species and their *k* values.

DISCUSSION

To assess the accuracy of our data, we compared the data, which refer only to the mature-seed TAGs (at the 107th DAP, **Tables 2** and **3**), with the results found by determining TAG composition of mature sea buckthorn seeds by capillary supercritical fluid chromatography−atmospheric pressure chemical ionization mass spectrometry (*7*). Because these results represented *molecular-species* composition of TAGs rather than their positional-species composition (see Materials and Methods), our data were recalculated accordingly (**Table 4**). To this end, as in the case of mesocarp TAGs (*8*), the concentrations of all positional species of the same qualitative and quantitative FA composition (**Tables 2** and **3**) were summed to obtain the content of a respective molecular species. The designations of the molecular species are shown in **Table 4**, where the species in parentheses indicate triacid TAGs. For example, (POL) = 2.8% signifies the combined concentrations of POL (1.3%) and PLO (1.5%) at the 107th DAP (**Table 2**).

A comparison of the qualitative TAG molecular-species composition reconstructed in this way with that established by Finnish researchers demonstrates that all TAG species found by lipase hydrolysis, except $StLe_2$, were also identified in the seeds using mass spectrometry (**Table 4**). Subsequently, the quantitative molecular-species composition of TAGs recalculated from the data of **Tables 2** and **3** was checked against the respective results obtained by us by triangulation of chromatographic peaks in Figure 3B in ref *7*. As shown in **Table 4**, there was fair agreement between the data found by lipase hydrolysis, on one hand, and by chromatography, on the other. Therefore, the qualitative and quantitative data presented in this paper can be considered as being accurate.

These data demonstrate that, in maturing sea buckthorn seeds, as in its mesocarp (*8*), the TAG composition can change under the action of a number of factors. A change in the concentration of certain FA species available for the TAG biosynthesis at a particular maturation stage was shown above to be one of these factors (*13*). In most cases, seed maturation is accompanied by an increase in the concentration of U and a decrease in the concentration of S (*14*−*16*). As a whole, sea buckthorn is similar to other plant species studied up to now in the pattern of changes of these indices in seeds (see Table 2 in ref *3*). At the same time, it differs from these species in an increased Le content (33−35%) in TAGs; the species-composition dynamics of TAGs, which, in mature seeds, contain more than 10% of Le residues, was not determined previously. Moreover, a sharp increase in StLeLe, StLLe, and StLL biosynthesis brought about by a rise in the St level at the terminal maturation stage was also observed here for the first time. It became possible for us to detect this phenomenon only because of the facts that, on one hand, the TAG composition was expressed, for the first time, not only as a percentage (as was the case in all previous works except ref *17*), but also as the absolute content values in nanomoles per seed (**Figures 2** and **3**) and, on the other hand, the composition of TAGs formed at each separate maturation stage was established (**Table 2**). Finally, the decrease in the level of L-containing TAG (**Table 3**) due to the cessation of L formation at the terminal stage was also brought about by this factor. Earlier, it was shown that the ripening of cruciferous seeds was accompanied by the formation of novel TAG species, which include erucic and eicosenoic acid residues; this was caused by the fact that the production of these FA species was started only after the onset of an intense TAG biosynthesis (*2*). However, in maturing sea buckthorn seeds, no qualitative shifts in TAG composition were observed.

Another factor of the dynamics of the TAG composition was a change in the positional specificity of TAG biosynthetic enzymes. This change was assessed by shifts in the affinity of particular FA species to the definite positions of the glycerol residue and expressed by the factor of selectivity (*8*). In the course of sea buckthorn seed maturation, these shifts affected the dynamics of TAG species level mostly in groups 2, 6, and 7. Previously, the changes in the affinity of O and L for the *sn*-2 position of TAGs were observed during the ripening of sunflower seeds, and at the 38th DAP, O exceeded L (*18*). However, in all other cases, including that of sea buckthorn seeds (**Figure 4**), this relationship between O and L was reversed. Meanwhile, the FA composition of the *sn*-2 position of soybean and corn seed TAGs remained constant throughout maturation, and the shifts in their positional-species compositions were caused only by changes in the composition of the FAs that were incorporated into the *sn*-1 and *sn*-3 positions of TAGs (*13*, *19*).

The decrease in the absolute content of a major group of highly unsaturated TAGs, group 5 (**Figure 6**), observed for the first time in this work, suggests that metabolization of TAGs deposited in the lipid bodies of cell can be regarded as yet another factor of the change in TAG composition of maturing sea buckthorn seeds. The evidence for the possibility of such metabolization prior to the end of ripening can be found in several papers published earlier, as detailed below.

First, before the onset of the exponential phase of oil accumulation, the seeds of sunflower (*16*, *18*), corn (*19*), soybean (*1*, *13*, *20*), and safflower (*21*) contained TAG species that, in their composition, were quite unusual for the oil of a particular plant. Because these TAG species, which most commonly included Le and S residues, were totally absent in mature seeds, it was concluded that they underwent metabolization during ripening (*22*).

Second, the maturation of grapes from the 18th to the 24th DAP was accompanied by a 2-fold decrease in the absolute

content of TAG species including L and O. This decrease was caused by the consumption of some TAG species in respiration and the others in phenolic compound biosynthesis (*17*). Finally, by the end of maturation of sunflower seeds, a decrease in the UUU concentration in total TAGs from 76.4 to 71.4% at the expense of the OOO, OLO, and OLL contents was accompanied by a certain increase in the LLL level (*16*). This decrease can be explained by the desaturation of O residues in TAGs with the formation of L-containing TAGs (*19*); a net decrease in the absolute content of *rac*-1,3-O TAG species in the maturing sea buckthorn mesocarp with the formation of respective *rac*-1,3-L species was explained in the same way (*8*). At the same time, the decrease in the group 5 L- and Le-containing TAGs during the maturation of the seeds of this plant species could not be brought about by their direct desaturation, because it was not accompanied by any significant increase in the content of LeLeLe, a putative product of this reaction (**Figure 6**).

An unusual mechanism of reserve TAG transformation was found in maturing seeds of *Lunnaria annua* (*23*). In the course of this process, $C_{22:1}$ and $C_{24:1}$ FAs were shown to be incorporated only in the *sn*-1 and *sn*-3 positions of the TAGs and equally divided between them. If the TAG biosynthesis in *L. annua* proceeded according to the classical Kennedy pathway, these FA species would be found in the *sn*-1 positions of the intermediates of this pathway, lysophosphatidic acids, phosphatidic acids, and *sn*-1,2-diacylglycerols. Actually, however, $C_{22:1}$ and $C_{24:1}$ FAs were absent in these positions. Thus, these FA species were initially present only in the *sn*-3 position of the TAGs and could be incorporated in their *sn*-1 position at the end of ripening only at the expense of an acyl exchange between the preformed TAG (*23*, *24*). Therefore, it is possible that the decrease in the absolute content of group 5 TAGs in sea buckthorn seeds was also brought about by the transfer of their acyl residues to other lipids.

In conclusion, it must be emphasized that this work is the first of its kind where seeds of the plant species with an oil-bearing mesocarp (*3*) were used for investigation of the dynamics of their TAG composition, and where the investigation itself involved the determination of the rate of TAG species biosynthesis, i.e., the *k* value.

ABBREVIATIONS USED

DAP, day after pollination; FA, fatty acid; H, L, Le, O, P, St, S, and U, hexadecenoic (total positional isomers), linoleic, linolenic, octadecenoic (total positional isomers), palmitic, stearic, total saturated, and total unsaturated acids, respectively, as well as the acyl residues of these FAs in triacylglycerols; *k*, kinetic constant of relative rate of TAG biosynthesis; TAG, triacylglycerol.

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