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Changes in Triacylglycerol Composition in Maturing Sea Buckthorn (*Hippophaë rhamnoides*) Seeds

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Abstract—Sea buckthorn (*Hippophaë rhamnoides* L.) seeds on the 29th, 53rd, 80th, and 107th day after pollination were used for determining, by lipase hydrolysis, the qualitative and quantitative composition of the triacylglycerol (TAG) positional types, groups, and positional species, as well as the factor of selectivity of incorporation of unsaturated fatty acids, octadecenoic, linoleic, and linolenic, into the *sn*-2-position of TAGs. Until the 80th day after pollination, there was a predominant formation of triunsaturated TAGs, which included linolenic and linoleic acid residues. After the 80th day, the absolute content of these major components of total TAGs markedly decreased, and an increase in total TAG content was mainly accounted for by the rise in the level of those TAG species, which included saturated fatty acids, palmitic and stearic (monosaturated–diunsaturated and disaturated–monounsaturated), as well as in the level of *sn*-2-octadecenoyl species belonging to the triunsaturated and palmito–diunsaturated types of TAGs. At each maturation stage, the quantitative dynamics of separate TAG species was determined by the content of fatty acid species available for TAG formation and the factor of selectivity of these species. The decrease in the content of a certain group of triunsaturated TAGs found here seems to be caused by their metabolization during seed maturation.

Key words: Hippophaë rhamnoides - selectivity factor - positional-type composition - positional-species composition - sn-2 acylation - maturation stages - kinetic constant of the relative rate of biosynthesis - monosaturated–diunsaturated triacylglycerols - triunsaturated triacylglycerols

INTRODUCTION

As distinct from other major reserve substances of seeds, quantitative and, frequently, qualitative composition of triacylglycerols (TAGs) is subjected to considerable changes in the course of fruit ripening. 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 of all 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 were investigated only at the level of their FA composition, and the changes in the species composition of the TAGs themselves remain little explored [2].

As the material for determining these changes, sea buckthorn (*Hippophaë rhamnoides*) fruits are of special interest because they are characterized by two different types of oil accumulation, which are characteristic for the mesocarp and seeds, respectively. In many respects, these fruits differ from the storage organs of other plants as regards the patterns of TAG biosynthesis and metabolism [3].

Earlier, the changes in the quantitative TAG species composition in the course of the sea buckthorn fruit maturation were determined only in their mesocarp [3], and the investigations of seed TAGs involved the determination of their composition and structure in mature fruits [4], changes in their FA composition during growth [5], and the pattern of this composition in separate geographic forms (climatypes) of *Hippophaë rhamnoides* [6, 7]. It was shown that these TAGs are in many respects quite different from those of the mesocarp [4–7].

These differences involve a higher rate of seed TAG biosynthesis and a shorter period of their accumulation [5]. Moreover, these TAGs are characterized by a prevalence of C_{18} FA residues, and, in this respect, are different from the mesocarp TAGs, where C_{16} FA residues predominate [4–7]. Finally, various climatypes of sea buckthorn are close 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 [7]. All these differences between the seeds and mesocarp might be caused by genetic factors, because, in all cases, seed embryo

Abbreviations and designations: DAP—day after pollination; FA—fatty acids; 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 residues of these FAs in triacylglycerols; *k*—kinetic constant of the relative rate of triacylglycerol biosynthesis; *rac*—racemate; *sn*—stereospecific numeration of carbon atoms in the glycerol molecule; TAG—triacylglycerol.

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includes equal portions of genes of both parents, while the sea buckthorn mesocarp is a somatic organ with only maternal genotype [5].

Thus, better understanding of direct causes of the differences observed and obtaining of a more detailed outline of TAG biosynthesis in sea buckthorn fruits would be of considerable interest. To this end, it was necessary to determine the changes in the principal indices of the quantitative species composition of TAGs not only in the mesocarp [3], 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 and other plant species with an oil-bearing mesocarp were not investigated earlier [5].

MATERIALS AND METHODS

As in our previous studies, the plants of sea buckthorn (*Hippophaë rhamnoides* L., cv. Dar Katuni) were used. Qualitative and quantitative TAG composition in seed samples were determined by lipase hydrolysis [3, 5–7]. We quantified the TAG positional types, which are characterized by a certain composition of saturated (S) and unsaturated (U) FAs, and, in addition, by a mid- or an extreme position of S and U in a TAG molecule ([SSU], [USU], [SUS], [SUU], and [UUU], wt % of total TAGs). Second, we estimated the concentrations of TAG positional species (wt % of total TAGs) characterized by a definite composition of individual FAs, viz., palmitic (P), stearic (St), hexadecenoic (H), octadecenoic (O), linoleic (L), and linolenic (Le), and, moreover, by the *sn*-2- or the *rac*-1,3-position of these FA residues in a TAG molecule [3].

All these compositional indices were determined on the *i*th day after pollination (DAP), where $i = 29, 53, 80$, and 107. The composition of TAGs formed at each of the successive stages of seed maturation, viz., at the 29–53 DAP stage (stage 1), 53–80 DAP stage (stage 2), and 80–107 DAP stage (stage 3), was also estimated [3]. The changes in TAG accumulation were expressed as changes in their concentration (wt % of total TAGs) and absolute content (*P*, nmol esterified FAs per seed) by the *i*th DAP. In this case, the *P* values were plotted on a logarithmic scale, because, at successive stages of seed development, the difference between these values could be as high as several orders of magnitude [5]. Changes in these values at the 3rd stage of maturation (ΔP, %) were calculated by the formula:

$$\Delta P = [(P_3 - P_2)P_2^{-1}] \times 100\%,$$

where P_2 and P_3 are the P values at the 2nd and 3rd stages, respectively.

The averaged intensity of formation of TAG types and species in the course of seed development was expressed by the kinetic constant of the relative rate (k, 1/day) of this process [3, 5]. The k constant was calculated by the equation:

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

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 on the *i*th DAP (see above); n is the number of maturation stages studied here, i.e., the number of seed samples of various age; P, P_0, and P_i (nmol esterified FAs per seed) are TAG absolute content values in mature seeds (107th DAP), green fruit seeds (29th DAP), and seeds at the *i*th DAP, respectively; T_i is the number of days between the initial (29th DAP) and the *i*th DAP [8]. Earlier, the equation (2) was mainly used in chemistry to characterize the kinetics of 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 [9, 10].

The affinity of U species of FAs (except hexadecenoic) for the *sn*-2-position of TAGs was characterized by a selectivity factor of incorporation of these species into the mid-position. This factor was calculated by the formula $([U]_{1,2,3} \times [U_j]_2)/([U_j]_{1,2,3} \times [U]_2)$, 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 contents of the total unsaturated FAs and the *j*th FA, wt %; 1, 2, and 3 designate total TAGs; 2 designates the *sn*-2-position of TAGs [3, 4, 7].

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

RESULTS

Formation of TAG Positional Types

Sea buckthorn seeds contain no more than 7% of total TAGs of the whole fruit, but, in the concentration of TAGs (% fr wt), they considerably exceed the mesocarp (13–22 vs. 2–4%, respectively) [5]. As shown in Table 1, TAG positional types containing U in the *sn*-2-position (class 2 TAGs) always predominated in total seed TAGs, and the types, which included S in this position (class 1 TAGs), usually did not comprise more than 1–2% of total TAGs.

The highest rate of the formation of total seed TAGs was achieved on the 68th–71st DAP [8], and, in the value of the rate constant of biosynthesis ($k \times 10^3 \sim$ 230 1/day), they were close to their total FAs, for which the k value was established earlier [5]. At the same time, Table 1 demonstrates that various TAG types were characterized by different rates of biosynthesis, and k values were lower for class 1 than class 2 TAGs. This difference in the biosynthesis rate was also confirmed by a greater steepness of the curves *5–7* for class 2 TAGs as compared to the curves *1–3* for class 1 TAGs in Fig. 1 [8]. It is interesting that, during the oil formation in the

Table 1. Dynamics and kinetics of triacylglycerol positional-type composition in maturing sea-buckthorn seeds

TAG positional types and classes	Triacylglycerol composition, wt %							$k \times 10^3$, 1/day
	by a given DAP				formed at successive stages of maturation			
	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	
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	

sea buckthorn mesocarp, class 1 TAGs exceeded 1.14-fold the class II TAGs in the *k* value [3].

After the 80th DAP, the intensity of total UUU accumulation was considerably decreased, and a major contribution to the TAG biosynthesis at the terminal stage (stage 3) of seed maturation was made by the TAG types, which contained S residues and comprised 76.2% of total TAG formed at this stage (Table 1). Such type-specific pattern of TAG accumulation at the stage 3 was brought about by an increased formation of S during this period (see Table 3 in [5]).

Formation of Major TAG Positional Species of SUU Type

As shown in Tables 2 and 3, the changes in the content of TAG positional species are presented only for the principal TAG types, that is, SUU and UUU, which together accounted for more than 97% of total TAGs in mature seeds. Moreover, only major positional species were taken into account, i.e., those species, the level of which, at least at some developmental stage, was equal to or exceeded 0.3% of total TAGs; the total content of these species ranged from 77 to 95% (Tables 2, 3). Thus, all data presented below are related only to major TAG species of SUU and UUU positional types.

As regards the TAGs of SUU type, their concentration on the 107th DAP was 20.6% (Table 2). As shown in Fig. 2, the accumulation of all these species was very active up to the 80th DAP. However, their biosynthesis continued until the 107th DAP, and, in the course of the stage 3 of development, their absolute content increased by about 25% (Table 2).

With respect to the pattern of the quantitative changes in the content of individual positional species of TAGs, total SUU could be divided into several groups, and each of them was characterized by a definite FA composition and structure (Table 2, Fig. 2). Group 1 consisted of TAG positional species, which included, along with P, only the L and Le residues. This group differed from other SUU groups in the highest concentration, the lowest rate of biosynthesis (*k*), and the least intensive formation at the maturation stage 3 (ΔP = 7.3%).

Next in absolute content after group 1 was group 2, which differed from the former in the presence of O residues and a higher formation rate (Table 2, Fig. 2). A considerable part of the total group 2 TAGs was formed at stage 3. As this took place, the absolute level of *sn*-2-

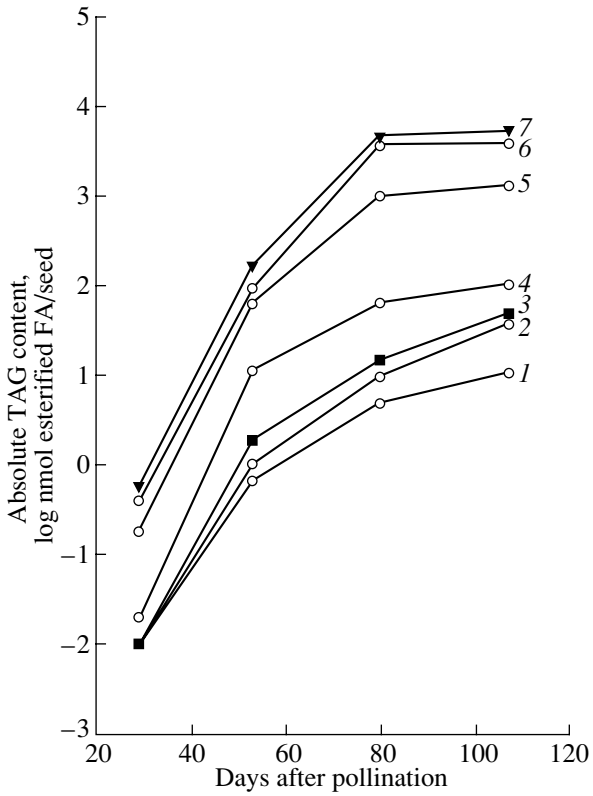

Fig. 1. Dependence of the absolute content of TAG types and their classes on DAP.
(*1*) SSU; (*2*) USU; (*3*) class 1; (*4*) SUS; (*5*) SUU; (*6*) UUU; (*7*) class 2.

Table 2. Dynamics and kinetics of TAG positional-species composition of SUU-type TAGs in maturing sea-buckthorn seeds

Major positional species of SUU TAGs and their groups	TAG composition, wt %							ΔP, %*	$k \times 10^3$, 1/day
	by a given DAP				formed at successive stages of maturation				
	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	15.0	235
PLLe	4.3	4.3	3.3	3.3	4.4	2.9	3.2	10.0	228
PLeL	3.9	3.6	2.4	2.3	3.7	2.1	1.4	6.0	229
PLL	5.1	2.7	3.7	3.4	2.7	3.3	0.4	1.0	235
Group 1	16.7	16.4	11.6	11.3	16.7	10.1	8.2	7.3	
POLe	0.8	2.5	0.9	1.3	2.6	0.7	5.1	60.0	241
POL	0.9	1.5	1.0	1.3	1.5	0.9	4.1	44.0	232
POO	0.3	1.3	0.4	0.6	1.3	0.3	2.6	68.0	246
Subgroup 2a	2.0	5.3	2.3	3.2	5.4	1.9	11.8	54.5	
PLeO	1.2	3.0	0.9	1.0	3.1	0.7	2.0	23.0	242
PLO	1.5	2.2	1.4	1.5	2.2	1.2	2.4	18.0	233
Subgroup 2b	2.7	5.2	2.3	2.5	5.3	1.9	4.4	20.0	
Group 2	4.7	10.5	4.6	5.7	10.7	3.8	16.2	37.2	
StLeLe	0.3	1.1	0.5	0.9	1.1	0.4	4.7	100.0	243
StLLe	0.3	0.8	0.8	1.3	0.8	0.7	6.1	82.0	242
StLL	0.4	0.5	0.8	1.4	0.5	0.7	7.1	95.0	229
Group 3	1.0	2.4	2.1	3.6	2.4	1.8	17.9	91.0	
Groups 1–3	22.4	29.3	18.3	20.6	29.8	15.7	42.3	24.6	

* See also Fig. 2.

O positional species, which comprised subgroup 2a, increased by more than one half, while that of *sn*-2-L + *sn*-2-Le species (subgroup 2b) augmented by only 20%.

Finally, SUU positional species, which included St instead of P (group 3), were characterized by the lowest concentration, and, in its formation rate ($k \times 10^3 \sim$ 238 1/day), group 3 was similar to group 2 ($k \times 10^3 \sim$ 239 1/day; Table 2, Fig. 2). At the stage 3, the absolute content of group 3 TAGs was almost doubled (ΔP = 91%). This sharp increase can be brought about by a drastic enhancement of St formation during the stage 3. Similarly, a rather weak increase in the group 1 content (see above) could be explained by a decline in the rate of L and Le synthesis during the same period (see Table 3 in [5]). In other words, in these cases, the pattern of quantitative changes in SUU species content at the maturation stage 3 was determined by differences in the amount of those FA species, which, at this stage, were available to produce a particular SUU species [3].

At the same time, at the stage 3, the content of group 2 TAG species, which included O residues, increased mostly at the expense of the subgroup 2a TAGs (see above), while the contribution of the subgroup 2b TAGs containing O only in their *rac*-1,3-positions to the ΔP value was far less important (Table 2, Fig. 2). It could be suggested that this pattern of changes was brought about by an enhanced O incorporation in the *sn*-2-position of TAGs at the maturation stage 3. Therefore, we determined the factor of selectivity of O, L, and Le incorporation at various DAP (Fig. 3). It can be seen that, at stage 3, the inclusion of O into the mid-position of TAGs was indeed increased considerably.

Formation of Major TAG Positional Species of UUU Type

In the course of maturation, the concentration of these species in total TAGs ranged from 58 to 72% (Table 3). The sum of major UUU species somewhat exceeded the major SUU species in their formation rate ($k \times 10^3 \sim 241$ 1/day).

Like SUU, UUU positional species could be divided into several groups (groups 4–7, Table 3). It can be seen that group 4 TAGs differed from other seed TAGs in the lowest formation rate ($k \times 10^3 \sim 202$ 1/day) and in the presence of the H residue in each of its species. Until the 53rd DAP, group 4 was one of the major UUU components, but, by the end of maturation, its concentration was reduced to less than 1%. Nevertheless, even at the stage 3, the absolute content of TAGs of this group rose by 10.8% (Table 3, Fig. 4).

Table 3. Dynamics and kinetics of TAG positional-species composition of UUU-type TAGs in maturing sea-buckthorn seeds

Major positional species of UUU TAGs and their groups	TAG composition, wt %							ΔP, %**	$k \times 10^3$, 1/day
	by a given DAP				formed at successive stages of maturation				
	29	53	80	107	29–53	53–80	80–107		
Group 4*	7.9	5.0	0.7	0.7	5.0	0.7	0.7	10.8	202
LLeLe	7.3	5.5	9.0	7.8	5.6	8.1	0	–4.6	224
LeLLe	4.0	3.3	6.1	5.5	3.4	5.5	0	–0.03	228
LLeL	4.3	1.7	5.1	4.1	1.7	4.6	0	–12.9	200
LLLe	9.4	4.1	13.8	11.5	4.2	12.6	0	–8.5	202
LLL	5.5	1.3	7.8	6.0	1.3	7.1	0	–17.6	175
OLL	3.4	2.1	5.9	5.3	2.2	5.4	0	–0.01	217
Group 5	33.9	18.0	47.7	40.2	18.4	43.3	0	–6.9	
LeOLe	0.7	1.9	1.7	2.1	1.9	1.5	5.7	35.0	251
LOLe	1.7	2.3	3.7	4.5	2.0	3.3	12.0	34.0	234
OOLe	0.5	1.9	1.4	2.0	1.9	1.2	7.7	58.0	254
LOL	1.0	0.7	2.1	2.3	0.7	1.9	4.1	21.0	223
OOL	0.6	1.2	1.6	2.1	1.2	1.4	6.7	44.0	239
Group 6	4.5	8.0	10.5	13.0	7.7	9.3	36.2	36.4	
LeLeLe	3.1	4.4	4.0	3.7	4.5	3.5	0.8	2.0	255
OLeLe	2.2	4.5	3.4	3.5	4.6	3.0	4.3	13.0	247
OLeL	2.6	2.8	3.8	3.6	2.9	3.4	1.8	5.0	241
OLLe	2.9	3.4	5.2	5.1	3.5	4.7	3.9	8.0	242
OLeO	0.4	1.2	0.7	0.8	1.2	0.6	1.8	27.0	255
OLO	0.5	0.9	1.1	1.2	0.9	1.0	2.2	21.0	243
Group 7	11.7	17.2	18.2	17.9	17.6	16.2	14.8	8.6	
Groups 4–7	58.0	48.2	77.1	71.8	48.7	69.5	51.7	2.8	

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

** See also Fig. 4.

Group 5 differed from group 4 in a somewhat higher formation rate (Table 3, Fig. 4). Moreover, the TAGs of group 5 (except OLL, see below) included only Le and L residues, and, in its concentration, this group exceeded other TAG groups (Tables 2, 3). Actually, by the 80th DAP, when the formation of Le and L was at its maximum [5], group 5 amounted to almost a half of total seed TAGs (Table 3).

However, the most prominent feature of the group 5 TAGs that distinguished them not only from other UUU, but also from all other seed TAGs, was the fact that their biosynthesis completely stopped by the 80th DAP, i.e., long before the end of maturation. What is more, at the stage 3, the absolute content of total group 5 TAGs decreased ($\Delta P \sim 7\%$). A phenomenon of the decrease in the level of a highly unsaturated TAG group in maturing seeds is not still explained.

All but one characteristics of the group 5 individual TAGs outlined above do not hold for the OLL species. The latter was included into group 5 only because, at the stage 3, its content also did not increase (Table 3).

UUU positional species of TAGs outside of groups 4 and 5 were characterized by a common structural feature, that is, the presence of O residues (Table 3); the only exceptions to this rule were OLL (see above), as well as LeLeLe discussed later. Nevertheless, these TAG positional species were divided here into two separate groups (6 and 7) according to the presence and the absence of O in their *sn*-2-position, respectively. Moreover, these groups significantly differed from each other in the rate of their formation at various maturation stages; the maxima of this rate were observed at the stages 3 and 2, respectively (Table 3, Fig. 4).

The *sn*-2-O positional species making up group 6 were produced throughout the entire maturation period.

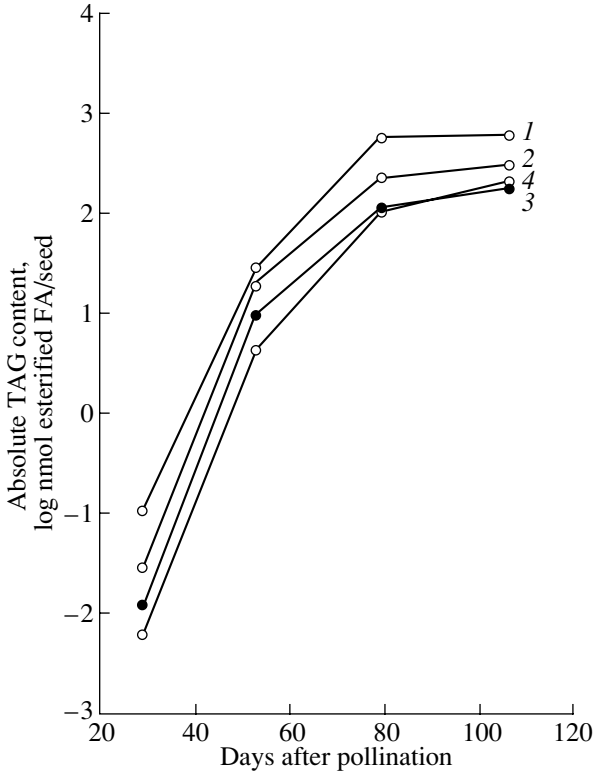

Fig. 2. Dependence of the absolute content of separate groups of SUU TAG positional species on DAP.
(*1*) Group 1; (*2*) group 2; (*3*) subgroup 2a; (*4*) group 3 (see Table 2).

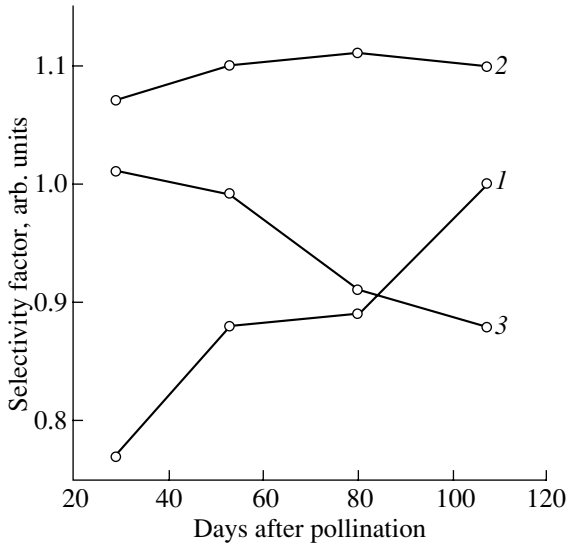

Fig. 3. Dependence of the selectivity factor of incorporation of various unsaturated FA species into the *sn*-2-position of TAGs on DAP.
(*1*) Octadecenoic acid; (*2*) linoleic acid; (*3*) linolenic acid.

However, their synthesis was particularly active after the 80th DAP (ΔP = 36.4%), when more than one third of total TAGs of this group were formed (Table 3, Fig. 4). It can be seen that, in the pattern of its accumulation, group 6 was similar to subgroup 2a (see below), and, in the rate of its biosynthesis ($k \times 10^3 \sim 240$ 1/day), it markedly exceeded groups 4 and 5 and, in this respect, was close to groups 1–3 (Table 2).

In turn, the group 7 TAGs were characterized by the presence of only Le or L in their *sn*-2-position, and the O residues occurred only in their *rac*-1,3-positions (Table 3). As mentioned above, the LeLeLe species, being devoid of O, is an exception to the other species of this group. Moreover, LeLeLe differed from all of them in the fact that, at the stage 3, an increase in its absolute content was as low as 2%. In this respect, LeLeLe was somewhat similar to certain group 5 TAG species, e.g., LeLLe, whose content, at the end of maturation, was also virtually constant (ΔP = –0.03%, Table 3).

Finally, group 7 differed from other seed TAGs in the highest rate of biosynthesis ($k \times 10^3 \sim 247$ 1/day). The peak of group 7 formation coincided with the stage 2, as was also the case for group 5 (see above). Meanwhile, at the growth stage 3, the absolute content of group 7 was increased only by less than 9% (Table 3, Fig. 4). This was caused by a decrease in the affinity of Le for the *sn*-2-position of TAGs (Fig. 3) and by the termination of L biosynthesis at this stage (see below).

Changes of the Positional-Species Composition of Triacylglycerols at Separate Maturation Stages

It can be seen that the UUU species predominated among the major TAG species of seeds, and they were followed by the SUU ones. All these species, except, possibly, OLL and LeLeLe, could be clearly divided into at least seven TAG groups differing in the structure and FA composition, the concentration throughout the growth period, the rate of biosynthesis, and the pattern of quantitative changes at the maturation stage 3 (ΔP).

A comparison of these data with quantitative changes in the FA composition of sea buckthorn seed TAGs detected by us earlier (see Table 3 and Figs. 1–3 in [5]) makes it possible to conclude that, at the stages 1 and 2, there was a prevalent formation of UUU species, whereupon their content remained, as a whole, almost unchanged. Moreover, at the stage 1, this process was accompanied by an intense accumulation of the TAG species containing P and H residues due to the selective formation of respective FA species [3]. At the stage 2, it was accompanied by an enhanced accumulation of the species rich in Le and L residues and belonging to the groups 1, 5, and 7; this, again, was caused by the preferential production of Le and L in the course of this period [3].

Of particular interest is the pattern of changes in the ΔP values, that is, in the TAG species levels at the maturation stage 3. Because of a general attenuation of TAG synthesis at this stage (see Figs. 1, 2, 4), the effect

of both regulatory factors of this process, that is, the concentration of certain FA species and the extent of their affinity for the *sn*-2 OH glycerol moiety, on the changes in the TAG composition could be traced more easily. Thus, it can be seen that the resumption of S and, especially, St formation brought about a sharp increase in the content of SSU, USU, and SUS, as well as group 2 and 3 TAGs. At the same time, a decrease in the concentration of Le and in its *sn*-2-OH affinity, together with the termination of L formation (see below), were responsible for a very moderate rise (ΔP) in the amount of groups 1 and 7. Meanwhile, this decrease could not account for the decline in the absolute content of the group 5 TAGs, which are predominant in total TAGs, and, as yet, there is no satisfactory explanation of this fact.

The termination of L synthesis after the 80th DAP (see Table 3 in [5]) resulted in considerable shifts in the changes in the L-containing TAG (L-TAG) species, and these shifts became more pronounced as the number of L residues in the L-TAGs increased. The shifts observed involved a modest rise and a considerable decrease in the L-TAG absolute content in groups 1 and 5, respectively. The selectivity factor of L did not affect the formation of L-TAG species, because, throughout the entire seed development, this factor was almost constant (Fig. 3). On the contrary, the affinity of O for the *sn*-2-position of TAGs rose steadily, bringing about an increase in the absolute contents of subgroup 2a and group 6 at the maturation stage 3 (ΔP = 54.5 and 36.4%, respectively).

As mentioned above, there is a direct relationship between the concentrations of separate TAG classes and their *k* values (Table 1). At the same time, there was no such relationship for the groups 1–7 considered above. Thus, groups 4 and 5 were similar in their $k \times 10^{-3}$ values (202 and ~208 1/day), but greatly differed from each other in their content in mature-seed TAGs (0.7 and 40.2%, respectively). Immediate reasons for the differences between separate TAG groups in their *k* values are unclear at present.

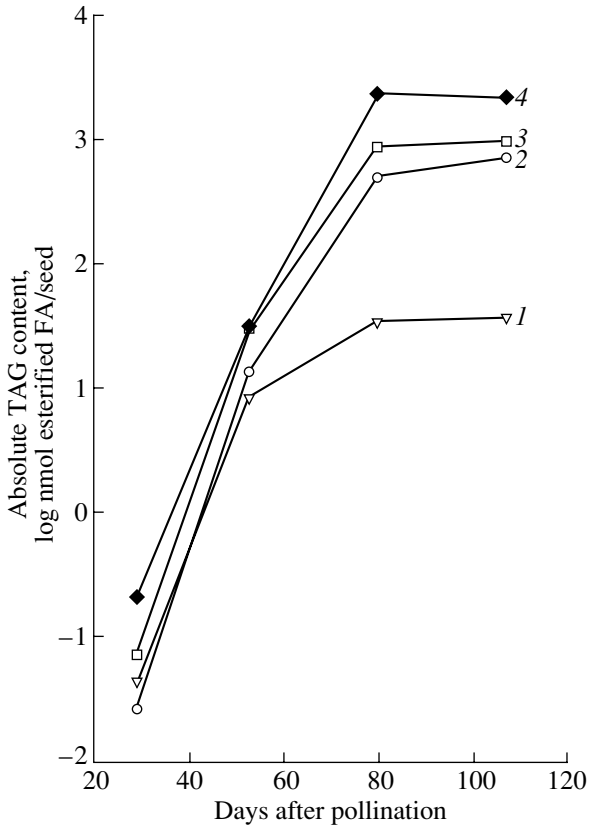

Fig. 4. Dependence of the absolute content of separate groups of UUU TAG positional species on DAP.
(*1*) Group 4; (*2*) group 6; (*3*) group 7; (*4*) group 5 (see Table 3).

DISCUSSION

The data reported here demonstrate that, in the maturing seeds of sea buckthorn, as also in its mesocarp [11], the composition of TAG positional species could change under the action of a number of factors. A change in the composition of FA species available at a particular maturation stage for TAG biosynthesis was shown to be one of these factors [12]. As a rule, seed maturation is accompanied by an increase and a decrease in U and S concentrations, respectively [13–15]. 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 [5]). At the same time, it differed from these species in an increased Le content (33–35%) in TAGs; the changes in the species composition of TAGs, which, in a mature seed, contain more than 10% of Le residues, were not determined previously. Moreover, a sharp increase in StLeLe, StLLe, and StLL biosynthesis brought about by a rise in St level at the terminal maturation stage was observed here for the first time. It became possible for us to detect this phenomenon only due to the facts that, first, the positional-species composition of TAGs was expressed, for the first time, not only as a percentage, as it was the case in all previous works except [16], but also as the absolute content values, nmol per seed (Fig. 2), and, second, the composition of TAGs formed at each separate maturation stage was established (Table 2). Finally, a decrease in the L-TAG level due to the cessation of L formation at the terminal stage (Table 3) 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 included 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, no qualitative shifts in TAG composition were observed in maturing sea buckthorn seeds.

Another factor of the changes in their composition consisted in a shift in the positional specificity of the

enzymes of TAG biosynthesis. This shift was expressed in the changes of affinity of separate FA species for definite positions of the glycerol residue [11]. In the course of sea buckthorn seed maturation, the value of this affinity affected the changes in TAG species level mostly in groups 2, 6, and 7. Previously, the changes in the affinity of unsaturated FAs for the *sn*-2-position of TAGs were observed during the ripening of sunflower seeds; in these TAGs, oleate, on the 38th DAP, exceeded linoleate in this regard [17]. At the same time, in all other cases, including that of sea buckthorn seeds (Fig. 3), oleate ranked much below linoleate in this respect. Meanwhile, the FA composition of the *sn*-2-position of soybean and corn seed TAGs remained constant throughout the entire maturation, and the shifts in their positional-species composition were only caused by changes in the composition of the FAs, which were incorporated into the *sn*-1- and *sn*-3-positions of TAGs [12, 18].

A decrease in the absolute content of one of the TAG groups, that is, group 5, demonstrates that metabolization of TAGs deposited in lipid bodies of the 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 also found in several works published earlier.

First, before the onset of the exponential phase of oil accumulation, the seeds of sunflower [15, 17], corn [18], soybean [1, 12, 19], and safflower [20] contained TAG species, which, in their composition, were quite unusual for the oils of these plants. Because these TAG species, which usually included Le and S residues, were totally absent in mature seeds, it was concluded that they underwent metabolization during ripening [21].

Second, a twofold decrease in the absolute content of TAG species, which included L and O, was observed in grapes from the 18th to the 24th DAP. This decrease was caused by the consumption of some TAG species in respiration, and the other ones, in phenolic compound biosynthesis [16]. Finally, by the end of sunflower seed maturation, a decrease in the UUU concentration in total TAGs from 76.4% to 71.4% at the expense of decreased OOO, OLO, and OLL contents was accompanied by a certain increase in the LLL level [15]. This decrease can be accounted for by the desaturation of O residues in TAGs with the formation of L-TAGs [18]; 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 has been explained similarly [11]. At the same time, the decrease in the L- and Le-TAGs in group 5 during seed maturation of this plant species could not be accounted for by their direct desaturation, because it was not accompanied by any significant increase in the content of LeLeLe, a putative product of this reaction (Table 3).

An unusual mechanism of reserve TAG transformation was found in maturing seeds of *Lunaria annua*. 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 TAGs and evenly divided between them. If the TAG biosynthesis in *L. annua* proceeded according to the classical Kennedy pathway [11], these FA species would be found in the *sn*-1-positions of the intermediates of this pathway, such as lysophosphatidic acids, phosphatidic acids, and *sn*-1,2-diacylglycerols. Actually, however, $C_{22:1}$ and $C_{24:1}$ FA residues were absent from these positions. Thus, these FA species were initially present only in the *sn*-3-position of TAGs and could be incorporated in their *sn*-1-position only at the expense of an acyl exchange between the preformed TAG at the end of ripening [22, 23]. 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.

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