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CHANGE ON STORAGE OF BIOLOGICAL ACTIVITY OF *Viburnum opulus* SEED COMPONENTS

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Data for changes on storage under natural conditions for 11 months of moisture, oil content, acid and hydroperoxide number, and protein content of ground seed of Viburnum opulus *were reported. The amino-acid and fatty-acid compositions were determined.*

Key words: seeds, *Viburnum opulus*, storage.

Prolonged storage of seeds causes various biochemical processes connected to the presence of pathogenic fungi, elevated temperature, and environmental effects. These factors cause, firstly, changes in the protective abilities of the seed casing and, as a result, more significant changes (hydrolysis and oxidation) of biologically active components found in the seeds [1]. Thus, hydrolysis causing an accumulation of free fatty acids (FA) in lipids and oxidation, both auto-oxidation and lipoxygenase-induced, to which unsaturated FA are most susceptible, can occur during prolonged storage of seeds.

Seeds of *Viburnum opulus* L. are a food-industry waste than can be used as a feed additive in a ground form together with the principal feed. Therefore, the effect of prolonged storage under natural conditions of the starting biological material in the ground form on the biological value of the natural components must be studied.

Thus, *Viburnum opulus* seeds (1 kg) were ground and placed in storage for 11 months at room temperature (20-25°C), natural humidity, and in the dark (sample I). Unground seeds were placed in storage under these same conditions in parallel (sample II). Changes of various biochemical parameters for sample I were recorded monthly for 11 months; for sample II, only once during the storage period. The control was ground seeds studied immediately after grinding. Moisture, oil content (free lipids), carotinoid content, and proteins were determined in seeds; acid and hydroperoxide number, in oil (Table 1).

Table 1 shows that the moisture in sample I changes unevenly. The starting moisture is 7.7% (seeds were placed in storage in February). It then decreases and from May, when the heating season ends and natural humidity increases, the natural moisture begins to increase. The acid number of the oil increases six times as a result of hydrolytic processes. Apparently the oil content increases due to liberation of fatty acids not only from free but also from bound and strongly bound lipids. Triacylglycerides are primarily hydrolyzed. This is consistent with TLC results of oils from all samples (solvent systems 1-3) and with the literature [2]. The percent active oxygen (hydroperoxide number) in oil changes insignificantly on storage with some tendency to increase. As we reported previously [3], this may be due to the presence of pigments that have anti-oxidant properties. The quantity of carotinoids during this same storage period decreases in both samples. However, according to TLC (solvent system 4), these are mainly carotinoids with oxygen-containing functional groups, possibly xanthophylls, in ground seeds. They change insignificantly in whole seeds. The protein content in seeds remains at 5-6%. The amino-acid composition of protein isolate from seeds that was determined at the start, middle, and end of storage (Table 2) is practically unchanged with the exception of an insignificant increase in the content of glutamic acid.

Determinations of the FA composition of all samples (Table 3) showed that the content of saturated FA in sample I increases during storage almost five times. Acid 16:0 increases 1.5-2 times; 22:0, from traces to 8.7%. The latter appears in measurable quantities after four months of storage. The content of acid 18:2 decreases significantly.

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TABLE 1. Properties of Seeds and Oil (Free Lipids) of *Viburnum opulus* on Storage under Natural Conditions

Month	Moisture	Oil content per abs. dry weight	Acid number, mg KOH	Hydroperoxide number, % active oxygen	Carotinoids, mg%		Protein in seeds, %
	%				in oil	in seeds	
				Sample I			
0	7.7	13.1	7.2	0.018	45.0	5.9	5.0
1	5.7	12.2	10.4	0.021	47.0	5.7	5.5
2	5.3	12.1	10.8	0.023	35.5	4.3	5.5
3	7.7	13.0	11.5	0.031	31.0	4.0	4.9
4	8.1	13.9	12.3	0.030	25.4	3.5	5.0
5	8.5	13.9	17.6	0.029	25.0	3.4	6.0
6	8.8	14.1	22.0	0.041	28.0	3.9	5.0
7	9.0	14.5	28.3	0.058	29.6	4.3	5.0
8	9.3	14.5	32.8	0.06	29.6	4.3	5.5
9	6.7	14.8	39.1	0.065	27.6	4.0	5.9
10	7.0	14.8	40.0	0.055	27.3	4.0	5.3
11	7.7	15.1	42.0	0.09	21.7	3.3	5.8
				Sample II			
11	7.1	15.3	9.8	0.08	17.0	2.6	5.6

TABLE 2. Amino-acid Composition of Protein Isolate from *Viburnum opulus* Seeds on Storage under Natural Conditions

Amino acid	**1**		**6**		**11**	
	mol, %	wt, %	mol, %	wt, %	mol, %	wt, %
Aspartic	8.35	8.78	8.95	9.45	9.19	9.71
Threonine	5.05	4.55	4.58	4.21	4.37	3.99
Serine	6.81	5.40	6.98	5.61	7.00	5.48
Glutamic	13.85	16.38	15.50	18.42	16.41	19.22
Proline	7.69	6.69	6.98	6.16	6.56	5.81
Glycine	8.35	4.32	8.95	4.70	8.97	4.73
Alanine	7.01	5.14	8.07	5.28	8.09	5.30
Cystine	0.87	0.75	0.65	0.71	0.87	0.76
Valine	7.03	6.42	6.55	5.90	6.34	5.68
Methionine	1.97	2.26	1.52	1.80	1.75	2.10
Isoleucine	4.39	4.5	4.14	4.20	3.93	4.12
Leucine	7.69	7.9	8.29	8.55	8.09	8.34
Tyrosine	2.19	3.09	1.74	2.60	1.96	2.94
Phenylalanine	5.05	6.81	4.80	6.34	4.37	5.97
Lysine	4.17	4.74	3.73	4.52	3.71	4.32
Cysteine	2.63	3.14	2.40	2.89	2.18	2.69
Arginine	6.37	9.09	6.11	8.60	6.12	8.76

The increased content of saturated FA on storage can be explained by hydrolysis not only of free (neutral lipids, NL) but also bound (polar lipids, PL) and strongly bound lipids. As a rule, FA of plant PL contain more saturated FA than those of NL. This was confirmed by exhaustive petroleum-ether extraction in a Soxhlet apparatus from whole *Viburnum opulus* seeds (stored whole for one year) of free lipids (NL), then $CHCl_3$:CH_3OH (2:1) extraction of bound lipids (PL), followed by hydrolysis by KOH (20%) of the remaining pulp and removal of strongly bound lipids (PL). The ratio of free, bound, and strongly bound lipids was 16.1, 8.6, and 1 (% of seed mass), respectively. The FA composition was determined for each of these lipid samples (Table 4). Table 4 shows that the saturation increases with increasing polarity of the isolated lipid groups. In our opinion, the results confirm the hypothesis stated above about the reasons for the increase of saturated FA and decrease of unsaturated ones during storage of ground seeds.

TABLE 3. Fatty-acid Composition of Free Lipids from *Viburnum opulus* Seeds on Storage under Natural Conditions (GC, %)

Month	Acid								
	14:0	16:0	17:0	18:1	18:2	18:3	22:0	Σ_{sat}	Σ_{unsat}
					Sample I				
0	0.2	2.3	-	42.3	54.0	1.2	Tr.	2.5	97.5
1	0.3	2.6	-	41.9	53.9	1.3	Tr.	2.9	97.1
2	0.1	1.0	-	39.0	59.9	Tr.	Tr.	1.1	98.9
3	Tr.	1.5	-	42.8	55.7	Tr.	Tr.	1.5	98.5
4	1.0	2.5	0.9	44.1	48.3	Tr.	3.2	6.6	93.4
5	0.5	2.2	0.5	42.6	51.2	-	3.0	6.2	93.8
6	1.0	2.7	0.8	42.9	49.1	-	3.5	8.0	92.0
7	1.6	3.7	1.2	41.9	47.3	-	4.3	10.8	89.2
8	Tr.	3.5	Tr.	42.8	49.2	-	4.5	8.0	92.0
9	Tr.	4.2	Tr.	45.6	42.8	Tr.	7.4	11.6	88.4
10	Tr.	3.9	Tr.	44.2	43.1	1.0	6.8	10.7	89.3
11	Tr.	4.2	Tr.	43.2	42.3	1.6	8.7	12.9	87.1
					Sample II				
11	0.2	1.1	-	44.2	51.1	0.9	2.5	3.8	96.2

TABLE 4. Fatty-acid Composition of Various Lipid Groups from *Viburnum opulus* Seeds (GC, %)

Acid	Lipid group		
	Neutral (free)	Polar (bound)	Strongly bound
12:0	1.4	1.2	1.3
14:0	1.2	0.9	2.1
15:0	Tr.	0.8	5.4
16:0	2.6	4.2	9.7
17:0	-	0.7	-
18:0	-	-	11.7
18:1	41.8	40.8	29.6
18:2	47.6	44.1	33.9
18:3	1.1	1.3	1.3
22:0	4.3	6.0	1.5
24:0	-	-	3.5
Σ_{sat}	9.5	13.8	35.2
Σ_{unsat}	90.5	86.2	64.8

EXPERIMENTAL

GC was performed on a Chrom-5 instrument using a flame-ionization detector at 160°C, a PDEGS (5%) column (1.2 m) on Chromaton N-AW-PMCS, He carrier gas, and 40 mL/min flow rate.

The contents in seeds of oil and moisture [4], acid number [5], and contents of hydroperoxide [6] carotinoids [7] were determined by the usual methods.

Free lipids were extracted exhaustively from air-dried and ground seeds by petroleum ether (40-60°C) at room temperature. The ether extracts were combined and evaporated in a rotary evaporator until solvent was completely removed.

Analytical TLC was performed on Silufol plates using the following solvent systems: petroleum ether (60-70°C):diethylether (9.5:05, 7:3, 5:5), hexane:acetone:benzene:isopropyl alcohol (6.95:2.5:0.4:0.15).

Alkaline hydrolysis was carried out as before [8]. Oil (0.1 g) was treated with methanolic KOH (1-1.5 mL, 20%). The mixture was shaken until a homogeneous transparent liquid was obtained and boiled for 1 h. The solution was diluted two times with water and acidified with H_2SO_4 (20%). The separated FA were extracted three times with diethylether. The ether extracts were combined and dried over Na_2SO_4. The solvent was removed in a rotary evaporator.

FA were esterified by diazomethane solution.

Protein content was determined using the biuret reaction [9]; amino-acid composition, at the Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow.

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