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FATTY ACID COMPOSITION OF *Oenothera biennis* SEED OIL DURING STORAGE. ANTIOXIDANT ACTIVITY

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In continuation of research on the lipid composition of seed oil from *Oenothera biennis* growing in Krasnodar Territory [1], we studied the effect of long-term (12 months) storage on the content of the principal fatty acids palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and γ-linolenic (18:3).

An oil sample from *Oenothera biennis* seeds (~1 mL) was placed in a tube (~2 mL), closed with a stopper, and stored in a refrigerator at 8°C. A sample was taken each month. The fatty acid composition was determined (Table 1). The composition and content of fatty acids in the samples were practically constant and within the uncertainty limits of the experiment.

The presence or absence of oxidized lipids was determined by hydrolyzing a sample of oil that was stored for one year, isolating the fatty acids, and preparing the methyl esters, which were analyzed for the presence of oxidized fatty acids by analytical TLC (ATLC) using solvents 1–3. The models were methyl esters of fatty acids from castor oil that contained monohydroxy- and dihydroxy-fatty acids. However, spots corresponding to oxidized fatty acids were not observed in the studied sample. Oil of *O. biennis* is known to contain tocopherols [2, 3], which we identified previously in the neutral lipids [1]. GC/MS of the unsaponified fraction of *O. biennis* oil identified the natural antioxidants α-, β-, and γ-tocopherol from their mass spectra using the AMDIS software [4].

The antioxidant activity of the oil was analyzed using a model system based on radical-chain oxidation of methyloleate initiated by azodiisobutyronitrile (AIBN). The advantage of this model system is that the oxidation process in it is very similar to the lipid oxidation process in living systems. Studies of natural compounds using this system and analogous apparatus are rather rare.

Figure 1 shows typical kinetic curves for oxygen absorption during initiated oxidation of methyloleate without an inhibitor and with *O. biennis* seed oil. It can be seen from the curves that the reaction occurred with an induction period. The rate of uninhibited oxidation of methyloleate and the oxidation rate of methyloleate inhibited by the oil components after complete consumption of the inhibitor were the same. Therefore, our experimental conditions did not reveal the contribution from oxidation of the oil itself. The reduced oxidation rate in the initial portion (induction period) indicated that an additional pathway for consumption of peroxide radicals had opened.

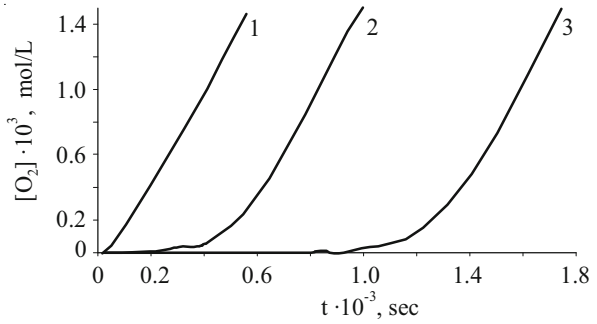

Fig. 1. Kinetic curves of oxygen absorption during methyloleate oxidation without inhibitor (1) and with 0.03 (2) and 0.05 (3) mL of fresh *Oenothera biennis* seed oil. 60°C, Wi = $6.3 \cdot 10^{-8}$ mol/(L·s).

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TABLE 1. Fatty Acid Composition of *Oenothera biennis* Seed Oil During Storage for 12 Months

Acid	Storage month											
	1	2	3	4	5	6	7	8	9	10	11	12
12:0	0.2	1.6	Tr.	Tr.	1.3	0.3	Tr.	–	0.2	0.2	0.3	1.6
14:0	Tr.	Tr.	Tr.	Tr.	Tr.	0.1	Tr.	–	0.1	Tr.	0.1	–
16:0	6.5	6.2	6.8	6.4	6.4	7.7	6.7	6.9	7.5	7.3	7.0	7.1
18:0	1.7	1.9	0.9	1.1	1.7	1.8	1.7	2.1	2.0	2.0	1.7	1.8
18:1	15.9	17.1	16.5	17.1	16.5	16.1	16.3	17.4	16.9	17.1	16.8	16.1
18:2	67.9	66.2	68.2	67.6	66.4	67.0	67.5	66.8	66.1	66.2	67.1	66.2
γ-18:3	**7.8**	**7.0**	**7.6**	**7.8**	**7.7**	**7.0**	**7.8**	**6.8**	**7.2**	**7.2**	**7.0**	**7.2**
$\Sigma_{sat.}$	8.4	9.7	7.7	7.5	9.4	9.9	8.4	9.0	9.8	9.5	9.1	10.5
$\Sigma_{unsat.}$	91.6	90.3	92.3	92.5	90.6	90.1	91.6	91.0	90.2	90.5	90.9	89.5

Tr.: traces.

TABLE 2. Induction Period as a Function of Storage Time and Conditions of *Oenothera biennis* Seed Oil

Volume of added oil, mL	τ_Σ, sec	$[InH]_\Sigma$, mol·L^{-1}
	Fresh oil	
0.0.2	572	$1.84 \cdot 10^{-3}$
0.03	657	$1.42 \cdot 10^{-3}$
0.05	1419	$1.86 \cdot 10^{-3}$
0.08	1865	$1.55 \cdot 10^{-3}$
	Oil stored for 3 months at 25°C	
0.01	110	$7.06 \cdot 10^{-4}$
0.02	220	$7.09 \cdot 10^{-4}$
	Oil stored for 15 months at 8°C	
0.023	151	$4.24 \cdot 10^{-4}$
0.03	225	$4.86 \cdot 10^{-4}$
	Oil stored for 18 months at 8°C	
0.02	115	$3.71 \cdot 10^{-4}$
0.045	180	$2.61 \cdot 10^{-4}$

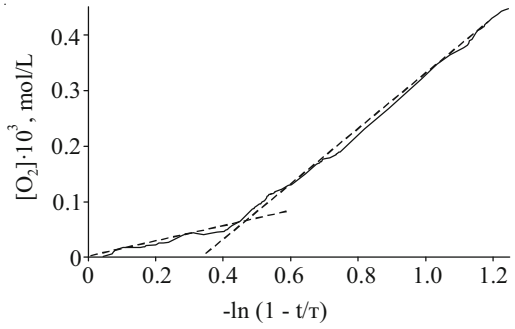

Fig. 2. Semi-logarithmic plot of oxygen absorption kinetic curve during methyloleate oxidation with 0.03 mL of fresh *Oenothera biennis* seed oil. 333 K, Wi = $6.3 \cdot 10^{-8}$ mol/(L·s).

TABLE 3. Inhibitor Content in *Oenothera biennis* Seed Oil and Methyloleate Oxidation Inhibition Rate Constants for Them

Oil storage time	$[InH]_{\Sigma}\cdot10^4$	$[InH]_1\cdot10^4$	$[InH]_2\cdot10^4$	$*k_7\cdot10^{-6}$	$**k_7\cdot10^{-4}$
	mol/L			$L\cdot(mol\cdot s)^{-1}$	
Fresh	17.3	5.7	11.6	4.6±0.2	5.6±1.0
3 months at 25°C	7.1	–	7.1	–	
15 months at 8°C	4.6	–	4.6	–	
18 months at 8°C	3.2	–	3.2	–	

*Average inhibition rate constant measured for first inhibitor; **average inhibition rate constant mesured for second inhibitor.

According to the results from the kinetic studies, *O. biennis* seed oil has good antioxidant activity that depends on the storage time and conditions (Table 2). We found that fresh oil contained at least two inhibitors with different inhibiting ability. This was evident from an inflection on a semi-logarithmic plot of the oxygen absorption kinetic curve upon oxidation of methyloleate in the presence of this sample (Fig. 2) [5]. The action time of the first inhibitor (τ_1) was determined from the inflection point on the semi-logarithmic curve. The total induction period (τ_Σ) was found graphically on the oxygen absorption kinetic curves. The induction period due to the second inhibitor was found from the difference $\tau_2 = \tau_\Sigma - \tau_1$. The total concentration of inhibitors ($[lnH]_\Sigma$), concentration of inhibitors with high antioxidant activity ($[lnH]_1$), and concentration of inhibitors with moderate antioxidant activity ($[lnH]_2$) were calculated using the values for τ_Σ, τ_1, and τ_2 (Table 3).

Results from the kinetic study showed that the content of first inhibitor in fresh oil was $(5.7 \pm 0.6)\cdot10^{-4}$ M; the rate constant for it, $k_7 = (4.6 \pm 0.2)\cdot10^6$ L/(mol·s). The k_7 value obtained by us agreed satisfactorily with the inhibition rate constant measured for α-tocopherol in a radical-chain system for styrene oxidation [$k_7 = 3.2\cdot10^6$ L/(mol·s)] at 30°C [6]. It has been reported that seed oil from this plant contains α- and γ-tocopherols totaling from 263 [2] to 351 [7] mg/kg with γ-tocopherol dominating. In our instance, the content of the fist inhibitor taking into account the molecular weight of α-tocopherol was 272 mg/kg. We observed these same tocopherols in unsaponified components of fresh oil. Thus, it can be presumed that the first induction period was due to the presence in *O. biennis* seed oil of α- and γ-tocopherols.

The inhibition rate constant of the second antioxidant was $(5.6 \pm 1.0)\cdot10^4$ L/(mol·s). This is significantly less than the first [$(4.6 \pm 0.2)\cdot10^6$ $L\cdot(mol\cdot s)^{-1}$]. Therefore, the stronger one is consumed first and then the weaker antioxidant (Table 3). The content of the second inhibitor in the seed oil was about twice that of the first and more active one (Table 3). Table 3 shows that it was present not only in fresh oil but also in that stored for 18 months at low temperature.

Based on these results it can be assumed that oxidation of lipids in *O. biennis* seed oil is inhibited by these endogenous antioxidants and that the fatty acid composition is practically constant during the whole storage period.

Tocopherols were studied using an AT6890N GC with an AT5973N mass-selective detector and a Vectra computer. Components were separated by chromatography over an HP-5MS (30,000 × 0.25 mm) column with temperature program 50–250°C, heating rate 25°C/min; 250–300°C, 5°C/min. Major components were identified from full mass spectra using a Probabalite Based Matching program.

GC of fatty acid methyl esters was performed on a GC-2014 chromatograph (Shimadzu) using an Omegawax 250 capillary column (30.0 m × 0.25 mm), polyethyleneglycol sorbent, column temperature 205°C, vaporizer 250°C, detector 260°C, He carrier gas, and flow rate 30 mL/min.

Analytical TLC was performed on Silufol plates using hexane:Et_2O (7:3, 1; 6:4, 2; 5:5, 3).

The isolation of oil from seeds and of fatty acids from oil and the preparation of methyl esters were carried out as before [8]; of unsaponified products, by the literature method [9].

Determination of Antioxidant Activity. Methyloleate (Alfa Aesar) was purified by vacuum distillation (3×) under Ar. Chlorobenzene was purified by shaking with conc. H_2SO_4, washed with water to remove acid, dried over $CaCl_2$, and distilled under Ar. AIBN was recrystallized from freshly distilled EtOH and dried in vacuo.

Kinetic experiments were carried out in a glass reactor that was charged with methyloleate (1.8 mL) and initiator in chlorobenzene (0.2 mL) and thermostatted. Seed oil from *O. biennis* (0.01–0.08 mL) was added. Oxygen absorption from the gas phase was monitored using a universal differential manometer. The measurement part of the apparatus was a differential

pressure sensor based on a high-sensitivity Si membrane element. Two reactors, one of which was the working reactor, were connected to the pressure sensor. The reactors were isolated during measurements. The membrane element and a microprocessor device were used to measure the pressure change in the working reactor and feed the data into the computer. The methyloleate oxidation rate (W) was determined from the initial portion of the oxygen absorption kinetic curve as described before [10].

Liquid-phase oxidation of methyloleate used atmospheric oxygen at 60°C. The initiation rate was calculated from the formula $W_i = 2ek_p \cdot [AIBN]$. We used the rate constant for AIBN decomposition in hydrocarbons ($k_p = 10^{15}\, e^{-127.5/(RT)}\ s^{-1}$, e = 0.5 [11]). In our experiments, the initiation rate was $6.3 \cdot 10^{-8}$ mol·(L·s)$^{-1}$.

The induction period was determined graphically from the intersection of two tangents to the kinetic curve [12]. The inhibitor concentrations [InH] were calculated using the equation

$$[InH] = \tau \cdot W_i \cdot f^{-1},$$

where *f* is the inhibition coefficient, equal to the number of chains terminated by the antioxidant. For phenol inhibitors, which usually occur in oil, *f* = 2.

The inhibition rate constant (k_7) was determined using the equation

$$[O_2] = -k_2 \cdot (k_7)^{-1} [RH] \cdot \ln(1 - t/\tau),$$

in the coordinates of which the oxygen absorption kinetic curves were processed. We used $k_2 = 4.34$ L·(mol·s)$^{-1}$, which was calculated from the ratio of constants $k_2 \cdot (k_6)^{-0.5}$ that was found by us in experiments on oxidation of methyloleate without inhibitors, for the calculations. It was assumed that $k_6 = 10^6$ L·(mol·s)$^{-1}$ [13].

The applicability of the used estimation method of the inhibiting activity of the compounds was checked using the classical oxidation inhibitor synthetic ionol. The value k_7 obtained by us for ionol was $(1.5 \pm 0.4) \cdot 10^4$ L·(mol·s)$^{-1}$, which agreed well with previous values of $1.4 \cdot 10^4$ [14] and $1.3 \cdot 10^4$ [15].

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