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INVESTIGATION BY PHOTOTHERMAL METHODS OF THE OILS FROM EVENING PRIMEROSE-SEEDS (*ONEOTHERA BIENNIS)* AND RAPESEEDS (*BRASSICA NAPUS*)

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We compare the photothermal properties of evening primerose-seeds oil with rapeseed oil. Both oils were investigated in the natural state in which some admixture of pheophytin (produced from chlorophyll) and carotenoids is present and in the bleached state. The yield of thermal deactivation of absorbed energy was obtained from steady state photoacoustic and absorption spectra. Steady state thermal deactivation in both natural oils was similar but in bleached oils was higher than in natural oils. The slow thermal deactivation of absorbed energy undergoing in microseconds time range was compared with quick deactivation using time-resolved photothermal spectroscopy. The slow component of thermal deactivation can be generated by the deactivation of triplet states as well as by the heat produced in photoreactions undergoing in investigated time range (from 0.4 μs till 5 μs). The evening primerose-seed oil exhibits more efficient slow thermal deactivation than the rapeseed oil what suggests that it is less photochemically stable than rapeseed oil. Bleaching causes the increase in the efficiency of both slow and total thermal deactivation in the oils what shows that the excitation energy is exchanged into heat rather in oil molecules than in pigment admixture.

INTRODUCTION

The changes in oil properties during storage and as a result of illumination are practical reasons for studying them intensively (Wan, 1991; Gunstrone & Norris, 1984; Porter, Caldwell & Mmills, 1995; Carlsson, Suprunchuk & Willes, 1976; Sherwin, 1976; Kowalski, Pieńkowska & Zadernowski, 1993). The presence of pigments such as pheophytin, chlorophylls and carotenoids influences autooxidation and photooxidation of the oils (Usuki, Endo & Kneda, 1984; Niewiadomski & Bratkowska, 1967). Chlorophylls and their derivative enhance the process of oil deterioration, whereas carotenoids prevent such process by quenching the singlet oxygen ($^1O_2$) (Truscott, 1990). Oils also possess other natural antioxidants (Sherwin, 1976; Brenes, Hildago, Garcia, Zamora & Garrido, 2000).

Evening primerose (*Oneothera biennis* L.) is important source of γ-linolenic acid which is applied in pharmacological and cosmetic products (Hudson, 1984). The rapeseed oil contains α-linolenic acid (Table 1). The reactions occurring in oils are investigated by means of several spectroscopic methods, but photothermal applications are scarce (Motylewski, Wiślicki, Walisiewicz-Niedbalska, Wiązowska & Kupińska, 2000). This method enables the measurements of the samples with very high absorption and scattering properties. In the present study the steady state photoacoustic spectroscopy and time resolved optoacoustic spectroscopy (LIOAS) (Braslavsky & Heibel, 1992) are applied in investigating natural and bleached oils from evening primerose-seeds (EPO) and rapeseeds (RSO).

MATERIAL

The oils were obtained from high quality biological material (seeds, 10% moisture) using a laboratory screw oil press. The seeds were not granulated and the head of the press was maintained at 70°C. The oil obtained from evening primerose-seeds (*Oneothera biennis*) will be denoted as EPO oil, whereas oil from rapeseeds (*Brassica napus* L.) as RSO oil. Fatty acids compositions of investigated oils were analyzed by gas chromatography using a Pay Unicam 4600 chromatograph with a flame ionization detector (FID). Results of this measurement are shown in Table 1. The content of γ-linoleic acid (γ- $C_{18:3}$) in EPO oil is 9.51% of weight, compared to 11.30% - as established by (Kowalski *et al.*, 1993). RSO oil lacked γ-linoleic acid. It contains the α-linoleic acid which not in all organisms can be converted into very important γ-$C_{18:3}$ (Table 1). Both types of oils contain some amount of unsaturated fatty acids ,but of course

Table 1. Fatty acids compositions of oils studied

Fatty acid ($C_{x:y}$)	Percentage content	
	EPO	RSO
$C_{16:0}$	5.81	4.44
$C_{16:1}$	0.08	0.22
$C_{18:0}$	1.71	1.11
$C_{18:1}$	5.43	62.63
$C_{18:2}$	76.08	20.62
α-$C_{18:3}$	0.15	9.01
γ-$C_{18:3}$	9.51	-
$C_{20:0}$	0.20	0.38
$C_{20:1}$	0.09	1.77
$C_{20:2}$	0.06	trace
$C_{22:0}$	0.16	trace
$C_{22:1}$	-	0.10

$C_{x:y}$ - x-number of carbon atoms, y-number of double bonds

the types of these acids and degree of unsaturation (number of double bonds) are different (Table 1). The unsaturation of EPO oil is higher than that of RSO. The fresh samples of oils were protected against uncontrolled autooxidation by storing them at 5°C in darkness in glass ampoules.

The pigment content in oils was evaluated from the sum of absorbance of diluted solution of oil in CCl_4 measured at 460nm (characteristic for carotenoids) and at 666nm (region of chlorophyll absorption). Values were multiplied by 1000 and are shown in Table 2 denoted as color. In the same Table 2 are also shown the peroxide values (POV).

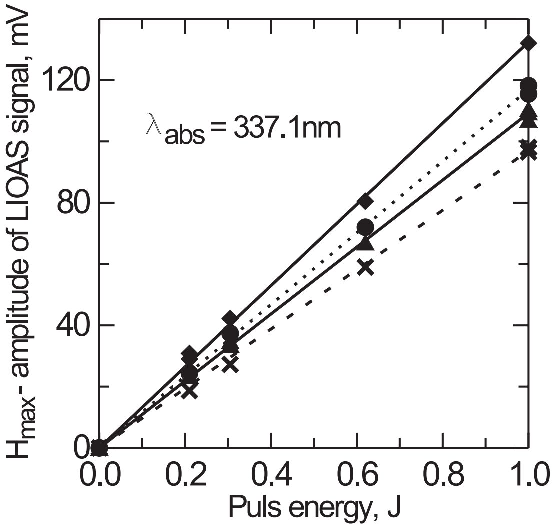

Fig. 1. The dependence of the first maximum of the photothermal LIOAS signal on the energy of the laser light. Curves up to down: 1 - RSO natural, 2 - RSO bleached, 3 - EPO natural, 4 - EPO bleached.

METHODS

The absorption spectra were measured with a Specord M40 recording spectrophotomether (Carl-Zeiss Jena, Germany).

To evaluate the part of excitation energy exchanged slowly into heat the laser induced optoacoustic spectroscopy (LIOAS) apparatus (Braslavsky & Heibel, 1992; Planner & Frąckowiak, 2001) was used. The approximation method proposed by Marti et al. (Marti, Nonell, Nicolau & Torres, 2000; Marti, Jurgens, Cuenca, Casals & Nonell, 1996) was applied, introducing further simplification necessary in investigation of very complex samples.

The part of energy exchanged into heat "promptly", it means in time shorter that time resolution of used arrangement (which is about 0.4μs — according calculation proposed in (Braslavsky & Heibel, 1992)) is denoted as α. It can be obtained from formula:

$$H_{max} = k\alpha E_{las}(1 - 10^{-A}) , \qquad (1)$$

where H_{max} is a high of first maximum of LIOAS signal, A — absorbance of sample at wavelength of

Table 2. Properties of investigated oils[a]

Sample	α/α_o	PVO	Color
EPO natural	0.82	2.00	336
EPO bleached	0.73	1.56	90
RSO natural	1.00	1.33	1103
RSO bleached	0.88	0.94	86

[a] α/α_o – relative value of parts of excitation exchanged into heat in time shorter than 0.4 µs (α_o – for natural RSO oil) POV — peroxide value, Color- the definition in text.

laser pulse (at 337.4nm), E_{las} — the energy of laser light beam, k — the coefficient related to apparatus optical geometry, electronic impedance and thermoelastic properties of sample.

The product of the yield of triplet state Φ_T and energy of triplet state E_T can be expressed (Marti, Nonell, Nicolau & Torres, 2000; Marti, Jurgens, Cuenca, Casals & Nonell, 1996):

$$\Phi_T \mathrm{E_T} = (1 - \alpha)E_{las} - \Phi_F E_F (2)\ ,$$

where α is this part of absorbed energy of the laser light (E_{las}) which is converted into heat in time shorter that time resolution of apparatus (which is about 0.4µ s according calculation proposed in (Braslavsky & Heibel, 1992), Φ_F and E_F are the yield and energy of fluorescence light. Fast thermal deactivation of excitation and fluorescence emission competes with slow thermal deactivation $(1 - \alpha)$ undergoing in time longer than 0.4 µs.

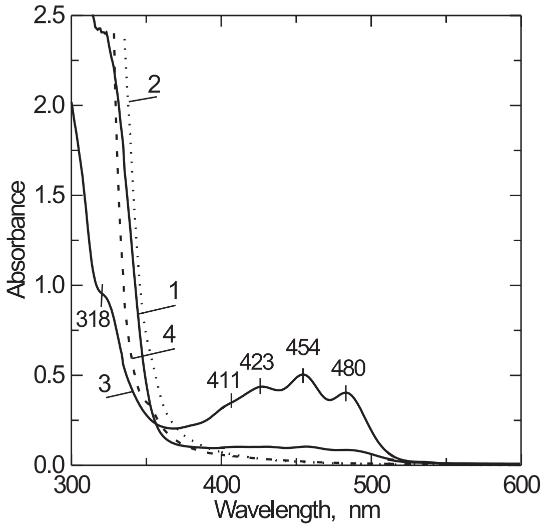

Fig. 2. Absorption spectra of investigated oils. Curves: 1 - EPO natural, 2 - EPO bleached, 3 - RPO natural, 4 - RPO bleached.

In natural pigmented sample crucial role in generation of triplet states can be played by carotenoids (Truscott, 1990). In our samples we do not know the type of molecule which is responsible for triplet state generation. To slow thermal deactivation (TD) in our sample can contribute also the heat produced in exothermic photochemical reactions undergoing in measured time range. For our samples instead formula (2) rather more general following formula can be applied:

$$\Sigma\Phi_R E_R = (1 - \alpha)E_{las} - \Phi_F E_F\ , \qquad (3)$$

where Φ_R and E_R are the yields and deactivated energies of all processes contributing to observed slow photothermal signal.

For all samples the first maximum of photothermal signal H_{max} was measured at several energy pulses of laser light changed by calibrated grey filters. For every one sample the dependence of H_{max} on laser energy is linear (Fig.1). From the slopes of these lines we obtained the ratio of values α for two samples, or to the compared amounts of energy converted “promptly” into heat. The steeper lines represent samples converting more energy in short times. It means that such samples are exchanging less energy into heat in longer times. Slow TD occurs predominantly from triplet states and photochemical reactions, because the thermal and radiative deactivations from singlet states are much faster. The relative values of fast TD are gathered in Table 2.

The systems were investigated at illumination by flashes (600 ps, 337.4 nm). At similar wavelength of excitation (331nm) was shown (Pieńkowska, Lipski & Kniat, unpublished results) that amount of energy emitted from singlet state as fluorescence of natural oil can be neglected. It makes our interpretation much easier because at the similar excitations intensities and wavelengths of emissions for natural and bleached oils were different.

Table 3. Thermal deactivation (TD) it means signal PAS divided by absorption (PAS/A) obtained from steady state PAS spectra in a.u.(TD accuracy about 0.1)

Sample	Wavelength of observation in nm		
	423_o	454	480
	Thermal deactivation		
EPO natural	0.4	0.4	0.5
EPO bleached	0.6	0.7	1.1
RSO natural	0.3	0.2	0.3
RSO bleached	0.5	0.8	1.2

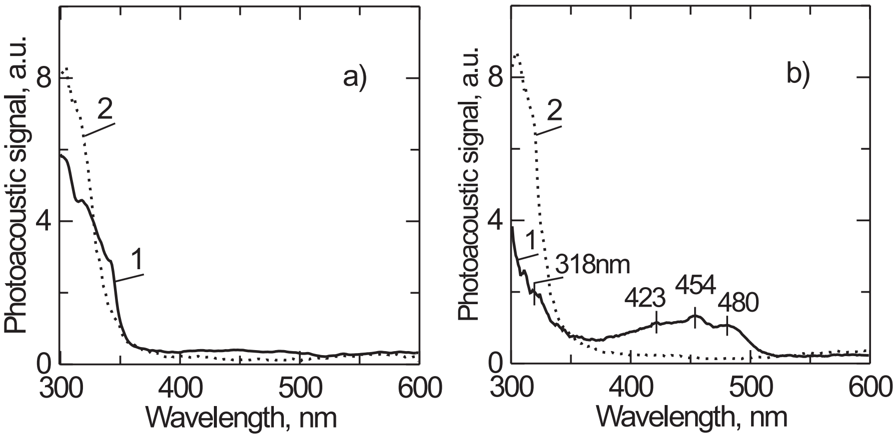

Fig. 3. Photoacoustic spectra of investigated oils: a) curves: 1 - EPO natural, 2 - EPO bleached, b) curves: 1- RSO natural, 2 - RSO bleached.

For our consideration is important that the emission of bleached oils is more intensive than that of natural samples and that it exhibits maximum at 411 nm independently on wavelengths of excitation. This maximum is probably due to some component of the oil not due to the presence of pigments.

All steady state photoacoustic spectra (PAS) were measured with a single beam photoacoustic spectrometer (Ducharme, Tessier & Leblanc, 1979; Frąckowiak, Cegielski, Niedbalska, Waloszek & Więckowski, 1996). The PAS were corrected by the division of obtained signals by carbon black photoacoustic signal making the PAS spectra independent of spectral distribution of the light source.

RESULTS

Fig.2 shows the absorption of natural (curve 1) and bleached (curve 2) EPO oil and the same data for RSO oil (curves 3 and 4). The content of carotenoids (three maxima in region of 400–500 nm) is highest in natural RSO oil. In RSO spectrum of absorption of pheophytin (about 411nm and at 670 nm – not shows in Fig.2) was found. The bleaching of oil diminishes the pigments content. Natural EPO oil contains much less pigments than natural RSO.

Fig.3 shows the steady state photoacoustic spectra (PAS) of the same set of the samples. In Table 3 are gathered the values of TD (it means the ratios of the PAS signal to absorbed energy). The values of TD are in the arbitrary units but the same for all set of samples. It is unexpected result that in a region of carotenoids absorption the bleached oil exhibit higher TD values than natural. Usually carotenoids which have very low yield of fluorescence exhibit high values of TD. It is diminished when carotenoids are engaged in efficient process of excitation energy transfer (Frąckowiak, Cegielski, Niedbalska, Waloszek & Więckowski, 1996; Truscott, 1990) or are using their excitation for some reaction.

From Table 2 and Table 3 follows that oil molecules are engaged in some photothermal reaction because the large difference in the pigment contents (Color in Table 2) gives rather low difference in TD (Table 3). It is known (Kowalski, Pieńkowska & Zadernowski, 1993) that oxidation of lipids is obviously exothermic process. The sequence of oxidative stability depends on the degree of unsaturation of oil (Sherwin, 1976; Kowalski, Pieńkowska & Zadernowski, 1993). Therefore we can expect that for bleached samples EPO will have higher TD than RSO, but the result is in a limit of accuracy similar. The pigment content diminishes the TD values (Table 3). It shows that pigments are less efficient in the exchange of excitation into heat than other molecules of the samples.

The content of various natural antioxidants such as α-tocopherol (Carlsson, Suprunchuk & Willes, 1976) and others, even not yet well knows oil ingredients (Brenes, Hildago, Garcia, Zamora & Garrido, 2000) can be in both investigated oils different but their common averaged action measured in steady state PAS in which are recorded the contributions from processes occurring with different kinetics can be similar. Therefore we decided to apply LIOAS - the time resolved photothermal method. The ratio of dH_{max} to dE_{las} is equal $k\alpha$ it means it is proportional to α in formula (1) and (2) which is a part of the absorbed energy converted into heat in time shorter than time resolution of the arrangement (0.4μs) (Marti, Nonell, Nicolau & Torres, 2000; Marti, Jurgens, Cuenca, Casals & Nonell, 1996). The factor k is the same for all lines in Fig.1. The product $\Phi_T E_T$ in formula (2) is for our samples exchanged into the sum taken for all contributing slow processes of the products of their yields and energies ($\Sigma\Phi_R E_R$) — formula (3). For complex system such as the oil we are not able to separate these contributions to $(1 - \alpha)$ which can be due to slow exothermic reactions as well as from thermal deactivation of triplet states. The molecular oxygen ground state is triplet ($^3O_2$) therefore it is of course not contributing to TD. Our observations that the bubbling of oxygen by oil samples practically has not influence on the slope of lines in Fig.1 suggest that the amount of atmospheric oxygen is high enough to run all measured processes. From fluorescence investigations (Pieńkowska, Lipski & Kniat, unpublished results) the bleached oils fluorescence is more efficient. The increase in the fluorescence yield causes the decrease in average yields of all slow processes of TD it means $(1 - \alpha)$ — formula (3). This can change a little the slope of curves 3 and 4 in Fig.1, but because of rather low yields of emission these changes are not very strong. The natural oils containing carotenoids exhibit lower exchange into heat in slow processes than bleached oils. It is reasonable because carotenoids quench the singlet oxygen responsible for several reactions. The amount of pheophytin or chlorophylls in investigated natural oils is very low, and does not cause photooxidation of the oils. The value of slow TD (the values of $(1 - \alpha)$) in EPO is higher than that in RSO (Table 2). It is in agreement in fact that oils with higher content of unsaturated acids are less photochemically stable (Hudson, 1984).

The LIOAS measurements are done after strong illumination of sample with laser pulse. In our experiments the wavelength of used laser light was 337.4 nm. In this region oil absorption is rather high comparing with absorption of pigment contamination. The laser light can start photooxidation. Part of these reactions can undergo in time range of our „slow” reactions (from 0.4 μs to 4 μs). The times of the destruction of some natural antioxidants by singlet oxygen are in μs range (Carlosson, Suprunchuk & Willes, 1976).

CONCLUSIONS

The presented results show that LIOAS results enable to compare the efficiency of destructive photoreactions undergoing in oil samples. To obtain more quantitative results we have to do some further approximation. In LIOAS, usually signal of measured dye is compared with reference dye having prompt thermal deactivation α equal one. Both sample and reference have to be in the same medium. In our case let us suppose that all excitation in natural RSO oil is exchanged into heat promptly and that all oils are non fluorescent. At such crude approximation the parts of slow deactivated energy $(1 - \alpha)$ will be in natural RSO equal zero and in bleached RSO, natural EPO and bleached EPO equal respectively: 0.12, 0.18 and 0.27 (Table 2). From these approximated values follows large difference between both investigated oils and strong influence of oil bleaching on their photochemical properties.

The fast component of TD is higher for RSO than that for EPO, what shows that slow TD processes are stronger for EPO, than for RSO (Table 2). Also POV is for EPO higher than for RSO (Table 2). As it follows from Table 1 EPO contains more unsaturated fatty acids than RSO. Some of these components have to be responsible for slow TD which can be due to their triplet states slow thermal deactivation or/and can produce heat in some exothermic photoreactions.

As it follows from Table 3 values of total TD increase with the oils bleaching and are for natural oils higher for EPO than that for RSO. The fact that the TD increases with the decrease in pigment content shows that TD is predominantly due to the thermal deexcitation of some other than pigments oil components.

The results suggest that RSO oil is photochemically more stable than EPO oil.

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