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The efficiency of the generation of photochemically active triplet states in oils containing various amounts of natural pigments

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Abstract

Absorption, fluorescence emission and excitation spectra as well as time resolved photothermal signals of oils, obtained by pressing the following types of seeds: evening primrose seeds, rapeseeds, borage seeds and viper's bugloss seeds, were measured. It was found that the oils differed in the compositions of fatty acids, acid values, peroxide values and contents of pigments. The oils contained not only various tocopherols and sterols but also different amounts of chlorophylls and carotenoid pigments. The chemical bleaching procedure used causes a decrease in the pigment content and changes acid values in the bleached oils in comparison with natural (unbleached) oils. The bleached oils exhibit predominantly fluorescence of fatty acids and products of their degradation. In natural oils, a high pigment and low fatty acid emission is observed, because of the excitation energy transfer from these acids to pigments that takes place. The ratios of fast thermal deactivations of natural oils to those of the bleached ones were different for various oils. This suggests that also the change in the efficiencies of triplet state generation due to bleaching differed for these oils. On the basis of the results presented it is possible to predict the influence of the oils' bleaching procedure on their stability during storage.

Keywords: Carotenoids; Chlorophyll-like pigment; Natural oils; Optical and photothermal spectroscopies

1. Introduction

The interest in the oils investigated has stemmed from different properties of their fatty acids enabling their diverse pharmaceutical, cosmetic, dietary and technical applications [1–5]. The vegetable, natural oils investigated are valuable dietary components and have been applied in pharmacy and for the production of cosmetics. Evening primrose oil is an important source of γ-linolenic acid, and rapeseed oil contains α-linolenic acid [1–3,5]. These acids play an important role in prevention and treatment of many diseases [1]. The process of production of oils and the conditions of their storage can cause chemical reactions of adverse effect on these acids. Especially in the presence of oxygen, vegetable oils undergo certain transformations, e.g. hydrolysis, auto-oxidation, photo-oxidation, etc. [1,2,4–6]. The practical reasons for their intense study are changes in the oil properties appearing during storage or as a result of illumination [1–6]. It is known that the presence of the natural pigments in the oil has a strong effect on auto-oxidation and photo-oxidation of oils occurring during their storage [6]. Chlorophyll-like pigments enhance oxidation, whereas the carotenoids prevent such processes by quenching the chlorophyll triplet states. Oils also contain other natural auto-oxidants and anti-oxidants [1]. The amounts of auto-oxidants and anti-oxidants in various types of oils are different [1]. Therefore, the processes of deterioration of oils depend

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on the type of oil, content of pigment and on the processes of its preparation [2,5,6].

The spectral and photochemical properties of the natural (unbleached) oil samples containing amounts of natural pigments, and bleached oils with very low pigment contents are compared. The bleaching procedure of the oil causes, not only the decrease in the amount of natural pigments, but can also destroy the oil. It is necessary therefore to establish the optimal bleaching condition that prevents oil damage.

Efficient generation of long lived triplet states enhances the reactions of oil degradation. In the present work several spectral properties, especially the ratios of efficiencies of the triplet states generation, obtained on the basis of laser induced optoacoustic spectroscopy (LIOAS), have been established for natural and bleached oils. The results enable estimation of the effect related mainly to the different pigment content in both type of oils on their stability, which can help establish proper conditions of production and storage of various oils.

2. Materials and methods

The subjects of the study were four types of oil (Table 1) obtained from the following sources: evening primrose seed (*Oneothera pradoxa* H.), rapeseeds (*Brassica napus* L.), viper's bugloss seeds (*Echium plantagineum* L.) and borage seeds (*Borage officinalis* L.). The labelling of the samples studied is shown in Table 1.

The oils to be studied were pressed from the seeds using a "Komet" expeller (model CA/59, Germany) (Table 1). Evening primrose seeds were pressed using a standard procedure applied for all other types of seeds (sample E) and on applying additional head cooling during extraction (sample EP, Table 1). Compositions of the fatty acid in the natural (unbleached) oil samples were determined by gas chromatography, using PYE Unicam 4600 apparatus with a flame-ionization detector (FID) and a computer integrator PU 4815. The results obtained are presented in Table 2. As follows from Table 2, the compositions of the fatty acids of oils obtained from different types of seeds are significantly different.

Some oil samples were chemically bleached by the addition of 4% (w/w) diatomaceous ("bleaching") earth also known as diatomite kieselgurh (Tonsil, Poland) and by heating to 40 °C. After 10 min of treatment, the earth was eliminated by the centrifugation on a centrifuge type T 24 Janetzki (15 min, 18 °C, 10 000 rotations/min).

Table 3 gives the following properties of the samples studied: acid values [11], peroxide values [12] (determined according to the International Standard—ISO) and the amount and type of natural pigments in the sample. In the field of oil technology this is referred to as "colour" [13]. The large amount of pigments, especially chlorophyll-type pigments, can cause the decrease in oil stability during storage as a result of irradiation by light. The carotenoids are able, in some extent, to prevent the photodegradation of oil. The parameter "colour" gives information about the presence of both types of

Table 1
Abbreviated notations of oil samples

Source of oil	Type of oil	Notation
Borage seeds,	natural	B
Borago officinalis L.	bleached	BB
Evening primrose seeds,	natural	E
Oenothera pradoxa H.	natural, cold press	EP
	bleached	EB
Rapeseeds, *Brassica napus* L.	natural	R
	bleached	RB
Viper's bugloss seeds,	natural	V
Echium plantagineum L.	bleached	VB

Table 2
Composition of fatty acids in oils (m, number of carbon atoms; n, number of double bonds)

Fatty acid, $C_{m,n}$	Percentage content of fatty acid in oils			
	E	R	B	V
$C_{16:0}$	5.81 ± 0.09	4.44 ± 0.02	11.04 ± 0.09	7.75 ± 0.09
$C_{16:1}$	0.08 ± 0.03	0.22 ± 0.09	0.47 ± 0.03	0.15 ± 0.03
$C_{18:0}$	1.71 ± 0.02	1.11 ± 0.02	4.83 ± 0.03	4.23 ± 0.03
$C_{18:1}$	5.43 ± 0.08	62.63 ± 0.02	18.97 ± 0.05	16.04 ± 0.05
$C_{18:2}$	76.08 ± 0.05	20.62 ± 0.09	35.80 ± 0.08	15.52 ± 0.05
$C_{18:3}$	0.15 ± 0.05	9.01 ± 0.02	trace	31.75 ± 0.08
γ-$C_{18:3}$	9.51 ± 0.03	–	21.18 ± 0.04	10.70 ± 0.02
$C_{18:4}$	–	–	–	12.61 ± 0.02
$C_{20:0}$	0.20 ± 0.05	0.38 ± 0.05	0.31 ± 0.03	–
$C_{20:1}$	0.09 ± 0.01	1.77 ± 0.01	4.17 ± 0.29	–
$C_{20:2}$	0.06 ± 0.01	trace	trace	–
$C_{22:0}$	0.16 ± 0.02	trace	0.19 ± 0.02	–
$C_{22:1}$	–	0.16 ± 0.01	2.31 ± 0.16	0.71 ± 0.05

$x \pm$ SD, where SD is standard deviation at 95% confidence level for $n = 5$, notations of oil samples as in Table 1.

Table 3
Properties of oils (definition of "colour" value is described in text, notations of oil samples as in Table 1)

Type of sample	Acid value[a]	Peroxide value[a]	Colour[b]
B	3.11 ± 0.07	2.15 ± 0.03	1156 ± 10
BB	2.83 ± 0.02	2.01 ± 0.06	414 ± 6
E	1.72 ± 0.04	1.25 ± 0.06	558 ± 8
EB	2.00 ± 0.04	1.22 ± 0.06	162 ± 5
R	1.71 ± 0.01	2.21 ± 0.09	1201 ± 10
RB	1.53 ± 0.01	1.45 ± 0.04	369 ± 8
V	1.52 ± 0.04	1.55 ± 0.06	933 ± 9
VB	0.92 ± 0.02	1.51 ± 0.02	132 ± 5

[a] $x \pm$ SD, where SD is standard deviation at 95% confidence level for $n = 4$.

[b] $x \pm$ u, where u is uncertainty that represents the combined effects of possible systematic and imprecision errors.

pigments but not about their influence on oil stability. The technologists are looking for the correlation of such a parameter with the oil properties without taking into account any photochemical processes involving the pigments.

The sample "colour" is calculated for oil samples diluted in CH_4 (in ratios (1:1, v:v) and (1:10, v:v) respectively, for estimation of chlorophyll and carotenoids absorbance in oil) by adding the absorption characteristic of chlorophyll-type pigment at 666 nm to that in the region of carotenoids (at 460 nm) and multiplying by 1000 [13]. As follows from Table 3, the bleaching procedure causes, as expected, a decrease in the pigment contents that is shown as a decrease in "colour" values. Additionally, changes in the sample acid values are observed whereas the peroxide values were less sensitive to the bleaching treatment (Table 3).

The absorption spectra were measured on a Specord M40 (Carl Zeiss, Germany). Fluorescence emission and excitation spectra were recorded by means of a Fluorescence Spectrophotometer F4500 (Hitachi, Japan).

The ratios of the excitation energy exchanged slowly into heat (TD_s) in natural (unbleached) and bleached oils were compared using the LIOAS method [7,8]. The TD_s values are related to the efficiency of the triplet state generation [7]. The samples were illuminated by nitrogen laser (337 nm, flash duration 0.2 ns). Usually as a reference sample a dye that exchanges whole absorbed energy into heat in time shorter than the time resolution of apparatus was used. The method of LIOAS signal analysis proposed by Marti et al. [9,10] was applied, with further approximations necessary when studying our complex samples. According to Marti et al. [9,10] the part of energy exchanged promptly into heat, that is in a time shorter than the time resolution of the experimental setup used (which for our apparatus was estimated as about 0.5 μs [7]) is denoted as α. It can be obtained from the formula:

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

where $H_{\max}$ is the height of the first maximum of the LIOAS signal, A, the investigated (or reference) sample absorbance at the laser pulse wavelength (at 337 nm), E_{las}, the energy of laser light beam, and k is the coefficient related to the optical geometry, electronic impedance and thermoelastic properties of sample. The coefficient k is usually eliminated by the measuring LIOAS signal for the reference sample whose total energy is promptly deactivated [7–10]. Unfortunately, for our purpose such an approach is unsuitable, therefore, the $H_{\max}$ values for natural (investigated) and bleached (reference) oil samples for the known E_{las} and A were measured. Then the appropriate equations, based on Eq. (1), for natural and bleached samples were divided to calculate the ratio of the promptly deactivated energies in the samples of natural (unbleached) (UB) and bleached (BL) oils: $\alpha_{\text{UB}}/\alpha_{\text{BL}}$. When this ratio is lower than one, the efficiency of the prompt deactivation increases as a result of oil bleaching (mainly related to the decolourization of oil), which means that the efficiency of the slow processes related to possible oil destruction decreases for bleached samples (with low pigment content). In this case, for the ratio being lower than 1, the bleaching procedure related to the removal of pigment will improve the oil stability during storage.

Absorption and fluorescence spectra as well as LIOAS signals were measured in 5% (v/v) oil solution in *n*-hexane.

3. Results

In order to establish the contents of various components present in natural (unbleached) and bleached oils the absorption and fluorescence spectra of samples were measured. Absorption in the region 300–350 nm (Fig. 1) is related to oxidized, polar components of oils, being the products of oil degradation [14]. This region comprises the absorption bands of various tocopherols (reported maxima of absorption at 298, 302, 304 and 306 nm [15] and fluorescence at about 345 nm) and sterols (absorption at 315 nm, emission at 365 nm). Exact positions of the maxima depend on several factors such as the molecule's conformation, the molecule's interactions with surroundings, etc. [16]. Moreover, it

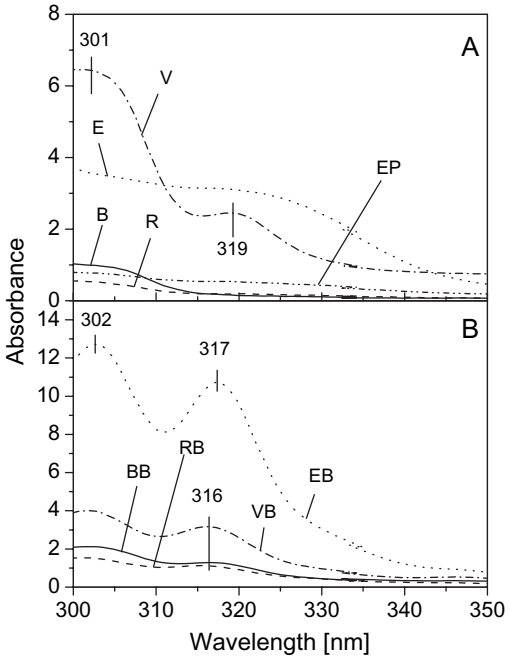

Fig. 1. Absorption spectra of natural: V, B, R, E, EP, R (A) and bleached: VB, BB, RB, EB (B) oils in *n*-hexane (5%, v/v) (notations of oil samples as in Table 1). Measured in a region of predominant absorption of oil components; absorption was recalculated on 1 cm optical path.

should be taken into account that hexane, used for oil dilution, also exhibits some absorption at 295 nm with fluorescence at 326 and 600 nm. Several products of fatty oil degradation emit fluorescence [17] and phosphorescence [18].

For the oils studied the absorption in the region 300–350 nm tends to change as a result of oil bleaching (Fig. 1A, B and Table 4) which shows that the process of oil bleaching involves some processes of oil degradation. It suggests that bleaching causes the degradation of fatty acids of oils [5]. The stability of lipids depends on the degree of oil unsaturation (Table 2) [2,19]. It can be expected that this process will be more efficient for bleached than for natural oils, however, it may not be so, because the influence of the pigments on oil stability depends on the amount and proportion of carotenoids and chlorophyll-type pigments and their interactions. Carotenoids can work as natural anti-oxidants. The degradation due to bleaching is stronger for E, EP and R samples, while weaker for V and B oils (Table 4). The degree of the changes in absorption in this region is different for different oils, which is in agreement with other effects of bleaching in the oil studied, as indicated in Table 3. The maxima at about 302 nm and about 320 nm are related to tocopherols and sterols as well as probably to some products of the oxidation of lipids. This process is exothermic. Similar maxima had been observed earlier [5] in the steady state photoacoustic spectra (PAS) of E and R oils.

The change in "colour" (Table 3) is predominantly related to the content of the pigments. Even for natural samples (Fig. 1A) the absorption in the fatty acids' components and their products (300–350 nm region) is much greater than that in the pigments' absorption range (350–700 nm) (Fig. 2A). The differences related to both the short and long wavelength regions are clearly seen by the comparison of the absorption spectra of bleached (Figs. 1B and 2B) and natural (Figs. 1A and 2A) oils.

The absorption spectra measured in 300–350 nm region are practically independent of temperature in the temperature range 5–20 °C (Fig. 3), but in the range due to pigment absorption, in the oxygen atmosphere the spectra exhibit stronger changes than in air atmosphere.

Table 4
The ratio of absorbances of bleached (A_{BL}) to natural (A_{UB}) oils calculated at 302 and 320 nm wavelengths (notations of oil samples as in Table 1)

Type of oil	A_{BL}/A_{UB}	
	λ = 302 nm	λ = 320 nm
B	1.7248	1.1068
V	0.6218	1.2881
E	1.9230	3.4045
EP	9.4204	8.4396
R	2.5395	6.4172

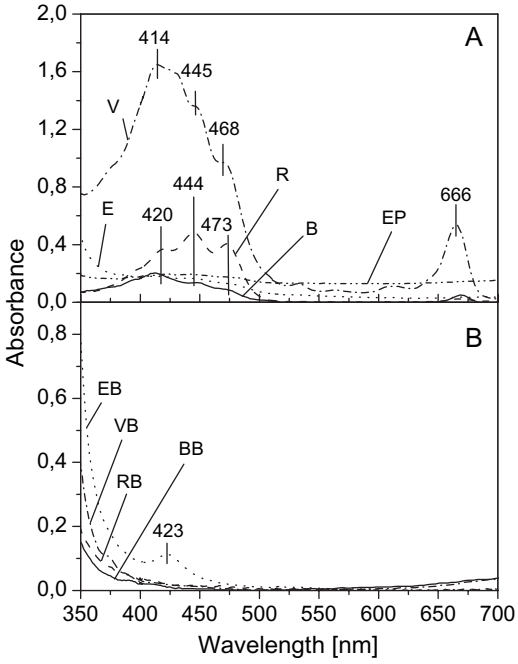

Fig. 2. Absorption spectra of natural (A) and bleached (B) oils in *n*-hexane (5%, v/v) measured in a region of pigments' absorption (notations of oil samples as in Table 1, absorption was recalculated on 1 cm optical path).

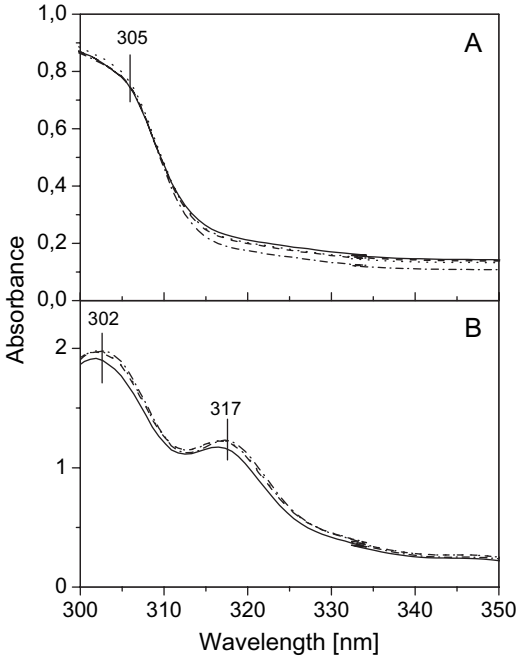

Fig. 3. Absorption spectra for natural (A) and bleached (B) oils from borage seeds (B and BB, natural and bleached, respectively) measured at various temperatures: solid line (20 °C), dashed (15 °C), dotted (10 °C) and dashed–dotted (5 °C). Oil sample diluted in *n*-hexane (5%, v/v); absorption was recalculated on 1 cm optical path.

Table 5
Quantum yield of fluorescence (Φ_F) of B and BB oils at different temperatures and in various atmospheres (λ_{exc} = 337 nm, the accuracy of Φ_F values ± 0.005, notations of oil samples as in Table 1)

Temperature (°C)	Atmosphere	B	BB
20	O_2	0.019	0.037
15		0.021	0.043
10		0.025	0.040
5		0.027	0.032
20	N_2	0.021	0.036
15		0.022	0.035
10		0.023	0.035
5		0.021	0.047
20	air	0.029	0.046
15		0.029	0.042
10		0.030	0.044
5		0.030	0.046

Table 6
The ratio of coefficients (α) related to the fast thermally deactivated energy and fluorescence yield (Φ_F) for natural (UB) and bleached (BL) oils (notations of oil samples as in Table 1)

Type of oil	Atmosphere	α_{UB}/α_{BL}	$\Phi_{F\ UB}/\Phi_{F\ BL}$
B	air	0.99	0.63
	N_2	1.21	0.59
V	air	0.41	0.75
	N_2	0.27	0.69
EP	air	0.21	1.71
	N_2	0.28	1.18

The absorption ratio of A (414 nm)/A (668 nm), in N_2 atmosphere at 20 °C, is about 13, whereas at 5 °C it is about 18. The chlorophyll-type pigments' absorption increases with decreasing temperature. This effect is superimposed by the changes in the light scattering, but it seems that the decrease in temperature causes changes in the pigment–lipid interactions and in the pigment interaction with gas molecules. These effects can influence the pigment absorption coefficient and the pigment degradation by oxygen, and also chlorophyll-type pigment fluorescence intensity (Table 5).

The fluorescence spectra excited in the region of oil component absorption (at 337 nm) (Fig. 4) show that the emission in the 350–600 nm region is lower for the natural (Fig. 4A) than the bleached samples (Fig. 4B) and also the emission in the 600–800 nm pigment fluorescence region is observed (Fig. 4C, D). This means that the excitation energy is transferred from the oil components to the pigment. The excitation transfer is lower for the samples containing less pigments (Fig. 4D) than for the coloured samples (Fig. 4C). The same conclusion follows from Table 5. The yield of fluorescence of B and BB oils excited at 337 nm, measured at the emission maximum (for natural B oil at 373 nm, for bleached BB oil at 405–409 nm) by a reference to the coumarine emission of the known yield of fluorescence was estimated [20] (Table 5). The intensity of the excitation light, at configuration of fluorescence spectrophotometer used, depends on optical density of samples. The necessary corrections of observed emission intensities were introduced before the calculation of the fluorescence yield (Table 6). The fluorescence yield of the bleached samples is higher than for the natural ones (Table 5) independent of the temperature and atmospheres used. Unexpectedly, the fluorescence yields in the air atmosphere are higher than in nitrogen, which suggests that some products of oxidation are efficiently fluorescent.

On excitation at 414 nm (Fig. 5) the emission observed at 669 nm is much more intensive for natural oils in the long (600–800 nm) (Fig. 5C) than in the short

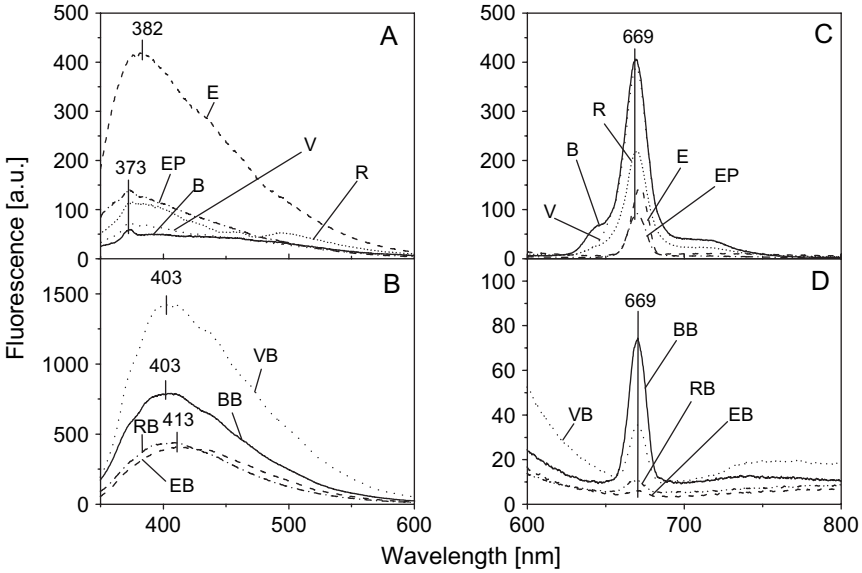

Fig. 4. Fluorescence spectra (λ_{exc} = 337 nm) of natural (A, C) and bleached (B, D) oils in *n*-hexane (5%, v/v) (oil notation—Table 1).

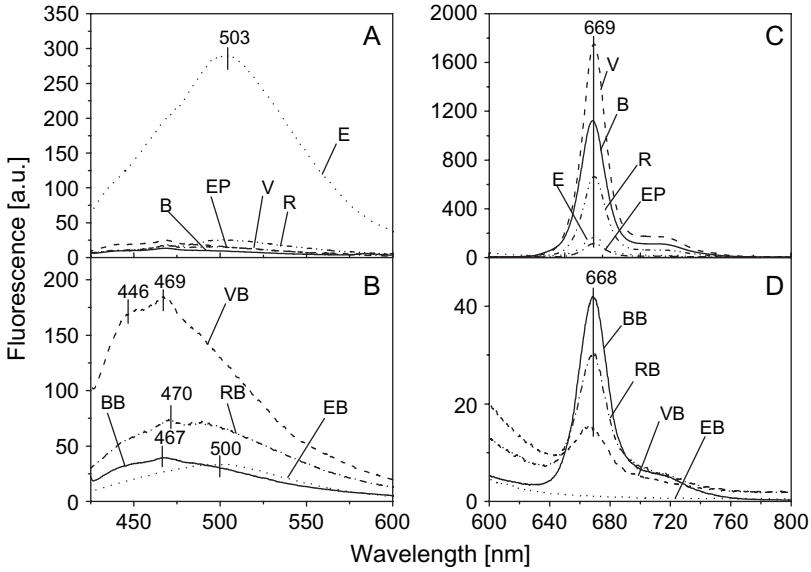

Fig. 5. Fluorescence spectra (λ_{exc} = 414 nm) of natural (A, C) and bleached (B, D) oils in *n*-hexane (5%, v/v) (notations of oil samples as in Table 1).

(425–600 nm) wavelength region corresponding to the emission of oil components (Fig. 5A). As follows from Fig. 2A and literature data, the absorption of pheophytins (414, 666 nm), chlorophylls (430, 662 nm) and carotenoids (425, 448, 476 nm) [21,22] can contribute to these spectra at the excitation wavelength used. The yield of the carotenoids' fluorescence is very low, therefore the emission observed at 669 nm is predominantly due to pheophytins and/or chlorophylls.

The fluorescence excitation spectra (Fig. 6) recorded in the oil emission (at 500 nm) and in pigment fluorescence (at 670 nm) ranges, differ in shape from the absorption spectra of the same samples (Figs. 1 and 2), which suggests that the samples contain components characterized by different yields of fluorescence. It has been found that the fluorescence excitation spectra of the bleached samples (Fig. 6B, D) differ from those of unbleached ones (Fig. 6A, C). A comparison of the fluorescence excitation spectrum of sample E (obtained without additional cooling during pressing) with that of sample EP (extra cooled during extraction) shows that the heating of oil during the pressing procedure strongly influences its properties. As a result of the bleaching procedure, the intensity of the bands at 349 nm, occurring

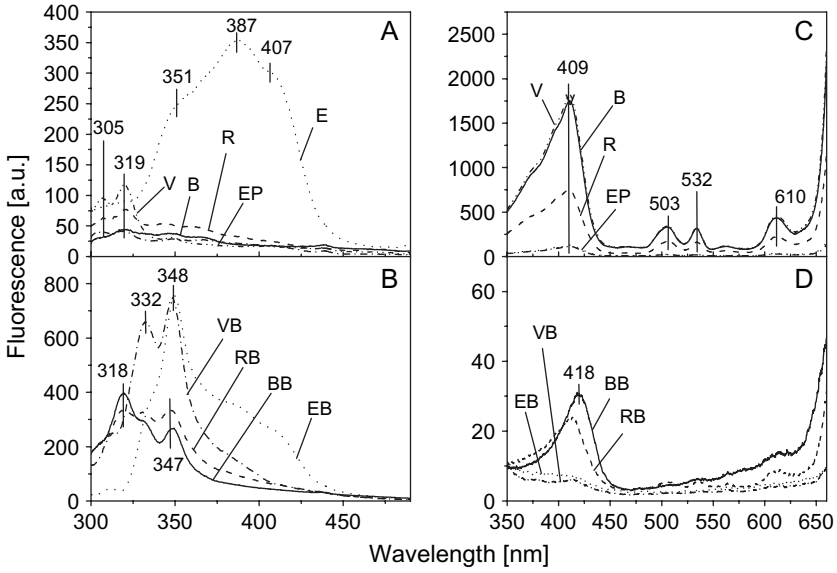

Fig. 6. Fluorescence excitation spectra of natural oils (A, C) and bleached oils (B, D). Observation wavelengths (λ_{obs}): (A, B) 500 nm, (C, D) 670 nm. Oils diluted in *n*-hexane (5%, v/v) (notations of oil samples as in Table 1).

also in the absorption spectrum of unbleached oil, significantly increases.

The calculated ratios of relatively fast thermally deactivated energy (α) for natural and bleached samples, established using the LIOAS method proposed by Braslavsky and Heibel [7] in the simplified Marti et al. [9,10] approximation, are shown in Table 6. The waveform LIOAS signal was measured for UB and BL samples. The ratio of the first maximum H_{UB} to H_{BL} can be obtained from the formula:

$$H_{UB}/H_{BL}=(\alpha_{UB}/\alpha_{BL})(E_{lasUB}/E_{lasBL})(1-10^{-A}_{UB})/(1-10^{-A}_{BL}) \quad (2)$$

where α_{UB} and α_{BL} are the parts of energy deactivated promptly, i.e. in time shorter than time resolution of the apparatus (0.5 μs). The ratio of these coefficients (α_{UB}/α_{BL}) gives the ratio of the fast thermal deactivations of energies of natural and bleached samples ($TD_{f\,UB}/TD_{f\,BL}$). This ratio gives the opportunity to evaluate the ratio of the yields of the triplet state generation in the natural (unbleached) (UB) and bleached (BL) oils. In most cases the TD_f of bleached oil is higher than that of the natural one (Table 6). This suggests strongly that bleaching causes the decrease in the efficiency of triplet state generation, and therefore should improve oil photochemical stability during storage. The ratio of TD_f of pigmented oil to TD_f of bleached oil differs significantly for different oils. The non typical behaviour is exhibited only with oil B in which, after bleaching, the content of chlorophyll-like pigment is still rather high (Figs. 4 and 5). For this oil the bleaching has a small influence on the efficiency of triplet state generation (α_{UB}/α_{BL} about one unit, Table 6). The ratios of fluorescence yield (Φ_F) of unbleached to the bleached samples are different for various types of oils (Table 6). This ratio depends not only on the contents of various pigments but also on the presence of products of oil degradation (Tables 3 and 4). From our results we can predict that the stability of the oils studied during storage can be improved by bleaching (caused mainly the oil decolourization). One can expect that the improvement of oil stability caused by bleaching in samples of EP and V oils is stronger than in oil B (Table 6). The influence of air or nitrogen is complex, because in the crude approximation used, we neglect the deexcitation by fluorescence and delayed luminescence emission, whereas oxygen also affects such radiative transitions. In the approximation assumed we are not able to calculate separately the absolute values of the efficiencies of triplet state generation for every sample studied. This will be done in the subsequent study by measuring the LIOAS signal with respect to a reference sample with a known, practically prompt, deactivation of excitation [7]. For such results it will be possible to analyze the LIOAS signals directly by the methods [9,10,23] and to obtain the yields of slow TD_s and the decay times of the TD_s components related to triplet states and eventually to some slow chemical exothermic reactions.

4. Conclusions

1. The properties of various oils change differently as a result of bleaching.
2. Bleaching procedure changes not only the amount of natural pigments present in oil but also causes the degradation of some other oil components.
3. For most types of oils the decrease in natural pigment content due to the oil bleaching procedure causes the decrease in the generation of the photochemically active triplet states, therefore it probably helps to improve the oil photochemical stability.

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