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The photochemical stability of oil from Evening Primrose seeds

Izabela Hanyż [a], Halina Pieńkowska [b], Alina Dudkowiak [a], Danuta Frąckowiak [a,*]

[a] Faculty of Technical Physics, Institute of Physics, Poznań University of Technology, Nieszawska 13a, 60-965 Poznań, Poland
[b] Institute of Technical Development, Warmia and Mazury University, Okrzeji 1a, 10-256 Olsztyn, Poland

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Abstract

Photochemical reactions taking place in oil to which was added chlorophyll *a* as well as β-carotene and subjected to illumination for different times, have been investigated by measuring the absorption, fluorescence emission and fluorescence excitation spectra. The addition of carotene was found to greatly lower chlorophyll degradation as a result of the quenching of chlorophyll triplet states; however, its presence also influence the generation of other oil photoproducts. A comparison of the photoreactions induced by the light absorbed predominantly by chlorophyll-like pigments with those induced by bright light covering practically the whole visible region implies that the majority of the photoreactions occur with the participation of excited chlorophyll molecules.

The singlet excitation energy of β-carotene is not transferred to chlorophyll. The quenching of photochemically active triplets of chlorophyll alters the extent of oil degradation imparted by this excitation. The absorption and emission spectra of the oil degradation products are superimposable and, therefore, the quantitative analysis of the products is not easy. The generation of malonic dialdehyde, which is the main product of oil degradation, is a little slower in the presence of carotenoids, which suggests that some components within the oil protect against photooxidation by the addition of carotenoids.

Keywords: β-Carotene; Chlorophyll-like pigment; Natural oils; Optical spectroscopy

1. Introduction

The oil obtained from Evening Primrose seeds is a source of γ-linoleic acid and, therefore, it enjoys widespread use in pharmacy and in the production of cosmetics [1,2]. The oils contain chlorophylls and carotenoids. The process of oil production, especially its bleaching, can cause several chemical reactions in the oil components, such as the degradation of γ-linoleic acid which is known to play an important role in the prevention and treatment of several diseases [1]. The bleaching process is carried out in order to lower the content of natural pigments in the oil and improve its photochemical stability. The photochemical role of the various pigments is different, in so far as chlorophyll-like pigments enhance the photodegradation of the oil components, whereas carotenoids diminish such processes by quenching the chlorophyll (Chl) triplet states [3], which are very active in photochemical reactions [3–5]. The carotenoids protect Chls against photooxidation, predominantly by quenching their triplet states; they also influence the Chls aggregation [6]. The interactions between these pigments depend on the aggregation of molecules and their mutual orientations [7–9]. The singlet excitation energy of carotenoids is transferred to Chls only in biological systems where both pigments are combined with biological macromolecules [9–11], whereas such a process in solution is usually inefficient [7,8]. The purpose of this study was to determine whether the efficiency of the oil degradation processes such as autooxidation and photooxidation, occurring on

* Corresponding author. Tel.: +48 61 665 3180; fax: +48 61 665 3178.
E-mail address: frackow@phys.put.poznan.pl (D. Frąckowiak).

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oil storage, could be reduced by changing the ratio of Chls/carotenoids (Cars) in the oil. The photooxidation of oil components occurs usually by the interactions of Chls in the triplet state directly with the surroundings and/or by the generation of photochemically active singlet oxygen. In this paper an attempt was made to improve oil stability by the addition of β-carotene without bleaching. To induce oil photodegradation, laser light absorbed predominantly by Chl-like pigments and xenon lamp light which covered the whole visible region, were used. Thus, it was possible to distinguish between the photoreactions occurring with Chl participation and those reactions due to illumination of other oil ingredients. The absorption, fluorescence emission and fluorescence excitation spectra of: (i) the natural (unbleached) oil diluted with chloroform, (ii) the same oil in chloroform solution pigmented by the addition of Chl *a* or (iii) by Chl *a* and β-carotene (Car) mixture were measured after different times of illumination. The kinetics of the reactions showing the increase in the amount of oil degradation products and in the bleaching of the pigments added as a function of illumination time were established.

2. Material and methods

The pressing of the Evening Primrose seeds and the establishment of the fatty acids composition in the oil were as described earlier [3]. For these experiments, the natural (unbleached) oil was diluted in chloroform (1/9, v/v). Chl *a* was obtained from dried nettle leaves using a standard chromatographic isolation method [4], β-carotene was purchased from Fluka AG. The concentrations of pigments in the samples were established spectrophotometrically by comparison of the oil absorption spectrum with known absorption spectra of dyes.

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 samples were illuminated separately by one of the two light sources. The first one was a high-pressure xenon lamp having almost a flat characteristic in the spectral region from 300 to 800 nm [5]. The measured light intensity of this lamp was 24.4 mW/cm^2. The second light source was a semiconducting laser SN 208990167 produced by Optel (Poland) emitting at 632.8 nm with the 8.3 mW/cm^2 intensity.

Fluorescence spectra were calculated for the same excitation energy, taking into account that in a mixture of both pigments added to oil, at the excitation wavelengths part of light is absorbed by Car, therefore, the amount of quanta absorbed by the Chl molecules decrease. In the calculations performed it was assumed that in solution the singlet excitation of Car is practically not transferred to Chl [6,7]. Accordingly, the yield of Chl *a* fluorescence should be approximately the same in the solution of Chl *a* alone and in the solution of both pigments (because the Car emission can be neglected) [6–8]. In such a situation the fluorescence intensity of Chl *a* can be calculated from the formula describing the ratio of the fluorescence yields of the two pigments dissolved in different solutions of different absorbencies [12]. In the calculations it was assumed that the absorbance of the oil diluted in chloroform is very low and therefore could be neglected, and the absorbance of Car in the solvent is denoted as μ_2, whereas the absorbance of Chl *a* as μ_1. In this approximation under additional supposition that the yield of Chl *a* fluorescence in both samples was the same, the real intensity of Chl *a* fluorescence (F) was obtained from the following simple formula:

$$F = F_m\left(1+\frac{\mu_2}{\mu_1}\right) \quad (1)$$

where F_m is the fluorescence intensity measured for the Chl and Car mixture in the oil solution.

The concentrations of Chl in both samples have to be equal or at least the fluorescence intensities have to be recalculated for the same number of Chl *a* molecules in the same sample volume. When obtaining similar Chl *a* fluorescence intensities in both samples, without and with Car addition, we can suppose that the simplifications used are at least approximately valid.

From the spectral data obtained at various times of illumination, using different wavelengths of emission detection for the fluorescence excitation spectra and various wavelengths of excitation for the fluorescence emission spectra, some conclusions concerning the stability of the oil components and the added pigments can be drawn. The emission spectra were measured in the region from 300 to 900 nm, while the fluorescence excitation spectra were from 250 to 800 nm.

The most interesting were the kinetics of the oil products generation related to the photodegradation and oxidation processes occurring in the presence of different amounts of Chl-like and carotenoids types of pigments added. These results were collected from a short wavelength spectral region. Analysis of such products separated by the chromatographic methods is usually performed by different spectral methods [1–5,13–17].

3. Results and discussion

Fig. 1 shows the absorption of the samples studied of the oil dissolved in chloroform without or with pigments added. The absorption spectra were recorded in two regions: 300–380 nm (Fig. 1A) and in 350–800 nm

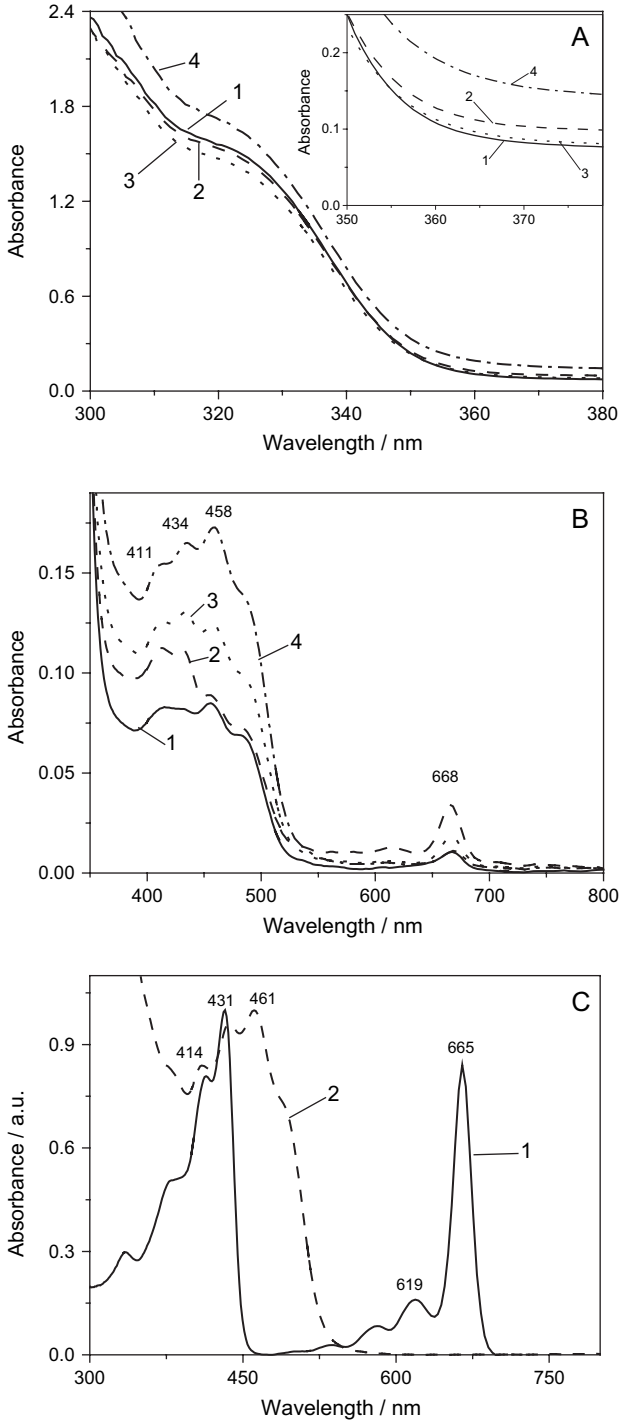

Fig. 1. (A, B) Absorption spectra of unbleached oil from Evening Primrose seeds in chloroform (1/9, v/v) without pigment addition (curves 1), with Chl *a* addition ($c = 3.6 \times 10^{-7}$ M) (curves 2) and with Chl *a* ($c = 2 \times 10^{-7}$ M) and Car ($c = 3 \times 10^{-7}$ M) (curves 3) with Car alone ($c = 6.6 \times 10^{-7}$ M) (curves 4); (C) the normalized absorption spectra of Chl *a* (curve 1) and Car (curve 2) solutions in chloroform.

(Fig. 1B). In the first of these regions the spectra reveal signals of the oil components and the products of its degradation [13–17], the second region contains the signals assigned to the absorption and possible photodegradation products of the pigments investigated [4,5]. The absorbencies of the oil components measured in the short wavelength region (Fig. 1A) are much higher than that observed in the long wavelength range (Fig. 1B). To facilitate the interpretation, Fig. 1C shows the normalized absorption spectra of both pigments, Chl *a* and β-carotene (Car) dissolved in chloroform. A comparison of Fig. 1C with Fig. 1B shows that the shapes of the absorption spectra of both pigments are not dramatically changed as a result of the oil addition. The concentrations of the pigments added to the oil were established spectrophotometrically. These concentrations were of an order of 10^{-7} M and because of them being so low the pigments aggregation can be neglected. As followed from Fig. 1A, the addition of the pigments has practically no effect on the oil absorption spectra measured in this region. It means that in the dark and in a short time the pigments do not cause additional oil degradation. Only small changes in the spectrum are observed in 350–380 nm region due to the Car absorption (Fig. 1A).

Fig. 2 shows the fluorescence excitation spectra of the oil without and with Chl *a* and Car added. A comparison of these spectra (Fig. 2A–C) with the absorption spectra (Fig. 1A, B) reveals significant differences. As the shapes of fluorescence excitation spectra depend on the wavelength of fluorescence observation, it can be concluded that complex processes of excitation energy migration between different chromophores occur. Particularly interesting are the fluorescence excitation spectra in the short wavelengths regions (Fig. 2A, B). In these regions the signals of the products of the oil degradation can be observed.

At the 364 nm observation wavelength (predominant emission of sterols, several lipid peroxidation products and unsaturated oil acids from exhydroperoxylinoleic acid [13–17]) a strong maximum of the emission excitation assigned to the sterols absorption appears at 315 nm (Fig. 2A). This emission is lower when the concentration of Chl is high and the content of Car is low. It increases with increasing Car concentration (Fig. 2A, curves 3 and 4), which suggests the occurrence of some process of the excitation energy transfer (ET) from several oil components to sterols, but participation of some carotenoids cannot be excluded. The presence of Chl *a* probably perturbs this transfer because an increase in its amount causes a decrease in the peak at 315 nm, whereas the presence of Car reduces this effect.

An emission signal is also observed at 507 nm (Fig. 2B), in the region of the fluorescence emission of malonic dialdehyde (MDA) being a product of several photochemical reactions of the oil degradation. This spectrum shows the following absorption maxima: at 315 nm (sterols), 360 nm (Chl) and, of course, at 388 nm (MDA) and 411 nm (pheophytine *a* (Phe)). These signals evidence the occurrence of the excitation transfer ET between different oil components, including also the

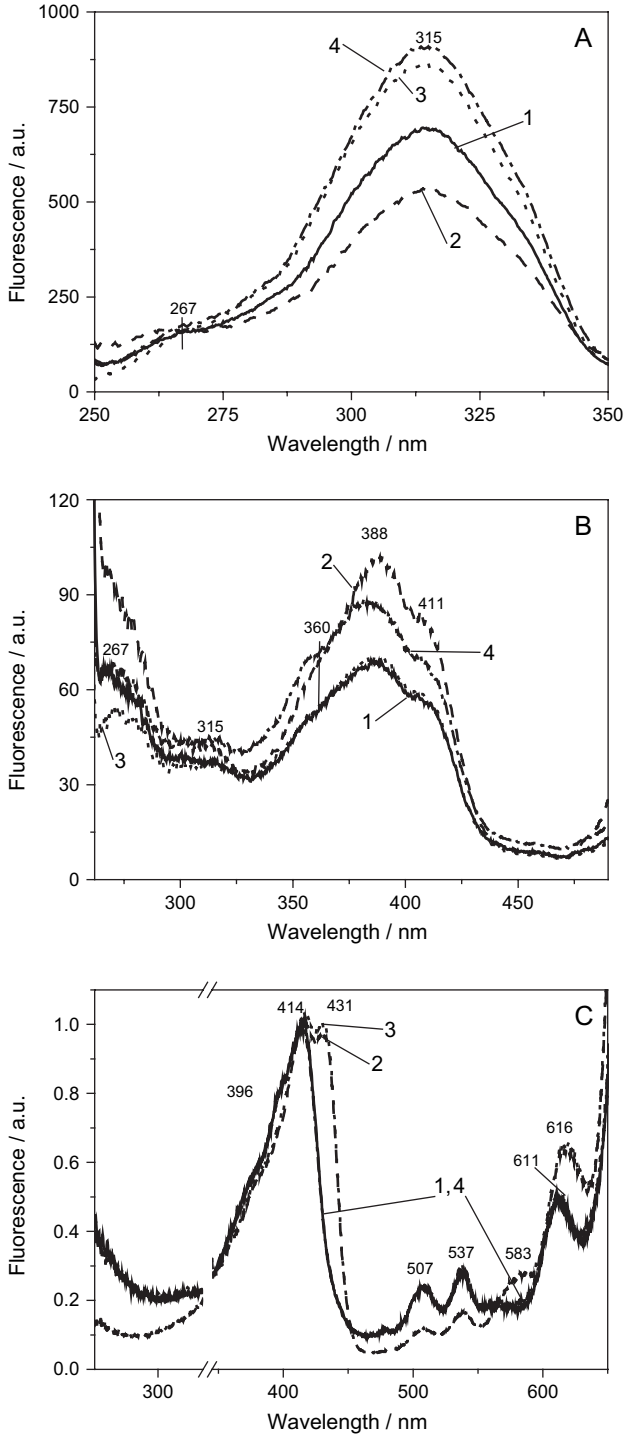

Fig. 2. Fluorescence excitation spectra of oil from Evening Primrose seeds in chloroform (1/9, v/v) (curves 1), with Chl *a* addition (curves 2) and with Chl *a* + Car mixture (curves 3) with Car alone (curves 4). Observation wavelengths (λ_{obs}): (A) 364 nm, (B) 507 nm, (C) 668 nm. (C) normalized at 414 nm (pigments concentrations as in Fig. 1).

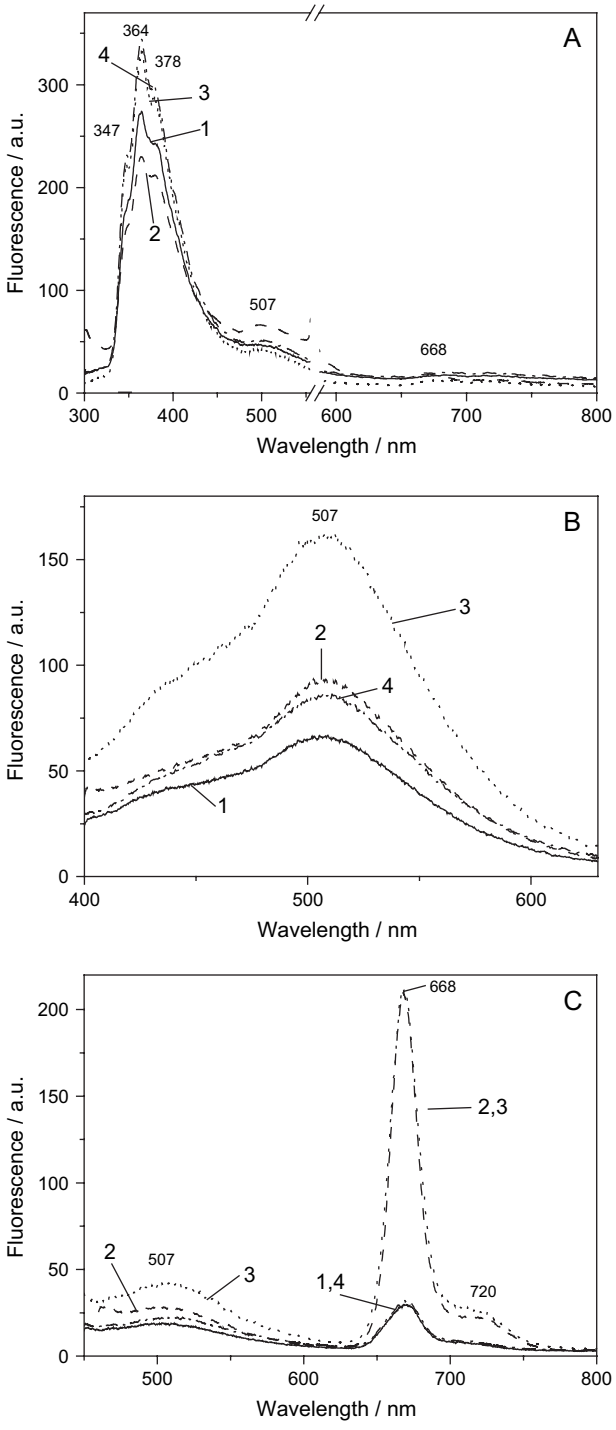

Fig. 3. Fluorescence spectra of oil from Evening Primrose seeds in chloroform (curve 1) with Chl *a* (curve 2) and with Chl *a* and Car (corrected on Car absorption, curve 3). Excitation wavelengths (λ_{exc}): (A) 287 nm, (B) 376 nm,(C) 431 nm (solvents ratio and pigments concentrations as in Figs. 1 and 2).

pigments added. Chl *a* addition causes an increase in the MDA emission, whereas Car addition reduces this increase. It suggests some participation of Chl triplets in the increase in MDA production. Fig. 2 also implies that the Chl added to the sample is to high degree pheophytinized, whereas the Chl originally present in the oil is more stable.

The fluorescence excitation spectra observed at 668 nm (at Chl *a* maximum emission) are normalized at 414 nm (maximum of the Soret band) (Fig. 2C).

The addition of Car does not give any additional maxima (e.g. at 461 nm) which could be due to the ET processes from Car to Chl-like pigments. It shows again that our supposition about the lack of transfer of the singlet Car excitation energy to Chl is fulfilled. The spectrum recorded at the 668 nm observation wavelength that is in the range of Chl *a* and Phe *a* emission shows only some maxima of absorption of these pigments. The excitation spectrum, observed at 720 nm in the fluorescence region of Q_y (0,1) Chl transition (not shown), has a character similar to that of the spectra shown in Fig. 2C, but of course the intensities are lower.

Fig. 3 shows the fluorescence spectra recorded at the 287 nm excitation, i.e. in the absorption region of MDA and other products of lipid peroxidation [13–17] (Fig. 3A), at 376 nm (absorption of polymerized MDA and lipids autooxidation and photooxidation products [13–17]) (Fig. 3B) and at 431 nm (absorption of Chl Soret [5]) (Fig. 3C). At 287 nm observation the maxima at 347, 364 and 378 nm assigned to triaenes, tetraenes and pentaenes lipid peroxidation products are observed. These maxima strongly overlap. Chl addition causes a decrease in the intensity of these maxima, whereas Car addition causes the opposite effect. At this stage of the

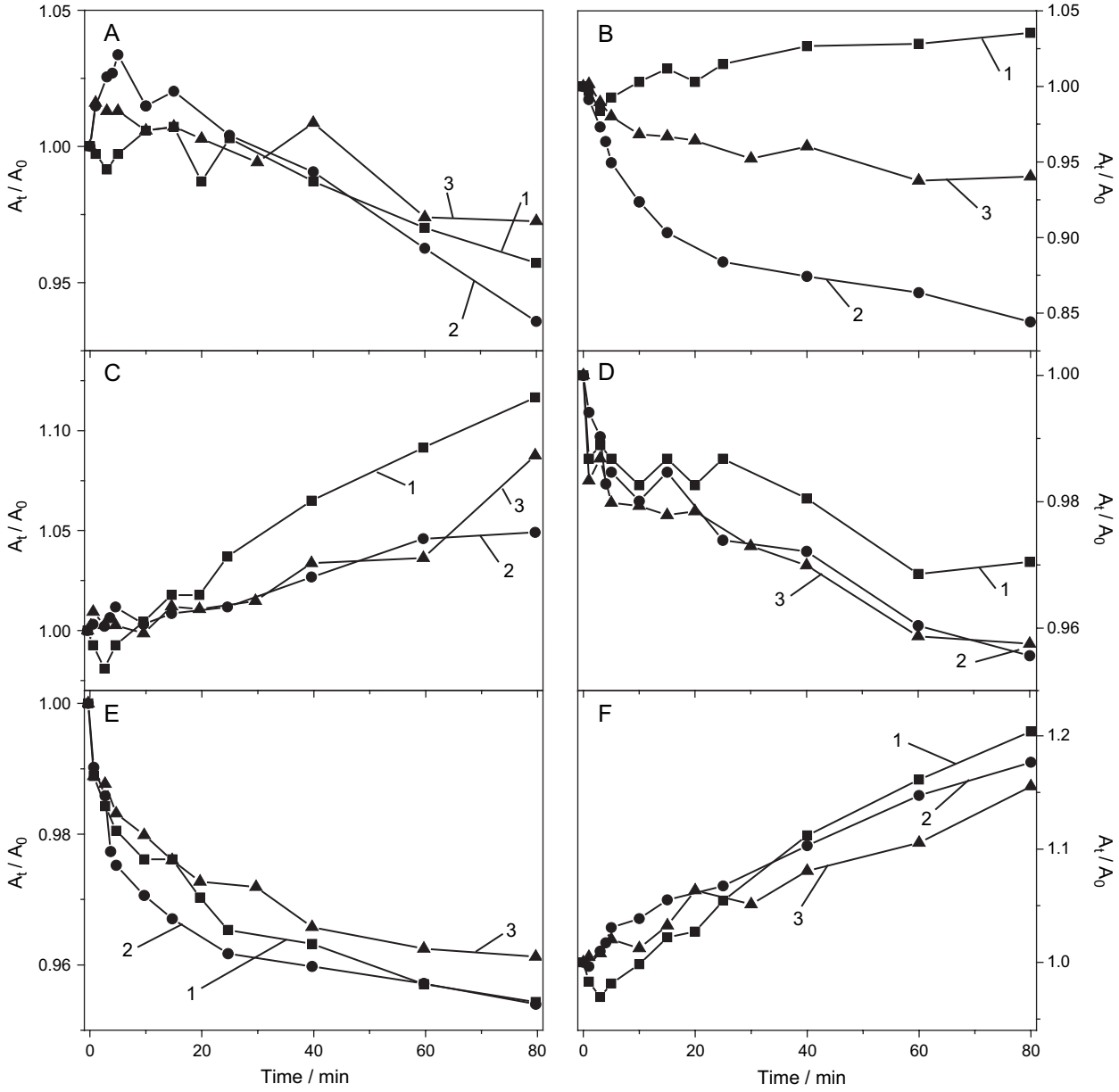

Fig. 4. Changes in relative absorption intensity as a function of the time of laser illumination measured at: (A) 458 nm, (B) 431 nm, (C) 414 nm, (D) 303 nm, (E) 315 nm, (F) 388 nm. A_0 – absorption before illumination, A_t – absorption after various time of illumination. Oil from Evening Primrose seeds with chloroform (curves 1), with Chl *a* (curves 2) and with Chl *a* and Car (curves 3).

investigation the molecular interpretation of these effects is not possible.

The fluorescence maximum at 507 nm, obtained at 376 nm excitation (Fig. 3B), is strong in the spectrum of the sample with Chl *a* added but the presence of Car causes its increase. The emission at 507 nm (due to MDA) increases after Chl addition and it is strong in the presence of both pigments. In parallel, the emission of Chl is not diminished (Fig. 3C), therefore, the changes in the MDA emission intensity are probably due to the effect of the pigments added on the interaction between the other oil components. At the excitation in the range of the Chl *a* Soret band (Fig. 3C), addition of Chl *a* causes a strong increase in the fluorescence intensity of Chl *a* Q_y (0,0) transition. A similar effect is observed upon the excitation in the range of the red band (at 619 nm) of the pigment absorption (not shown). The Car addition causes a decrease in the observed emission (not shown), when excited at 431 nm, because the Car absorbs part of the incident light. After introducing the correction for this effect [12], Chl emission at both wavelengths of excitation is almost unchanged by Car addition (Fig. 3C, curves 2 and 3), which means that Car singlet excitation energy is not transferred to Chl *a*.

On the basis of spectra measured after different times of the sample illumination by the laser light absorbed predominantly by Chl-like pigments, the kinetics of changes in the absorption and fluorescence intensities presented in Fig. 4 could be obtained. The changes in the relative absorption values (described as A_t/A_0, where A_0 is the value before the illumination) take place as a result of laser light illumination in different spectral regions. From Fig. 4A, it follows that the kinetics of the absorption decay in the region of predominant Car absorption (at 458 nm) is similar in the samples with Chl and with Chl and Car added and for the sample containing the oil with the natural content of both the pigments. In this region Chl pigments absorb, therefore these changes can be due to Chl-pigments bleaching. The Chls decomposition is very probable because the laser light is strongly absorbed by these pigments. The laser emits in the region of Chl and Phe absorption (at 632.8 nm). As a result of the illumination the prooxidant components causing oil degradation could be produced, but the influence of Car addition on this absorption region can be neglected.

Fig. 4B shows clearly, a strong protecting effect of Car on the Chl added against bleaching. The difference between the results presented in Fig. 4A and B can be due to different absorption ratios of both pigments occurring in the natural oil. The Car addition has practically no influence on the pheophytinization kinetic of the Chl added (Fig. 4C). The increase in the Phe absorption region is caused by the pheophytinization of Chl. This process is slower for the added Chl than for the Chl naturally present in the oil.

The process of tocopherols destruction is stronger in the Chl presence, whereas the additional Car has no influence on this process (Fig. 4D). A similar situation is observed for sterols (Fig. 4E) and for generation of MDA (Fig. 4F). The obtained absorption results suggest that Car has stronger influence on Chl protection against photobleaching and on oil components photodegradation.

A comparison of the results obtained upon laser (Fig. 4) and xenon lamp (Figs. 5 and 6) illumination on the oil absorption indicates that at the same time

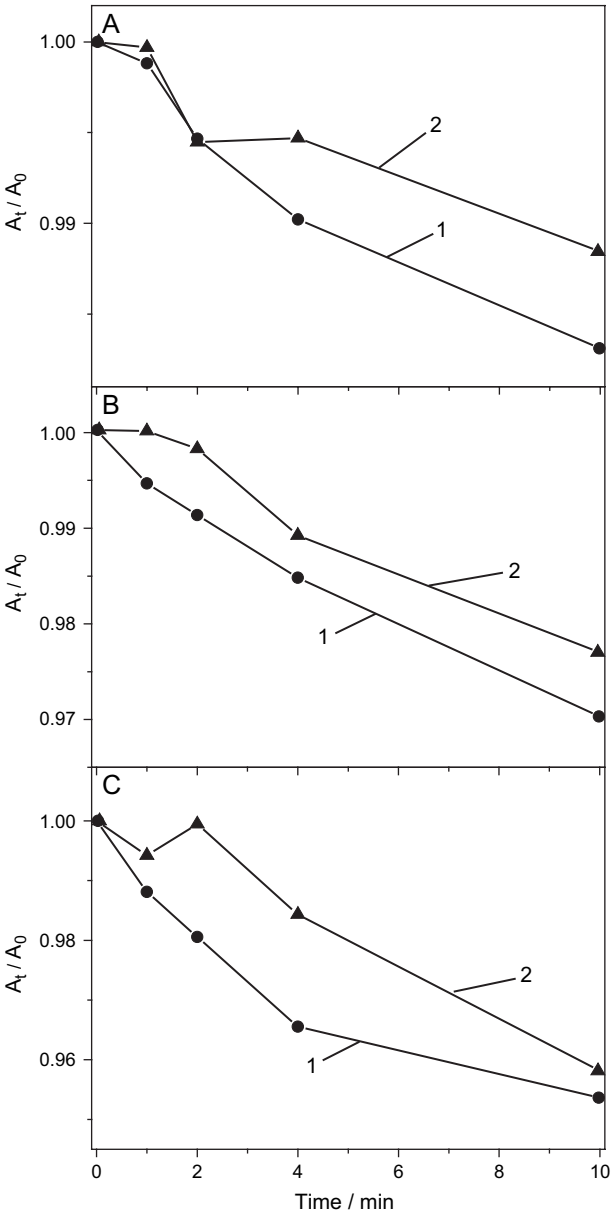

Fig. 5. Changes in relative absorption spectra as a function of time of xenon lamp illumination measured at: (A) 286 nm, (B) 303 nm, (C) 315 nm; A_0, A_t – description as in Fig. 4. Oil from Evening Primrose seeds with chloroform with Chl *a* (curves 1) and with Chl *a* and Car (curves 2).

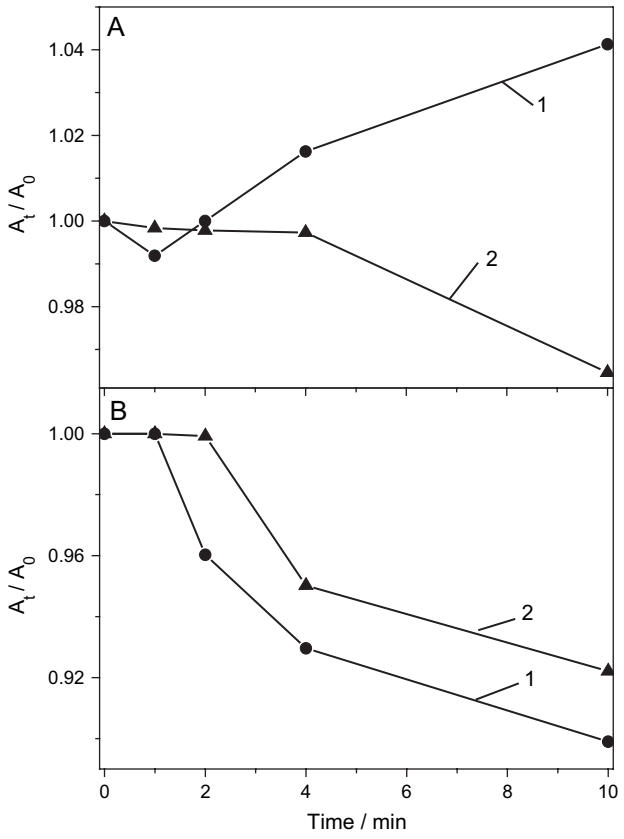

Fig. 6. Changes in relative absorption spectra as a function of time of xenon lamp illumination measured at: (A) 411 nm, (B) 434 nm; A_0, A_t – description as in Fig. 4. Oil from Evening Primrose seeds with chloroform with Chl *a* (curves 1) and with Chl *a* and Car (curves 2).

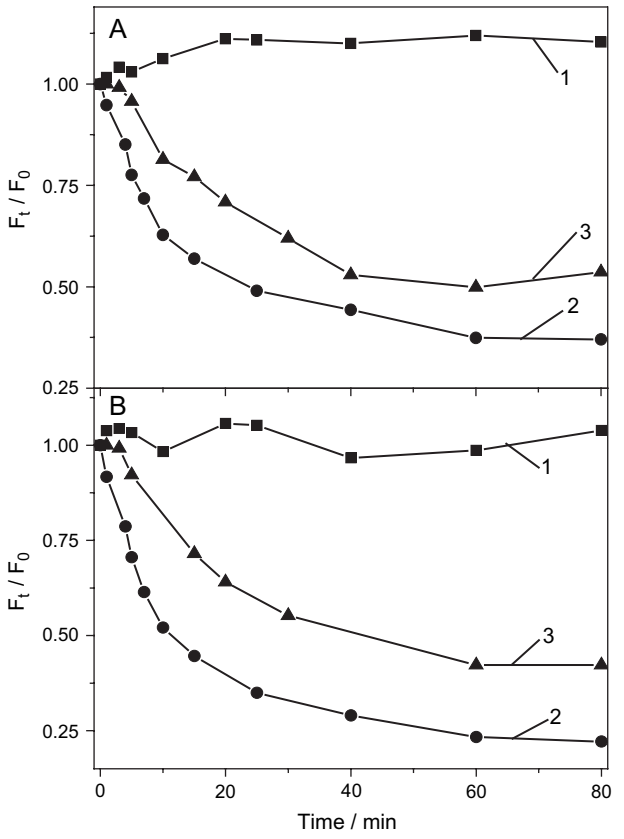

Fig. 7. Changes in relative fluorescence intensity as a function of time of laser illumination measured: at 668 nm and λ_{exc}: 619 nm (A), 431 nm (B);. F_0 – fluorescence intensity before illumination, F_t – fluorescence intensity after various time of illumination. Oil from Evening Primrose seeds in chloroform (curves 1), with Chl *a* (curves 2) and with Chl *a* and Car (curves 3).

of illumination and a higher global xenon lamp light intensity, the laser illumination is more effective in destruction of the oil containing Chl-like type pigments. Fig. 6A shows that on addition of Car at the xenon lamp illumination the pheophytinizations of some components diminish. The kinetics of degradation due to Chl addition is different for the curves in Fig. 6A, B. A similar, but weaker effect is observed as a result of laser illumination. Illumination of the other oil component affects the pheophytinization process (Fig. 4C and Fig. 6A).

The protection of Chl *a* by Car also follows the kinetics of fluorescence decay obtained on the sample illumination by laser (Fig. 7A, B). Similar effects are observed for Phe and for Chl at excitation in various regions of these pigments absorption and at observation in various emission ranges (not shown). Some protection of steroids by Car can be suggested (Fig. 8A) showing the changes in the fluorescence excited at 287 nm (absorption of lipid peroxidation products [10]) and emission at 507 nm (emission of several oil products). Fig. 8B shows an unexpected result that addition of Chl may also prevent sterol degradation. The emission observed at 507 nm and at 389 nm excitation (Fig. 8C), suggests the decrease in MDA generation by Car. It shows that Car can diminish the oil degradation.

The results presented show that the addition of Car affects not only Chl-like pigment degradation but also several other oil components. It seems that the photoreactions involving the other oil components occur, in great part, with the participation of Chl excitation energy.

4. Conclusion

On the basis of the results presented it is not easy to decide in which degree the Car addition can improve the oil stability, because the absorption and emission spectra of oil components and their products of destruction are strongly overlapped, and because some products cause an increase in the absorption or emission values in a given spectral region, whereas others cause a decrease in these values. Different results obtained from the kinetics of absorption and fluorescence can be explained by different yields of fluorescence of various oil products. It has been established that the addition of

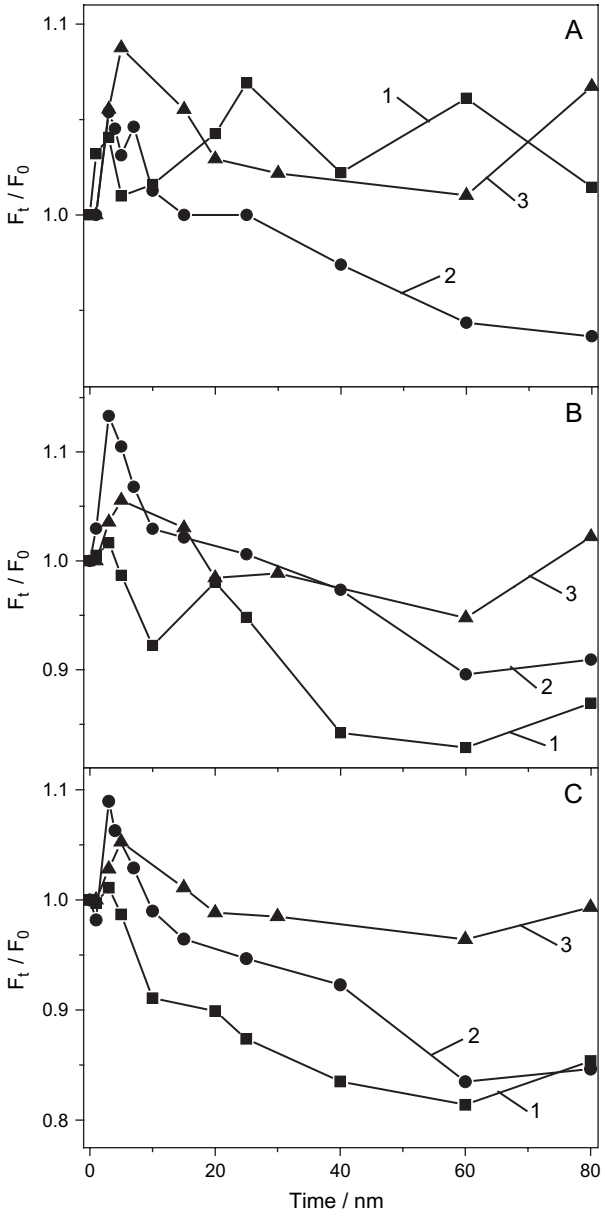

Fig. 8. Changes in relative fluorescence intensity as a function of time of laser illumination measured: at 507 nm and λ_{exc}: 287 nm (A), 376 nm (B), 388 nm (C). F_0, F_t – description as in Fig. 7. Oil from Evening Primrose seeds in chloroform (curves 1), with Chl *a* (curves 2) and with Chl *a* and Car (curves 3).

Car diminishes the Chl photodegradation and probably should also diminish the oxidation of oil due to the action of these pigments. It was shown that in oil the singlet excitation of Car is not transferred to Chl, therefore Car could not cause an increase in the efficiency of photoreactions followed after Chl illumination. The quenching of the Chl triplet by Car makes the Chl presence less threatening to the oil stability. The generation of MDA, which is a product of oil degradation (Fig. 4F), is slightly slower in the presence of Car and also the sterol degradation (Fig. 4E) is weaker in the presence of Car. The question if it is possible to improve the oil stability by Car addition instead of oil bleaching on a technological scale is still open and needs further investigation.

Acknowledgements

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