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The influence of carotene and bacteriopheophytin *c* addition on paths of nonradiative and radiative deexcitation of the oil from *Amarantus* seeds

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Abstract

The absorption, fluorescence emission and excitation as well as steady state photoacoustic spectra (PAS) of the oil from *Amarantus* seeds, without and with carotene and bacteriopheophytin *c* (BPhe *c*) addition, have been measured. The oil investigated originally contains only some amount of carotene but no admixtures of chlorophyll-like pigments. As follows from the dependence of the photoacoustic signal multiplied by the frequency of light modulation on this frequency, the kinetics of thermal deactivation (TD) of oil depends on the pigment contents. The relative values of thermal deactivation calculated for the samples with pigments added to that of the original oil depend on the type of the pigment added. The relative yields of fluorescence have been also calculated. The results suggest that also the photochemical reactions taking place in various illuminated samples could be different. This suggestion has been experimentally confirmed. The photochemical stability of the oil sample increases when carotene is added. The information about the oil stability is important in order to establish proper conditions for oil storage and transportation.

Keywords: *Amarantus*; Bacteriopheophytin *c*; β Carotene; Photochemical stability; Thermal deactivation

1. Introduction

As it follows from our earlier study [1–4] carried out for the oil obtained from various seeds [5] the content of the pigments in oil has strong influence on its photochemical stability [6–9]. The information about the oil stability is important in order to establish proper conditions for oil storage and transportation [10,11].

Previously investigated oils contained two types of pigments: carotenoids and chlorophyll-like pigments [4]. The natural oil from *Amarantus* contains low amount of carotenoids [12]. No amounts of chlorophyll-like types of pigments have been experimentally established on the basis of the absorption spectra. Therefore, it is possible to investigate the influence of chlorophyll-like pigment addition on the oil photochemical and spectral properties.

It is known [13–15] that carotenoids can play a triple role: they can work as light harvesting pigments transferring the energy absorbed to chlorophyll-type pigments, they can protect the organism against photodestruction by the quenching of the chlorophyll-type pigments triplets, they can quench dangerous singlet oxygen [14–16]. The paths of the interactions between chlorophyll-type pigments and Car are shown in Fig. 1. Therefore, carotenoids usually improve the photochemical stability of oil especially when occur in great amount [4]. Triplet energy quenching influences the kinetics of the process of thermal deactivation (TD) [1].

In order to investigate the spectral properties of oil with additions of different pigments, the absorption (*A*), fluorescence emission and fluorescence excitation spectra as well as steady state photoacoustic spectra (PAS) at various frequencies of light modulation were measured. The thermal deactivation (TD = PAS/*A*) of the samples investigated was calculated. The kinetics of TD of the samples investigated in different spectral

Abbreviations: *A*, absorption; PAS, photoacoustuic spectroscopy/signal; TD, thermal deactivation; β Car, β carotene; BPhe *c*, bacteriopheophytin *c*.

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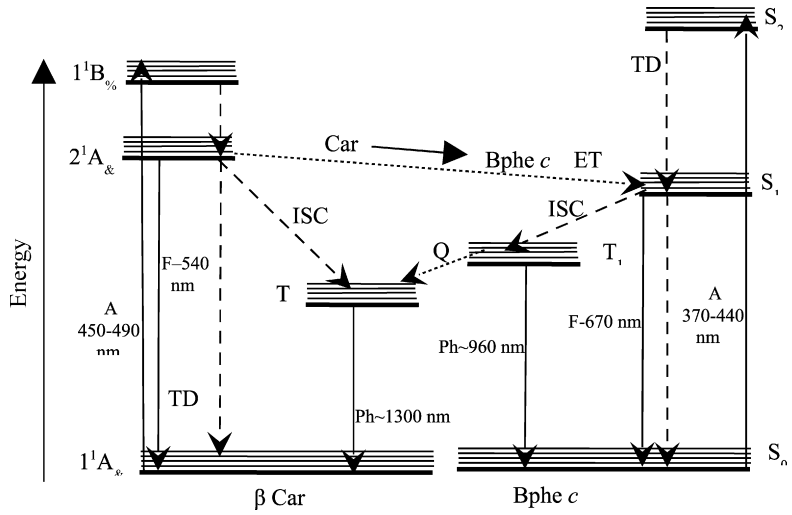

Fig. 1. Diagram of energy levels of BPhe and β Car.

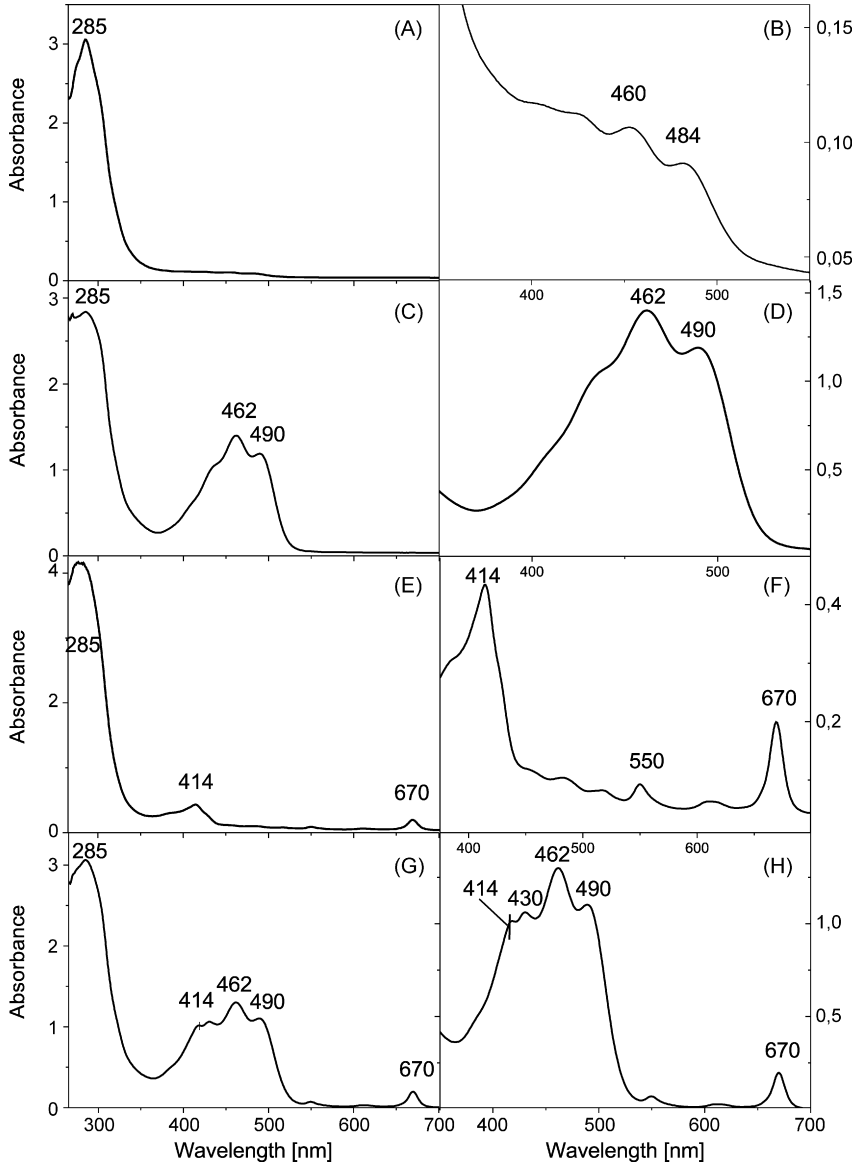

Fig. 2. Absorption spectra of oil (A and B), oil with added β Car (C and D), oil with added BPhe *c* (E and F) and oil with both pigments added (G and H).

Table 1
Values of relative thermal deactivation the samples studied calculated in respect to that of oil, for different frequencies of light modulation and different spectra region

	Frequencies									
	5.2 Hz				20 Hz			40 Hz		
	λ = 414 nm	λ = 460 nm	λ = 490 nm	λ = 670 nm	λ = 414 nm	λ = 460 nm	λ = 490 nm	λ = 670 nm	λ = 414 nm	λ = 460 nm	λ = 490 nm	λ = 670 nm
Oil + β Car/oil	0.42	0.26	0.31	0.38	0.32	0.14	0.16	0.79	0.06	0.03	0.03	0.61
Oil + BPhe *c*/oil	0.65	1.80	0.20	0.50	0.37	1.23	0.14	0.43	0.03	0.24	0.14	0.13
Oil + β Car + BPhe *c*/oil	0.24	0.19	0.22	0.33	0.16	0.13	0.14	0.28	0.02	0.03	0.03	0.12

regions were compared on the basis of the method proposed by Moore [17] and Poulet's laboratory [18]. From the spectra measured at various frequencies of light modulation the mean decay time of the "slow" component of thermal deactivation can be obtained and the intensity ratio of the slow to fast component can be evaluated [1,17,18]. The relative yield of the fluorescence of samples with pigments added to that of the original oil was calculated for the samples with different contents of the pigments and at various wavelengths of fluorescence excitation.

2. Materials and methods

The oil from *Amarantus* seeds was obtained by the method similar to that proposed for other oils [5]. Bacteriopheophytin *c* (BPhe *c*) was obtained from *Prosthecochloris aestuarii* and separated chromatographically according to the method described in literature [19] and used previously [20]. The β carotene (β Car) used in the study was from Fluka AG.

In order to introduce additional pigments into the oil samples, the pigments were dissolved in acetone, then mixed with the known amount of oil and acetone was evaporated under nitrogen atmosphere, at 20 °C for 45 min. The concentrations of the dyes were established from absorption in acetone. The absorption spectra were measured using a Cary 4000 (Varian) spectrophotometer. Fluorescence emission and fluorescence excitation spectra were recorded by means of a fluorescence spectrophotometer F 5000 (Hitachi). The samples were illuminated by a xenon lamp during the measurements of the steady state PAS spectra taken by means of a single beam photoacoustic spectrophotometer constructed in our laboratory [21]. The following frequencies of light modulation in PAS measurements were used 5.2, 20 and 40 Hz.

Photochemical sensitivity of the samples was measured by monitoring the oil absorption changes induced by the samples illumination by xenon lamp (150 W) for several minutes, as described in Ref. [4].

3. Results and discussion

Fig. 2A–G show the absorption spectra of the samples investigated measured before illumination. Fig. 2A and B present the absorption of the pure oil investigated; Fig. 2A shows the absorption in the whole spectral region investigated over which the β Car absorption is almost invisible, therefore the region of β Car absorption is shown in a different scale in Fig. 2B. The absorption at 285 nm is due to the fatty acids (predominantly linoleic acids but probably folic acids as well [22]). Fig. 2 C–H show similar absorption spectra for other samples (after β Car addition in Fig. 2C and D, after BPhe *c* addition in Fig. 2E and F and in Fig. 2G and H for samples with both pigments added).

The kinetics of absorption changes due to the sample illumination is better seen in Fig. 3 (Fig. 3A for pure oil, Fig. 3B for the oil with β Car, Fig. 3C for the sample with BPhe *c*, Fig. 3D for the sample with both pigments). The changes are normalized in respect to the absorption before illumination. The photochemical changes in absorption are lower for the sample with β Car added than for the original oil (Fig. 3A and B). The effects are

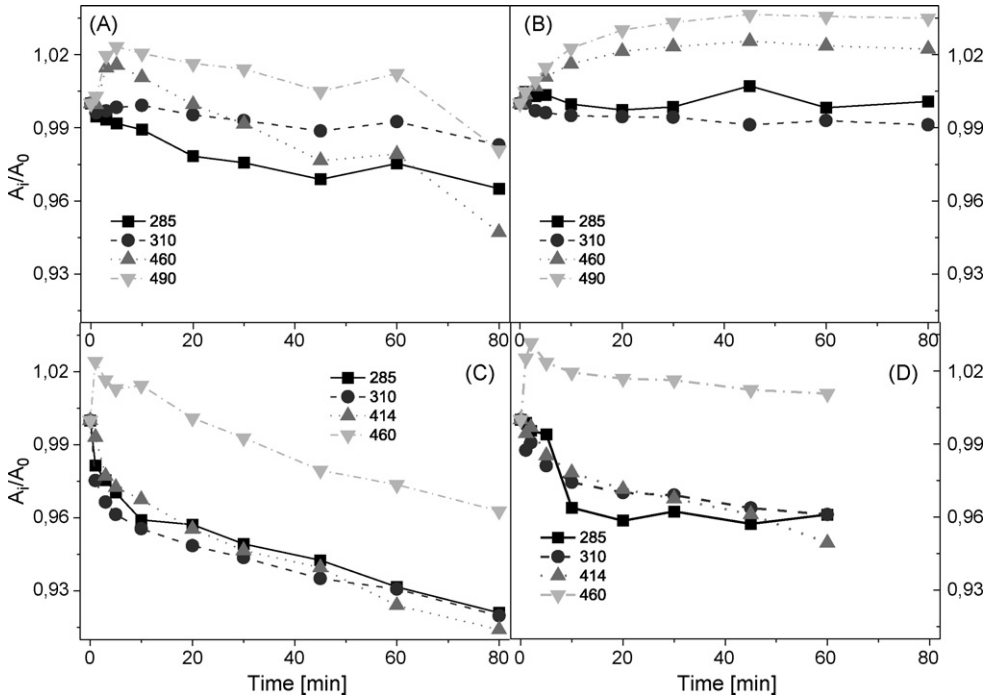

Fig. 3. Normalized kinetic of absorption changes as a function of time of illumination observed at different wavelength; for samples: (A) oil, (B) oil with β Car, (C) oil with BPhe *c* and (D) oil with both pigments added.

different for observations at various wavelengths. The sample with Bphe *c* (Fig. 3C) is photochemically sensitive. In the region of BPhe *c* absorption and also at other wavelengths of absorption, strong photochemical changes are observed in the sample with this pigment added (Fig. 3C). These changes are diminished as a result of β Car addition (Fig. 3D), which shows that an additional amount of β Car can improve the photochemical stability of the oil containing chlorophyll-like pigments.

Interactions between chlorophyll-like pigments and carotenoids are complicated even when these pigments are in the solvents not taking part in photoreactions, therefore, it is not easy to follow their interaction mechanisms in complex and anisotropic biological systems [23–25]. In order to investigate these mechanisms in complex and photochemically unstable solvents such as oil, it is necessary to investigate several paths of such system's deexcitation [5,25]. In oils it is particularly important as oils contain fluorescent species [5,26,27]. Fig. 4 shows PAS of the samples investigated. The shape of PAS spectra depends strongly on the pigment content. The shapes of PAS are different from those of the absorption spectra (Fig. 2) which shows that different types of pigments exhibit different efficiency of thermal deactivation. The highest value of PAS is observed for the sample with β Car added, the lowest for pure oil, but the PAS amplitude depends on the absorption of the sample measured. More informative are the results showing thermal deactivation that is the ratio of PAS to absorption.

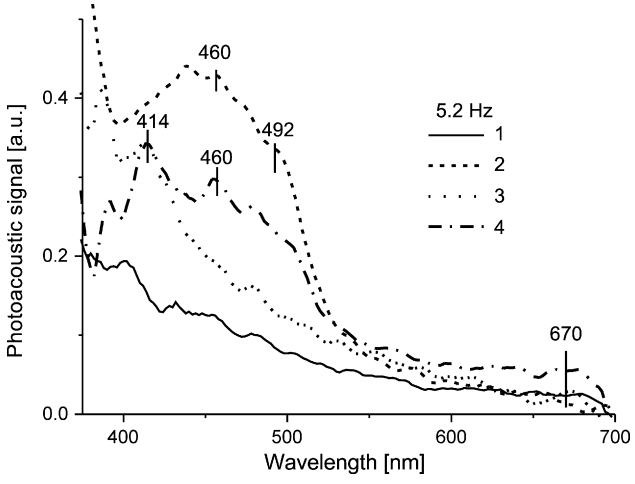

Fig. 4. Photoacoustic signal for oil (curve 1), oil with added β Car (curve 2), oil with added BPhe *c* (curve 3) and oil with both pigments added (curve 4), frequency 5.2 Hz.

PAS measurements have been made at three frequencies of illuminating light modulations. The shapes and intensities of PAS depend on the frequency of the light modulation (results not shown). Such a dependence is predicted by the Rosencwaig-Gersho theory [28]. In Ref. [1] the thermal diffusion lengths for similar samples (oils) have been estimated. The values of the thermal diffusion length have shown that the samples are transparent (absorption length is longer than sample thickness) and thermally thick. These parameters determined indicate which variant of the Rosencwaig-Gersho theory [28] should be applied. Table 1 presents the relative values of TD of the samples studied calculated in respect to that of pure oil, obtained on the basis of PAS and the absorption results. The values were calculated for the regions of Soret band of BPhe *c* (at 414 nm), red band of the same pigment (at 670 nm) and at two wavelengths located in the carotenoids absorption range (at 460 nm and at 490 nm). The

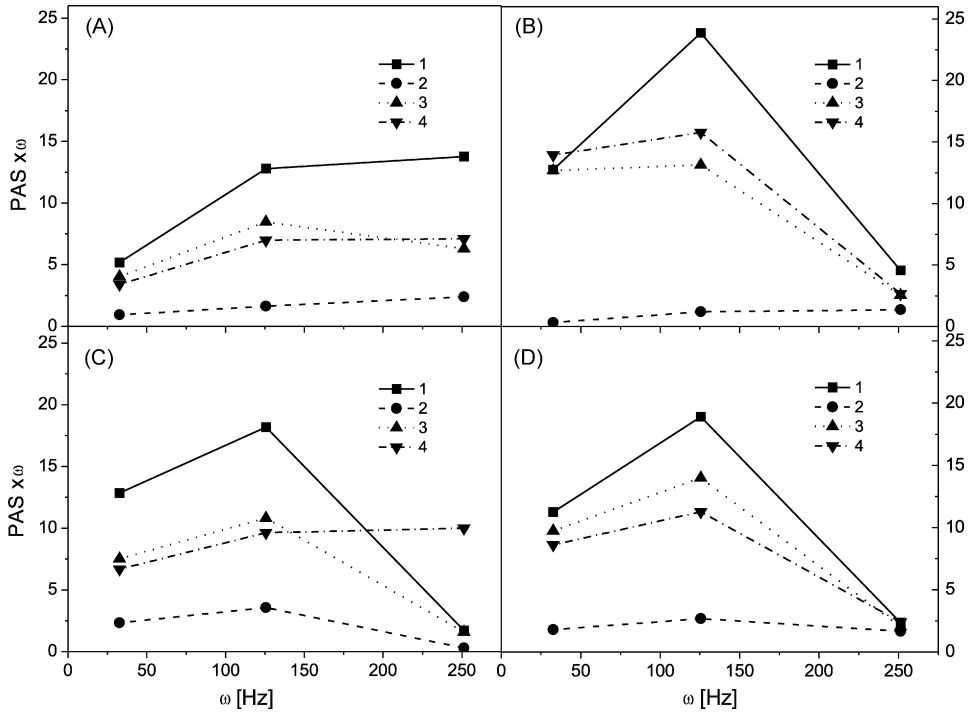

Fig. 5. Dependence of the signal of PAS multiplied by frequency of the light modulation *versus* this frequency. (A) Oil, (B) oil with β Car, (C) oil with BPhe *c*, (D) oil with β Car and BPhe *c*. Curve (1) 414 nm, (2) 670 nm, (3) 460 nm, (4) 490 nm.

measurements were performed in the region of low oil absorption (Fig. 2A) because in this region the pigments absorption bands occur and because our PAS apparatus is not able to measure in the shorter wavelengths (200–350 nm) in which the main band of oil absorption is located. As it follows from Table 1, TD in the region of the Soret band (at 414 nm) is in most cases the highest for the sample with BPhe *c* but the β Car addition results in its decrease. A similar situation is observed in the region of β Car absorption (at 460 and 490 nm). The addition of β Car causes a decrease in the relative TD, which becomes lower than that of BPhe *c* alone. When both pigments are added, the relative TD is the lowest for almost all cases also in the region of BPhe *c* red band (Table 1). In interpretation of the data from Table 1 we have to remember that there is a competition between TD

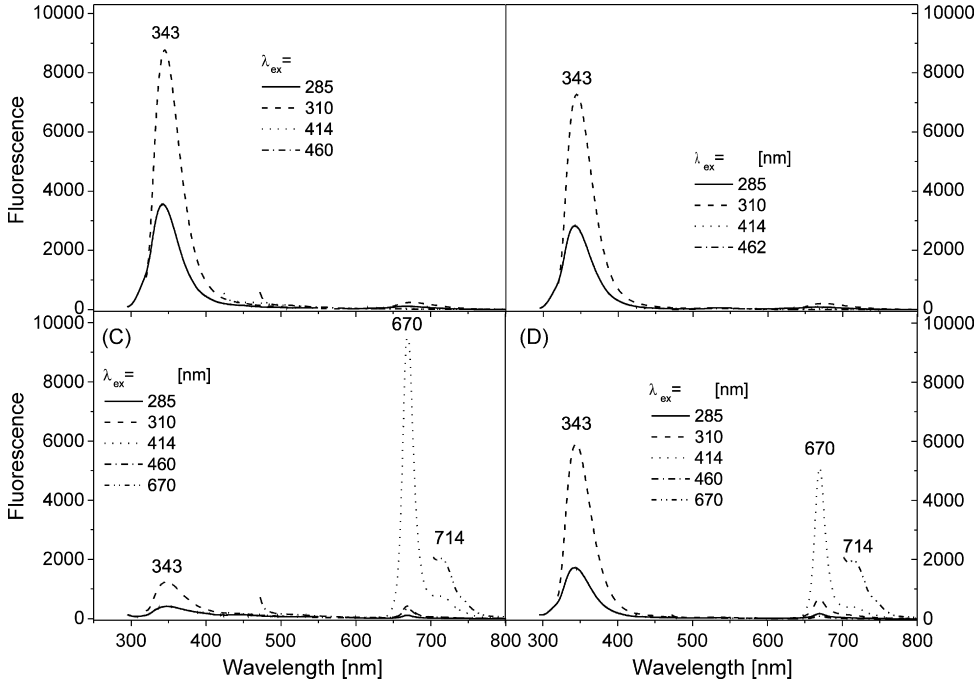

Fig. 6. Fluorescence spectra of (A) oil, (B) oil with β Car, (C) oil with BPhe *c*, (D) oil with β Car and BPhe *c*.

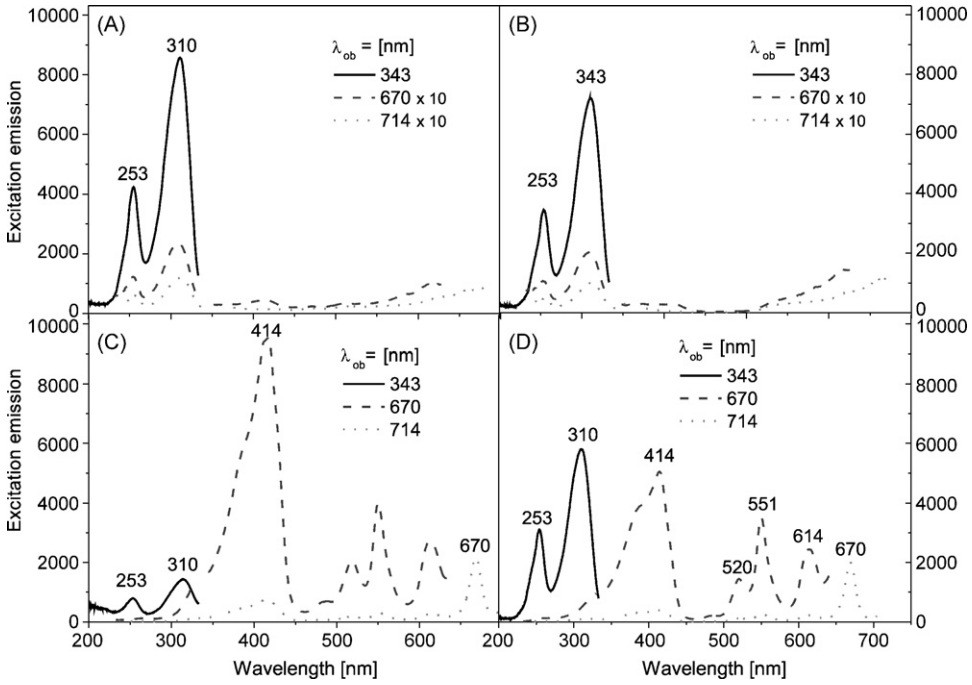

Fig. 7. Excitation emission spectra of (A) oil, (B) oil with β Car, (C) oil with BPhe *c*, (D) oil with β Car and BPhe *c*, (×10) intensity of excitation emission multiplied by 10.

and the radiative deexcitation-fluorescence emission. For the oil with BPhe *c*, the TD calculated for the Soret band region is different than that in the red band. It is partially due to a higher TD of the pure oil in the region of shorter wavelengths as well as different paths of possible deactivations of Chl-like pigments excitation at these two bands. TD of the samples with β Car added is usually higher.

Fig. 5 presents PAS multiplied by the frequency of the light modulation *versus* this frequency. As follows from this plot, the kinetics of the PAS decays is different for the sample with BPhe *c* added than for the sample with a high amount of β Car added. This kinetics depends also on the wavelength of observation. It is known that the deactivation occurring with participation of the triplet state is slower and the changes revealed in Fig. 5 are due to different kinetics of the deactivation process [5,17,18,25]. The β Car addition changes the shape of the curves in Fig. 5. Small changes are observed also in the region of the Soret band (at 414 nm), which indicates interactions between both types of pigments. Different shape of the curve for the Soret and the red band regions informs about different processes of excitation energy transfer occurring between BPhe *c* excited into these two states and other pigments or oil molecules.

The fluorescence spectra of the samples investigated at various wavelengths of excitation are presented in Fig. 6. As follows from Fig. 6A, the pure oil exhibits emission at 343 nm, and its fluorescence intensity is practically unchanged after β Car addition (Fig. 6B). The decrease in the emission intensity can be due to the absorption of some of the exciting light quanta by unfluorescent β Car. The BPhe *c* addition causes a generation of fluorescence of this pigment with the main band maximum at 670 nm and decrease in the oil emission (at 343 nm). It shows that the excitation of oil is transferred to BPhe *c*. When both pigments are added, the oil emission at 343 nm region increases and the intensity of the BPhe *c* band at 670 nm decreases. It suggests that the energy transfer from β Car to BPhe *c* is not efficient and that both pigments interact with the oil molecules.

Fig. 7 shows the excitation spectra of the samples studied. For oil and oil with β Car (Figs. 7A and B) two maxima at 343 and 670 nm occur. These two spectra are similar. The excitation spectrum of the sample with BPhe *c*, exhibits a maximum characteristic of the absorption of BPhe *c* spectra. Addition of β Car causes a decrease in the fluorescence emission spectra, and similarly it causes a decrease in the intensity of the excitation emission spectra. Table 2 shows the yields of fluorescence of the samples investigated relative to the yield of the pure oil sample. The β Car addition causes a decrease in the sample fluorescence yield observed in the region of the oil excitation maximum (at 310 nm). The addition of BPhe *c* causes a further decrease in this yield of fluorescence but the addition of both pigments causes an increase in this yield of emission. Fluorescence yields calculated for the BPhe *c* absorption regions (at 414 nm) is of course higher for the sample with BPhe *c* and lower for the sample with both pigments. In the β Car absorption region (at 450 nm) the sample with the both pigments added exhibits a lower yield of

Table 2
Values of fluorescence yield of the samples investigated relative to the yield of the pure oil sample at different wavelength

	λ (nm)		
	310	414	460
Oil + β Car/oil	0.72	0.061	0.008
Oil + BPhe *c*/oil	0.16	22.7	5.289
Oil + β Car + BPhe *c*/oil	0.56	4.97	0.111

fluorescence than that with only BPhe *c*, which suggests that β Car quenches the BPhe *c* emission. The law of energy conservation permits a comparison of the sum of both relative yields in three types of sample. The sum of relative yields of TD and the yield of fluorescence is the highest for the sample with BPhe *c* added, whereas the sum of the relative yields is the lowest for the sample with only β Car added.

At 414 and 460 nm of BPhe *c* and β Car absorption, the sum of relative yield of TD and fluorescence is the highest for the oil with BPhe *c*. It is due to high yield of fluorescence BPhe *c*. The calculated yield of fluorescence is lower and more energy can be used for photoreaction and TD.

4. Conclusions

A simple relation between the relative TD values for the samples studied has not been found, but this ratio usually decreases with increasing sample photochemical stability. The chlorophyll-like pigments, such as BPhe *c*, cause a decrease in the photochemical stability of the oil studied, whereas the β Car presence improves the oil stability. The type and amount of pigments present in the oil have a strong influence on its fluorescence emission and thermal deactivation yields, but even high TD is not always accompanied by a low stability of the sample. The results depend on the mutual interactions of the pigments and their interactions with the oil molecules.

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