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A marginal contribution of selected carotenoids to the supression of UV-irradiation-induced lecithin peroxidation in hexane solution

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Abstract: The aim of this work was to study the anticipated antioxidant role of four selected carotenoids in mixtures with lecithin lipoidal compounds in hexane solution, under continuous UV-irradiation in three different ranges (UV-A, UV-B and UV-C). Two carotenes (β-carotene and licopene) and two xantophylls (lutein and neoxanthin) were cmployed to control the lipid peroxidation process generated by UV-irradiation, by scavenging the involved free radicals. The results show that while carotenoids undergo a substantial, structural dependent destruction (bleaching), which is highly dependent on energy of the UV-photons, their contribution to the expected suppression of lecithin peroxidation is of marginal importance, not exceeding a maximum of 20%. The marginal antioxidant behaviour has been attributed to a highly unordered hexane solution, where the scavenging action of the carotenoids becomes less competitive.

Keywords: carotenoids, lipids, UV-irradiation, bleaching, peroxidation.

INTRODUCTION

The recent depletion of the stratospheric ozone layer has resulted in increased levels of ultraviolet (UV) radiation at the Earth's surface. The depletion of the ozone shield is caused by a huge release of atmospheric pollutants, such as chlorofluorocarbons, chlorocarbons and organobromides. The ozone destruction has led to an increase of biologically damaging UV-light at ambient levels (mainly UV-B light, 280–320 nm). It induces consequences which affect many crucial biologically important processes of global importance, such as DNA replication,[1,2] photosynthesis,[3,4] etc. Although UV-light can generally influence the whole human immune system,[5,6] it has been especially recognized as one of the major agents initiating many harmful, free-radical-mediated processes, such as lipid peroxidation (LP). Hence, UV-light plays a triggering role in the initiation of the very complex (LP) process, leading ultimately to cancer,[7] melanoma skin cancer[8] included.

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As with free radicals mediated chain reaction, lipid peroxidation* consists of an initiation step (formation of lipid radicals – $L^{\bullet}$, through the abstraction of allylic and doubly allylic H-atoms from the hydrophobic anticonjugated polyunsaturated moieties of a lipid), a propagation step (where lipid radicals react with oxygen to form lipid peroxy radicals – $LOO^{\bullet}$) and a termination step (formation of lipid hydroperoxides – LOOH – with a dienes type of structure, in which the anti-conjugated moieties no longer exist.[10–14]

Typical lipid peroxidation initiators are Reactive Oxygen Species (ROS), such as hydroxy radicals ($OH^{\bullet}$) or peroxy radicals ($ROO^{\bullet}$). They can be created through a variety of chemical reactions,[13] some of which include typical lipid radical producers.[15–17] Additionally, they can be induced through a variety of external stresses,[9] implying very commonly external radiation,[18,19] which, in case of UV-irradiation, may include a special type of LP initiators, photosensitisers, in very different media.[12,20–22]

Lipid peroxidation is mostly controlled by the action of antioxidants *in vivo;* many biomolecules (and classes of biomolecules) serve as antioxidants, such as enzymes, tocopherols (vitamin E), L-ascorbic acid (vitamin C), retinol (vitamin A), thiamin and riboflavin (vitamin B), flavonoids, etc.[23–28]

In recent years, carotenoids have received wide research interest as potential antioxidants, based on studies which reported that a higher consumption of carotenoids leads to a lower risk of cancer and cardiovascular disease. The antioxidant action of carotenoids is documented in a number of studies[11,29–32] and it is related to the conjugated chemical structures with multiple potential sites approachable for attack by ROS species. Concerning the LP process, all biological antioxidants are grouped into two categories: preventive antioxidants, which reduce the initiation of peroxidation by suppressing the generation of chain-initiating radicals, and chain-breaking antioxidants, which disrupt the chain propagation by trapping the chain-initiating and/or chain-propagating peroxyl radicals.[10,33] Carotenoids are generally classified as preventive antioxidants (physical or chemical quenching of toxic singlet oxygen leading to LP *via* a non-radical mechanism) but also act as chain-breaking antioxidants. Carotenoids react with radical species in three different types of mechanisms: (I) radical addition, (II) electron transfer to radicals and (III) allylic hydrogen abstraction.[10,29,32,34]

Hence, to understand the basic mechanisms of the interaction of carotenoids with lipid radicals produced by UV-light, a mixture of lecithin/pigments and lecithin only were irradiated with UV-light (254, 300 and 350 nm) in this work. The irradiation was performed in hexane solution, for different irradiation periods, thus providing the possibility for kinetics analysis, by monitoring the increase of the absorbance at 234 nm (an indication of peroxidative diene structures), and a de-

* When mediated and promoted by free radicals; LP can also be achieved through a non-radical pathway by direct lipids reaction with singlet oxygen; in such a case, the presence of a photosensitizer is necessary to create singlet oxygen.[9]

crease of the absorbance at the wavelength of the maximum of the absorption of carotenoids for each carotenoid in the lecithin/pigment mixtures.

EXPERIMENTAL

The pigments were isolated from plant material (β-carotene, lutein and neoxanthin from spinach, and lycopene from tomato fruits) purchased at the local market. All experiments and experimental procedures, beginning with the extraction, were performed under dim light as far as possible, and inside vessels and equipment covered with aluminum foil or black cloth, to prevent possible chlorophyll photooxidation.[35]

Lecithin Epikuron 100 P, a mixture of phospholipids, was a gift from "ICN Galenika", Belgrade. It was manufactured by "Degussa Texturant Systems", Hamburg, Germany. According to the accompanying declaration, the mixture contained 97.4 % of acetone insoluble substances and had an acid value of 30, a moisture content of 0.66 %, a peroxide value of 0.88, an iodine number of 78.68 and a pH value of 6.6. The lipoidal content was: phosphatidylethanolamine 18.0 %, phosphatic acid 8.3 %, phosphatidylinositol 14.1 %, phosphatidylcholin 21.7 %.* The lecithin mixture was kept in the dark to prevent at least the photooxidation process. The dark autooxidation, however, could not be eliminated in this manner; however, it was taken into consideration during the calculation of the LP yield.

Extraction of the pigments from spinach (Spinacia oleracea)

The photosynthetic pigments were extracted from spinach leaves using a modified method proposed by Svec.[36] Fresh spinach leaves free of midribs (0.030 kg) were dropped into boiling water, which was quickly replaced (after 1–2 min) with cool water. Hot water inactivates the enzymes thus preventing pigment alteration and permitting the coagulation of proteins and water-soluble substances. After drying between paper towels, the leaves were separated and placed in mixture of methanol (60 cm^3) and 40–75 °C petroleum ether (30 cm^3), which was occasionally agitated during the following 30 min. Methanol removes water from the plant material and the petroleum ether extracts the pigments before they undergo secondary reactions. The deep-green extract was decanted through a cotton pad. The leaves were re-extracted twice with the same quantities of methanol and 40–75 °C petroleum ether (2:1). The extracts were diluted with 120 cm^3 of saturated NaCl solution, which keeps most of the pigments in the petroleum ether layer. The remaining aqueous methanol layer was re-extracted with 40 cm^3 of a mixture containing 40–75 °C petroleum ether and diethyl ether (1:1), which ensures the solubility of the pigments in the organic phase. The successive extracts were treated in the same manner. The final extract was a mixture of pigments containing various forms of chlorophyll, as well as accessory pigments, carotenoids (carotenes and xanthophylls).

Isolation of carotenoids from the spinach extract by column chromatography

The carotenoid-fractions were isolated using a modified procedure of Svec[37] and Brockmann,[38] *i.e.,* column chromatography with silica gel (silica gel 60, Merck, 0.063–0.200 mm) as the adsorbent and a benzene/acetone mixture as the eluent. The benzene/acetone ratio was changed from the initial 1:0 to the final 1:1, to permit an easier elution of the polar fractions. β-Carotene appeared first (eluted by benzene only), followed by chlorophylls (benzene:acetone 7:1) and xanthophylls fractions, lutein and neoxanthin (benzene:acetone 6:1–1:1). The fractions were dried and redissolved in hexane. The fractions were identified by comparing their Vis spectra with standards spectra.

Extraction of the pigments from tomato fruits

Ground tomato fruit (8 g) was thoroughly mixed with 40 cm^3 of ethanol. The slurry was stirred until the tomato paste material was no longer sticky (about 3 min). The ethanol was removed by vacuum filtration. The retained tomato residue was mixed with 60 cm^3 of a mixture of acetone and petroleum ether (1:1). The extract was collected by vacuum filtration, and the filter residue was

* The acid value, peroxide number and iodine number were rechecked and found to be true.

rewashed with the solvent mixture (20 cm^3) in order to improve the yield. The filtrate was transferred to a small separatory funnel and mixed with 50 cm^3 of saturated NaCl solution. The organic layer was rewashed twice, repeatedly, first with 50 cm^3 of 10 % potassium carbonate and then with 50 cm^3 of water. Finally, approximately 1 g of anhydrous magnesium sulfate was added to dry the organic layer. After 10–15 min, the solution was vacuum filtered to remove the drying agent.

Isolation of carotenoids from the tomato extract by column chromatography

The lycopene fraction was isolated by column chromatography with alumina (aluminium oxide 90, Merck, 0.063–0.200 mm) as the adsorbent and a petroleum ether/acetone mixture as the eluent. The mixture ratio was changed from the initial 10:0.1 to the final 9:1, to permit the easier elution of lycopene. β-Carotene appears first (eluted by a petroleum ether/acetone mixture of 10:0.1), followed by the lycopene fraction (eluted by a 9:1 mixture). The fractions were dried and redissolved in hexane.

HPLC analysis of the carotenoids fractions

The high percentage of carotenoids in the separated fraction was proved by (Hewlett Packard) HPLC analysis. The analysis was done under isocratic conditions; column: Zorbax Eclipse XDB-C18, mobile phase: acetonitrile/methanol/ethyl acetate, 60:20:20; flow rate: 0.5 ml/min. The monitoring wavelengths were: 445 nm for β-carotene and lycopene, 438 nm for lutein and 447 nm for neoxanthine.

UV-treatment

Continuous irradiations of the carotenoids, lecithin and carotenoids/lecithin mixtures in hexane were performed in a cylindrical photochemical reactor "Rayonnet", with 14 symmetrically placed lamps with emission maxima in three different ranges: 254 nm (UV-C), 300 nm (UV-B) and 350 nm (UV-A). The samples were irradiated in quartz cuvettes (1 cm × 1 cm × 4.5 cm) placed on a rotating circular holder. The total measured energy flux was about 25 W/m^2 for 254 nm, 21 W/m^2 for 300 nm and 18 W/m^2 for 350 nm light.

Vis spectroscopy

The Vis spectra of lecithin dissolved in hexane, and lecithin mixed with the carotenoids fractions, before and after irradiation with UV-light, were recorded in the wavelength range 200–800 nm on a Varian Cary-100 Spectrophotometer.

Spectrometry for conjugated dienes

The peroxidative dienes structures were determined by measuring the absorbance at 234 nm[13,39–42] in hexane solution. Both lecithin and the pigments were dissolved separately in hexane and then mixed in a 10:1 ratio. The initial concentrations of lecithin and carotenoids in hexane were 2.56×10^{-4} mol/dm^3 and 4×10^{-6} mol/dm^3, respectively (being a mixture of lipoidal compounds, "an average" molecular weight of lecithin was taken for the calculation; on the other hand, the following molar extinction coefficients were used for the calculation of the concentration of the carotenoids: for β-carotene in hexane at 453 nm, 1.39×10^5 dm^3/mol cm; for lycopene in hexane at 503 nm, 1.72×10^5 dm^3/mol cm; for lutein in diethyl ether at 445 nm, 1.41×10^5 dm^3/mol cm and for neoxanthin in ethanol at 438 nm, 1.36×10^5 dm^3/mol cm.[43–45]) The peroxidation of the phospholipids of lecithin was generated by UV-irradiation at 254 nm (UV-C), 300 nm (UV-B) and 350 nm (UV-A). Hexane solutions of pure lecithin, as a kind of blank, were irradiated simultaneously with the lecithin/pigments mixtures. The increase in the absorbance at 234 nm, as an indication of the formation of peroxidative diene structures, was determined by Vis measurements. The maximal carotenoids absorbances were recorded as a function of the UV-irradiation time to follow the rate of their destruction, *i.e.,* their bleaching (observed as a loss of colour).

RESULTS

The structures of carotenes (β-carotene and lycopene), the change in the absorption specta of lecithin/carotenes mixtures after continuous prolonged irradiation with UV-B light (300 nm) in hexane, and kinetic logarithmic plots for increasing irradiation intervals are shown in Figs. 1 and 2, respectively. The changes in absorption spectra were followed for a pure lecithin solution in hexane (not shown) and for lecithin/carotenes mixtures at 234 nm (peroxides), as well as at the maximum absorption wavelengths of the carotenes (450 nm for β-carotene and 470 nm for lycopene) (Figs. 1B, 2B). The abosption spectra showed very similar behaviour

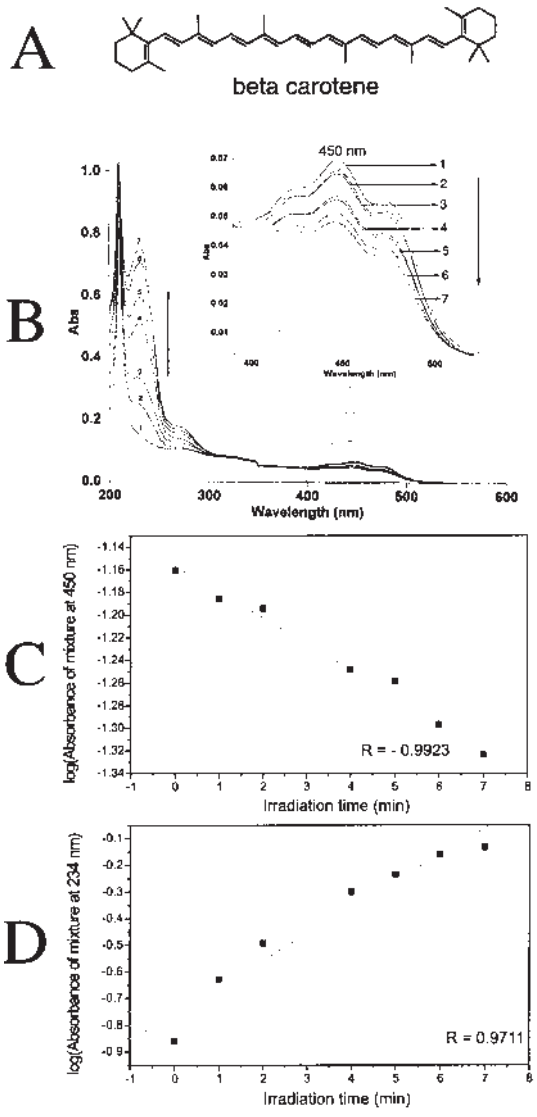

Fig. 1. (A) Structure of β-carotene; (B) Changes of the absorption spectrum of a lecithin/β-carotene (10:1) mixture exposed to UV-B radiation at 300 nm in hexane. The exposure time periods, min: (1) 0; (2) 1; (3) 2; (4) 4; (5) 5; (6) 6; (7) 7. The initial concentrations of β-carotene and lecithin were 4×10^{-6} mol dm^{-3} and 2.5×10^{-4} mol dm^{-3}, respectively; (C) Kinetic logarithmic plot of β-carotene bleaching in the mixture, with time of UV-B irradiation. The absorbance of β-carotene was followed at 450 nm; (D) Kinetic logarithmic plot of the production of peroxides, obtained by measuring the absorbance of the lecithin/β-carotene mixture at 234 nm during various times of UV-B irradiation.

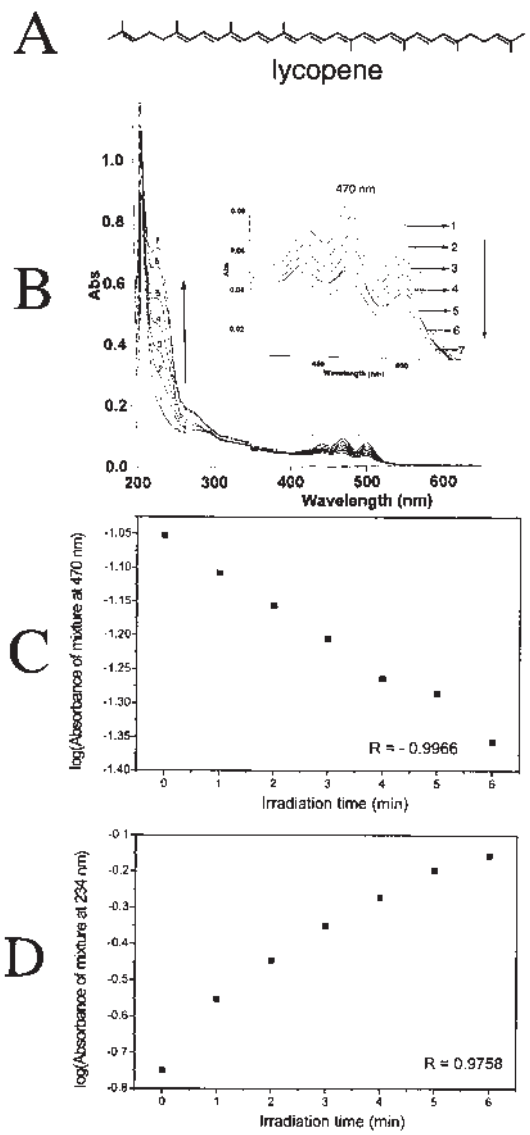

Fig. 2. (A) Structure of lycopene; (B) Changes of the absorption spectrum of a lecithin/lycopene (10:1) mixture exposed to UV-B radiation at 300 nm in hexane. The exposure time periods, min: (1) 0; (2) 1; (3) 2; (4) 3; (5) 4; (6) 5; (7) 6. The initial concentrations of lycopene and lecithin were 4×10^{-6} mol dm^{-3} and 2.5×10^{-4} mol dm^{-3}, respectively; (C) Kinetic logarithmic plot of lycopene bleaching in the mixture, with time of UV-B irradiation. The absorbance of lycopene was followed at 470 nm; (D) Kinetic logarithmic plot of the production of peroxides obtained by measuring the asorbance of the lecithin/lycopene mixture at 234 nm after various times of UV-B irradiation.

during similar irradiation regimes with UV-A and UV-C light (not shown). The kinetic logarithmic plots obtained using UV-B light showed a linear behaviour with average *R* values of about 0.98. The kinetics of the bleaching of carotenes and the production of peroxides both seem to obey first-order law (Figs. 1C, 2C and 1D, 2D, respectively). The kinetic logarithmic plots obtained with UV-A and UV-C had very similar shapes to the presented ones.

The structures of the xanthophylls (lutein and neoxanthin), the change of the absorption spectra of lecithin/xanthophylls mixtures after continuous prolonged irradiation with UV-B light (300 nm), and a kinetic logarithmic plots for increasing

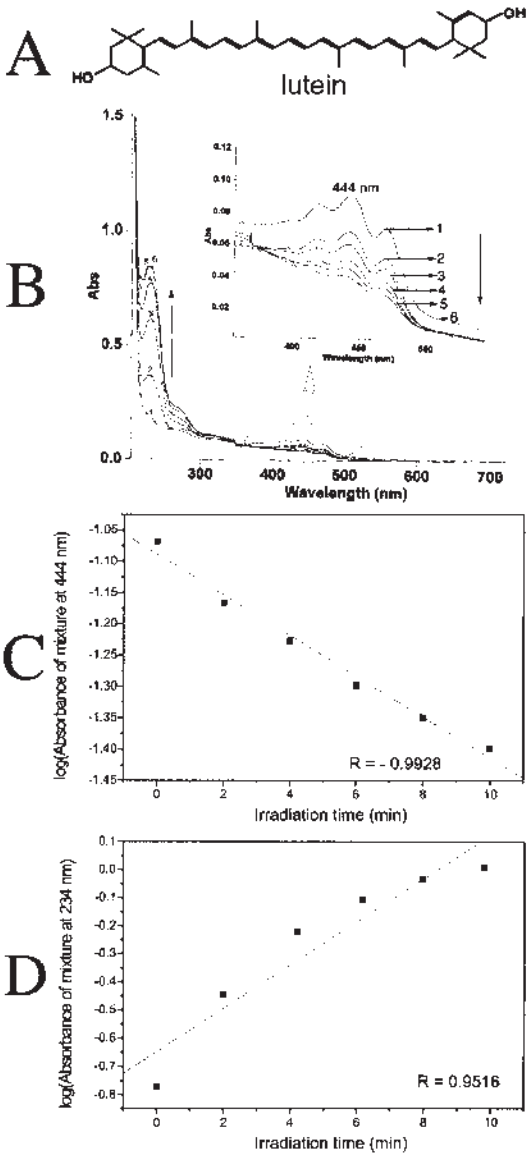

Fig. 3. (A) Structure of lutein; (B) Changes of the absorption spectrum of a lecithin/lutein (10:1) mixture exposed to UV-B radiation at 300 nm in hexane. The exposure time periods, min: (1) 0; (2) 2; (3) 4; (4) 6; (5) 8; (6) 10. The initial concentrations of lutein and lecithin were 4×10^{-6} mol dm^{-3} and 2.5×10^{-4} mol dm^{-3}, respectively; (C) Kinetic logarithmic plot of lutein bleaching in the mixture with time of UV-B irradiation. The absorbance of lutein was followed at 444 nm; (D) Kinetic logarithmic plot of the production of peroxides obtained by measuring the absorbance of the lecithin/lutein mixture at 234 nm after various times of UV-B irradiation.

irradiation times are shown in Figs. 3 and 4, respectively. The changes of the absorption spectra of pure lecithin solution in hexane (not shown) and for lecithin/xanthophylls mixtures at 234 nm (peroxides), as well as at the maximum absorption wavelengths of the xanthophylls (444 nm for lutein and 435 nm for neoxanthin) were followed (Figs. 3B, 4B). The absorption spectra showed very similar behavior during irradiation with UV-A and UV-C light (not shown). The kinetic logarithmic plots obtained with UV-B light showed a linear dependence with an average *R* value of about 0.98. The kinetics of the bleaching of the xanthophylls

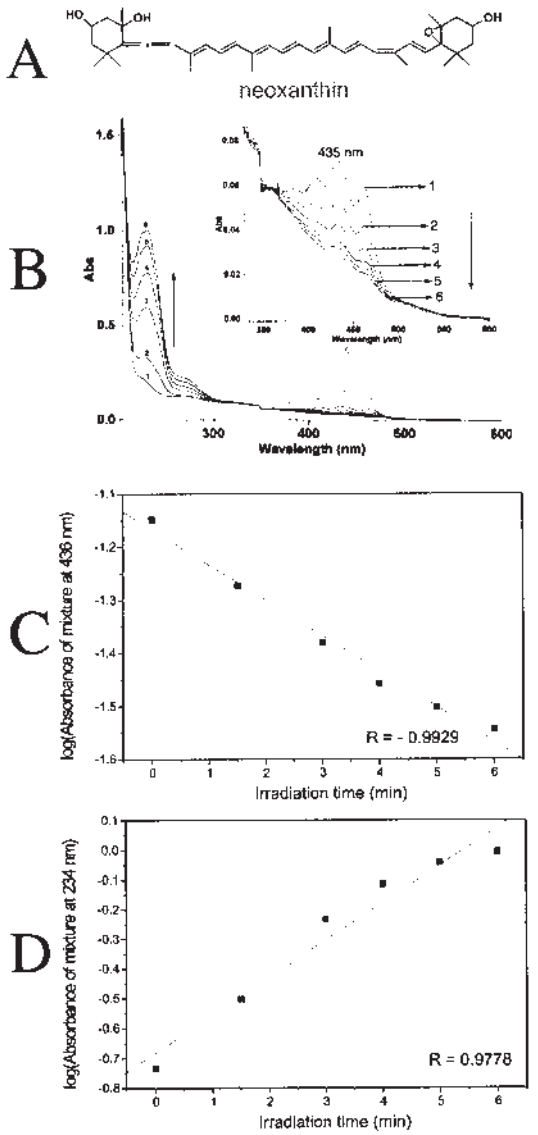

Fig. 4. (A) Structure of neoxanthin; (B) Changes of the absorption spectrum of a lecithin/neoxanthin (10:1) mixture exposed to UV-B radiation at 300 nm in hexane. The exposure time periods, min: (1) 0; (2) 1.5; (3) 3; (4) 4; (5) 5; (6) 6. The initial concentrations of neoxanthin and lecithin were 4×10^{-6} mol dm^{-3} and 2.5×10^{-4} mol dm^{-3}, respectively; (C) Kinetic logarithmic plot of neoxanthin bleaching in the mixture with time of UV-B irradiation. The absorbance of neoxanthin was followed at 435 nm; (D) Kinetic logarithmic plot of the production of peroxides obtained by measuring the absorbance of the lecithin/neoxanthin mixture at 234 nm after various times of increasing UV-B irradiation.

and the production of peroxides both seem to obey a first-order law (Figs. 3C, 4C and 3D, 4D, respectively). The kinetic logarithmic plots obtained with UV-A and UV-C had very similar shapes to the presented ones.

The slopes calculated from kinetic logarithmic plots shown in Figs. 1C–4C and 1D–4D, representing the rates of UV-induced bleaching of the carotenoids and the production of lipid peroxides of lecithin, respectively, are given in Table I. k_1 represents the rate constants of the bleaching of the carotenoids while k_2 and k_3 represent the rate constants of the production of lipid peroxides of lecithin in the absence and in the presence of the four carotenoids, respectively. Such a presenta-

tion provides a comparison of the slopes (rates constants), reflecting the changes in the production of peroxides and the kinetics of the bleaching of the pigments for a pure lecithin solution and lecithin/pigments mixtures, for all the investigated carotenoids, in all three UV-irradiation ranges. It allows an insight into the protective function of the involved carotenoids toward UV-induced lecithin peroxidation.

TABLE I. Kinetics of the bleaching of the pigments and the production of peroxides in pure lecithin solution and lecithin/pigments mixtures in hexane, during increasing UV-irradiation intervals in three UV-ranges: 254 nm (UV-C), 300 nm (UV-B) and 350 nm (UV-A). The absorbances of β-carotene, lycopene, lutein and neoxanthin were followed at 450 nm, 470 nm, 444 nm and 435 nm, respectively. The kinetics obey linear plots: $y = k_1 x + n$; $y - \log \varepsilon_{max,1}$; $\varepsilon_{max,1}$ is the absorption maxima of the pigment in lecithin/pigment mixtures at 450 nm (β-carotene), 470 nm (lycopene), 444 nm (lutein) and 435 nm (neoxanthin), x – UV-irradiation time, k_1 – first order rate constant for the bleaching of the pigment in the lecithin/pigment mixtures. $y = k_2 x + n$; $y - \log \varepsilon_{max,2}$; $\varepsilon_{max,2}$ is the pure lecithin absorption maximum at 234 nm, x – UV-irradiation time, k_2 – first order rate constant for the production of peroxides. $y = k_3 x + n$; $y - \log \varepsilon_{max,3}$; $\varepsilon_{max,3}$ is the absorption maximum of the lecithin/pigment mixtures at 234 nm, x – UV-irradiation time, k_3 – first order rate constant for the production of peroxides.

Wavelength nm	k_1/min^{-1} (carotenoids bleaaching)	k_2/min^{-1} (pure lecithin peroxidation)	k_3/min^{-1} (lecithin peroxidation in the mixtures with carotenoids)
		β-Carotene	
254	–0.10065	0.09465	0.07689
300	–0.02274	0.11050	0.09959
350	–0.00301	0.01979	0.01829
		Lycopene	
254	–0.18553	0.08774	0.07363
300	–0.04926	0.11189	0.09519
350	–0.01055	0.02357	0.02088
		Lutein	
254	–0.11370	0.08840	0.06702
300	–0.03249	0.10190	0.08527
350	–0.00251	0.01160	0.01079
		Neoxanthin	
254	–0.08504	0.09062	0.01047
300	–0.06680	0.13187	0.12662
350	–0.00246	0.01209	0.01211

DISCUSSION

Carotenoids are usually C_{40} tetraterpenoids built from eight C_5 isoprenoid units. The basic linear and symmetrical skeleton can be cyclized at one or both ends. Cyclization, hydrogenation, dehydrogenation, double-bond migration, chain shortening or extension, rearrangement, isomerization, introduction of oxygen

functions, or combinations of these processes, result in the countless structures of carotenoids.[46] A significant characteristic is a long conjugated double-bond system, providing an extended π-delocalization, leading to a substantial bathochromic shift in the Vis region. The shift is responsible for yellow, orange or red color of these compounds, including carotenes (made of carbon and hydrogen only) and xanthophylls (containing also oxygen).

Due to their structural features, carotenoids have many functions in nature. One of their major functions is to protect the photosynthetic apparatus from an excess of light, or against UV-light by preventing a huge reduction of the photosynthetic capacity and changes in the photosynthetic apparatus associated with the action of UV-light.[3,4] For this reason, some plants, such as soybean, increase the synthesis of photosynthetic pigments in the chloroplasts, especially carotenoids, as a protection mechanism against UV-irradiation.[47] A controversy of the behavior of these plants arises from the fact that carotenoids are not strong UV-absorbers but, nevertheless, are still able to have a protective function against UV-light.

Another very important function of carotenoids, of much more global character than the one related to photosynthesis (but including it!), is their antioxidant function (this is one of the reasons for the wide use of carotenoids in the food industry.[48,49] For such a purpose, carotenoids can act in a preventive manner: they may inhibit the formation of ROS species by reacting directly with oxygen, or, if radicals have already been created, they may scavenge them acting as chain-breaking antioxidants.[10,11,29] There are three possible mechanisms for carotenoids (CAR) – radicals ($R^{\bullet}$) interaction: (I) radical addition or adduct formation (CAR–$R^{\bullet}$), (II) electron-transfer reaction resulting either in a cation-radical ($CAR^{\bullet+}$), an anion-radical ($CAR^{\bullet-}$) or in a neutral alkyl-radical formation ($CAR^{\bullet}$), and (III) the hydrogen-abstraction mechanism, mostly related to the presence of carbonyl chromophores in the involved radicals (CAR + >C=O → $CAR^{\bullet}$ + >C–O–H).[32,50–52] The cation-radicals ($CAR^{\bullet+}$) and the anion-radicals ($CAR^{\bullet-}$) absorb strongly in the near-IR, with maxima in the 900–1000 nm range;[30,53–55] on the other hand it is very difficult to characterize the neutral carotenoid-radical ($CAR^{\bullet}$) since it has no distinctive strong absorption, as is observed for $CAR^{\bullet+}$ or $CAR^{\bullet-}$.[51] The particular involvement of any of the cited mechanisms (I–III) with carotenoids in hexane solution certainly depends on the chemical structure of the radicals.

Since carotenoids are not efficient UV-absorbers, including the four studied in this report, their increased destruction (bleaching) during prolonged UV-irradiation (Figs. 1B–4B, the right parts) must be free radical mediated.[32,50–52] In a recently prepared report,[56] it was shown that the (same) bleaching of carotenoids in hexane in the absence of lecithin, or any other potential protective target, obeyed pseudo first-order kinetics, implying only the electron-transfer mechanism (II), which, in an ionized hexane solution, may lead to the production of the carotenoid anion-radical ($CAR^{\bullet-}$) or the carotenoid radical-cation ($CAR^{\bullet+}$):

$$CAR + e^- \rightarrow CAR^{\bullet -} \quad (1)$$

$$CAR + RH^{\bullet +} \rightarrow CAR^{\bullet +} + RH \quad (2)$$

where $RH^{\bullet +}$ represents the hexyl radical-cation. The other two possibilities, adduct formation (I) and hydrogen abstraction (III) were excluded: the former one because the Vis absorbance (*i.e.,* the carotenoids spectra) should have remained qualitatively and quantitatively unaltered, which was not the case, since the CAR–radical adduct ($CAR^{\bullet}–R^{\bullet}$) has the same spectrum as CAR itself; the latter one (III) because there were no carbonyl moieties in the hexane solution (containing carotenoids only) capable of performing H-abstraction. Hence, the electron-transfer mechanism (II) was suggested as the only realistic option, ending with either $CAR^{\bullet -}$ or $CAR^{\bullet +}$ formation. In both cases, an extended conjugation, *i.e.,* an additional delocalization, occurs in the π-electronic system of the carotenoids (compared to the one that already exists in neutral carotenoids), moving the maximum absorption (λ_{max}) values of the carotenoids further into the near-IR range (900–1000 nm).

The system studied in this work was more complex since it contained lecithin, *i.e.*, mixture of lipoidal compounds, although from the lipid peroxidation point of view, they could be treated as a single, more or less alternated chemical species. The only moieties relevant for LP in the involved lipoidal molecules are the long-chain hydrocarbon moieties; the lipid polar heads, which are actually the major difference between them in chemical terms (*i.e.,* as chemical species: phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, phosphatidic acids) are completely irrelevant for the LP process, at least in solution.* Furthermore, the system is more complex in that the UV-irradiation may affect both the species present: carotenoids and lipids. For this reason, pure lecithin was irradiated as a blank, in order to estimate the control of LP by the four studied carotenoids during irradiation.

Table I gives a comparative review of the bleaching of the four pigments (k_1 rate constants) with the simultaneous production of lipid peroxides in the absence and in the presence of the studied carotenoids (rate constants k_2 and k_3, respectively), in the three investigated UV-ranges. The 2nd column shows a huge drop in the k_1 values when going from UV-C to UV-B and from UV-B to UV-A photons, which was also observed for the same pigment in the absence of lecithin.[56] However, while the ratios of the bleaching rates of the four carotenoids ($k_{1,UV\text{-}C}$ / $k_{1,UV\text{-}B}$ and $k_{1,UV\text{-}B}$ / $k_{1,UV\text{-}A}$), in the absence of lecithin, averaged about 10 (*i.e.,* one order of magnitude difference between the bleaching rate constants related to the three UV-ranges), this work shows that in the presence of lecithin, the bleaching ratios were two-step regulated. The $k_{1,UV\text{-}C}$ / $k_{1,UV\text{-}B}$ ratios were 4.4 for β-carotene, 3.8 for lycopene, 3.5 for lutein, and just 1.3 for neoxanthin, while the $k_{1,UV\text{-}B}$ / $k_{1,UV\text{-}A}$ ratios were 7.6 for β-carotene, 4.7 for lycopene, 12.9 for lutein and 27.5

* In micellar or more complex system, the polar heads play a role in the organization of assemblies, influencing therefore, in an indirect way, the LP process itself.

for neoxanthin. Thus in all cases, the $k_{1,UV\text{-}B} / k_{1,UV\text{-}A}$ ratio was larger than the corresponding $k_{1,UV\text{-}C} / k_{1,UV\text{-}B}$ ratio. However, the relationship (*i.e.,* the ratio of the two ratios, $[(k_{1,UV\text{-}B} / k_{1,UV\text{-}A})]/[(k_{1,UV\text{-}C} / k_{1,UV\text{-}B}])$ was quite different for the involved carotenoids: the $[(k_{1,UV\text{-}B} / k_{1,UV\text{-}A})]/[(k_{1,UV\text{-}C} / k_{1,UV\text{-}B})]$ ratio was 1.7 for β-carotene, 1.2 for lycopene, 3.6 for lutein and 21.3 for neoxanthin. Both these facts confirm two things. Firstly, the bleaching of the carotenoids is highly dependent on the energy input of the UV-photons (ranging from 18–25 W/m^2), which (converted to eV s) widely encompasses (a couple of orders of magnitude) the ionization potentials of all the chemical species present in this system (5–10 eV). A higher energy input generally means more radicals are present in the system, primarily hexyl radicals ($RH^{\bullet}$) and lipid radicals ($L^{\bullet}$), as well as lipid peroxy radicals ($L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$), since oxygen is certainly present in the hexane solution.[57] Secondly, the ratios of the bleaching rates of the carotenoids are dependent on their chemical structures. Just based on the $[(k_{1,UV\text{-}B} / k_{1,UV\text{-}A})] / [(k_{1,UV\text{-}C} / k_{1,UV\text{-}B})]$ values, it is evident that the xanthophylls (neoxanthin and lutein) are more sensitive to changes in the energy of incident UV-photons, going from UV-A *via* UV-B to UV-C, than the carotenes. Furthermore, neoxanthin is more sensitive than lutein (the $[(k_{1,UV\text{-}B} / k_{1,UV\text{-}A})] / [(k_{1,UV\text{-}C} / k_{1,UV\text{-}B})]$ values of 21.6 and 3.6, respectively). The corresponding values for the carotenes (β-carotene and lycopene) are smaller (compared to the xantophylls) and mutually closer (1.7 for β-carotene and 1.2 for lycopene). Obviously, since none of the carotenoids are efficient UV-absorbers, the observed sensitivity with changing UV-energy is not directly related. In this complex system, not only electron-transfer mediated bleaching (mechanism II, Eqs. (1, 2), with consequential production of carotenoid radicals, $CAR^{\bullet-}$ and $CAR^{\bullet+}$) must be taken into account but also, at least, possible H-abstraction (mechanism III). The lipid peroxy-radicals ($LOO^{\bullet}$) are not only chain-propagation agents, but, although not containing carbonyl chromophores, possible H-abstractors, too.[15] The adduct formation (at least $CAR\text{–}L^{\bullet}$), mechanism (I), can not be strongly substantiated based on the presented results: the absorption spectra changes are too strong and too evident (Figs. 1B–4B). Thus, in the studied system, the carotenoids might be bleached possibly through (a) the electron-transfer mechanism (II) and H-abstraction (III) and/or (b) their free-radical scavenging ($L^{\bullet}$ and $LOO^{\bullet}$), chain-breaking antioxidative function. To distinguish between the two options (a) and (b), the rate constants of the production of lipid peroxides were determined (based on the absorbance measurements performed at 234 nm) in the absence and the presence of the four carotenoids. The changes in the absorption at 234 nm for different UV-B irradiation periods recorded in the presence of β-carotene, lycopene, lutein and neoxanthin are shown in Figs. 1B–4B, respectively (the left parts). Very similar curves were obtained with UV-A and UV-C irradiation. The k_2 and k_3 rate constants obtained for all three UV-ranges (the latter ones calculated from logarithmic plots obtained in the presence of carotenoids, Figs. 1D–4D) are presented in Table I.

The k_2 rate constants obtained with UV-B and UV-C irradiation are very similar. On the contrary, the k_2 values obtained with UV-A irradiation are about one order of magnitude smaller. Hence, energy of the UV-photons plays again a crucial role in the production of peroxides, as in the case of the bleaching of the carotenoids. The slightly higher k_2 values obtained with UV-B ($k_{2,\,\text{UV-B}}$) compared to the ones obtained with UV-C ($k_{2,\,\text{UV-C}}$) may be explained by the fact that UV-C not only produces lipid peroxides, but is simultaneously strongly absorbed by the same diene peroxative structures.[58] Thus a (dis)balance between the creation and destruction of peroxides exists, whereby the balance is shifted to the left at the beginning of the irradiation, but later a steady-state level is established. For this reason the higher energy input (of UV-C compared to UV-B) does not result in correspondingly higher k_2 values; the $k_{2,\,\text{UV-B}}$ values are just a little higher, since UV-B light is less destructive toward the created diene structures than UV-C.[58]

The most crucial point of this work, reflecting the antioxidant activities of the four carotenoids in hexane solution in the presence of lecithin, comes from the comparison of the k_2 and k_3 rate constants. Even a brief look shows that these values (obtained for the same UV-range) are generally very close. The $k_{2,\,\text{UV-A}}$ and $k_{3,\,\text{UV-A}}$ are especially close (equal in the case of neoxanthin, and about 10 % different in the case of the other three carotenoids, Table I), while the $k_{3,\text{UV-B}}$ and $k_{3,\,\text{UV-C}}$ rate constants are approximately 20 % smaller than the corresponding k_2 rate constants ($k_{2,\,\text{UV-B}}$ and $k_{2,\,\text{UV-C}}$). The latter fact is of exceptional importance for the anticipated antioxidant role that the four carotenoids are expected to play in preventing or significantly diminishing lecithin peroxidation. While they substantially undergo pronounced bleaching, their contribution in decreasing lecithin peroxidation does not exceed 20 % (for UV-B and UV-C). In other words, their antioxidative control of the LP process of lecithin is of marginal importance. The techniques used in this work did not allow a detailed explanation of this behaviour to be offered, *i.e.,* to propose the exact mechanism(s) which govern the bleaching of carotenoids and the simultaneous peroxidation of lecithin under the direct action of UV irradiation. Laser flash photolysis or pulse radiolysis techniques[59] (as used in the other studies[12,20,21] have to be employed for such a purpose. Nevertheless, a reasonable explanation might be offered based on the presented results.

CONCLUSIONS

The results presented in this work suggest two things: (i) the scavenging of lipid radicals ($L^{\bullet}$ and/or $LOO^{\bullet}$) by carotenoids is a marginal event compared to their own bleaching, (ii) this fact does not negate a general antioxidant function of carotenoids.[60,61] Instead, it changes their scavenging (antioxidant) capacity in a (highly unordered) homogeneous solution where all the radicals present are free to move in any direction. This certainly leads to smaller probabilities for the occurrence of at least some radical interactions and consequentially scavenging actions. However, it is reasonable to expect that in a very constrained, space-limited sys-

tems, such as micelles or monolayers, where the movement of the free radicals is highly sterically dependent due to the predominant "cage effect",[12,21,22,62] the scavenging capabilities of the employed carotenoids toward lipid radicals should be more expressed.[41,63,65]

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ИЗВОД

МАРГИНАЛНИ ДОПРИНОС ОДАБРАНИХ КАРОТЕНОИДА У СУЗБИЈАЊУ ПЕРОКСИДАЦИЈЕ ЛЕЦИТИНА ИНДУКОВАНЕ УЛТРАВИОЛЕТНИМ ЗРАЧЕЊЕМ У ХЕКСАНУ

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Циљ овог рада је проучавање антиоксидационе улоге 4 изабрана каротеноида у смеши са лецитинским липидима у хексану, изложеној дејству ултравиолетног зрачења из три различита опсега (UV-A, UV-B и UV-C). Два каротена (бета-каротен и ликопен) и два ксантофила (лутеин и неоксантин) су коришћена да контролишу процес липидне пероксидације, генерисан дејством UV-зрачења, "хватањем" слободних радикала укључених у овај процес. Резултати показују да док каротеноиди подлежу базичној, структурно зависној деструкцији (обезбојавању), која је врло зависна од енергије улазних UV-фотона, њихов допринос очекиваном сузбијању пероксидације лецитина је од маргиналног значаја, не премашујући 20 %. Ово маргинално антиоксидантно понашање каротеноида приписано је неуређеном раствору хексана у коме је "хватање" слободних радикала од стране каротеноида слабо ефикасно.

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