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UV-induced change in the antioxidant activity of quercetin toward benzophenone-initiated lipid peroxidation

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Abstract: The aim of this work was to estimate the degradation and change in the antioxidant activity of quercetin in the presence of two different mixtures of phospholipids in methanolic solution, under continuous UV-irradiation from three different sub-ranges (UV-A, UV-B and UV-C), in the presence and in the absence of the selected UV-absorbing photosensitizer, benzophenone. Quercetin was employed to control the lipid peroxidation process generated by UV-irradiation, by absorbing part of the UV incident light, and/or by scavenging the involved, created free radicals. The results showed that quercetin undergoes irreversible destruction, which was highly dependent on the energy input of the UV-photons, and was more expressed in the presence than in the absence of benzophenone. Simultaneously, quercetin expressed a suppression effect on lipid peroxidation processes in UV-irradiated phospholipid mixtures in both the absence and presence of benzophenone (more or less effective, respectively). In the UV-C-irradiated mixtures, photosensitizing function of benzophenone was significantly reduced due to its strong absorption in the UV-C spectral range, therefore affecting lower antioxidant activity of the remaining quercetin.

Keywords: quercetin; phospholipids; benzophenone; UV-irradiation; lipid peroxidation.

INTRODUCTION

Ultraviolet (UV) radiation is part of the natural sunlight spectrum that reaches the Earth's surface. Most of the solar UV energy incident on human skin derives from the deeply penetrating UV-A region (>95 % from 320 to 400 nm),[1] while the rest belongs to the more energetic UV-B radiation (280–320 nm); UV-C radiation (200–280 nm) is completely absorbed by the atmosphere. In addition to being the obligatory driving force of photosynthesis, light may also be a damaging factor in the biosphere:[2] recent depletion of the stratospheric ozone layer

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has led to an increase of the biologically most damaging UV-B portion at ambient levels. It triggers events that affect many crucial biological processes of global importance, such as DNA replication[3,4] and photosynthesis.[5,6] It has been especially recognized as one of the major agents that initiate many harmful free radical-mediated processes, such as lipid peroxidation (LP).

Lipid peroxidation occurs either by a free radical chain reaction (Type I), or, through a non-radical pathway (Type II), by direct reaction with singlet oxygen, created in the presence of a photosensitizer.[7–10] As for every free radical-mediated process – in the form of a chain reaction – lipid peroxidation consists of an initiation step (formation of lipid radicals – $L^{\bullet}$), a propagation step (in which lipid radicals react with oxygen to form lipid peroxy radicals – $LOO^{\bullet}$), and a termination step (formation of lipid hydroperoxides – LOOH – with a diene type of structure).[10,11] 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, some of which include typical lipid radical "producers", such are a variety of external stresses,[10] implying very commonly an external radiation;[11–13] in case of UV-irradiation, LP may include a special type of LP initiators, *e.g.*, photosensitizers such as benzophenone (BZP), in very different media.[14–16]

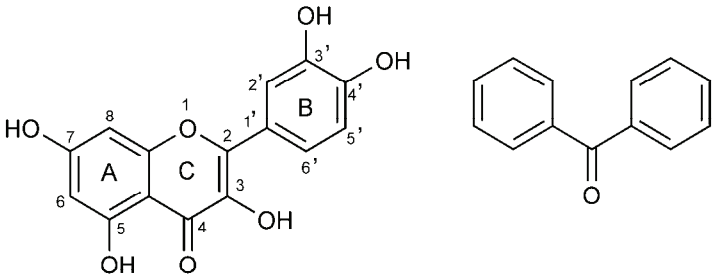
Benzophenone (Fig. 1) has been long recognized for its most known organic photochemical reaction, *i.e.*, H-abstraction by its long lived triplet state, ^{3}BZP.[14–16] The photochemistry of benzophenone and BZP-related compounds is very complex and depends on the particular solvent in homogeneous solutions,[17–19] or on a particular BZP-interactive compound in a given solvent.[20,21] By absorbing UV-light, benzophenone (photosensitizer) can be promoted into a long-lived triplet state (^{3}BZP) which is very reactive toward its surroundings (*e.g.*, phospholipid mixture and quercetin), including the crucial interaction for this study, the direct reaction with lipids resulting in the production of lipid radicals ($L^{\bullet}$) in the initiation step of the lipid peroxidation (LP) chain reaction:[22]

$$^3\text{BZP} + \text{LH} \rightarrow \text{BZPH}^{\bullet} + \text{L}^{\bullet} \quad (1)$$

where ^{3}BZP is the triplet state of benzophenone, $BZPH^{\bullet}$ is the BZP-ketyl-radical, LH is the unsaturated lipid and $L^{\bullet}$ is the lipid radical.

On the other hand, lipid peroxidation is mostly controlled *in vivo* by the action of the antioxidants. Many biomolecules (and classes of biomolecules) serve as antioxidants, including flavonoids.[23–25] Flavonoids are benzo-γ-pyrone derivatives consisting of pyran and phenolic rings. The most important structural elements related to their functions are: the *o*-dihydroxy group in the B-ring (catechol structure) as a potential radical target, the double bond between positions 2 and 3 of the C-ring conjugated with a keto group in position 4 (because of its capacity to delocalize the unpaired electron of a flavonoid radical), and the C-3,

C-5 and C-7 hydroxyl groups (of the C- and A-rings), as potential scavengers of free radicals (Fig. 1).[26–28] Quercetin (flavonol) is a member the flavonoids family, having the structure shown in Fig. 1. Recent studies connect the high antioxidant activity of flavonols with the presence of the OH-group in position 3 of the C-ring in combination with the catechol structure of the B-ring.[25–29]



Quercetin Benzophenone

Fig. 1. Structure of quercetin and benzophenone.

Quercetin and its glycosides are reportedly synthesized in plants as a part of their total response toward UV-radiation to prevent extended induced damage.[30,31] Quercetin absorbs UV radiation with absorbance maxima in the UV-A (λ_{max} = 365 nm, ε = 28,400 mol^{-1} dm^3 cm^{-1}) and UV-C range (λ_{max} = 256 nm, ε = 28,300 mol^{-1} dm^3 cm^{-1}). However, flavonoids can also act as free radical scavengers to prevent oxidative skin damage[32–35] and their topical application has met with considerable interest.[36–38] Thus, quercetin may provide protection against UV radiation either through absorption (preventive, inhibition mode) or scavenging activities ("chain-breaking", antioxidant mode). In the former case, the absorbed UV energy may be dissipated as heat[39] or converted into quercetin decomposition products, both *in vitro* and *in vivo*.[29,40,41] In the latter case, quercetin (as well as other flavonoids) scavenges already created free radicals (such as lipid peroxy radicals, LOO$^\bullet$, or some ROS species present), mostly by the hydrogen-atom-transfer mechanism, shown in Eq. (2):[42–44]

$$\text{Fl–OH} + \text{L}^\bullet \rightarrow \text{Fl–O}^\bullet + \text{LH} \qquad (2)$$

The oxidized Fl–O$^\bullet$ radical (which can be stabilized by one intramolecular H-bonding in the B-ring: –O$^\bullet\cdots$H–O–),[44] may react with a second radical (L$^\bullet$), acquiring a quite stable quinone structure.[45]

In a recent publication,[46] the stabilities of two flavonoid components, quercetin and rutin, in solution toward UV-irradiation (from the three sub-ranges, UV-A, UV-B and UV-C), as well as their antioxidant activities in the presence of a lipoidal mixture (*i.e.*, lecithin), as the "protection target", were compared. Despite the lower stability of quercetin against UV-irradiation, its antioxidant ability

to protect the lipid mixture from peroxidation was still found to be higher than that of rutin. In addition, it was recently found that continuous UV-irradiation of quercetin and rutin in solution was followed by the creation of products that absorb in spectral region between 250 and 350 nm.[47]

The system studied in this work was more complex compared to the previous one[46] due to the presence of benzophenone. The investigated system (lipids + BZP + quercetin, in methanol) was planned to provide studies of both protective actions of quercetin during prolonged continuous UV-irradiation: the preventive one (studied through UV-induced degradation), as well as the antioxidant one (studied indirectly, by tracing the proliferation of UV-induced LP process, expressed through creation of diene structures of LP peroxides in the presence and absence of benzophenone. Due to a greater complexity of the studied system, pure phospholipids (of higher "purity", compared to the previous report[46]) and pure BZP were irradiated as blanks (separately and their mixture), in order to estimate in the next step, LP control by quercetin during UV-irradiation, and estimate the differences between the rates of lipid peroxide production (LPP) in the presence and absence of quercetin, as well as in the presence and absence of the photosensitizer (BZP).

The system was studied by using prolonged irradiation from three UV-subranges (UV-A, 320–400 nm; UV-B, 290–320 nm; UV-C, 200–280 nm). The changes of the BZP-initiated lipid peroxidation were estimated in the light of quercetin presence and action in this system, under the same UV-irradiation regime.

EXPERIMENTAL

Phospholipids were gifted by Phospholipid GmbH, Cologne, Germany (Phospholipon® 80 and Phospholipon® 90). According to the Manufacture's declaration, the composition is for Phospholipon® 80 (PL80), phosphatidylcholine 78 %, lyso-phosphatidylcholine 3.3 %; fatty acid composition: palmitic acid 10–15 %, stearic acid 1.5–4 %, oleic acid 6.0–13.0 %, linoleic acid 61–71.0 %, linolenic acid 4.0–7.0 %, peroxide value max. 2.1, and for Phospholipon® 90 (PL90), phosphatidylcholine 98 %, lyso-phosphatidylcholine 2.1 %; fatty acid composition: palmitic acid 12±2 %, stearic acid 3±1 %, oleic acid 10±3 %, linoleic acid 66±5 %, linolenic acid 5±2 %, peroxide value max. 1.3. The phospholipids mixtures were kept in the dark to prevent at least the photooxidation process. Benzophenone (BZP) was obtained from Sigma. (St. Louis, CA, USA). The quercetin standard was purchased from Merck.

UV-irradiation

Continuous irradiation of the samples in methanol were performed in a cylindrical photochemical reactor "Rayonnet", with 10 symmetrically placed lamps with emission maxima in three different ranges: 350 (UV-A), 300 (UV-B) and 254 nm (UV-C). The samples were irradiated in quartz cuvettes (1 cm×1 cm×4.5 cm) placed on the rotating circular holder. The total measured energy flux was about 12.9 W m^{-2} for 350 nm, 15.0 W m^{-2} for 300 nm and 17.9 W m^{-2} for 254 nm at 10 cm distance from the lamps.

UV–Vis spectroscopy

The UV–Vis spectra of the phospholipids dissolved in methanol, and the phospholipids mixed with flavonoids and benzophenone, before and after irradiation with UV-light, were recorded on a Varian Cary-100 spectrophotometer. All spectra were recorded in the spectral range 200 to 600 nm.

Spectrometry for conjugated dienes

The peroxidative dienes structures were determined by measuring the absorbance at 234 nm in methanol solution.[22,48,49] The phospholipids, quercetin and benzophenone were dissolved separately in methanol and then mixed. The final concentrations of phospholipids, quercetin and BZP in mixture were 3.5×10^{-3} g dm^{-3}, 2.5×10^{-5} and 1.1×10^{-5} mol dm^{-3}, respectively. The peroxidation of phospholipids was generated by UV-irradiation at 350 (UV-A), 300 (UV-B) and 254 nm (UV-C). Methanolic solutions of BZP alone and phospholipids (with and without BZP), as kind of blanks, were irradiated simultaneously with the phospholipids/quercetin and phospholipids/quercetin/BZP mixtures. The increase in the absorbance at 234 nm, as an indication of the formation of peroxidative diene structures, was determined by UV–Vis measurements. The maximum quercetin absorbances were recorded as a function of UV-irradiation time to follow the rate of its degradation.

RESULTS

The changes of the absorption spectra of BZP after continuous prolonged irradiation in methanol with UV-B light (300 nm) are shown in Fig. 2A. The analogue spectra obtained for BZP with UV-A (350 nm) and UV-C (254 nm) radiation expressed very similar shapes (data not shown). The corresponding kinetic ln-plots (of BZP absorbance maximum at 253 nm, $A_{253\ nm}$) for increasing irradiation intervals ($t_{irr.}$), for all three UV-subranges (UV-A, -B and -C), are shown in Fig. 2B and the corresponding degradation (*i.e.*, bleaching) rate constants ($k_{BZP\text{-}bleach.}$ in min^{-1}), determined as the slopes of linear plots shown in Fig. 2B are displayed on the corresponding graphs.

The changes in the absorption spectra of the mixture of BZP with quercetin after continuous prolonged irradiation in methanol with UV-B light (300 nm) are shown in Fig. 3A. The analogue spectra obtained for the BZP + quercetin mixture with UV-A (350 nm) and UV-C (254 nm) radiation expressed very similar shapes (not shown). The corresponding kinetic ln-plots (of the mixture absorbance at 372 nm, $A_{372\ nm}$, (the maximum absorbance of quercetin) for increasing irradiation intervals ($t_{irr.}$), for all three UV-sub-ranges (UV-A, -B and -C), are shown in Fig. 3B, and the corresponding degradation rate constants ($k_{(BZP+Querc.)\text{-}bleach.}$ in min^{-1}), determined as the slopes of linear plots shown in Fig. 3B, are displayed on the corresponding graphs.

The changes in the absorption spectra of PL80 and the PL80/BZP mixture in methanol, followed at 234 nm for peroxide formation, during continuous prolonged irradiation with UV-B light are shown in Figs. 4A and 4B, respectively. The corresponding kinetic ln-plots (of absorbance recorded at 234 nm, $A_{234\ nm}$) for increasing UV-B irradiation periods ($t_{irr.}$), with linear fitting (average R va-

lues of about 0.98), are shown in Fig. 4C. The changes in the absorption spectra and corresponding kinetic ln-plots obtained for UV-A and UV-C irradiated PL80 and PL80/BZP, as well as for UV-A, -B and -C irradiated PL90 and PL90/ /BZP mixtures in methanol expressed very similar shapes to the ones presented.

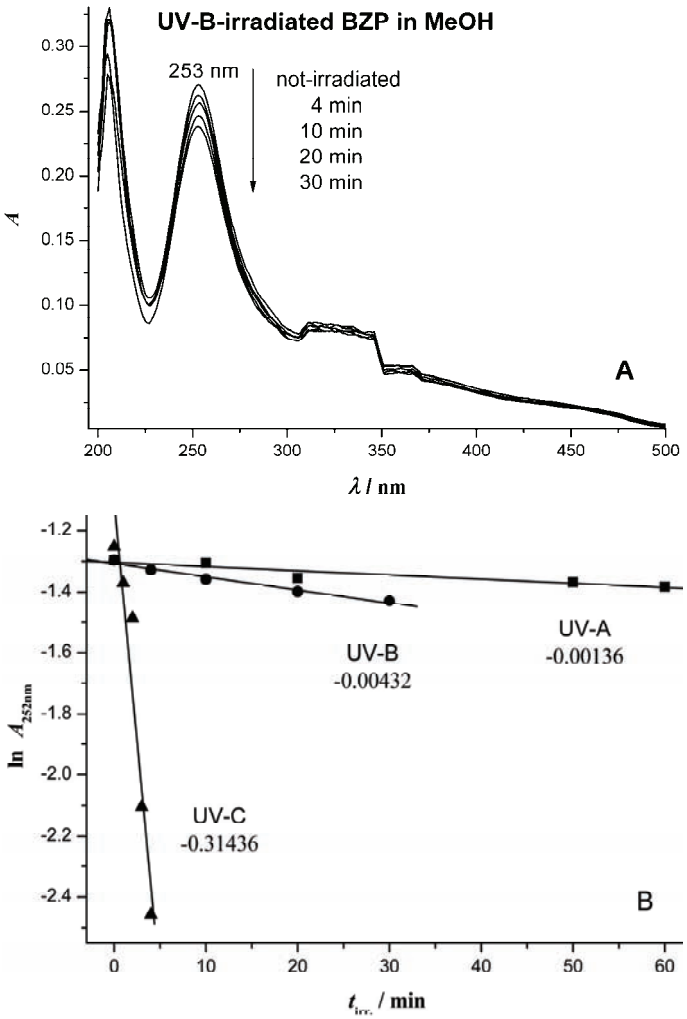


Fig. 2. Changes in the absorption spectrum of benzophenone in methanol during exposure to UV-B radiation at 300 nm. The initial concentration of benzophenone was 1.1×10^{-5} mol dm^{-3} (A). Kinetic ln plot of benzophenone bleaching induced by UV-A, -B and -C irradiation; the absorbance of benzophenone was followed at 253 nm; the corresponding bleaching rate constants ($-k_{\text{BZP-bleach.}}$ in min^{-1}) are displayed for all three UV-irradiation ranges (B).

The changes in absorption spectra of PL90/quercetin and PL90/BZP/quercetin mixtures in methanol, with lipid peroxides production (LPP) followed at

234 nm and quercetin degradation followed at 372 nm after continuous prolonged irradiation with UV-B, are shown in Fig. 5A and 5C, respectively. The augmented parts of the same spectra, focusing only on the quercetin absorption at 372 nm, are shown in the insets in Fig. 5A and 5C, respectively. The corresponding kinetic ln-plots of peroxide production followed at 234 nm ($A_{234\ nm}$) and quercetin degradation followed at 372 nm ($A_{372\ nm}$) showed linear dependences (with average R values of about 0.98), Fig. 5B and 5D, respectively).

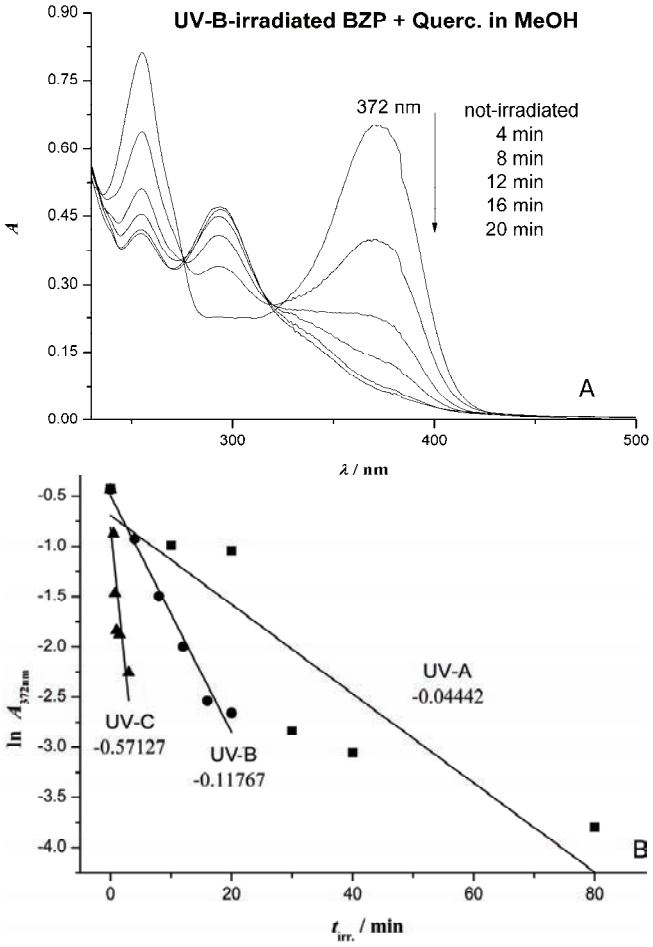


Fig. 3. Changes in the absorption spectrum in the mixture of benzophenone with quercetin in methanol exposed to UV-B radiation at 300 nm. The initial concentration of benzophenone was 1.1×10^{-5} mol dm^{-3} and the quercetin concentration was 2.5×10^{-5} mol dm^{-3} (A). Kinetic ln plot of the (BZP + querc.) mixture bleaching induced by UV-A, -B and -C irradiation; the absorbance of the mixture was followed at 372 nm (the absorbance maximum of quercetin); the corresponding degradation-bleaching rate constants ($-k_{(BZP+Querc.)\text{-}bleach.}$ / min^{-1}) are displayed for all three UV-irradiation ranges (B).

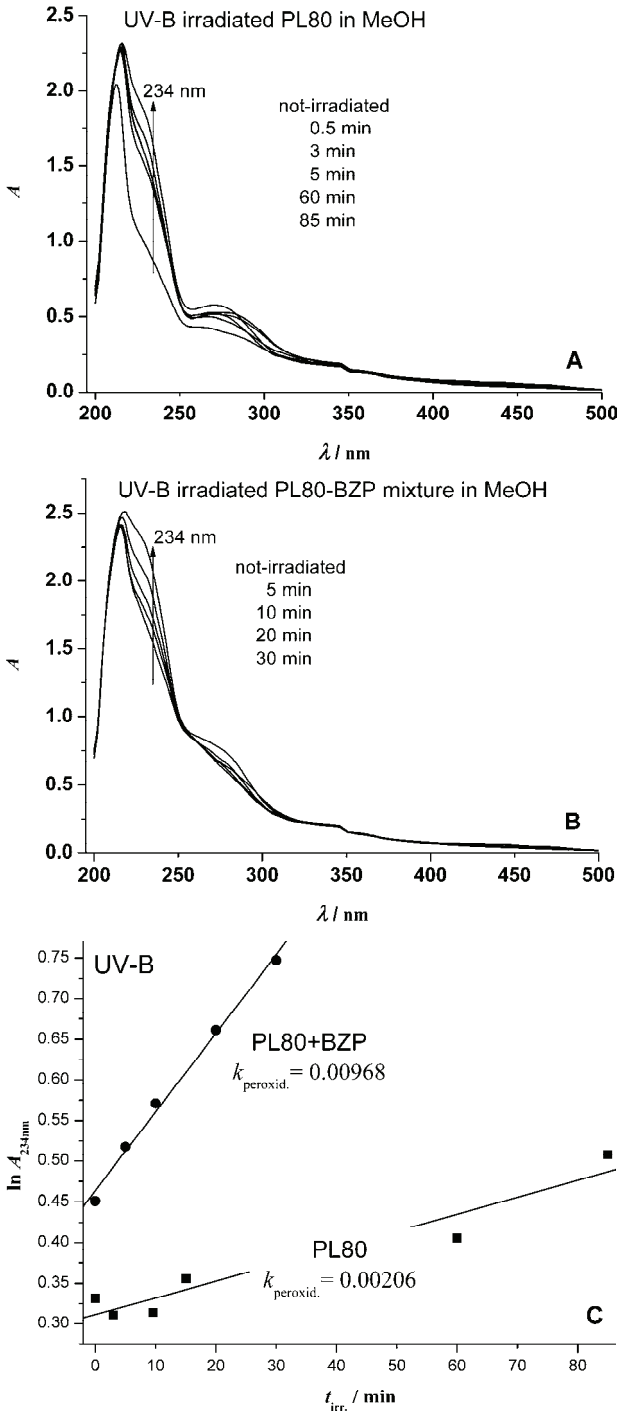
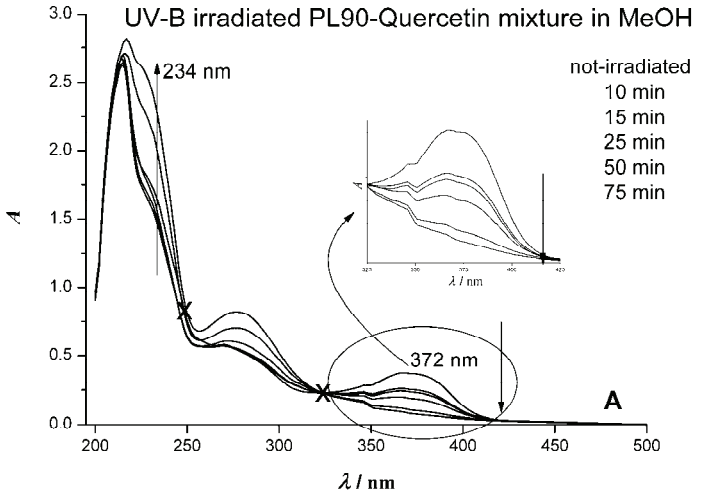
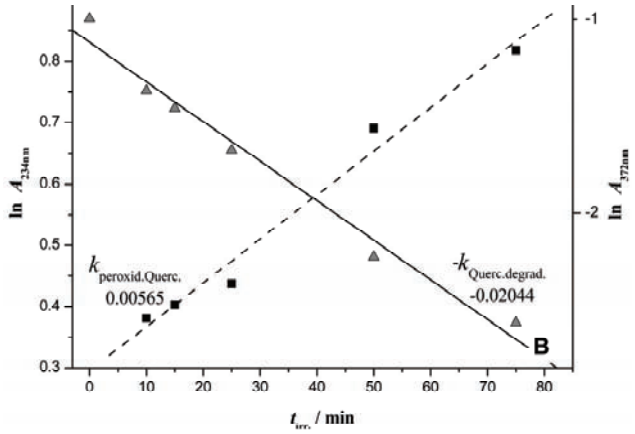
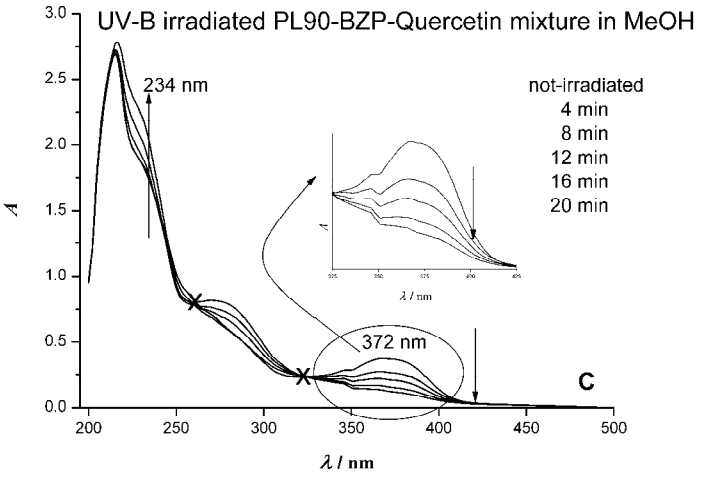


Fig. 4. Changes in the absorption spectrum of PL80 in the phospholipid and phospholipid/ /BZP mixture, respectively, exposed to UV-B radiation (300 nm) in methanol. The initial concentrations of BZP and PL80 phospholipids were 1.1×10^{-5} mol dm^{-3} and 3.5×10^{-3} g dm^{-3}, respectively (A and B); kinetic ln plots for the production of lipid peroxides, obtained by measuring the absorbance of the PL80 and PL80/ /BZP mixture at 234 nm in methanol, following different, increasing periods of UV-B irradiation; the corresponding degradation rate constants ($k_{peroxid.}$ / min^{-1}) are displayed also (C).







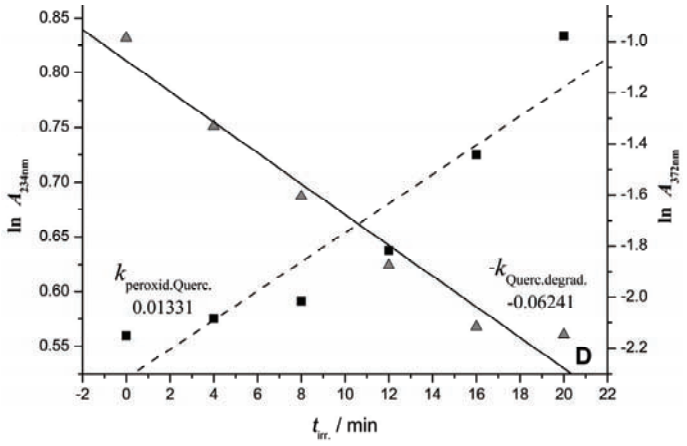


Fig. 5. Changes in the absorption spectrum of PL90 in the phospholipid/quercetin (A) and phospholipid/BZP/quercetin (C) mixtures exposed to UV-B radiation (300 nm) in methanol; the augmented part of the same spectra, focusing only on the quercetin absorption with maximum at 372 nm, is shown as the insets of graphs A and C, respectively. The initial concentrations of BZP, PL90 phospholipids and quercetin were 1.1×10^{-5} mol dm^{-3}, 3.5×10^{-3} g dm^{-3} and 2.5×10^{-5} mol dm^{-3}, respectively. Kinetic logarithmic plots obtained from the lipid peroxides absorbance data at λ_{max} = 234 nm and quercetin degradation data in the same mixture at 372 nm, following increasing periods of UV-B irradiation for phospholipids/quercetin (B) and phospholipids/BZP/quercetin (D) mixtures in methanol; the corresponding LP-production and quercetin UV-induced degradation rate constants ($k_{peroxid.Querc.}$ and $k_{Querc.degrad.}$, respectively, in min^{-1}) are displayed on the graphs B and D.

The changes of the absorption spectra and the kinetic ln-plots obtained with UV-A and -C for these mixtures, as well as for PL80 related mixtures irradiated with UV-A, -B and -C, expressed very similar shapes as the presented ones.

The slopes calculated from kinetic ln-plots shown in Fig. 4, representing the rates of UV-induced production of lipid peroxides (followed at 234 nm, $k_{peroxid.}$) in both investigated mixtures of phospholipids (PL80 and PL90), in the presence and absence of benzophenone, are given in Table I. The $k_{peroxid.}$ rate constants were calculated for all three UV-irradiation ranges. Thus, the $k_{peroxid.}$ rate constants (calculated in min^{-1}) represent some kind of blank indicators for the peroxidation of phospholipids (in absence of quercetin as an antioxidant).

The slopes calculated from kinetic ln-plots shown in Fig. 5, representing the rates of UV-induced lipid peroxides production (followed at 234 nm, $k_{peroxid.Querc.}$) and quercetin degradation (followed at 372 nm, $k_{Querc.degrad.}$) in the investigated phospholipid/quercetin and phospholipid/quercetin/BZP mixtures, are given in Table II for both investigated types of phospholipids (PL80 and PL90). The $k_{peroxid.Querc.}$ and $k_{Querc.degrad.}$ rate constants (in min^{-1}) were calculated for all three UV-irradiation ranges.

TABLE I. Kinetics of phospholipids (PL80 and PL90) peroxidation ($k_{peroxid.}$ / min^{-1}) in the presence or in the absence of BZP in methanol during increasing UV-irradiation intervals ($t_{irr.}$) for the three UV-ranges: 350 (UV-A), 300 (UV-B) and 254 nm (UV-C). The absorbance of phospholipids and phospholipids/BZP mixture were followed at 234 nm. The initial concentrations of BZP and phospholipids were 1.1×10^{-5} mol dm^{-3} and 3.5×10^{-3} g dm^{-3}, respectively

UV-Range	PL80		PL90	
	without BZP	with BZP	without BZP	with BZP
UV-A (350 nm)	0.00156	0.00592	–	0.00590
UV-B (300 nm)	0.00206	0.00968	0.00371	0.01420
UV-C (254 nm)	0.03152	0.00910	–	0.02026

TABLE II. Kinetics of UV-induced quercetin degradation and lipid peroxides production (LPP) in phospholipids (PL80 & PL90)/quercetin and (PL80 and PL90)/BZP/quercetin mixtures in methanol ($k_{Querc.degrad.}$ and $k_{peroxid.Querc.}$ in min^{-1}, respectively) during increasing UV-irradiation intervals for three UV-ranges: 350 (UV-A), 300 (UV-B) and 254 nm (UV-C). Quercetin degradation and phospholipids peroxidation were followed at 372 and 234 nm, respectively. The initial concentrations of BZP, phospholipids, and quercetin were 1.1×10^{-5}, 3.5×10^{-3} and 2.5×10^{-5} mol dm^{-3}, respectively

UV-Range	PL80 + quercetin				PL90 + quercetin			
	without BZP		with BZP		without BZP		with BZP	
	λ / nm							
	372	234	372	234	372	234	372	234
UV-A	–	–	0.01545	0.00244	0.00177	0.00070	0.02554	0.00229
UV-B	0.01804	0.00127	0.05031	0.00742	0.02044	0.00565	0.06241	0.01331
UV-C	0.04796	0.00377	0.07152	0.00515	0.05765	0.02019	0.07439	0.01918

DISCUSSION

Bearing in mind that the all results were obtained by UV spectrophotometry, it is important to define "clarity criteria" for their interpretation, based on the positions of the λ_{max} values. Benzophenone absorbs in the spectral range 225–300 nm, with the absorbance maximum at 253 nm in methanol (Fig. 2). The UV-induced formation of lipid peroxides could be followed at 234 nm (see experimental section and Figs. 4 and 5), while quercetin in methanol has absorption maxima at 256 nm and at 372 nm (at which wavelength the degradation kinetics quercetin was followed) (Fig. 5).

UV-induced bleaching of benzophenone, and the benzophenone + quercetin mixture in methanol

Prolonged continuous irradiation of benzophenone (BZP) in methanol caused a gradual decrease in the absorbance during increasing irradiation periods over the whole spectral range (200–500 nm) for all the three applied UV-sub-ranges (Fig. 2A). These changes measured at 253 nm followed first order kinetics, as shown in Fig. 2B. The BZP bleaching constant, $k_{BZP\text{-}bleach.}$, in methanol in-

creased with increasing UV-irradiation energy input, from UV-A to -C (Fig. 2B); the ratios of the bleaching rates for UV-C and -B and UV-C and -A irradiated BZP in methanol showed differences of two orders of magnitude ($k_{\text{BZP-bleach.(UV-B)}}/k_{\text{BZP-bleach.(UV-A)}}$ = 3.2 and $k_{\text{BZP-bleach.(UV-C)}}/k_{\text{BZP-bleach.(UV-B)}}$ = = 72.8, respectively, and the ratio $k_{\text{BZP-bleach.(UV-C)}}/k_{\text{BZP-bleach.(UV-A)}}$ = 231.2), as shown in Fig. 2B. Thus, the energy input of the UV-incident photons appeared to be the dominant factor governing BZP bleaching.

The same was the case for prolonged continuous irradiation of BZP in the mixture with quercetin in methanol, for both the spectral (Fig. 3A) and kinetic behavior (Fig. 3B) (the absorbance data were in this case obtained at 372 nm, the maximum absorbance of quercetin). The ratios of the bleaching rates $k_{\text{(BZP+Querc.)-bleach.}}$ in min^{-1}, obtained from the slopes from Fig. 3B, reconfirmed the dominant role of the energy input of the UV-incident radiation in the bleaching of the BZP + quercetin mixture, which at 372 nm practically means quercetin degradation (BZP has a negligible absorbance at this wavelength). Compared to BZP (Fig. 2B, UV-B), quercetin undergoes a much faster bleaching in the mixture (Fig. 3B, UV-B), even faster than its own bleaching in methanol (Figs. 6A and 6B): 0.11767 min^{-1}/0.03488 min^{-1} = 3.37. Thus, it seems that BZP contributes to the faster degradation of quercetin (however, the photochemistry of the complete mixture is out of scope of this paper).

The blank experiment: UV-induced peroxide formation in the absence of quercetin – the influence of BZP

The kinetics data corresponding to UV-induced lipid peroxide formation in phospholipid as well as in phospholipid / BZP mixtures in methanol (for both, PL80 and PL90) are listed in Table I. Similar to the UV-induced bleaching of BZP, the results of UV-induced peroxide formation also suggest that the rates of LPP are also highly dependent on the energy of the applied UV radiation since the rate constants increase from UV-A to UV-C irradiation (Table I). Hence, generally, the incident UV-energy also plays a governing role in the production of peroxides. On the other hand, lipid peroxidation in both mixtures of phospholipids was faster in the presence of benzophenone than in its absence, both for UV-A and -B irradiated phospholipid mixtures (Table I). The corresponding rate constant ratios for PL80 in methanol, with and without BZP, $k_{\text{peroxid.BZP-PL80}}/k_{\text{peroxid.PL80}}$, were 3.79 and 4.70 for UV-A and -B, respectively, and for PL90, the ratio $k_{\text{peroxid.BZP-PL90}}/k_{\text{peroxid.PL90}}$ was 3.83 for UV-B. However, the LP rate constant for the UV-C-irradiated PL80/BZP mixture in methanol was smaller than the one for PL80 in methanol without BZP, the corresponding rate constant ratio for the PL80 mixtures in methanol (without and with BZP), $k_{\text{peroxid.PL80}}/k_{\text{peroxid.BZP-PL80}}$, was 1.27 (Table I). In addition, the LP rate constant value for UV-C was similar (even slightly smaller) when compared to the one

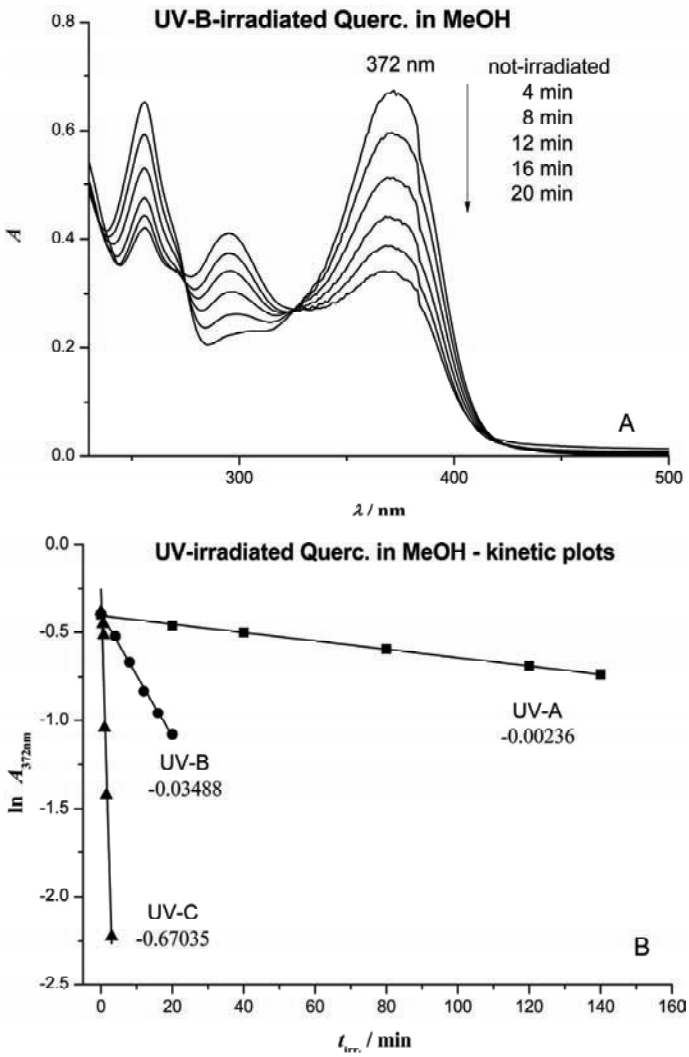


Fig. 6. Changes in the absorption spectrum of quercetin in methanol during exposure to UV-B radiation (300 nm). The initial concentration of quercetin was 2.5×10^{-5} mol dm^{-3} (A). Kinetic ln plot of quercetin degradation induced by UV-A, -B and -C irradiation; the absorbance of quercetin was followed at 372 nm; the corresponding degradation rate constants ($-k_{\text{Querc.degrad.}}$ in min^{-1}) are displayed for all three UV-irradiation ranges (B).

obtained for UV-B irradiated PL80/BZP mixture, $k_{\text{peroxid.BZP-PL80}} = 0.00910$ and 0.00968 min^{-1}, respectively (Table I). Thus, it is obvious that the BZP-initiating role was inefficient when using continuous UV-C irradiation for the PL80/BZP mixture. The BZP-initiating role was the most efficient when using UV-A irradiation: as it was shown that UV-A photons had the smallest impact on benzophenone bleaching (Figs. 2and 3) and thus, the LPP was the highest in the case of

UV-A. The same conclusion was reached in recent publication: BZP-initiating role for LP was the most dominant in the case of UV-A irradiated phospholipid/BZP mixtures in *n*-hexane.[22] However, the LPP kinetic data for PL90 mixtures in methanol were not so consistent although they followed an increasing trend of absorbances at 234 nm (the corresponding UV-spectra are not shown).

UV-induced peroxide formation in the presence of quercetin

A comparative list of the LPP rate constants values, $k_{\text{peroxid.Querc}}$, for both phospholipids mixtures in the presence of quercetin as well as the degradation rates of quercetin itself, $k_{\text{Querc.degrad.}}$, in the absence and in the presence of benzophenone, in methanol, for all three investigated UV sub-ranges are given in Table II. Similar to the UV-induced BZP degradation in methanol (Fig. 3) as well as for UV-induced LPP in PL80 and PL90 mixtures, with and without BZP, in methanol (Table I), the results of UV-induced peroxide formation in phospholipids/quercetin mixtures, with and without BZP, once again suggest that the LP rates were dependent on the applied UV energy, *i.e.*, in general, the rate constants increased from the UV-A to the UV-C irradiation regime (Table II). Hence, the energy input of the incident UV photons plays a major role in the production of peroxides, as well as in the synchronous degradation of quercetin, the $k_{\text{Querc.degrad.}}$ rate constants also increased from UV-A to UV-C, as can be seen in Table II).

Quercetin-BZP competition in the phospholipid mixtures

The effect of BZP on LP production is evident: LP production was more expressed in the presence of BZP than in its absence. For example, the calculated $k_{\text{peroxid.Querc.BZP}}$ (in the presence of BZP)/$k_{\text{peroxid.Querc.}}$ (without BZP) ratios for UV-B-irradiated (PL80 and PL90)/quercetin mixtures in methanol were 5.84 and 2.36, respectively (Table II). This means that BZP-sensitization maintained its important role also in the presence of the quercetin, as it had in the absence of quercetin. Similar to the already cited behavior of UV-C-irradiated (PL80 and PL90)/BZP mixtures in methanol (Table I), BZP was not very efficient for UV-C-irradiated (PL80 and PL90)/quercetin/BZP mixtures in methanol, $k_{\text{peroxid.Querc.BZP}} \approx k_{\text{peroxid.Querc.}}$ (Table II). This could be related to the fact that the rate constant for UV-C-induced BZP bleaching was much higher than in the case of UV-A and -B. Hence, sensitization capacity of benzophenone was significantly reduced under UV-C continuous irradiation (Tables I and II).

On the other hand, the influence of quercetin on LP production in UV-irradiated PL80 and PL90 methanol solutions cannot be neglected. Compared to the "blank-experiments" ($k_{\text{peroxid.BZP}}$ and $k_{\text{peroxid.}}$), LP-production was generally suppressed when quercetin was present. The suppression effect was the most expressed in the case of UV-C-irradiated PL80/quercetin mixture (without BZP): $k_{\text{peroxid.PL80}}/k_{\text{peroxid.Querc.PL80}} = 3.07$ (Tables I and II). The presence of querce-

tin in the phospholipid mixtures both with and without BZP suppressed LP production by a factor between 2.58 and 1.06 for UV-A and -C irradiated PL90/ /quercetin/BZP mixtures, respectively (Tables I and II). The suppression effects of quercetin on LP production were more expressed in the PL80/quercetin than in the PL80/quercetin/BZP mixtures in methanol, which was evidenced by the increasing trend of the corresponding rates ratios with increasing UV-incident energy from UV-B to -C (the $k_{\text{peroxid.PL80}}/k_{\text{peroxid.Querc.PL80}}$ values were 1.62 (UV-B) and 3.07 (UV-C), and the corresponding $k_{\text{peroxid.BZP-PL80}}/k_{\text{peroxid.Querc.BZP-PL80}}$ values were 1.30 (UV-B) and 1.77 (UV-C), Tables I and II).

UV-induced quercetin degradation in methanolic phospholipid and phospholipids/BZP mixtures

The previously published results of UV-induced quercetin degradation in methanol solutions[43] were confirmed by the data presented herein, *i.e.*, quercetin stability against prolonged continuous UV-irradiation decreases with increasing energy input of UV-irradiation, from UV-A to -C (Table II). Thus, in more complex systems such as the ones presented in this work (phospholipid/quercetin and phospholipid/quercetin/BZP in methanol), quercetin still undergoes degradation during continuous UV-irradiation (insets of Fig. 5A and 5C), following first order kinetics (Table II). The sensitizing role of BZP affects quercetin degradation during UV-irradiation in methanolic phospholipid/quercetin/BZP mixtures, *i.e.*, the degradation is faster in the presence than in the absence of BZP (Table II). For example, the calculated $k_{\text{Querc.degrad.BZP-PL90}}$ (with BZP)/ $/k_{\text{Querc.degrad.PL90}}$ (without BZP) ratios for the UV-A, -B and -C irradiated PL90/ /quercetin mixtures were 14.43, 3.05 and 1.29, respectively (Table II). Once again, it could be concluded that BZP had the highest photosensitizing effect with UV-A radiation, since its role in the UV-C-irradiated mixtures appeared to be negligible (Table II). The reason is certainly related to the strong BZP absorption in the UV-C spectral range, having an absorption maximum at 253 nm.

As reported, quercetin has two absorption maximums, in the UV-A (λ_{max} = = 372 nm) and UV-C (λ_{max} = 260 nm) spectral ranges[46] which overlap with the applied UV-irradiation range(s) used in this work. In a recently published investigation, Fahlman and Kroll[50] studied UV-A and -B irradiation of quercetin in BZP-containing methanol solutions, in which irreversible degradation followed by formation of several C-ring-opened photoproducts were registered. They registered BZP-impact on both quercetin degradation and the formation of photoproducts.[50]

On the other hand, in a more complex, organized system, *i.e.*, liposomes, the same authors, Fahlman and Kroll (2009)[40] established quercetin as a strong inhibitor of lipid oxidation induced by UV-A and -B irradiation. However, it was found that the absorption of UV-A and UV-C irradiated quercetin (at the exci-

tation wavelengths of 365 and 255 nm) may lead to greater levels of the excited state species, as reported by Smith and Markham,[51] which may result in an overall decrease in the antioxidant behavior of quercetin toward LP processes. In a recently published paper, suppression effect of quercetin on lipid peroxidation processes (with a lecithin mixture as the "protection target") was clearly registered by the thiobarbituric acid–malon-dialdehyde (TBA–MDA) test.[46]

CONCLUSIONS

To conclude, in the studied complex UV-irradiated system, at least three UV-initiated, more or less synchronous processes occur: BZP bleaching, quercetin degradation and lipid peroxidation. All three processes are highly affected by the incident UV-photons input. A suppression effect of quercetin on the LP process (as a measure of its antioxidant capacity) was proven. The suppression effect is less effective when BZP is present. The reason could lie in more favored degradation of quercetin in the presence of BZP.

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И З В О Д

УТИЦАЈ КОНТИНУАЛНОГ УЛТРАЉУБИЧАСТОГ ОЗРАЧИВАЊА НА АНТИОКСИДАТИВНУ АКТИВНОСТ КВЕРЦЕТИНА У ОДНОСУ НА ЛИПИДНУ ПЕРОКСИДАЦИЈУ ИНИЦИРАНУ БЕНЗОФЕНОНОМ

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Циљ овог рада је праћење деградације као и промена антиоксидативне активности кверцетина у присуству две различите смеше фосфолипида у метанолу, у условима континуалног ултраљубичастог (UV) озрачивања из три различита под-опсега, UV-A, UV-B и UV-C, и то, у присуству и одсуству изабраног UV–апсорбујућег фотосензора, бензофенона. Кверцетин служи да контролише процес липидне пероксидације инициране UV-зрачењем, апсорпцијом дела упадне UV светлости, и/или „скупљањем" том приликом створених слободних радикала. Резултати показују да кверцетин подлеже иреверзибилној деструкцији индукованој UV-зрачењем која веома зависи од енергије упадних UV-фотона, при чему је више изражена у присуству бензофенона. Истовремено, кверцетин показује ефекат сузбијања процеса липидне пероксидације у UV-озраченим смешама фосфолипида у оба случаја – у одсуству или присуству бензофенона (више или мање ефикасно, редом). У смешама фосфолипида озрачиваних UV-C зрачењем, фотосензитивна функција бензофенона је знатно редукована као последица његове снажне апсорпције у истом UV-C опсегу спектра, утичући тако на мању антиоксидативну активност преосталог кверцетина.

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