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Irreversible UV-induced quercetin and rutin degradation in solution studied by UV spectrophotometry and HPLC chromatography

JELENA B. ZVEZDANOVIĆ, JELENA S. STANOJEVIĆ, DEJAN Z. MARKOVIĆ* and DRAGAN J. CVETKOVIĆ

University of Niš, Faculty of Technology, Bulevar oslobodjenja 124, 16000 Leskovac, Serbia

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Abstract: The irreversible degradation of quercetin and rutin, dissolved in methanol and water, induced by continuous UV-irradiation from two different sub-ranges (UV-B and UV-C) were studied in this work. The degradation of both flavonoids is related to the formation of UV-induced degradation products: both processes follow first-order kinetics. The degradation and rate constants of the formation of the products are both dependent on the involved UV-photons energy input in both solvents.

Keywords: quercetin; rutin; UV-irradiation; degradation; products; kinetics.

INTRODUCTION

Ultraviolet (UV) radiation is part of the natural sunlight spectrum that reaches the Earth's surface. Depending on the source, the reported UV-portion of the total sunlight does not exceed 5–6 %, of which, under normal circumstances, >95 % is UV-A (320–400 nm) radiation, the rest being UV-B (290–320 nm) light;[1] UV-C radiation (200–280 nm) is completely absorbed by the atmosphere. Besides being the obligatory driving force of photosynthesis, light may also be damaging factor in the biosphere.[2] The recent depletion of the stratospheric ozone layer has led to an increase on the biologically most damaging UV-B portion of light at ambient levels. Plants generally respond to an increase in UV-B radiation through very different mechanisms, including the synthesis of UV-absorbing, protective pigments, such as flavonoids.[3,4]

Flavonoids are a large class of natural polyphenol compounds of low molecular mass, widely distributed in plant world, where they perform several very important functions, including anti-oxidation.[5–7] They are benzo-γ-pyrane derivatives consisting of pyrane and phenolic rings. The most important structural

* Corresponding author. E-mail: dejan_markovic57@yahoo.com
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elements related to their functions are: an *o*-dihydroxy group in the B ring (catechol structure) as a potential radical target, a double bond between positions 2 and 3 of the C-ring conjugated with keto group in position 4 (because of its capacity to delocalize the uncoupled electron of the flavonoid radical) and C-3, C-5 and C-7 hydroxyl groups (of the C and A rings) as potential free radical scavengers (Fig. 1).[8–10] Quercetin (flavonol) and rutin (flavon) are members of the flavonoid family, with the structures shown in Figs. 1A and 1B, respectively. The main difference in their structures lies in position C-3, where instead of an OH group (quercetin), a glycoside residue has been inserted (rutin). Updated studies connect the antioxidant activity of flavonoids with presence (or absence) of an OH group at position 3 of the C-ring, in combination with a catechol B-ring structure.[5–11] The importance of this position may be estimated – among other ways – by comparing the stabilities of quercetin and rutin toward an externally induced stress, such as UV-irradiation.

(A) (B)

Fig. 1. Structure of A – quercetin and B – rutin.

Quercetin and its glycosides (such as rutin) are believed to be responsible for prevention of extended UV radiation-induced damage to a variety of plants. Quercetin absorbs UV radiation with absorbance maxima in the UV-A (λ_{max} = = 365 nm, ε = 28400 M^{-1} cm^{-1}) and UV-C range (λ_{max} = 256 nm, ε = 28300 M^{-1} cm^{-1}), suggesting that one plausible photoprotective mechanism could be direct absorption of UV radiation, thereby preventing the formation of reactive oxygen species (ROS) and consequent direct DNA damage.[12] The UV energy absorbed by quercetin may be dissipated as heat[13] or converted into decomposition products.[11] UV-irradiation of flavonoids in solution and in liposome systems (*in vitro*), as well as *in vivo*, results in their irreversible breakdown, accompanied by the appearance of a number of decomposition products.[14,15]

In the recently accepted report,[16] the stability of quercetin and rutin in solution toward UV-irradiation from the three ranges (UV-A, UV-B and UV-C)

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were compared, as were their antioxidant activities in the presence of a lipoidal mixture as a protection target. It was concluded that: 1) rutin expressed higher stability than quercetin toward UV-irradiation, while the stability of both flavonoids was found to be affected by the energy of incident photons; 2) however, the higher stability of rutin had only a partial effect on its antioxidant ability to protect a lipid mixture from peroxidation, *i.e.*, its dominance in stability over quercetin (towards UV-irradiation) was not supported by its antioxidant ability compared to that of quercetin under the same experimental conditions.

This study is a continuation of the previous investigation.[16] Thus, quercetin and rutin were again irradiated in solution but this time reversed phase HPLC techniques in combination with absorption spectrophotometry were employed to analyze the formation of the degradation products quercetin and rutin induced by continuous UV-irradiation to provide the data for a kinetic analysis to follow.

EXPERIMENTAL

Samples preparations

Quercetin and rutin standards were purchased from Merck. Quercetin was dissolved in methanol and rutin in both methanol and water to give a concentration of 0.1 mM.

UV-Irradiation treatment

The irradiation of the pigments in solution was performed in a cylindrical photochemical reactor "Rayonnet", with 7 symmetrically placed lamps, with emission maxima in two different UV sub-ranges, 300 (UV-B) and 254 nm (UV-C). The total emitted energy flux (measured using a UV-meter, Solarmeter, model 8.0 UV meter, Solartech) was about 10.5 W m^{-2} for 300 nm and 12.5 W m^{-2} for 254 nm at a 10 cm distance from the lamps. The samples (2 ml) were irradiated in quartz cuvettes placed on a rotating circular holder.

UV Spectroscopy

The UV spectra of rutin and quercetin in different solvents were recorded on a Varian Cary-100 spectrophotometer. All spectra, before and after irradiation with UV-light, were recorded in the range from 200 to 500 nm.

High performance liquid chromatography – photodiode array detector (HPLC–DAD)

The HPLC analyses were performed at 15 °C on an Agilent 1100 Series system, Waldbron, Germany, using a diode array detector set at 257, 295 and 372 nm. Aliquots of 20 μl were injected into a 4.6×250 mm RPC-18 column (Zorbax Eclipse XDB-C18) with 5 μm particle size. Elution was realized in the gradient mode proposed by Veit *et al.* using two mobile phase components: A = 0.15 % phosphoric acid in water–methanol (77:23 v/v) mixture, B = methanol.[17] The gradient mode was set as follows: 0 to 3.6 min, isocratic 100 % A; 3.6–24.0 min, linear gradient from 100 to 80.5 % A; 24–30 min, isocratic 80.5 % A; 30–60 min, linear gradient from 80.5 to 51.8 % A; 60–67.2 min, linear gradient to 100 % B; 67.2–90.0 min, isocratic 100 % B. The flow rate was 1.0 cm^3 min^{-1}. The HPLC peaks (areas) recorded at 372 nm were used for the determination of quercetin and rutin degradation (bleaching) induced by UV-irradiation, *i.e.*, kinetic analysis. New peaks were detected at 295 and 257 nm because of quercetin and rutin UV-irradiation in solution; their areas were plotted against irradiation time, providing possibilities for kinetic analysis.

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RESULTS

The changes in chromatograms of UV-irradiated quercetin (in methanol) and rutin (in methanol and in water) are shown in Figs. 2 and 3, respectively. The changes of absorption spectra of quercetin and rutin exposed to UV-irradiation are shown in the related inserted spectra in Figs. 2 and 3 (right), respectively.

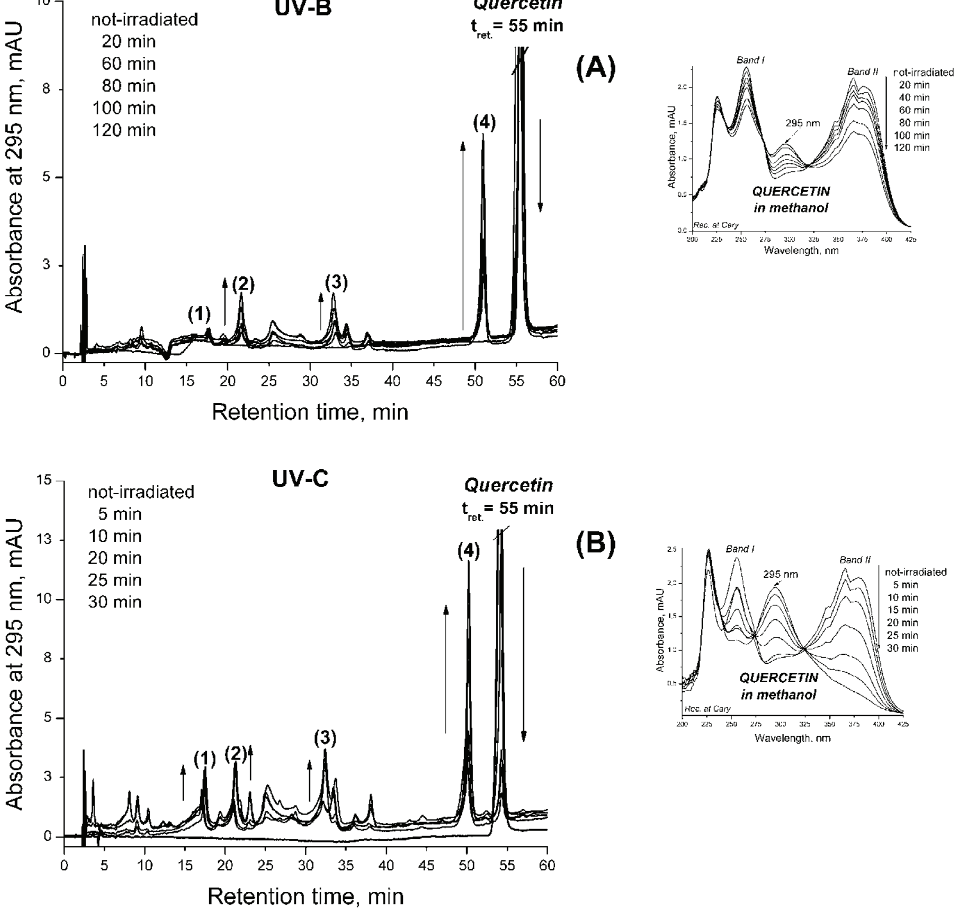

Fig. 2. Quercetin in methanol, irradiated with continuous UV-B (A) and UV-C (B) radiation. The changes in HPLC-chromatograms recorded at 295 nm (A, B: left); the corresponding changes in the samples absorption spectra, recorded in the spectral range 200–425 nm (A, B: right). The exposure times are displayed on the graphs and the arrows show the change in direction: down (↓) in the case of quercetin, and up (↑) in the case of the degradation products. Main observed products of UV-induced degradation of quercetin (at different retention times, $t_{ret.}$: 17, 21, 32 and 50 min) were numerated with the numbers 1–4, respectively, in the chromatograms. mAU – mili (10^{-3}) relative absorbance units.

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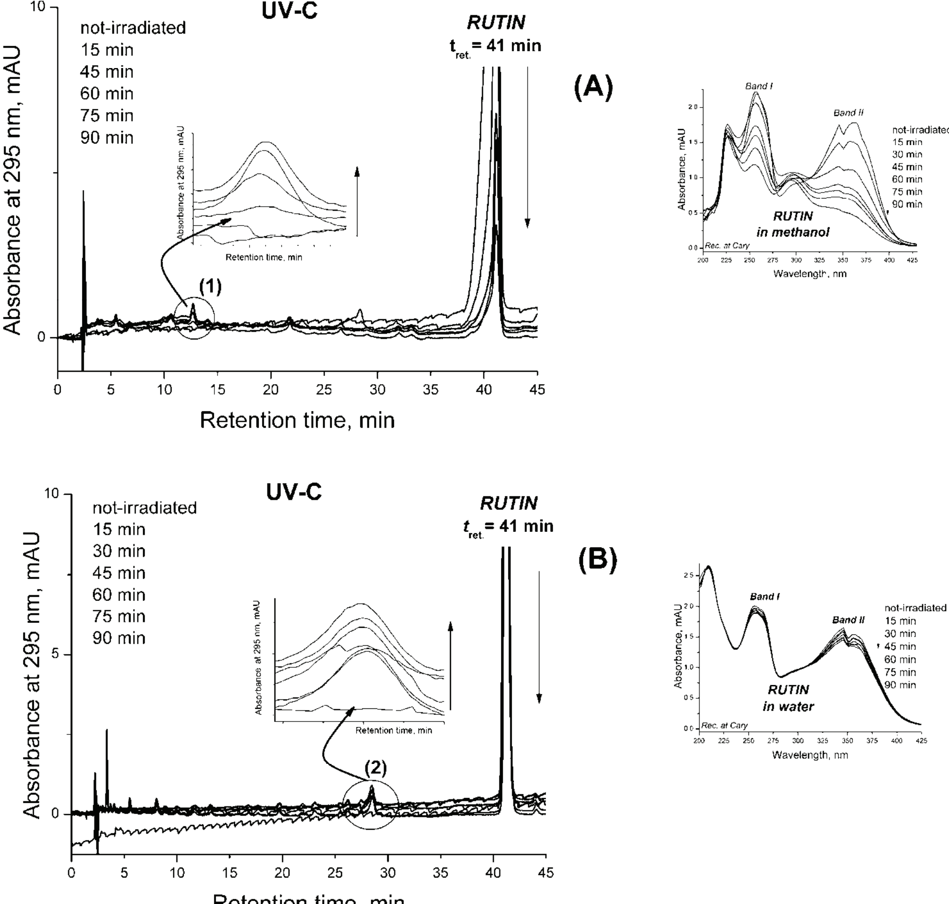

Fig. 3. Rutin in methanol (A) and water (B), irradiated with continuous UV-C irradiation. The chromatograms explanation is the same as for Fig. 2. The only observed products (at different retention times, $t_{ret.}$, 13 and 28 min, respectively) were numerated with numbers 1 and 2, respectively, in the chromatograms.

Irradiation of quercetin in methanol with either UV-B or UV-C radiation resulted in a decrease in quercetin ($t_{ret.}$ = 55 min) and the appearance of new peaks, *i.e.*, four major peaks (corresponding to UV-induced products) at the following retention times: 1) 17, 2) 21, 3) 32 and 4) 50 min (Fig. 2). Irradiation of rutin in methanol and in water with UV-C radiation resulted in rutin degradation ($t_{ret.}$ = = 41 min) and the appearance of two new peaks, at 1) 13 and 2) 28 min, respectively (Fig. 3). The absorption spectra of the four observed UV-induced degradation products of quercetin and the two of rutin are shown in Figs. 4A and 4B, respectively. The proposed, possible structures of the degradation products of quercetin are shown in Fig. 4A. These compounds, together with their names,

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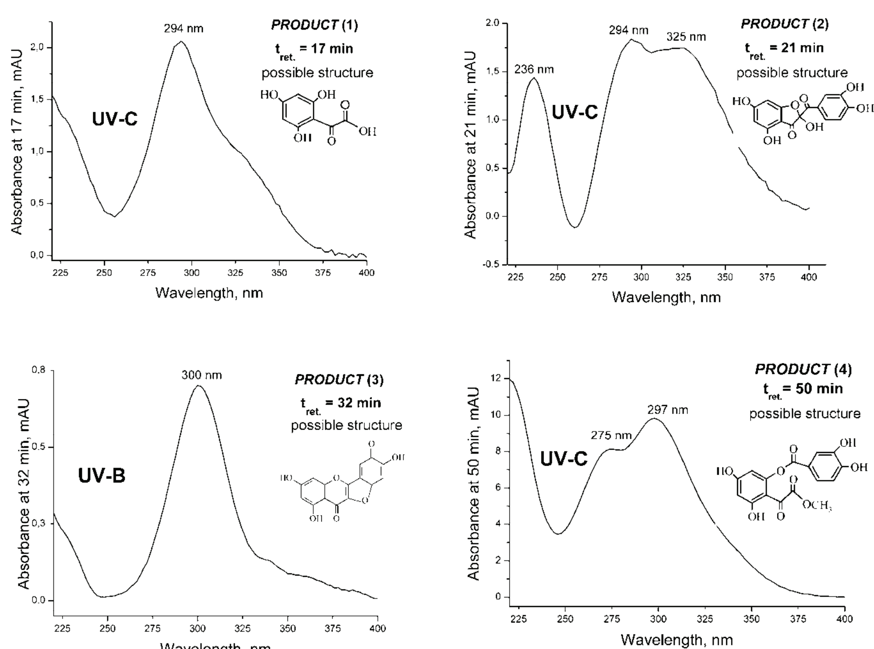
positions of the absorption maxima (λ_{max}) and related references are given in Table I.[18–23]

(A) - Quercetin in methanol, the degrad. products



(B) - Rutin, the degrad. products

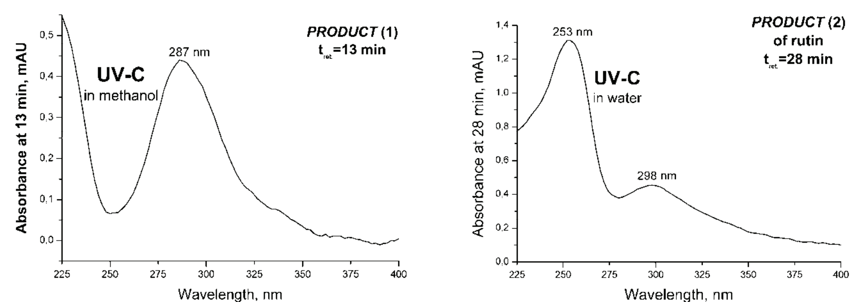


Fig. 4. Absorption spectra of UV-B and UV-C induced quercetin degradation products (A), as well as UV-C induced rutin degradation products (B), corresponding to the main observed peaks in the related HPLC chromatograms (the retention times are marked).

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TABLE I. Possible structures of the products formed as the result of UV-B and UV-C irradiation of quercetin in methanol, detected by HPLC. The proposals are based on comparisons of the absorption spectra of the products with literature spectral data

Product	$t_{ret.}$ min	Name of product	Structure, obtained by	λ_{max} nm	References cited
1	17	2,4,6-Trihydroxyphenylglyoxylic acid	[structure], electrochemical oxidation	292	Spectrum taken from Zhou *et al.*[18] The compound is also cited in refs.[19–21]
2	21	2-(3,4-Dihydroxybenzoyl)-2,4,6-trihydroxy-3(2*H*)-benzofuranone	[structure], electrochemical, enzymatic, 2,2'-azoisobutyronitrile oxidation and auto-oxidation	294, 325	Taken from Zhou and Sadik.[19] The compound is also cited in ref.[19]
3	32	1,3,8-Trihydroxy-9*aH*,11*H*-benzofuro[3,2-*b*]-[1]benzopyran-7,11-dione cyclic ether connecting AC-system with B-ring	[structure], electrochemical oxidation	300	Taken from Timbola *et al.*[22]
4	50	Methyl 2-{2-[(3,4-dihydroxybenzoyl)oxy]-4,6-dihydroxyphenyl}-2-oxoacetate	[structure], γ-irradiation-induced degradation	275, 297	Taken from Marfak *et al.*[23]

Decreasing kinetic plots of quercetin and rutin degradation obtained using the quercetin ($t_{ret.}$ = 55 min) and rutin ($t_{ret.}$ = 41 min) peaks from the chromategrams recorded at 372 nm (not shown), ln $S_{372nm} = f(t_{irr})$, are shown in Fig. 5. The plots show a good linear fit, with R^2 values 0.95–0.99. The degradation kinetics seems to obey a first-order law. The calculated rate constants, k_{degrad} (min^{-1}), for the UV-B and UV-C sub-ranges in the two solvents, are given in Table II.

The analogue kinetic ln plots obtained using the peaks areas recorded at 295 nm, ln $S_{295nm} = f(t_{irr.})$ for the four observed major products of UV-irradiated quercetin, **1**, **2**, **3** and **4**, are shown in Fig. 6A–D, respectively. The plots show good linear fits, with R^2 values 0.92–0.99. The calculated rate constants for the

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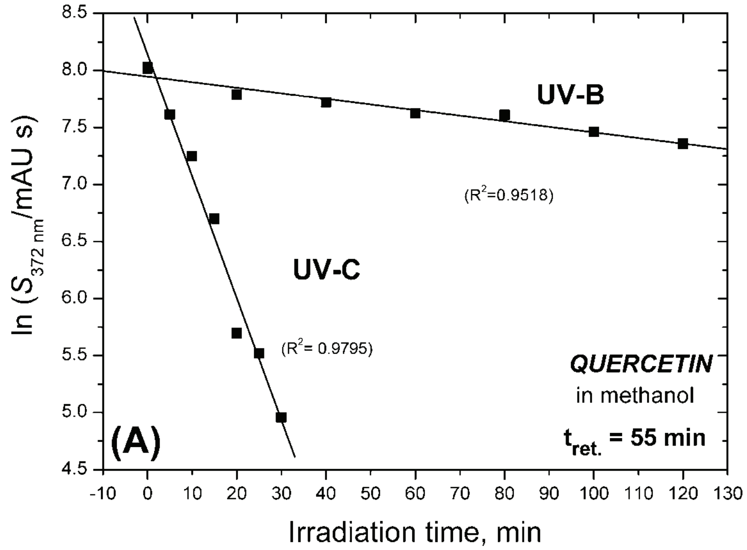
formation of the UV- induced degradation products of quercetin, for UV-B and UV-C sub-ranges in methanol are given in Table III.



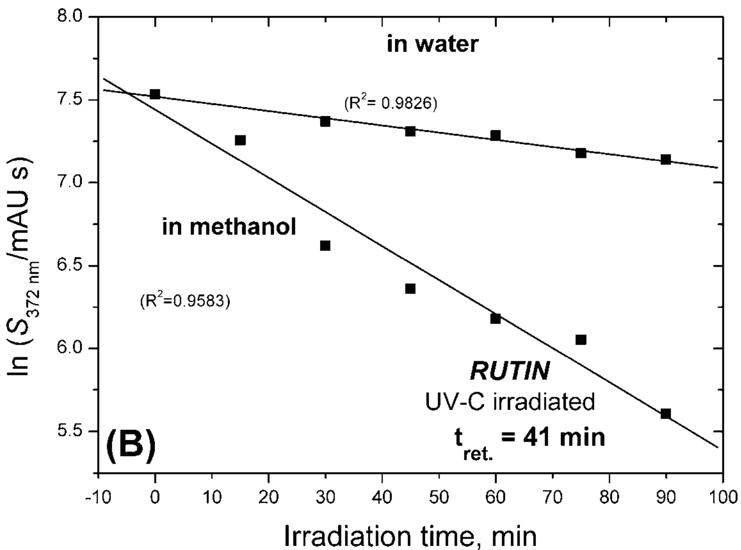


Fig. 5. Kinetic ln plots of UV-B and UV-C induced quercetin (A) and UV-C induced rutin (B) changes, obtained from the HPLC-chromatograms recorded at 372 nm, as a function of UV-B and C irradiation time, ln $S_{372nm} = f(t_{irr.})$. The plots show good linear fits, with R^2 values 0.95–0.99. The corresponding rate constants calculated from the slopes of the linear plots are given in Table II.

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TABLE II. Kinetics of quercetin and rutin degradation in methanol and water (shown in Fig. 5), during increasing UV-irradiation time in two different UV sub-ranges: 300 (UV-B) and 254 nm (UV-C). Peak areas were taken from the corresponding HPLC-chromatograms at 372 nm (analogues to Figs. 2 and 3, not shown). ln $S_{372\text{nm}} = k_{\text{degrad.}}\ t_{\text{irr.}} + n$, where $S_{372\text{nm}}$ – peak area of quercetin and rutin from the corresponding chromatograms recorded at 372 nm, $t_{\text{irr.}}$ – UV-irradiation time, $k_{\text{degrad.}}$ – rate constants for UV-induced degradation of quercetin and rutin in methanol and water, given as the slopes of the linear plots in Fig. 5, in min^{-1}; $c = 1.0\times10^{-4}$ M

UV-irradiation wavelength, nm	Quercetin, $t_{\text{ret.}}$=55 min	Rutin, $t_{\text{ret.}}$=41 min	
	In methanol		In water
300 (UV-B)	–0.0049	–	–
254 (UV-C)	–0.1069	–0.0206	–0.0043

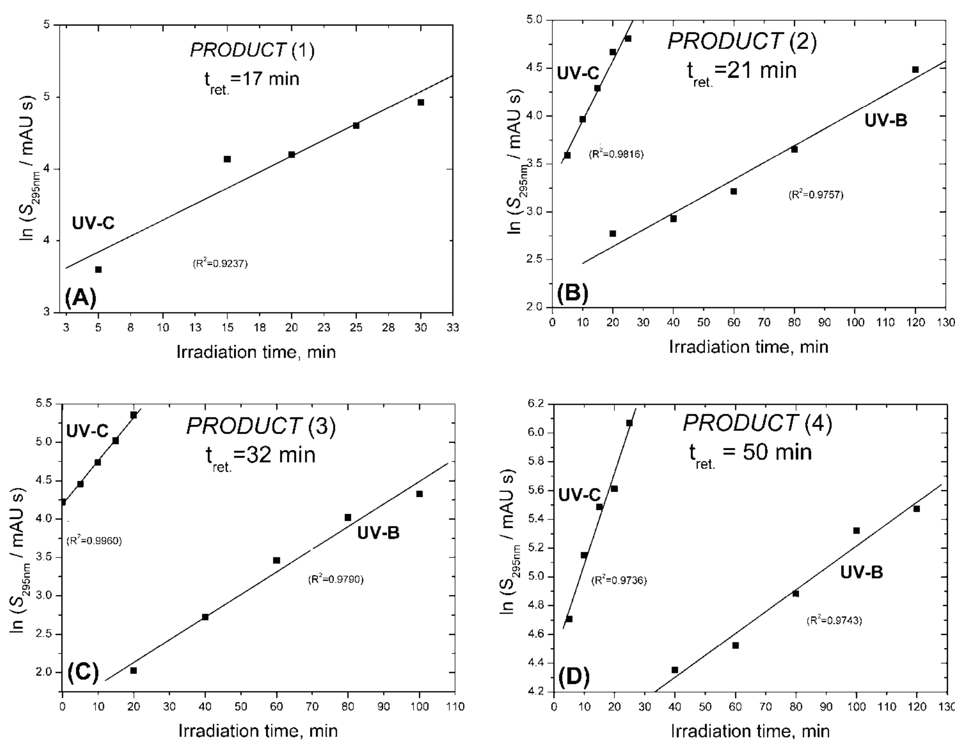

Fig. 6. Kinetic ln plots of UV-B and C induced quercetin degradation products, obtained from the HPLC-chromatograms recorded at 295 nm, as a function of UV-B and C irradiation time, ln $S_{295\text{nm}} = f(t_{\text{irr.}})$. The plots show good linear fits, with R^2 values 0.95–0.99. The corresponding rate constants calculated from the slopes of the linear plots are are given in Table III.

DISCUSSION

Absorption spectra

The absorption spectra of flavonoids generally consist of two distinctive bands, in a broad range of 240–400 nm.[24] Band I, covering the range 300–380

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nm, is attributed to the B-ring (with the A_{max} position around 350–370 nm), while band II, covering the range of 240–280 nm (with the A_{max} position around 260–270 nm) is attributed to the A–C benzoyl system; a weak band with an absorption maximum around 300 nm is also detected, which is attributed to the C-ring only – shown in Fig. 1 and in the related inserted spectra in Figs. 2 and 3. Both the investigated flavonoids expressed clear sensitivity to UV-irradiation, from both UV-ranges. Continuous UV-irradiation of quercetin and rutin in methanol induced a gradual decrease of the absorbance intensity, *i.e.*, a hypochromic effect, as shown in the related inserted spectra in Figs. 2A, 2B and 3A; similar spectral behavior of the absorption spectra was also observed for rutin in water under UV-C-irradiation (Fig. 3B). Thus, continuous irradiation of quercetin and rutin in solution resulted in their degradation, which increased with increasing irradiation time. However, rutin underwent a slower degradation in methanol, compared to quercetin (Figs. 3A and 2B**,** respectively). These results again confirmed that presence or absence of 3-OH group (quercetin, rutin, respectively) plays a major role in the stability of the flavonoids toward UV-irradiation.[11,16]

TABLE III. Kinetics of the formation of UV-B and UV-C-induced quercetin degradation products in methanol (the analogues plots to the ones for Table II). The peak areas were taken from the corresponding HPLC-chromatograms recorded at 295 nm (Fig. 2), *i.e.* the products A_{max} value. $k_{degrad.prod.}$ – rate constant for the formation of the degradation products in methanol was calculated from Fig. 6, in min^{-1}

UV-irradiation wavelength, nm	Product			
	1, $t_{ret.}$ = 17 min	**2**, $t_{ret.}$ = 21 min	**3**, $t_{ret.}$ = 32 min	**4**, $t_{ret.}$ = 50 min
300 (UV-B)	–	0.0176	0.0295	0.0152
254 (UV-C)	0.0405	0.0628	0.0568[a]	0.0637

[a]Until 20 min of UV-C irradiation

A clear increase in the absorbance was observed between 270 and 325 nm for quercetin in methanol, with a maximum at 295 nm (the related spectra are inserted in Fig. 2) for the two UV-ranges (UV-B and UV-C). For UV-C-irradiated rutin in methanol and especially rutin in water, the analogue increase in the absorbance was not so clear (the related spectra are inserted in Fig. 3). The existence of $A_{max,295nm}$ indicates the formation of product(s) as a result of UV-induced quercetin degradation that absorb in the spectral region 270–325 nm (for this reason, a monitoring wavelength of 295 nm was chosen in the HPLC–DAD experiments to estimate the UV-induced changes of quercetin and rutin in solution). The same spectral behaviors of UV-irradiated quercetin and rutin in solution were previously observed, but under slightly different conditions of UV-irradiation compared to the present experiments, *i.e.*, with about two times larger UV-B and UV-C incident energy.[16]

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HPLC–DAD Analysis

The results of the HPLC analysis proved quercetin and rutin underwent ireversible UV-induced bleaching: a clear decrease of the corresponding main peaks areas at t_{ret} = 55 min and 41 min, assigned to quercetin and rutin, respectively, at all three monitoring wavelengths was observed (at 257 nm, 372 nm – not shown, and at 295 nm, as can be seen in Figs. 2 and 3). Irradiation of quercetin in methanol with either UV-C radiation for 30 min or with UV-B light for 120 min resulted in about 95 and 50 % decreases in the quercetin content, respectively, and its conversion into four major products (Figs. 2 and 4A). For both UV-B and UV-C radiation, the peak observed at t_{ret} = 50 min was dominant on the chromatograms (Fig. 2). However, the product corresponding to the peak observed at $t_{ret.}$ = 17 min was formed in a better yield as a result of UV-C compared to UV-B irradiation (Fig. 2). The UV-spectra corresponding to the four observed products induced by UV-irradiation (either by UV-B or by UV-C) of quercetin in methanol are shown in Fig. 4A. The proposed, possible structures of the products, based on a comparison of their absorption spectra with the ones found in the respective literature related to different methods of quercetin oxidation (Table I), are inserted in Fig. 4A.

The absorption spectra of the products **1** and **3** (in the mobile phase, acidic methanol–water solution, see experimental section), obtained from the peaks at t_{ret} = 17 min and 32 min in chromatograms of UV-irradiated quercetin in methanol, are shown in Fig. 4A. These products have absorbance maximums positioned at 294 nm (product **1**) and 300 nm (product **3**) Similar absorption spectra to that of product **1** were found for di- and tri-hydroxy-phenylglyoxylic acid, products obtained by electrochemical oxidation of quercetin, with maximums at about 288 and 292 nm, respectively (Table I).[18] This proposed, possible structure requires a C-ring opening (product **1**). In the case of product **3**, an additional molecular rearrangement might occur finishing in a closed structure again *via* a furan type of chromophore as a "bridge".[18,19]

Absorption spectra of the products **2** and **4** (in the mobile phase) obtained from the peaks registered at 21 and 50 min are shown in Fig. 4A and the proposed structures are given in Table I.[18–23] Product **2** has two unresolved bands in spectral range between 250 and 400 nm (with broad, "shoulder-like" A_{max} at 294 and 325 nm) and another clear A_{max} at 236 nm, as shown in Fig. 4A. Product **4** has two unresolved bands with A_{max} at 275 and 297 nm, as shown in Fig. 4A. A comparison with literature data suggests again C-ring opening for product **4**, while possible structure for product **3** appears to be of a similar type as product **2**, Table I.[18–23] Hence, it may be concluded that although obtained by different manners of quercetin oxidation (Table I), the formation of the degradation products were very probably initiated by C-ring opening, followed by stabilization

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of such structures (products **1** and **4**) or further molecular rearrangements (products **2** and **3**).

However, to date, none of the four proposed (quercetin-related) structures (Table I) have been induced by UV-irradiation. In a similar study by Fahlman and Krol (2009),[12] it was shown that UV-A and UV-B irradiated quercetin in methanol (under energy flux conditions very similar to those used in the present study, *i.e.*, 7.4 W m^{-2} at 365 nm, and 13.3 W m^{-2} at 310 nm, respectively) underwent decomposition as the result of C-ring opening[12] followed by the formation of the three products (independent of the presence of oxygen in the system), which were identified as 2,4,6-trihydroxybenzaldehyde, 2-[(3,4-dihydroxybenzoyl)-oxy]-4,6-dihydroxybenzoic acid and 2(3,4-dihydroxyphenyl)ethanol. Unlike in the present study, they also used the well-known UV-photosensitizer benzophenone to observe its effect on quercetin decomposition*, and found that the photoconversion yield was increased in the presence of benzophenone, *i.e.*, in the absence of benzophenone, only 20 % of the quercetin underwent decomposition over 11 h, whereas 90 % of the quercetin was decomposed under both UV-A or UV-B irradiation in less than 1 h when benzophenone was present, by the same C-ring opening mechanism as in the absence of benzophenone.[12] Unfortunately, the authors did not present the absorption spectra of their proposed degradation products, for their comparison with those presented in the present work.[12]

Irradiation of rutin (0.1 mM) in methanol and water with UV-C radiation for 90 min resulted in about 85 % and 30 % decrease of the initial rutin content and its conversion to two barely visible products, with retention times of **1** 13 min and **2** 28 min, respectively, as determined by the chromatograms recorded at 295 nm (Fig. 3). Absorbance spectrum of product **1** in the mobile phase, which is related to the peak with $t_{ret.}$ = 13 min in the chromatogram of UV-C-irradiated rutin in methanol (Fig. 3A), is shown in Fig. 4B. The spectrum shows one clear band with absorbance maximum at 287 nm (Fig. 4B). The absorbance spectrum of product **2** (in the mobile phase), which is related to the peak with $t_{ret.}$ = 28 min in the chromatogram of UV-C-irradiated rutin in water (Fig. 3B), is shown in Fig. 4B. The spectrum shows two bands: the first one is intensive with an absorbance maximum at 253 nm and the second one has a significantly lower A_{max} value at 298 nm (Fig. 4B).

While UV-induced decomposition of quercetin and rutin seem to be unquestionable,[11] the possible mechanisms are still much disputed and very rarely published. For the three detected products as a result of quercetin irradiation in methanol, Fahlman and Krol (2009) proposed two distinct mechanisms, both requiring C-ring opening:[12] one occurred by direct reaction with singlet oxygen (the creation of which was assured in the used aerated system by energy transfer from

* Benzophenone was recently employed with similar idea to study its effect on bleaching of carotenoids in a similar *in vitro* system.[25]

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the sensitizer, *i.e.*, the benzophenone triplet state, to a normal, triplet oxygen),[26] while in the case of the other two products, a precedent molecular rearrangement occurred; in both cases, the quercetin AC–B system (Fig. 1) was decomposed, and very simple, one-ring benzoyl-type molecules were finally formed. The spectra of the four separated quercetin degradation products in methanol obtained in this work (Fig. 4A), as well as of the two rutin degradation products in methanol and water (Fig. 4B), with a clear absence of the flavonoids band I, attributed to the B-ring of the flavonoids AC–B system (Fig. 1), suggest that the flavonoid structure was irreversibly decomposed and probably smaller-sized molecules were formed. The results obtained in the pre-work might support these suggestions, since the formation of all four quercetin-degradation products were possibly initiated by C-ring opening.

Flavonoids degradation: kinetic analysis

Both flavonoids undergo substantial degradation expressed through the proportional decrease of peak areas obtained from the related HPLC-chromatograms recorded at 295 nm (Figs. 2 and 3), and at 257 and 372 nm (data not shown), as a function of the UV-irradiation time ($t_{irr.}$), *i.e.*, ln $S_{372nm} = f(t_{irr.})$. This dependence is expressed as a linear plot (Fig. 5), indicating first order kinetics of the degradation of the flavonoids, as already reported.[16,27]

The values of the calculated quercetin and rutin degradation rate constants, $k_{degrad.}$ (min^{-1}), are comparable but also different in comparison to already published k values,[16] which is in agreement with the somewhat different conditions of continuous UV-irradiation (such as energy flux and distance from the lamps), as well as with the different employed techniques (UV-spectrophotometry *vs.* HPLC). The ratio of degradation rate constant values for quercetin irradiated with UV-C and UV-B, $k_{Querc.,degrad.UVC}$ / $k_{Querc.,degrad.UVB}$, is about 21 (Table II). This trend was already observed in a previous study:[16] thus, the degradation of flavonoids is highly dependent on the UV-energy input. This behavior was observed in another study related to the continuous UV-irradiation of a very similar compound, 3-hydroxyflavone.[28]

However, rutin was generally more stable against UV-irradiation than quercetin (by a factor of 5), as shown in Table II. Obviously, the substituted position 3 in the C-ring (Fig. 1) makes a major difference in the structures of quercetin and rutin; rutin reportedly has two intra-molecular hydrogen bonds related to the B-ring and the glycoside residue, *i.e.*, interactions of O-3' with H-4', and O-2''' with H-3' (Fig. 1B),[29] while in the case of quercetin, three hydrogen-bonds were reported, *i.e.*, the interaction of O-3' with H-4', and =O-4 with H-5 and H-3 (Fig. 1A).[30] Hence, it is evident that the 3-OH position makes the main difference in the response of quercetin and rutin to continuous UV-irradiation, making the gly-

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coside residue in quercetin more susceptible to the action of UV radiation than the glycoside residue in rutin.[11]

In the case of rutin exposed to continuous UV-C radiation, the degradation rate constant in methanol was higher than in water: 0.0206 *vs*. 0.0043 min^{-1}, as given in Table II. The solubility of rutin in methanol is slight higher than in water (the corresponding free energies of solvation are –56.35 and –49.42 kcal mol^{-1}, respectively).[31] However, the influence of oxygen can not be neglected because of the formation of singlet oxygen[26] that could potentially play a certain role in the UV-induced degradation of rutin and quercetin. In such circumstances, flavonoids would need to act as sensitizers (for singlet oxygen creation if other sensitizers are not present) while, on the other hand, they could become objects of degradation themselves. The relevant data to be born in mind when referring to the susceptibility of rutin *vs*. quercetin to UV-irradiation are: the solubility of oxygen in methanol and water at 20 °C are 2.12 and 0.265 g dm^{-3}, respectively; the viscosity under the same conditions are 0.597 mPa s for methanol and 1.002 mPa s for water; the lifetimes of singlet oxygen in methanol and water are 10 and 4 μs, respectively.[31] Thus, during UV-C irradiation, the higher solubility of oxygen, the longer lifetime of singlet oxygen and the smaller viscosity of methanol than water[31] makes rutin more susceptible to UV-induced degradation in methanol than in water, which is documented by the corresponding $k_{degrad.}$ values given in Table II.

Kinetic analysis of the formation of the UV-induced products

The kinetics of the formation of the main quercetin degradation products seem to be first order (Fig. 6). The formation of the products was faster when quercetin was irradiated with UV-C than with UV-B radiation (Table III). For example, UV-C-induced formation of quercetin product **2**, detected at $t_{ret.}$ = 21 min in HPLC chromatogram recorded at 295 nm, was about 3.6 times faster than when UV-B radiation was used (Table III). Similarly, UV-C-induced formation of the quercetin products **3** and **4**, detected at $t_{ret.}$ 32 and 50 min, respectively, were about 1.9 and 4 times faster than for their formation induced by UV-B radiation (Table III). The obvious reason again lies in the increased energy input. A comparison of the rate constants revealed at least: 1) three of the four products, *i.e*., products 2–4 (Table III) were formed during continuous irradiation of quercetin with both UV-B and UV-C radiation, in other words changing the energy input (from UV-B to UV-C) did not lead to a significant qualitative change in the outcome; 2) however, change in the energy input was reflected in significantly higher formation rates, by factors of 3.5 (product **2**), 1.9 (product **3**) and 4.2 (product **4**) – Table III; 3) this undoubtedly indicates that the formation of the products was directly related to quercetin degradation, which was also energy dependent (Table II).

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When rutin was exposed to UV-C radiation in methanol and water, formation of degradation products was also observed over irradiation time (chromatograms in Fig. 3), but their formation kinetics was not so clear (not shown). The reasons for this behavior could be either in already cited higher stability of rutin to UV-irradiation compared to quercetin,[16] or in the insufficiently long irradiation time used in the related experiments. However, extension of the irradiation times could possibly make comparison of quercetin and rutin responses toward UV-irradiation more difficult and less relevant.

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И З В О Д

ИРЕВЕРЗИБИЛНА UV-ИНДУКОВАНА ДЕГРАДАЦИЈА КВЕРЦЕТИНА И РУТИНА У РАСТВОРУ, ИСПИТИВАНА UV-СПЕКТРОФОТОМЕТРИЈОМ И HPLC ХРОМАТОГРАФИЈОМ

ЈЕЛЕНА Б. ЗВЕЗДАНОВИЋ, ЈЕЛЕНА С. СТАНОЈЕВИЋ, ДЕЈАН З. МАРКОВИЋ и ДРАГАН Ј. ЦВЕТКОВИЋ

Универзитет у Нишу, Технолошки факултет, Булевар ослобођења 124, 16000 Лесковац

У овом раду је проучавана иреверзибилна деградација кверцетина и рутина, растворених у метанолу и води, индукована континуалним UV-озрачивањем из две подобласти, UV-A и UV-B. Деградација оба флавоноида је повезана са UV-индукованим формирањем деградационих продуката: оба процеса прате кинетику првог реда. Константе брзина и деградације флавоноида и формирања продуката у оба растварача су зависне од енергије упадних UV-фотона.

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