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ORIGINAL ARTICLE

Antioxidant and iron-chelating properties of taxifolin and its condensation product with glyoxylic acid

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Abstract The condensation of taxifolin with glyoxylic acid was examined, and the properties of the resulting product were compared with those of taxifolin. The structure of the product was determined by NMR spectroscopy. The ability of the polyphenols to scavenge reactive oxygen species (ROS) was estimated by luminol-dependent chemiluminescence. The iron-chelating and iron-reducing activities were studied using absorption spectrophotometry. It was shown that the condensation leads to the formation of a dimer consisting of two taxifolin units linked through a carboxymethine bridge at the C-6 and C-8 positions of the A ring. The dimer exhibited a somewhat higher ROS scavenging activity than taxifolin. The iron-binding capacity of the compounds was proportional to the number of polyphenol units. The iron-reducing ability of the dimer was lower than that of taxifolin. Thus, the dimer possessed a higher antioxidant activity than the parent flavonoid. The data obtained may be useful for a better understanding of processes occurring in foods and beverages and in a search for new active compounds.

Keywords Taxifolin · Glyoxylic acid · Condensation · ROS scavenging activity · Iron-chelating and iron-reducing activities

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Introduction

Flavonoids are a large group of plant polyphenols widely distributed in nature and present in human diets. The consumption of flavonoids or their sources might be associated with reduced risk of a number of diseases, including some forms of cancer, cardiovascular and neurodegenerative diseases (Vauzour et al. 2010). Flavonoids exhibit a broad range of biological activities, including antioxidant, anti-inflammatory, antibacterial, anti-atherosclerotic, and anticarcinogenic activities (Wang and Ng 1999; Li et al. 2014). Therefore, numerous quantitative structure–activity relationship studies aimed at a search for, and design of new active polyphenolic compounds have been carried out (Roy and Mitra 2009; Gunda et al. 2014; Jung et al. 2015). Flavonoids can be ascribed to the category of biologically relevant antioxidants (Roy and Mitra 2009) and are considered in recent years as capable not only to scavenge reactive oxygen species (ROS) but also prevent their generation by binding transition metal ions (such as iron and copper ions) and inhibiting the Fenton reaction (Jomova and Valko 2011). However, under certain conditions, flavonoids exert a pro-oxidant action (Jomova and Valko 2011), probably due to their metal-reducing activity: after metal ion binding and subsequent reduction by flavonoids, these metal ions can participate in the Fenton reaction. Thus, the pro- and antioxidant action of flavonoids is related to both their ROS scavenging and metal-binding activities and metal-reducing ability.

Taxifolin is one of the most abundant dihydroflavonols, which displays different biological activities, including the ROS scavenging and the metal-binding activities (Mladěnka et al. 2011; Shubina and Shatalin 2013; Říha et al. 2014). This compound is a minor component in complex

preparations such as silymarin (Legalon®), Pycnogenol®, and Venoruton® (Weidmann 2012). In nature taxifolin is found in French maritime bark (Rohdewald 2002), Douglas fir bark (Kiehlmann and Li 1995), and Siberian larch wood (Ivanova et al. 2012) and can be extracted on a commercial scale from the bark and wood, which are the waste products of the forest industry (Ostronkov and Lashin 2012). From this point of view, the use of taxifolin as a lead compound for the design of new biologically active molecules seems to be economical and promising. In addition, taxifolin is present in human diet and is found in cherry liqueur (Rødtjer et al. 2006), wines (Baderschneider and Winterhalter 2001; De Simón et al. 2014), and vinegars (Cerezo et al. 2010) aged in cherry wood. According to the literature data, during the storage and aging of flavonoid-rich foods and beverages, polyphenolic compounds are condensed with aldehydes, such as acetaldehyde and glyoxylic acid (Es-Safi et al. 2000) to form dimers, oligomers and polymers. It has been reported that the antioxidant activity of oligomeric polyphenols positively correlates with the degree of polymerization (Jerez et al. 2007; Zhou et al. 2014). In the present study, primary attention was focused on the reaction of taxifolin with glyoxylic acid and a comparison of ROS scavenging, iron-binding and iron-reducing activities of the resulting product with those of taxifolin. The results obtained may advance our understanding of processes occurring in food and beverages and their impact on the antioxidant properties of food products. In addition, these results reveal the structure–activity relationship of the polyphenols being examined, which can assist in the development of new effective antioxidant agents.

Materials and methods

Chemicals

Glyoxylic acid monohydrate, ascorbic acid, dimethyl sulfoxide-d_6 (d_6-DMSO), benzene, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4′-disulfonic acid monosodium salt hydrate (ferrozine), 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol), and horseradish peroxidase (150 U/mg) were purchased from Sigma-Aldrich (USA). Taxifolin was kindly provided by Flamena (Russia). Phosphate-buffered saline was from Paneco (Russia). Iron(II) sulfate, iron(III) chloride, hydrogen peroxide, hydrochloric acid, acetic acid, and sodium acetate were obtained from Reakhim (Russia). Tetrahydrofuran, acetone, and ethanol were purchased from Panreac (Spain). All chemicals were of analytical grade. All solutions were prepared using distilled water purified by a Milli-Q system (Millipore USA).

Synthesis and characterization

Briefly, 148 mg of glyoxylic acid and 20 µl of concentrated hydrochloric acid were added to the solution of taxifolin (608 mg) in 5 ml of tetrahydrofuran. The mixture was refluxed for 2 h. The progress of the reaction was monitored by thin-layer chromatography [R_f for the resulting product and taxifolin was 0.242 and 0.387, respectively; the eluent was a benzene–acetone–ethanol mixture 8:2:1 (v/v/v)]. The solvent was removed under reduced pressure, resulting in the formation of oil. Then the condensation product was isolated in the form of a sodium salt, washed by heated ethanol to remove unreacted starting compounds, and dried under vacuum.

The structural features of the resulting product were determined by NMR spectroscopy (^{1}H, ^{13}C, HSQC, HMBC). ^{1}H, ^{13}C, HSQC, and HMBC spectra were measured on a Bruker Avance-III 600 spectrometer at 600 (for proton) and 150 MHz (for carbon). The product was dissolved in d_6-DMSO.

ROS scavenging activity

The ROS scavenging activity of polyphenols was estimated using a luminol–H_2O_2–horseradish peroxidase system. Measurements were carried out on a Tecan Infinite F200 microplate reader (Austria) in 96-well plates (Greiner). The effect of compounds being examined was assessed as follows. Solutions containing NaCl (137 mM), KCl (2.7 mM), Na_2HPO_4 (10 mM), KH_2PO_4 (1.76 mM), luminol (100 µM), horseradish peroxidase (1.25 U), and polyphenols (0.05–0.56 µM) or ascorbic acid (0.08–20 µM) in different concentrations were prepared (pH 7.4). Hydrogen peroxide (at a final concentration of 10 µM) was added to the mixtures immediately prior to registration. Luminol-dependent chemiluminescence (LCL) signals were recorded at 37 °C until LCL returned to the baseline. The integral LCL was calculated as a sum of LCL values recorded during the measurements. Changes in LCL by the action of the compounds were estimated relative to the control LCL values as follows: $\int_{LCL} = 100\% - (\int_{compound}/\int_{control}) \times 100\%$, where $\int_{LCL}$ is the inhibition of integral LCL, %; $\int_{compound}$ is the integral LCL in the presence of compounds (polyphenols or ascorbic acid); $\int_{control}$ is the integral LCL without addition of compounds. Ethanol was used for the preparation of a stock taxifolin solution, and its concentration in samples did not exceed 0.2%. It was shown that ethanol did not inhibit LCL at this concentration.

Interaction of polyphenols with iron ions

Due to the low solubility of iron hydroxides formed in the solution during the hydrolysis of iron salts, the study was performed in acetate buffer at pH 5.4

Iron-chelating activity

The ability of polyphenols to chelate Fe^{2+} was studied by absorption spectrophotometry in the UV–Vis region. The appearance of new absorption bands was detected during titration experiments in a 1-cm quartz cuvette: 0.25–5 μl of a solution of iron(II) sulfate (0.1 M stock solution in 0.5 M HCl) was sequentially added to 1 ml of polyphenols (at final concentrations of 0.25 and 0.5 mM). UV–Vis spectra were recorded on a Cary 100 Scan spectrophotometer (Varian, Australia) at room temperature. Because the mixture was not equilibrated during titration, the stoichiometric ratio of complexes formed between polyphenols and iron ions was determined on the basis of kinetic data on absorption changes (at 495, 550, and 620 nm) at different [Fe^{2+}]/[polyphenol] ratios. Absorbance was measured on a Tecan Infinite F200 microplate reader (Austria) in 96-well plates (Greiner) at 37 °C. Changes in the molar ratio of polyphenols to iron ions, and vice versa, in the mixture led to changes in the concentrations of the complexes, which made it possible to determine their stoichiometric ratio. During the experiments, the ratios of the molar concentrations of iron ions to polyphenols were varied from 0.1 to 18.4 (the final concentration of polyphenols was 0.25 mM). The experiments were carried out in the absence or presence of ascorbic acid.

Iron-reducing activity

The iron-reducing activity of polyphenols was estimated using a modified ferrozine method (Mira et al. 2002). The complex formed between ferrozine and Fe^{2+} was detected on a Tecan Infinite F200 microplate reader (Austria) in 96-well plates (Greiner) at 562 nm at 37 °C. Briefly, solutions containing ferrozine (1 mM) and polyphenols or ascorbic acid at different concentrations (12, 18, 26, 40, 59, 90 μM) were prepared. Then, a solution of iron(III) chloride (at a final concentration of 100 μM) was added to the mixtures. Absorbance was recorded after 0.5, 1, 2, 3, and 4 h. The concentration of Fe^{2+} was evaluated by a calibration curve obtained by using $FeSO_4$ solutions (a concentration range from 0.8 to 120 μM).

Ascorbic acid, which is able to scavenge ROS and reduce transition metal ions, was used as a standard antioxidant.

Statistical processing of the data was carried out using the program MS Excel 2003. The values are presented as the mean ± standard error of the means of three to six independent experiments.

Results

Characterization of the resulting product

As a result of condensation, a light yellow powder was obtained. The UV spectrum of the product dissolved in PBS showed the absorption band maxima at 224, 290, and 332 nm. These absorption bands are typical for flavonoids. The absorbance of the product at a concentration of 21.28 μg/ml at 332 nm was the same as that of taxifolin at a concentration of 9.12 μg/ml. As the concentration of the product was increased to 64.5 μg/ml and higher, a new absorption band centered at 512 nm appeared in the spectrum. In contrast, taxifolin solutions did not absorb in the visible region. The solubility of the product in water solutions was more than 10 times higher than that of taxifolin. The logarithm of the partition coefficient (logP) for the product was 1.13 ± 0.12, whereas logP for taxifolin was 1.64 ± 0.17.

The structure of the product was determined by the methods of NMR spectroscopy (^1H, ^{13}C, HSQC, HMBC). The ^1H NMR spectrum showed a singlet signal of proton of the glyoxylic acid residue at δ 5.11. The integration of signals gave the areas under the peaks that are proportional to the number of detected protons. The ratio of integrated signal areas for H-6 (δ 5.57), H-8 (δ 5.63), and $HCCOO^-$ (δ 5.11) to those for H-2 (δ 4.85), H-3 (δ 4.34), H-2′ (δ 6.72), H-5′ (δ 6.75), and H-6′ (δ 6.88) is 1:2. These data suggest that the product consists of two taxifolin units linked through a carboxymethine bridge at the C-6 and C-8 positions of the A ring. The spectrum also had singlet signals of hydroxyl groups at δ 9.0, δ 12.11, and δ 12.80 (Table 1). The ^{13}C NMR spectrum showed a signal of carbon of the carboxymethine group at 68.49 ppm, which is absent in the spectra of the precursors (taxifolin and glyoxylic acid), indicating the presence of an aliphatic bridge in the structure of the product. The spectrum also had two signals of the carboxyl group at 176.35 and 173.40, suggesting the formation of two stereoisomers of 6-8 dimer. The structure of the product was further examined by the methods of two-dimensional NMR spectroscopy (HSQC, HMBC). The HSQC experiment showed that C-2 (δ 83.02), C-3α (δ 71.87), and $CHCOO^-$ (δ 68.49) correlate with H-2 (δ 4.85), H-3 (δ 4.34), and $CHCOO^-$ (δ 5.11), respectively. Additional information from HMBC correlations confirmed the position of the glyoxylic acid residue at C-8, which was determined from the correlation of $HCCOO^-$ (δ 5.11) to C-8a (δ 160). It was also shown that $HCCOO^-$ (δ 5.11) correlates with C-7 (δ 163), which could be due to the position of the carboxymethine bridge at both C-8 and C-6. The ^{13}C, ^1H NMR and HMBC

Table 1 NMR (^{1}H, ^{13}C, HMBC) characteristics of the resulting product

Position	δC	δH	HMBC
2	83.02; 83.28 (CH)	4.85 (2H, m)	C-2, C-3, C-1′, C-2′, C-6′
3α	71.87; 71.97 (CH)	4.34 (2H, m)	C-2, C-3, C-1′
4	196.91;196.09 (C=O)	–	–
$HCCOO^-$	68.49 (CH)	5.11 (1H, s)	$HCCOO^-$, C-4a, C-7, C-8a, COO^-
4a	108.48	–	–
5	161.76;161.87 (C)	12.11 (s, OH)	–
6	97.63; 97.48 (CH)	5.57 (1H, s)	C-4a, C-6, C-7
7	163.18 (C)	12.80 (s, OH)	–
8	98.71; 98.51 (CH)	5.63 (1H, s)	C-4a, C-8
8a	160.11; 160.25 (C)	–	–
1′	128.92 (C)	–	–
2′	115.58; 115.66 (CH)	6.72 (2H, m)	C-2, C-1′, C-2′, C-3′
3′	145.39 (C)	9.00 (br s, OH)	–
4′	146.10; 146.17 (C)	9.00 (br s, OH)	–
5′	115.71; 115.79 (CH)	6.75 (2H, m)	C-4′, C-5′, C-6′
6′	119.52; 119.70 (CH)	6.88 (2H, m)	C-4′
COO^-	176.35; 173.40 (COO^-)	–	–

spectral data are presented in the Table 1. The data obtained suggest that the resulting product consists of two taxifolin units bridged by a carboxymethine group at the C-6 and C-8 positions (Fig. 1), and we designated this 6-8 dimer as Df-Tf.

ROS scavenging activity

The ROS scavenging activity of polyphenols was compared using a luminol–H_2O_2–horseradish peroxidase system. The results showed that taxifolin and Df-Tf displayed high ROS scavenging activity (Fig. 2a). The integral LCL in the presence of both polyphenols decreased in a dose-dependent manner (Fig. 2b). The half maximal inhibitory concentration ($IC_{\int 50}$) for taxifolin and Df-Tf was 0.58 ± 0.04 and 0.42 ± 0.03 μM, respectively. The $IC_{\int 50}$ value of ascorbic acid used as a standard antioxidant was 4.60 ± 0.05 μM (data not shown).

Iron-chelating activity

The metal-chelating properties of taxifolin have been studied previously (Mladěnka et al. 2011; Říha et al. 2014; Shatalin and Shubina 2014; Shubina and Shatalin 2013; Teixeira et al. 2005). It was found that taxifolin forms complexes with iron(II) ions, as evidenced by the appearance of new absorption bands in the visible region (Shatalin and Shubina 2014; Shubina and Shatalin 2013). The stoichiometric ratio and absorption spectra of the complexes depend on test conditions (pH, solvent, ion strength) (Shatalin and Shubina 2014; Shubina and Shatalin 2013). In the present study, polyphenols were titrated by a Fe(II) solution in acetate buffer at pH 5.4. The absorption spectra showed new absorption bands centered at 425 and 685 nm for taxifolin and at 460 nm for Df-Tf appeared, indicating the formation of polyphenol–iron complexes (Fig. 3). At the same time, the intensity of absorption changed

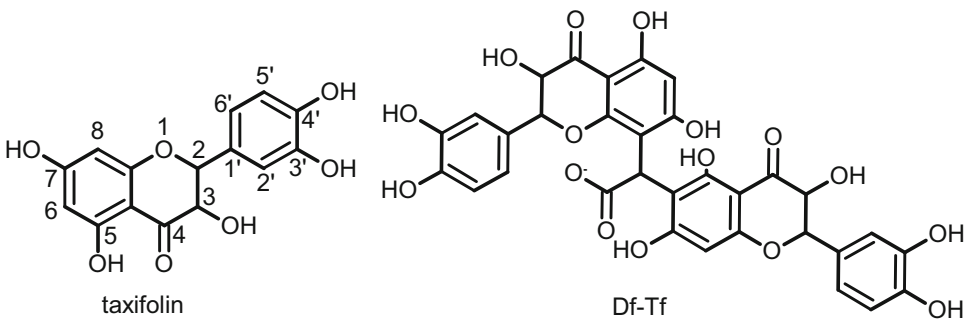

Fig. 1 Structures of taxifolin and the resulting product

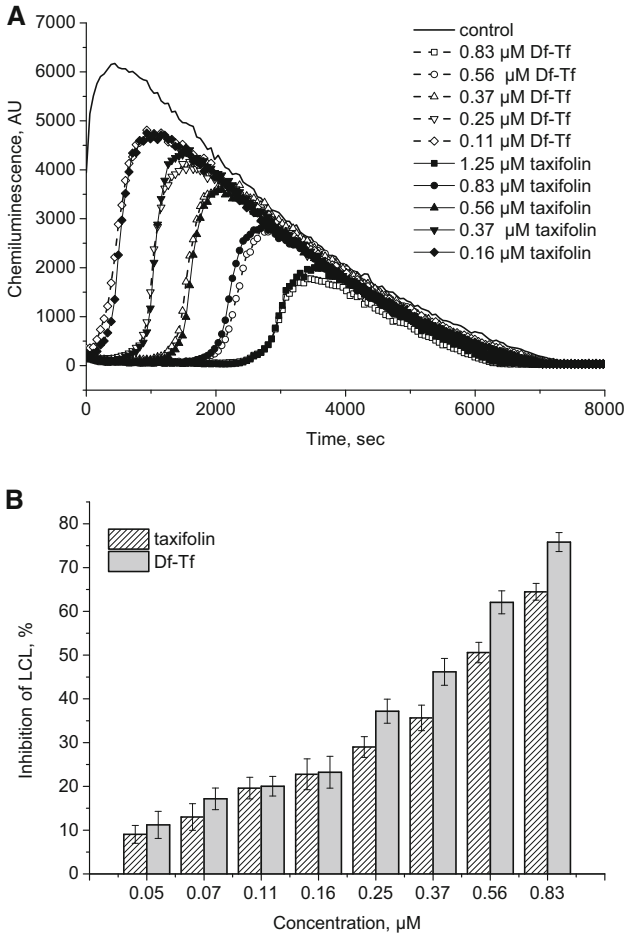

Fig. 2 Effect of polyphenolic compounds on chemiluminescence. **a** Amplitudes and kinetics of chemiluminescence in control and in the presence of polyphenols. **b** Comparison of the effect of polyphenols on integral chemiluminescence. The values are the means ± standard errors of three to six independent experiments

depending on time (Fig. 4). This suggests that the mixture was not equilibrated. Therefore, the stoichiometric ratio of the complexes was determined on the basis of the kinetic data on absorption changes at different [Fe^{2+}]/[polyphenol] ratios (Fig. 4). Our results demonstrate that the stoichiometric ratio of the polyphenol–Fe^{2+} complexes is 1:4 and 1:2 for Df-Tf and taxifolin, respectively.

Iron-reducing activity

To examine the ability of the polyphenols to reduce iron ions, the dependence of Fe^{2+} concentration on polyphenol concentration was determined at pH 5.4 after 0.5, 1, 2, 3, and 4 h of incubation with a $FeCl_3$ solution (Fig. 5). Ascorbic acid, which is capable of reducing two equivalents of transition metal ions, was used as a standard reductant. It was shown that both polyphenols reduced Fe(III) ions; the concentration of Fe(II) ions in the presence of Df-Tf was lower than that in the presence of taxifolin and was slightly dependent on the concentration of Df-Tf in the system (in the concentration range from 12 to 90 μM). In particular, an increase in the Df-Tf concentration from 12 to 90 μM resulted in the reduction of iron ions from 33 ± 3 to 38 ± 3 μM after 4 h, whereas an increase in taxifolin concentration from 18 to 90 μM led to the reduction of iron ions from 48 ± 4 to 93 ± 8 μM. A comparison of the reducing activity of taxifolin with that of ascorbic acid showed that, although ascorbic acid at low concentrations reacts more rapidly with iron ions than taxifolin, taxifolin eventually reduces more iron ions (Fig. 5).

Discussion

The results of the study indicated that the condensation of taxifolin with glyoxylic acid led to the formation of a dimer consisting of two taxifolin units linked through a carboxymethine bridge at the C-6 and C-8 positions of the A ring. This finding agreed well with the earlier reported data. Thus, es-Safi and coworkers showed that flavanols, such as catechin and epicatechin, react with some aldehydes. In particular, the condensation of these flavanols with glyoxylic acid occured at the C-6 and C-8 positions of the A ring, giving carboxymethine-linked dimers (Es-Safi et al. 2002). Similarly, unsubstituted phenol reacted with glyoxylic acid at the *ortho* and *para* positions. At the beginning of the reaction, hydroxymandelic acid was formed, which consists of one phenol unit and one unit of glyoxylic

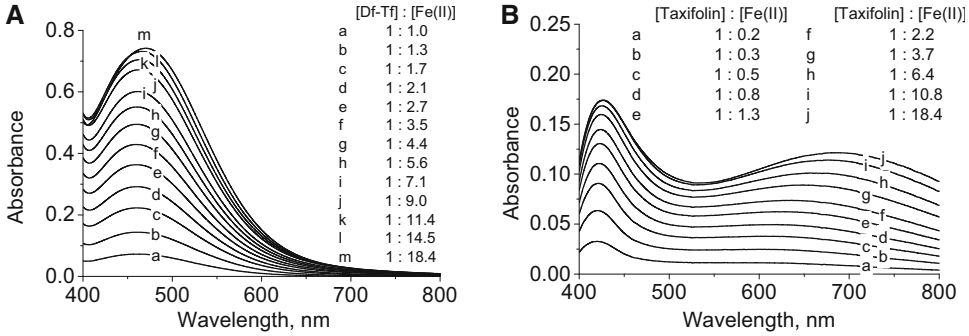

Fig. 3 Absorption spectra of polyphenol–Fe^{2+} complexes in acetate buffer, pH 5.4. **a** Absorbance of taxifolin in the presence of different Fe^{2+} concentrations. **b** Absorbance of the resulting product in the presence of different Fe^{2+} concentrations

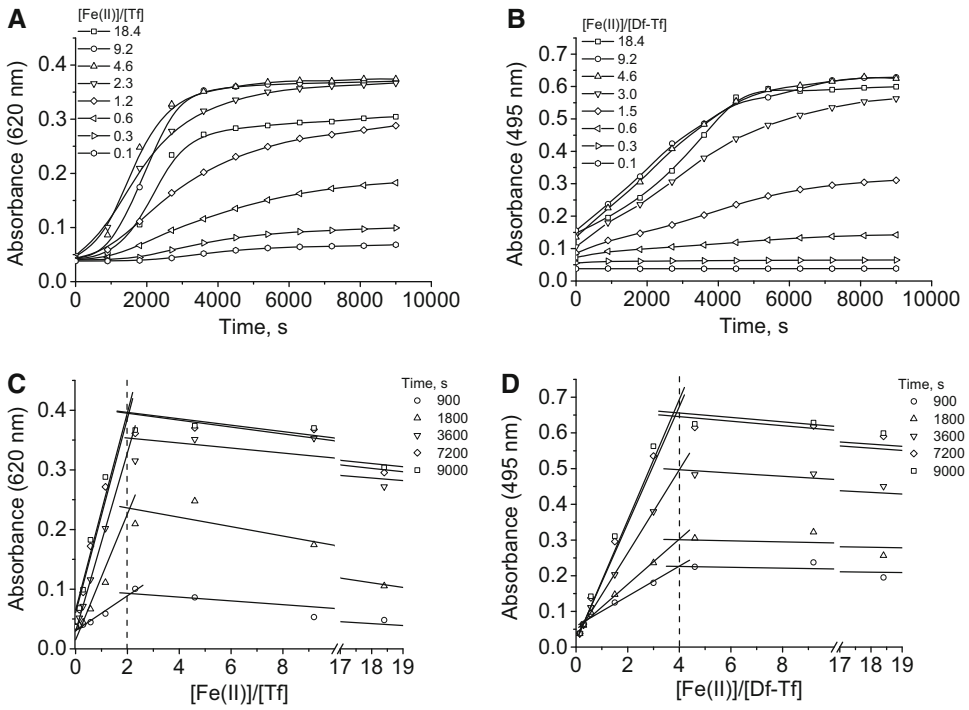

Fig. 4 Absorbance of polyphenol–Fe^{2+} complexes depending on time. **a** Taxifolin–Fe^{2+} complexes; **b** Df-Tf–Fe^{2+} complexes; **c** absorbance of the taxifolin–Fe^{2+} complexes at 620 nm versus the molar Fe^{2+}/taxifolin ratio; **d** Absorbance of Df-Tf–Fe^{2+} complexes at 495 nm versus the molar Fe^{2+}/Df-Tf ratio. Fe^{2+}/polyphenol ratios were varied from 0.1 to 18.4. The final concentration of polyphenols was 250 μM. The final concentration of ascorbic acid was 100 μM

acid. Its subsequent condensation with starting compounds leads to the formation of dimers, trimers and oligomers (Hoefnagel 1993). Our results suggested that the reactions mentioned above occured by a similar mechanism: at the first stage the aldehyde acted as an electrophile, displacing a hydrogen atom in the aromatic ring of a polyphenol. Then, the adduct was attacked by another phenolic molecule to yield a dimer. Probably, this type of reaction takes place during the storage and aging of flavonoid-rich foods and beverages. The products of the condensation of catechin and epicatechin with acetaldehyde were detected in wine samples (Saucier et al. 1997; Drinkine et al. 2007; Wollmann and Hofmann 2013). Taxifolin was also found in wines. Its level changed depending on conditions (De Simón et al. 2014), which suggests its transformation. We assume that taxifolin reacts with aldehydes, including glyoxylic acid, which is formed from one of the most concentrated organic acids in wine, tartaric acid, and this interaction results in the formation of condensed polyphenolic derivatives. Presumably, similar reactions occur in other food products. Therefore, a comparison of the properties of newly formed compounds and parent flavonoids provides a better understanding of the impact of these processes on the antioxidant properties of food and beverages. In addition, the study of the structure–activity relationship of polyphenols may facilitate the development of new effective antioxidants.

The antioxidant activity of polyphenols depends on the number and location of aromatic hydroxyl groups (Chen et al. 1996). In particular, high radical scavenging activity is observed for polyphenols containing catechol moiety (Rice-Evans et al. 1996). The presence of hydroxyl groups in the *orto*- position in the B ring provides the delocalization of electron density and enhanced stability of the radical formed as a result of the interaction of the compounds with ROS (Foti et al. 1996). The presence of 5- and 7-OH groups in the A ring also contributes to the antioxidant activity of polyphenols (Rice-Evans et al. 1996). Taxifolin and Df-Tf have these structural features and display high ROS scavenging activity, supporting the idea that these groups are responsible for the ROS scavenging activity of polyphenols (Rice-Evans et al. 1996). A comparison of ROS scavenging properties of taxifolin and Df-Tf shows that the molar concentration required for 50% inhibition of LCL for Df-Tf is 1.4 times less than that for taxifolin. These results are also in good agreement with the literature data, which demonstrate that the antioxidant activity of oligomeric polyphenols positively correlates with the degree of polymerization (Jerez et al. 2007; Zhou et al. 2014).

In addition to ROS scavenging activity of polyphenols, these compounds are able to prevent ROS generation by binding transition metal ions and inhibiting the Fenton reaction (Jomova and Valko 2011). In accordance with our previous results and the available literature data, taxifolin chelates transition metal ions, such as iron and copper ions (Mladěnka et al. 2011; Říha et al. 2014; Shatalin and Shubina 2014; Shubina and Shatalin 2013), and the composition of the complexes depends on experimental conditions (solvent, ionic strength, and pH) (Shatalin and

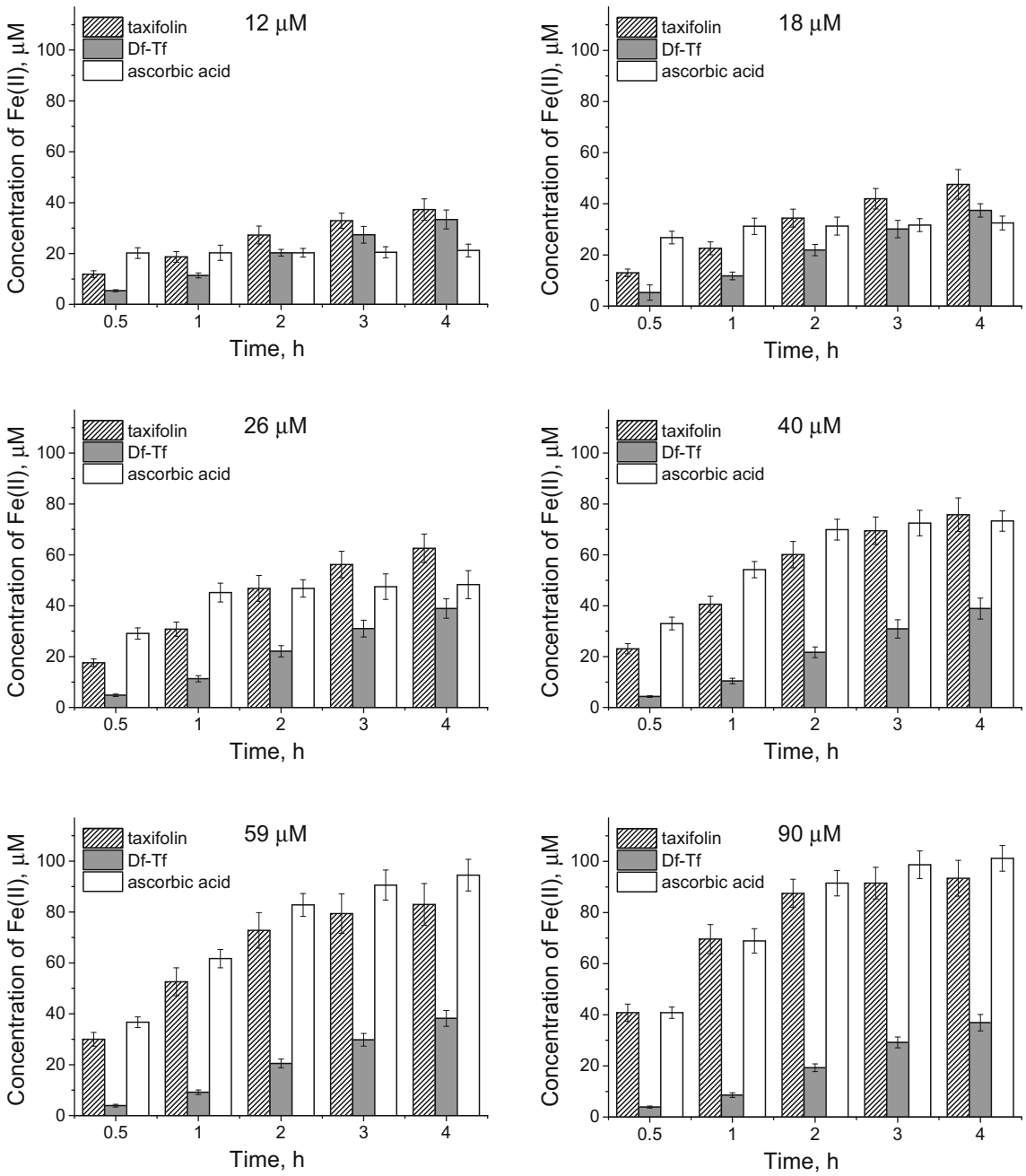

Fig. 5 Reduction of iron ions in the presence of polyphenols and ascorbic acid at different concentrations. The concentration of $FeCl_3$ is 100 μM. The values are the mean ± standard deviation of three independent experiments

Shubina 2014; Shubina and Shatalin 2013). The data obtained in this work demonstrate that the stoichiometric ratio of polyphenol:Fe^{2+} complexes in acetate buffer at pH 5.4 is 1:2 and 1:4 for taxifolin and Df-Tf, respectively. Consequently, the iron-binding ability of the compounds is proportional to the number of polyphenol units. Based on the structure–activity relationship, the most important sites for the chelation of metal ions were proposed: two adjacent hydroxyl groups in the B ring, the 5-hydroxy-4-keto site, and the 3-hydroxy-4-keto site (even in the absence of the 2,3-double bond) (Ř́ha et al. 2014). All above-mentioned groups are inherent in the structures of the compounds being tested in this study and may participate in the interaction of polyphenols with iron ions.

Thus, the results indicate that taxifolin and Df-Tf can act as dual-function antioxidants able to both scavenge ROS and prevent ROS formation by chelating iron ions. However, the interaction of polyphenols with transition metal ions can also underlie their pro-oxidant effect. Flavonoids can reduce metal ions, which promote the Fenton reaction.

Our experiments showed that taxifolin reduces a greater amount of Fe^{3+} than Df-Tf and ascorbic acid after 4 h at equal concentrations. These data partially agree with our previous results demonstrating that taxifolin may act as a pro-oxidant under certain conditions (Shatalin and Shmarev 2010). In turn, the effective reduction of transition metal ions has been earlier shown for flavonoids containing the 2,3-double bond in the C ring, 3-hydroxyl group, and adjacent hydroxyl groups in the B ring (Mira et al. 2002). The polyphenols being examined lack the 2,3-double bond, suggesting that they exhibit a moderate metal reducing activity. It should be noted that the 2,3-double bond provides the delocalization of electron density between A, B, and C rings, and the stabilization of the radical formed during redox reactions of polyphenol with transition metal ions or ROS. In the absence of this bond, the one-electron oxidation of the hydroxyl group of catechol moiety by ROS or metal ions results in the formation of the radical with a delocalized electron density in the catechol part of a molecule. In any case, both ROS scavenging and metal-reducing activities of polyphenols depend on the structure of compounds and their redox potentials (Navas Díaz et al. 1998). Taking this into account, the lower, as compared with taxifolin, iron-reducing ability of Df-Tf can be explained, at least partially, by differences in the redox potentials, the rates of iron reduction, and the metal-binding properties of these polyphenols. Because the ROS scavenging activity of the polyphenols being tested was close enough, it was assumed that the interactions of polyphenols with metal ions play a key role in this case. Probably, Df-Tf chelates iron ions more effectively than taxifolin. Our results show that the concentration of iron ions in the presence of Df-Tf is slightly dependent on the concentration of the polyphenol in the system (in the concentration range from 12 to 90 μM). On this basis, we hypothesize that the binding constants of complexes formed between Df-Tf and Fe(II/III) are high enough, and the release of iron ions from these complexes is a slow process, which prevents the detection of Fe(II) ions by the ferrozine method.

One important thing should be also noted. As our results show, Df-Tf is dissolved more readily in water solutions than taxifolin, indicating that the condensation of polyphenols with aldehydes leads to the formation of products that are differently distributed between hydrophobic and hydrophilic phases than parent flavonoids. A high solubility of a polyphenol in one of two phases leads to its accumulation inside this phase. Therefore, the hydrophobic/hydrophilic properties and the content of condensed polyphenols in food and beverages will influence the bioavailability and properties of the polyphenolic fraction. According to the literature data, hydrophilic polyphenols may act as strong antioxidants in oil-in water emulsion systems (Pazos et al. 2005). It has been suggested that highly water-soluble polyphenols may interact with the hydrophobic phase via their hydrophobic core, resulting in the location of these compounds at the lipid interface where they can more effectively protect lipids from oxidation (Pazos et al. 2005). To estimate the action of Df-Tf in lipid-containing systems, further studies are required.

Conclusion

The results obtained in the present study indicated that the reaction of taxifolin with glyoxylic acid led to the formation of 6-8 dimer (Df-Tf), which consisted of two taxifolin units linked through the carboxymethine bridge at the C-6 and C-8 positions of the A ring. The comparison of the properties of Df-Tf and taxifolin showed that Df-Tf exhibited higher ROS scavenging and metal-binding activities than taxifolin. In addition, the concentration of Fe(II) ions in the presence of Df-Tf was lower than that in the presence of taxifolin. All together, these results indicated that Df-Tf possessed a higher antioxidant activity than the parent flavonoid. Possible interactions of the product with hydrophilic and hydrophobic phases must also be taken into account in further investigations for a more comprehensive estimation of its action.

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