DOI:10.1021/jf902395k

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Simultaneous Detection of Pro- and Antioxidative Effects in the Variants of the Deoxyribose Degradation Assay

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Interest in the redox properties of natural products has led to the development of various assays for the detection of antioxidant activities and ROS-scavenging properties. Here, additional modifications of the 2-deoxy-D-ribose degradation assay are introduced that specifically allow the determination of interactions of the test compound with the autoxidation of ascorbic acid and the autoxidation of the test compound itself. To illustrate this, juglone and quercetin were used as examples. The modified assay systems provide insights into their specific antioxidative and pro-oxidative properties. In additional, an extensive characterization of the redox properties of their complex with iron is possible, if iron ions are added in the free form or complexed with EDTA. The juglone−iron complex proved to be pro-oxidative in a wider range of milieus than the quercetin−iron complex.

KEYWORDS: Hydroxyl radical; juglone; quercetin; ROS; chelation of iron ions

INTRODUCTION

Interest in the redox properties of natural products has stimulated many studies focusing on natural products that prevent the destructive effects caused by reactive oxygen species (ROS) on many biomolecules (*1*). ROS are also involved in controlling the pathogenesis of many degenerative diseases (*2*,*3*) and may activate redox sensitive transcription factors (*4*). As a result, the maintenance of redox homeostasis is a crucial asset for survival of stress scenarios, in the tissues of both plants (*5*) and animals (*2*).The maintenance of physiological redox homeostasis is as essential for a cell as that of osmosis or pH. During evolution, cells have developed complex interacting regulatory mechanisms that include enzymes and reducing metabolites that help to maintain the redox homeostasis (*6*). Consequently, natural products have been and are screened for their antioxidant activity and, in this context, especially for their ROS-scavenging properties. This broad interest has led to the development of various assays to determine antioxidant activity (*7*−*10*).

The hydroxyl radical (•OH) occupies an exceptional position among ROS because of its extreme reactivity and oxidative potential; it attacks even inert compound such as alkanes (*11*) which are normally considered to be stable under physiological conditions. One route to hydroxyl radicals is outlined by the Fenton reaction (reaction **1**) which is catalyzed by transition metals (e.g., Fe, Cr, Cu, or Mn). These catalysts are regenerated then in a Haber−Weiss reaction (reaction **2**).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH \quad (1)$$

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \quad (2)$$

Gutteridge and co-workers introduced an assay that allowed the detection of interactions of the test compounds with the formation of hydroxyl radicals by the Fenton reaction (*12*−*14*). This assay uses 2-deoxy-D-ribose as detection molecule, and it is utilized in phytochemistry and food chemistry to assess antioxidant properties of various compounds or extracts (*10*, *15*−*17*). 2-Deoxy-D-ribose is degraded by hydroxyl radicals that are generated in the reaction mixture by the Fenton reaction. The rate constant of the reaction is $3.1 \times 10^9\ M^{-1}\ s^{-1}$ (*14*). The amount of the degradation product, malonyldialdehyde (MDA), can be determined photometrically after a reaction with 2-thiobarbituric acid yielding a pink pigment. Benzoic or formic acids, rhodamine B and other substances were used as substrates to detect hydroxyl radical attack (*18*, *19*); they show, however, no special advantage compared to 2-deoxy-D-ribose. The originally described reaction mixture contains hydrogen peroxide, iron(III) ions, ascorbic acid and 2-deoxy-D-ribose. Iron is added in two forms into the reaction mixture, either as $FeCl_3$ that can be chelated by tested substances or in the form of a Fe(III)−EDTA complex. The complex of iron with EDTA avoids complex formation with the tested substances, 2-deoxy-D-ribose (*13*) or ascorbic acid (*20*), but does not prevent the participation of the iron in the Fenton reaction. According to Gutteridge (*12*, *13*), redox active scavengers inhibit efficiently 2-deoxy-D-ribose degradation by hydroxyl radicals that were formed in the solution: the iron ions were complexed by EDTA. In the absence of EDTA, a portion of the iron ions is complexed by 2-deoxy-D-ribose. The hydroxyl radicals arise close to the 2-deoxy-D-ribose molecule. Accordingly, compounds with ligand properties compete for iron ions with the 2-deoxy-D-ribose molecules and thus decrease 2-deoxy-D-ribose degradation that is caused by iron-catalyzed hydroxyl radical attack . The reactants can be dissolved only in water or inorganic buffers, because the hydroxyl radical reacts with most of the organic solvents and substances employed for buffer preparations (*21*). Ascorbic acid initializes the Fenton reaction by reduction of iron(III) ions. The kinetics of this iron(III) reduction may be the decisive factor

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 Published on Web 01/28/2010

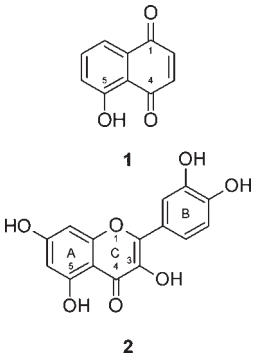

Figure 1. Chemical structures of juglone (**1**) and quercetin (**2**).

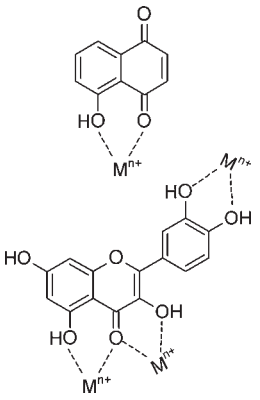

Figure 2. Chelates of juglone and quercetin with ions of transition metals.

(*22*, *23*). Thus, if ascorbic acid is omitted in the reaction mixture, the capability of the test compound itself to reduce iron(III) and start the Fenton reaction can be also assessed (*10*).

The aim of this paper is to introduce additional modifications of the 2-deoxy-D-ribose degradation assay that facilitate even more detailed insights into the possible reactions of the test compound than those that are already described in the literature. The main innovations that these modifications offer are that (i) the omission of hydrogen peroxide allows determination of interactions with the autoxidation of ascorbic acid, and (ii) if ascorbic acid is also left out, the possible autoxidation of the tested substance itself may become evident. The quality of the information that may be obtained by the newly introduced modifications of the 2-deoxy-D-ribose degradation assay are exemplified by juglone and quercetin (**Figure 1**). For comparative reasons, the chosen pH is exactly the same as of the cytoplasm (pH = 7.4).

Juglone (5-hydoxy-1,4-naphthoquinone) is known as a phytotoxin and redox cycler (*17*, *24*), and quercetin is a flavonoid aglycon widespread within the plant kingdom and well-known for its antioxidative and iron chelating properties (*25*, *26*). Reputedly, quinones are good acceptors of electrons (*27*) whereas phenols are donors of electrons (*28*). Juglone has both a quinone and a phenolic functional group. Quercetin has five hydroxyl groups and one keto group. The *o*-hydroxyl groups of ring B are usually the initial target of oxidants. Structural features that are required for complex stability with transition metals include the 3-hydroxy-4-keto grouping in ring C, the 5-hydroxy-4-keto arrangement of rings A and C, and the *o*-hydroxyl groups of ring B (**Figure 2**) (*26*, *29*). Consequently, the antioxidant activities of flavonoids depend on their redox properties and chelation of transition metals.

MATERIALS AND METHODS

Chemicals. Juglone, hydrogen peroxide, and 2-deoxy-D-ribose (deoxyribose) were obtained from Fluka (Buchs, Switzerland). All other chemicals and organic solvents used were of analytical grade and purchased from Sigma-Aldrich Inc. (St. Louis, MO). Water had Milli-Q quality.

H_2O_2/Fe^{3+}/Ascorbic Acid System. Juglone or quercetin was dissolved in an aqueous KH_2PO_4/KOH buffer solution (50 mM, pH 7.4) to yield final concentrations from 2 to 500 μM; to 125 μL of this solution, 25 μL of a 10.4 mM 2-deoxy-D-ribose solution in the same buffer system and 50 μL of an aqueous solution of $FeCl_3$ (50 μM) were added. In one series, those 50 μL contained 52 μM EDTA dissolved in buffer, which was premixed with the aqueous $FeCl_3$ solution (1:1 v/v). In the other series, the EDTA solution was replaced by the same volume of the buffer. In the first series, EDTA complexed the iron ions, preventing them from being chelated by the test compound; in the second series, the iron ions were complexed by the test compound. To start the Fenton reaction, various reactants dissolved in the above-mentioned buffer systems or in water were added: 25 μL of 10.0 mM aqueous solution of H_2O_2 and 25 μL of 1.0 mM ascorbic acid in buffer. Standard 1.5 mL sample vials (La-Pha-Pack, Werner Reifferscheidt GmbH, Langerwehe, Germany) were used as reaction vials. The mixture was vortexed and incubated at 27 °C for 60 min. Thereafter, 10 μL of 2.5% ethanolic butylated hydroxytoluene solution (w/v) followed by 250 μL of 1.0% 2-thiobarbituric acid dissolved in 3% trichloroacetic acid (w/v) was added to each vial to detect malonyldialdehyde, the decomposition product of 2-deoxy-D-ribose caused by the attack of hydroxyl radicals. The vials were vortexed and heated in a water bath at 85 °C for 30 min. The reaction was stopped by transferring the vials into an ice water bath for 3 min. To extract the reaction product of MDA and thiobarbituric acid, 600 μL of *n*-butanol was added, and the mixture was rigorously vortexed. The butanol layers of the vials, each 350 μL, were pipetted into flat bottomed 96 well plates (Greiner, Kremsmünster, Austria) and the absorbance was determined with a microplate reader (Tecan Infinite M200, Männedorf, Switzerland) at 532 nm. Assays were performed in triplicate. Reaction mixtures lacking the test compound served as positive control (100% MDA). The blank contained the full reaction mixture except 2-deoxy-D-ribose (negative control).

H_2O_2/Fe^{3+} System. This modification was carried out without the addition of ascorbic acid, which was replaced by the same volume of the buffer. Scoring was performed after 1 h. The blank contained the full reaction mixture except 2-deoxy-D-ribose (negative control). The positive control was the H_2O_2/Fe^{3+}/ascorbic acid system mixture lacking the test compound (100% MDA).

Fe^{3+}/Ascorbic Acid System. Hydrogen peroxide was replaced by the same volume of water. Deoxyribose degradation strongly depended on the diffusion of air oxygen into the liquid. Consequently, scoring was performed only after 16 h. The blank (negative control) contained the full reaction mixture without 2-deoxy-D-ribose (0% MDA). The positive control was the H_2O_2/Fe^{3+}/ascorbic acid system mixture lacking the test compound (100% MDA).

Fe^{3+} System. This modification of the deoxyribose assay was carried out without the addition of H_2O_2 and ascorbic acid, which were replaced by the same volume of the buffer or water. Scoring was performed after 16 h (diffusion of air oxygen). The blank contained the full reaction mixture except 2-deoxy-D-ribose (negative control). The positive control was the H_2O_2/Fe^{3+}/ascorbic acid system mixture lacking the test compound (100% MDA).

For all systems, possible interferences of tested compounds with the MDA detection procedure in the assay systems were checked before the experiments (*8*).

Statistical Analyses. Statgraphics Plus 5.0 (Statistical Graphics Corp., Rockville, MD) was used to perform analyses of variance (ANOVA) with Duncan's multiple range tests at a confidence level of 95%.

RESULTS AND DISCUSSION

All reactions that are presented in the ongoing text are hypotheses that aim to explain the results which were obtained from the deoxyribose degradation assay. These reactions, however, represent only a portion of possible ones.

H_2O_2/Fe^{3+}/Ascorbic Acid System. The Fenton reaction generates hydroxyl radicals after reduction of iron(III) by ascorbic acid (*21*). Juglone and quercetin proved to be strong inhibitors of 2-deoxy-D-ribose degradation in both variants of this experiment, with and without addition of EDTA (**Figure 3a,b**). Additionally, juglone showed a very weak pro-oxidative effect in the

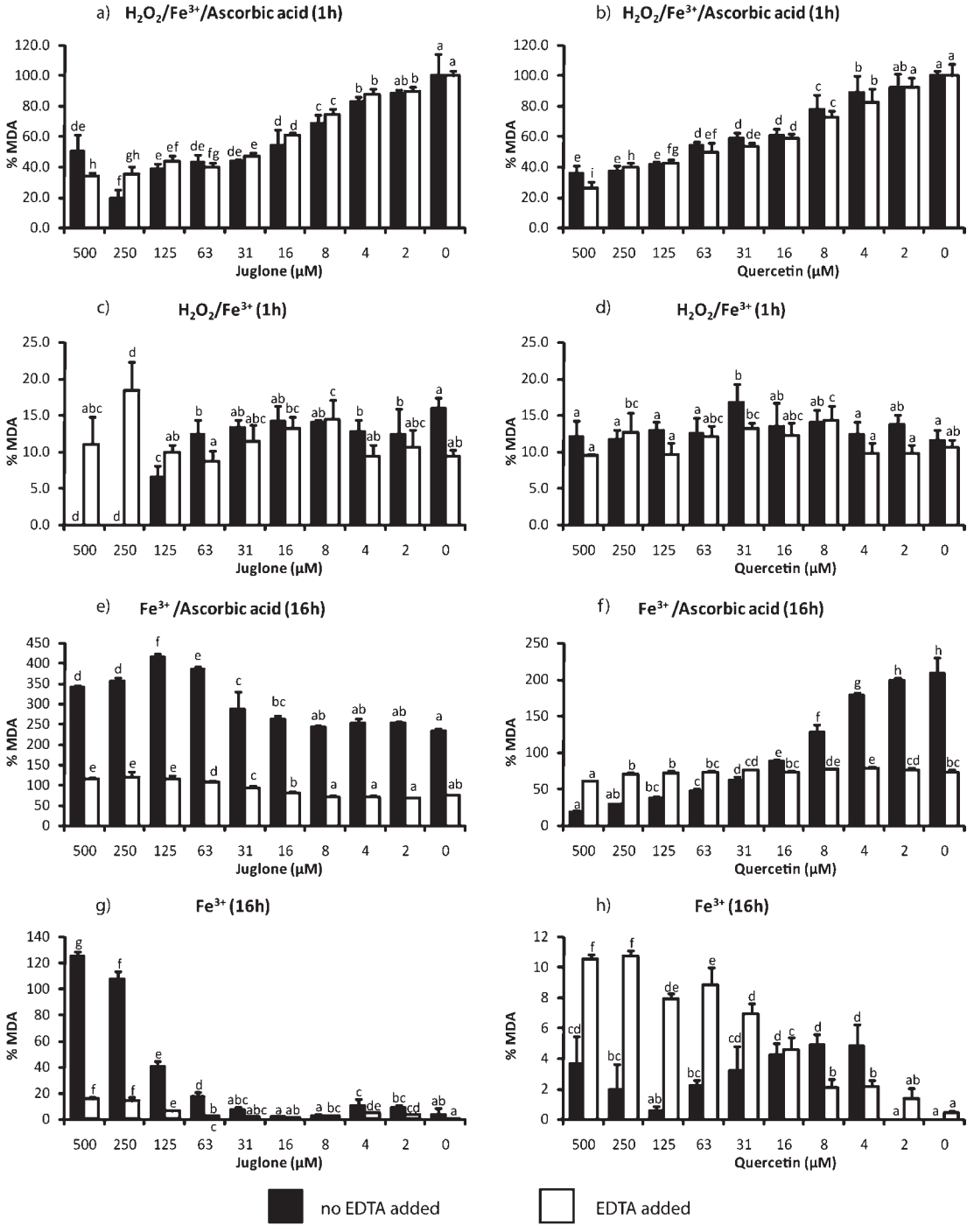

Figure 3. Activities of juglone and quercetin in various systems of the deoxyribose degradation assay, quantified in % malonyldialdehyde, an oxidative decomposition product of 2-deoxy-D-ribose; bars are means; error bars, standard deviation; $N = 3$, letters indicate different levels of significance (95% probability, Duncan's multiple range test).

concentration 500 μM in the variant without EDTA (**Figure 3a**). Juglone inhibited the 2-deoxy-D-ribose degradation. Several interactions are possible: (i) the hydroxyl group can reduce hydroxyl radical to water by an one electron transfer (**Figure 4**); (ii) the quinone part can oxidize hydrogen peroxide to molecular oxygen and/or ascorbic acid to dehydroascorbic acid by two electron transfers (**Figure 4**); (iii) juglone may be reduced to trihydroxynaphthalene by two electron transfers, a further reducing agent that scavenges any ROS by either one or two electron transfers (**Figures 4** and **5**). The absence of hydrogen peroxide and ascorbic acid prevented the initialization of Fenton reaction in both variants (with and without EDTA) when the scoring was performed within one hour. Juglone showed pronounced antioxidative effects by decreasing the deoxyribose degradation in both systems, either with or without addition of EDTA. In the latter system, however, in 500 μM, juglone again increased the concentration of MDA compared to the concentration of 250 μM. The following chemistry is possible: In the concentrations 2−250 μM juglone removes the iron ions from the competive ligand 2-D-deoxyribose and concomitantly scavenges arising ROS. Juglone is a 5-hydroxy-1,4-naphthoquinone, and the 5-hydroxy-4-carbonyl moiety of the juglone molecule complexes iron ions (**Figure 2**) (*29*). Most flavonoids show the same combination of functional groups that are well-known for chelation of transition metals (*30*).

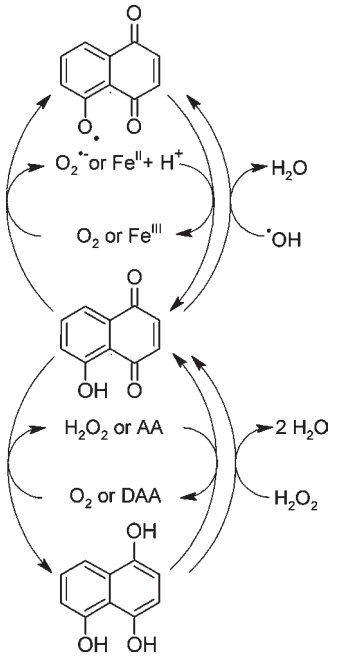

Figure 4. Selected possible one electron and two electron redox reactions of phenolic and quinone groups of juglone. AA, ascorbic acid; DAA, dehydroascorbic acid.

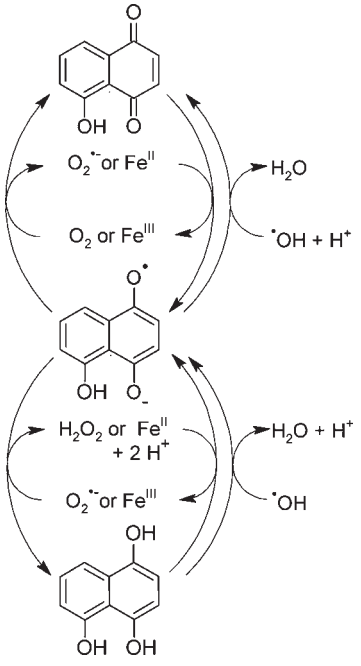

Figure 5. Selected possible one electron redox reactions of quinone and semiquinone groups of juglone.

The weak pro-oxidative effect observed (**Figure 3a**) in the concentration 500 μM in the variant without addition of EDTA most probably is caused by the different redox properties of iron−juglone complex. The reduction of iron(III) in the iron−juglone complex might proceed more easily compared to the complex of Fe(III)−EDTA if trihydroxynaphthalene or higher concentrations of juglone are present (**Figure 6**). This interpretation offers a possible explanation for the different behavior of juglone in the two variants of the assay at the highest concentration tested, 500 μM. Low solubility of higher concentrations than that precluded the testing of higher dosages of juglone. Possible one electron and two electrons reactions of juglone are shown in **Figures 4** and **5**.

Quercetin is well-known as an efficient reducing agent and chelator of transition metals (*31*). Both characteristics are visible in the results of the assay: The decrease of the relative MDA concentrations depends on the quercetin concentrations in both variants of the assay (**Figure 3b**) (*12*, *13*). Quercetin can reduce not only the highly reactive hydroxyl radical but also the more stable hydrogen peroxide to water (**Figures 7** and **8**). Phenols also form complexes with iron ions (**Figure 2**). Scavenging of free radicals and chelation of iron both contribute to the antioxidant activity of flavonoids. This assay suggests that the redox properties of quercetin are more responsible for the observed antioxidative effect than the chelation of iron. The protection of deoxyribose against attack by hydroxyl radicals was more efficient in the variant with EDTA added (**Figure 3b**). Oxidation products of quercetin, such as phenolic acids, their esters and other derivatives (*32*) with similar antioxidant and/or chelation properties, however, could also participate in the previously outlined reactions.

H_2O_2/Fe^{3+} System. This assay system explores if the tested compound can substitute for the function of ascorbic acid and start the Fenton reaction by reduction of iron(III) (*10*). Hydrogen peroxide may also reduce iron(III) ions (reaction **3**) and initiate the Fenton reaction.

$$H_2O_2 + Fe^{3+} \rightarrow Fe^{2+} + O_2^{\bullet -} + 2H^+ \quad (3)$$

Only low concentrations of MDA were observed. Consequently, this pathway is negligible (**Figure 3c,d**). Superoxide anion radicals ($O_2^{\bullet -}$) undergo spontaneous dismutation to hydrogen peroxide (reaction **4**). The speed of this reaction may be increased by phenolic complexes of the metals that function as catalysts (*33*).

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (4)$$

Phenols may also interact with superoxide anion radicals. The product, hydrogen peroxide, is further reduced to water (reactions **5** and **6**).

$$HO{-}Ar{-}OH + O_2^{\bullet -} \rightarrow {}^{-}O{-}Ar{-}O^{\bullet} + H_2O_2 \quad (5)$$

$$HO{-}Ar{-}OH + H_2O_2 \rightarrow O{=}Ar{=}O + 2H_2O \quad (6)$$

Juglone demonstrated negligible antioxidant activity in the variant of the assay without addition of EDTA, especially in the concentrations 500 and 250 μM (**Figure 3c**). In the variant with addition of EDTA, the concentration of 250 μM indicates the emergence of a weak pro-oxidative effect. Quercetin definitely had no effects in either variant of this assay (**Figure 3d**). In summary, the obtained results suggest that neither juglone nor quercetin can promote the 2-deoxy-D-ribose degradation in the presence of hydrogen peroxide.

Fe^{3+}/Ascorbic Acid System. Here, ascorbic acid undergoes autoxidation in the presence of transition metals and, as a result, produces ROS (reactions **7** and **8**) (*20*, *34*), but compared to the previously described systems the speed of the whole process is slower because it depends on the diffusion rate of atmospheric oxygen into the reaction liquid. The time point for scoring thus was extended to 16 h.

$$2Fe^{3+} + \text{ascorbic acid} \rightarrow 2Fe^{2+} + \text{dehydroascorbic acid} \quad (7)$$

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet -} \quad (8)$$

If the iron ions form a complex with ascorbic acid—this is the case if no EDTA is added—the reduction of iron ions and

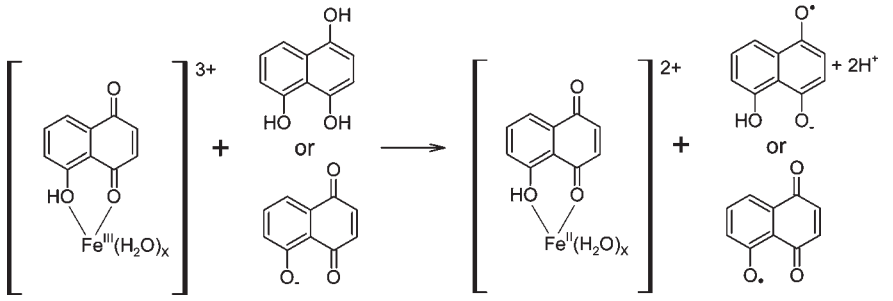

Figure 6. Reduction of the iron(III)−juglone complex by trihydroxynaphthalene or juglone.

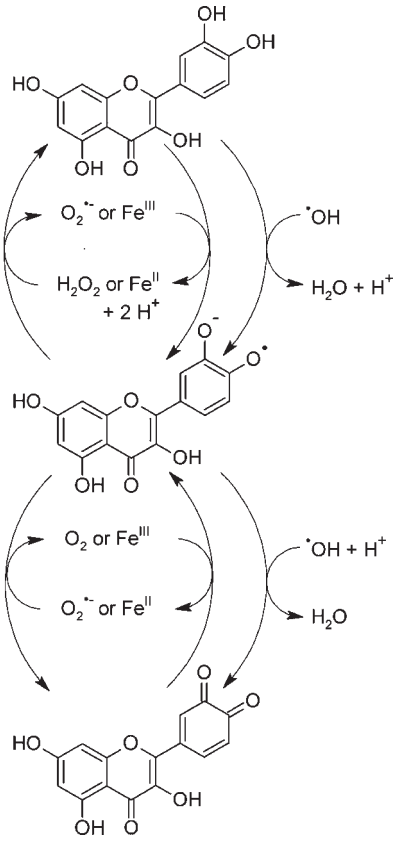

Figure 7. Selected possible one electron redox reactions of quercetin.

molecular oxygen increases (0 concentrations in **Figure 3e,f**), and, thus, more ROS are formed that may enter the Haber−Weiss and Fenton reactions. By contrast, Buettner reported the opposite phenomenon (*20*): The iron(III)−EDTA complex increased the speed of the ascorbic acid autoxidation reaction. This can be explained by different reaction times—Buettner used 15−30 min—and also a different end point. It is important to pay attention to the fact that Buettner studied the decrease of ascorbic acid, not that of 2-deoxy-D-ribose. 2-Deoxy-D-ribose most probably competes with ascorbic acid as ligand for iron. This affects the portion of the iron ions that are complexed by the 2-deoxy-D-ribose molecules (*12*). If this is the case, then the possibility that formed hydroxyl radicals directly attack 2-deoxy-D-ribose increases (*13*).

Juglone boosted the pro-oxidative effect of ascorbic acid in both variants of the assay. The amplification of the activity was stronger in the variant without addition of EDTA (**Figure 3e**). The effect increased in concentrations 63−125 μM and slightly decreased in concentrations from 250 to 500 μM. The redox properties of the iron−juglone complex and the reduction activity

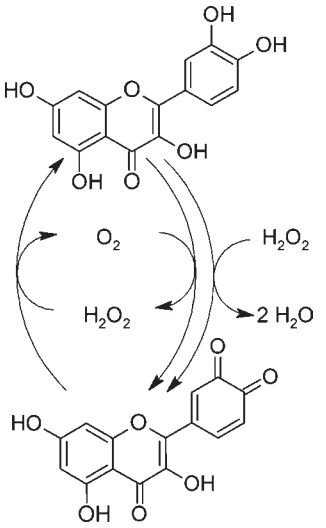

Figure 8. Selected possible two electron redox reactions of quercetin.

of the hydroxyl group of juglone and the potentially formed trihydroxynaphthalene contribute most probably to the pro-oxidative effect that is evident in this system of the assay. Trihydroxynaphthalene may arise following reduction by ascorbic acid, superoxide anion radical or hydrogen peroxide. Juglone and its reduced derivative, trihydroxynaphthalene, participate in the redox cycling of iron(III)/iron(II) besides of ascorbic acid (**Figures 4** and **5**). This effect was visible in both of the variants with and without addition of EDTA. These results suggest that the most important contribution was the more easy reduction of the iron−juglone complex compared to that of the Fe−EDTA complex. This effect, however, is not fully visible in the above-mentioned systems because of the presence of hydrogen peroxide that may undergo similar reactions from the start of the assay. In this system, hydrogen peroxide is generated by concomitant activities of juglone, ascorbic acid and iron ions. By contrast, this is not the case in the classic system of the 2-deoxy-D-ribose degradation assay. There exists, however, an assay system that uses phospholipid liposomes for detection of hydroxyl radicals (*34*, *35*); it uses a similar setup as in this modified system of the 2-deoxy-D-ribose degradation assay. In the variant without addition of EDTA, the potential shift in the redox properties may cause the strong pro-oxidative effects observed in the 63 and 125 μM concentrations where iron might be better available for Fenton reaction. The decrease of the pro-oxidative effect in the higher concentrations may be caused by changing concentrations of reactants and reaction products. This affects the redox potentials of the reactions and alters their speed. Thus, specific phenomena are only apparent at certain concentrations.

Quercetin inhibited the pro-oxidative activity of ascorbic acid more or less acting as an antioxidant. In contrast to juglone, this

effect was more apparent in the variant without addition of EDTA (**Figure 3f**). The results suggest that quercetin blocked the degradation of 2-deoxy-D-ribose. It is possible that both mechanisms, scavenging of ROS and chelation of iron ions, contributed to the observed effect. The results from the variant without addition of EDTA indicate that the contribution of complexation of iron ions may be higher; iron−quercetin complexes decrease the activity of iron ions in the Fenton reaction as their reduction seems to be more difficult (*36*). Further studies are required to explore if quercetin protects the iron ions against reduction by ascorbic acid by acting as more efficient competitor in their chelation.

The coupling reactions between semiquinones and monodehydroascorbic acid also may influence the activities of both juglone and quercetin (*37*). The occurrence of complex parallel reactions prevents an unambiguous clarification of the detailed mechanisms.

Fe^{3+} System. This system tests the possible autoxidation potential of the assayed substances. The principle is similar to the system where ascorbic acid generates ROS, but here the test compound replaces ascorbic acid (reactions **9**−**11**).

$$HO{-}Ar{-}OH + Fe^{3+} \rightarrow Fe^{2+} + {}^{-}O{-}Ar{-}O^{\bullet} + 2H^{+} \quad (9)$$

$${}^{-}O{-}Ar{-}O^{\bullet} + O_2 \rightarrow O_2^{\bullet -} + O{=}Ar{=}O \quad (10)$$

Alternatively, the following reaction is also possible:

$$HO{-}Ar{-}OH + 2Fe^{3+} \rightarrow 2Fe^{2+} + O{=}Ar{=}O + 2H^{+} \quad (11)$$

Iron(II) ions can reduce molecular oxygen into superoxide anion radicals (reaction **8**). Complex formation between the tested substance and iron ions plays a key role; in the various complexes, iron ions possess variable redox properties that affect their participation in oxidation or reduction reactions (reactions **12** and **13**).

$$HO{-}Ar{-}OH + Fe^{III}\text{-complex} \rightarrow Fe^{II}\text{-complex} + {}^{-}O{-}Ar{-}O^{\bullet} + 2H^{+} \quad (12)$$

$$Fe^{II}\text{-complex} + O_2 \rightarrow Fe^{III}\text{-complex} + O_2^{\bullet -} \quad (13)$$

Similar to the last system described, scoring was performed after 16 h because the system also depends on the diffusion of molecular oxygen from the air into the reaction mixture.

Juglone showed notable pro-oxidant activity that was more pronounced in the variant without EDTA addition. The efficient concentrations were 63−500 μM for the variant without EDTA addition and 125−500 μM for variant with EDTA addition. The results suggest that juglone reduced iron(III) to iron(II) ions, either directly by its hydroxyl group or indirectly by superoxide anion radicals that were formed by reduction of molecular oxygen (**Figures 4** and **5**). The reduction of molecular oxygen to the superoxide anion radical and its subsequent dismutation generates hydrogen peroxide which initiates the Fenton reaction (**Figure 4**). The reduction activity of juglone explains the pro-oxidative effect in both variants of this assay system. The higher pro-oxidative effect in the variant without addition of EDTA suggests that iron ions are more easily reduced in the complex with juglone than in the complex with EDTA.

The pro-oxidative effect of quercetin was more evident in the variant with addition of EDTA than in the variant without addition of EDTA (**Figure 3h**). In the variant without addition of EDTA, the pro-oxidative effect was only apparent in the lower concentrations (4−63 μM). No significant effects were shown at the higher concentrations (125−500 μM). The results suggest that quercetin affects the degradation of 2-deoxy-D-ribose in this system assay by chelation of iron ions and strong redox properties. In the variant without the addition of EDTA, iron was chelated by quercetin and this prevented the reduction of iron(III) to iron(II). In the variant with addition of EDTA, quercetin behaved similarly to juglone. It reduced the iron ions in the complex with EDTA (**Figure 7**). Quercetin may also interact with molecular oxygen by transfer of either one or two electrons (**Figures 7** and **8**); this generates hydrogen peroxide. It is suggested that hydrogen peroxide may arise mainly through the superoxide anion radical intermediate (*28*, *38*). Superoxide anion radicals may also reduce iron(III) ions. Reduction of molecular oxygen and iron(III) started the Fenton reaction in the variant without the addition of EDTA.

Conclusive Assessment. All modified systems of the 2-deoxy-D-ribose degradation assay demonstrated that the chosen test compounds, juglone and quercetin, enter complex redox reactions depending on the presence and absence of other components in the assay system. In the author's view, this set of assays provides easy and cost-effective characterization of the potential pro- and antioxidative effects of the test compound in changing milieus, a highly critical issue in the characterization of the redox chemistry of a test compound. Electron transfer reactions determined by Fenton chemistry are highly milieu-dependent and this especially merits attention in the classification of antioxidative properties of tested compounds or compound mixtures.

ABBREVIATION USED

EDTA, ethylenediaminetetraacetic acid; Fe−EDTA, chelate of EDTA with iron ions; MDA, malonyldialdehyde; ROS, reactive oxygen species.

ACKNOWLEDGMENT

The manuscript benefited from suggestions and comments of G. Bachmann, F. Hadacek (University of Vienna, Vienna, Austria) and L. Kubicova (University of Leipzig, Leipzig, Germany). The author is indebted to Sharon Robinson (University of Wollongong, Wollongong, Australia) for linguistic corrections.

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Received for review July 11, 2009. Revised manuscript received December 22, 2009. Accepted January 14, 2010. The research was supported by a Lise Meitner fellowship of the Austrian Science Fund (FWF M920-B03).