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Heat-Induced, Metal-Catalyzed Oxidative Degradation of Quercetin and Rutin (Quercetin 3-*O*-Rhamnosylglucoside) in Aqueous Model Systems

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The oxidative degradation of quercetin and rutin in phosphate buffer solutions, pH 8.0, at 97 °C, was studied by means of UV−vis spectroscopy and reversed-phase high-performance liquid chromatography (HPLC). The effect of the transition metal ions Fe^{2+} and Cu^{2+} on degradation rate and browning development was also assessed. It was shown that both flavonols are very labile to thermally induced degradation under oxidative conditions. Fe^{2+} and Cu^{2+} caused an increase in the degradation rate, as well as an increase in browning (A_{420}). Significant differences were observed in the degradation mechanisms, as implied by HPLC analyses. It is postulated that metal ions promote flavonol oxidation through reactive oxygen species formation, whereas increases in browning could be ascribed to oxidation and metal−polyphenol interactions.

Keywords: *Aqueous model systems; flavonol oxidation; metal-catalyzed; quercetin; rutin; thermal degradation*

INTRODUCTION

Flavonoids constitute a group of naturally occurring antioxidants, which over the past years have become of increasing interest because of their possible beneficial biological properties (Havsteen, 1983; Middleton, 1984, 1996; Cook and Samman, 1996). Among the most common and important flavonoids are flavonols, a particular class of compounds that exhibit a remarkable antioxidant activity (van Acker et al., 1996). Flavonols usually occur in plant foodstuffs as a mixture of *O*-glycosides and may be present in amounts up to a few hundred milligrams per kilogram of fresh weight in soft fruits and juices made from them (Pierpoint, 1986).

Recent studies estimate that the daily intake of flavonols and related flavonoids is 23−27 mg (Bravo, 1998), based mainly on the composition of plant foods currently consumed in The Netherlands and Denmark. However, it is widely believed that accurate estimation of total polyphenol intake is not available. This is mainly attributed to the fact that in a specific plant tissue more than one type of polyphenol may occur; the concentrations of these polyphenols are dependent upon genetic and environmental factors. However, other factors such as germination, degree of ripeness, storage, and processing may be significant in this respect (Bravo, 1998).

Flavonols have been a subject of increasing research concerning their potency for use as natural antioxidants in food systems. Early studies (Hudson and Mahgoub, 1980) showed that flavonols such as quercetin, kaempferol, and rutin exhibit good stabilizing activity when added to lard as leaf lipid extracts. Quercetin and myricetin were also found to efficiently inhibit oxidation of ground fish lipids (Ramanathan and Das, 1992). Furthermore, quercetin and morin were demonstrated as very good stabilizers for fish oil (Nieto et al., 1993), whereas studies with canola and marine oils (Wanasundara and Shahidi, 1994, 1998) revealed that myricetin, quercetin, and morin are superior antioxidants compared to synthetic butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

In some cases it has been observed that the levels of polyphenols decline when plant foods and products undergo thermal processing. Common domestic processes such as boiling and frying can affect to some extent flavonol content in onions (Crozier et al., 1997; Price et al., 1997; Hirota et al., 1998), tomatoes, lettuce, celery (Crozier et al., 1997), and broccoli florets (Price et al., 1998). Also, cooking can cause a decrease in total polyphenols of chickpea and blackgram (Jood et al., 1987), tannins in pulses (Rao and Deosthale, 1982) and legume seeds (Chau et al., 1997), and isoflavones in soybean (Coward et al., 1998) and soy products (Mahungu et al., 1999). An important decline during drying has also been reported for plum anthocyanins and flavonol glycosides (Raynal and Moutounet, 1989; Raynal et al., 1989) and grape pomace polyphenols (Larrauri et al., 1997). However, despite the quite large volume of research on the antioxidant activity of flavonols in biological and food systems, little is known about their thermostabilities and their interactions with other naturally occurring compounds. To further elucidate flavonol availability and the beneficial effects of fruit and vegetable consumption on human health, as well as to assess flavonol potency for use as food antioxidants, basic knowledge is required on the degradation behavior of these compounds when foods undergo thermal processing. Additionally, the structure elucidation of flavonol degradation and/or reaction products will further clarify the role of flavonols as antioxidants.

Preliminary investigations based on alkaline aqueous media (Makris and Rossiter, 2000) consisted of the first approach to flavonol thermal degradation and provided

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evidence concerning the role of heating, oxygen, the glycosidic linkage between quercetin and rutinose, and the sugar units as such. The high solubility of both quercetin and rutin at alkaline pH permitted the establishment of reliable time courses of decay, and the use of reversed-phase high-performance liquid chromatography (RP-HPLC) elucidated some aspects of the degradation mechanisms involved. It is irrefutable, however, that to have a better understanding of the principles that govern heat-induced flavonol degradation in aqueous media, studies over a wider pH range are required. Furthermore, the effect of iron(II) and copper(II), which are the most abundant transition metal ions in plant tissues (Weigert, 1991), was thought to be of particular interest with respect to flavonol oxidative degradation.

Efforts to establish a suitable liquid model for such an examination indicated a pH value of 8.0, which provided a very good solubility of both flavonols and enabled reliable calculations of degradation kinetics. Following boiling of asparagus spears, a plant food rich in rutin, the pH of water was 8.04 (Makris and Rossiter, 2000; unpublished data), and thus it was considered that pH 8.0 could, in some cases, represent a typical value of plant food processing conditions. Phosphate was preferred over other buffers because phosphate species are abundant in biological fluids and apparently do not interact with the flavonols under examination. This model was used to evaluate the effect of Fe^{2+}− and Cu^{2+}−citrate complexes on the rate of quercetin and rutin oxidative degradation. Citrate−iron and −copper were chosen as representative transition metal ion chelates because they are abundant in biological and food systems and their role in catalyzing free radical formation is well-known.

MATERIALS AND METHODS

Chemicals. Water used for HPLC analyses was distilled, purified by an EasyPure RT ultrapure water system, and filtered through 0.45 μm filters (Millipore). Acetonitrile (MeCN), ethanol (EtOH), and methanol (MeOH) were from BDH Chemicals Ltd. (Poole, U.K.). MeCN was of HPLC grade. MeOH and EtOH were of analar grade. 2,4,6-Trihydroxybenzoic acid (phloroglucinol carboxylic acid) was from Aldrich Chemical Co. Ltd. (Gillingham-Dorset, U.K.). 3,4-Dihydroxybenzoic acid (protocatechuic acid), 2,2′-dipyridyl, 1-(2-pyridylazo)-2-naphthol (PAN), quercetin, and rutin (quercetin 3-*O*-rhamnosylglucoside) were from Sigma Chemical Co. (St. Louis, MO). Citric acid, cupric sulfate, ferrous sulfate, orthophosphoric acid, sodium sulfite (Na_2SO_3), and phloroglucinol were from BDH Chemicals Ltd.

Treatment of Flavonols in Phosphate Buffer Solutions (pH 8.0). A 250-mL triple-neck, round-bottom flask, equipped with a water-cooled reflux condenser, was used. Heating was accomplished by means of a magnetically stirred oil bath. One hundred milliliters of 0.05 M phosphate buffer solution, pH 8.0, containing 1 mM quercetin or rutin was refluxed (97 °C). Flavonols were added to the solution just before the initiation of each treatment. For the treatments under nonoxidative conditions, solutions were purged with argon to eliminate oxygen. Oxidative conditions were established by bubbling air with a sparger into the solution during refluxing. The flow rate of air was kept constant throughout treatments. Degradation rates of the flavonols were calculated as first-order kinetics.

To evaluate Fe^{2+} and Cu^{2+} effects, flavonols were treated in the same buffer containing $FeSO_4$ or $CuSO_4$ at salt-to-flavonol millimolar ratios of 1:20, 1:10, and 1:5. Salts, along with flavonols, were added to the solution just before the initiation of each treatment. Blanks containing only salts were also run. Samples were taken after 15, 30, 60, 120, and 240 min. Ferrous and cupric sulfate stock solutions were prepared in 0.1 M citric acid. Citric acid concentration in the final solutions was 0.5 mM.

Table 1. RP-HPLC Elution Program Used for Quercetin and Rutin Determination

time (min)	eluent A (%)	eluent B (%)
0	100	0
1	100	0
41	60	40
51	50	50
60	50	50

Quercetin and Rutin Determination. *Sample Preparation.* Ten milliliters of sample was cooled and adjusted to pH 3.0 with HCl. The sample was concentrated in a rotary vacuum evaporator ($T \leq 40$ °C), dissolved in 10 mL of 50% aqueous methanol (in cases when insoluble material was observed, 70% aqueous methanol was used), and filtered through Millex HV_{13}, 0.45 μm, syringe filters (Millipore). This solution was used for HPLC analysis. Analyses were carried out immediately after sample preparation.

Analytical HPLC Procedure. A Waters 600E gradient pump with an Applied Biosystems 757 detector set at 260 nm was used. The system was computer-controlled by JCL 6000 software. Chromatography was carried out on a Waters Symmetry C_{18}, 3.9 × 150 mm, 5 μm, column with an Opti-Guard guard column, C_{18}, 3 mm. Columns were thermostatically controlled to maintain a temperature of 40 °C. Eluents were (A) 4.34 mM aqueous orthophosphoric acid (pH 2.5) and (B) MeCN/eluent A (6:4), and the flow rate was 1 mL min^{-1}. Injection was made by means of a Rheodyne injection valve with a 20 μL fixed loop. The elution program used is shown in Table 1. The column was washed with 100% MeCN and re-equilibrated with 100% eluent A before the next injection. Identification of quercetin and rutin was based on retention times of the original compounds. Quantitation was made by external standard method. Standard solutions were prepared in absolute EtOH and kept at −20 °C.

UV−Vis Spectroscopy. *Sample preparation* was made as for the previously described quercetin and rutin HPLC procedure. Samples were diluted 1:10 with MeOH prior to analysis, unless elsewhere specified. All samples were analyzed shortly after preparation.

Analytical Procedure. A Shimadzu UV−vis scanning spectrophotometer, interfaced with UV-2101PC software, was used. Absorbance was read at 420 nm, and spectra were obtained throughout a wavelength range varied from 230 to 600 nm, in a 1.0-cm path length cuvette.

Determination of Iron(II). Samples were prepared as for HPLC analysis, and the evolution of the Fe^{2+} ions was monitored by means of spectrophotometry. For this reason, a protocol based on the method described by Drysdale and Munro (1965) was established. The reaction mixture (1 mL) contained 0.15 mL of Na_2SO_3 (500 mM), 0.1 mL of dipyridyl (0.5%), 0.65 mL of aqueous acetic acid (10%), and 0.1 mL of sample, placed in a 1.5-mL Eppendorf tube. The mixtures were incubated in a water bath at 100 °C for 1 h and then left to cool to ambient temperature. The concentration of Fe^{2+} was calculated from the absorbance at 520 nm, and a calibration curve was established by plotting A_{520} against concentration of standard solutions, containing 2.43−19.44 μM Fe^{2+} (r^2 = 0.9985).

Determination of Copper(II). A spectrophotometric method based on that described by Gallardo Melgarejo et al. (1989) was used. In a 1.5-mL Eppendorf tube were placed 0.25 mL of PAN (0.1% in EtOH), 0.5 mL of EtOH, 0.25 mL of sodium acetate buffer (pH 4.8), and 0.1 mL of sample (total volume = 1.1 mL), and the mixture was vortexed. After 5 min, the absorbance was read at 595 nm. The concentration of copper(II) was calculated from a calibration curve, established by plotting A_{595} against known concentrations of copper(II), ranging from 2.93 to 23.48 μM (r^2 = 0.9971). For both iron(II) and copper(II) determinations, aqueous solutions were prepared in distilled water. For the blanks, 0.1 mL of distilled

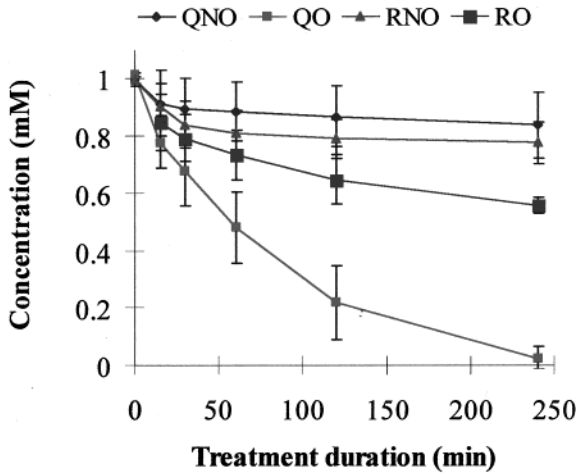


Figure 1. Time courses of quercetin (Q) and rutin (R) degradation, in phosphate buffer, pH 8.0, at 97 °C, under nonoxidative (NO) and oxidative (O) conditions.

water was used instead of sample. Measurements were carried out with an LKB Biochrom 4049 spectrophotometer and a 1-cm path length cuvette.

Statistics. In all cases experiments were run at least in triplicate, unless elsewhere specified, and the values obtained were averaged. When necessary, standard deviation was also calculated. The evaluation of the effect of the metal ions on R_D was based on the comparison of the values obtained. Comparisons were made on the basis of Student's test, at a 95% significance level. The relationship between R_D values and metal ion concentration was established with linear regression and determination of the correlation coefficients.

RESULTS

Effect of Oxygen. Figure 1 shows the time course of quercetin and rutin decay in phosphate buffer, at pH 8.0. Under nonoxidative conditions (elimination of oxygen), both flavonols exhibited excellent stability, and after 240 min at 97 °C, the decline in concentration was 16% for quercetin and 22% for rutin. The degradation rates (R_D) calculated under these conditions were 4.3 $\times$ 10^{-5} and 6.3 $\times$ 10^{-5} s^{-1} for quercetin and rutin, respectively. When oxidative conditions were used, quercetin concentration decreased by almost 98% and that of rutin by 45%, during the same time period, the corresponding R_D values being 18.7 $\times$ 10^{-5} and 10.3 $\times$ 10^{-5} s^{-1}. With regard to quercetin, the HPLC analysis showed the major degradation product to be protocatechuic acid (QB1); relatively small amounts of other products were also detected (Figure 2A). Protocatechuic acid has been detected during quercetin and rutin degradation under alkaline conditions (Makris and Rossiter, 2000) and identified on the basis of mass spectrometric and ^{1}H NMR analyses.

Oxidative conditions did not give rise to any other major product. Following degradation by UV−vis spectroscopy (Figure 3) revealed a considerable increase in absorbance at 261 and 291 nm, something that could be mainly attributed to protocatechuic acid (λ_{max} = 258 and 292 nm) (van Sumere, 1989). A small increase in absorbance was also seen at 445 and 469 nm. Increases at 291 nm have been observed in the polyphenol oxidase-catalyzed oxidation of quercetin (Jiménez and García-Carmona, 1999). An increase in absorbance at 440−460 nm during quercetin oxidation with cerium ammonium nitrate in DMF, in the presence of tBuOK, has been reported and ascribed to quinone formation (Dangles et al., 1998).

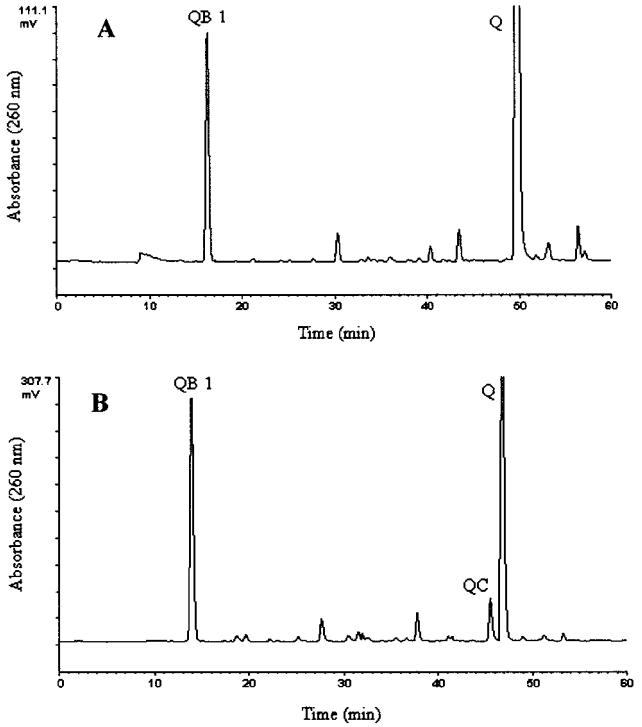


Figure 2. HPLC trace of a quercetin solution (1 mM) treated in phosphate buffer, pH 8.0, at 97 °C, for 240 min, under nonoxidative conditions (A) and in the presence of 0.2 mM $CuSO_4$, for 15 min, under oxidative conditions (B). Detection was performed at 260 nm. QB1, protocatechuic acid; QC, unknown; Q, quercetin.

Rutin breakdown under nonoxidative conditions was very limited, and thus only limited amounts of degradation products were found. On the other hand, oxidative conditions that provoked a more extended degradation enabled the formation of a series of products, as illustrated in Figure 4A. The important findings derived from the spectrophotometric studies were a decrease at 359 nm and an increase at 299 and 258 nm, wavelengths that correspond to rutin's λ_{max} (data not shown).

Effect of Fe^{2+} and Cu^{2+} Addition. Both ions were applied as sulfate salts at flavonol-to-salt millimolar ratios varying from 20:1 to 5:1. It can be clearly seen in Figure 5 that upon addition of Fe^{2+} or Cu^{2+}, the decay of both flavonols was accelerated (Table 2), a fact that indicated the occurrence of redox reactions, as judged by the evolution of concentration of both Fe^{2+} and Cu^{2+} (Figure 8). The manner in which Cu^{2+} acted was dose-dependent, because increasing amounts of ions caused increased degradation rates. The same was observed for rutin/Fe^{2+} solutions, but no such correlation was found for quercetin/Fe^{2+} solutions. When R_D values were plotted against metal ion concentration, the correlation coefficients (r^2) for Cu^{2+} treatments were 0.95 and 0.94 for quercetin and rutin solutions, respectively, whereas the corresponding values for Fe^{2+} treatments were 0.44 and 0.49 (Figure 6).

The presence of metal ions did not alter the HPLC profile of quercetin degradation products. However, the addition of Cu^{2+} at a 5:1 ratio gave rise to a peak termed QC (Figure 2B), which was not formed in any other case examined. The spectrophotometric investigation showed that the spectrum of quercetin solutions after 240 min of treatment exhibited maxima at 260−262 and 290−292 nm and a less profound maximum at 441−443 nm, irrespective of the metal ion applied (Figure 3). Maxima

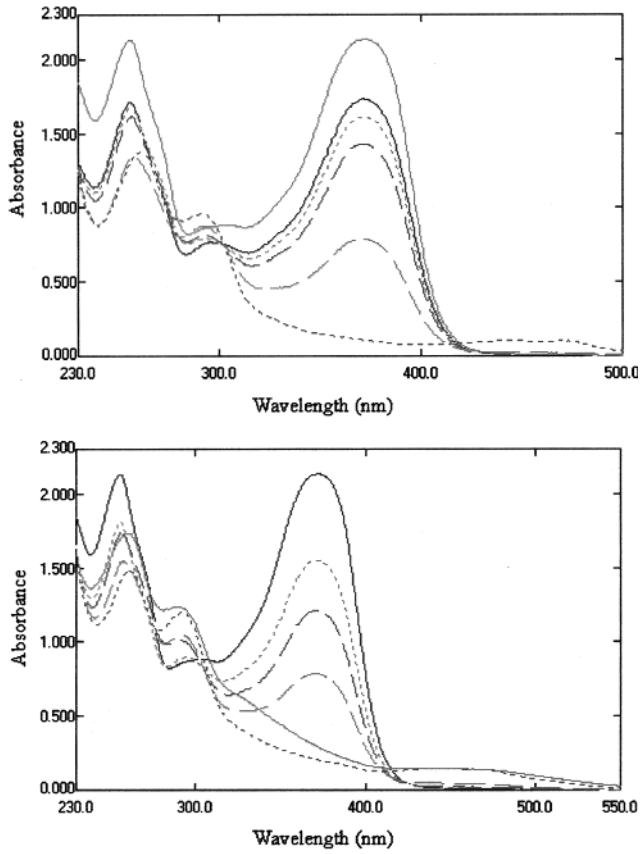


Figure 3. Degradation of quercetin (1 mM) in phosphate buffer solution, pH 8.0, at 97 °C, under oxidative conditions (top) and in the presence of 0.2 mM $FeSO_4$ (bottom), as shown by UV−vis spectroscopy. Samples were diluted 1:10 prior to analysis. Scanning was performed from 230 to 600 nm, in a 1-cm path length cuvette.

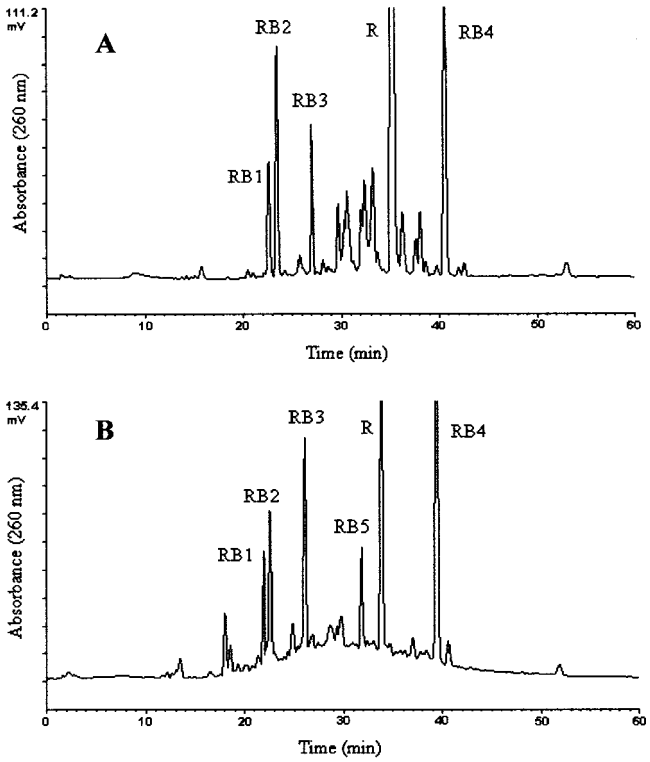


Figure 4. HPLC profiles of rutin solutions (1 mM) treated in phosphate buffer, pH 8.0, at 97 °C, for 240 min under oxidative conditions (A) and in the presence of 0.2 mM $CuSO_4$ (B). Detection was carried out at 260 nm. R, rutin. The rest of the peaks are degradation products.

at 290−292 and 441−443 nm tended to disappear when 1:5 ratios were used.

The HPLC profile of rutin initially was identical with that of the metal-free solution, but after 240 min of treatment, five major peaks could be distinguished (Figure 4B). None of these peaks corresponded to products derived from quercetin degradation. At the same time, no considerable changes were observed on its spectrum, apart from small increases at 258 and 290 nm (data not shown).

The effect of metal ion addition on browning development is shown in Figure 7. Although in every case metal ions caused a dramatic increase in browning, a relationship between the degree of browning and metal ion concentration could not be established. Increasing concentrations of Fe^{2+} appeared to inversely affect browning development in quercetin solutions, but the opposite was observed for rutin. Moreover, addition of increasing concentrations of Cu^{2+} did not provoke an analogous browning in quercetin solutions, but a positive dose-dependent phenomenon was observed for rutin. It should be highlighted that quercetin solutions gave higher final browning values than rutin solutions (Table 3), but in some cases rutin solutions exhibited much greater relative browning increases (Table 4).

DISCUSSION

As in alkaline solutions (Makris and Rossiter, 2000), quercetin and rutin degraded very differently in the presence of metal ions, due to the glycosylation of the 3-hydroxyl group. The application of either Fe^{2+} or Cu^{2+} could increase significantly R_D values of both flavonols under oxidative conditions, with respect to metal-free solutions, with the exception of rutin−Fe^{2+} and quercetin−Cu^{2+} (20:1), ($P < 0.05$). These increases were found to be highly correlated with Cu^{2+} but not with Fe^{2+} concentration. These results indicate different interactions among flavonols, metal ions, and oxygen. Metal ion concentrations during treatments indicate that both ions might have undergone redox cycles (Figure 8). In all experiments, it was shown that the metal ions could interact with quercetin more profoundly than with rutin, yielding higher R_D values, and thus the hydroxyl group at the 3-position appears to be a key parameter with respect to degradation.

Because metal ions are involved in the oxidation of the flavonols, this in turn facilitates decomposition. This could occur primarily through a hydroxyl free radical (HFR) or other reactive oxygen species (ROS) formation (Figure 9). It can be postulated that, under the conditions used, generation of HFRs or ROS may take place via more than one mechanism. When Fe^{2+} was used, ROS could be derived as shown in reaction i of Figure 9 (Aust et al., 1990; Halliwell and Gutteridge, 1990).

Furthermore, if oxidation of the B-ring is considered, formation of H_2O_2 may occur (reaction ii of Figure 9) (Singleton, 1987).

If so, then generation of HFRs is possible (reaction iii of Figure 9) (Halliwell and Gutteridge, 1984, 1986, 1990; Crichton, 1991).

In addition, if both reactions ii and iii take place, then further reaction is possible (reaction iv of Figure 9).

Moreover, $^{\bullet}OH$ produced from reaction iii could promote further Fe^{2+} oxidation (reaction v of Figure 9) (Aust et al., 1990).

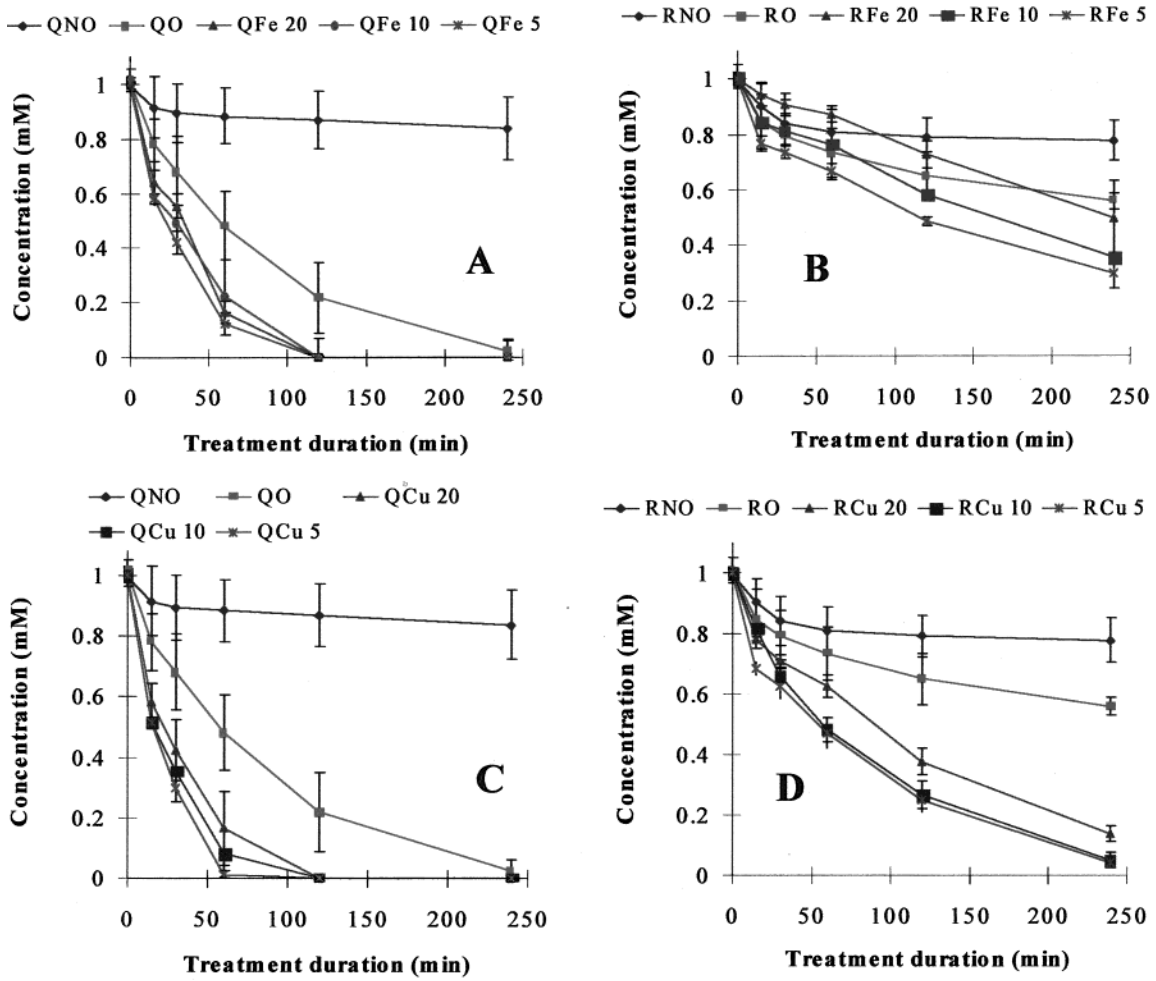


Figure 5. Time course of quercetin (Q) and rutin (R) decay in phosphate buffer, pH 8.0, at 97 °C. NO and O, nonoxidative and oxidative conditions, respectively (metal-free solutions); A and B, iron(II) effect; C and D, copper(II) effect; 20, 10, 5, flavonol-to-salt millimolar ratios 20:1, 10:1, and 5:1, respectively.

Table 2. Effect of Various Conditions on Flavonol Degradation Rate[a]

condition	quercetin	rutin
nonoxidative	4.3	6.3
oxidative	18.7	10.3
Fe (1:20)	57.8	4.0
Fe (1:10)	47.7	10.8
Fe (1:5)	55.8	16.2
Cu (1:20)	44.0	17.5
Cu (1:10)	66.8	21.0
Cu (1:5)	88.0	26.3

[a] Values are expressed as s^{-1} ($\times 10^{-5}$) and represent means of triplicate determinations.

Cu^{2+} is able to catalyze similar reactions as well, yielding HFRs (reactions vi and vii of Figure 9).

In phosphate buffers and at pH values >7.0, the autoxidation of Fe^{2+} is very rapid (Lambeth et al., 1982). This might explain the decline in Fe^{2+} concentration initially observed in all cases (Figure 8). In solutions containing only Fe^{2+}, the concentration of Fe^{2+} declined and remained at low levels throughout treatment, but in the presence of quercetin a gradual recovery was found. It is likely that Fe^{3+} oxidized quercetin and concomitantly was reduced back to Fe^{2+}. In contrast, in rutin solutions Fe^{2+} concentration remained fairly constant, thus implying that rutin did not allow Fe^{2+} oxidation. Rutin can bind Fe^{2+} more effectively than quercetin, thereby preventing Fe^{2+} autoxidation and

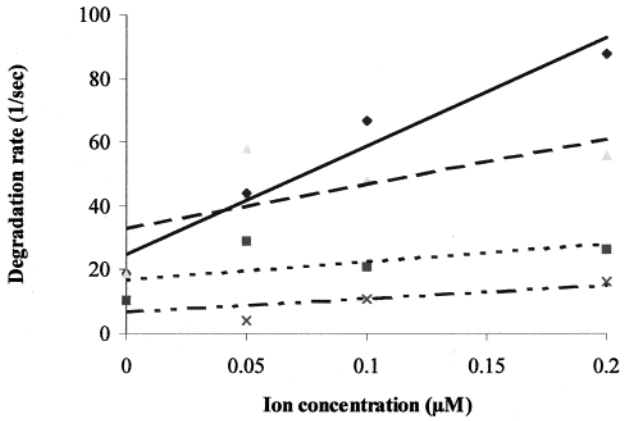


Figure 6. Correlation between degradation rate (R_D) values and metal ion concentration: (—) quercetin/Cu^{2+} solutions; (- - -) quercetin/Fe^{2+} solutions; (···) rutin/Cu^{2+} solutions; (-···-) rutin/Fe^{2+} solutions. Values represent means of triplicate analysis. R_D values are $\times 10^{-5}$.

ROS formation to a significant extent (Afanas'ev et al., 1989). That is, the application of Fe^{2+} in rutin solutions did not increase the R_D considerably, but Cu^{2+} did ($P < 0.05$), with respect to metal-free solutions. Such phenomena rather account for the low correlation found between flavonol R_D and metal ion concentration, indicating that chelation of Fe^{2+} or Fe^{3+} with flavonols results in inhibition of flavonol oxidation.

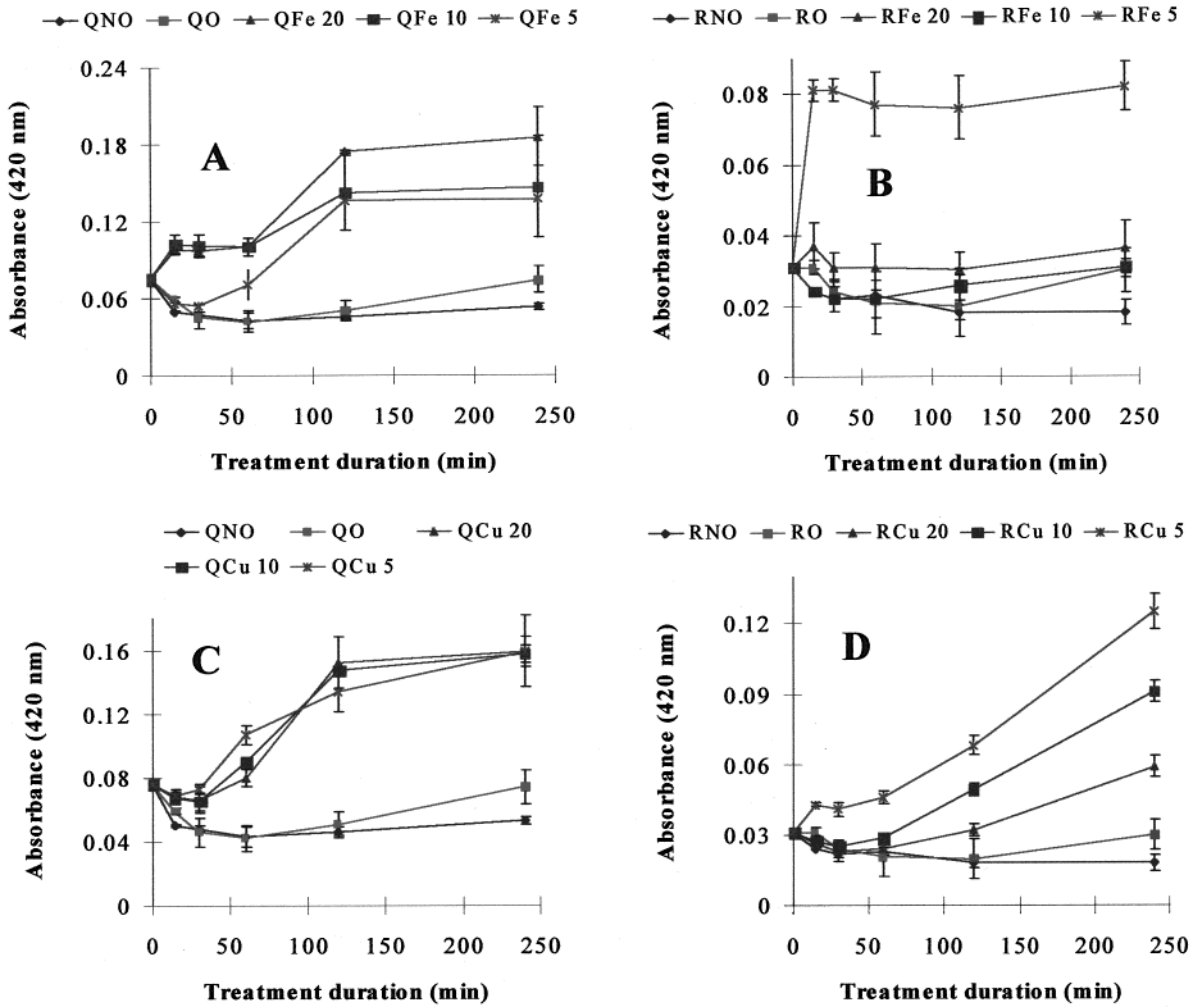


Figure 7. Browning development during treatment of quercetin (Q) and rutin (R) in phosphate buffer solutions, pH 8.0, containing $FeSO_4$ or $CuSO_4$. NO and O, nonoxidative and oxidative conditions, respectively (metal-free solutions); (A and B) iron(II) effect; (C and D) copper(II) effect; (20, 10, 5) flavonol-to-salt millimolar ratios of 20:1, 10:1, and 5:1, respectively.

Table 3. Browning (A_{420}) Development after 240 min of Treatment, in Quercetin and Rutin Solutions Containing Fe^{2+} or Cu^{2+}[a]

condition	quercetin	rutin
nonoxidative	0.053 ± 0.001	0.018 ± 0.003
oxidative	0.074 ± 0.008	0.030 ± 0.005
Fe (1:20)	0.185 ± 0.017	0.036 ± 0.006
Fe (1:10)	0.146 ± 0.027	0.031 ± 0.002
Fe (1:5)	0.137 ± 0.009	0.082 ± 0.016
Cu (1:20)	0.159 ± 0.007	0.059 ± 0.004
Cu (1:10)	0.157 ± 0.008	0.091 ± 0.010
Cu (1:5)	0.159 ± 0.017	0.125 ± 0.005

[a] Salt-to-flavonol ratios are indicated in parentheses. Samples were diluted 1:10. Values are means of triplicate analyses.

Table 4. Relative Browning Increase of Flavonol Solutions in Phosphate Buffer, pH 8.0, Treated for 240 min in the Presence of either $FeSO_4$ or $CuSO_4$[a]

	% increase in browning	
condition	quercetin	rutin
nonoxidative	−30.26	−41.94
oxidative	−2.63	−3.23
Fe (1:20)	143.42	16.13
Fe (1:10)	92.11	0.00
Fe (1:5)	80.26	164.52
Cu (1:20)	109.21	90.32
Cu (1:10)	106.58	193.55
Cu (1:5)	109.21	303.23

[a] Salt-to-flavonol ratios applied are indicated in parentheses. Values represent means of triplicate analysis. Samples were diluted 1:10.

Chelation of rutin with Cu^{2+} is reversible and can be overcome by the addition of a strong chelator, such as EDTA, but chelation of quercetin with Cu^{2+} may result in its oxidation (Brown et al., 1998). Thus, it appears that the 3-OH group is the crucial factor concerned with quercetin−Cu^{2+}/Fe^{2+} interactions, and it is presumably associated with the oxidation of the flavonol. This hypothesis could explain the greater stability of rutin, which may be mainly ascribed to the occupation of the 3-position of the flavonol skeleton by rutinose.

The initial decrease in Cu^{2+} concentration is evidence that chelation with quercetin oxidized the latter and reduced the former to Cu^{+}. Subsequent gradual oxidation of Cu^{+} back to Cu^{2+} occurred probably due to decomposition of quercetin. As in the case of Fe^{2+}, Cu^{2+}−rutin interactions were much less pronounced. However, in both cases increasing amounts of Cu^{2+} were highly correlated with increased R_D values, suggesting that chelation of either quercetin or rutin with Cu^{2+} cannot inhibit Cu^{2+}-mediated oxidation. In contrast, in all cases, except quercetin−Cu^{2+} (20:1), Cu^{2+} was found to provoke very important R_D increases ($P < 0.05$) with respect to metal-free solutions.

The effect of metal ions on browning development was also an important issue. It was found that upon addition of either Fe^{2+}or Cu^{2+}, quercetin and rutin solutions

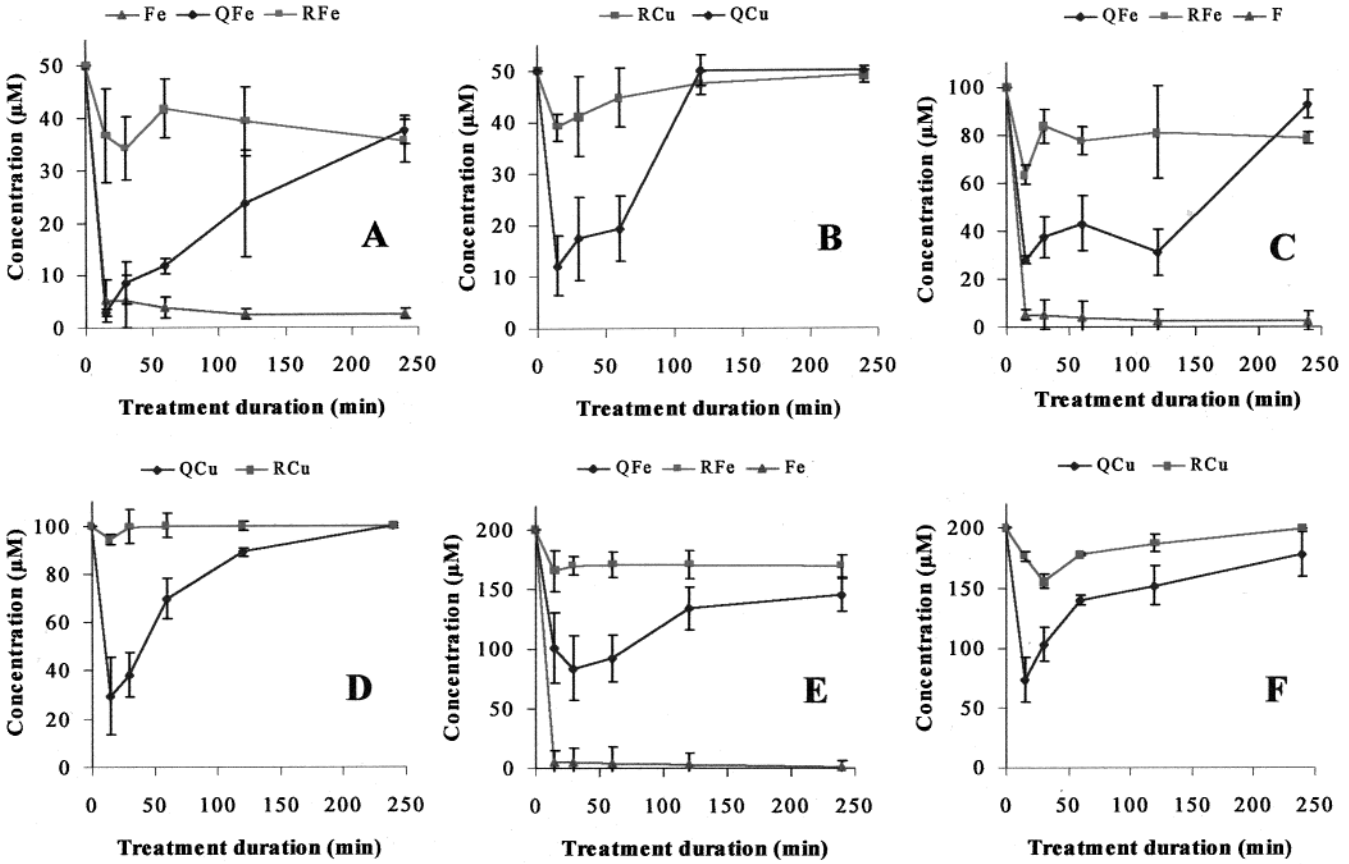
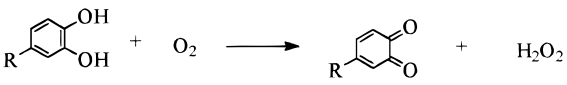


Figure 8. Evolution of iron(II) and copper(II) during treatment in phosphate buffer solutions, pH 8.0. Cu, copper(II); Fe: iron(II); Q, in the presence of 1 mM quercetin; R: in the presence of 1 mM rutin; (A and B) millimolar ratio of 20:1; (C and D) millimolar ratio of 10:1; (E and F) millimolar ratio of 5:1. In solutions containing only copper(II), no changes in concentration throughout treatment were observed.

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



(ii)

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

$$^{\bullet}OH + H_2O_2 \rightarrow H_2O + H^+ + O_2^- \quad \text{(iv)}$$

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

$$Cu^{2+} + H_2O_2 \rightarrow Cu^+ + HO_2^{\bullet} + H^+ \quad \text{(vi)}$$

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + {}^{\bullet}OH + OH^- \quad \text{(vii)}$$

Figure 9. Reactions leading to HFR and other ROS formation.

developed intense brown shades. The examinations showed that discoloration does not always appear at the beginning of the treatment, but there is a gradual increase in absorbance at or around 420 nm. However, in Fe^{2+}−rutin (1:5) solution browning appeared as soon as rutin was added, probably due to a strong and stable chelate. Similar iron−polyphenol effects have been observed (Cheng and Crisosto, 1994, 1997).

According to the data presented in Table 3, quercetin solutions exhibited very intense browning after 240 min of treatment, whereas in the corresponding rutin solutions browning was always at lower levels. However, in some cases rutin was shown to give a much higher relative increase, a finding that suggests that during rutin degradation, formation of compounds that are potentially browning agents in response to metal addition takes place. Taking into consideration that the increase at 420 nm was time-dependent, it could be concluded that browning of quercetin solutions was due to advanced oxidation. Nevertheless, it is believed that the key parameter concerned with browning is composition of the solutions. Because quercetin and rutin do not yield the same degradation products, it would be reasonable to assume that the relative amounts of quercetin and rutin, and the degradation products thereof, define at any time the coloration of the solutions. For quercetin it is obvious that after a period, only protocatechuic acid participates in browning development because quercetin is completely degraded. On the other hand, in rutin solutions the coloration should be mainly produced from rutin−metal interactions. It is known that chelation of flavonols with metal ions can cause bathochromic shifts to their spectrum (Markham and Mabry, 1975; Boudet et al., 1998; Brown et al., 1998). In fact, in the case when rutin solution was added to Fe^{2+} at a 5:1 ratio, a profound increase in browning was observed. This possibly happened because rutin chelated with Fe^{2+}, and thereby a bathochromic shift occurred. Similar effects were seen for rutin−Cu^{2+} (5:1) and quercetin−Fe^{2+} (20:1 and 10:1). However, the contribution of the other compounds formed during rutin decomposition cannot be excluded.

CONCLUSIONS

The study presented herein elucidated to a certain extent the effect that oxygen and transition metal ions can have on a flavonol and a glycoside thereof at near neutral pH and temperatures normally attained during boiling of an aqueous solution. It is believed that, as far as this was possible, the conditions used are representative of situations that could be encountered during food processing, either industrial or domestic.

Certainly, foodstuffs contain numerous substances that could inhibit the action of transition metals, but it is also plausible that in food systems other agents may facilitate destruction of valuable, in terms of nutrition, molecules, such as flavonoids. For example, it has been shown that, under specific conditions, aluminum salts may enable acceleration of iron-induced lipid peroxidation, although do not themselves stimulate peroxidation (Gutteridge et al., 1985; Oteiza, 1994). Therefore, the ability of flavonols to resist degradation during processes involving heating is thought to be highly associated with their structure, but it is also substantially dependent upon other factors, such as the presence of oxygen or oxidizing agents. These factors may also be important with respect to food browning. Given the importance that flavonols have gained in recent years, it is anticipated that similar studies will contribute toward understanding the behavior of flavonols during food processing. This is essential in evaluating flavonol losses in processed foods and estimating the actual flavonol intake through diet.

Currently, work is in progress concerning the study of more complex model systems, which will further clarify flavonol behavior at high temperatures as well as interactions with other natural food components. In addition, efforts are being expended on the isolation and structure elucidation of some of the major rutin degradation products. It is expected that knowledge of the nature of such compounds will greatly facilitate the examination of flavonol glycoside degradation and will provide clues about their potential biological activities.

LITERATURE CITED

Afanas'ev, I. B.; Dorozhko, A. I.; Brodskii, A. V.; Kostyuk, V. A.; Potapovitch, A. I. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem. Pharmacol.* **1989**, *38*, 11, 1763−1769.

Aust, S. D.; Miller, D. M.; Samokyszyn, V. M. Iron redox reactions and lipid peroxidation. *Methods Enzymol.* **1990**, *186*, 457−463.

Boudet, A. C.; Cornard, J. P.; Merlin, J. C. Complexation of the quercetin molecule with aluminium. *Polyphenol Commun.* **1998**, *98*, 211−212.

Bravo, L. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.* **1998**, *56*, 11, 317−333.

Brown, J. E.; Khodr, H.; Hider, R. C.; Rice-Evans, C. A. Structural dependence of flavonoid interactions with Cu^{2+} ions: implications for their antioxidant properties. *Biochem. J.* **1998**, *330*, 1173−1178.

Chau, C.-F.; Cheung, P. C.-K.; Wong, Y.-S. Effects of cooking on content of amino acids and antinutrients in three Chinese indigenous legume seeds. *J. Sci. Food Agric.* **1997**, *75*, 447−452.

Cheng, G. W.; Crisosto, C. H. Development of dark skin discoloration on peach and nectarine fruit in response to exogenous contaminations. *J. Am. Soc. Hortic. Sci.* **1994**, *119*, 529−533.

Cheng, G. W.; Crisosto, C. H. Iron-polyphenol complex formation and skin discoloration in peaches and nectarines. *J. Am. Soc. Hortic. Sci.* **1997**, *122*, 95−97.

Cook, N. C.; Samman, S. Flavonoids—Chemistry, metabolism, cardioprotective effects, and dietary sources. *Nutr. Biochem.* **1996**, *7*, 66−76.

Coward, L.; Smith, M.; Kirk, M.; Barnes, S. Chemical modification of isoflavones in soyfoods during cooking and processing. *Am. J. Nutr.* **1998**, *68* (Suppl.), 1486S−1491S.

Crichton, R. R. Iron and oxidative damage. In *Inorganic Biochemistry of Iron Metabolism*; Ellis Horwood: U.K., 1991.

Crozier, A.; Lean, M. E. J.; McDonald, M. S.; Black C. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *J. Agric. Food Chem.* **1997**, *45*, 590−595.

Dangles, O.; Fargeix, G.; Bret, S.; Dufour, C. Spectroscopic investigation of quercetin oxidation. *Polyphenol Commun.* **1998**, *98*, 147−148.

Drysdale, J. W.; Munro, H. N. Small-scale isolation of ferritin for the assay of the incorporation of ^{14}C-labeled amino acids. *Biochem. J.* **1965**, *95*, 851−858.

Gallardo Melgarejo, A.; Gallardo Céspedes, A.; Cano Pavón, J. M. Simultaneous determination of nickel, zinc, and copper by second-derivative spectrophotometry using 1-(2-pyridylazo)-2-naphthol as reagent. *Analyst* **1989**, *114*, 109−111.

Gutteridge, J. M. C.; Quinlan, G. J.; Clark, I.; Halliwell, B. Aluminium salts accelerate peroxidation of membrane lipids stimulated by iron salts. *Biochim. Biophys. Acta* **1985**, *835*, 441−447.

Halliwell, B.; Gutteridge, M. C. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* **1984**, *219*, 1−14.

Halliwell, B.; Gutteridge, M. C. Role of iron in oxygen radical reactions. *Methods Enzymol.* **1984**, *105*, 47−56.

Halliwell, B.; Gutteridge, M. C. Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. *Arch. Biochem. Biophys.* **1986**, *246*, 501−514.

Halliwell, B.; Gutteridge, M. C. Role of free radicals and catalytic metal ions in human disease: An overview. *Methods Enzymol.* **1990**, *186*, 1−88.

Havsteen, B. Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol.* **1983**, *32*, 1141−1148.

Hirota, S.; Shimoda, T.; Takahama, U. Tissue and spatial distribution of flavonol and peroxidase in onion bulbs and stability of flavonol glucosides during boiling of the scales. *J. Agric. Food Chem.* **1998**, *46*, 3497−3502.

Hudson, B. J. F.; Mahgoub, S. E. O. Naturally occuring antioxidants in leaf lipids. *J. Sci. Food Agric.* **1980**, *31*, 646−650.

Jimenez, M.; Garcia-Carmona, F. Oxidation of the flavonol quercetin by polyphenol oxidase. *J. Agric. Food Chem.* **1999**, *47*, 56−60.

Jood, S.; Chauhan, B. M.; Kapoor, A. C. Polyphenols of chickpea and blackgram as affected by domestic processing and cooking methods. *J. Sci. Food Agric.* **1987**, *39*, 145−149.

Lambeth, D. O.; Ericson, G. R.; Yorek, M. A.; Ray, P. D. Implications for in vitro studies of the autoxidation of ferrous ion and the iron-catalyzed autoxidation of dithiothreitol. *Biochim. Biophys. Acta* **1982**, *719*, 501−508.

Larrauri, J. A.; Rupérez, P.; Saura-Calixto, F. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. *J. Agric. Food Chem.* **1997**, *45*, 1390−1393.

Mahungu, S. M.; Diaz-Mercado, S.; Li, J.; Schweck, M.; Singletary, K.; Faller, J. Stability of isoflavones during extrusion processing of corn/soy mixture. *J. Agric. Food Chem.* **1999**, *47*, 279−284.

Makris, D. P.; Rossiter, J. T. Quercetin and rutin (quercetin 3-*O*-rhamnosylglucoside) thermal degradation in aqueous media under alkaline conditions. In *Functional Foods 99—Claims and Evidence*; Buttriss, J., Saltmarsh, M., Eds.; Royal Society of Chemistry Press: London, U.K., 2000; pp 216−238.

Middleton, E., Jr. The flavonoids. *Trends Pharmacol. Sci.* **1984**, 335−338.

Middleton, E., Jr. Biological properties of plant flavonoids: An overview. *Int. J. Pharmacol.* **1996**, *34*, 344−348.

Nieto, S.; Garrido, A.; Sanhueza, J.; Loyola, L. A.; Morales, G.; Leighton, F.; Valenzuela, A. Flavonoids as stabilizers of fish oil: An alternative to synthetic antioxidants. *J. Am. Oil Chem. Soc.* **1993**, *70*, 773−778.

Oteiza, P. I. A mechanism for the stimulatory effect of aluminium on iron-induced lipid peroxidation. *Arch. Biochem. Biophys.* **1994**, *308*, 374−379.

Pierpoint, W. S. Flavonoids in the human diet. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure−Activity Relationships*; Publisher: Location, 1986; pp 125−140.

Price, K. R.; Bacon, J. R.; Rhodes, M. J. C. Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (*Allium cepa*). *J. Agric. Food Chem.* **1997**, *45*, 938−942.

Price, K.; Casuscelli, F.; Colquhoun, I.; Rhodes, M. J. C. Composition and content of flavonol glycosides in broccoli florets (*Brassica olearacea*) and their fate during cooking. *J. Sci. Food Agric.* **1998**, *77*, 468−472.

Ramanathan, L.; Das, N. P. Studies on the control of lipid oxidation in ground fish by some polyphenolic products. *J. Agric. Food Chem.* **1992**, *40*, 17−21.

Rao, P. U.; Deosthale, Y. G. Tannin content of pulses: Varietal differences and effects of germination and cooking. *J. Sci. Agric.* **1982**, *33*, 1013−1016.

Raynal, J.; Moutounet, M.; Souquet, J.-M. Intervention of phenolic compounds in plum technology. 1. Changes during drying. *J. Agric. Food Chem.* **1989**, *37*, 1046−1050.

Raynal, J.; Moutounet, M. Intervention of phenolic compounds in plum technology. 2. Mechanism of anthocyanin degradation. *J. Agric. Food Chem.* **1989**, *37*, 1051−1053.

Singleton, V. L. Oxygen with phenols and related reactions in musts, wines, and model systems: Observations and practical implications. *Am. J. Enol. Vitic.* **1987**, *38*, 69−77.

van Acker, S. A. B. E.; de Groot, M. J.; van de Berg, D.-J.; Tromp, M. N. J. L.; den Kelder, G. D.-O.; van der Vijgh, W. J. F.; Bast, A. A quantum chemical explanation of the antioxidant activity of flavonoids. *Chem. Res. Toxicol.* **1996**, *9*, 1305−1312.

van Sumere, C. F. Phenols and Phenolic Acids. In *Methods in Plant Biochemistry, Vol. 1: Plant Phenolics*; Harborne, J. B., Ed.; Academic Press: New York, 1989.

Wanasundara, U. N.; Shahidi, F. Stabilization of canola oil with flavonoids. *Food Chem.* **1998**, *50*, 393−396.

Wanasundara, U. N.; Shahidi, F. Stabilization of marine oils with flavonoids. *J. Food Lipids* **1994**, *5*, 183−196.

Weigert, P. Metal loads of food of vegetable origin including mushrooms. In *Metals and Their Compounds in the Environment*; VCH: Weinheim, Germany, 1991.

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