Antioxidant Activities of Six Natural Phenolics Against Lipid Oxidation Induced by Fe^{2+} or Ultraviolet Light

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ABSTRACT: The mechanisms and antioxidant activities of six natural phenolics against lipid oxidation induced by ultraviolet (UV) radiation or Fe^{2+} were studied. An oil emulsion was prepared with flax oil and the thiobarbituric acid-reactive substances (TBARS) method was used to determine lipid oxidation. The antioxidant activities of the six phenolics against UV-induced lipid oxidation were as follows: quercetin > rutin = caffeic acid = ferulic acid = sesamol > catechin. The inhibitory concentrations (IC_{50}) showed that the effectiveness of these antioxidants against Fe^{2+}-induced lipid oxidation was in the order quercetin (1.7 μM) > rutin (10.3 μM) > catechin (14.9 μM) > sesamol (18.5 μM) > caffeic acid (19 μM) > ferulic acid (>250 μM), and quercetin was more efficient than butylated hydroxytoluene (BHT) (2.9 μM). Quercetin and rutin had absorption maxima at the UV-A (320–380 nm) region, while the other phenolics tested had absorption maxima near (catechin, 278 nm) or at the UV-B (280–320 nm) region. The stoichiometric ratios of quercetin, rutin, catechin, and caffeic acid to Fe^{2+} were 3:1, 2:1, 1:1, and 1:1, respectively. Although free-radical scavenging capability of antioxidants was the most critical, UV absorption and/or Fe^{2+}-chelation properties of natural phenolics also contributed significantly to the control of lipid oxidation induced by UV or Fe^{2+} in oil systems.

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KEY WORDS: Iron chelation, lipid oxidation, phenolic antioxidants, UV radiation.

There has been great interest in natural phenolic antioxidants in recent years owing to their occurrence in edible plants, their health benefits, and their potential use as natural food preservatives. Natural phenolics such as flavonoids (e.g., quercetin, rutin, and catechin) and hydroxycinnamic acid derivatives (e.g., caffeic acid and ferulic acid) are widely distributed both in edible plants and in foodstuffs derived from plants, and represent substantial constituents of the nonenergetic part of human diet. The estimated daily intake of the natural phenolics by an adult ranges from about 20 mg to 1 g (1), and dietary flavonoids were reported to be recovered in plasma in nonnegligible concentrations, as studied in an animal model (2). Such intake and bioavailability may be important to human health. It has been reported that natural phenolics play an important role in the prevention of coronary heart diseases (1,3,4) and hypercholesterolemia (5), and possess antiallergic, antiinflammatory, antiviral, anticarcinogenic, and antiproliferative properties (6). Food researchers are also interested in natural phenolics because of their potent quality-preserving effects as antioxidants in edible oils (7,8,9) and ground fish (10).

The antioxidant effects of natural phenolics have been studied extensively by using *in vitro* lipid oxidation models. Although the metal-chelating (4,11,12) and ultraviolet (UV)-absorbing characteristics (13) of natural phenolics have been discussed as an important factor for their antioxidant activities, few reports demonstrate the contribution of metal-chelating as well as UV-absorbing characteristics of phenolics to their antioxidant activities. In addition, few studies have been conducted to compare the antioxidant activities of flavonoids with hydroxycinnamic acid derivatives (14).

The objective of this study was to investigate the antioxidant activities of six natural phenolics (quercetin, rutin, catechin, sesamol, ferulic acid, and caffeic acid) against lipid oxidation induced by UV-radiation or Fe^{2+}, and the mechanisms involved.

MATERIALS AND METHODS

Chemicals and equipment. Flax seed oil was obtained from Omega Nutrition Inc. (Vancouver, Canada). Catechin (±), caffeic acid, ferulic acid, sesamol, and butylated hydroxytoluene (BHT) were purchased from Sigma (St. Louis, MO), and quercetin and rutin were from Aldrich (Milwaukee, WI). All the chemicals used were of reagent grades. UV light was generated by a UV lamp (8 W, 300 nm) purchased from Fotodyne Inc. (Hartland, WI), and the Beckman DU-600 (Beckman Instruments Inc., Fullerton, CA) was used for spectral analysis.

UV-induced lipid oxidation (UV-LO). Flax seed oil emulsion was prepared with a potassium phosphate buffer (pH 7.4, 50 mM) as described by Yin *et al.* (15). Phenolics were dissolved in 100 μL ethanol and added to oil emulsion samples to create a final concentration of 50 μM. Ethanol, which was used to dissolve phenolics (0.5%, final), did not influence the assay and was also added in the control. In the absence or presence of phenolics, 20 mL oil emulsion was irradiated by a UV lamp at a distance of 10 cm with constant stirring for 30 min. At each 5-min interval, 0.5 mL of the oil emulsion sample was taken and lipid oxidation was determined by the thiobarbituric acid-reactive substances (TBARS) method

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(16). The results were shown as nmole malonaldehyde (MDA)/mg lipid.

Fe^{2+}-induced lipid oxidation (Fe^{2+}-LO). Fe^{2+}-LO was studied by incubating reaction mixtures containing the following reagents: 0.1 mL $FeCl_2$ (1 mM), 0.5 mL flax-oil emulsion (prepared as above), 0.4 mL potassium phosphate buffer (pH 7.4, 50 mM), and 25 µL phenolic compounds with different concentrations. Solvents (25 µL) used to dissolve phenolics were added in control samples. Quercetin and rutin were dissolved in dimethylsulfoxide (DMSO); sesamol and the rest of the phenolics were dissolved in water and ethanol, respectively. The reaction mixtures were then incubated at 37°C for 30 min with shaking. Lipid oxidation was determined by measuring the TBARS that formed in the oil emulsion mixtures. The results were shown as the percentage of inhibition of the controls.

UV-absorbing and Fe^{2+}-chelating assay. The absorption spectra of these phenolics were recorded between 250 and 700 nm by using a Beckman DU-600. In the Fe^{2+}-chelating assay, quercetin was dissolved in 50% ethanol; caffeic acid and ferulic acid were dissolved in 17% ethanol; rutin was dissolved in 3.2% DMSO; and sesamol and catechin were dissolved in cold (room temperature) and hot (70°C) water, respectively. Solutions with different molar ratios of phenolic to $FeCl_2$ were prepared, and the absorption spectra were recorded against references prepared by the respective solvent solution. Stoichiometry was obtained from the spectra. $FeCl_2$ did not interfere with absorption above 250 nm.

Statistical analysis. Values represent means of duplicated analysis. Data were analyzed by the analysis of variance ($P < 0.05$), and means were separated by the Student-Newman-Keuls' (SNK) multiple range test (17).

RESULTS AND DISCUSSION

UV radiation-induced lipid oxidation and TBARS values increased with UV radiation time (Fig. 1). As suggested by McCord and Fridovich (18) the production of $\cdot O_2^-$ and ·OH by UV radiation should have increased lipid oxidation in oil emulsion. Figure 1 shows that all six phenolics (50 µM, final concentrations) inhibited lipid oxidation in the UV-LO system in the following order: quercetin > rutin = caffeic acid = ferulic acid = sesamol > catechin. Quercetin was the strongest antioxidant among the phenolics tested. It kept the TBARS values of oil emulsions at their initial levels throughout the 30-min testing period. Catechin was the weakest antioxidant tested.

Flavonoids and hydrocinnamic acid derivatives were reported to scavenge free radicals in an activity-structure related manner by donating hydrogen atoms (7,13,14,19,20). Rice-Evans *et al.* (14) studied the free radical-scavenging capability of many phenolic antioxidants and quantified it as the TEAC (Trolox-equivalent antioxidant activities) value. The TEAC values of the phenolics evaluated by Rice-Evans *et al.* (14) were: 4.7, 2.4, 2.4, 1.9, and 1.26 for quercetin, rutin, catechin, ferulic acid, and caffeic acid, respectively.

Catechin had the same TEAC value as rutin (2.4), but the inhibition efficiency of UV-LO by catechin was extremely low (Fig. 1). This can be explained by the low absorption at 300 nm of catechin (Table 1), which was less than 1% of rutin. Similarly, ferulic acid and caffeic acid, which had lower TEAC values than catechin, showed stronger UV-LO inhibition than catechin because the absorption of ferulic acid and caffeic acid at 300 nm were about 150-fold that of catechin (Table 1). Quercetin had great TEAC and UV-absorbing ca-

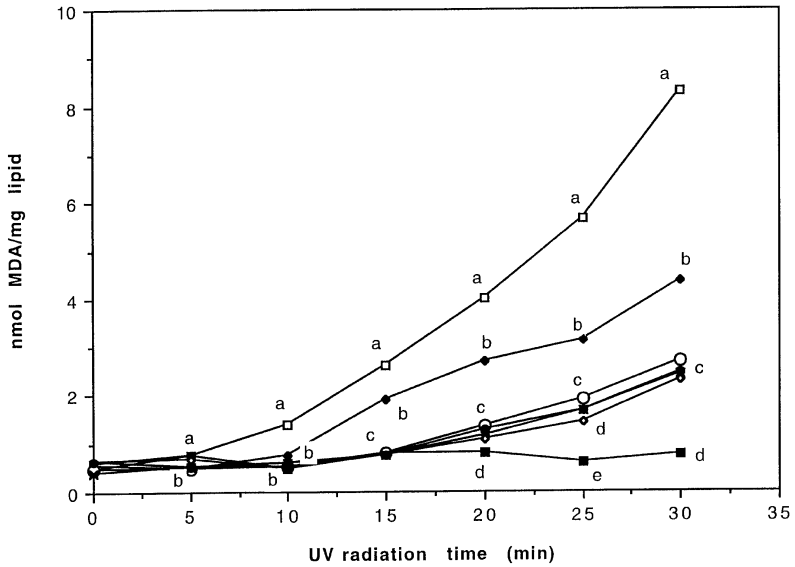

FIG. 1. Inhibition of ultraviolet (UV)-induced lipid oxidation by quercetin, rutin, catechin, caffeic acid, ferulic acid, and sesamol. □, Control; ■, quercetin; ◇, rutin; ◆, catechin; ○, ferulic acid; ●, caffeic acid; ×, sesamol. [a-d]Different letters within the same concentration are significantly different ($P < 0.05$). MDA: malonaldehyde.

pability at 300 nm, so it inhibited UV-LO effectively. Among rutin, ferulic acid, and caffeic acid, rutin has the highest TEAC, caffeic acid the lowest, and ferulic acid is in the middle. However, their UV-absorbing capabilities at 300 nm were the reverse of the TEAC values (Table 1), probably due to the combined effect of free radical-scavenging (shown as TEAC) and UV-absorbing capabilities (Fig. 1). So it could be concluded that UV-absorbing, besides free radical-scavenging capability, also contributed to the inhibition of UV-LO.

Phenolics are found in leaf epidermis, and their presence provides a significant UV-filtering effect and protects cell membranes from lipid oxidation (13,21). Highest levels of ferulic acid and similar hydroxycinnamate derivatives occur in leaves, seeds, and other plant organs of large surface-to-volume ratios (13). Flavonoids in leaves are many times higher than those in other tissues of the same plant, and outer green leaves contain a much higher level of flavonoids than inner leaves. The content of flavonoids, particularly in outer leaves, was reduced greatly when the plants were grown in a greenhouse (22).

Table 1 shows that at pH 5–6, quercetin and rutin had absorption maxima at the UV-A (320–380 nm) region, whereas the other phenolics had absorption maxima near (catechin, 278 nm) or at the UV-B (280–320 nm) region. The presence of phenolics with high TEAC and high UV-absorbing characteristics may reduce microcidal effects of UV treatment, but may also inhibit lipid oxidation of UV-radiated foods. Also, such phenolics, if used in sunscreens, may be useful in preventing the lipid peroxidation of skin exposed to UV radiation. In Japan ferulic acid has already been an active ingredient in many skin lotions and sunscreen creams designed for photoprotection (23).

All the phenolics displayed increasing trends of inhibition when their final concentrations were increased in the Fe^{2+}-LO system (Fig. 2). The IC_{50} (minimal concentration required to obtain 50% inhibition) calculated from Figure 2 is listed in Table 2. Quercetin had the lowest IC_{50} and was the most effective inhibitor of the Fe^{2+}-LO among these natural phenolics tested. Rutin, catechin, caffeic, and sesamol were also effective inhibitors of the Fe^{2+}-LO at 100 μM or greater (Fig. 2). Ferulic acid showed the greatest IC50 and was the least effective inhibitor of the Fe^{2+}-LO.

Afanas'ev *et al.* (11) demonstrated that the Fe^{2+}-rutin complex was stable in a 50 mM phosphate buffer (pH 7.4) with a 1:2 stoichiometry. Based on the UV absorption spectra, Crawford *et al.* (24) suggested that one molecule of quercetin would complex with three molecules of Cu^{2+}, two of which were intramolecular and the third intermolecular. Morel *et al.* (12) reported that catechin and quercetin could remove intracellular iron from Fe^{2+}-loaded rat hepatocytes by chelation. Nardini *et al.* (4) observed caffeic acid:copper complex formation by UV-visible spectra when caffeic acid was incubated with cupric ions. In this study, we found that all the phenolics but sesamol and ferulic acid acted as Fe^{2+}-chelators. The stoichiometric ratios of quercetin, rutin, catechin, and caffeic acid to chelate Fe^{2+} were 3:1, 2:1, 1:1, and 1:1, respectively (Fig. 3). Caffeic acid was a weak chelator. It could not chelate Fe^{2+} tightly and it probably formed a temporary complex with Fe^{2+} (data not shown).

Part of the Fe^{2+}-LO inhibiting effect could be derived from the Fe^{2+}-chelating properties of the phenolics, because IC_{50} was shown to relate to the combined effect of TEAC and Fe^{2+}-chelating capacity. Among the phenolics tested in Fe^{2+}-LO, quercetin had the lowest IC_{50}, probably due to its high TEAC and high Fe^{2+}-chelating capabilities. Catechin, which had the same TEAC as rutin, showed a greater IC_{50} than rutin. The high IC_{50} of catechin may be caused by its low Fe^{2+}-chelating capacity (Table 2). Ferulic acid displayed greater TEAC than caffeic acid but was less efficient in inhibiting the Fe^{2+}-LO. This low antioxidant efficiency of ferulic acid could be caused by the incapability of ferulic acid to chelate Fe^{2+} in the Fe^{2+}-LO system. Therefore, the results indicate that the iron-chelating capability played an important role in preventing lipid oxidation induced by Fe^{2+}. However, Fe^{2+}-chelating was not the most critical factor for phenolics to inhibit Fe^{2+}-LO, because sesamol and BHT, not capable of chelating Fe^{2+}, had low IC_{50}.

TABLE 1
Extinction Coefficient (ε) of Quercetin, Rutin, Catechin, Caffeic Acid, Ferulic Acid, and Sesamol at Their Absorption Maxima and at 300 nm*

	ε of peak 1 ($M^{-1}cm^{-1}$)	ε of peak 2 ($M^{-1}cm^{-1}$)	ε at 300 nm ($M^{-1}cm^{-1}$)
Quercetin	28400 ± 1700 (374 nm)	28300 ± 1700 (256 nm)	10500 ± 300[d]
Rutin	18800 ± 900 (358 nm)	25300 ± 1400 (265 nm)	11700 ± 600[c]
Catechin	4500 ± 340 (278 nm)		110 ± 10[f]
Ferulic acid	17100 ± 400 (310 nm)	16300 ± 1100 (286 nm)	15900 ± 400[b]
Caffeic acid	18300 ± 800 (312 nm)	18200 ± 1000 (287 nm)	172000 ± 700[a]
Sesamol	4700 ± 30 (294 nm)		3950 ± 20[e]

[a-f]Means within a column with no common superscript differ significantly ($P < 0.05$).
*pH: 5–6.

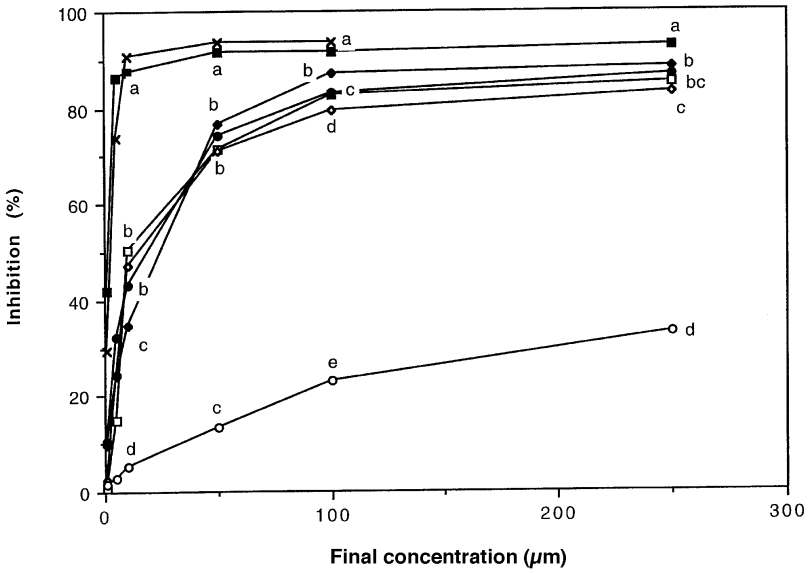

FIG. 2. Inhibition of Fe^{2+}-induced lipid oxidation by quercetin, rutin, catechin, caffeic acid, ferulic acid, and sesamol. ■, Quercetin; □, rutin; ●, catechin; ○, ferulic acid; ◆, caffeic acid; ◇, sesamol; ×, BHT. [a-d]Different letters within the same concentration are significantly different ($P < 0.05$).

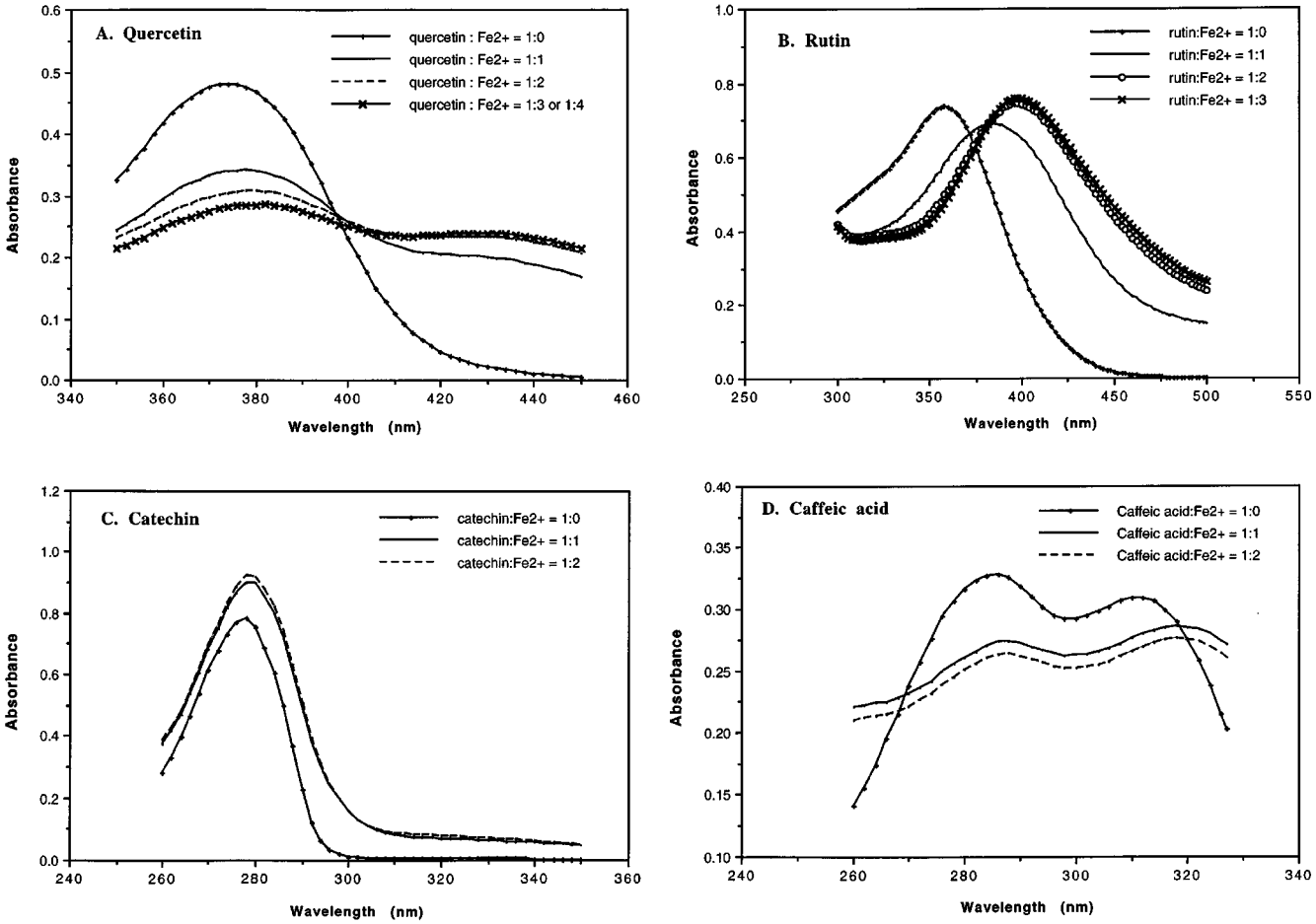

FIG. 3. Absorption spectra of quercetin, rutin, catechin, caffeic acid, and their complexes with ferrous ion.

TABLE 2
IC_{50} and Iron Chelating Capabilities of Quercetin, Rutin, Catechin, Caffeic Acid, Ferulic Acid, and Sesamol

	Quercetin	Rutin	Catechin	Ferulic acid	Caffeic acid	Sesamol	BHT
Iron chelation*	1:3	1:2	1:1	none	weak	none	ND
IC_{50} (μM)	1.7 ± 0.5^{d}	10.3 ± 0.1^{c}	14.9 ± 0.3^{b}	>250	19 ± 2.8^{a}	18.5 ± 0.6^{a}	2.9 ± 0^{d}

$^{a-d}$Means within a row with no common superscript differ significantly ($P < 0.05$).
*Phenolic:Fe^{2+} ratio at pH 5-6. ND, not detremined. BHT: butylated hydroxytoluene.

The results suggest that quercetin is the most potent antioxidant among the six phenolics tested in UV-LO and Fe^{2+}-LO systems because it had high TEAC, UV-absorbing characteristics, and iron-chelating capacity. Rutin ranked second. Although free radical-scavenging capability was the most critical, UV-absorbing and Fe^{2+}-chelating characteristics contributed significantly to the prevention of lipid oxidation in oil systems when lipid oxidation was induced by UV light and/or ferrous iron.

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