# Benzoic and Cinnamic Acid Derivatives as Antioxidants: Structure−Activity Relation

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The antioxidant activity of four derivatives of benzoic acid was systematically compared with the activity of the four homologous derivatives of cinnamic acid. The couples of compounds differed for the kind of aromatic substitution (*p*-hydroxy, *p*-hydroxymethoxy, *p*-hydroxydimethoxy, dihydroxy). The antioxidant activity was measured using (i) a competition kinetic test, to measure the relative capacity to quench peroxyl radical and (ii) the in vitro oxidative modification of human low-density lipoprotein (LDL), initiated by 2,2′-azobis(amidinopropane) dihydrochloride or catalyzed by Cu(II). In both models, cinnamic acids were more efficient than their benzoic counterparts. As for the influence of the aromatic substitution, in the kinetic test the antioxidant activity increased in the sequence *p*-hydroxy < *p*-hydroxymethoxy < dihydroxy < *p*-hydroxydimethoxy. In contrast, in the LDL system, the dihydroxy acids had an antioxidant capacity equal to or higher than that of the *p*-hydroxydimethoxy acids.

**Keywords:** *Benzoic acids; cinnamic acids; antioxidants; low-density lipoprotein*

## INTRODUCTION

Recently much attention has focused on the role and mechanism of several flavonoids as inhibitors of oxidative processes (van Acker et al., 1996; Bors et al., 1990, 1997). Minor attention, however, has been directed to the antioxidant activity of the simple phenolic acids, the derivatives of benzoic and cinnamic acids. These compounds are ubiquitous in plant food (i.e., fruits, vegetables, coffee) (Herrmann, 1989), and therefore a certain quantity of them is consumed in our daily diet. A specific absorption of phenolic acids has been demonstrated both in rat and in man, and specific metabolites have been identified in human and rat urine (Jacobson et al., 1983; Booth et al., 1957; Jung et al., 1983; Wolffram et al., 1995); consequently, they may have an action in vivo. Recently, a role in the antioxidant defense in vivo has been demonstrated for caffeic acid in rat (Nardini et al., 1997).

In the past few years, the antioxidant activity of some of these small monomeric phenols was studied in different model systems. Some phenolic derivatives of cinnamic and benzoic acids were studied for their capacity to reduce ferrylmyoglobin (Laranjinha et al., 1995a) and to inhibit LDL oxidative modification induced by azoinitiators and metal catalysts (Laranjinha et al., 1994; Nardini et al., 1995) or by ferrylmyoglobin and metmyoglobin (Laranjinha et al., 1995b; Castelluccio et al., 1995). Methodical studies on a number of phenolic acids reported their activity in the inhibition of methyl linoleate (Cuvelier et al., 1992) and lard oxidation (Marinova et al., 1992), in strong oxidation conditions, and their relative ability to scavenge the radical cation of 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) in aqueous phase (Rice-Evans et al., 1996).

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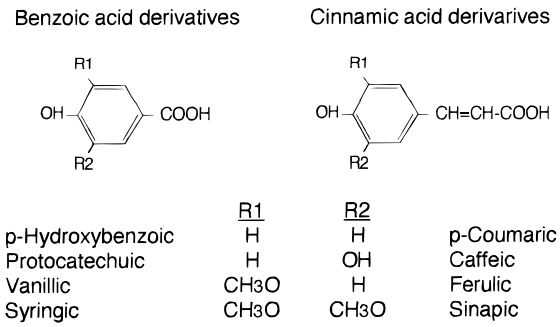


| | R1 | R2 | |
|---|---|---|---|
| p-Hydroxybenzoic | H | H | p-Coumaric |
| Protocatechuic | H | OH | Caffeic |
| Vanillic | $CH_3O$ | H | Ferulic |
| Syringic | $CH_3O$ | $CH_3O$ | Sinapic |

**Figure 1.** Chemical structures of phenolic acids, derivatives of cinnamic and benzoic acids.

All of these studies rather consistently indicated that the antioxidant activity of phenolic acids is reasonably related to their structure, namely, the substitutions on the aromatic ring and the structure of the side chain (Shahidi and Wanasundara, 1992). Still, the data are scanty (in most of the studies only a limited number of compounds was considered) or not comparable because of the different model systems used to determine the antioxidant capacity.

In this study, we systematically compared the capacity of four derivatives of benzoic acid and their four homologous derivatives of cinnamic acid, predominant in foods of plant origin, in their quenching activity toward peroxyl radical, measured by a competition kinetic procedure (Tubaro et al., 1996) and in modulating the in vitro resistance of human low-density lipoprotein (LDL) to oxidative modification initiated by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) or catalyzed by Cu(II). The eight compounds, apart from the carboxyl and propenoic groups in their side chain, differ in pattern of hydroxylation and methoxylation of their aromatic ring (Figure 1).

## METHODS

**Competition Kinetic Test.** Crocin was isolated from saffron (Bors, 1984). The concentration of crocin was calculated

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from the absorption coefficient in methanol ($E = 1.33 \times 10^5$ $M^{-1}$ $cm^{-1}$ at 443 nm). The antioxidant activity was measured using a competition kinetic test (Tubaro et al., 1996), adapted from that described by Bors et al. (1984). In brief, the reaction mixture contained 12 $\mu$M crocin and increasing amounts of phenolic acids (from 6 to 30 $\mu$M) in 10 mM phosphate buffered saline (PBS), pH 7.4. The reaction was started by the addition of 10 mM AAPH to the reaction mixture pre-equilibrated at 40 °C. The bleaching rate of crocin ($V_0$), that is, the rate of its reaction with peroxyl radicals, was calculated by measuring the decrease of its absorption at 443 nm in the first 10 min of reaction. In the presence of various concentrations of phenolic acids, the corresponding bleaching rates were termed $V$. The slopes, calculated by linear regression analysis of the plot [antioxidant]/[crocin] versus $V_0/V$, indicate the relative capacities of the different molecules to interact with ROO•. The antioxidant capacity of the phenolic acids, relative to the activity of trolox, was calculated by dividing the slope of each compound by the slope of trolox.

In another set of experiments, to study the mechanism of reaction of syringic acid with Cu(II), the above-mentioned crocin method was modified, using a more complex initiating system in which the transition metal ion Cu(II) (25 $\mu$M) decomposes cumene hydroperoxide (10 mM final concentration, from a stock ethanol solution) to produce hydroperoxyl and alkoxyl radicals.

The oxidation of syringic and sinapic acids was also monitored by measuring (i) the oxygen consumption, using a Clark electrode, and (ii) their disappearance by an HPLC method with electrochemical detector, during AAPH-initiated or Cu(II)-catalyzed oxidation.

**LDL Preparation and Oxidation.** Human LDL ($d$ = 1.019−1.063 g/mL) was isolated from fasting plasma collected in ethylenediaminetetraacetic acid (EDTA) (1 mg/mL) by sequential ultracentrifugation in salt solutions, according to the method of Havel et al. (1955), using a Beckman T-100 benchtop ultracentrifuge (T-100.3 rotor) (Fullerton, CA). LDL solution was flushed with $N_2$, stored at 4 °C, and used 1 week from the time of preparation. Protein was measured according to the method of Lowry et al. (1951), using bovine serum albumin as a standard.

For oxidation experiments, LDL was dialyzed in the dark for 24 h at 4 °C against three changes of 1 L each of 0.01 M PBS, 0.0027 M KCl, and 0.138 M NaCl, pH 7.4.

Dialyzed LDL (200 $\mu$M protein/mL) was oxidized in PBS at 37 °C for 4 h in the presence of 5 $\mu$M $CuCl_2$ or 4 mM AAPH. The oxidation of LDL was performed in the presence and in the absence of the phenolic acids at 5 $\mu$M final concentration. This concentration was selected on the basis of our previous experience in vitro (Nardini et al., 1995), and it is close to the concentration of caffeic acid found in postprandial plasma of rats fed a caffeic acid-enriched diet (Nardini et al., 1997).

Oxidation was stopped by refrigeration and addition of 3 mM EDTA and 100 $\mu$M butylated hydroxytoluene (BHT).

The oxidative modifications of LDL were evaluated through the measurement of conjugated dienes (CD) formation, lipid hydroperoxide production, tryptophan residue destruction, electrophoretic mobility shift, and α-tocopherol consumption.

The kinetics of CD formation was followed by continuously monitoring the absorbance at 234 nm, using a Beckman DU 70 spectrophotometer (Fullerton, CA) thermostated at 37 °C. Lipid hydroperoxides were measured iodometrically, according to the method of El-Saadani et al. (1989), at different time points. Tryptophan residues were determined by the measurement of intrinsic fluorescence in cetyltrimethylammonium bromide (Reyftmann et al., 1990). LDL electrophoresis was performed at pH 8.6 in 0.05 M barbital buffer on 0.5% agarose gels, using a Beckman Paragon apparatus (Fullerton, CA). The gels were stained with Sudan B black. The increased electrophoretic mobility of LDL (REM) was expressed relative to the mobility of native LDL. α-Tocopherol was measured, after extraction, by reversed phase HPLC (Perkin-Elmer, Norwalk, CT) with fluorescence detection (Bieri et al., 1979).

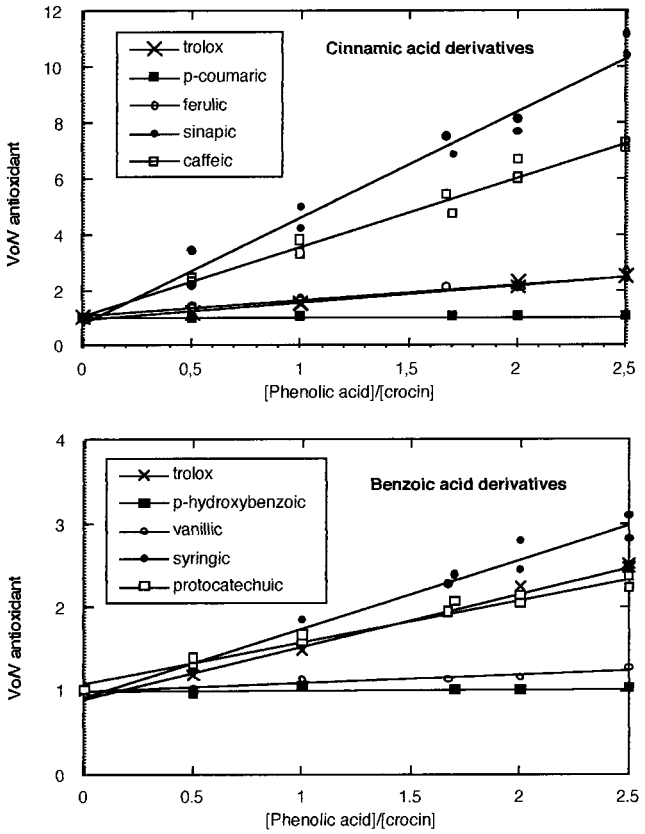


**Figure 2.** Antioxidant capacity of the phenolic acids, relative to the activity of trolox. The slope of linear regression of $V_0/V$ ($y$) on [antioxidant]/[crocin] ($x$) is an index of the relative capacity of the different molecules to interact with ROO•. $V_0$ indicates the rate of reaction of crocin with peroxyl radical, and $V$ is the bleaching rate in the presence of the phenolic acid. The reaction mixture contains 12 $\mu$M crocin, 10 mM AAPH, and different concentrations of phenolic acids (from 6 to 30 $\mu$M). Linear regression equations: trolox, $y = 0.76 + 0.64x$, $r = 0.99$; *p*-coumaric acid, $y = 1.02 + 0.03x$, $r = 0.99$; ferulic acid, $y = 0.84 + 0.8x$, $r = 0.97$; sinapic acid, $y = 0.43 + 4.11x$, $r = 0.99$; caffeic acid, $y = 1.09 + 2.44x$, $r = 0.99$; *p*-hydroxybenzoic acid, $y = 1 + 0.01x$, $r = 0.97$; vanillic acid, $y = 0.95 + 0.15x$, $r = 0.96$; syringic acid, $y = 0.7 + 1.01x$, $r = 0.99$; protocatechuic acid, $y = 1.26 + 0.39x$, $r = 0.97$.

**Table 1. Antioxidant Activity of Benzoic and Cinnamic Acid Derivatives Relative to That of Trolox**

| aromatic substitution | benzoic acid derivatives | cinnamic acid derivatives | $P$ |
|---|---|---|---|
| *p*-hydroxy | 0.02 ± 0.001 | 0.04 ± 0.003 | 0.0069 |
| *p*- hydroxymethoxy | 0.15 ± 0.001 | 0.90 ± 0.060 | 0.0056 |
| *p*- hydroxydimethoxy | 1.30 ± 0.050 | 6.03 ± 0.030 | 0.0002 |
| dihydroxy | 0.79 ± 0.030 | 3.97 ± 0.050 | 0.0003 |
| $P$ | <0.001 | <0.001 | <0.001 |

[a] Results are mean ± standard error of at least three experiments.

**Statistical Analysis.** Data were expressed as mean and standard error. Statistical analysis was performed by one- or two-factor analysis of variance (ANOVA) and Scheffe's method for multiple comparison.

## RESULTS AND DISCUSSION

The reactivity with the peroxyl radical of the eight phenolic acids, measured by the competition kinetic test and expressed as trolox activity, is shown in Figure 2 and Table 1. The phenolic acids were used at concentrations ranging from 6 to 30 $\mu$M (crocin = 12 $\mu$M, AAPH = 10 mM).

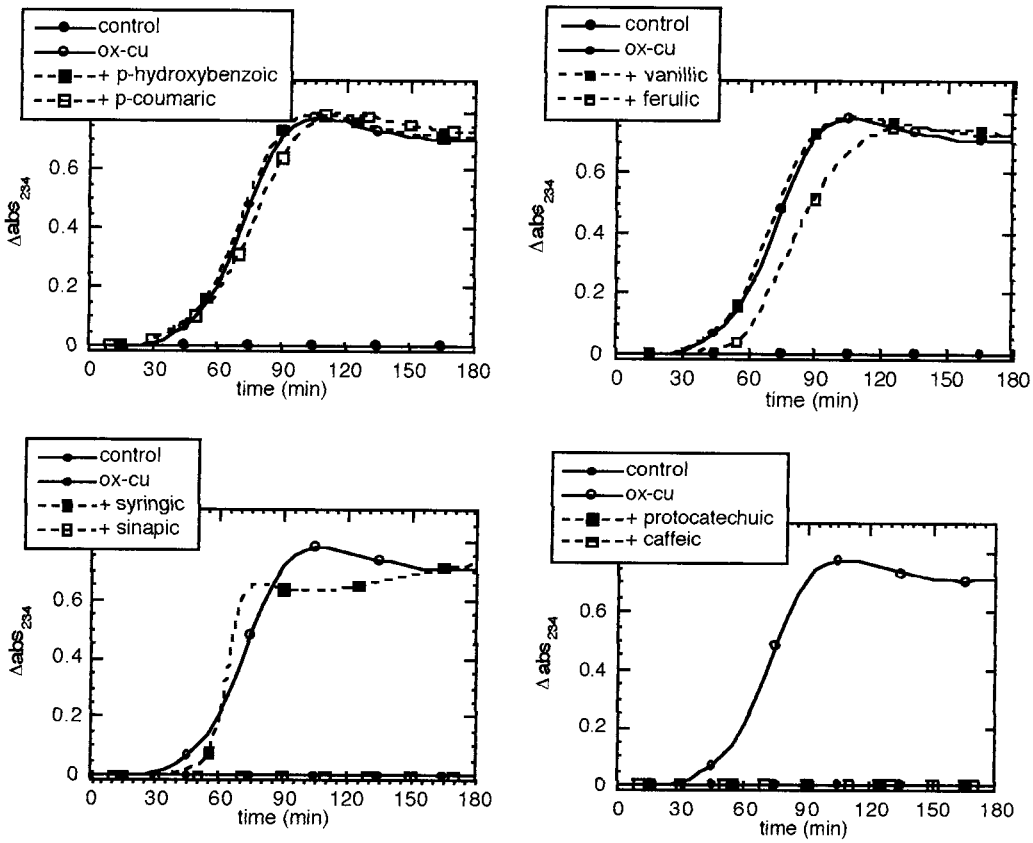


**Figure 3.** Inhibition of Cu(II)-catalyzed LDL oxidative modification by phenolic acids and kinetics of CD formation in the presence of 5 $\mu$M phenolic acids. LDL (50 $\mu$M/mL) was oxidized in PBS at 37 °C with 5 $\mu$M Cu(II), and absorbance was continuously monitored at 234 nm.

The antioxidant capacity relative to trolox indicated that the cinnamic acid derivatives are more efficient than their benzoic counterparts. The double bond of propenoic derivatives probably participates in stabilizing the radical by resonance. In addition, the electron-withdrawing carboxylic group has a negative influence on the H-donating ability of the phenolic ring.

With regard to the aromatic substitution, the order of effectiveness within class (benzoic or cinnamic) was *p*-hydroxydimethoxy > dihydroxy > *p*-hydroxymethoxy > *p*-hydroxy. The increase of methoxy groups or the catechol structure substantially increased the antioxidant activity of the compounds by further stabilizing the phenoxyl radical. An overall view indicates that sinapic acid is 6 times as efficient as trolox, followed by caffeic (4 times), syringic (1.3 times), ferulic (0.9 times), and protocatechuic acid (0.79 times). *p*-Coumaric, *p*-hydroxybenzoic, and vanillic acids were hardly effective.

The effect of the eight phenolic acids on the LDL resistance to oxidative modification was tested using two oxidative systems: the thermal decomposition of the azocompound AAPH and the classical copper-catalyzed oxidation. AAPH produces peroxyl radicals at constant rate through its spontaneous thermal decomposition (Niki, 1990); Cu(II) probably decomposes pre-existing lipid hydroperoxides into the LDL particle to give peroxyl and alkoxyl radicals (Thomas and Jackson, 1991; Esterbauer et al., 1992; Patel et al., 1997). Therefore, the inhibition of the oxidation by AAPH represents the simple scavenging of peroxyl radicals, whereas the inhibition of the Cu(II)-catalyzed oxidation represents the association of both chelation of metal ions and scavenging of different free radical species.

When oxidation was catalyzed by 5 $\mu$M Cu(II), *p*-hydroxybenzoic, *p*-coumaric, ferulic, and vanillic acids failed to reduce CD formation (Figure 3). Syringic acid seemed to have a pro-oxidant effect, and only protocatechuic, sinapic, and caffeic acids were able to strongly inhibit the CD formation. Caffeic and sinapic acids also significantly reduced both lipid hydroperoxide formation and change in the electrophoretic mobility of LDL and preserved tryptophan. *p*-Hydroxy- and *p*-hydroxymethoxybenzoic and cinnamic acids were ineffective (Table 2). Analyzing the pairs of compounds, cinnamic acid derivatives tended to be more efficient than their benzoic acid counterparts.

In the case of AAPH-initiated oxidation, none of the compounds was able to completely inhibit CD formation in the first 3 h, even if all of them showed a tendency to reduce oxidation, with the exception of *p*-hydroxybenzoic acid (Figure 4).

When the other indicators of LDL oxidative modification—lipid hydroperoxides, relative electrophoretic mobility (REM), and tryptophan (Table 3)—were analyzed, the difference between cinnamic and benzoic acid derivatives, observed in the Cu(II)-catalyzed oxidation, decreased and all of the compounds exerted a certain protection against peroxyl radical, with the exception of *p*-hydroxybenzoic acid. Syringic acid in this system behaved similarly to the other phenolic acids, slightly protecting LDL from oxidation. The tendency to an increase of the antioxidant efficiency with increasing number of hydroxy and methoxy substitutions needs to be emphasized, caffeic, protocatechuic, and sinapic acids being the more active.

Using both oxidation systems, LDL α-tocopherol disappeared in the first 2 h, regardless of the presence of the phenolic acids (data not shown). Caffeic acid represented the only exception, showing a sparing effect

**Table 2. Inhibition of LDL Oxidative Modification by Phenolic Acids Belonging to the Benzoic (B) and Cinnamic (C) Series: Copper-Catalyzed Oxidation[a]**

| | LPO (nmol/mg of P) | tryptophan (% residue) | | |
|---|---|---|---|---|
| | 2 h | 2 h | 4 h | REM[b] 2 h |
| control | 76 ± 25 | 100 | 97 ± 1 | 1 |
| oxidized LDL | 405 ± 80 | 65 ± 4 | 36 ± 6 | 2.1 ± 0.1 |
| + *p*-hydroxybenzoic (B) | 320 ± 78 | 69 ± 2 | 38 ± 10 | 1.9 ± 0.1 |
| + *p*-coumaric (C) | 283 ± 88 | 61 ± 3 | 35 ± 3 | 1.8 ± 0.1 |
| + vanillic (B) | 324 ± 83 | 64 ± 4 | 38 ± 7 | 2.0 ± 0.1 |
| + ferulic (C) | 344 ± 85 | 67 ± 7 | 35 ± 4 | 1.8 ± 0.2 |
| + syringic (B) | 735 ± 166* | 45 ± 6** | 34 ± 2 | 3.0 ± 0.2*** |
| + sinapic (C) | 97 ± 54* | 87 ± 7** | 62 ± 19 | 1.4 ± 0.2*** |
| + protocatechuic (B) | 179 ± 87 | 68 ± 1 | 36 ± 4 | 1.8 ± 0.1* |
| + caffeic (C) | 22 ± 19** | 89 ± 7** | 64 ± 15 | 1.3 ± 0.1*** |

[a] LDL (200 $\mu$M protein/mL) was incubated at 37 °C in 10 mM PBS for 4 h with 5 $\mu$M Cu(II) in the presence and absence of phenolic acids (5 $\mu$M). Results represent mean ± SE of three independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ from oxidized LDL by one-factor ANOVA (Scheffe's test). [b] Electrophoretic mobility, relative to native LDL.

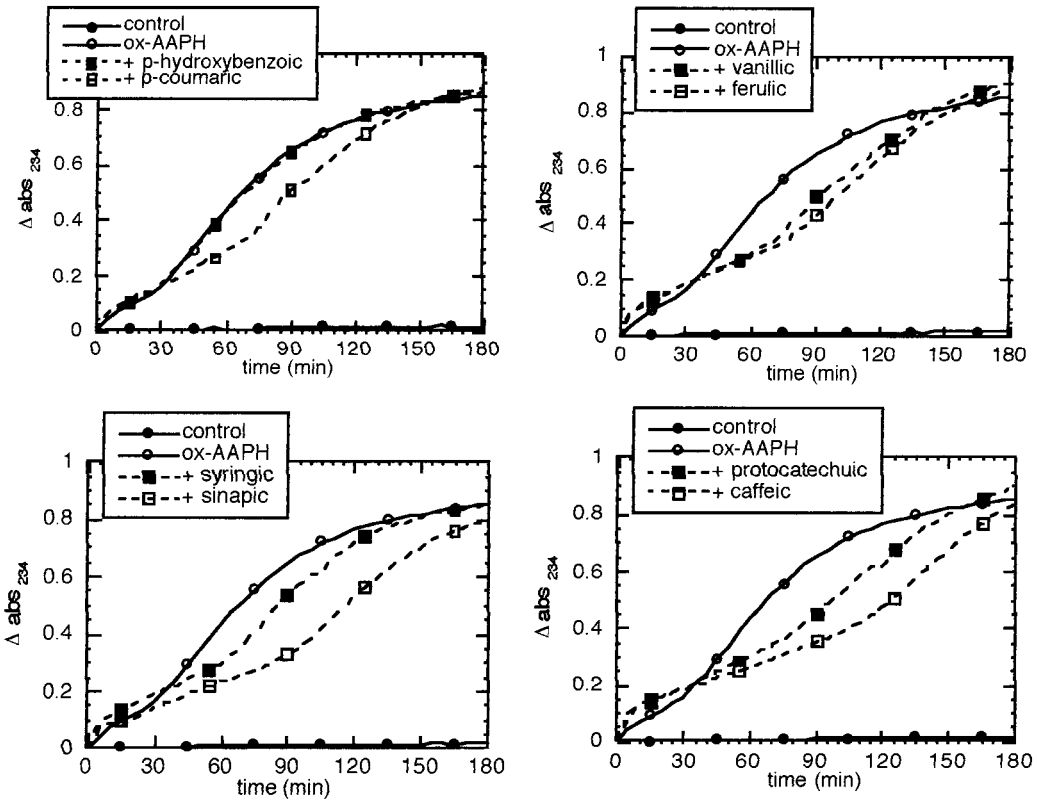


**Figure 4.** Inhibition of AAPH-initiated LDL oxidative modification by phenolic acids and kinetics of CD formation in the presence of 5 $\mu$M phenolic acids. LDL (50 $\mu$M/mL) was oxidized in PBS at 37 °C with 4 mM AAPH, and absorbance was continuously monitored at 234 nm.

on α-tocopherol [60 and 7% of time 0 in Cu(II) and AAPH oxidation, respectively].

The relative contributions of aromatic substitution and of propenoic or carboxylic side chain in the inhibition of LDL modification by the eight compounds in the two different oxidation systems was discriminated by a two-factor ANOVA (Table 4).

In copper-catalyzed oxidation, the effect of the side chain prevailed, mainly due to the difference in the capacity of inhibiting LDL modification observed between the pairs syringic/sinapic and protocatechuic/caffeic. An evident effect of the aromatic substitution was also observed, due to the higher antioxidant efficiency of the two dihydroxy derivatives. This effect is linked to the capacity of caffeic and protocatechuic acids to complex copper ions.

The antioxidant capacity of phenols is generally ascribed to the reaction with oxidants to form resonance-stabilized phenoxyl radicals (Baum and Perun, 1962). This activity is strengthened by the presence of a second hydroxy group, as in caffeic and protocatechuic acids, through the formation of an intramolecular hydrogen bond. Moreover, in the case of copper-catalyzed oxidation, only the presence of the two hydroxy groups in the ortho position (caffeic and protocatechuic acid) produced the formation of the Cu(II)−phenolic acid complex, evidenced by the shift of their spectra (Figure 5), resulting in a chelating effect of copper, as already described for caffeic acid (Nardini et al., 1995).

In the case of AAPH-initiated oxidation, the significance of the contribution of the two factors (aromatic

**Table 3. Inhibition of LDL Oxidative Modification by Phenolic Acids Belonging to the Benzoic (B) and Cinnamic (C) Series: AAPH-Initiated Oxidation**[a]

| | LPO (nmol/mg of P) | tryptophan (% residue) | | |
|---|---|---|---|---|
| | 2 h | 2 h | 4 h | REM[b] 2 h |
| control | 58 ± 7 | 100 ± 1 | 97 ± 1 | 1 |
| oxidized LDL | 600 ± 45 | 49 ± 1 | 26 ± 2 | 2.2 ± 0.2 |
| + *p*-hydroxybenzoic (B) | 481 ± 109 | 53 ± 2 | 33 ± 1 | 2.0 ± 0.4 |
| + *p*-coumaric (C) | 275 ± 28*** | 61 ± 2 | 37 ± 3* | 1.5 ± 0.2** |
| + vanillic (B) | 265 ± 28*** | 58 ± 1 | 37 ± 2* | 1.4 ± 0.1** |
| + ferulic (C) | 293 ± 48*** | 60 ± 4 | 40 ± 3** | 1.5 ± 0.2** |
| + syringic (B) | 230 ± 15*** | 61 ± 4 | 38 ± 3* | 1.4 ± 0.1** |
| + sinapic (C) | 237 ± 6*** | 71 ± 3** | 41 ± 5** | 1.3 ± 0.1*** |
| + protocatechuic (B) | 250 ± 13*** | 72 ± 8** | 37 ± 2* | 1.3 ± 0.1** |
| + caffeic (C) | 199 ± 16*** | 82 ± 9*** | 50 ± 6*** | 1.2 ± 0.1*** |

[a] LDL (200 $\mu$M protein/mL) was incubated at 37 °C in 10 mM PBS for 4 h with 4 mM AAPH in the presence and absence of phenolic acids (5 $\mu$M). Results represent mean ± SE of three independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ from oxidized LDL by one-factor ANOVA (Scheffe's test). [b] Electrophoretic mobility, relative to native LDL.

**Table 4. Relative Effect of Aromatic Substitution and Side Chain on the Modulation of LDL Oxidative Modification by Phenolic Acids**

| | LPO (2 h) | REM (2 h) | tryptophan (2 h) |
|---|---|---|---|
| LDL Oxidation by Cu(II) | | | |
| aromatic substitution | $P < 0.005$ | $P < 0.0005$ | $P < 0.05$ |
| side chain | $P < 0.005$ | $P < 0.0001$ | $P < 0.001$ |
| substitution × side chain | $P < 0.005$ | $P < 0.0001$ | $P < 0.001$ |
| LDL Oxidation by AAPH | | | |
| aromatic substitution | ns[b] | ns | $P < 0.005$ |
| side chain | $P < 0.05$ | ns | $P < 0.05$ |
| substitution × side chain | ns | ns | ns |

[a] Data were analyzed by using two-factor analysis of variance (ANOVA). [b] ns, nonsignificant.

substitution and side chain) to the inhibition of LDL modification strongly decreased; in fact, all of the compounds exerted a certain protection against oxidation, independently of their chemical structure.

The results obtained in LDL oxidation by AAPH are in partial disagreement with the kinetic competition test, because the antioxidant capacity of sinapic acid (*p*-hydroxydimethoxypropenoic acid) was higher than that of caffeic acid, the dihydroxy-substituted compound.

Caffeic acid is reported to act synergistically with α-tocopherol, both delaying α-tocopherol oxidation and recycling α-tocopherol from the α-tocopheroxyl radical (Laranjinha et al., 1995b; Nardini et al., 1995). In the LDL experiments, using both oxidation systems, only caffeic acid showed a sparing effect on α-tocopherol. In addition, in an in vivo study on rats, we found that dietary supplementation with caffeic acid produced a significant and dose-dependent increase in α-tocopherol plasma and lipoprotein concentration (Nardini et al., 1997). For these reasons, we hypothesize that the different behaviors of caffeic and sinapic acids in the LDL and in the crocin system could be linked to the capacity of caffeic acid to maintain the LDL−tocopherol in the reduced form. This can be the mechanism also in the hypothesis that α-tocopherol (in vitro and at low radical flux) acts as promoter of the transfer of radicals into the lipoprotein particle through its radical form (Neuzil et al., 1997).

To clarify the pro-oxidant effect of syringic acid in the LDL oxidation catalyzed by Cu(II) (see Figure 2 and Table 2), the activity of syringic acid has been tested in the cumene hydroperoxide/Cu(II) system, compared with sinapic acid. In this system, copper decomposes

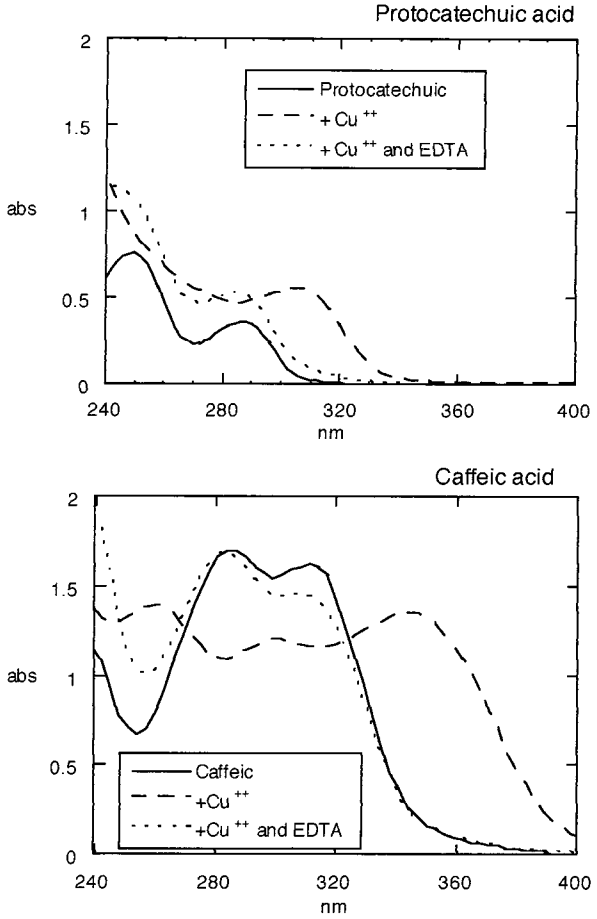


**Figure 5.** Chelating effect of caffeic and protocatechuic acids. Spectra of caffeic and protocatechuic acids (100 $\mu$M) alone were recorded in 10 mM PBS, pH 7.4. The two phenolic acids (100 $\mu$M) were incubated with $CuCl_2$ (100 $\mu$M) in 10 mM PBS, pH 7.4; spectra were registered immediately after Cu(II) addition (zero time) and after 1 h of incubation. For the spectra in the presence of Cu(II) and EDTA, EDTA (1 mg/mL final concentration) was added 2−3 min after Cu(II) addition and spectra were recorded.

hydroperoxides, yielding peroxyl and alkoxyl radicals. Although the antioxidant activity of sinapic acid was proportional to its concentration, syringic acid at low concentrations acted as an antioxidant, switching to pro-oxidant when present at concentrations higher than those of copper (Figure 6). In addition, a direct interaction of syringic acid with Cu(II) was not observed: in

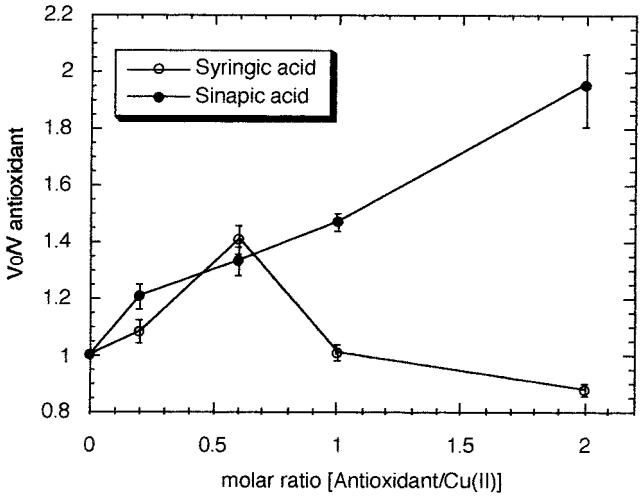


**Figure 6.** Concentration-dependent effect of syringic and sinapic acids on the bleaching of crocin initiated by 10 mM cumene hydroperoxide and 25 $\mu$M Cu(II). The phenolic acids were added in increasing amount from 5 to 50 $\mu$M. $V_0$ indicates the bleaching rate of crocin in absence of phenolic acid, and $V$ is the bleaching rate in the presence of the compounds. Each point represents the mean of at least three measurements.

fact, differently from sinapic acid, syringic acid was not subjected to oxidation by copper in the absence of hydroperoxides, as evidenced by (i) the lack of oxygen consumption (data not shown) and (ii) no change in syringic acid concentration (measured by HPLC-ECD) after 2 h of incubation with copper (data not shown).

These results suggest that the pro-oxidant effect of syringic acid in the presence of Cu(II) and hydroperoxides could be linked to a different stability of the phenoxyl radicals derived from its oxidation. It can be assumed that, at high concentrations, the phenoxyl radical of syringic acid leads to propagation reactions, as reported for other phenolic compounds (Gordon, 1990). The lack of pro-oxidant effect of syringic acid in the case of AAPH oxidation could be linked to the different rate of peroxyl radical generation by the thermodecomposition of the azoinitiator and/or to different relative concentration of radicals.

## CONCLUSIONS

Our study, using LDL as a substrate and with both oxidation systems, confirmed that the antioxidant efficiency of monophenols is strongly enhanced by the introduction of a second hydroxy group and is increased by one or two methoxy substitutions in position ortho to the −OH group.

In the kinetic test, caffeic acid (dihydroxycinnamic) had an antioxidant capacity lower than that of sinapic acid (*p*-hydroxydimethoxycinnamic). The discrepancy with the LDL model could be linked to the capacity of caffeic acid to act synergistically with α-tocopherol, regenerating the α-tocopheroxyl radical.

In all systems, the greatest antioxidant capacity of hydroxycinnamic acid derivatives is linked to the presence of the propenoic side chain, instead of the carboxylic group of benzoic acid derivatives; the conjugated double bond in the side chain could have a stabilizing effect by resonance on the phenoxyl radical, thus enhancing the antioxidant activity of the aromatic ring.

Remarkably, hydroxycinnamic acid derivatives are the most widely represented phenolic acids in food vegetables, strengthening their potential role as nutritional antioxidants.

## ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; BHT, butylated hydroxytoluene; CD, conjugated dienes; EDTA, ethylenediaminetetraacetic acid; HPLC/ECD, high-performance liquid chromatography/electrochemical detector; LDL, low-density lipoprotein; PBS, phosphate-buffered saline; REM, relative electrophoretic mobility; ROO•, peroxyl radicals.

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