Antioxidant Activity and Partitioning of Phenolic Acids in Bulk and Emulsified Methyl Linoleate

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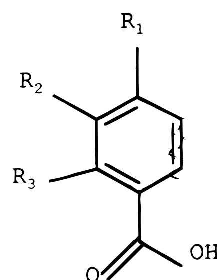
To evaluate the effect of colloidal parameters on the activity of natural antioxidants, the effect of selected phenolic acids on both bulk and emulsified methyl linoleate oxidation (in the dark at 40 °C) was examined. Oxidation was monitored by determining the formation of hydroperoxides; their isomer distribution and ketodiene (oxodiene) products were monitored by using high-performance liquid chromatography. This study showed the system- and concentration-dependent antioxidant activity of phenolic acids. The scavenging of α,α-diphenyl-β-picrazylhydrazyl radicals reflected the antioxidant activity in a bulk oil system but not in an emulsion. Specific interactions of the antioxidant with other compounds, for example, the emulsifier, and intramolecular hydrogen bonds may play an important role in reducing the antioxidant activity. Furthermore, these interactions of antioxidants with emulsifier have a strong influence on the partitioning of antioxidants. Thus, the proportion of the antioxidant solubilized in the lipid phase and particularly in the interface did not necessarily reflect the efficiency of the antioxidant.

Keywords: *Antioxidant; emulsion; methyl linoleate; partition; phenolic acid*

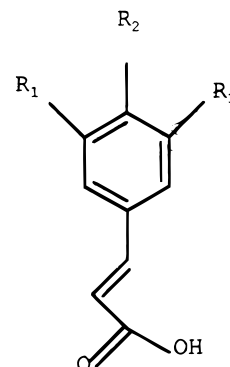
INTRODUCTION

Phenolic acids are shown to have antioxidant activity in different lipid systems. The potency to inhibit lipid oxidation was tested in bulk lipids such as bleached and deodorized olive oil (Satué et al., 1995), purified sunflower oil (Yanishlieva and Marinova, 1995), and lard (Dziedric and Hudson, 1984; Chen and Ho, 1997). Furthermore, the activity of phenolic acids was tested in dispersed lipid systems such as oil-in-water emulsions (Chen and Ho, 1997), low-density lipoprotein (Laranjinha et al., 1994, 1995; Frankel et al., 1995; Nardini et al., 1995; Teissedre et al., 1996; Meyer et al., 1998), and liposome-containing systems (Osawa et al., 1987; Scott et al., 1993). The antioxidative activity of phenolic acids is generally governed by their chemical structures (Figure 1); the activity improves as the number of hydroxyl (OH) (Dziedric and Hudson, 1984; Nardini et al., 1995; Teissedre et al., 1996; Chen and Ho, 1997) and methoxy groups increases (Dziedric and Hudson, 1984), the number of OH groups being more important (Dziedric and Hudson, 1984). Thus, caffeic acid is more active than ferulic acid, which in turn is more active than coumaric acids. However, only few results have been reported on the antioxidant activity of sinapic acid (Thumann and Herrmann, 1980), although sinapic acid is the major phenolic acid in certain plants, such as Brassiceae seeds (Pokorny, 1995).

Free radical scavenging is a generally accepted mechanism for phenolic antioxidants to inhibit lipid oxidation (Bors and Saran, 1987). The free radical scavenging activity is dependent on the number and arrangement of the OH groups and on the extent of structural



2,3-Dihydroxy benzoic acid: R_1=H, R_2=R_3=OH
3-Hydroxy benzoic acid: R_1=R_3=H, R_2=OH
Vanillic acid: R_1=OH, R_2=OCH_3, R_3=H



Caffeic acid: R_1=R_2=OH, R_3=H
Ferulic acid: R_1=H, R_2=OH, R_3=OCH_3
Sinapic acid R_1=R_3=OCH_3, R_2=OH

Figure 1. Structures of phenolic acids used in the study.

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conjugation, as well as on the presence of electron-donating and electron-withdrawing substituents in the ring (Bors et al., 1990). Measurement of the scavenging effect on stable free radicals can be used as a method to evaluate in a relatively short time the antioxidant activities of standard compounds (Chen and Ho, 1997) and of natural extracts (Costantino et al., 1992; Yen and Chen, 1995; Yamaguchi et al., 1998).

The higher antioxidant activity of hydroxycinnamic acids compared to that of the corresponding hydroxybenzoic acids (Satué et al., 1995) is due to the greater delocalization of the unpaired electron of the antioxidant radical caused by the conjugated side chain (Graf, 1992). Furthermore, the electron-withdrawing properties of the carboxyl group attached to the aromatic ring have a negative influence on the hydrogen-donating abilities of hydroxybenzoic acids. Furthermore, the activity of certain phenolic acids, for example, caffeic acid, is due to the metal chelating property (Nardini et al., 1995).

Several authors have shown that the activity of antioxidants is highly dependent on the test system used to determine lipid oxidation (Frankel et al., 1994, 1996; Huang et al., 1994, 1996; Frankel and Huang, 1996; Hopia et al., 1996b; Chen and Ho, 1997). Most of the earlier studies have proved the "Polar Paradox" phenomenon (Porter, 1993) of antioxidants; hydrophilic antioxidants are more effective than lipophilic antioxidants in bulk oil, whereas lipophilic antioxidants show greater activity in emulsions. This has been reported with α-tocopherol and ascorbic palmitate and their respective water soluble analogues Trolox and ascorbic acid (Frankel et al., 1994) as well as with phenolic diterpenes in rosemary extract (Frankel et al., 1996; Hopia et al., 1996b). However, Chen and Ho (1997) showed opposite results with caffeic and ferulic acids and their corresponding phenethyl esters. Only a few phenolic acids, among them ferulic and caffeic acids, have been studied for their solubilization in emulsions so far (Schwarz et al., 1996). However, their chemical structure and chromatographic properties indicate that they are more hydrophilic than tocopherols.

Radical scavenging is an important parameter that affects the antioxidant activity. However, in food lipids other parameters, such as partition of active compounds into different phases, may affect the activity (Porter, 1993). The aim of this study was to evaluate the antioxidant activity of caffeic, sinapic, ferulic, vanillic, and 3-hydroxy- and 2,3-dihydroxybenzoic acids on the basis of their radical scavenging activity and ability to inhibit lipid oxidation in hydrophobic (bulk lipids) and hydrophilic lipid (emulsions) systems. To study the importance of partition behavior of antioxidants, the solubilization capacity of the lipid phase in emulsions was analyzed.

MATERIALS AND METHODS

Methyl Linoleate, Standards, and Reagents. Methyl linoleate (purity = 99%) was obtained from Nu Chek Prep Inc. (Elysian, MI) and Sigma Chemical Co. (St. Louis, MO). The initial hydroperoxide content as measured by HPLC was 5−6 mmol/kg of methyl linoleate. Phenolic acids were supplied by Extrasynthese (Genay Cedex, France). α-Tocopherol were supplied by Merck (Darmstadt, Germany). Organic solvents of HPLC grade were purchased from Rathburn Chemicals (Walkerburn, U.K.).

Determination of the Radical Scavenging Activity (RSA). α,α-Diphenyl-β-picrazylhydrazyl radical (DPPH) was used as a stable radical. As the odd electron of DPPH becomes paired off, the absorption vanishes and the resulting decolorization is stoichiometric with respect to the number of electrons taken up (Blois, 1958). RSA was tested by measuring the decrease of the absorption at 516 nm of DPPH solution after addition of the antioxidant solution. In a cuvette, 2960 μL of 0.1 mM ethanolic DPPH solution was mixed with 40 μL of a 1 mM antioxidant solution, and the absorption was monitored at the start and after 10 min. The resulting difference is expressed as the number of radicals scavenged.

Preparation of Bulk Oil and Emulsion Samples. Methyl linoleate was found to be free of tocopherol by HPLC using a fluorescence detector (Haila and Heinonen, 1994) and was used without further purification. The 10% oil-in-water emulsions were prepared using 0.625 g of methyl linoleate and 5.56 mL of distilled Milli-Q water (Millipore Corp., Bedford, MA) with 0.0625 g of Tween 20 (polyoxylene sorbitan monolaurate; Sigma Chemical Co.). Emulsions were prepared by sonicating the solution for 3 min in an ice bath with a U 50 Control Ikasonic sonicator (Janke & Kunkel GmbH & Co. KG, Staufen, Germany).

Oxidation Experiments. Bulk oil samples (0.5 g each) were autoxidized in the dark at 40 °C in clear open glass vials (volume = 11 mL). Emulsion samples (6.25 g each) with magnetic stirring in glass vials (volume = 20 mL) with a cap were autoxidized in similar conditions. Antioxidants were added at levels of 50 or 1000 μM in oil. To ensure mixing of antioxidants in methyl linoleate, antioxidants were dissolved in ethanol, which was then evaporated under nitrogen. Samples for oxidation analyses were taken periodically.

Analyses of Oxidation Products. Hydroperoxide content, the distribution of *cis,trans* and *trans,trans* isomers, and the formation of ketodiene compounds were monitored by HPLC (Hopia et al., 1996a) using a Supercosil LC-SI 57930 column (250 × 2.1 mm, particle size = 5 μm; Supelco, Bellefonte, PA) with the exception that hexane was replaced by heptane. The HPLC system was made up of an autosampler (Waters 700 Satellite WISP, Millipore Corp.), a pump (Waters 501), a UV−vis photodiode array detector (Waters 996 PDA) set at 234 nm (hydroperoxides) and 268 nm (ketodiene compounds), and a computer work station with Millenium 2010 software (Waters, Milford, MA). An HPLC chromatogram of methyl linoleate oxidation products in a sample containing 50 μM ferulic acid is shown in Figure 2.

Partitioning of Phenolic Acids. Emulsion samples for partitioning studies were prepared as for oxidation experiments, but commercial corn oil was used instead of methyl linoleate. To determine the proportion of antioxidant in the aqueous phase an ultrafiltration method was used as described earlier by Schwarz et al. (1996) using tubes with a molecular cutoff of 3000 Da (Millipore, Eschborn, Germany). The antioxidant concentration in the aqueous phase was determined by HPLC according to the method of Stöckmann and Schwarz (1999). The stationary phase was a chemically modified (LiChrosorb 100, CN, 5 μm; 250 × 4 mm i.d., Knauer, Berlin, Germany). The eluent was composed of hexane, 2-propanol, ethyl acetate, and acetic acid (25%) in the ratio 55:35:5:5 with a flow rate of 0.7 mL/min. Solutes were detected at their absorbance maximum using a UV detector (UVIS-206, Latek, Eppelheim, Germany). All samples for HPLC were diluted by a mixture of 2-propanol and hexane (70:30).

Statistical Analyses. Each oxidation and partition analysis was done in duplicate. The results are expressed as an average of duplicate analyses, and each experiment was done in duplicate. DPPH radical scavenging tests were run in triplicate and averaged. The one-way analysis of variance was done at the endpoint of oxidation by using Minitab Statistical software from Addison-Wesley (Reading, MA). Correlation coefficients (r) between DPPH RSA and inhibition of hydroperoxide formation were calculated to estimate the effect of different parameters on the results of oxidation experiments.

RESULTS AND DISCUSSION

DPPH Radical Scavenging Activity. Table 1 lists the electron-donating activity for the hydroxycinnamic

Table 1. Radical Scavenging Ability of Antioxidants (13.3 μM) in 0.3 mM Ethanolic DPPH Solution

antioxidant	RSA[a] (%) (±SD)	antioxidant	RSA[a] (%) (±SD)
2,3-dihydroxybenzoic acid	39.0 (±1.46)[a]	α-tocopherol	28.4 (±0.46)[d]
sinapic acid	33.2 (±0.58)[b]	ferulic acid	15.7 (±0.62)[e]
caffeic acid	30.5 (±0.31)[c]	vanillic acid	2.4 (±0.15)[f]
Trolox	28.4 (±1.24)[d]	3-hydroxybenzoic acid	1.6 (±0.09)[f]

[a] RSA (%) = ($Abs_{516\ nm}$ [start] − $Abs_{516\ nm}$ [10 min]) × 100/$Abs_{516\ nm}$ [start]. Values with the same superscript are not significantly different at $p < 0.05$.

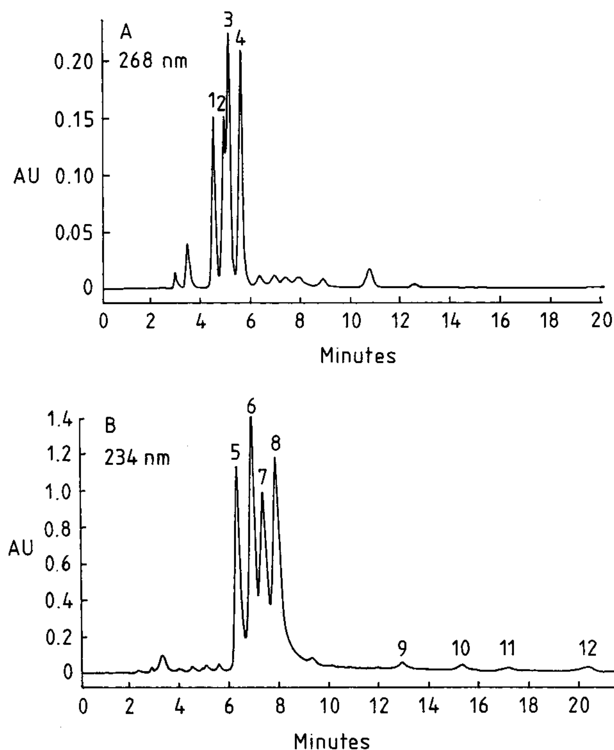

Figure 2. HPLC chromatogram of oxidation products of bulk methyl linoleate in sample containing 50 μM ferulic acid. Chromatographic conditions: silica column (5 μm, 25 cm × 2.1 cm); heptane/diethyl ether (88:12, v/v); flow rate = 0.3 mL/min. Autoxidation products were monitored at 234 nm (hydroperoxides) and 268 nm (ketodiene compounds). Elution order of oxidation products: (a) peaks 1−4, ketodienes; (b) peaks 5−8, hydroperoxides, 13-*cis,trans,* 13-*trans,trans,* 9-*cis,-trans*, and 9-*trans,trans*; peaks 9−12, hydroxy compounds.

acids (caffeic, ferulic, and sinapic acid), hydroxybenzoic acids (2,3-dihydroxybenzoic, 3-hydroxybenzoic, and vanillic acid), Trolox, and α-tocopherol. The results concerning Trolox and α-tocopherol indicate that solubility of antioxidant does not affect DPPH RSA. As expected, the electron-donating ability within the group of hydroxycinnamic acids was higher for two OH groups (caffeic acid) attached to the aromatic ring system than for one hydroxyl or methoxy group (ferulic acid). In addition to one hydroxyl group, a second methoxy group (sinapic acid) caused an increase in hydrogen donation, leading to a higher activity than caffeic acid. Within the group of benzoic acids, a single hydroxyl group (3-hydroxybenzoic acid) attached to the aromatic ring showed no RSA, but the second OH group (2,3-dihydroxybenzoic acid) had a marked effect, which resulted in an RSA higher than that of caffeic acid. Vanillic acid possesses a methoxy group in addition to one OH group; however, this does not increase the RSA. These results were in good agreement with the DPPH scavenging activity measured by Chen and Ho (1997).

Formation of Hydroperoxides Bulk Oil. The formation of hydroperoxides in oxidizing bulk lipid was more effectively inhibited by hydroxycinnamic acids (caffeic, ferulic, and sinapic acid) than by hydroxybenzoic acids (Figure 3B). The order of activity of phenolic acids (1000 μM) for the inhibition of hydroperoxide formation after 7 days of oxidation was caffeic acid ≈ sinapic acid > α-tocopherol > ferulic acid > 2,3-dihydroxybenzoic acid > 3-hydroxybenzoic acid and vanillic acid. Caffeic acid, sinapic acid, and α-tocopherol inhibited the hydroperoxide formation in bulk methyl linoleate dose dependently, at concentrations from 50 to 1000 μM. The concentration-dependent effect of the least active hydroxycinnamic acid derivative, ferulic acid, was similar to that of the most active hydroxybenzoic acid, 2,3-dihydroxybenzoic acid (Figure 3). These data are in agreement with results reported by Satué et al. (1995), who compared the activities of ferulic acid and vanillic acid in olive oil oxidation.

Table 2. Correlation Coefficients (r) between Inhibition (Percent) of Hydroperoxide Formation and DPPH RSA

oxidation expt	r	oxidation expt	r
bulk oil, 50 μM	0.65	emulsion, 50 μM	0.32
bulk oil, 1000 μM	0.88	emulsion, 1000 μM	0.24

[a] Inhibition (%) = 100 × ($hydroperoxides_{control}$ − $hydroperoxides_{sample}$)/$hydroperoxides_{control}$. Values with the same superscript are not significantly different at $p < 0.05$.

The DPPH RSA correlated well with the relative antioxidant activity in the bulk oil system (Table 2). The orders of activity were in both experiments similar except for 2,3-dihydroxybenzoic acid, which showed markedly lower activity in bulk oil than in the RSA test (Table 1; Figure 3). The formation of intramolecular hydrogen bonding between the carboxyl group and the 2-hydroxyl group in 2,3-dihydroxybenzoic acid may have given rise to a discrepancy between the low activity in the bulk oil system and the high activity in the RSA test carried out in ethanol. According to Joesten and Schaad (1974), 2-hydroxybenzoic acid forms an intramolecular hydrogen bond between the carbonyl oxygen and the OH-group H atom, which would explain a lower activity in inhibiting hydroperoxide formation than expected. These intramolecular hydrogen bondings are likely more prevalent in water-containing systems than in bulk oil systems; it can be assumed that hydrogen bonds between water or ethanol and the carbonyl group are competitively formed. The correlation coefficients between the results of DPPH RSA and inhibition (percent) of hydroperoxide formation at antioxidant concentrations of 50 and 1000 μM were 0.65 and 0.88, respectively (Table 2), corresponding to coefficients of determination (r^2) of 0.42 and 0.77. The result indicates that in a bulk lipid system the antioxidant activity of phenolic acids is 42 and 77% attributable to their RSA. However, excluding the values of 2,3-dihydroxybenzoic acid would raise the coefficient to 0.95 (50 μM) and 0.92 (1000 μM).

Hydroxylation increased the activity of 2,3-dihydroxy- compared to 3-hydroxybenzoic acid. Hydroxylation also increases the antioxidant activity of cinnamic acids

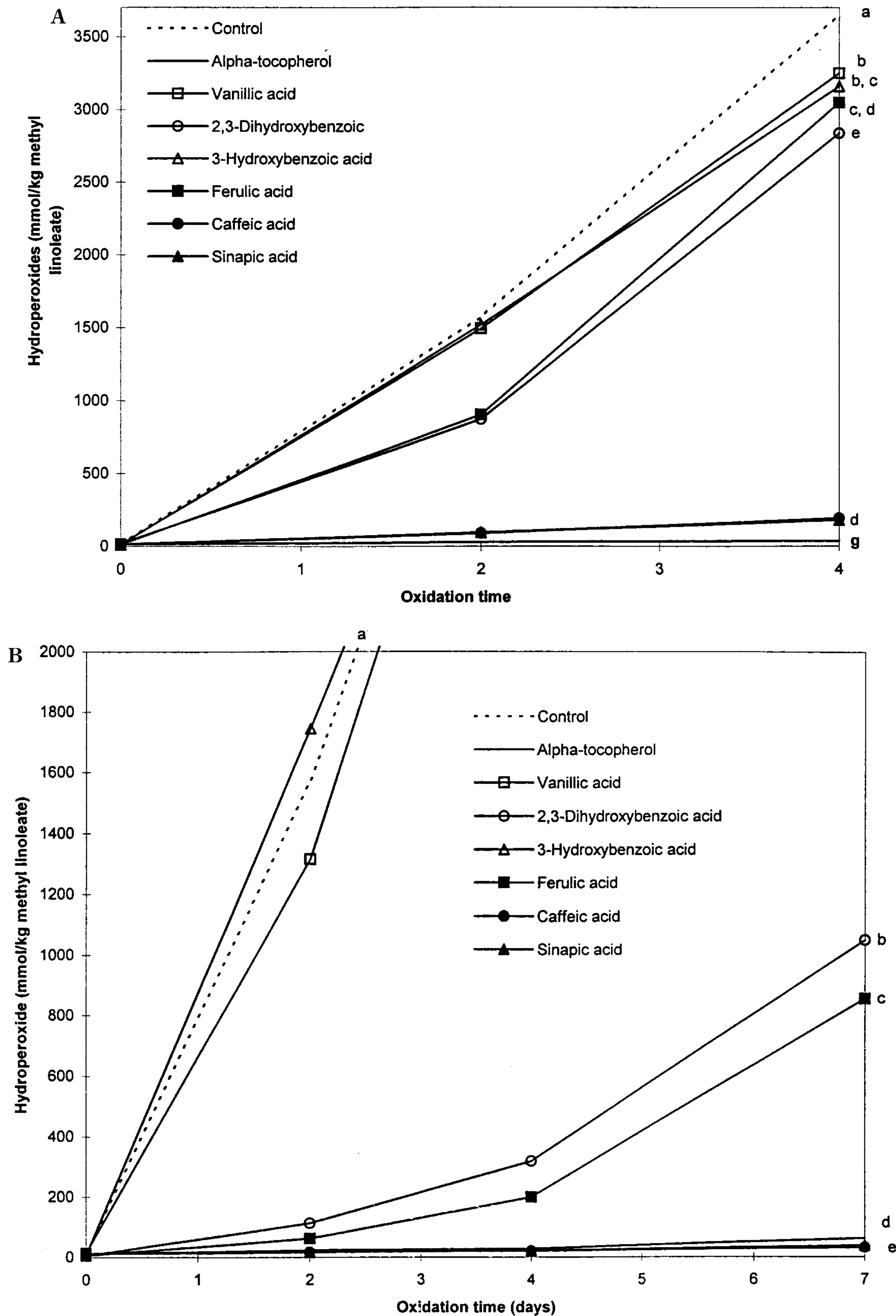

Figure 3. Effect of phenolic acid or α-tocopherol on the oxidation of bulk methyl linoleate: (A) 50 μM; (B) 1000 μM. Values marked by the same letter or no letter are not significantly different at $p < 0.05$ at the endpoint of oxidation.

according to Satué et al. (1995). Methoxylation enhanced the antioxidative activity of hydroxycinnamic acids; sinapic acid with two methoxy groups was more effective than ferulic acid with one methoxy group. Two methoxy groups in ortho position to the OH group in sinapic acid raised the activity to that of caffeic acid, which contains two OH groups. However, methoxylation did not increase the activity of hydroxybenzoic acids as the activity of vanillic acid was in the same range compared to 3-hydroxybenzoic acid. The relationship between the degree of hydroxylation or methoxylation and antioxidant activity is in good agreement with results pub-

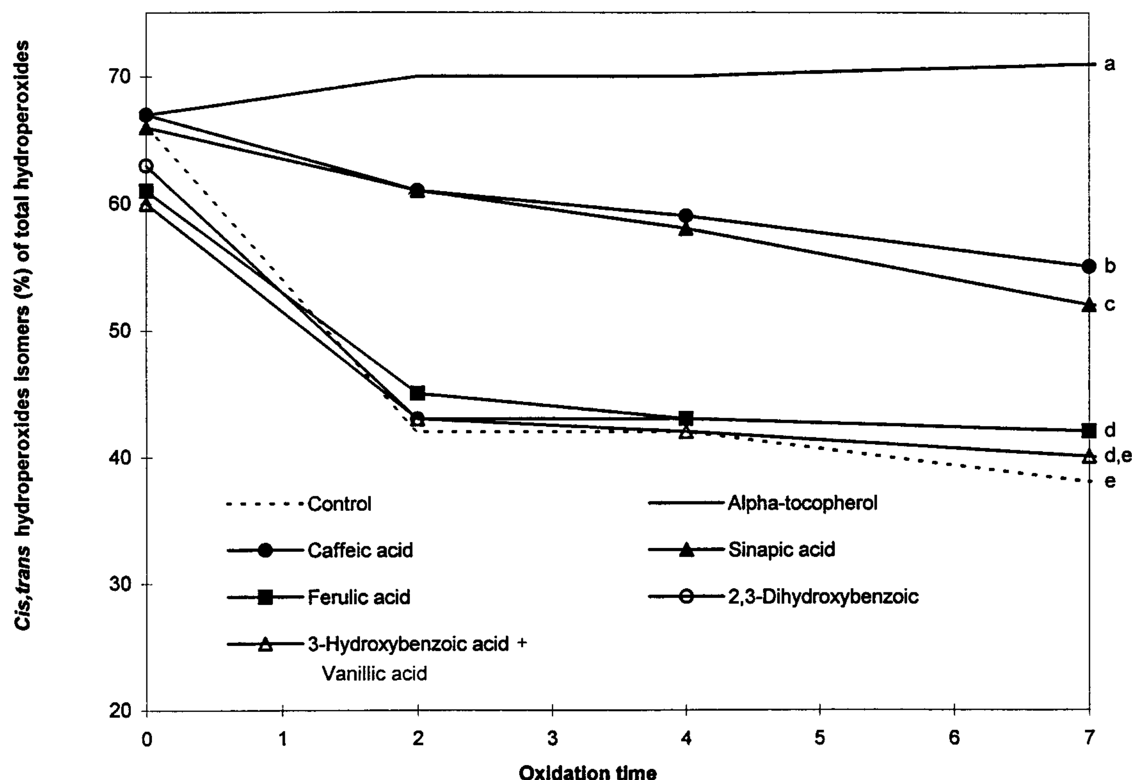

Figure 4. Effect of phenolic acid or α-tocopherol (1000 μM) on the ratio of *cis,trans*-hydroperoxide (percent) isomers to total hydroperoxides in bulk methyl linoleate oxidation. Values marked by the same letter or no letter are not significantly different at $p < 0.05$ at the endpoint of oxidation.

lished by Dziedzic and Hudson (1980), Yanishlieva and Marinova (1995), Chen and Ho (1997), and Satué et al. (1995).

Formation of Ketodiene Compounds. The effect of antioxidants on hydroperoxide decomposition was followed by monitoring the levels of ketodiene compounds during methyl linoleate oxidation. No enhanced effect of hydroperoxide formation was found by the tested antioxidants, and the formation of ketodiene compounds followed the order of hydroperoxide formation (data not given).

Hydroperoxide Isomer Distribution. In a homogeneous system, caffeic, sinapic, ferulic, and 2,3-dihydroxybenzoic acids affected the pattern of the primary autoxidation products of methyl linoleate. In the control, 38% of the hydroperoxide isomers were *cis,trans* hydroperoxides after 7 days of oxidation. At a level of 1000 μM α-tocopherol, 71% of hydroperoxides were *cis,trans* hydroperoxide isomers, whereas at the same hydroperoxide level caffeic acid produced 55%, sinapic acid 52%, and both ferulic and 2,3-hydroxybenzoic acids 42% *cis,trans* hydroperoxide isomers after 7 days of oxidation. Vanillic and 3-hydroxybenzoic acids had no effect on the distribution of hydroperoxide isomers (Figure 4).

Porter et al. (1980) demonstrated that the ratio of *cis,trans* to *trans,trans* hydroperoxide isomers depends on the ability of the medium to donate hydrogen atoms to the radical. In the presence of a better hydrogen donor more *cis,trans* hydroperoxide isomers are formed. On the basis of the *cis,trans* hydroperoxide isomer distribution all phenolic acids showed lower hydrogen-donating activity than α-tocopherol. However, sinapic and caffeic acids more effectively inhibited the formation of hydroperoxides in methyl linoleate than α-tocopherol. Thus, phenolic acids are slower hydrogen donors than α-tocopherol, and the speed of hydrogen donation does not necessarily reflect the amount of radicals scavenged. These data are in agreement with our earlier studies on flavonoids and on α-tocopherol in methyl linoleate (Hopia and Heinonen, 1999; Pekkarinen et al., 1999). The flavonoids quercetin and myricetin inhibited the hydroperoxide formation more strongly, but both were weaker hydrogen donors than α-tocopherol. The ability to produce mainly *cis,trans* isomers seems to be unique for α-tocopherol.

Hydroperoxide Formation in an Emulsified System. The activity of phenolic acids decreased markedly with respect to the activity of α-tocopherol when their activities in bulk oil and emulsion were compared. In an emulsified system only methoxylated phenolic acids acted as antioxidants. In contrast to the activity in bulk oil, caffeic acid did not improve the oxidative stability of methyl linoleate emulsion. The order of activity of phenolic acids in inhibiting hydroperoxide formation after 4 days of oxidation was α-tocopherol > sinapic acid > ferulic acid > vanillic acid. 2,3-Dihydroxybenzoic, 3-hydroxybenzoic, and caffeic acid slightly promoted hydroperoxide formation or did not show any inhibiting effect. The antioxidant activity of sinapic acid and especially that of ferulic acid were concentration dependent. α-Tocopherol strongly inhibited hydroperoxide formation at both 50 and 1000 μM levels (Figure 5).

In the emulsified lipid system only α-tocopherol affected the hydroperoxide isomer distribution, whereas the effect of phenolic acids did not differ from the control. After 4 days of oxidation, 39% of hydroperoxides were *cis,trans* isomers in the control and 45 and 69% in samples containing 50 and 1000 μM α-tocopherol, respectively (data not given).

The effect of bulk and emulsified systems on the antioxidant activity of phenolic acids has been earlier reported by Chen and Ho (1997). They found an anti-

Table 3. Partitioning of Phenolic Acids in Biphasic Water−Oil (9:1) Systems, 1% Tween 20 Solution, and Emulsion [1% Tween 20, Proportion in the Aqueous Phase (Percent)]

antioxidant	proportion in the lipid phase (%)		
	water−oil	Tween 20	emulsion
vanillic acid	3.7[d]	24.7[f]	29.3[d]
2,3-dihydroxybenzoic acid	5.6[b]	20.2[e]	29.9[d]
3-hydroxybenzoic acid	4.5[c]	28.1[d]	34.4[c]
ferulic acid	10.4[a]	46.9[b]	51.9[a]
caffeic acid	1.2[e]	48.8[a]	52.3[a]
sinapic acid	5.6[b]	36.9[c]	41.6[b]

oxidant effect of caffeic and ferulic acids in 3% stripped corn oil-in-water Triton X-100 emulsion oxidation at 60 °C and that both phenolic acids were more effective inhibitors of emulsion oxidation than α-tocopherol, caffeic acid being the most effective. In bulk corn oil they reported the effect on retarding the lipid oxidation in the order caffeic acid > α-tocopherol > ferulic acid. These discrepancies may be due to experimental differences, such as antioxidant concentration (150 μM), oil and emulsifier as well as an oxidation temperature of 60 °C.

The order of activity in emulsion differed from the order in bulk oil. The correlation coefficient between results of DPPH RSA and inhibition (percent) of emulsified methyl linoleate oxidation was 0.32 at the level of 50 μM and 0.24 at the level of 1000 μM (Table 2). This result is supported by earlier findings, because no correlation was found between inhibition of corn oil-in-water emulsion and DPPH RSA (Ho and Chen, 1997). Thus, measurement of the free radical scavenging activities cannot be used when the antioxidant activity of phenolic acids in emulsified systems is evaluated. In heterophasic systems the antioxidant activity is affected by several parameters, which are so far only poorly understood. Partitioning, and interactions with other compounds, may widely affect the antioxidant activity. The kinetics of radical scavenging is markedly influenced by the solvent (Avila et al., 1995).

Partitioning of Phenolic Acids. To investigate whether the partitioning of the antioxidant in emulsions has a significant effect on their activity, the proportion of antioxidant solubilized in the lipid phase and the aqueous phase was determined. Emulsion systems are formed by three or four different environments; the oil phase is dispersed in the continuous aqueous phases. The surfactant forms aggregate at the oil−water interface and micelles in the aqueous phase. Schwarz et al. (1996) suggested that the partition of antioxidants into these different environments of emulsion is mainly dependent on the polarity of the antioxidant and on specific interactions with different constituents of the emulsion. As lipid oxidation occurs in the lipid phase and its surface, the proportion of antioxidants solubilized in these environments is considered to be active as a radical chain-breaking antioxidant. Therefore, the antioxidant effect of hydrophilic antioxidants in emulsified systems is also dependent on the partitioning behavior (Huang et al., 1996; Schwarz et al., 1996).

Table 3 compares the partitioning of phenolic acids in the lipid phase, in water−oil, Tween 20, and emulsion systems. The lipid phase is composed of oil and emulsifier in emulsion, but in biphasic water−oil systems and in Tween 20 solutions the lipid phase consists of oil or emulsifier. In biphasic water−oil systems the antioxidant proportion solubilized in oil increased in the order caffeic acid < vanillic acid < 3-hydroxybenzoic acid < 2,3-dihydroxybenzoic acid < sinapic acid < ferulic acid. However, in emulsions the order of lipid solubility is markedly different, revealing the following order: vanillic acid < 2,3-dihydroxybenzoic acid < 3-hydroxybenzoic acid < sinapic acid < ferulic acid < caffeic acid. These disparities are attributable to the solubilization capacity and specific interaction of the Tween 20 emulsifier with the antioxidants, because the order of antioxidant proportion solubilized in the lipid phase is in the same order as for Tween 20 micellar solutions. The solubilization capacity of Tween 20 for antioxidants is strongly elevated compared to oil. Despite its 10-fold lower concentration, Tween 20 solubilizes at least a 4-fold amount of hydroxybenzoic and -cinnamic acids. Moreover, Tween 20 shows different solubilization capacities for the phenolic acids investigated.

Caffeic acid demonstrates the lowest lipid solubility in water−oil systems, whereas in emulsion it showed the highest proportion in the lipid phase. This indicates that the emulsifier Tween 20 exhibits a higher solubilization capacity for caffeic acid than other acids. As shown in Table 3 the proportion of caffeic acid solubilized by Tween 20 in micellar solution is higher than for other acids.

The intramolecular hydrogen bond of 2,3-hydroxybenzoic acid may explain the unexpected high proportion in lipid phase despite its second OH group. In contrast, the lipid solubility in emulsions was lower than would have been expected from the water−oil partitioning, which is clearly attributable to the solubilization by Tween 20.

The proportion of the antioxidant solubilized in the lipid phase has a marked effect on the antioxidant activity of phenolic acids in heterophasic lipid systems. However, it does not necessarily reflect the antioxidant activity, because the specific interaction of antioxidant with other minor compounds, such as emulsifier, have also a marked effect on the activity of individual compounds. They can cause a higher proportion of the antioxidant in the lipid phase but lead to negative effects on the antioxidant activity. The results suggest that the high proportion of caffeic acid associated with Tween 20 micelles is due to hydrogen bonds between the ether oxygen of the polyoxyethylene chain and the OH groups of caffeic acid (Mulley and Metcalf, 1956). These strong interactions may probably explain the decrease in the activity of caffeic acid in emulsion despite its high concentration solubilized in the lipid phase. According to Avila et al. (1995), the hydrogen donation depends on the strength of the hydrogen bonds between the antioxidant and the solvent, which will be represented by the emulsifier in this case.

Comparing the solubilization of antioxidant in the lipid phase of emulsions and the sum of proportion solubilized in water−oil and Tween 20 solutions, a slight but significant ($p < 0.05$) increase was observed for 2,3-dihydroxybenzoic acid, 3-hydroxybenzoic acid, and caffeic acid. This behavior may be explained by an increase of the solubilization capacity of Tween 20 when aggregates are formed in the oil−water interface of emulsions, thereby changing the geometry (O'Haver and Harwell, 1995). In contrast, for ferulic acid a significant decrease was observed, which is most likely due to the higher lipid solubility favoring different locations in the emulsifier-formed aggregates (Myers, 1992).

This study demonstrated that specific interactions of the antioxidant with other compounds, for example, the

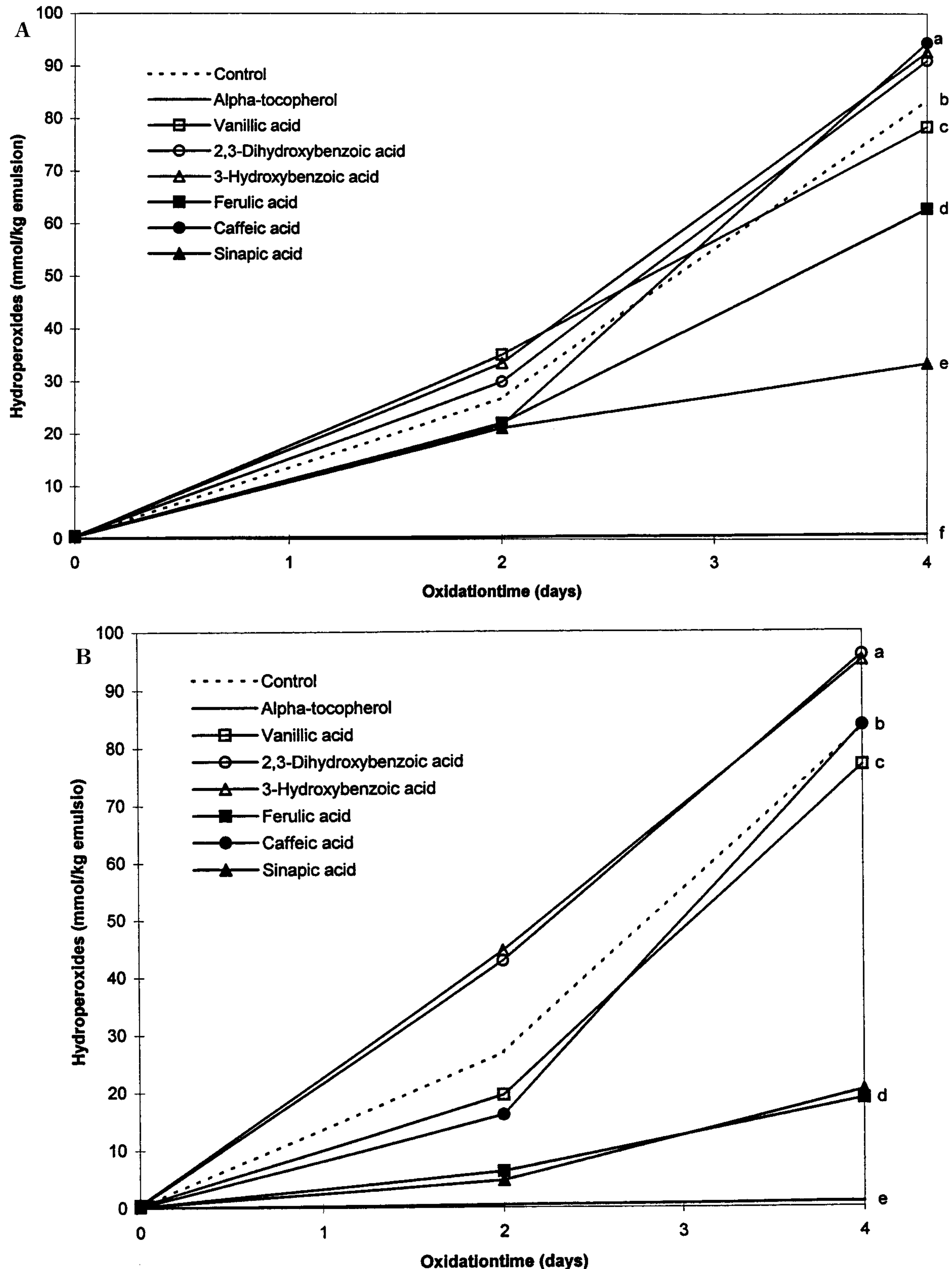

Figure 5. Effect of phenolic acid or α-tocopherol on the oxidation of emulsified methyl linoleate: (A) 50 μM; (B) 1000 μM. Values marked by the same letter or no letter are not significantly different at $P < 0.05$ at the endpoint of oxidation.

emulsifier, and the intramolecular hydrogen bond may play an important role in antioxidant activity. The specific interaction between emulsifier and other minor compounds in the system, as well as the intramolecular bond, may also have a marked effect on partitioning of antioxidants by causing a higher proportion of the antioxidant in the lipid phase but lead to negative effects on the antioxidant activity. Thus, the proportion of the antioxidant solubilized in the lipid phase and in the interface did not necessarily mirror the antioxidant activity of the compound.

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