Effect of Protein on the Antioxidant Activity of Phenolic Compounds in a Lecithin−Liposome Oxidation System

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The effect of bovine serum albumin (BSA) was investigated on the antioxidant activity of phenolic compounds, grape extracts, and red wines in a lecithin−liposome system oxidized at 37 °C with copper. In the absence of BSA, the phenolic compounds inhibited hydroperoxide formation in decreasing order: ferulic acid, epicatechin, catechin, rutin, malvidin, caffeic acid, quercetin, and propyl gallate. Hexanal formation was inhibited in the following decreasing order: ferulic acid, epicatechin, catechin, malvidin, caffeic acid, quercetin, rutin, and propyl gallate. Gallic acid and delphinidin promoted hydroperoxide and hexanal formation. In the presence of 20% BSA, liposome oxidation was much slower. Ferulic acid followed by malvidin and rutin were the most efficient in inhibiting lipid and protein oxidation. Two grape extracts and two red wines inhibited hydroperoxide and hexanal formation more efficiently without BSA. With BSA, the red wines were more active than the grape extracts in inhibiting lipid oxidation but were not different in inhibiting protein carbonyls.

Keywords: *Liposome oxidation; proteins; phenolic compounds; antioxidants; flavonoids; lecithin; liposome; hydroperoxides; hexanal; tryptophan; lysine; protein carbonyls; fluorescence*

INTRODUCTION

A wide range of phenolic compounds inhibit the in vitro oxidation of human low-density lipoproteins (LDL) (DeWhalley et al., 1990; Mangiapane et al., 1992; Frankel et al., 1993, 1995; Nardini et al., 1995; Vinson et al., 1995; Pearson et al., 1997; Teissedre et al., 1996). Polyphenolic compounds in the diet may enhance the stability of LDL to oxidation, which is implicated in the pathogenesis of coronary heart disease (Steinberg et al., 1989; Esterbauer et al., 1992). Phenolic compounds are also effective antioxidants in other food-related systems, including lecithin−liposomes (Yuting et al., 1990; Terao et al., 1994; Saija et al., 1995; Huang and Frankel, 1997; Yi et al., 1997) and aqueous emulsions (Frankel et al., 1997; Huang and Frankel, 1997; Rice-Evans et al., 1996). Lecithin−liposomes are suitable model systems in which to test the antioxidant activity of the polar hydrophilic phenolic compounds found in grape extracts and green tea (Meyer et al., 1997; Yi et al., 1997; Frankel et al., 1997; Huang and Frankel, 1997). The antioxidant activity of various phenolic compounds is greatly influenced by the lipid system used as substrate (Hopia et al., 1996; Huang et al., 1996). The results in the literature on the antioxidant activity of different phenolic compounds are confounded because a wide range of systems are used as substrates (Frankel, 1993).

Apolipoprotein B (apo B), associated with LDL, has specific sites for copper binding (Gieseg and Esterbauer, 1994). In oxidized LDL, lysine residues of apo B are derivatized by lipid peroxide decomposition products (Steinbrecher, 1987). The copper-mediated oxidation of tryptophan residues in the LDL apo B moiety plays an important role in initiating lipid oxidation in LDL particles (Giessauf et al., 1995). Hypochlorite preferentially oxidizes lysine and tryptophan residues of apo B-100 in LDL (Hazell and Stocker, 1993). Polyphenols can act as multidentate ligands binding with protein surfaces (Haslam, 1989) and with bovine serum albumin (BSA) (Muralidhara and Prakash,1995; Bartholome et al., 1996). Protein binding of phenolic compounds in wine and grapes may explain in part the different antioxidant activities of these compounds in the LDL oxidation assay (Teissedre et al., 1996). The antioxidant activity of phenolic compounds in grape extracts is higher in LDL than in lecithin−liposomes (Meyer et al., 1997; Yi et al., 1997). Thus, the effect of proteins on lipid oxidation might prove important in clarifying the antioxidant action of phenolic compounds in food and other biological systems.

While the literature on the effect of phenolic compounds on lipid oxidation continues to accumulate, the data are sparse on the effect of proteins, other than lipoproteins, on their antioxidant activities. This paper reports a study of the effect of BSA on the antioxidant activity of structurally related pairs of phenolic compounds shown in Figure 1, as well as grape extracts and red wines, in a lecithin−liposome oxidation system. As a readily available protein, BSA was chosen because it is most abundant in blood. The antioxidant activity was evaluated in this study on the basis of both lipid and protein oxidation.

MATERIALS AND METHODS

Materials. (+)-Catechin, quercetin, rutin (quercetin 3-rutinoside), malvidin, and delphinidin were obtained from Extrasynthèse (Genay, France). Caffeic acid, ferulic acid, gallic

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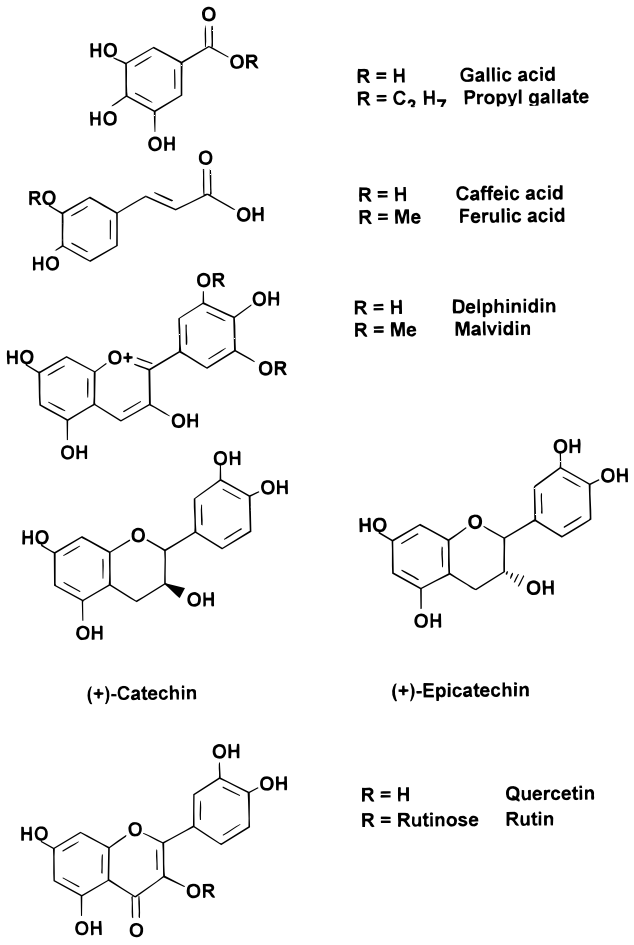

Figure 1. Structures of the phenolic compounds tested.

acid, (−)-epicatechin, hexanal, fluorescamine, and L-α-phosphatidylcholine (lecithin from soybean), with a phosphatidylcholine (PC) content of ∼40%, were purchased from Sigma Chemical Co. (St. Louis, MO). Propyl gallate and BSA (96−99%) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Cupric acetate monohydrate was obtained from EM Science (Cherry Hill, NJ). The buffer was made of succinic acid and succinic acid sodium salt adjusted to pH 4.7, and all reagents were obtained from Sigma Chemical Co. Acetone, ACS grade, was obtained from Fisher Scientific (Fair Lawn, NJ).

Grapes and Wines. Petite Sirah and Cabernet Sauvignon red grapes were harvested at the University of California, Davis, and the corresponding red wines were obtained from local producers. Dealcoholized wine was prepared by removing alcohol with a rotating evaporator at 50 °C under vacuum, followed by purging with nitrogen at room temperature, and replacing the volume lost with distilled water. The phenolic extract from grapes was obtained by extraction with aqueous methanol (60%, v/v). Total phenols were determined, as previously described (Meyer et al., 1997), according to the Folin−Ciocalteu method (Singleton and Rossi, 1965), and expressed as gallic acid equivalents (GAE).

Liposome Oxidation. The liposomes were prepared as described by Huang and Frankel (1997) to a final PC concentration of 0.8 by weight. The average particle size of the liposomes was 0.1 μm as measured with a Microtrac Ultrafine particle analyzer (Leeds & Northrup, North Wales, PA). The lecithin liposomes were oxidized at 37 °C with 3 μM cupric acetate, on the basis of our previous work with phenolic compounds from grapes (Yi et al., 1997), using a 25 mM succinate buffer at pH 4.7, without or with 0.16% BSA in the presence of 5 and 10 μM gallic acid, propyl gallate, caffeic acid, ferulic acid, malvidin, delphinidin, (+)-catechin, (−)-epicatechin, rutin, and quercetin. Rutin and quercetin were dissolved in 50% ethanol in succinic acid buffer, malvidin and delphinidin in ethanol, and all other phenolics in the buffer. Ethanol was then evaporated by purging with nitrogen. Extracts of Petite Sirah and Cabernet Sauvignon grapes and wines were tested at total phenol concentration of 20 μM GAE. Oxidation samples were analyzed spectrophotometrically for conjugated diene hydroperoxides at 234 nm and gas chromatographically for hexanal measurements, as described previously (Frankel et al., 1994). Duplicate oxidation samples were taken daily for 5 days. Antioxidant activity toward lipid oxidation was calculated as percent inhibition of conjugated diene and hexanal production and expressed as % inhibition = $[(C - S)/C] \times 100$, where C is the amount of conjugated diene hydroperoxides or hexanal formed in the control sample without antioxidant and S is the amount of conjugated diene hydroperoxides or hexanal formed in the antioxidant sample.

Protein Oxidation. A Perkin-Elmer (Norwalk, CT) fluorescence spectrophotometer 650-40 was used to measure independently the decrease in tryptophan and lysine and the formation of protein carbonyls in duplicate sample aliquots taken daily for 5 days. The excitation and emission wavelengths were 283 and 338 nm for tryptophan, 390 and 475 nm for lysine, and 350 and 450 nm for protein carbonyls. Tryptophan was determined according to the method of Giessauf et al. (1995) and lysine according to the method of Böhlen et al. (1973), as modified by Hazell and Stocker (1993). The percent inhibition of formation of protein carbonyls was calculated using the same formula as for the inhibition of lipid oxidation, where C is the fluorescence of the control sample and S is the fluorescence of the antioxidant. The percent inhibition of loss of tryptophan and of lysine was calculated as $[(C_0 - C_t)/[C_0 - (S_0 - S_t)/S_0)]]/[(C_0 - C_t)/C_0] \times 100$, where C_0 is the initial fluorescence of the control sample, C_t is the fluorescence of the control sample at time t, S_0 is the initial fluorescence of the antioxidant sample, and S_t is the fluorescence of the antioxidant sample at time t.

Statistical Analysis. Differences among antioxidant activities were tested by one-way analysis of variance using Minitab Statistical Software (Addison-Wesley, Reading, MA). The significance level was $P < 0.05$.

RESULTS

Liposome Oxidation in the Absence of BSA. Oxidation of the lecithin liposome (PC) samples showed that the formation of conjugated diene hydroperoxides accelerated after 1 day and the formation of hexanal after 2 days (Figure 2). In the PC control, the formation of hydroperoxides reached a maximum after 2 days, followed by a decrease (Figure 2a), while hexanal formation increased when the amount of hydroperoxides decreased (Figure 2b). On this basis, percentage inhibition values were calculated at the propagation phase of the oxidation, on day 2 for hydroperoxides and on day 3 for hexanal. When phenolic compounds were compared at 5 μM concentration on the basis of hydroperoxide formation, ferulic acid had the highest antioxidant activity, followed by epicatechin, catechin, rutin, and malvidin, caffeic acid and quercetin, and propyl gallate with the lowest activity. Gallic acid and delphinidin acted as prooxidant in increasing hydroperoxide formation (Table 1). The antioxidant activity increased at 10 μM, and ferulic acid and catechin were again the most active antioxidants, followed by malvidin, epicatechin, rutin, caffeic acid, and quercetin (Figure 2). Hydroperoxide formation was higher in the presence of gallic acid and delphinidin (10 μM) than the control PC and reached a maximum between 1 and 2 days (Figure 2a).

On the basis of hexanal formation at 5 μM, ferulic acid was the most active antioxidant, followed by epicatechin and catechin, malvidin, caffeic acid, quercetin, rutin, and propyl gallate (Table 1). Delphinidin

Table 1. Inhibition of Lipid Oxidation in Lecithin Liposome Oxidation with 5 μM Phenolic Compounds in the Absence (PC) or Presence of Bovine Serum Albumin (PC-BSA)[a]

phenolic compound	hydroperoxides (2 days)		hexanal (3 days)	
	PC	PC-BSA	PC	PC-BSA
control	0.0 ± 0.4 g	0.0 ± 0.4 f	0.0 ± 1.5 g	0.0 ± 1.0 cd
gallic acid	−34.6 ± 0.3 i	−23.8 ± 0.8 h	−53.8 ± 2.2 i	−248.5 ± 75.4 e
propyl gallate	5.6 ± 0.2 f	−6.8 ± 0.3 g	34.1 ± 0.1 f	−38.0 ± 1.3 d
caffeic acid	19.2 ± 0.4 e	4.7 ± 3.6 e	51.2 ± 0.2 d	−8.3 ± 0.4 cd
ferulic acid	54.6 ± 0.3 a	57.8 ± 0.4 a	79.1 ± 0.3 a	89.7 ± 0.2 a
malvidin	23.7 ± 0.3 d	40.7 ± 0.1 b	64.3 ± 0.5 c	81.9 ± 0.1 ab
delphinidin	−18.6 ± 0.7 h	−28.3 ± 2.6 h	−3.9 ± 1.6 h	21.7 ± 0.4 bcd
catechin	44.0 ± 0.0 c	18.5 ± 0.1 d	68.6 ± 0.5 b	66.3 ± 1.3 ab
epicatechin	47.5 ± 0.9 b	16.0 ± 0.1 d	70.8 ± 0.1 b	67.3 ± 5.2 ab
rutin	23.3 ± 0.4 d	31.7 ± 1.5 c	32.4 ± 1.6 f	82.7 ± 0.6 ab
quercetin	19.1 ± 0.5 e	16.5 ± 1.7 d	44.8 ± 0.3 e	40.8 ± 1.7 abc

[a] Inhibition values are shown as mean values ± standard deviation. Values in the same column followed by the same letter are not significantly different ($P < 0.05$).

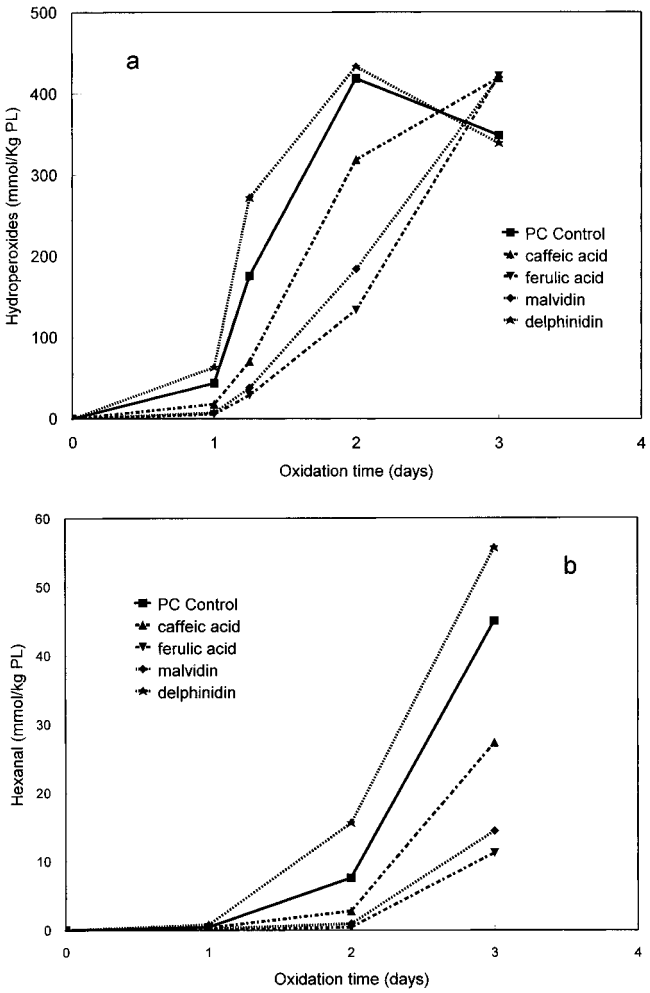

Figure 2. Effect of 10 μM phenolic compounds on oxidation of lecithin liposome (PC): (a) hydroperoxides and (b) hexanal.

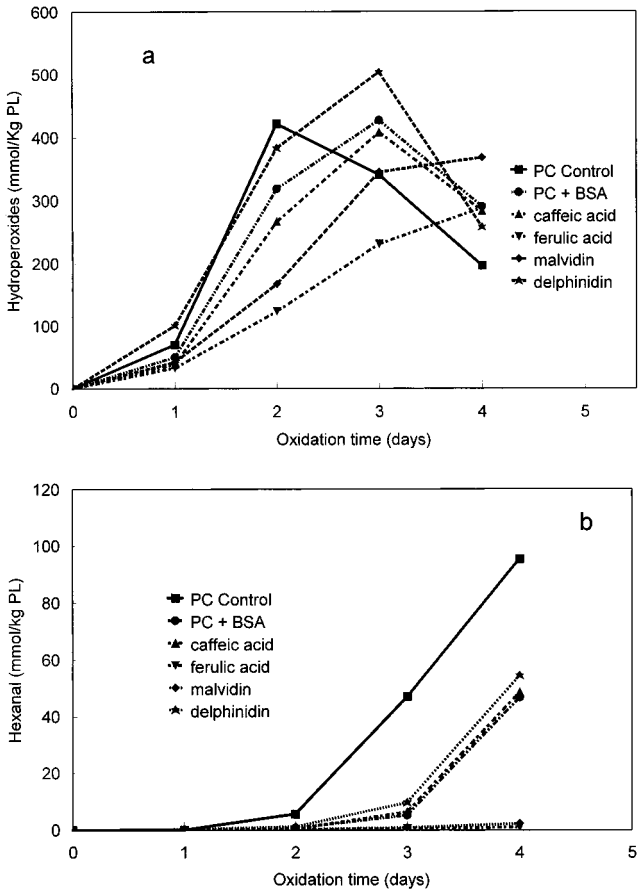

Figure 3. Effect of 10 μM phenolic compounds on oxidation of lecithin liposome in the presence of BSA (PC + BSA): (a) hydroperoxides and (b) hexanal.

and gallic acid were prooxidant in promoting hexanal formation. The inhibition of hexanal formation did not increase at 10 μM as much as the inhibition of hydroperoxide formation. The same trends for hexanal were observed after 3 days of oxidation as for hydroperoxides after 2 days of oxidation. At 10 μM, the trend of antioxidant effectiveness was similar, with ferulic acid, catechin, epicatechin, and malvidin being the most active, followed by rutin, caffeic acid, quercetin, and propyl gallate with the lowest activity. The prooxidant activity of gallic acid and delphinidin increased on the basis of hexanal formation (Figure 2b).

Liposome Oxidation in the Presence of BSA. In the presence of 20% BSA, the rates of hydroperoxide and hexanal formation were much slower, but the induction periods for the controls remained the same as in the absence of BSA (Figure 3). The antioxidant activities of phenolic compounds were difficult to compare. The antioxidant efficiency of the phenolic compounds was also less evident because the oxidation of PC was slower, and the formation of hydroperoxides reached a maximum, followed by a decrease. In the PC plus BSA control, the maximum formation of hydroperoxides was reached later than in the PC control (Figure 3a). In the presence of BSA at 5 μM, ferulic acid was the most active antioxidant, followed by malvidin, rutin, the catechins and quercetin, caffeic acid,

Table 2. Inhibition of Protein Oxidation in Lecithin Liposome with Phenolic Compounds (at 5 or 10 μM) in the Presence of Bovine Serum Albumin[a]

phenolic compound	tryptophan loss (2 days)	lysine loss (4 days)	carbonyl gain (4 days)
5 μM			
control	0.0 ± 0.1 d	0.0 ± 1.8 ef	0.0 ± 9.3 cd
gallic acid	−18.8 ± 0.1 f	−35.9 ± 3.6 h	−15.7 ± 3.9 e
propyl gallate	−10.1 ± 0.9 e	−25.2 ± 3.4 g	−9.3 ± 5.4 de
caffeic acid	−6.2 ± 0.4 e	−7.0 ± 0.0 f	−4.2 ± 3.1 cd
ferulic acid	30.2 ± 2.0 a	97.5 ± 0.0 a	40.9 ± 0.8 a
malvidin	19.3 ± 0.7 b	57.1 ± 5.2 b	21.5 ± 0.8 b
delphinidin	−18.6 ± 0.9 f	−37.4 ± 10 h	−31.9 ± 1.7 f
catechin	2.2 ± 2.8 d	21.5 ± 6.9 c	5.9 ± 0.6 c
epicatechin	−0.6 ± 1.7 d	11.7 ± 3.5 cd	−1.2 ± 2.3 cd
rutin	7.5 ± 1.5 c	87.1 ± 0.0 a	31.0 ± 3.9 ab
quercetin	1.1 ± 1.3 d	6.8 ± 3.5 de	2.0 ± 1.2 cd
10 μM			
control	0.0 ± 0.1 f	0.0 ± 2.0 c	0.0 ± 2.9 de
gallic acid	−14.8 ± 0.3 h	−40.7 ± 2.5 d	−2.2 ± 2.8 f
propyl gallate	0.9 ± 0.5 f	0.7 ± 13 c	−7.9 ± 2.2 f
caffeic acid	−0.7 ± 0.7 f	37.8 ± 10 b	−2.6 ± 1.5 ef
ferulic acid	39.2 ± 2.0 a	98.2 ± 2.5 a	51.0 ± 2.2 a
malvidin	21.8 ± 2.4 b	96. 3 ± 5.2 a	38.1 ± 0.3 b
delphinidin	−7.8 ± 0.6 g	−5.5 ± 15 c	−1.8 ± 2.9 ef
catechin	12.6 ± 0.5 d	41.4 ± 5.2 b	5.4 ± 1.5 d
epicatechin	5.9 ± 0.9 e	12.1 ± 18 bc	−1.2 ± 1.5 e
rutin	19.4 ± 0.3 c	83.3 ± 13 a	10.8 ± 0.9 c
quercetin	5.8 ± 1.5 e	22.6 ± 5.2 bc	−2.3 ± 2.2 ef

[a] See Table 1.

and propyl gallate, on the basis of hydroperoxide formation (Table 1). Delphinidin and gallic acid had the same prooxidant activity, and propyl gallate had weak prooxidant activity. On the basis of hexanal formation, ferulic acid was the most active antioxidant, followed by malvidin, the catechins and rutin, which were not significantly different, and quercetin. Although delphinidin was a prooxidant in promoting hydroperoxide formation, it was a weak antioxidant in inhibiting hexanal formation (Table 1). Although caffeic acid promoted hexanal formation, it was not significantly different from the control. Gallic acid and propyl gallate were stronger prooxidants on the basis of hexanal formation than on the basis of hydroperoxide formation.

In the presence of 10 μM ferulic acid, malvidin, and rutin, hydroperoxide formation continued to increase at a lower rate after 3 days, but the rate decreased after 3 days in the presence of the other phenolic compounds tested (Figure 3a). Hexanal formation continued to increase in all samples tested during the entire oxidation period. Similar trends in antioxidant and prooxidant activities were observed on the basis of hydroperoxide and hexanal formation after 3 and 4 days (Figure 3b). Ferulic acid and malvidin were the most active antioxidants followed by catechin and rutin.

To maximize the differences observed in protein oxidation, the percentage inhibition values were calculated on day 2 for tryptophan and on day 4 for lysine and for protein carbonyls (Table 2). At 5 μM, ferulic acid was the most efficient antioxidant, followed by malvidin and rutin, in retarding protein oxidation on the basis of losses of both tryptophan and lysine and the formation of protein carbonyls. The catechins and quercetin had little or no activity, while caffeic acid, propyl gallate, and gallic acid were prooxidant in promoting protein oxidation. At 10 μM, ferulic acid and malvidin were the most active antioxidants in preventing protein oxidation, followed by rutin and the catechins. Although caffeic acid and quercetin were active in inhibiting loss of lysine, they were not active in inhibiting loss of tryptophan or formation of carbonyls. Gallic acid promoted the loss of tryptophan and lysine and the formation of carbonyls at 10 μM; propyl gallate was inactive toward the loss of tryptophan and lysine and promoted the formation of protein carbonyl (Table 2).

Effect of Grape Extracts and Wine on Oxidation of Liposomes. Extracts of Petite Sirah and Cabernet Sauvignon grapes and the corresponding red wines were better inhibitors of hydroperoxides and hexanal in the absence of BSA than in the presence of BSA (Table 3). In the absence of BSA, the Cabernet Sauvignon wine showed the highest antioxidant activity, followed by the extract of Petite Sirah grapes and the corresponding wine and the extract of Cabernet grapes. In the presence of BSA, and on the basis of hydroperoxide formation, the two wines had more antioxidant activity than the two grape extracts; on the basis of hexanal formation, the extract of Petite Sirah grapes had the highest activity followed by the Cabernet Sauvignon wine, the Petite Sirah wine, and the extract of Cabernet Sauvignon grapes. On the basis of tryptophan loss, the Cabernet Sauvignon wine was the most effective inhibitor, followed by the Petite Sirah wine, which had the same activity as the Cabernet Sauvignon grape extract, and the extract of Petite Sirah grapes. The two wines and the two grape extracts were not significantly different as inhibitors of protein carbonyl formation (Table 3).

DISCUSSION

With the lecithin−liposome system used in the present study, ferulic acid was the best antioxidant in inhibiting lipid oxidation, followed by the catechins, rutin, malvidin, caffeic acid, and quercetin. In the presence of 20% BSA, ferulic acid was also the best antioxidant in inhibiting both lipid and protein oxidation, followed by malvidin and rutin. Gallic acid, propyl gallate, and delphinidin were prooxidants in most evaluations. Some of the less polar phenolic compounds tested appear to be more effective antioxidants than their more polar

Table 3. Inhibition of Lecithin Liposome Oxidation with Grape Extracts and Wines at 20 μM GAE Total Phenols, in the Absence (PC) or Presence of Bovine Serum Albumin (PC-BSA)[a]

	% inhibition of lipid oxidation				% inhibition of protein oxidation	
	hydroperoxides (2 days)		hexanal (4 days)		tryptophan loss (2 days)	carbonyl gain (4 days)
grape extract/wine	PC	PC-BSA	PC	PC-BSA	PC-BSA	PC-BSA
control	0.0 ± 0.3 d	0.0 ± 3.5 c	0.0 ± 2.1 e	0.0 ± 0.7 d	0.0 ± 0.5 d	0.0 ± 3.6 b
Petite Sirah extract	43.1 ± 2.3 b	28.5 ± 0.7 b	50.8 ± 0.1 c	51.5 ± 0.6 a	3.9 ± 0.4 c	41.5 ± 0.3 a
Cabernet Sauvignon extract	36.2 ± 0.6 c	24.8 ± 1.3 b	47.1 ± 0.0 d	42.9 ± 0.1 c	6.4 ± 0.2 b	46.6 ± 1.0 a
Petite Sirah wine	39.7 ± 1.8 bc	36.3 ± 1.9 a	56.7 ± 0.2 b	42.9 ± 1.5 c	6.3 ± 0.4 b	45.7 ± 7.9 a
Caberenet Sauvignon wine	49.4 ± 0.9 a	40.4 ± 0.1 a	65.3 ± 0.3 a	47.5 ± 1.8 b	15.0 ± 0.4 a	49.9 ± 1.0 a

[a] see Table 1.

counterparts in Figure 1. Thus, ferulic acid is less polar and more active antioxidant than caffeic acid. Similarly, malvidin and propyl gallate are less polar and more effective antioxidants than their respective counterparts, delphinidin and gallic acid.

Ferulic acid and malvidin may be more active antioxidants in inhibiting both lipid and protein oxidation by their increased hydrogen donor ability and by forming more stable phenoxyl radical intermediates due to their methoxyl substituents. The lower polarity of ferulic acid and malvidin may also impart a better affinity for the liposome interface and better inhibit their oxidation. The enhanced antioxidant activity of ferulic acid and malvidin in the presence of BSA may also be explained by their better access to the liposome interface undergoing oxidation.

The prooxidant activity of gallic acid, delphinidin, and propyl gallate, which have three ortho hydroxyl groups (Figure 1), is apparently related to their high polarity, which is consistent with our previous results (Huang and Frankel, 1997; Satué-Gracia et al., 1997). These polar compounds may have a higher reducing activity in converting copper ions to a lower valence state, causing them to become prooxidant. Propyl gallate, gallic acid, and delphinidin may also bind strongly to BSA and contribute to the marked loss of tryptophan fluorescence (Table 2).

Different mechanisms to explain the action of phenolic antioxidants include free radical scavenging activity (Bors and Saran, 1987), metal chelation (Hudson and Lewis, 1983), and protein binding (Muralidhara and Prakash, 1995). Our understanding of the antioxidant mechanisms is further compounded by complex interfacial phenomena in heterogeneous systems where hydrophilic polyphenolic compounds vary in their partition behaviors between the water and oil phases and their interface (Schwarz et al., 1996; Huang et al., 1997). The antioxidant activity of phenolic compounds is related not only to structural characteristics but also to their ability to interact with the interface where oxidation takes place. Flavonoids are known to anchor through chemical bonds to the polar head of phospholipids, forming reversible physicochemical complexes (Bombardelli and Spetta, 1991). Thus, depending on their liposolubility, there is a relationship between flavonoid interaction with membranes and formation of flavonoid−phospholipid complexes (Saija et al., 1995). In the liposome system tested, the polar catechins were good antioxidants in the absence of BSA due to their higher affinity toward the polar liposome surface, as reported previously (Huang and Frankel, 1997). BSA inhibits peroxidation of liposomes induced by azo initiators (Dean et al., 1991). The stabilizing effect of BSA on liposome oxidation was shown also in the present study as the control PC sample oxidized more slowly in the presence of the protein.

In the liposome system used in this study, in the presence of BSA caffeic acid was either prooxidant or inactive in inhibiting lipid oxidation. Caffeic acid was shown to strongly bind to serum albumin, inducing a conformational change in the protein as indicated by quenching of fluorescence emission intensity, mainly due to tryptophan (Muralidhara and Prakash, 1995).

According to Bartholome et al. (1996), the *o*-dihydroxyphenolic acids interact more strongly than monohydroxyphenolic acids or catechin with BSA. Thus, in the present study, binding of caffeic acid to BSA may promote oxidation of the lecithin−liposomes.

Strong protein binding of phenolic acids or polyphenolic compounds may promote oxidation in the lecithin−liposome system.

The antioxidant activities of Petite Sirah and Cabernet Sauvignon grape extracts were similar to those of the corresponding wines, confirming previous findings (Meyer et al., 1997; Yi et al., 1997). In the absence of BSA, wines containing high amounts of catechins and lower amounts of rutin and malvidin (Frankel et al., 1995) exerted higher antioxidant activity than the extracts of the corresponding grapes, which are rich in anthocyanins, such as malvidin glycosides (Meyer et al., 1997). In the presence of BSA, the two red wines were more active antioxidants than the grape extracts in inhibiting lipid oxidation, but they showed no difference in inhibiting protein carbonyls. The relative antioxidant potency of phenolic compounds in liposomes is thus mediated to different extents by the presence of protein.

LITERATURE CITED

Bartholome, B.; Estrella, I.; Hernandez, T. Evaluation of the capability of low molecular weight phenolic compounds to interact with proteins. *Polyphenolic Commun.* **1996**, *July 15−18*, 439−440.

Böhlen, P.; Stein, S.; Dairman, W.; Udenfriend, S. Fluorometric assay of proteins in the nanogram range. *Arch. Biochem. Biophys.* **1973**, *155*, 213−220.

Bombardelli, E.; Spetta, M. Phospholipid-polyphenol complexes: A new concept in skin care ingredients. *Cosmet. Toiletries* **1991**, *106*, 69−76.

Bors, W.; Saran, M. Radical scavenging by flavonoid antioxidants. *Free Radical Res. Commun.* **1987**, *2*, 289−294.

Dean, R. T.; Hunt, J. V.; Grant, A. J.; Yamamoto, Y.; Niki, E. Free radical damage to proteins: the influence of the relative localization of radical generation, antioxidants, and target proteins. *Free Radical Biol. Med.* **1991**, *11*, 161−168.

DeWhalley, C.; Rankin, S. M.; Hoult, J. R.; Jessup, W.; Leake, D. Flavonoids inhibit the oxidative modification of low-density lipoproteins by macrophages. *Biochem. Pharmacol.* **1990**, *39*, 1743−1750.

Esterbauer, H.; Gebicki, J.; Puhl, H.; Jürgens, G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biol. Med.* **1992**, *13*, 341−390.

Frankel, E. N. In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends Food Sci. Technol.* **1993**, *4*, 220−225.

Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454−457.

Frankel, E. N.; Huang, S.-W.; Kanner, J.; German, J. B. Interfacial phenomena in the evaluation of antioxidants: bulk oil *vs* emulsions. *J. Agric. Food Chem.* **1994**, *42*, 1054−1059.

Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890−894.

Frankel, E. N.; Huang, S. W.; Aeschbach, R. Antioxidant activity of green teas in different lipid systems. *J. Am. Oil Chem. Soc.* **1997**, *74*, 1309−1315.

Gieseg, S. P.; Esterbauer, H. Low-density lipoprotein is saturable by pro-oxidant copper. *FEBS Lett.* **1994**, *343*, 188−194.

Giessauf, A.; Steiner, E.; Esterbauer, H. Early destruction of tryptophan residues of apolipoprotein B is a vitamin E-independent process during copper-mediated oxidation of LDL. *Biochim. Biophys. Acta* **1995**, *1256*, 221−232.

Haslam, E. Polyphenol complexation. In *Plant Polyphenols*; Cambridge University Press: Cambridge, U.K., 1989; pp 154−214.

Hazell, L. J.; Stocker, R. Oxidation of low-density lipoprotein with hypochlorite causes transformation of the lipoprotein into a high-uptake form for macrophages. *Biochem. J.* **1993**, *290*, 165−172.

Hopia, A. I.; Huang, S.-H.; Schwarz, K.; German, J. B.; Frankel, E. N. Effect of different lipid systems on antioxidant activity of rosemary constituents carnosol and carnosic acid with and without α-tocopherol. *J. Agric. Food Chem.* **1996**, *44*, 2030−2036.

Huang, S.-W.; Frankel, E. N. Antioxidant activity of tea catechins in different lipid systems. *J. Agric. Food Chem.* **1997**, *45*, 3033−3038.

Huang, S.-W.; Hopia, A.; Frankel, E. N.; German, J. B. Antioxidant activity of α-tocopherol and Trolox in different lipid substrates: bulk oils vs oil-in-water emulsions. *J. Agric. Food Chem.* **1996**, *44*, 444−452.

Huang, S.-W.; Frankel, E. N.; German, J. B.; Aeschbach, R. Partition of selected antioxidants in corn oil−water model systems. *J. Agric. Food Chem.* **1997**, *45*, 1992−1994.

Hudson, B. J. F.; Lewis, J. I. Polyhydroxy flavonoid antioxidants for edible oils. Structural criteria for activity. *Food Chem.* **1983**, *10*, 47−55.

Mangiapane, H.; Thomson, J.; Salter, A.; Brown, S.; Bell, G. P.; White, D. A. The inhibition of low density lipoproteins by (+)-catechin, a naturally occurring flavonoid. *Biochem. Pharmacol.* **1992**, *43*, 445−450.

Meyer, A. M.; Yi, O.-S.; Pearson, D. A.; Waterhouse, A. L.; Frankel, E. N. Inhibition of human low-density lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). *J. Agric. Food Chem.* **1997**, *45*, 1638−1643.

Muralidhara, B. K.; Prakash, V. Interaction of 3′-*O*-caffeoyl D-quinic acid with human serum albumin. *Int. J. Pept. Protein Res.* **1995**, *46*, 1−8.

Nardini, M.; D'Aquino, M.; Tomassi, G.; Gentilli, V.; Di Felice, M.; Scaccini, C. Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. *Free Radical Biol. Med.* **1995**, *19*, 541−552.

Pearson, D. A.; Frankel, E. N.; German, J. B.; Aeschbach, R. Inhibition of endothelial cell induced oxidation of LDL by rosemary and plant phenolic antioxidants. *J. Agric. Food Chem.* **1997**, *45*, 578−582.

Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

Saija, A.; Scalese, M.; Lanza, M.; Marzullo, D.; Bonina, F.; Castelli, F. Flavonoids as antioxi dant agents: importance of their interactions with biomembranes. *Free Radical Biol. Med.* **1995**, *19*, 481−186.

Satué-Gracia, M. T.; Heinonen, M.; Frankel, E. N. Anthocyanins as antioxidants on human LDL and lecithin liposome systems. *J. Agric. Food Chem.* **1997**, *44*, 3362−3367.

Schwarz, K.; Frankel, E. N.; German, J. B. Partition behaviour of antioxidative phenolic compounds in heterophasic systems. *Fett/Lipid* **1996**, *98*, 115−121.

Singleton, V. L.; Rossi, J. A., Jr. Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. Beyond cholesterol. Modification of low-density lipoproteins that increase its atherogenicity. *N. Engl. J. Med.* **1989**, *320*, 915−924.

Steinbrecher, U. P. Oxidation of human low-density lipoprotein results in derivatization of lysine residues of apolipoprotein B by lipid peroxide decomposition products. *J. Biol. Chem.* **1987**, *262*, 3603−3608.

Teissedre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of *in vitro* human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* **1996**, *70*, 55−61.

Terao, J.; Piskula, M.; Yao, Q. Protective effect of epicatechin, epicatechin gallate and quercetin on lipid peroxidation in phospholipid bilayers. *Arch. Biochem. Biophys.* **1994**, *308*, 278−284.

Vinson, J. A.; Dabbagh, Y. A.; Serry, M. M.; Jang, J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using a *in vitro* oxidation model for heart disease. *J. Agric. Food Chem.* **1995**, *43*, 2800−2802.

Yi, O.-S.; Meyer, A. S.; Frankel, E. N. Effect of phenolic grape extracts on the oxidation of lecithin liposomes. *J. Am. Oil Chem. Soc.* **1997**, *74*, 1301−1307.

Yuting, C.; Rongliang, Z.; Zhongjian, J.; Young, J. Flavonoids as superoxide scavengers and antioxidants. *Free Radical Biol. Med.* **1990**, *9*, 19−21.

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