Lipid Oxidation in Emulsions As Affected by Charge Status of Antioxidants and Emulsion Droplets

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The influence of charge status of both lipid emulsion droplets and phenolic antioxidants on lipid oxidation rates was evaluated using anionic sodium dodecyl sulfate (SDS) and nonionic polyoxyethylene 10 lauryl ether (Brij)-stabilized emulsion droplets and the structurally similar phenolic antioxidants gallamide, methyl gallate, and gallic acid. In nonionic, Brij-stabilized salmon oil emulsions at pH 7.0, gallyol derivatives (5 and 500 μM) inhibited lipid oxidation with methyl gallate > gallamide > gallic acid. In the Brij-stabilized salmon oil emulsions at pH 3.0, low concentrations of the galloyl derivatives were prooxidative or ineffective while high concentrations were antioxidative. In SDS-stabilized salmon oil emulsions, oxidation rates were faster and the galloyl derivatives were less effective compared to the Brij-stabilized emulsions. Differences in antioxidant activity were related to differences in the ability of the galloyl derivatives to partition into emulsion droplets and to increase the prooxidant activity of iron at low pH.

Keywords: *Lipid oxidation; antioxidants; emulsions; salmon oil; phenolics*

INTRODUCTION

Phenolic compounds are commonly added to foods to inhibit lipid oxidation. However, their effectiveness is often difficult to predict because there are several distinctly different mechanisms by which phenolic compounds influence lipid oxidation rates. Phenolics can scavenge free radicals thereby inhibiting lipid radical chain reactions (Nawar, 1996). Phenolics can also chelate transition metal ions hence reducing metal-induced oxidative reactions (Moran et al., 1997). However, phenolic compounds also reduce Fe^{3+} to Fe^{2+}. Since Fe^{2+} rapidly decomposes peroxides into free radicals (Dunford, 1987), the metal-reducing properties of phenolics can increase oxidative reactions. Finally, the physical location of phenolics will influence their ability to influence lipid oxidation. In bulk oils, polar phenolics concentrate at the oil−air interface where oxidation is most prevalent, making them more effective than structurally similar nonpolar counterparts which are more evenly distributed throughout the oil. The opposite trend is observed in oil-in-water emulsions where nonpolar phenolics, which are retained in the lipid droplets, are more effective antioxidants than their more polar counterparts (Huang et al., 1997).

In oil-in-water emulsion systems, there are several mechanisms by which charge status of emulsion droplets and antioxidants influence lipid oxidation, including the following.

(1) Attractive/repulsive electrostatic interactions between charged emulsion droplets and charged prooxidants greatly affects the location and hence activity of transition metals. Previous work (Mei et al., 1998a) showed that Fe^{3+} and Fe^{2+} are strongly bound to negatively charged sodium dodecyl sulfate (SDS)-stabilized emulsion droplets, but not to positively charged dodecyltrimethylaminmonium bromide (DTAB)- nor uncharged polyoxyethylene 10 lauryl ether (Brij)-stabilized emulsion droplets. Consequently, lipid oxidation occurred much faster in negatively charged emulsions in the presence of positively charged transition metal ions (Mei et al. 1998a,b). Similar results have also been reported in fatty acid micelle systems (Yoshida and Niki, 1992).

(2) Attractive/repulsive electrostatic interactions between charged emulsion droplets and charged antioxidants can affect the location and hence activity of antioxidants. Superior antioxidant action was found when electrostatic interactions concentrate antioxidants at the surface of dispersed lipids. For instance, the antioxidant activity of ascorbic acid (negatively charged) increases dramatically in the presence of positively charged lipid micelles (Pryor et al., 1993), spermine's (positively charged) ability to inhibit lipid oxidation increases in the presence of negatively charged phospholipids (Kogure et al., 1993), and the antioxidant activity of Trolox (negatively charged) is higher in the presence of positively charged phospholipid liposomes (Barclay and Vinqvist, 1993).

(3) pH can affect the location of ionic antioxidants by altering their charge and thus their solubility. Charged antioxidants have a greater tendency to partition into the water phase of biphasic systems while their uncharged counterparts have a higher tendency to partition into organic phases. Increasing the concentration of antioxidants in the organic phase increases their antioxidant activity in oil-in-water emulsions (Huang et al., 1997). Changes in the charge status and partitioning behavior of an antioxidant would occur at pHs near its pK_a.

(4) Ionic interactions between prooxidants and other food components can also influence oxidation rates. Chelators can bind transition metals thus decreasing their chemical reactivity (Dunford, 1987) and interactions with anionic emulsion droplets (Mei et al., 1998a). Other ionic food components capable of binding transi-

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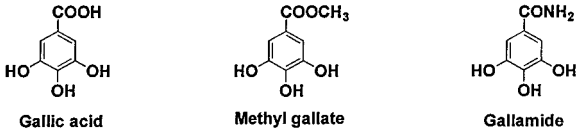

Figure 1. Structures of gallic acid, methyl gallate, and gallamide.

tion metals (e.g., phenolics, proteins and polysaccharides) could also influence the chemical reactivity and physical location of metals thus influencing their ability to promote oxidation.

While numerous publications have investigated the ability of phenolics to inhibit lipid oxidation in both experimental model systems and foods, much more needs to be understood about the structure−function relationship of these antioxidants. The purpose of this research was to determine how emulsion droplet charge, antioxidant charge, and pH influence lipid oxidation rates in oil-in-water emulsions. Gallamide, methyl gallate, and gallic acid were used in this study to represent antioxidants with different charge status (Figure 1). These structurally similar galloyl derivatives were chosen because they have the same antioxidant functional groups and they are analogues of some commonly used food antioxidants. SDS and Brij were used to produce negatively charged and noncharged emulsions, respectively. Better understanding the interactions between antioxidants, prooxidants, and emulsion droplets as a function of ionic state and pH may help in the design of more effective antioxidative systems which minimize lipid oxidation in food emulsions.

MATERIALS AND METHODS

Gallic acid, methyl gallate, porphyridium cruentum β-phycoerythrin (β-PE), polyoxyethylene 10 lauryl ether [Brij, $CH_3(CH_2)_{11}(OCH_2CH_2)_{10}OH$], and SDS [SDS, $CH_3(CH_2)_{11}OSO_3Na$] were obtained from Sigma Chemical Co. (St. Louis, MO). Gallamide (3,4,5-trihydroxybenzamide) was from Lancaster Synthesis Inc. (Windham, NH). 2,2′-Azobis(2-amidinopropane)-dihydrochloride (AAPH) was from Wako Chemicals USA, Inc. (Richmond, VA), and bathophenanthrolinedisulfonic acid was from Aldrich (Miilwaukee, WI). All other chemicals were of reagent grade or purer.

Preparation and Characterization of Emulsions. Salmon oil, containing 99.5 ± 0.2% triacylglycerols, 0.034 μmol of thiobarbituric acid reactive substances/g of oil, and 0.12 μmol of lipid peroxides/g of oil, was isolated from minced muscle by centrifugation at 10000*g* for 20 min (Mei et al., 1998a). Twenty grams of an aqueous solution of 1.6 mM SDS or Brij was mixed with 0.4 g of salmon oil (oxidation studies) or hexadecane (ζ potential and antioxidant partitioning studies) and was sonicated at a power level of +200 and a repeating duty cycle of 0.4 s for 110 s using an ultrasonic generator (Braun-Sonic 2000 U, Braun Biotech, Allentown, PA). These sonication conditions did not increase the concentrations of lipid oxidation products in the salmon oil. Both imidazole and acetate buffers were added at a final concentration of 10 mM each (based on total emulsion volume), and then the emulsion was adjusted to pH 7 or 3 with HCl. The size distribution of the emulsions was determined by static light scattering (Weiss et al., 1996) using a Horiba LA-900 instrument (Irvine, CA). Galloyl derivatives were added to the pH adjusted emulsions at a final concentration of either 5 or 500 μM.

Lipid Oxidation Studies. Emulsions were incubated at 22 °C, and samples were taken periodically to determine thiobarbituric acid reactive substances (TBARS) and lipids peroxides. TBARS were measured according to the method of McDonald and Hultin (1987) using 2 mL of the TBA reagent, 1 mL of H_2O, and 20 μL of emulsion. After heating and centrifugation, the solution was scanned from 580 to 520 nm. Absorbance was calculated as $A_{532nm} - A_{580nm}$. Absorbance at 580 nm was used to correct for any potential light scattering since 580 nm represents the closest non-TBARS absorbing wavelength to 532 nm. Concentrations of TBARS were determined from a standard curve prepared using 1,1,3,3-tetraethoxypropane. Lipid peroxides were measured using a modified method of Shantha and Decker (1994). Three milliliters of methanol/1-butanol (2:1, v:v) was mixed with 20 μL of emulsion sample and 30 μL of thiocyanate/Fe^{2+} solution. The thiocyanate/Fe^{2+} solution was made immediately before use by mixing 1 vol of thiocyanate solution (3.94 M ammonium thiocyanate) with 1 vol of Fe^{2+} solution (obtained from the supernatant of a mixture of 3 mL of 0.144 M $BaCl_2$ in 0.4 M HCl and 3 mL of freshly prepared 0.144 M $FeSO_4$). Twenty minutes after Fe^{2+} addition, absorbance was measured at 510 nm. Lipid peroxides were quantitated from a standard curve using H_2O_2.

Characterization of the Physical and Chemical Properties of the Galloyl Derivatives. The capability of the galloyl derivatives to scavenging free radicals was tested by their ability to prevent the peroxyl radical [originating from 2,2′-azobis(2-amidinopropane)dihydrochloride; AAPH] initiated decay of porphyridium cruentum β-phycoerythrin (β-PE) fluorescence according to Cao et al. (1993). The galloyl derivatives (0.1 μM) and β-PE (1.5 μg/mL) were mixed into 75 mM phosphate buffer (pH 3.0 or 7.0). β-PE concentration was determined at 545 nm according to the method of Gantt and Lipschultz (1974). Fluorescence decay was monitored after addition of AAPH (6.5 mM) for 50 min using an excitation and emission wavelengths of 545 and 575 nm, respectively, on a Hitachi F-2000 Fluorometer (Tokyo, Japan). Fluorescent decay was calculated as the area between the curve of β-PE alone and the sample in question. Percent inhibition was calculated as [1 − (curve area of galloyl + AAPH + β-PE)/(curve area of AAPH + β-PE)] × 100.

The ability of the galloyl derivatives to reduce Fe^{3+} to Fe^{2+} was determined spectrophotometrically at 535 nm at pH 3.0 and 7.0 (Diehl et al., 1965) using 0.2 μM bathophenanthrolinedisulfonic acid, 0.1 μM $FeCl_3$ and 0.01 μM of the galloyl derivatives. Bathophenanthrolinedisulfonic acid and $FeCl_3$ were incubated for 5 min followed by the addition of the galloyl derivatives. A_{535nm} was monitored continuously for 1.5 and 15 min for pH 3.0 and 7.0, respectively. These incubation times fall within the period of linear increases in absorbance for each pH. Fe^{2+} concentration was calculated from a standard curve using $FeSO_4 \cdot 7H_2O$.

The ability of the galloyl derivatives to influence the binding of iron to emulsion droplets was determined by measuring changes in ζ potential (Mei et al., 1998a). SDS- and Brij-stabilized hexadecane emulsions were diluted 500-fold into acetate/imidazole buffer (10 mM each; pH 3.0 and 7.0) and were then mixed with $FeSO_4 \cdot 7H_2O$ (500 μM) followed by the galloyl derivatives. The ζ potential of the emulsions were determined by injection into a Malvern ZEM5002 Zetamaster (Worcestershire, U.K.).

The partitioning of the galloyl derivatives (500 μM) was determined after incubation of the emulsions at 22 °C for 1 h (preliminary tests showed that equilibrium had been reached prior to this time period). The emulsion was then centrifuged at 2000*g* at room temperature for 1.5 h, and approximately 0.5 mL of the continuous phase of the emulsion from the bottom of the centrifuge tubes was removed using a syringe. Removal of the emulsion droplets from the continuous phase of the emulsion by centrifugation was determined by light scattering to be ≥99%. Samples (50 μL) were then mixed into 1 mL of methanol/butanol (2:1), and absorbance was measured from 650 to 250 nm against a blank containing 1 mL of methanol/butanol (2:1) and 50 μL of the continuous phase of an emulsion without the galloyl derivatives. Concentration of the galloyl derivatives in the aqueous phase was determined as $A_{peak} - A_{650nm}$. The A_{peak} for gallic acid, gallamide, and methyl gallate were 265, 270, and 275 nm, respectively. A_{650nm} was used as a correction for any potential light scattering caused by surfactant micelles. Continuous phase concentrations were expressed on a percentage basis using the absor-

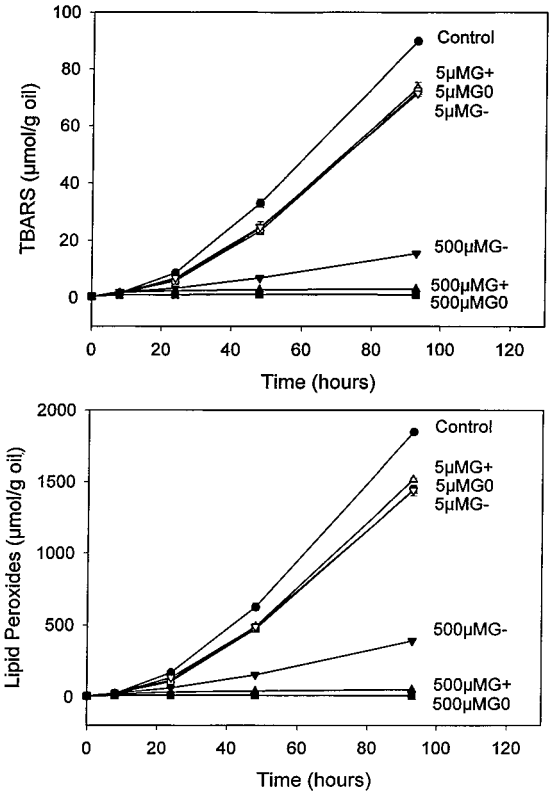

Figure 2. Oxidative stability of SDS-stabilized salmon oil (2.0%) emulsions at pH 7.0 in the presence and absence of gallic acid (G−), methyl gallate (G0), and gallamide (G+). Lipid oxidation was measured using (a) thiobarbituric acid reactive substances (TBARS) and (b) lipid peroxides.

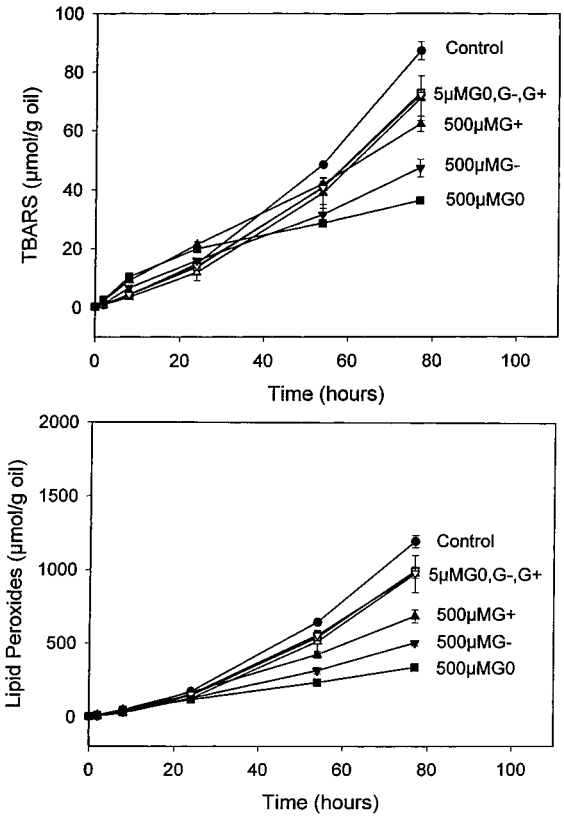

Figure 3. Oxidative stability of SDS-stabilized salmon oil (2.0%) emulsions at pH 3.0 in the presence and absence of gallic acid (G−), methyl gallate (G0), and gallamide (G+). Lipid oxidation was measured using (a) thiobarbituric acid reactive substances (TBARS) and (b) lipid peroxides.

bance of a 500 μM solution (no emulsion droplets) of the respective galloyl derivative as 100%.

All data represent averages of duplicate measurements of two samples in two separate trials.

RESULTS

Effect of Surfactant Type on Lipid Oxidation. The influence of the interfacial ionic characteristics of oil droplets on lipid oxidation rates was determined in salmon oil-in-water emulsions stabilized by anionic (SDS) and nonionic (Brij) surfactants. Dispersion of the lipid by sonication was carefully controlled in order to produce emulsion droplets of similar sizes (0.94−1.01 μm). SDS-stabilized emulsions were less oxidatively stable than Brij stabilized emulsions (Figures 2−5). Compared to the Brij-stabilized emulsion, the SDS-stabilized emulsion had TBARS concentrations that were 4.8- and 6.0-fold greater at pH 3.0 and 7.0, respectively, and lipid peroxide concentrations that were 13- and 19-fold greater at pH 3.0 and 7.0, respectively, after 48−54 h of oxidation. Lipid peroxide formation in the SDS-stabilized emulsions was greater at pH 3.0 than at 7.0 while TBARS formation was similar at both pHs (Figures 2 and 3). A pH dependence of oxidation was also observed in the Brij-stabilized emulsions with higher oxidative stability being observed at pH 3 than 7 as determined by both TBARS and lipid peroxides (Figures 4 and 5).

Effect of Galloyl Derivatives and Surfactant Type on Lipid Oxidation. In the SDS-stabilized emulsion at pH 7.0, 5 μM of the galloyl derivatives was able to decrease TBARS and lipid peroxides 22−29% after 48−54 h of oxidation with little or no apparent differences among the galloyl derivatives (Figure 2). When the concentration of galloyl derivatives was increased to 500 μM, lipid oxidation rates decreased dramatically with inhibition of TBARS formation ranging 88−97% and inhibition of lipid peroxide formation ranging 75−98% after 48−54 h of oxidation. At 500 μM concentrations, differences in the effectiveness of the different galloyl derivatives became apparent with gallic amide and methyl gallate being more effective than gallic acid.

At pH 3.0, 5 μM of the galloyl derivatives was found to inhibit TBARS and lipid peroxide formation throughout the entire oxidation period (Figure 3). When 500 μM of the galloyl derivatives was tested at pH 3.0, the overall antioxidant activity of the galloyl derivatives was less than pH 7.0, and the galloyl derivatives were found to initially accelerate TBARS formation (1.4-, 1.3-, and 1.1-fold higher after 24 h oxidation for gallamide, methyl gallate, and gallic acid, respectively). After 48 h of oxidation, the galloyl derivatives inhibited TBARS formation (13−41%). The galloyl derivatives decreased lipid peroxide formation in the SDS-stabilized emulsion at pH 3.0 (34−64% inhibition); however, this decrease was much less than occurred at pH 7.0 (75−98% inhibition).

In the Brij-stabilized emulsion at pH 7.0, all of the galloyl derivatives inhibited TBARS and lipid peroxide formation at both 5 and 500 μM (Figure 4). The order of effectiveness at 5 μM was gallic acid < gallamide < methyl gallate. Addition of galloyl derivatives at 500 μM resulted in complete inhibition of TBARS and lipid peroxide formation. At pH 3.0, gallic acid and gallamide were strong prooxidants at 5.0 μM increasing TBARS

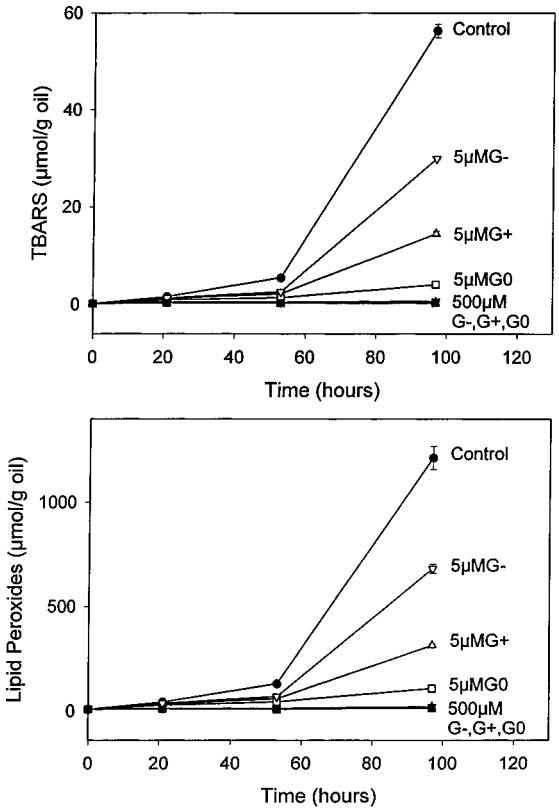

Figure 4. Oxidative stability of Brij-stabilized salmon oil (2.0%) emulsions at pH 7.0 in the presence and absence of gallic acid (G−), methyl gallate (G0), and gallamide (G+). Lipid oxidation was measured using (a) thiobarbituric acid reactive substances (TBARS) and (b) lipid peroxides.

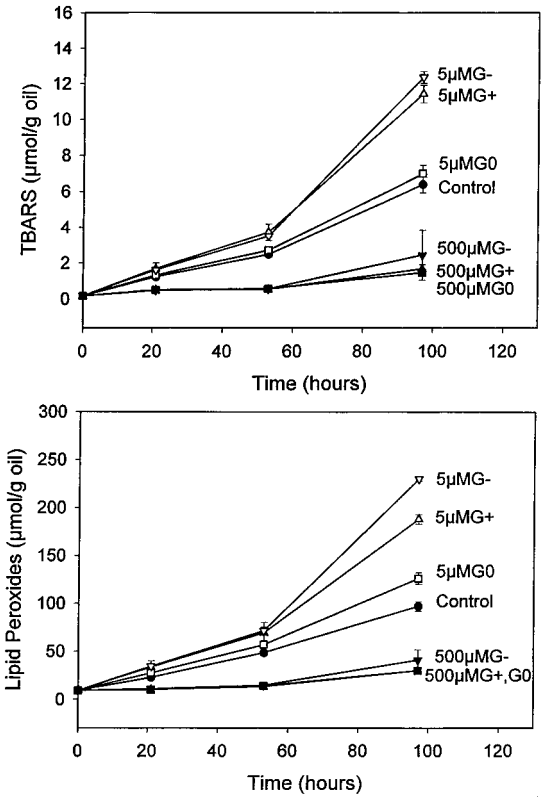

Figure 5. Oxidative stability of Brij-stabilized salmon oil (2.0%) emulsions at pH 3.0 in the presence and absence of gallic acid (G−), methyl gallate (G0) and gallamide (G+). Lipid oxidation was measured using (a) thiobarbituric acid reactive substances (TBARS) and (b) lipid peroxides.

and lipid peroxides 1.9−2.6-fold after 97 h of oxidation (Figure 5). Methyl gallate (5 μM) was slightly prooxidative, increasing TBARS 1.1-fold and lipid peroxides 1.3-fold. At 500 μM, pH 3.0, inhibition of TBARS and lipid peroxides was observed with little differences occurring among the galloyl derivatives.

Free-Radical Scavenging Activity of Galloyl Derivatives. The ability of galloyl derivatives to scavenge peroxyl radicals was tested in a nonlipid system using the AAPH-initiated fluorescent decay of β-PE as a marker of free-radical attack. All of the galloyl derivatives were strong free-radical scavengers with concentrations as low as 0.1 μM showing significant inhibition at pH 3.0 and 7.0 (Table 1). At both pH 3.0 and 7.0, gallic acid was the best radical scavenger while methyl gallate and gallamide had similar scavenging activity.

Iron Reducing Activity of Galloyl Derivatives. Phenolics are capable of reducing iron, thus increasing their prooxidant activity (Moran et al., 1997). The ability of the galloyl derivatives to reduce ferric ions was determined using bathophenanthrolinesulfonic acid, a ferrous ion specific chromophore (Diehl et al., 1965). All of the galloyl derivatives were able to reduce Fe^{3+} (Table 1). Iron reducing activity increased with decreasing pH with 44−55-fold more iron being reduced per min at pH 3.0 than 7.0. Increasing iron reduction with decreasing pH also occurs with soybean phenolics (Moran et al., 1997). Fe^{3+} reducing rates at pH 3.0 were gallic acid > methyl gallate > gallamide, while pH 7.0 was gallic acid > methyl gallate = gallamide.

Ability of Galloyl Derivatives To Alter Emulsion Droplet Charge and Iron-Emulsion Droplet Interactions. Addition of the galloyl derivative to SDS- and Brij-stabilized hexadecane emulsion had no effect on droplet charge (Table 2). The pyrogallol and the catechol (*ortho*-dihydroxybene) groups of phenolics have iron-binding capacity (Moran et al., 1997; Brune et al., 1991). Since iron associated with emulsion droplets is a strong prooxidant and compounds which decrease iron-emulsion droplet interaction (e.g., chelators) effectively inhibit lipid oxidation (Mei et al., 1998a), phenolics could potentially inhibit lipid oxidation via interactions with iron. The extent of iron interactions with emulsion droplets can be determined by changes in ζ potential (Mei et al., 1998a). $FeCl_2$ (500 μM) reduced the net negative charge (made the ζ potential more positive) of the SDS-stabilized emulsion droplets, indicating iron-surfactant interactions (Table 2). $FeCl_2$ had no influence on the weak negative charge of the Brij-stabilized emulsion droplets, suggesting no iron-surfactant interactions. None of the galloyl derivatives (500 μM) were able to alter the association of Fe^{2+} (500 μM) with the SDS-stabilized emulsion droplets.

Table 1. Ability of the Galloyl Derivatives To Scavenge Peroxyl Radicals [As Determined by the 2,2′-Azobis-(2-amidinopropane) Dihydrochloride Initiated Decay of *Porphyridium cruentum* β-Phycoerythrin Fluorescence] and Reduce Fe^{3+} (As Determined Spectrophotometrically Using Bathophenanthrolinedisulfonic Acid)

	peroxyl radical scavenging (% inhibition)		Fe^{3+} Reduction (μM/min)	
	pH 3.0	pH 7.0	pH 3.0	pH 7.0
control	0	0	0	0
gallamide	34 ± 8	9 ± 3	7.95 ± 0.57	0.18 ± 0.08
methyl gallate	41 ± 7	10 ± 5	9.56 ± 0.31	0.17 ± 0.03
gallic acid	49 ± 2	15 ± 3	11.6 ± 0.89	0.24 ± 0.05

Table 2. Ability of the Galloyl Derivatives (500 μM) To Alter Emulsion Droplet Charge and Interactions between Fe^{2+} (500 μM) and Emulsion Droplets As Determined by Changes in ζ Potential[a]

	ζ potential (mV)	
	SDS-stabilized emulsion	Brij-stabilized emulsion
emulsion alone	−105.0 ± 1.0	−2.9 ± 0.1
emulsion + Fe^{2+}	−94.0 ± 0.7	−2.6 ± 0.2
emulsion + gallamide	−105.8 ± 1.3	−3.1 ± 0.1
emulsion + gallamide + Fe^{2+}	−92.4 ± 1.3	−3.0 ± 0.1
emulsion + methyl gallate	−105.4 ± 0.3	−3.0 ± 0.1
emulsion + methyl gallate + Fe^{2+}	−94.4 ± 0.4	−2.9 ± 0.1
emulsion + gallic acid	−106.2 ± 0.3	−3.1 ± 0.1
emulsion + gallic acid + Fe^{2+}	−95.0 ± 0.4	−2.9 ± 0.1

[a] Emulsions were stabilized with sodium dodecyl sulfate (SDS) and polyoxyethylene 10 lauryl ether (Brij).

Table 3. Relative Concentrations (%) of the Galloyl Derivatives in the Continuous Phase of Sodium Dodecyl Sulfate (SDS)- and Polyoxyethylene 10 Lauryl Ether (Brij)-Stabilized Hexadecane Emulsions

	SDS emulsion		Brij emulsion	
	pH 3.0	pH 7.0	pH 3.0	pH 7.0
gallamide	100 ± 0	97 ± 3	98 ± 0	90 ± 7
methyl gallate	98 ± 0	101 ± 0	93 ± 0	93 ± 1
gallic acid	102 ± 0	99 ± 1	91 ± 7	98 ± 4

Physical Interactions between the Galloyl Derivatives and Emulsion Droplets. The ability of the galloyl derivatives to physically interact with the emulsions was determined by centrifugal separation of emulsion droplets from the continuous phase of the emulsion. In this method, surfactant micelles would not be separated and thus would be found in the continuous phase; therefore, partitioning values only represent association of the galloyl derivatives with the emulsion droplets. Table 3 shows that galloyl derivatives did not associate with SDS-stabilized emulsion droplets at pH 3.0 or 7.0. In the Brij-stabilized emulsions, the galloyl derivatives were found to associate with emulsion droplets and micelles, and this association was dependent on the type of galloyl derivative and pH. At pH 7.0, methyl gallate (7%) and gallamide (10%) both associated with the Brij-stabilized emulsion droplet while gallic acid did not. At pH 3.0, methyl gallate (7%) and gallic acid (9%) but not gallamide was observed to associate with the emulsion droplets.

DISCUSSION

Factors Influencing the Ability of Surfactants and Galloyl Derivatives To Accelerate Lipid Oxidation. The oxidative stability of emulsion droplets is determined by factors which influence the activity of both prooxidants and antioxidants. Previous results from a corn-oil emulsion where oxidation was accelerated with iron and ascorbic acid showed that anionic (SDS) emulsion droplets oxidized faster than nonionic (Brij) or cationic (DTAB) droplets (Mei et al., 1998b). Transition metals such as iron also seem to be important prooxidants in the salmon oil emulsions used in these experiments since the SDS-stabilized emulsion was less oxidative stable than the Brij-stabilized emulsions (Figures 2-5). Evidence that transition metals are active prooxidants in the salmon oil emulsions used in these experiments is also supported by previous work which showed that EDTA inhibits oxidation in the absence of added iron (Mei et al., 1998a).

Galloyl derivatives were found to be strong reducing agents at pH 3.0 (Table 1) which would likely increase prooxidant activity of iron since Fe^{2+} is more reactive than Fe^{3+} (Dunford, 1987). The strong metal reducing potential of the galloyl derivatives explains why they are observed to be less effective antioxidants at low pH in both the Brij- and SDS-stabilized salmon oil emulsions (Figures 3 and 5), an observation which has also been reported with the phenolic antioxidant, Trolox, whose activity decreases with decreasing pH in a Tween 20-stabilized corn oil emulsion (Huang et al., 1996).

The physical location of the galloyl derivatives could also influence their ability to increase the prooxidant activity of metals. Iron at or near the surface of emulsion droplets is strongly prooxidative (Mei et al., 1998a). Gallamide was not observed to directly bind to the SDS-stabilized emulsions as determined in the partitioning studies (Table 3). However, this does not mean that electrostatic attraction would not concentrate gallamide near the emulsion droplet surface (without direct binding to the emulsion surface). Concentration of gallamide near the emulsion droplet surface could result in increased reduction of iron associated with the emulsion droplet, especially at low pH where the metal reducing potential of gallamide is high. The iron reduction by gallamide near the surface of the SDS-stabilized emulsion droplets could explain why it was less effective at inhibiting oxidation than gallic acid and at pH 3.0.

Factors Influencing the Ability of Galloyl Derivatives To Inhibit Lipid Oxidation. Phenolics can be effective antioxidants due to their ability to scavenge free radicals (Nawar, 1996) and to chelate metals (Moran et al., 1997). The galloyl derivatives were not strong enough chelators to remove iron from the surface of the SDS-stabilized emulsion droplets, indicating that they do not inhibit interactions between iron and emulsion droplets (Table 2). The galloyl derivatives were capable of scavenging free radicals with gallic acid having slightly higher peroxyyl radical scavenging than gallamide and methyl gallate (Table 1). One of the major factors which influences the effectiveness of phenolics in lipid emulsions is their partitioning behavior, with increasing association between the phenolics and the emulsion droplets resulting in increasing antioxidant activity (Huang et al., 1997). At pH 7.0, the galloyl derivatives were not observed to associate with the SDS-stabilized emulsion droplet (Table 3), suggesting that the interfacial surface packing properties of SDS could be physically preventing the galloyls from entering the emulsion droplets or that the partial negative charge of the galloyls was preventing association by electrostatic repulsion. Additionally, phenolics can partition into Tween micelles (Huang et al., 1997), suggesting that they may be soluble in the surfactant headgroups. Lack of partitioning of galloyl derivatives into the SDS-stabilized emulsion droplets could be due the small headgroup size of SDS which is unable to solubilize the phenolics.

Gallamide was not observed to associate with the SDS-stabilized emulsions droplets at pH 3.0 (centrifugation method and ζ potential; Tables 2 and 3) or 7.0 (centrifugation method; Table 3). The inability of gallamide to associate with the SDS-stabilized emulsion droplets suggests that gallamide does not bind to SDS through electrostatic interactions. This could be due to the low charge density of gallamide which does not allow it to compete effectively with other cations (e.g., Na^+)

present in the emulsion. Lack of gallamide binding to negatively charged emulsion droplets may explain why its activity did not increase dramatically in the SDS-stabilized emulsion compared to other published reports were the antioxidant activity of ascorbate, spermine, and Trolox was dramatically increased in the presence of oppositely charged lipid dispersions (Pryor et al., 1993; Kogure et al., 1993; Barclay and Vinqvist, 1993).

The galloyl derivatives were able to partition into the Brij-stabilized emulsion droplets (Table 3). Association of the phenolics with lipids and surfactants increases with increasing hydrophobicity (Huang et al., 1997). The partitioning behavior of gallic acid and gallamide changed dramatically with pH with gallic acid partitioning into the Brij-stabilized emulsion droplets only at pH 3.0 and gallamide partitioning only being observed at pH 7.0. These changes in partitioning behavior are likely due to the increased protonation and thus hydrophobicity of gallic acid at low pH and the decreased protonation and thus hydrophobicity of gallamide at high pH. Methyl gallate, whose hydrophobicity would not be influence by pH, exhibited similar partitioning behaviors at both pH 3.0 and 7.0.

The influence of the galloyl derivatives on the oxidative stability of the salmon oil emulsions is a balance between their ability to increase the prooxidant activity of transition metals and scavenge free radicals. Therefore, it is not surprising that the galloyl derivatives (5 μM) were less effective antioxidants and even prooxidants at pH 3.0 where their ability to increase the prooxidative activity of metals is high. The free-radical scavenging activity of the galloyl derivatives can overcome its prooxidative activity resulting in antioxidant activity at high concentrations (500 μM). This could be due to the fact that maximum iron reduction rates had been reached with the excess galloyl derivatives scavenging any radicals generated by the reduced iron. The ability of the galloyl derivatives to inhibit lipid oxidation generally increased as their association with the emulsion increased. The low amount of association of the galloyl derivatives with the SDS-stabilized emulsion droplets could explain why the galloyl derivatives did not inhibit lipid oxidation as effectively as observed in the Brij-stabilized emulsions (Figures 2−5).

Methyl gallate's partitioning behavior was equal to or better than the ionic galloyls at both pH 3.0 and 7.0 in the Brij-stabilized emulsion. When the effectiveness of the galloyl derivatives were compared in the Brij-stabilized emulsions at 5.0 μM (comparison at 500 μM were difficult since the galloyl derivatives almost completely inhibited lipid oxidation), methyl gallate was consistently observed to have the lowest prooxidant (e.g., 5 μM, pH 3.0) or highest antioxidant (e.g., 5 μM, pH 7.0) activity. At pH 7.0, gallamide, which partitioned into the emulsion, was more effective than gallic acid. At pH 3.0, no significant differences in the activity of gallic acid and gallamide was observed even though gallic acid partitioned into the emulsion. Lack of differences between gallic acid and gallamide at pH 3.0 could be due to the stronger iron reducing potential of gallic acid at pH 3.0 (Table 1) which would increase the prooxidant activity of metals and thus could mask the ability of gallic acid to inhibit lipid oxidation by scavenging free radicals in the lipid phase of the emulsion.

It should be noted that the galloyl derivatives were able to inhibit lipid oxidation even when they did not associate with the emulsions [e.g., all galloyl derivatives at pH 7.0 in the SDS-stabilized emulsion; gallic acid (5 and 500 μM) at pH 7.0 and gallamide (500 μM) at pH 3.0 in the Brij-stabilized emulsion]. The ability of the galloyl derivatives to inhibit oxidation without associating with the emulsions suggests that radical scavenging in the aqueous phase also results in the protection of emulsified lipids.

CONCLUSIONS

Phenolics are commonly used as food additives to inhibit lipid oxidation. In recent years, numerous publications have touted the antioxidant benefits of phenolics from natural plant sources. However, the antioxidant potential of these compounds in foods is difficult to predict because the structure−function relationship of phenolic antioxidants is unclear.

This research shows that several factors influence the ability of phenolic antioxidants to inhibit lipid oxidation in emulsions. In the emulsion systems studied in these experiments, the ability of structurally similar phenolic antioxidants to increase the reactivity of prooxidant metals and partition into the emulsion droplets where they can scavenge free radicals seem to be the most important factors in predicting the antioxidant activity of phenolics. Therefore, the observed antioxidant activity of phenolics in oil-in-water emulsions actually represents the net sum of its prooxidative (metal reduction) and antioxidative (free radical scavenging) activity. This prooxidative/antioxidative balance is highly dependent on pH since the metal reducing capacity of phenolics increases dramatically with decreasing pH. Evaluation of the prooxidative/antioxidative balance of phenolics could provide useful information in predicting their antioxidant behavior in lipid dispersions.

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