The Influence of Various Emulsifiers on the Partitioning and Antioxidant Activity of Hydroxybenzoic Acids and Their Derivatives in Oil-in-Water Emulsions

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ABSTRACT: The partitioning behavior of a series of hydroxybenzoic acids and their derivatives was determined in biphasic water-oil systems, emulsifier solutions, and oil-in-water (O/W) emulsions. The effect of gradually decreasing polarity on partitioning behavior and antioxidant efficiency in O/W emulsions was investigated by using gallic acid and its esters as antioxidants. Sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), polyoxyethylene 20 cetyl ether (Brij 58), and partially hydrolyzed soybean lecithin (PHLC, Emultop®) were used to investigate the influence of different classes of emulsifiers on the partitioning behavior. The antioxidant activity of gallic acid and its methyl, ethyl, propyl, butyl, and octyl esters showed markedly different trends in O/W emulsions depending on the emulsifier used. The results are discussed with respect to the properties of the emulsifiers, such as hydrogen bond basicity, hydrophobic interactions, and structural properties.

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KEY WORDS: Antioxidant, hydroxybenzoic acid, gallates, emulsifier, SDS, CTAB, Brij 58, phospholipid, emulsion, partitioning.

Oil-in-water (O/W) emulsions are a major type of dispersed lipid system in foods, cosmetics, and pharmaceutical products. Antioxidants are frequently employed to prevent lipid oxidation, but factors that may influence their activity in emulsions are poorly understood. In some studies (1–4), partitioning of the antioxidants between the water and lipid phases is related to their effectiveness. Also there are some indications that the emulsifier may have a strong influence on antioxidant activity in dispersed lipid systems (4,5). Due to their amphiphilic nature, emulsifiers accumulate at oil-water interfaces where oxidation is considered to occur (6,7). It has been shown that solubilization of the antioxidant by the emulsifier in emulsions is strongly dependent on the type of emulsifier (8). Thus, the proportion of the antioxidant associated with the emulsifier at or near the lipid surface will vary widely. This may in turn determine the effectiveness of chain-breaking antioxidants, as they are considered to exert their activity at or close to the lipid surface (4).

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The difference in the antioxidant activity using emulsifiers with positive, negative, or amphiphilic head groups was studied in phospholipid liposome-containing systems (4) and in systems containing linoleic acid emulsified by cetyltrimethylammonium bromide (CTAB) or sodium dodecyl sulfate (SDS) (5). Both systems showed a decrease in antioxidant activity related to the repulsive effect of negatively charged head groups and an increase in antioxidant activity related to the attractive force of positively charged head groups, as these different forces may lead either to an increase or to a decrease, respectively, in the antioxidant concentration at the water–lipid interface. Differences, however, were not compared with the partitioning behavior and were not discussed in view of the nature of the hydrogen bonds, which contribute to the solubilization capacity of the emulsifier. Several authors (9–13) suggested that cationic emulsifiers may act as hydrogen bond acceptors *via* their negative counter-ion. This may explain the attracting force of the emulsifiers toward polar phenolic antioxidants, which act as hydrogen bond donors resulting in a lower hydrogen abstraction kinetic rate. The influence of different solvents on the hydrogen donation activity was demonstrated in several studies (14–16). Valgimigli *et al.* (15) hypothesized that the solvent molecules forming a complex with the hydroxylic H-atom of the antioxidant must first be removed and replaced by a radical. In this way, strong interactions of the solvent molecules with the antioxidant prevent abstraction of the hydrogen.

The polarity of the antioxidants in dispersed lipid systems was considered by Porter (17) to determine their activity. They observed a higher activity of nonpolar compared to polar antioxidants in phospholipid-containing dispersed systems and named this effect the polar paradox. Conversely, polar antioxidants showed higher activity than nonpolar antioxidants in bulk oils. Based on these results, the partitioning behavior of antioxidants was investigated in emulsions containing a polyoxyethylated emulsifier (Tween 20) and related to the difference in antioxidant activity (1–3). The results supported the polar paradox. However, the comparison was based only on the difference in polarity of two antioxidant analogs, such as Trolox and α-tocopherol, carnosic acid and methyl carnosate, and ascorbic acid and ascorbyl palmitate.

This study is aimed at systematically investigating the re-

lationship between the partitioning behavior of antioxidants and their antioxidant activity in O/W emulsions. The effect of different emulsifiers was tested with antioxidants of gradually decreasing polarity, as opposed to previous studies where two antioxidant analogs of different polarities were investigated. In contrast to previous studies using mass balance (1,2,8), a mathematical model was employed to accurately determine the proportion of antioxidant solubilized in the oil and by the emulsifier in the lipid phase of the emulsion (18).

EXPERIMENTAL PROCEDURES

Materials. SDS, polyoxyethylene 20 cetyl ether (Brij 58), gallic acid, propyl gallate, sodium acetate (anhydrous), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma (St. Louis, MO). CTAB, methyl gallate, ethyl gallate, butyl gallate, octyl gallate, *p*-hydroxybenzoic acid methyl ester (PHBME), and *p*-hydroxybenzoic acid ethyl ester (PHBEE) were purchased from Fluka (Buchs, Switzerland). 3,4-Dihydroxybenzoic acid ethyl ester (DHBEE) and 3,4-dihydroxybenzoic acid (DHB) were obtained from Aldrich (Steinheim, Germany). Sudan red B and ferrous (II) chloride-hexahydrate were obtained from Merck (Darmstadt, Germany). *p*-Hydroxybenzoic acid (PHB), ethanol, *n*-hexane, isopropanol, ethyl acetate, and acetic acid were of analytical grade from Carl Roth (Karlsruhe, Germany). Partially hydrolyzed soybean lecithin (PHLC; Emultop®, 27–30% hydrolyzed) was a gift from Lucas Meyer (Hamburg, Germany). Barium chloride dihydrate and ammonium thiocyanate were obtained from Riedel-de Haën (Hannover, Germany). Commercial corn oil was used for partitioning studies, and corn oil stripped of tocopherol (Acros, Gelnhausen, Germany) was used for oxidation experiments. The water used was deionized with a conductivity of <5 μS.

Preparation of O/W emulsions for oxidation. Emulsifiers were dissolved in 20 mL of acetate buffer (0.2 M, pH 5). After addition of the oil, emulsification was carried out by sonication with a Sonoplus HD 200 from Bandelin (Berlin, Germany) equipped with an MS73 probe (90% pulse, 200 W, 20 kHz, 40%). After 60 s of sonication, the remaining acetate buffer was added in portions of 5 mL to a final volume of 50 mL. The antioxidants were added from stock solutions to a final concentration of 100 μmol kg^{-1} related to the oil. Afterward, the emulsion was sonicated for 30 s resulting in 2 min of sonication time in total. For Brij 58 emulsions, a small amount of the buffer containing the emulsifier was heated to approximately 70°C to solubilize the emulsifier. After cooling to room temperature, the emulsions were prepared as described previously.

Oxidation. The emulsions were stored at 37°C in sealed glass vessels for 3–4 wk in the dark. The oxidation process was monitored by measuring the concentration of conjugated dienes, hydroperoxides, and hexanal at regular intervals.

Hydrogen donation ability. The absorbance at 516 nm of 2470 μL 0.1 mM ethanol solution of DPPH was measured before and 15 min after adding 30 μL of a 1 mM ethanolic antioxidant solution. The decrease in absorbance is stoichiometric with respect to the number of electrons taken up (19,20).

Determination of hydroperoxides. The concentration of hydroperoxides was determined by measuring the concentration of conjugated dienes (CD) and by color reaction with thiocyanate and ferrous (II). CD were measured at 234 nm in isopropanol and hydroperoxide concentration was calculated using a molar extinction coefficient for methyl linoleate hydroperoxides of 26,000 (21). Determination of the hydroperoxide content by color reaction with thiocyanate and ferrous(II) was carried out according to Pardun (22) but used isopropanol instead of a benzene/methanol mixture as the solvent.

Determination of hexanal. The formation of hexanal was measured by static headspace gas chromatography (HSGC) using the same parameters as described by Frankel *et al.* (23). Before injection, the samples were incubated for 15 min at 60°C.

Particle size determination. Physical stability of the emulsions was tested by particle size measurement based on dynamic light scattering (Zetaplus; Brookhaven, Holtsville, NY).

Zeta potential. The emulsions were diluted 1:5000 with acetate buffer (1 mM, pH 5) prior to determination of the zeta potential (Zetaplus, Brookhaven).

Partitioning behavior. The details are described elsewhere (18). The partitioning behavior was determined using ultrafiltration (SDS-, CTAB-, and Brij 58-containing systems) and dialysis (PHLC systems). A small proportion of the aqueous phase was separated from emulsions, emulsifier solutions, or biphasic water-oil systems by ultrafiltration. The antioxidant concentration of the filtrate was measured by high-performance liquid chromatography (HPLC). After dialysis, the concentration in the aqueous phase was determined by the difference in hydroxybenzoic acid concentration between the two compartments separated by a membrane. The proportions of the antioxidant solubilized by the oil and by the emulsifier in the lipid phase of the emulsion were computed with a mathematical model (18).

Statistical analysis. All oxidation experiments were carried out at least twice, and samples were analyzed in triplicate. Each oxidation experiment showed the same trend and the standard deviations (SD) are within-run SD. One-way analysis of variance and Fisher's comparison tests, at a significance level of 0.05, were calculated using a Minitab software program (Addison-Wesley Publishing Company, Reading, MA).

RESULTS AND DISCUSSION

Our study demonstrates the influence of different emulsifiers on the partitioning behavior and antioxidant activity of hydroxybenzoic acids and their esters in emulsions. The oil-water interface appears to be particularly relevant as antioxidants functioning as effective chain-breakers are considered to exert their activity near or at the oil droplet surface (4). Due to their amphiphilic properties, emulsifiers accumulate at the oil-water interface in emulsions and thus are considered to dominate the properties of the interface in terms of solubilization capacity for antioxidants.

Surface properties and droplet size of emulsions. The charge at the surface, formed by the emulsifier surrounding the oil droplets, is characterized by the zeta potential (24,25). For emulsions containing 20% oil and 1% emulsifier, the zeta potential was, as expected, negative for SDS (–57 mV), slightly negative for Brij 58 emulsions (–13 mV), and positive for CTAB emulsions (+54 mV). For PHLC emulsions a negative potential (–57 mV) was obtained, which may be attributable to partial hydrolysis (27–30%) of the phospholipids (soybean lecithin). The addition of gallates did not change the zeta potential. The mean oil droplet diameter was around 580 nm for SDS and CTAB, 650 nm for Brij 58, and 1,200 nm for PHLC emulsions and remained unchanged during storage for 3 wk at 37°C.

Partitioning behavior of hydroxybenzoic acid derivatives. The partitioning behavior of hydroxybenzoic acids was investigated in emulsions containing 1% SDS and 20% oil (Table 1). The proportion of antioxidant solubilized in the lipid phase ($[S]_{Lipid,Em.}$) of SDS emulsions was smaller than the sum of that solubilized in the oil phase ($[S]_{Oil}$) of the biphasic water-oil system and that incorporated into SDS micelles ($[S]_{Mic.}$) of the SDS solution. Provided that the emulsifier is predominantly localized at the oil-water interface and that there is only a small amount of SDS micelles in the aqueous phase, most of the hydroxybenzoic acid derivatives associated with the emulsifier are located at or near the oil-water interface. The partition behavior strongly depends on the polarity of the antioxidant, which is governed by its structural properties. The aromatic ring and the alkyl chain cause a decrease in polarity whereas the hydroxyl and carboxyl groups exert an opposite effect (26). Consequently, with an increasing number of hydroxyl groups the proportion of hydroxybenzoic acid derivatives in the aqueous phase of biphasic water-oil systems increases. When the alkyl chain increases, the compounds become more lipophilic, resulting in an increase in the proportion solubilized in the oil phase. The antioxidant proportion in the lipid phase in all three systems (i.e., biphasic water-oil systems, emulsifier solutions, and emulsions) was in the same order. This indicates that in SDS emulsions the partitioning behavior is largely determined by the polarity of the antioxidant and particularly by the length of the alkyl chain. Fewer hydroxybenzoic acid derivatives were solubilized in the lipid phase of emulsions compared to the sum of the proportion solubilized in oil and by the emulsifier in the two-component systems (Table 1). This is because of the equilibrium of hydrophobic interactions between oil and emulsifier with the antioxidant. Thus, the driving force for transferring the antioxidant from the aqueous phase into the lipid phase is reduced. It is interesting that the proportion of butyl gallate and DHBEE solubilized in the oil phase of biphasic water-oil system was in the same range. However, the proportion of butyl gallate associated with SDS micelles was higher than that of DHBEE (Table 1). This may be due to stronger hydrophobic interactions of SDS with increasing alkyl chain length of the gallates.

TABLE 1
Partitioning of Hydroxybenzoic Acid Derivatives in 1% SDS Solution, Biphasic Water–Oil Systems (20% oil), and O/W Emulsions (20% oil, 1% SDS)[a]

	Proportion [% ± SD][b]		
Antioxidant	$[S]_{Mic.}$	$[S]_{Oil}$	$[S]_{Lipid,Em.}$
Gallic acid	< 1	< 0.5	< 1
Methyl gallate	30.1 ± 0.91	2.8 ± 0.76	31.6 ± 0.96
Ethyl gallate	49.8 ± 0.66	5.9 ± 1.26	50.4 ± 0.79
Propyl gallate	67.6 ± 1.17	24.1 ± 0.69	71.5 ± 0.39
Butyl gallate	82.4 ± 0.34	49.6 ± 0.70	86.2 ± 0.24
DHB	< 1	< 0.5	< 1
DHBEE	66.6 ± 0.39	53.1 ± 0.79	76.0 ± 0.32
PHB	5.0 ± 0.52	0.7 ± 0.49	1.4 ± 1.06
PHBME	58.3 ± 0.50	69.5 ± 0.27	78.9 ± 0.33
PHBEE	75.2 ± 0.35	85.6 ± 0.23	91.7 ± 0.16

[a]$[S]_{Oil}$, proportion [%] of antioxidant in the oil phase of water–oil system; $[S]_{Lipid,\ Em}$, proportion [%] of antioxidant in the lipid phase of O/W emulsion; $[S]_{Mic}$, proportion [%] of antioxidant in the micellar pseudophase of SDS solution. Concentration of the micellar pseudophase ([SDS]) = SDS concentration total ($[SDS]_{Total}$) – SDS concentration at critical micellar concentration (CMC, $[SDS]_{CMC}$); SDS concentration at CMC corresponds to 0.02%; DHB, 3,4-dihydroxybenzoic acid; DHBEE, 3,4-dihydroxybenzoic acid ethyl ester; PHB, *p*-hydroxybenzoic acid; PHBME, *p*-hydroxybenzoic acid methyl ester; PHBEE, *p*-hydroxybenzoic acid ethyl ester; SDS, sodium dodecyl sulfate; O/W, oil-in-water.
[b]*n* = 4; SD = standard deviation.

Partition behavior of gallates in SDS emulsions. Table 2 shows the partitioning behavior of gallates in SDS emulsions. The lipid phase of SDS emulsions is represented by the oil phase and the emulsifier. To differentiate between gallate solubilized by each component, a mathematical model was applied (18) that considers the mutual influence of oil and emulsifier on the solubilization capacity of the emulsion. With increasing chain length, the amounts of gallate solubilized by the oil and by SDS increased. The greater increase in solubilization of propyl gallate compared to ethyl gallate in the oil phase of the emulsion (Table 2) can be related to the higher solubilization capacity of oil compared to SDS in biphasic water-oil systems and SDS solutions (Table 1). Gallic acid was almost completely solubilized in the aqueous phase, i.e., the proportion solubilized by SDS or oil can be ignored.

Effect of different emulsifiers on the partitioning behavior. To cover a wide range of emulsifiers, the partitioning behavior of ethyl gallate and gallic acid was investigated in four dif-

TABLE 2
Influence of the Alkyl Chain on the Partitioning of Gallates Between the SDS Emulsifier Environment, Aqueous and Oil Phases in O/W Emulsions (1% SDS, 20% oil)

	Proportion of antioxidant in the (pseudo)phase [% ± SD][a]		
Antioxidant	SDS emulsifier	Oil	Aqueous
Gallic acid	< 1	Trace	> 99
Methyl gallate	29.4 ± 0.37	2.1 ± 0.23	68.5 ± 0.44
Ethyl gallate	48.9 ± 1.27	2.1 ± 0.44	49.0 ± 1.34
Propyl gallate	65.0 ± 0.58	7.0 ± 0.68	28.0 ± 0.89
Butyl gallate	76.2 ± 0.53	10.2 ± 0.70	13.6 ± 0.88

[a]*n* = 24. For abbreviations see Table 1.

TABLE 3
Influence of Different Emulsifiers on the Partitioning of Ethyl Gallate or Gallic Acid Between Emulsifier Environment, Aqueous and Oil Phase in O/W Emulsions (1% emulsifier, 20% oil)

	Proportion of antioxidant in the (pseudo)phase [% ± SD][a]			
(Pseudo)phase	PHLC[b]	SDS[b]	Brij 58[b]	CTAB
Ethyl gallate				
Emulsifier	39.5 ± 0.81	48.9 ± 1.27	66.0 ± 0.44	95.4 ± 4.72
Oil	5.5 ± 0.31	2.1 ± 0.44	5.2 ± 0.47	0.1 ± 4.78
Aqueous	55.0 ± 0.86	49.0 ± 1.34	28.8 ± 0.64	4.6 ± 6.73
Gallic acid				
Emulsifier	5.9 ± 0.71	< 1	21.8 ± 0.54	54.5 ± 0.87
Oil	Trace	Trace	Trace	Trace
Aqueous	94.1 ± 0.71	> 99	78.2 ± 0.54	45.5 ± 0.87

[a]*n* = 24, SD = standard deviation
[b]PHLC, partially hydrolyzed soybean lecithin; Brij 58, polyoxyethylene 20 cetyl ether; CTAB, cetyltrimethylammonium bromide; see Table 1 for other abbreviations.

ferent emulsions containing SDS, CTAB, Brij 58, and PHLC (Table 3). The proportion of ethyl gallate in the lipid phase increased in the order PHLC < SDS < Brij 58 < CTAB. Conversely, the concentration in the aqueous phase decreased in the same order.

The proportion of ethyl gallate solubilized in the oil phase was highest in PHLC emulsions and decreased with increasing solubilization capacity of the emulsifier, except for Brij 58.

Less than 1% of gallic acid was solubilized in the lipid phase of SDS emulsions while the solubilization capacity of the emulsifiers increased in the order PHLC < Brij 58 < CTAB (same as for ethyl gallate). Only traces of the gallic acid were solubilized in the oil phase of biphasic water-oil systems, and thus it was ignored when computing the proportion of gallic acid in emulsions.

The effect of emulsifier concentration on the partitioning behavior of ethyl gallate was examined (Table 4). Increasing the SDS and PHLC concentration from 1 to 2% increased the proportion of ethyl gallate associated with the emulsifier. In contrast, the proportion of ethyl gallate in the oil phase decreased when the amount of SDS increased, but no effect was found in PHLC emulsions. The differences are clearly related to the ability of the emulsifier to solubilize the antioxidant (18).

Hydrophobic interactions and hydrogen bonds are considered to contribute strongly to the solubilization capacity of the emulsifier. Hydrophobic interactions are likely to contribute to the same extent in the case of SDS, CTAB, and Brij 58. Differences in the hydrogen bond basicity, compared to water, give rise to the observed discrepancies in the solubilization capacity of the emulsifier (11–13).

The high concentration of gallates associated with CTAB compared to SDS (Table 3) can be attributed to the negative counter-ion (i.e., bromide) of CTAB. Unlike CTAB, the counter-ion in SDS (i.e., sodium) is positively charged, thus, the basicity follows the order CTAB > water > SDS. Interactions of the gallates with CTAB negative counter-ions are possible *via* hydrogen bonds with the hydroxyl groups of the gallates, as counter-ions of CTAB may act as hydrogen bond acceptors (10).

Interactions of CTAB with aromatic rings are weak for substituted aromatic compounds (27) and may contribute little to the observed solubilization capacity. The acidity of the solute does not appear to influence solubilization in SDS, i.e., the hydrophobic interaction between SDS and antioxidants is the main driving force for antioxidant incorporation (11).

The hydrophilic moiety of the nonionic Brij 58 is constituted by bulky polyoxyethylene chains providing a polar environment (28) due to the free electron doublets of oxygen. Therefore, Brij 58 has a higher solubilization capacity than SDS (Table 3). The polyoxyethylene chains of Brij 58 form a palisade layer, which constitutes a diffuse polar microenvironment. Quina *et al.* (11) suggested that the distinctive solubilization properties of Brij 58 are due to the polarizability of the micellar solubilization site. In addition, oxygen atoms of

TABLE 4
Influence of Emulsifier Concentration on the Partitioning Behavior of Ethyl Gallate in SDS and PHLC Emulsions[b]

	Proportion of ethyl gallate in the (pseudo)phase [% ± SD][a]			
(Pseudo)phase	1% SDS	2% SDS	1% PHLC	2% PHLC
Emulsifier	48.9 ± 1.27	65.9 ± 1.13	39.5 ± 0.81	56.1 ± 0.55
Oil	2.1 ± 0.44	0.8 ± 0.22	5.5 ± 0.31	5.4 ± 0.80
Aqueous	49.0 ± 1.34	33.3 ± 1.15	55.0 ± 0.86	38.5 ± 0.97

[a]n = 24.
[b]See Tables 1 and 3 for abbreviations.

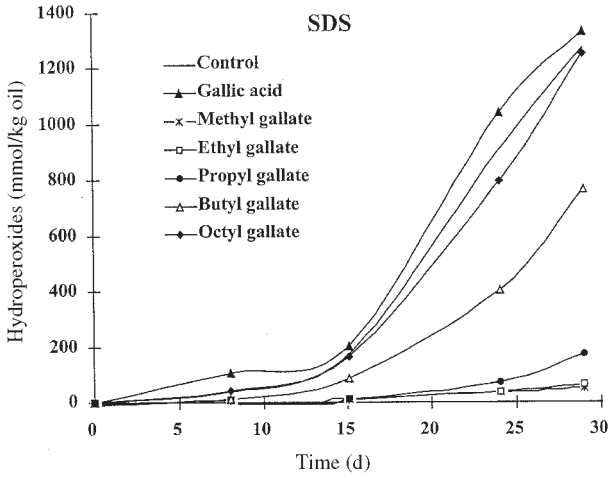

FIG. 1. Antioxidant activity of gallates in sodium dodecyl sulfate (SDS) emulsions (1% SDS; 20% stripped corn oil) at 37°C.

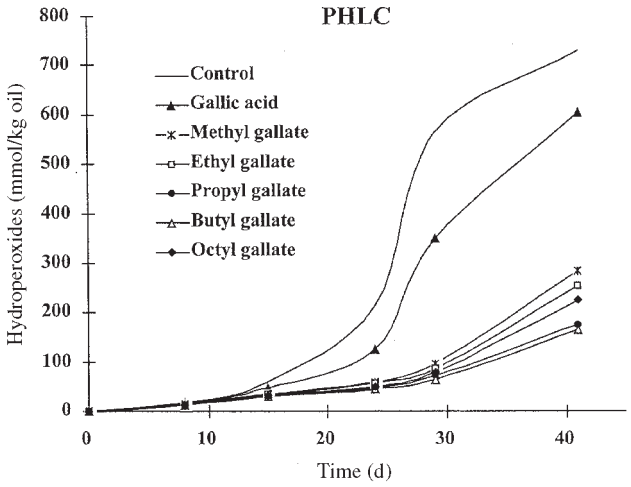

FIG. 3. Antioxidant activity of gallates in partially hydrolyzed soybean lecithin (PHLC) emulsions (1% PHLC; 20% stripped corn oil) at 37°C.

the polyoxyethylene chain are considered to take part in the formation of hydrogen bonds, as shown by enhanced hydrogen bond basicity (11,29).

PHLC showed the lowest solubilization capacity for ethyl gallate and higher solubilization capacity for gallic acid than SDS (Table 3). Water sheaths can be incorporated between the layers of lamellar structures formed by phospholipids (30) and may thus contribute to the solubilization of gallic acid despite its low lipid solubility. The zeta potential determination indicated a negative charge at the oil droplet surface, which may account for the low tendency to build hydrogen bonds with the antioxidant.

The type of emulsifier affected the solubilization capacity of the oil in the lipid phase of emulsions. The markedly lower solubilization capacity of oil for ethyl gallate in CTAB and SDS emulsions compared to biphasic water-oil systems may be due to competition between the hydrophobic interaction, which causes solubilization of ethyl gallate in oil and in the emulsifier environments (Table 3). By contrast, a slight decrease in solubility was observed in Brij 58 emulsions, which may be attributable to the polarizability of the solubilization site. In the case of PHLC, the lower solubilization capacity explains the relatively low decrease in the proportion solubilized by the oil compared to biphasic water-oil systems.

Antioxidant activity of gallates in emulsion. The antioxidant activity was investigated by monitoring the evolution of hydroperoxides and hexanal. The formation of hydroperoxides was determined by measuring the concentration of CD and by color reaction in the presence of thiocyanate and Fe^{2+}. Only data for the latter method are shown, as both methods showed the same results with respect to the order of antioxidant activity. It is worth noting that the hydroperoxide concentrations, as determined by thiocyanate-ferric ion color reaction shown in Figures 1–3, were four times higher than those determined by CD.

Antioxidant activity of ethyl gallate and gallic acid. The inhibition of hydroperoxide and hexanal formation by ethyl gallate increased in the following order: Brij 58 < PHLC < SDS (Table 5), and no activity was observed for CTAB (data not shown). Gallic acid showed antioxidant activity in the PHLC emulsion but not in the other emulsions. With respect to the partitioning behavior (Table 3), a reversed order of activity would have been expected, as several studies have suggested that the attracting forces of the emulsifier head groups for the antioxidant result in an improved activity (4,5). Hydrogen bonds, which contribute to the increased partitioning of the gallates into the lipid phase (emulsifier + oil phase), may counteract the hydrogen-donating ability and, thereby, lower the antioxidant activity. The marked effect of hydrogen bonds on the kinetics of hydrogen abstraction was evidenced by Avila *et al.* (14) and MacFaul *et al.* (16). As demonstrated by these authors, the solvent acting as a Lewis base (hydrogen bond acceptor) can interact with the antioxidant and the strength of these interactions determines the hydrogen-donating activity of the antioxidant. This mechanism may also

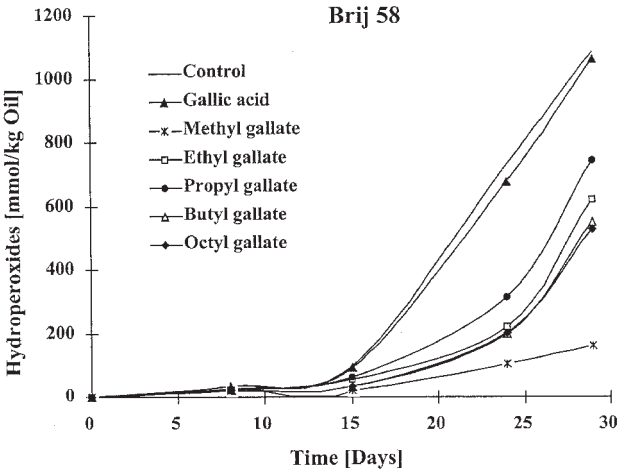

FIG. 2. Antioxidant activity of gallates in Brij 58 (polyoxyethylene 20 cetyl ether) emulsions (1% Brij 58; 20% stripped corn oil) at 37°C

TABLE 5
Inhibition of Hydroperoxide and Hexanal Formation by Gallates in SDS, PHLC, and Brij 58 Emulsions (20% stripped corn oil and 1% emulsifier) After 24 d at 37°C

	Inhibition [% ± SD][b]					
	PHLC		SDS		Brij 58	
Antioxidant	Hexanal	Hydroperoxide	Hexanal	Hydroperoxide	Hexanal	Hydroperoxide
Gallic acid	36.3 ± 1.91[c]	40.4 ± 1.64[d]	–7.4 ± 0.25[d]	–14.1 ± 0.44[e]	25.1 ± 0.14[d]	7.2 ± 1.13[e]
Methyl gallate	90.7 ± 0.65[b]	72.5 ± 1.64[c]	95.8 ± 0.15[a]	96.0 ± 0.12[a]	77.3 ± 0.87[a]	85.7 ± 0.21[a]
Ethyl gallate	91.8 ± 0.04[b]	73.9 ± 0.59[c]	95.3 ± 0.37[a]	95.6 ± 0.15[a]	61.3 ± 0.57[c]	69.8 ± 0.18[c]
Propyl gallate	95.7 ± 0.36[a]	78.6 ± 0.82[a]	89.2 ± 0.06[b]	92.0 ± 0.06[b]	61.1 ± 0.45[c]	56.8 ± 0.28[d]
Butyl gallate	96.8 ± 0.16[a]	79.0 ± 0.14[a]	73.2 ± 0.10[c]	55.7 ± 0.08[c]	60.6 ± 0.57[c]	72.9 ± 0.32[b]
Octyl gallate	97.3 ± 0.06[a]	76.7 ± 0.49[b]	70.8 ± 0.98[c]	12.6 ± 2.14[d]	67.0 ± 0.34[b]	72.5 ± 0.04[b]

[a]Values in the same column followed by different superscript letters are significantly different ($P < 0.05$).
[b]$n = 4$. See Table 1 and 3 for abbreviations.

apply for CTAB and Brij 58 where the bromide counter-ion and the ether oxygen may act as hydrogen bond acceptors (11,13). Also, in particular with CTAB, the bromide is "naked" because it is well separated from the ammonium positive charge, and its basicity is thus enhanced. As a consequence, its interaction by hydrogen bonding with the hydroxyl group of ethyl gallate might result in the formation of hydrogen bromide in minute amounts. Hydrogen bromide acts as a radical scavenger and can generate bromide radicals. These may promote and propagate oxidation by radical mechanism, and thus explain the prooxidant behavior of gallates in CTAB emulsions. Bromide and chloride, but not fluoride and iodide salts of alkali and alkaline earth metals were reported to exhibit prooxidative effects (31,32).

Antioxidant activity with increasing emulsifier concentration. The importance of the amounts of antioxidant solubilized by the emulsifier was demonstrated by increasing the SDS concentration in emulsions. Increasing the emulsifier concentration results in an increase of the antioxidant concentration associated with the emulsifier. The emulsifier accumulates at the water-oil interface where the lipid oxidation occurs. In the SDS emulsion, the activity of ethyl gallate increased from 76.4 to 89.0% inhibition of hydroperoxide formation as the SDS concentration increased from 1 to 2%, respectively. Since the proportion of ethyl gallate solubilized by SDS is enhanced, the proportion solubilized in the aqueous phase and oil is decreased (Table 4). A significant decrease in antioxidant activity from 52.4% inhibition of hydroperoxide formation, at a PHLC concentration of 1%, to 41.7% inhibition, for PHLC at 2%, was observed in the PHLC emulsions. This is probably because PHLC exerts some antioxidant activity itself, and therefore lowers the extent to which ethyl gallate can reduce hydroperoxides.

Antioxidant activity of gallates. The hydrogen donation activity of gallic acid and its esters toward a stable radical (DPPH) was measured in ethanol solution. The number of radicals reduced by one molecule of gallate ranged from 3.7 to 4.1. No significant differences were observed between the activities of the individual compounds, i.e., differences in the activity observed in emulsions can be related to characteristic parameters of the system. The course of oxidation in SDS, Brij 58, and PHLC emulsions in the presence of different esters of gallic acid with increasing chain length is shown in Figures 1–3. Table 5 compares the inhibition of hydroperoxide and hexanal formation after 24 d, whereas Table 6 demonstrates the inhibition of hydroperoxide formation due to gallates when controls were at the same degree of oxidation. Several studies (1,2,17,23,33) suggested that a decrease in antioxidant polarity would result in solubilization of a higher proportion in the lipid phase and, thereby, improve antioxidant activity in dispersed lipid systems. However, except for the increase from gallic acid to methyl gallate, the order of the antioxidant activity of gallates followed markedly different trends in SDS, CTAB, Brij 58, and PHLC emulsions (data for CTAB not shown). In SDS emulsions, all gallates except gallic acid inhibited the formation of hydroperoxides (Fig. 1). The activity increased as follows: gallic acid < octyl gallate < butyl gallate < propyl gallate < ethyl gallate = methyl gallate. In contrast, no antioxidant activity, or a slight prooxidant activity, was observed for gallates in CTAB emulsions (data not shown). In Brij 58 emulsions, the antioxidant activity decreased from methyl gallate to propyl gallate, but increased again for butyl gallate and octyl gallate (Fig. 2). By contrast, in PHLC emulsions the order of activity increased with increasing alkyl chain length, except for octyl gallate, which was less active than propyl and butyl gallates (Fig. 3).

The order of antioxidant activity after 24 d (Table 5) was similar to the order of activity when all controls reached an oxidation level of 213 mmol hydroperoxides/kg oil (Table 6). At this level of oxidation, the difference between the activity of the individual antioxidants was less pronounced than after 24 d. The inhibition of hexanal formation (Table 5) measured after 24 d followed the same trends as found for inhibition of hydroperoxides.

There was a nonlinear relationship between decreasing polarity of the gallates and antioxidant activity in SDS, Brij 58, and PHLC emulsions (Table 6), indicating that different parameters influence the antioxidant action. However, it is rather difficult to relate these trends to specific interactions. Interphase transport rates may limit antioxidant activity as po-

TABLE 6
Inhibition of Hydroperoxide Formation by Gallates in SDS, PHLC, and Brij 58 Emulsions at an Oxidation Level of 213 mmol Hydroperoxides per kg Oil Related to the Controls at 37°C (20% stripped corn oil and 1% emulsifier)[a]

	Inhibition of hydroperoxide formation[b] [%]		
Antioxidant	PHLC	SDS	Brij 58
Gallic acid	40.4	–14.1	–9.4
Methyl gallate	72.5	94.7	81.4
Ethyl gallate	73.5	94.7	59.5
Propyl gallate	78.4	93.1	48.5
Butyl gallate	78.9	54.0	70.4
Octyl gallate	76.8	7.0	70.4

[a]See Tables 1 and 3 for abbreviations.
[b]Values were derived by interpolation from the oxidation data shown in Figures 1–3.

larity decreases (34). Castle and Perkins (35) suggested that this effect determines the low activity of the lipophilic α-tocopherol in SDS-linoleic acid mixed micelles. However, only small differences were observed between Trolox and its C_1–C_{10} hydrocarbon esters.

In SDS emulsions, methyl and ethyl gallates are the most efficient antioxidants among alkylgallates (Fig. 1), although they were the least soluble in the oil phase (Table 2). Several hypotheses could explain this apparent contradiction: (i) It is possible that increased hydrophobic interactions may lower the diffusion of gallates into the SDS-enriched environment resulting in a reduced antioxidant activity. According to Fendler (36), the mobility of the hydrocarbon chain is reduced with increasing hydrocarbon chain length. Therefore, the lower activity of propyl, butyl, and octyl gallates may be attributable to an increase in hydrophobic interactions with SDS. This is supported by the increased proportion of butyl gallate in SDS solution compared to DHBEE, in spite of their similar partitioning behavior in biphasic water-oil systems (Table 1). As the hydration of the SDS micelles is suggested for the first two methylene groups of SDS (13,36), it can be assumed that gallic acid requires at least a C_3 hydrocarbon ester to penetrate into the palisade layer. This would explain the highest activity observed for methyl and ethyl gallates.

(ii) Another explanation might be an interaction between the headgroup of SDS and the antioxidant, as solubilization in the hydrated part of the micelles is associated with replacement of water molecules (37). This may cause formation of hydrogen bonds between the negatively charged headgroup and the antioxidant, which may explain the decreasing activity with increasing alkyl chain length of the gallates.

(iii) On the other hand, it might be speculated that intramolecular interactions between the hydrogen atoms of the first methylene group of the alkyl ester chain and the oxygen atom of the hydroxyl group in the meta position of the gallate can occur by forming an eight-member ring transition. This may increase the hydrogen donor capacity of the hydroxyl group by stabilizing the resulting phenoxy radical and thus increasing the antioxidant capacity of alkyl gallates. This intramolecular interaction is possible with the bending of the alkyl ester chain toward the hydroxyl group. With increasing length of the alkyl ester chain of gallates, this bending capacity could be reduced because a longer alkyl chain will penetrate more easily into the hydrocarbon region of SDS-enriched environments through hydrophobic interactions.

By contrast, in PHLC-containing emulsions antioxidant activity continuously increased from methyl gallate to butyl gallate, but activity for octyl gallate was lower (Table 6). Several mechanisms may account for the different behavior compared to SDS. First, PHLC themselves may contribute to the inhibition of lipid oxidation (38) thereby altering the antioxidant mechanism and hence the effect of different polarity. Second, the unsaturated fatty acid residues may account for higher lateral diffusion in the phospholipid bilayers compared to the saturated alkyl chains of SDS. Aranda *et al.* (39) showed that nonpolar α-tocopherol preferentially partitions into the most fluid domains, i.e., most unsaturated regions, of model phospholipid membranes. The stronger antioxidant effect of gallic acid in PHLC emulsions compared to the other emulsifiers is probably related to solubilization of gallic acid in water sheaths, which are incorporated between phospholipid bilayers and thus are located close to the interface (30). The solubilization of noncharged antioxidants is not influenced by the nature of the head group of the emulsifier (10, 27), i.e., there is no direct interaction between the head group and the antioxidant. However, *via* the hydrating water molecules near the head group, we may expect an influence as the water molecules are increasingly replaced by lipophilic antioxidants. This indirect effect may in turn differ between SDS and PHLC emulsifier.

In Brij 58 emulsions, the antioxidant activity decreased from methyl gallate to propyl gallate; gallates with longer alkyl chains had higher antioxidant activities in Brij 58 emulsions (Fig. 2). In contrast to SDS emulsions, gallates are attracted by the diffuse environments constructed of the oxygen atoms of the polyoxyethylene chains in Brij 58. Phenols are solubilized in the palisade layers of the SDS aggregates with their hydroxyl groups closely oriented to the polar head groups. Solutes in ethoxylated emulsifiers are considered to penetrate deeper into the polyoxyethylene environment as their solubility in ether increases, thus altering their antioxidant activity.

The results for the PHLC emulsions were in agreement with the study of Porter *et al.* (33) on gallates in soybean lecithin dispersions. However, the results for SDS and Brij 58 emulsions were quite unexpected in view of the polar paradox, which states, according to Porter *et al.* (33), that the efficiency of an antioxidant increases with decreasing polarity in dispersed lipid systems and decreases with decreasing polarity in bulk oils. These results could mean that the polar paradox is limited to emulsions containing emulsifiers with properties similar to phospholipids.

REFERENCES

1. Huang, S.W., E.N. Frankel, K. Schwarz, and J.B. German, Effect of pH on Antioxidant Activity of α-Tocopherol and Trolox in Oil-in-Water Emulsions, *J. Agric. Food Chem. 44*:2496–2502 (1996).

2. Huang, S.W., A. Hopia, K. Schwarz, E.N. Frankel, and J.B. German, Antioxidant Activity of α-Tocopherol and Trolox in Different Lipid Substrates: Bulk Oils vs. Oil-in-Water Emulsions, *Ibid. 44*:444–452 (1996).
3. Huang, S.W., E.N. Frankel, R. Aeschbach, and J.B. German, Partition of Selected Antioxidants in Corn Oil-Water Model Systems, *Ibid. 45*:1991–1994 (1997).
4. Barclay, L.R.C., and M.R. Vinqvist, Membrane Peroxidation: Inhibiting Effects of Water-Soluble Antioxidants on Phospholipids of Different Charge Types, *Free Radical Biol. Med. 16*:779–788 (1994).
5. Pryor, W.A., J.A. Cornicelli, L.J. Devall, B. Tait, B.K Trivedi, D.T. Witiak, and M. Wu, A Rapid Screening Test to Determine the Antioxidant Potencies of Natural and Synthetic Antioxidants, *J. Org. Chem. 58*:3521–3532 (1993).
6. Fessenden, R.W., A. Hitachi, and V. Nagarajan, Measurement of the Dipole Moment of a Peroxyl Radical by Microwave Dielectric Absorption, *J. Phys. Chem. 88*:107–110 (1984).
7. Boyd, S.L., R.J. Boyd, and L.R.C. Barclay, A Theoretical Investigation of the Structures and Properties of Peroxyl Radicals, *J. Am. Chem. Soc. 112*:5724–5730 (1990).
8. Schwarz, K., E.N. Frankel, and J.B. German, Partition Behavior of Antioxidative Phenolic Compounds in Heterophasic Systems, *Fett/Lipid 98*:115–121 (1996).
9. Abraham, M.H., H.S. Chadha, J.P. Dixon, C. Rafols, and C. Treiner, Hydrogen Bonding. Part 40. Factors That Influence the Distribution of Solutes Between Water and Sodium Dodecylsulfate Micelles, *J. Chem. Soc. Perkin Trans. II*:887–894 (1995).
10. Abraham, M.H., H.S. Chadha, J.P. Dixon, C. Rafols, and C. Treiner, Hydrogen Bonding. Part 41. Factors that Influence the Distribution of Solutes Between Water and Hexadecylpyridinium Chloride Micelles, *Ibid.* 19–24 (1997).
11. Quina, F.H., E.O. Alonso, and J.P.S. Farah, Incorporation of Nonionic Solutes into Aqueous Micelles: A Linear Solvation Free Energy Relationship Analysis, *J. Phys. Chem. 99*:11708–11714 (1995).
12. Vitha, M.F., J.D. Weckwerth, K. Odland, V. Dema, and P.W. Carr, Study of the Polarity and Hydrogen Bond Ability of Sodium Dodecyl Sulfate Micelles by Kamlet-Taft Solvatochromic Comparison Method, *J. Phys. Chem. B. 100*:18823–18828 (1996).
13. Vitha, M.F., and P.W. Carr, Study of the Polarity and Hydrogen-Bond Ability of Dodecyltrimethylammonium Bromide Micelles by Kamlet-Taft Solvatochromic Comparison Method, *Ibid. 102*:1888–1895 (1998).
14. Avila, D.V., K.U. Ingold, and J. Lusztyk, Dramatic Solvent Effects on the Absolute Rate Constants for Abstraction of the Hydroxyl Hydrogen Atom from *tert*-Butyl Hydroperoxide and Phenol by the Cumyloxyl Radical. The Role of Hydrogen Bonding, *J. Am. Chem. Soc. 117*:2929–2930 (1995).
15. Valgimigli, L., J.T. Banks, K.U. Ingold, and J. Lusztyk, Kinetic Solvent Effects on Hydroxylic Hydrogen Atom Abstractions are Independent of the Nature of the Abstracting Radical. Two Extreme Tests Using Vitamin E and Phenol, *J. Am. Chem. Soc. 117*:9966–9971 (1995).
16. MacFaul, P.A., K.U. Ingold, and J. Lusztyk, Kinetic Solvent Effects on Hydrogen Atom Abstraction from Phenol, Aniline, and Diphenylamine. The Importance of Hydrogen Bonding on Their Radical-Trapping (antioxidant) Activities, *J. Org. Chem. 61*: 1316–1321 (1996).
17. Porter, L.P., Paradoxical Behavior of Antioxidants in Food and Biological Systems, *Toxicol. Ind. Health 9*:1–2 (1993).
18. Stöckmann, H., and K. Schwarz, Partitioning of Low Molecular Weight Compounds in O/W Emulsions, *Langmuir 15*:6142–6149 (1999).
19. Blois, M.S., Antioxidant Determination by the Use of a Stable Free Radical, *Nature 181*:1199–1200 (1958).
20. Pekkarinen, S.S., H. Stöckmann, K. Schwarz, I.M. Heinonen, and A.I. Hopia, Antioxidant Activity and Partitioning of Phenolic Acids in Bulk and Emulsified Methyl Linoleate, *J. Agric. Food Chem. 47*:3036–3043 (1999).
21. Chan, H.W.S., and G. Levett, Autoxidation of Methyl Linoleate. Separation and Analysis of Isomeric Mixtures of Methyl Linoleate Hydroperoxides and Methyl Hydroperoxylinoleate, *Lipids 12*:99–104 (1977).
22. Pardun, H., *Analyse von Nahrungsfetten*, Parey, Berlin, 1976, pp. 227–228
23. Frankel, E.N., S.W. Huang, J. Kanner, and J.B. German, Interfacial Phenomena in the Evaluation of Antioxidants: Bulk Oils vs. Emulsions, *J. Agric. Food Chem. 42*:1054–1059 (1994).
24. Tsybyshev, V.P., and V.A. Lifshits, Microelectrophoretic Investigation of Surfactant Adsorption on the Interface in an Oil-Water Emulsion, *Russ. J. Phys. Chem. 68*:1345–1350 (1994).
25. Dalgleish, D.G., M. Srinivasan, and H. Singh, Surface Properties of Oil-in-Water Emulsion Droplets Containing Casein and Tween 60, *J. Agric. Food Chem. 43*:2351–2355 (1995).
26. Hansch, C., and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, John Wiley & Sons, New York, 1979, pp. 15, 69–167
27. Treiner, C., A.K. Chattopadhay, and R. Bury, Heat of Solution of Various Alcohols in Aqueous Micellar Solutions of Hexadecyltrimethylammonium Bromide as a Function of Surfactant Concentration: The Preferential Solvation Phenomenon, *J. Colloid Interface Sci. 104*:569 (1985).
28. Myers, D., *Surfactant Science and Technology*, VCH Publishers, New York, 1992, p. 136.
29. Mulley, B.A., and A.D. Metcalf, Non-Ionic Surface-Active Agents, *J. Pharm. Pharmcol. 8*:774–779 (1956).
30. Krog, N.J., Food Emulsifiers and Their Chemical and Physical Properties, in *Food Emulsion*, edited by S.E. Friberg and K. Larsson, Marcel Dekker, New York, 1997, pp. 774–779.
31. Wettasinghe, M., and F. Shahidi, Oxidative Stability of Cooked Comminuted Lean Pork as Affected by Alkali and Alkali-Earth Halides, *J. Food Sci. 61*:1160–1164 (1996).
32. Kanner, J., and J.E. Kinsella, Lipid Deterioration Initiated by Phagocytic Cells in Muscle Foods: β-Carotene Destruction by a Myeloperoxidase-Hydrogen Peroxide-Halide System, *J. Agric. Food Chem. 31*:370–376 (1983).
33. Porter, W.L., E.D. Black, and A.M. Drolet, Use of Polyamide Oxidative Fluorescence Test on Lipid Emulsions: Contrast in Relative Effectiveness of Antioxidants in Bulk vs. Dispersed Systems, *Ibid. 37*:615–624 (1989).
34. van de Waterbeemd, H., P. van Bakel, and A. Jansen, Transport in Quantitative Structure-Activity Relationships VI: Relationship Between Transport Rate Constants and Partition Coefficients, *J. Pharm. Sci. 70*:1081–1082 (1981).
35. Castle, L., and M.J. Perkins, Inhibition Kinetics of Chain-Breaking Phenolic Antioxidants in SDS Micelles. Evidence That Intermicellar Diffusion Rates May Be Rate-Limiting for Hydrophobic Inhibitors Such as α-Tocopherol, *J. Am. Chem. Soc. 108*:6381–6382 (1986).
36. Fendler, J.H., *Membrane Mimetic Chemistry*, John Wiley & Sons, New York, 1982.
37. Cabane, B., Structure of Some Polymer-Detergent Aggregates in Water, *J. Phys. Chem. 81*:1639–1645 (1977).
38. Saito, H., and K. Ishihara, Antioxidant Activity and Active Sites of Phospholipids as Antioxidants, *J. Am. Oil Chem. Soc. 74*: 1531–1536 (1997).
39. Aranda, F.J., A. Coutinho, M.N. Berberan-Santos, M.J.E. Prieto, and J.C. Gómez-Fernández, Fluorescence Study of the Location and Dynamics of α-Tocopherol in Phospholipid Vesicles, *Biochim. Biophys. Acta 985*:26–32 (1989).

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