Activities of Antioxidants Are Affected by Colloidal Properties of Oil-in-Water and Water-in-Oil Emulsions and Bulk Oils

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The activity of α-tocopherol, Trolox, propyl gallate, gallic acid, methyl carnosoate, and carnosic acid was studied in two oil-in-water (o/w) emulsions, in two water-in-oil (w/o) emulsions, and in bulk oil with and without added emulsifiers. All antioxidants had either moderate or higher activity in bulk oil than in the emulsions. In most emulsions, the most polar antioxidants, propyl gallate and gallic acid, exhibited either prooxidant activity or no antioxidant activity. Methyl carnosoate was the most active antioxidant in w/o emulsions but was less active than Trolox in o/w emulsions. α-Tocopherol was less active in bulk oil than in emulsions, but its activity in bulk oil was markedly enhanced by the addition of o/w emulsifiers. Partitioning of antioxidants, hydrogen bonding, interphase transport, surface accessibility, and interaction of emulsifier with antioxidants are considered to be important parameters that determine antioxidant activity in lipid-containing systems.

Keywords: *Water/oil emulsions; oil/water emulsions; bulk oil; antioxidants; α-tocopherol; Trolox; propyl gallate; gallic acid; carnosic acid; methyl carnosoate; emulsifiers*

INTRODUCTION

Emulsions represent a major group of colloidal systems relevant for foods, cosmetics, and pharmaceuticals. Antioxidants are useful to prevent lipid oxidation in emulsions, but their activity may vary widely depending on the composition of the system. The importance of the colloidal properties of the oil phase has been recognized, and several investigators have demonstrated differences in the efficiency of antioxidants between oil-in-water (o/w) emulsions and bulk oils (Uri, 1961; Porter et al., 1989; Roginski, 1990; Frankel et al., 1994, 1996; Huang et al., 1996a, 1996b). However, only little information has been published on the activity of antioxidants in water-in-oil (w/o) emulsions (Szelag et al., 1990).

The lower activity of hydrophilic antioxidants in o/w emulsions than in bulk lipids could be related in part to the partition behavior of the antioxidants between the aqueous phase and the lipid phase (Frankel et al., 1994; Huang et al., 1996a, 1996b). The proportion of radical chain-breaking hydrophilic antioxidants partitioning into the aqueous phase is considered to be inactive (Castle and Perkins, 1986) because lipid oxidation occurs in the lipid phase and at the lipid surface (Fessenden et al., 1984; Boyd et al., 1990). Therefore, hydrophilic antioxidants are in general less effective than lipophilic antioxidants in protecting lipids in o/w emulsions (Frankel et al., 1994; Schwarz et al., 1996). According to Roginsky (1990) and Castle and Perkins (1986), the antioxidant activity of nonpolar antioxidants is decreased in the presence of water because the reactants antioxidants and radicals are statistically distributed in the lipid particles and, therefore, may be separated by the surrounding aqueous phase.

The lower activity of α-tocopherol than that of its derivative 2,2,5,7,8-pentamethyl-6-chromanol, and the lower activity of 2,6-di-*tert*-butyl-4-methylpropionate than that of 2,6 di-*tert*-butyl-4-methyl phenol (BHT), was related to their lower inter-membrane transfer in the presence of a lipid-soluble radical initiator 2,2′-azobis(2,4-dimethylvaleronitrile) in phospholipid liposomes (Niki et al., 1985). Kagan et al. (1990) demonstrated that α-tocopherol with a phytyl chain length of either 11 or 6 carbon atoms had greater antioxidant activity than α-tocopherol with a phytyl chain of 16 carbon atoms. However, when a methyl group was attached to the tocol structure, the molecule was less active as an antioxidant because it partitioned largely into the aqueous phase.

Several investigators have related the activity of antioxidants to the colloidal properties of emulsions. Ruben and Larson (1985) observed higher antioxidant activity of α-tocopherol when incorporated into lecithin bilayers rather than when incorporated into oil droplets in o/w emulsions. On the other hand, Huang et al. (1997) concluded from their partition studies that antioxidants favorably solubilized by the surfactant Tween 20 to form micelles in o/w emulsions were not as active as highly oil-soluble antioxidants (Frankel et al., 1994; Frankel et al., 1996).

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10.1021/jf991289a CCC: $19.00
Published on Web 09/01/2000

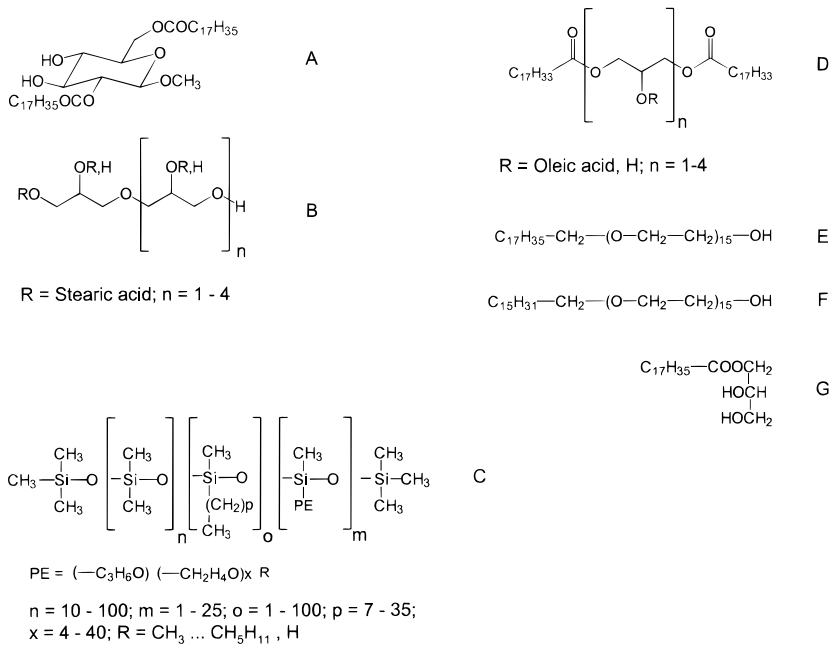

Figure 1. Structure of emulsifiers. Polyglyceryl glucose methyl distearate (PGMS) is a mixture of A and B. C is polysiloxan polyalcohol polyether colpolymer (PPPC). D is polyglyceryl-3 oleate (PGO). Cetheareth-15 and glyceryl stearate (CGS) is a mixture of E, F, and G.

The presence of the aqueous phase generally decreases the activity of antioxidants because hydrogen-bonded complexes are ineffective hydrogen donors to lipid radicals (Roginsky, 1990). Frankel et al. (1994) hypothesized that the higher activity of polar antioxidants than of nonpolar antioxidants in bulk oils is caused by the accumulation of highly polar antioxidants at the air-oil interface. Uri (1961) concluded that antioxidants of low solubility suspended in the liquid phase is not disadvantageous for a good antioxidant provided that the rate of diffusion and dissolution are not rate limiting.

Several investigators demonstrated the synergistic effect of phospholipids with α-tocopherol in bulk oils, but this effect was attributed mainly to the regenerating effect of the tocopheryl radical (Niki et al., 1986; Segawa et al., 1995; Totani, 1997). In contrast, Koga and Terao (1995) related the higher radical-scavenging activity of α-tocopherol for the water-soluble radical initiator 2,2′-azobis(2-aminodinopropyl) dihydrochloride than for the lipid-soluble radical initiator 2,2′-azobis(2,4 dimethylvaleronitrile) to the enhanced accessibility to the aqueous environment in reversed phospholipid micelles in bulk oils.

Although much attention has been given to comparisons of antioxidant activity between bulk oils and dispersed lipids in a continuous aqueous phase, comparisons of antioxidant action between w/o and o/w emulsions have received little attention in the literature. Also, the effect of different emulsifiers in bulk oil has been investigated only for phospholipids (Koga and Terao, 1995).

This paper reports on the effects of the colloidal properties of the oil phase of emulsions and bulk oil on the activities of antioxidants of different properties. The antioxidants investigated were mono-, di-, and triphenolic compounds and their derivatives with a free carboxylic acid: α-tocopherol and Trolox, methyl carnosoate and carnosic acid, and propyl gallate and gallic acid. Their polarity decreased in the following order: gallic acid > propyl gallate > Trolox > carnosic acid > methyl carnosoate > α-tocopherol (Huang et al., 1996b; Schwarz et al., 1996; Huang et al., 1997).

MATERIALS AND METHODS

Materials. Tocopherol-stripped corn oil was purchased from Acros (Gelnhausen, Germany). Trolox was obtained from Aldrich Chemical Co. (Milwaukee, WI), α-tocopherol, gallic acid, and propyl gallate were purchased from Sigma Chemical Co. (St. Louis, MO), and hexanal was purchased from Fluka Chemical Corp. (Ronkonkoma, NY). Carnosic acid was isolated from sage leaves and methylated as described previously (Djarmati et al., 1993; Huang et al. 1996b). The purities of carnosic acid and methyl carnosoate were checked by high-pressure liquid chromatography. Commercial emulsifiers were gifts from Goldschmidt, Essen, Germany: Tegocare 215 (cetheareth-15 and glyceryl stearate; CGS), Tegocare 450 (polyglyceryl glucose methyl distearate; PGMS), Abil EM 90 (polysiloxan polyalcohol polyether colpolymer; PPPC), and Isolan Go 33 (polyglyceryl-3 oleate; PGO). All other chemicals were of analytical grade.

Preparation of the Emulsions. O/w and w/o emulsions (60 g) consisted of 30% oil phase and 70% aqueous phase and contained 100 μM antioxidant based on the oil phase. The aqueous phase consisted of 0.5% $MgSO_4$ in distilled water. The oil phase was composed of 72% tocopherol-stripped corn oil, 8% beeswax, and 20% emulsifier: o/w CGS emulsion contained cetheareth-15 and glyceryl stearate (HLB value = 12.1), o/w PGMS emulsion contained polyglyceryl glucose methyl distearate (HLB value = 11.5), w/o PPPC emulsion contained polysiloxan polyalcohol polyether copolymer (HLB value = 5.1), and w/o PGO emulsion contained polyglyceryl-3 oleate (HLB value = 5.1) (Figure 1).

O/w emulsions were prepared with an Ultra-turrax homogenizer (IKA Works, Inc., Cincinnati, OH) run at 13 500 rpm, and the aqueous phase at 70 °C was added in one step to the oil phase at 60 °C. The final temperature of the o/w emulsions was 60 °C. To prepare w/o emulsions, the aqueous phase at 60 °C was added in 10 proportions to the oil phase at 60 °C. An Ultra-turrax homogenizer run at 8000−13 500 rpm was used to emulsify the first four portions, and a blender run at 250 rpm was used for the remaining aqueous phase. The emulsification procedure duration was 30 s per portion. The oils and emulsions (60 g) were stored in 150-mL bottles with gastight seals at 37 °C in the dark. Antioxidants were added

in methanol, and the solvent was evaporated under nitrogen before adding the constituents of the emulsion and starting the emulsification process.

Determination of Hydroperoxides. Hydroperoxides were determined by measuring conjugated dienes in 2-propanol at 234 nm using 26 000 as the molar extinction coefficient for methyl linoleate hydroperoxide (Chan and Levett, 1977).

Determination of Hexanal. Hexanal was measured by static headspace gas chromatography as described by Frankel (1994), and samples were incubated at 60 °C for 15 min. To avoid the interfering effects for the determination of hexanal in the headspace due to partitioning of hexanal between the water and oil phase in emulsion as described by Roozen et al. (1994), an external calibration was carried out with hexanal as reference compound for each system. Aliquots of 20-μL hexanal solutions of different concentrations were added to 0.5 g oil, o/w emulsion, or w/o emulsion. Oil and o/w emulsions were agitated with a vortex mixer for 20 s in sealed gas chromatography vials before headspace analysis, whereas w/o emulsions were stored for 24 h after hexanal addition to allow equilibrium to be reached in the emulsion.

Transelectron Microscopy. The freeze-fracture electron microcoscopy (FFEM) involves a number of subsequent preparation steps. The samples were cryofixed in liquid propane cooled by liquid nitrogen. After freeze fracturing and etching at −100 °C for 60 s in the freeze fracture apparatus (BAF 400 from Balzers, Liechtenstein), replicas (Pt/C) were obtained by electron beam evaporation. The replicas were cleaned in sulfuric acid and distilled water, mounted on uncoated copper grids, and examined in an EM 902 (Zeiss, Germany) at 90 kV.

Statistical Analysis. The software program Minitab (Addison-Wesley Publishing Company, Menlo Park, CA) was used for the determination of one-way analysis of variance. ANOVA and Fisher's comparison test were considered significant at $p < 0.05$.

RESULTS

Transelectron Microscopy of Emulsions. Transelectron microscopy photographs of o/w and w/o emulsions are shown in Figure 2a−d. Both the o/w CGM and o/w PGMS emulsions showed lamellar structures that formed a partial three-dimensional network in the aqueous phase. Spherical droplets and nonspherical areas ranging predominantly from 0.3 to 4 μm were formed by the lipophilic phase. The droplets were surrounded by several layers. In addition, multilamellar vesicles were present in the o/w PGMS emulsion, which incorporated bulk water. The spherical water droplets in w/o emulsions with a mean size of approximately 0.5−1.5 μm were surrounded by single layers and dispersed in the continuous lipophilic phase.

Transelectron microscopy photographs of bulk oil with an addition of emulsifier in the absence of dispersed water are shown in Figure 2e−h. In the presence of CGS and PGMS emulsifiers lamellar structures were observed, but the addition of PPPC and PGO did not result in any detectable colloidal structures.

Oxidation Rate of Bulk Oil and Emulsions. *Hydroperoxide Formation*. Figure 3 compares hydroperoxide formation in bulk oil with that of o/w emulsions and that of w/o emulsions, without added antioxidants. The rate of hydroperoxide formation varied among the various systems. In bulk oil, the hydroperoxide concentration increased to 39 mmol/kg oil in five weeks, whereas in o/w CGS and o/w PGMS emulsions, the amounts ranged from 25 to 29 mmol/kg oil. Hydroperoxide formation was lowest (15 mmol/kg oil) in w/o PPPC and highest (75 mmol/kg oil) in the w/o PGO emulsion.

Hexanal Formation. Hexanal formation followed markedly different trends than hydroperoxide formation (Figure 4). Bulk oil formed the lowest amount of hexanal (55 μmol/kg oil after 5 weeks). Hexanal formation in o/w PGMS emulsion was 10 times higher (3000 μmol/kg oil) than in o/w CGS emulsion (320 μmol/kg oil) despite forming similar amounts of hydroperoxide. The order of hexanal formation in the w/o emulsions was the same as the order for hydroperoxide formation. After 5 weeks, w/o PPPC emulsion had formed the lowest amount of hexanal (140 μmol/kg oil), while w/o PGO emulsion formed 870 μmol/kg oil.

To determine whether the emulsifiers had a prooxidative or antioxidative effect on the oil, bulk oil was oxidized in the presence of 20% emulsifier, which corresponds to the composition of the oil phase in emulsions. Oxidation was carried out at 60 °C to keep the oil liquid. Under these conditions the order of oxidizability between emulsions and bulk oil changed markedly. Hydroperoxide formation in bulk oil with emulsifiers increased between 68 and 86 mmol/kg oil after 10 days compared to an increase of 69 mmol/kg oil in bulk oil without emulsifier. The hydroperoxide concentration increased in the following order: PGMS < bulk oil without emulsifier = PPPC < CGS < PGO (Figure 5). Hexanal formation was measured after 10 days and ranged from 257 to 661 μmol/kg oil, increasing in the order PPPC < PGO < PGMS < CGS, and the amount of hexanal was 622 μmol/kg in bulk oil without emulsifier (data not shown).

Antioxidant Inhibition in Emulsions and Bulk Oil. The activities of antioxidants in bulk oil and emulsions were compared with respect to the formation of lipid oxidation products in bulk oil and emulsions without added antioxidants (controls) when the hydroperoxide formation ranged from 22 to 33 mmol/kg oil and hexanal formation from 407 to 867 μmol/kg oil in the controls (Tables 1 and 2). The extent of oxidation was reached after 3 to 6 weeks for hydroperoxides and after 2 to 9 weeks for hexanal formation. The oxidation time corresponded approximately to the length of the induction periods of the individual controls based on the slope of the curves shown in Figures 3 and 4. The antioxidant activities of α-tocopherol, Trolox, propyl gallate, gallic acid, methyl carnosoate, and carnosic acid differed strongly among the systems investigated.

In bulk oil, the antioxidants Trolox, propyl gallate, gallic acid, carnosic acid, and methyl carnosoate inhibited hydroperoxide formation over 85%, whereas α-tocopherol caused only 39% inhibition (Table 1). Similarly, Trolox, carnosic acid, and methyl carnosate inhibited hexanal formation by over 85%, gallic acid and propyl gallate by around 60%, and α-tocopherol by 30% (Table 2).

Trolox, carnosic acid, and methyl carnosoate were markedly less effective in inhibiting hydroperoxide formation (11−52%) in all emulsions than in bulk oil. Gallic acid was a slight to strong prooxidant in all emulsions. In most emulsions, propyl gallate was either a prooxidant or had no activity. In o/w PGMS emulsion, however, inhibition by propyl gallate was in the same range as that in bulk oil. In contrast, α-tocopherol inhibited hydroperoxide formation in w/o PPPC and o/w PGMS emulsions to the same extent as in bulk oil, but in o/w CGS and w/o PGO emulsions, inhibition by α-tocopherol was clearly less.

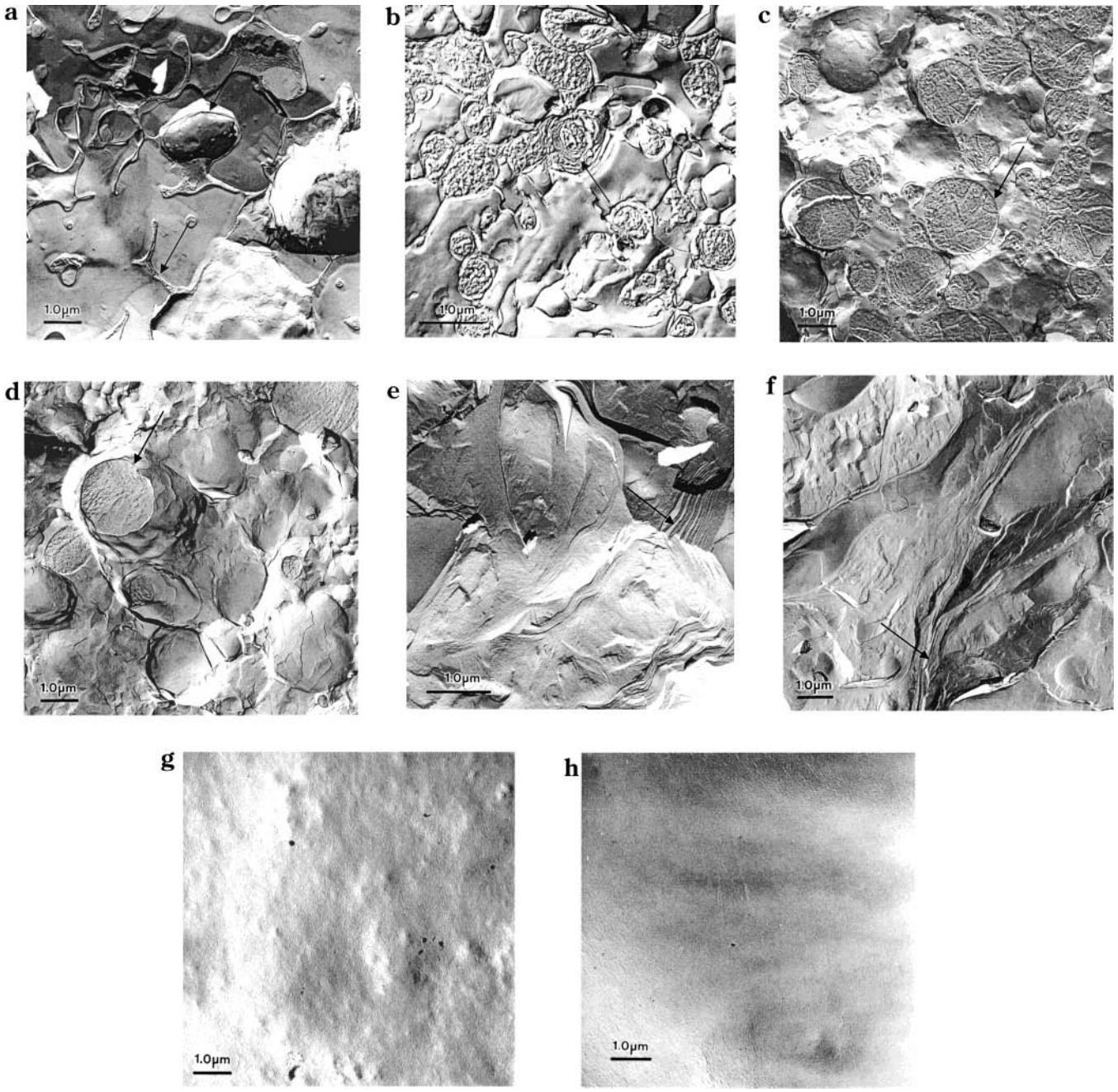

Figure 2. Transelectron microscopy photographs of o/w CGS emulsion (a), o/w PGMS emulsion (b), w/o PPPC emulsion (c), w/o PGO emulsion (d), bulk oil with CBS emulsifier (e), bulk oil with PGMS emulsifier (f), bulk oil with PPPC emulsifier (g), and bulk oil with PGO emulsifier (h).

The antioxidant activities of the individual antioxidants were compared with those of their carboxylic derivatives. Hydroperoxide formation in bulk oil was inhibited to the greatest extent (98%) by Trolox, while α-tocopherol was the least effective antioxidant (Table 1). In emulsions, Trolox showed higher activities than α-tocopherol except for w/o PPPC emulsion. In general, the differences between the activities of α-tocopherol and Trolox in emulsions were smaller than in bulk oil.

Trolox showed high activity in inhibiting hexanal in bulk oil whereas α-tocopherol was the least effective of the antioxidants (Table 2). In emulsions, α-tocopherol and Trolox inhibited hexanal formation in most cases significantly, but the order of activity between Trolox and α-tocopherol changed (Table 2).

Propyl gallate and gallic acid inhibited strongly hydroperoxide and hexanal formation in bulk oil but had either no activity or strong prooxidant activity in all emulsions, except for the o/w PGMS emulsion, where propyl gallate was the strongest inhibitor of hydroperoxide and hexanal formation (Table 1).

Although methyl carnosate was more effective than carnosic acid in inhibiting hexanal in both w/o emulsions, the activities between these antioxidants showed no significant differences in the o/w emulsions and in the bulk oil.

Antioxidant Inhibition in Bulk Oils with Emulsifier. To further investigate the influence of the emulsifier on antioxidant action, bulk oil containing emulsifier was oxidized in the presence of α-tocopherol, Trolox, propyl gallate, and gallic acid (Tables 3 and 4). The emulsifier−oil ratio in bulk oils was equal to that in emulsions. Oxidations were carried out at 60 °C in order to keep the oils fluid. However, the turbidity of

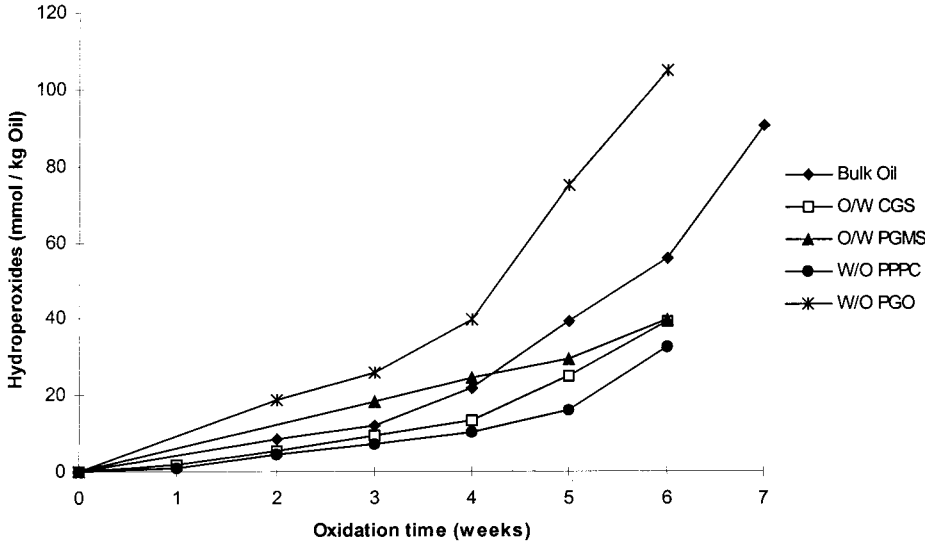

Figure 3. Hydroperoxide formation in bulk oil, o/w emulsions, and w/o emulsions at 37 °C.

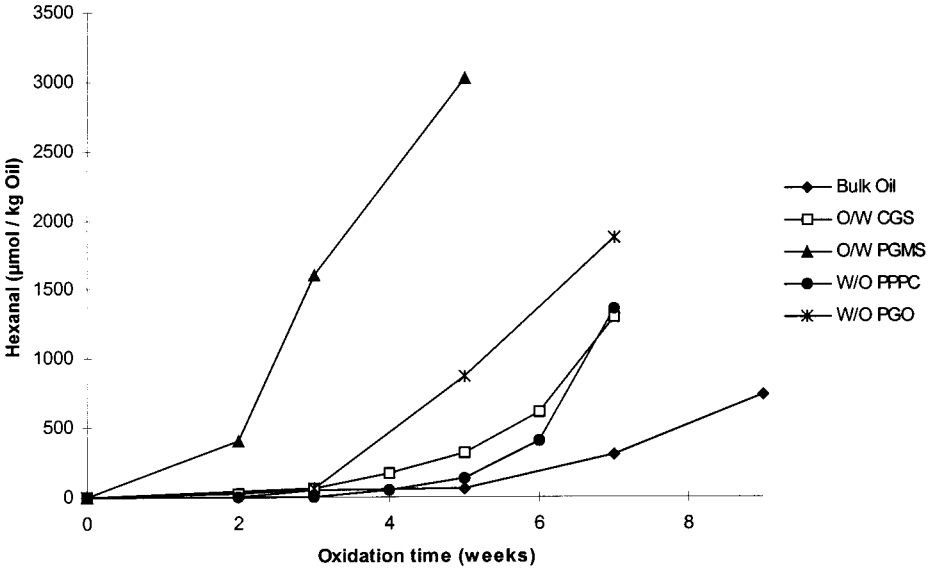

Figure 4. Hexanal formation in bulk oil, o/w emulsions, and w/o emulsions at 37 °C.

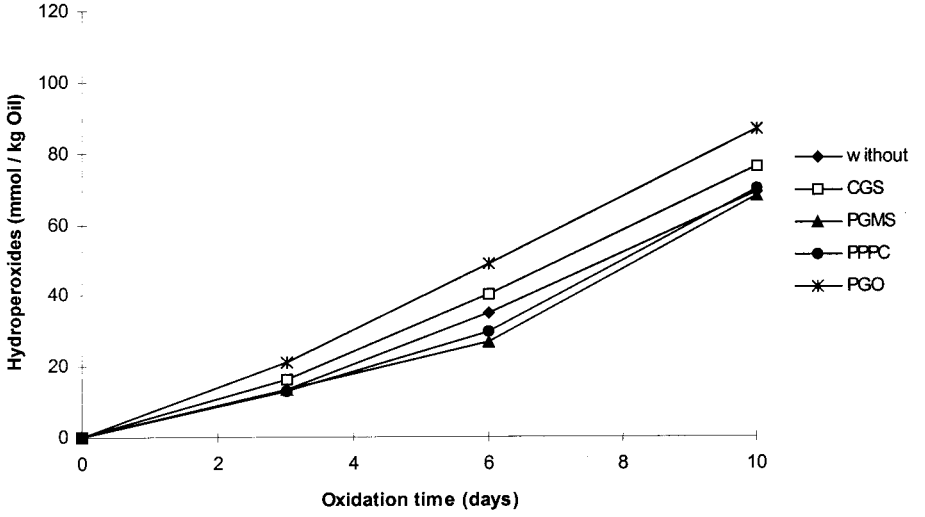

Figure 5. Hydroperoxide formation in bulk oil with and without emulsifier at 60 °C.

bulk oil with o/w emulsifiers CGS and PGMS indicated partial crystallization of the oil, whereas bulk oils with w/o emulsifiers PPPC or PGO were even liquid at ambient temperature. As the differences in oxidation of bulk oils with emulsifiers at 60 °C were smaller compared to the different emulsion at 37 °C, it was

Table 1. Inhibition of Hydroperoxide Formation by Antioxidants in Bulk Oils and Emulsions after 3−6 Weeks at +37 °C (Percent Mean Inhibition ± SD)[a,b]

	systems														
antioxidants	bulk oil			O/W CGS			O/W PGMS			W/O PPPC			W/O PGO		
tocopherol	38.9	±4.55	a	19.5	±3.71	cd	31.5	±4.79	bc	34.7	±2.76	d	7.5	±9.23	c
Trolox	98.2	±4.11	c	21.1	±0.81	d	43.2	±6.61	c	15.5	±1.73	c	22.2	±6.60	d
propyl gallate	86.7	±1.13	b	−20.0	±2.24	b	89.9	±0.80	d	−11.1	±2.58	b	−7.1	±2.82	b
gallic acid	87.2	±1.09	b	−61.1	±2.18	a	−9.7	±13.13	a	−148.6	±11.39	a	−45.8	±13.97	a
carnosic acid	85.6	±2.49	b	11.3	±0.73	c	37.6	±1.22	b	37.0	±1.44	d	23.7	±4.55	d
methyl carnosate	90.7	±0.57	cb	15.5	±3.09	cd	20.7	±5.38	c	52.0	±0.91	e	36.2	±3.00	e
oxidation time (weeks)	4			5			5			6			3		
hydroperoxide formation (mmol/kg oil) without antioxidant	22			25.1			29.1			32.7			25.7		

[a] Inhibition = $[C - S)/C] \times 100$, where C = hydroperoxide formation in the control and S = hydroperoxide formation in the sample with antioxidant. Negative values represent prooxidant activity. SD (standard deviation), $n = 3$. [b] Values within each column followed by the same letter are not significantly different ($p < 0.05$).

Table 2. Inhibition of Hexanal Formation by Antioxidants in Bulk Oils and Emulsions after 2−9 Weeks at +37 °C (Percent Mean Inhibition ± SD)[a,b]

	systems														
antioxidant	bulk oil			O/W CGS			O/W PGMS			W/O PPPC			W/O PGO		
tocopherol	30.2	±4.37	a	−8.2	±1.53	c	87.6	±0.06	b	26.7	±2.05	c	33.7	±0.31	c
Trolox	88.3	±0.36	c	44.8	±6.14	e	93.1	±0.32	b	68.7	±1.50	e	16.5	±1.09	cb
propyl gallate	61.3	±0.23	b	−115.0	±14	b	91.3	±0.09	b	−54.3	±5.90	b	0.8	±14.79	ab
gallic acid	60.5	±0.60	b	−260.1	±10	a	−105.0	±8.59	a	−264.2	±8.72	a	−9.1	±2.49	a
carnosic acid	86.0	±0.33	c	32.0	±9.39	de	90.3	±2.07	b	60.0	±3.40	d	14.0	±0.78	b
methyl carnosoate	91.3	±0.53	c	16.8	±1.89	d	82.9	±0.99	b	89.9	±1.35	f	75.4	±0.62	d
oxidation time (weeks)	9			6			2			6			5		
hexanal formation (μmol/kg oil) without antioxidant	739.5			613.8			398.8			407.0			866.9		

[a] Inhibition = $[C - S)/C] \times 100$, where C = hexanal formation in the control and S = hexanal formation in the sample with antioxidant. Negative values represent prooxidant activity. SD (standard deviation), $n = 3$. [b] Values within each column followed by the same letter are not significantly different ($p < 0.05$).

possible to calculate the inhibition of hydroperoxides and hexanal formation by antioxidants for all systems after the same oxidation time. After 10 days the oils with emulsifiers reached a level of 70−86 mmol hydroperoxides/kg and 257−661 μmol hexanal/kg. The inhibition of hexanal and hydroperoxide formation showed similar trends. The CGS and PGMS emulsifiers increased the α-tocopherol activity, whereas the activity of Trolox was not changed. In contrast, the activities of propyl gallate and gallic acid were both decreased. Although PPPC emulsifier decreased the activity of propyl gallate, this emulsifier did not affect the activity of the other antioxidants as compared to their activities in bulk oil without emulsifier. PGO had a strong negative effect on the activities of all antioxidants. The activity of Trolox was strongly depressed by PGO, but this antioxidant still demonstrated the highest activity of all the antioxidants.

DISCUSSION

The composition and colloidal characteristics of the lipid phase influenced the efficiency of antioxidants in bulk oils, o/w emulsions, and w/o emulsions. For emulsification different commercial surfactants were added to stabilize the emulsion by surrounding the oil or water droplets, thereby dominating the structure of the water/oil interface (Bergenstahl and Claeston, 1997). Waxes added to emulsions form crystals at 37 °C in the oil phase, thereby enhancing the viscosity of w/o emulsions. According to Junginger (1997) w/o emulsifiers form a monomolecular layer at the water−oil interface. In contrast, o/w emulsifiers PGMS or CGS are able to form multiple layers (Hameyer, 1993) which is in accordance to the TEM in Figure 2. In addition, multilamellar vesicles were present in the o/w PGMS emulsion which incorporate bulk water as described by Müller-Goymann (1984). In both o/w emulsions lamellar structures in the aqueous phase are due to crystallization of the emulsifier, which may form mixed hydrated crystals with waxes. Thereby, three-dimensional structures are built which can incorporate bulk water, producing a hydrophilic gel phase (Junginger, 1997).

O/w and w/o emulsions and bulk oil behaved differently on oxidation. The formation of greater amounts of hexanal in all emulsions than in bulk oil indicates that the presence of water caused extensive formation of hexanal. This effect may be due to different mechanisms of hexanal formation because in aqueous systems proton-catalyzed hexanal formation is favored (Ohloff, 1978). The pronounced disparities between the oxidation behavior of the emulsions and the similarity of the oxidation behavior of bulk oils with the corresponding emulsifier (but no water) added, indicate that the differences in the oxidation behavior in emulsions are caused mainly by the interaction of emulsifiers and minor compounds with water. Interaction of emulsifiers at the water/oil interface result in lyotrophic mesomorphism, i.e., structures formed by the emulsifier in the presence of water (Krog, 1997; Junginger, 1997).

Table 3. Inhibition of Hydroperoxide Formation by Antioxidants in Bulk Oils with and without Emulsifier Addition after 10 d at +60 °C (Percent Mean Inhibition ± SD)[a,b]

	emulsifier														
antioxidant	without			CGS			PGMS			PPPC			PGO		
tocopherol	10.8	±0.34	d	55.3	±3.01	ab	32.4	±1.93	c	18.7	±0.67	c	−4.7	±1.22	c
Trolox	58.0	±0.45	c	59.6	±0.53	a	59.5	±1.03	a	66.7	±1.32	a	7.2	±2.41	a
propyl gallate	66.7	±0.39	a	29.3	±3.14	b	12.3	±1.17	d	44.4	±0.94	b	1.1	±2.11	b
gallic acid	63.7	±0.41	b	47.2	±0.36	c	44.4	±0.37	b	67.8	±0.24	a	−8.0	±1.85	c
hydroperoxide formation (mmol/kg oil) without antioxidant	69			75.9			67.9			69.9			86.3		

[a] Inhibition = $[C - S)/C] \times 100$, where C = hydroperoxide formation in the control and S = hydroperoxide formation in the sample with antioxidant. The calculations are based on the ratio between the control and the sample with antioxidant at the same oxidation time. Negative values represent prooxidant activity. SD (standard deviation), $n = 3$. [b] Values within each column followed by the same letter are not significantly different ($p < 0.05$).

Table 4. Inhibition of Hexanal Formation by Antioxidants in Bulk Oils with and without Emulsifier Addition after 10 d at +60 °C (Percent Mean Inhibition ± SD)[a,b]

	emulsifier														
antioxidant	without			CGS			PGMS			PPPC			PGO		
tocopherol	7.9	±0.64	c	74.4	±1.17	a	58.0	±3.60	b	12.9	±0.49	d	−0.6	±0.57	b
Trolox	77.1	±0.79	a	69.1	±0.87	b	71.1	±3.43	a	81.7	±0.79	a	11.0	±1.40	a
propyl gallate	71.7	±0.08	b	44.7	±15.1	c	45.7	±1.10	c	58.1	±1.08	c	−22.9	±0.00	d
gallic acid	76.6	±0.08	a	44.1	±0.04	c	44.0	±2.83	c	81.0	±1.37	b	−6.0	±2.82	c
hexanal formation (μmol/kg oil) without antioxidant	622			661			523			257			286		

[a] Inhibition = $[C - S)/C] \times 100$, where C = hexanal formation in the control and S = hexanal formation in the sample with antioxidant. The calculations are based on the ratio between the control and the sample with antioxidant at the same oxidation time. Negative values represent prooxidant activity. SD (standard deviation), $n = 3$. [b] Values within each column followed by the same letter are not significantly different ($p < 0.05$).

The relatively low susceptibility of the w/o PPPC emulsion compared to that of the w/o PGO emulsion may be explained by the high molecular weight of approximately 1.4×10^4 g/mol of PPPC, which is several magnitudes higher than that of PGO. Silicone is a well-known inhibitor of lipid oxidation at high temperature due to the formation of a monomolecular film at the air-oil interface (Freeman et al., 1973). In w/o emulsion, PPPC may produce a hydrophobic layer that impedes the penetration of initiators from the aqueous phase into the continuous oil phase as PPPC exhibits strong absorption at the oil/water interface (Hameyer and Gould, 1990). Although the specific oil surface was considerably increased by forming oil droplets, both of the o/w emulsions formed about the same amount of hydroperoxides as bulk oil formed. Structure-related properties of the emulsifier that may account for this effect include the formation of protective multilayers at the water−oil interface and an increase in viscosity that results from formation of hydrophilic gel structures (Sim et al., 1979). On the other hand, o/w PGMS emulsions produced the greatest amount of hexanal. Whether the methylglucose headgroups of the PGMS emulsifier or any byproducts may be prooxidative, as reported for monosaccharides (Mabrouk and Dugan, 1961), was not clarified in this study.

In contrast to the activities of antioxidants in emulsions, in bulk oil all antioxidants showed moderate or high activity and no prooxidant activity. This trend can be explained by several mechanisms. First, in emulsions, the active proportion of polar antioxidants functioning as radical chain breakers is decreased due to the partitioning into the aqueous phase. (Huang et al., 1996a). Second, the lower activity of antioxidants in emulsions may be related to the formation of H-bonded complexes between antioxidants and water molecules (Roginski, 1990). The formation of H-bonded complexes requires the presence of antioxidants at the water−oil interface or in the aqueous phase. Thus, with increasing polarity, the formation of H-bonded complexes will rise.

Gallic acid and propyl gallate, the most polar antioxidants, showed no activity or even functioned as prooxidants in most emulsions, but exhibited high activity in bulk oil. Gallic acid partitions almost completely and propyl gallate partitions partly into the aqueous phase, as indicated by their water−oil partition coefficients ($P_{oil-water}$) of 0.112 and 0.895, respectively (Schwarz et al., 1996). Also, gallic acid and propyl gallate radicals, due to their nonhindered phenol structure, show low resonance stability compared to hindered phenols. Thus, gallic acid and propyl gallate radicals may promote the oxidation process (Roginski, 1990; Mahoney, 1969). At the same time, propyl gallate and gallic acid show a strong reducing activity in aqueous systems by converting traces of metals (e.g., Fe and Cu) into more active catalysts at the lower valence state.

The high activity of the polar gallates suspended in bulk oil, on the other hand, may be due to their low solubility (Uri, 1961) and their accumulation at the surface as has been hypothesized by Frankel et al. (1994). This hypothesis can be supported by the strong decrease in antioxidant activity of gallates when PGO, CGS, or PGMS is added as an emulsifier. The addition of such emulsifiers leads to a higher solubilization of polar antioxidants in nonpolar systems (Luisi, 1988). Only the activity of gallic acid was not affected in the presence of PPPC emulsifier.

The antioxidant action of α-tocopherol, the most lipophilic antioxidant in this study, in emulsion and bulk oil contrasted the behavior of all other antioxidants. Whereas α-tocopherol was the least active of all antioxidants in bulk oil, its activity in emulsions was

in some cases higher than that of the other antioxidants. This result is in agreement with results of our previous studies in which α-tocopherol had lower activity than Trolox in bulk oil and the opposite trend in o/w emulsions using Tween 20 as an emulsifier (Frankel et al., 1994; Huang et al., 1996a). This behavior may be explained by the lipophilic character of α-tocopherol leading to a low surface activity. Huang et al. (1997) showed that 12.5% of α-tocopherol partitions into the emulsifier phase in a biphasic corn oil−Tween 20 system (9:1). Therefore, it can be assumed that the emulsifier in the emulsion is also capable of solubilizing a portion of the antioxidant, thereby enhancing the surface accessibility of α-tocopherol. Also, in bulk oils the addition of o/w emulsifiers CGS and PGMS increased the activity of α-tocopherol over that in bulk oil without emulsifier. These results are in accordance with those of Koga and Terao (1995) who observed an increase in the activity of tocopherol in bulk oil due to the addition of phospholipids and those of Ruben and Larson (1985) who demonstrated high activity of α-tocopherol when incorporated into lecithin bilayers rather than into the oil droplet in o/w emulsions.

The separation of lipid radicals and lipophilic antioxidant molecules by the continuous aqueous phase was considered by Castle and Perkins (1986) to be the main reason for the relatively low activity of α-tocopherol in micellar solutions in contrast to the more polar derivatives. Emulsifiers applied to o/w emulsions in this study build numerous discrete environments in addition to the oil droplets due to multilamellar structures that incorporate low amounts of water layers (Müller-Goymann, 1984; Krog et al., 1997). Therefore, antioxidants with low polarity, i.e., those with a high water−oil partition coefficient, such as carnosic acid and methyl carnosoate (Huang et al. 1996b), in addition to α-tocopherol (essentially not soluble in water), may show reduced activity.

The high activity of methyl carnosoate and carnosic acid compared to α-tocopherol in bulk oil and a Tween 20-containing o/w emulsion was demonstrated in an earlier study (Huang et al., 1996b). Methyl carnosoate had the highest activity in inhibiting hydroperoxide and hexanal formation in all w/o emulsions, whereas in o/w emulsions, inhibition by methyl carnosoate was less than that in w/o emulsions and was always lower than that by Trolox. This behavior may be due to the fact that oil constitutes a continuous phase in w/o emulsions where the lipophilic antioxidants move freely. In contrast to o/w emulsions, gel structures formed by waxes in the oil phase of w/o emulsions are not hydrated (Junginger, 1997).

No consistent change in the order of activity between o/w and w/o emulsions was observed when comparing α-tocopherol vs Trolox and methyl carnosoate vs carnosic acid. Also, propyl gallate and gallic acid in o/w CGS emulsion and w/o PPPC and PGO emulsions had no antioxidant activity or prooxidant activity, but propyl gallate had the highest activity of all antioxidants in o/w PGMS emulsion. Therefore, it may be assumed that differences in the antioxidant activity for the same type of emulsion may be additionally influenced by specific interaction with the emulsifier dominating the oil/water interfaces.

ABBREVIATIONS USED

$P_{oil/water}$, partition coefficient for corn oil/water systems; w/o emulsion, water-in-oil emulsion; o/w emulsion, oil-in-water emulsion; CGS, ceteareth-15 and glyceryl stearate; PGMS, polyglyceryl glucose methyl distearate; PPPC, polysiloxan polyalcohol polyether colpolymer; PGO, polyglyceryl-3 oleate; HLB, hydrophilic−lipophilic-balance.

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Received for review November 29, 1999. Revised manuscript received June 1, 2000. Accepted June 1, 2000. The authors acknowledge the Feodor-Lynen Scholarship to K.S. from the Alexander-von-Humboldt-Foundation.

JF991289A