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Partition of Selected Antioxidants in Corn Oil−Water Model Systems

Keywords: *Partition coefficient; antioxidants; α-tocopherol; Trolox; carnosol; carnosic acid; methyl carnosate; rosmarinic acid; gallic acid; propyl gallate; catechin; corn oil triglycerides; emulsion; Tween 20*

INTRODUCTION

In multiphasic food systems, antioxidants partition into different phases thermodynamically according to their affinities toward these phases (Castle and Perkins, 1986; Pryor, 1988; Wedzicha, 1988). The proportions of antioxidants residing in different phases depend on the relative polarity of the antioxidants and the lipid substrates, surfactants, pH, and temperature as well as the composition of the phases (Cornell et al., 1970; Pryor et al., 1993; Barclay and Vinqvist, 1994; Schwarz et al., 1996; Huang et al., 1996a). The environment where the antioxidant is located affects its hydrogen-donating ability and oxidative stability (Roginsky, 1990; Hopia et al., 1996; Huang et al., 1996a,b). Also, the location of the antioxidant influences its accessibility and diffusion rate to oxidation initiators or chain-carrying species in multiphase systems (Castle and Perkins, 1986; Yi et al., 1991; Koga and Terao, 1994, 1995). However, the relationship between partition of antioxidants and their effectiveness in multiphase systems is not yet clearly understood.

In general, hydrophilic antioxidants were more effective than lipophilic antioxidants in bulk edible oils, whereas lipophilic antioxidants were more effective in emulsions (Porter, 1980, 1993; Porter et al., 1989; Frankel et al., 1994). Gallic acid, ascorbic acid, and Trolox (a water-soluble analogue of α-tocopherol) were better antioxidants than their lipophilic analogues, gallic esters, ascorbyl palmitate, and α-tocopherol, in bulk oils, but less active in emulsions. The changes in the effectiveness of antioxidants in different systems were explained by interfacial phenomena (Frankel et al., 1994). The apparent activity of antioxidants in multiphasic systems depends in part on their effective concentrations in the respective phases. Accordingly, in bulk oils, hydrophilic antioxidants were assumed to locate preferentially at the oil−air interfaces and better protect lipids from oxidation than lipophilic antioxidants that are dispersed in the oil phase. In oil-in-water emulsions, lipophilic antioxidants would concentrate in the oil−water interfaces and inhibit lipid oxidation more effectively than hydrophilic antioxidants that partition into the water phase. This was illustrated in previous studies showing that Trolox is a less effective antioxidant than α-tocopherol in corn oil or methyl linoleate emulsified with polyoxyethylene sorbitan monolaurate (Tween 20) due to its solubility in the water phase (Huang et al., 1996b). The antioxidant activities of carnosic acid and rosmarinic acid were also lower in emulsions than in bulk corn oil (Frankel et al., 1996a). In contrast, α-tocopherol and carnosol were better antioxidants in emulsions than in bulk oils. Similar trends were also observed between bulk soybean oil, peanut oil, fish oil, and the corresponding emulsions (Frankel et al., 1996b). Methyl carnosate was a more effective antioxidant than its more polar analogue, carnosic acid, in bulk corn oil as well as in emulsions (Huang et al., 1996c). In contrast, the less polar carnosol (γ-lactone of carnosic acid) was a less effective antioxidant than carnosic acid in bulk oil and emulsions (Hopia et al., 1996).

The oxidative stability of these phenolic diterpenes and the ability of oxidation products of certain phenolic compounds to act as antioxidants further complicate the relationship between antioxidant activity and distribution of antioxidants. The distribution of antioxidants in multiphase systems is thus an important parameter necessary to clarify the mechanism of antioxidant and their relative effectiveness. A previous study in this laboratory demonstrated that in oil-in-water emulsions, hydrophilic antioxidants (ferulic acid, caffeic acid, propyl gallate, gallic acid, Trolox, and catechin) partitioned to various extents in the water phase (Schwarz et al.,

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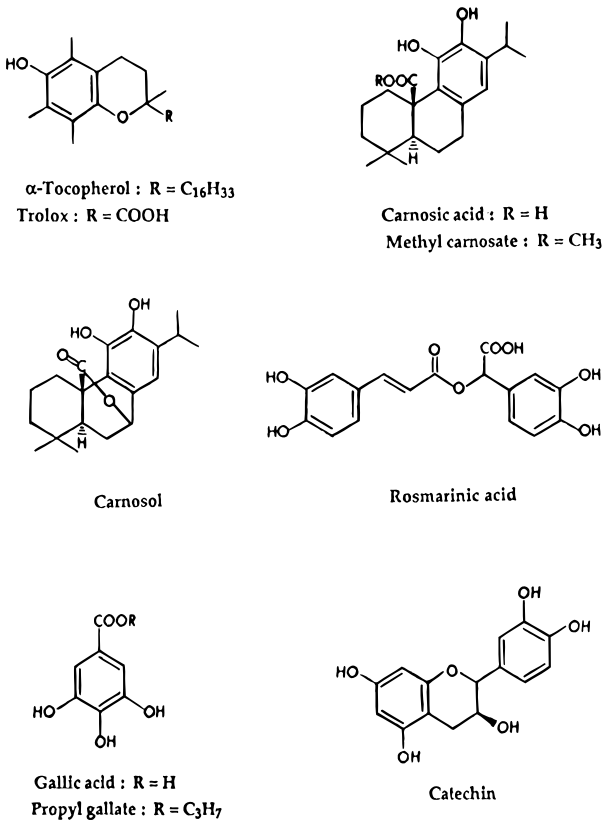

Figure 1. Structures of antioxidants.

1996). However, only limited information is available on the partition of lipophilic antioxidants in multiphase systems.

The present study compared the partition behavior of selected lipophilic and hydrophilic antioxidants and defined the relationship between the phase distribution of these antioxidants and their antioxidant activity in emulsions. The distribution of antioxidants was determined in corn oil−water mixtures, in oil−Tween 20 mixtures, in Tween 20 micellar solutions, and in emulsified corn oil.

MATERIALS AND METHODS

Corn oil triglycerides stripped of tocopherols were purchased from Eastman Kodak Co., Rochester, NY. α-Tocopherol was obtained from Fluka Chemical Co., Ronkonkoma, NY, and propyl gallate was purchased from Aldrich Chemical Co., Milwaukee, WI. Gallic acid, (±)-catechin, and Tween 20 (density = 1.095 g mL^{-1}) were obtained from Sigma Chemical Co., St. Louis, MO. Carnosic acid and canosol were obtained at >98% by purification of rosemary extracts (Aeschbach et al., 1990), and rosmarinic acid was purchased from Carl Roth GmbH, Karlsruhe, Germany. Methyl carnosate was synthesized as described previously (Huang et al., 1996c). The structures of antioxidants used in this study are shown in Figure 1.

Three model systems were used for measuring partition coefficients of lipophilic antioxidants between different phases as follows: (1) 1:1 (w:w) oil−water mixtures, corn oil (1 g) containing 100 ppm of antioxidant was mixed with deionized water (1 g) by vortexing two times for 20 s after purging with nitrogen; (2) 9:1 (w:w) oil−Tween 20 mixtures, corn oil (3 g) containing 100 ppm of antioxidant was mixed with Tween 20 (0.3 g) by vortexing two times for 20 s after purging with nitrogen; (3) 1% (w/w) Tween 20 aqueous micellar solutions containing 100 ppm of antioxidants. The concentration of antioxidant in the oil phase in model systems 1 and 2 was determined quantitatively by extraction and high-performance liquid chromatography (HPLC) as described previously (Huang et al., 1996c). The partition coefficients of lipophilic antioxidants between oil and water were calculated as follows: partition coefficient = $(V_w/V_l)[W_l/(W_t - W_l)]$, where V_w is the volume of water, V_l is the volume of oil, W_t is the total amount of the antioxidant, and W_l is the amount of the antioxidant in the oil phase; the oil density (g mL^{-1}) used for calculating the volume was 0.916. The same formula was used to calculate the partition coefficients of lipophilic antioxidants between oil and Tween 20, of which the density was 1.095 (g mL^{-1}). The concentrations of lipophilic antioxidants in the water phase in model system 3 were determined by the same centrifugation−membrane filtration method as was used below to determine partition coefficients of hydrophilic antioxidants. Three model systems containing 100 ppm of antioxidants were prepared and used to determine partition coefficients of hydrophilic antioxidants: (a) 10% (w/w) corn oil−water mixtures; (b) 1% Tween 20 aqueous micellar solutions; (c) 10% oil-in-water emulsions with 1% Tween 20. The concentrations of hydrophilic antioxidants in the water phase in these three systems were determined after centrifugation and membrane filtration as described previously (Huang et al., 1996b), and their partition coefficients were calculated as follows: partition coefficient = $(V_w/V_l)[(W_t - W_w) - 1]$, where V_w is the volume of water, V_l is the volume of oil, W_t is the total amount of the antioxidant, and W_w is the amount of the antioxidant in the water phase.

Antioxidants in the water phases and in the methanol extracts derived from the oil were analyzed on a Hewlett-Packard 1090 HPLC system using a Supelcosil LC-18-DB column (particle size, 5 μm; 2.1 mm i.d. × 25 cm; Supelco, Inc., Bellefonte, PA) and UV detection at 230 nm (Huang et al., 1996a,b). The elution solvent was 100:1 methanol/2 M citric acid for α-tocopherol; 85:15:1 methanol/water/2 M citric acid for carnosol, carnosic acid, and methyl carnosate; 50:50:1 methanol/water/2 M citric acid for rosmarinic acid and propyl gallate; 25:75:1 methanol/water/2 M citric acid for catechin; and 10:90:1 methanol/water/2 M citric acid for gallic acid. The isocratic flow rate was 0.3 mL min^{-1}.

Table 1. Partition of Lipophilic Antioxidants (Percent Mean in Oil Phase ± SD, n = 2, 3)[a]

antioxidant	1:1 corn oil−water	partition coeff	9:1 corn oil−Tween 20	partition coeff
α-tocopherol			87.5 ± 0.8	0.60
methyl carnosate	96.2 ± 0.4[b]	23.4	70.1 ± 1.1	0.20
carnosol	94.5 ± 0.5	15.9	70.4 ± 1.1	0.20
carnosic acid	91.6 ± 0.8[b]	10.2	13.9 ± 0.4	0.013

[a] Percent in oil phase was calculated on the basis of its partition ratio between phases in each system at a concentration of 100 μg/g of oil. [b] Huang et al. (1996c).

RESULTS

The partition coefficients of lipophilic and hydrophilic antioxidants were determined in different systems by analyzing their concentrations in the oil and the water phases after equilibrium was reached. In 1:1 corn oil−water mixtures, 91.6−96.2% of the lipophilic antioxidants partitioned into the oil phase (Table 1). α-Tocopherol was water-insoluble because of its long phytyl chain. The partition coefficients between oil and water were calculated to be 23.4 for methyl carnosate, 15.9 for carnosol, and 10.2 for carnosic acid (Table 1). Although carnosic acid possesses a carboxyl group, it has an affinity toward the oil phase in unbuffered oil−water mixtures without emulsifier. In 9:1 corn oil−Tween 20 mixtures, 87.5% α-tocopherol, 70.1% methyl carnosate, 70.4% carnosol, and 13.9% carnosic acid partitioned into the oil phase. The corresponding calculated partition coefficients of these lipophilic antioxidants ranged from 0.01 to 0.6 (Table 1). Results indicate that these four lipophilic antioxidants have a higher affinity for the Tween 20 phase than for the oil

Table 2. Partition of Hydrophilic Antioxidants (Percent Mean in Water Phase ± SD, $n = 2, 3$)[a]

antioxidant	10% corn oil−water	partition coeff	1% Tween 20−water	10% emulsified corn oil
Trolox[b]	68.3 ± 1.0	3.83	43.3 ± 0.8	36.3 ± 1.5
propyl gallate	90.7 ± 0.3	0.85	25.6 ± 0.2	16.8 ± 0.2
rosmarinic acid	94.5 ± 0.4	0.48	20.4 ± 0.8	17.0 ± 0.5
catechin	99.6 ± 0.3	0.033	27.5 ± 0.1	21.5 ± 0.4
gallic acid	99.8 ± 1.2	0.017	76.1 ± 1.8	70.1 ± 2.0

[a] Percent in water phase was calculated on the basis of its partition ratio between phases in each system at a concentration of 100 μg/g of water. [b] Huang et al. (1996a).

phase. Although methyl carnosate is more lipophilic than carnosol in corn oil−water mixtures, both have the same apparent lipophilicity in corn oil−Tween 20 mixtures. In 1% Tween 20 aqueous micellar solutions, these lipophilic antioxidants were not detected in the water phase. Therefore, in 10% oil-in-water emulsions with 1% Tween 20, these lipophilic antioxidants partition completely into the oil phase, Tween 20 micelles, and the oil−water interfaces.

In 10% oil−water mixtures, >90% of the hydrophilic antioxidants, propyl gallate, rosmarinic acid, catechin, and gallic acid, equilibrated in the water phase, compared to 68% of Trolox. The partition coefficients of propyl gallate, rosmarinic acid, catechin, and gallic acid between oil and water were <1, whereas Trolox had a partition coefficient of 3.8 (Table 2). Trolox and the hydrophilic antioxidants tested were very soluble in Tween 20 and were almost completely retained in the Tween 20 phase of 9:1 corn oil−Tween 20 mixtures (data not shown). Less hydrophilic antioxidants partitioned into the water phase in 1% Tween 20 aqueous micellar solutions (20.4−76.1%) and in 10% corn oil emulsified with 1% Tween 20 (16.8−70.1%) (Table 2). Rosmarinic acid had a higher affinity toward Tween 20 than the other hydrophilic antioxidants tested. The highly water soluble gallic acid partitioned mostly into the water phase of the 1% Tween 20 micellar solution and 10% emulsified corn oil.

DISCUSSION

This research was initiated to understand the varying activity of antioxidants in bulk oils and emulsions. These two types of oil-bearing materials differ primarily in the total area of surface and in a discrete interfacial phase made up of amphiphilic emulsifiers. Previous studies have shown that much of the variation in the antioxidant behavior of compounds that differ in polarity can be explained by their tendency to distribute into the interface of emulsified oils. The present results showing that lipophilic antioxidants are more distributed in the oil phase and at the oil−water interfaces (Table 1) are consistent with past results showing that they are more effective than hydrophilic antioxidants in emulsions. In 10% corn oil emulsified with 1% Tween 20, α-tocopherol was a better antioxidant than Trolox (Frankel et al., 1994; Huang et al., 1996a,b), and the lipophilic carnosol and carnosic acid were better antioxidants than the hydrophilic rosmarinic acid (Frankel et al., 1996a). The higher activity of methyl carnosate and carnosol relative to that of the more hydrophilic analogue carnosic acid in oil-in-water emulsions (Huang et al., 1996c; Frankel et al., 1996a) may thus be explained by the partition of carnosic acid into Tween 20 micelles.

The presence of an interfacial phase between oil and air was proposed to explain the better antioxidant activity of the polar Trolox and gallic acid relative to that of their respective nonpolar analogues, α-tocopherol and propyl gallate, in bulk corn oil (Frankel et al., 1994; Huang and Frankel, 1977). This concept is supported by the fact that in oil−water mixtures the polar antioxidants concentrate in the water phases and at the oil−water interface. In bulk oil systems, in the absence of the water phase, these polar antioxidants by virtue of their interfacial tension tend to accumulate at the surface of the oil−air interface (Becher, 1966).

Tween 20 had an important effect on the partition of hydrophilic compounds tested in corn oil emulsions, as shown by the marked decrease in their concentrations in the water phase of the corn oil emulsions compared to the corresponding corn oil−water mixtures (Table 2). We previously calculated that in the same corn oil emulsified with Tween 20, about 26−68% of the Tween 20 was at the oil−water interface and 32−74% formed micelles (Huang et al., 1996b). Thus, the low antioxidant or prooxidant activities of Trolox (Frankel et al., 1994), rosmarinic acid (Frankel et al., 1996b), and catechin, propyl gallate, and gallic acid (Huang and Frankel, 1997) can be explained by the higher partition of these hydrophilic compounds into the Tween 20 micelles and the oil−water interface.

Antioxidants may also show different properties in different discrete phases. Antioxidants had different oxidative stabilities in different lipid systems (Hopia et al., 1996; Huang et al., 1996c). Carnosic acid was less stable than carnosol and methyl carnosate in bulk corn oil and in Tween 20 micellar solutions. Carnosic acid and methyl carnosate remained active as antioxidants even after they were completely depleted. Therefore, the oxidation products of carnosic acid and methyl carnosate retained antioxidant activity and increased their effectiveness. At pH 3−4, more Trolox partitioned into the oil phase and the oil−water interfaces, but its antioxidant activity was lower than at pH 5−6, even though Trolox and α-tocopherol were more stable in Tween 20 micellar solutions at pH 3 (Huang et al., 1996a). Thus, the antioxidant behavior of Trolox and α-tocopherol is affected not only by their distribution in emulsions but also by their hydrogen-donating ability and relative oxidative stability in different phases.

The oxidative stability of foods varies according to the location of lipid components in multiphase systems because of their exposure to prooxidant and antioxidant factors affecting autoxidation. The effectiveness of antioxidants of different polarity will therefore be influenced by their distribution in food systems. The determination of antioxidant partitioning between different phases is an important parameter by which to select antioxidants to favor their distribution toward the microenvironment that is most susceptible to oxidation. However, in the complex process of lipid oxidation in foods, other factors must be considered, including their relative oxidative stability and hydrogen-donating ability, in addition to the multiphase partition of antioxidants. While the role of discrete phases in lipid oxidation can now be recognized, other anomalous behaviors of antioxidants can be studied to better understand the oxidative stability of foods.

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