Antioxidant Activity of Carnosic Acid and Methyl Carnosate in Bulk Oils and Oil-in-Water Emulsions

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This study was aimed at evaluating the antioxidant activities of carnosic acid and its methyl ester, methyl carnosate, in inhibiting the formation and decomposition of hydroperoxides in bulk and emulsified corn oil triglycerides at 60 °C. In both lipid systems, methyl carnosate was a better antioxidant than carnosic acid, and both were more active than α-tocopherol. The difference in antioxidant activity between methyl carnosate and carnosic acid was greater in emulsion than in bulk systems. Carnosic acid was less stable than methyl carnosate and α-tocopherol in bulk corn oil and 1% Tween 20 micelle solution. The partitioning of carnosic acid into the water phase may explain its low activity in emulsions. The measurement of antioxidant depletion may not be a valid method to evaluate antioxidant activities because the antioxidant activities of carnosic acid and methyl carnosate were not related to their oxidative stability in bulk oil and Tween 20 solution.

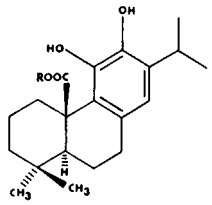
Keywords: *Antioxidants; carnosic acid; methyl carnosate; α-tocopherol; corn oil triglycerides; emulsion; mechanism; interfacial oxidation; hydroperoxides; hexanal; partition coefficient; oxidative stability of antioxidants*

INTRODUCTION

Chipault et al. (1952) investigated the antioxidant activities of 32 common spices in lard under conditions of the active oxygen method (AOM) at 100 °C, and rosemary and sage exhibited the greatest activity. Both were found to contain similar antioxidants (Nakatani, 1989; Nakatani and Inatani, 1981, 1983, 1984; Cuvelier et al., 1994). The antioxidant activity of rosemary extracts was reported to be dependent on the concentration of phenolic diterpenes during storage and thermal stress (Schwarz et al., 1992).

Methyl carnosate, the methyl ester of carnosic acid (Figure 1), was first reported in a petroleum ether extract of *Salvia lanigera* (Al-Hazimi, 1986). Methyl carnosate and carnosic acid showed a stronger antioxidant activity than carnosol, rosmadial, and rosmanol, other major antioxidant compounds of both spices, based on the disappearance of methyl linoleate in a lipophilic solvent oxidized by bubbling pure oxygen at 110 °C (Cuvelier et al., 1994). Under these conditions, methyl carnosate and carnosic acid were reported to exhibit similar antioxidant activity. Methyl carnosate has received less attention than carnosic acid in the literature.

Rosemary and sage were less effective in corn oil-in-water emulsions than in lard (Chipault et al., 1955). The antioxidant activities of a rosemary extract, carnosic acid, and rosmarinic acid were also less 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 emulsions of soybean oil and peanut oil but not in emulsified fish oil (Frankel et al., 1996b). In fish oil emulsions, the rosemary compounds inhibited the formation of conjugated dienes and pentenal but not that of propanal. The lower antioxidant activity of the polar hydrophilic rosemary compounds in the emulsion systems may be explained by their partitioning into the water phase, thus being less effective than interfacially located antioxidants.



R=H CARNOSIC ACID
R=CH_3 METHYL CARNOSATE

Figure 1. Structures of carnosic acid and methyl carnosate.

In fresh rosemary, carnosic acid is the major phenolic diterpene (Aeschbach, 1990; Löliger, 1991; Schwarz and Ternes, 1992a), but it is degraded during solvent extraction (Schwarz and Ternes, 1992b). In methanol, carnosic acid is unstable and appears to oxidize into phenolic compounds with either a δ- or γ-lactone structure (Wenkert et al., 1965; Schwarz and Ternes, 1992b; Cuvelier et al., 1994) and methyl carnosate (Cuvelier et al., 1994). γ-Lactones (rosmanol, epirosmanol, and 7-methylepirosmanol) are formed from carnosol, which is derived from carnosic acid (Schwarz and Ternes, 1992b). When in contact with oxidized methyl oleate in the absence of air, the first radical species of carnosic acid produced between 50 and 110 °C is a hydroxyl-phenoxy radical, and the second radical produced above 110 °C has an ESR spectrum identical with that of carnosol (Geoffroy et al., 1994). However, carnosol, rosmanol, epirosmanol, and 7-methylepirosmanol also show antioxidant activities when evaluated by different methods in different lipid systems (Nakatani and Inatani, 1981, 1984; Inatani et al., 1983; Chen et al., 1992; Cuvelier et al., 1994; Frankel et al., 1996). The oxidation of bulk corn oil triglycerides containing

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added carnosic acid was inhibited after the complete depletion of carnosic acid (Hopia et al., 1996). The mechanism for the antioxidant activity of carnosic acid is thus complicated because its degradation products can act as antioxidants.

The literature comparing the relative activities of natural antioxidants is confusing because different methods have been used with different lipid systems under drastic conditions (Frankel, 1993). Few investigators have evaluated antioxidant activity systematically with respect to the interactions of different variables that affect the relative activities. Frankel et al. (1994, 1996) and Huang et al. (1994, 1996) showed that the relative effectiveness of lipophilic and hydrophilic antioxidants was dependent on the lipid substrate, physical state, antioxidant concentration, oxidation time, and method used to determine lipid oxidation. Although triglycerides are the major lipids in food systems, polyunsaturated fatty acids (PUFA) and their esters are used widely as lipid substrates to evaluate antioxidant activity. Huang et al. (1996) and Hopia et al. (1996) demonstrated that linoleic acid is not a valid substrate for evaluating food antioxidants because it forms unique mixed micelles with surfactants.

As a lipophilic analogue of carnosic acid, methyl carnosate is suitable to study the difference in interfacial phenomena between bulk and emulsion systems. No information is available in the literature on the stability or degradation of methyl carnosate. In this paper, the effectiveness of carnosic acid and methyl carnosate in bulk corn oil and corn oil-in-water emulsions was compared by systematically studying the interactive effects of three variables, antioxidant concentration, physical state, and oxidation stage, on antioxidant activities at 60 °C. α-Tocopherol was used as a reference. The antioxidant effectiveness was evaluated at different stages of oxidation by measuring the formation of hydroperoxides (conjugated dienes) and the decomposition of hydroperoxides (hexanal) in both lipid systems. To define the relationship between the antioxidant activity and phase distribution, the partition coefficients of carnosic acid and methyl carnosate between oil and water phases were also measured. The stability of these antioxidants in bulk oil and Tween 20 micelle solution was determined by measuring their depletion during oxidation using the high-performance liquid chromatography method (HPLC).

MATERIALS AND METHODS

Materials. Corn oil triglycerides stripped of tocopherols were purchased from Eastman Kodak Co., Rochester, NY. Corn oil was found to be free of tocopherols by HPLC (Handelman et al., 1985), and its peroxide value was less than 5. At a peroxide value of 5, linoleic hydroperoxides present as initiators of oxidation correspond to 2.5 mmol/kg of oil. The fatty acid composition determined by gas chromatography (GC) of the methyl esters was 16:0 10.5%, 18:0 2.1%, 18:1 25.5%, 18:2 60.8%, and 18:3 1.1%. α-Tocopherol was obtained from Fluka Chemical Co., Ronkonkoma, NY, and Tween 20 (polyoxyethylene sorbitan monolaurate) was obtained from Sigma Chemical Co., St. Louis, MO. Carnosic acid was obtained at >98% by purification of rosemary extracts (Aeschbach et al., 1990).

Synthesis of Methyl Carnosate. Carnosic acid was methylated as described by Djarmati et al. (1993). Diazomethane in diethyl ether solution was added dropwise to a solution of carnosic acid (100 mg) in diethyl ether (25 mL). The purity of the crude methyl ester (pale yellow product) was checked by HPLC. A 25 mg sample of crude product dissolved in 2 mL of petroleum ether was chromatographed on a column of 25 g of silica gel (particle size, 10−40 μm; Sigma Chemical Co., St. Louis, MO) using petroleum ether−chloroform gradient elution by a modification of the procedure described by Al-Hazimi (1986). The column was washed with 30 mL of petroleum ether−chloroform (1:1), and then a yellowish methyl carnosate was eluted with petroleum ether−chloroform (1:3). This purified methyl carnosate was recrystallized and identified by comparing the retention time by HPLC with that of an authentic standard compound.

Preparation of Bulk Oil and Emulsion Samples. Oil samples (10 g) were prepared with or without added 150 or 300 μM antioxidants. These concentrations are equivalent to 50 and 100 ppm of carnosic acid or methyl carnosate and 65 and 130 ppm of α-tocopherol, respectively. Antioxidants were dissolved in corn oil by warming to 60 °C for 10 min; 10% oil-in-water emulsions (30 g) were prepared in 50 mL Erlenmeyer flasks as described by Huang et al. (1996). Emulsification was carried out by sonicating for a total of 6 min at high power (sonicator, cell disruptor, model W-10, Heat Systems, Ultrasonics, Inc., NY). The particle sizes of emulsions were determined with a Microtrac ultrafine particle analyzer (Leeds & Northrup, North Wales, PA). The average particle size in freshly prepared emulsions was 0.2−0.25 μm. The pH of emulsions with and without antioxidants ranged between 3.5 and 3.9.

Oxidation. Bulk oil samples (6 g in 25 mL screw-capped flask) and emulsion samples were oxidized at 60 °C in a shaker oven (Lab-Line Instrument, Inc., Melrose Park, IL). Oxidation was followed by measuring conjugated diene hydroperoxides spectrophotometrically and hexanal by static headspace GC. All oxidation reactions and analyses were done in duplicate, and the results were calculated by one-way analysis of variance (Wagner, 1992).

Measurement of Conjugated Diene Hydroperoxides. Measurements of conjugated dienes in oil samples were carried out according to the same procedures described previously (Frankel et al., 1994). For emulsions, samples (0.1 g) were dispersed in 5 mL of methanol and then diluted with more methanol to a measurable absorbance. The absorbance was measured at 234 nm, and concentrations of hydroperoxides were calculated on the same basis as the oil samples.

Measurement of Hexanal by Static Headspace GC. The procedures used for hexanal measurements were those described previously (Frankel et al., 1994), with the exception that all oil and emulsion samples were equilibrated at 60 °C for 15 min. Hexanal, one of many important volatile products of lipid hydroperoxide decomposition, is a useful marker for the decomposition of *n*−6 PUFAs (Frankel, 1982).

Measurement of Partition Coefficients between Oil and Water Phases. Corn oil (0.5 or 1.0 g) was weighed into a 10 mL screw-capped test tube containing 100 ppm antioxidants dissolved in methanol and dried under nitrogen. Deionized water (1.0 g) was weighed into the oil samples (1.0 g) and vortexed twice for 20 s after purging with nitrogen. The samples were then centrifuged at 3000 rpm for 15 min. The oil layer (0.5 g) was weighed and extracted five times with 1 mL of methanol containing citric acid (50 ppm) and erythorbic acid (50 ppm) by vortexing 20 s and then centrifuging at 2000 rpm for 3 min. The methanol extracts were evaporated to dryness under nitrogen, dissolved in 0.5 mL of methanol, vortexed, and centrifuged at 2000 rpm for 3 min. The clear methanol extracts were analyzed by HPLC (method given below). To measure the original antioxidant concentration, the oil samples (0.5 g) containing antioxidants were also extracted by the same method. The partition coefficient of the antioxidant between oil and water was calculated as follows:

$$\text{partition coefficient} = (V_w/V_l)[W_l/(W_t - W_l)]$$

where V_w = volume of water, V_l = volume of oil, W_t = total amount of the antioxidant, and W_l = amount of the antioxidant in the oil phase; the oil density (g mL^{-1}) used for calculating the volume was 0.916.

The methanol extracts 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. The elution

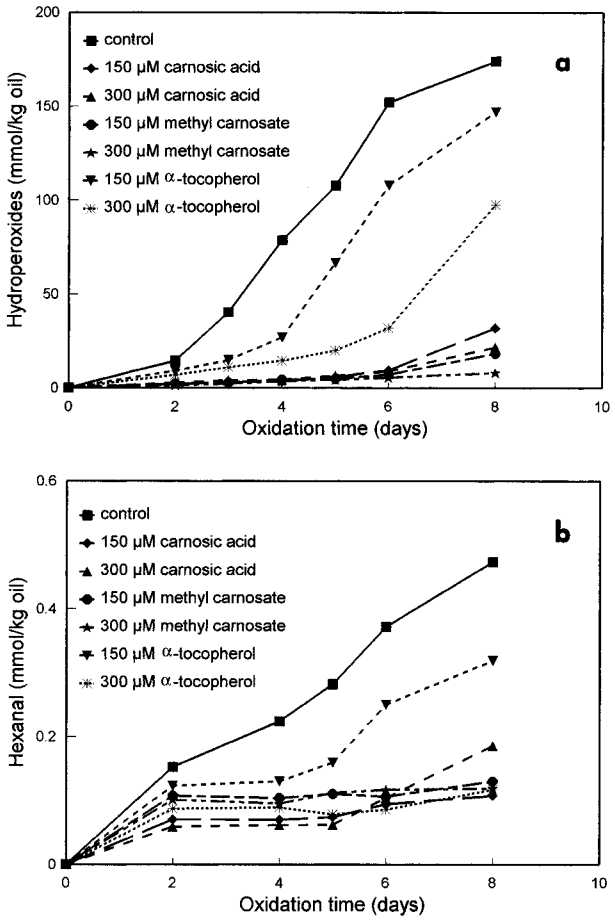

Figure 2. Effects of carnosic acid, methyl carnosate, and α-tocopherol on oxidative stability of bulk corn oil triglycerides at 60 °C: (a) hydroperoxide formation and (b) hexanal formation.

solvent was 85:15:1 methanol:water:2 M citric acid for carnosic acid and methyl carnosate and 100:1 methanol:2 M citric acid for α-tocopherol. The flow rate was 0.3 mL min^{-1}.

Oxidative Stability of Antioxidants. The concentration of antioxidants in bulk corn oil triglycerides during oxidation was determined by HPLC after the methanol extraction as described above. The oxidative stability of antioxidants in 1% Tween 20 micelle solutions at 60 °C was determined by measuring the concentration of antioxidants directly in Tween 20 solutions by the same HPLC method.

RESULTS

Effects of Antioxidants in Bulk Corn Oil Triglycerides. *Formation of Hydroperoxides.* Carnosic acid, methyl carnosate, and α-tocopherol inhibited hydroperoxide formation at 150 and 300 μM (Figure 2a and Table 1). Methyl carnosate and carnosic acid were more effective inhibitors of hydroperoxide formation than α-tocopherol at both concentrations during 8 days of oxidation. Although methyl carnosate inhibited hydroperoxide formation better than carnosic acid at both concentrations, the difference in antioxidant activity was small (89−95% vs 82−88%, Table 1, day 8).

Decomposition of Hydroperoxides. Carnosic acid and methyl carnosate inhibited hexanal formation less effectively than hydroperoxide formation (compare graphs a and b in Figure 2, Table 1). Carnosic acid and methyl carnosate were better inhibitors of hexanal formation than α-tocopherol at 150 μM. Carnosic acid inhibited hexanal formation better than methyl carnosate and α-tocopherol at the same concentration between 0 and 4 days (Figure 2b). Methyl carnosate inhibited hexanal formation better than carnosic acid at 300 μM on day 6 and at 150 and 300 μM on day 8 (Table 1). In contrast to hydroperoxide formation, carnosic acid decreased hexanal formation more effectively at 150 μM than at 300 μM after 6 days.

Effects of Antioxidants in Emulsified Corn Oil Triglycerides. *Formation of Hydroperoxides.* The emulsions with and without carnosic acid oxidized more rapidly and had a shorter induction period than the corresponding bulk oils (Figures 2a and 3a). Methyl carnosate and α-tocopherol inhibited hydroperoxide formation more effectively in emulsions than in bulk oils (Tables 1 and 2). The inhibition of hydroperoxide formation by methyl carnosate was greater at 300 μM than at 150 μM (Figure 3a and Table 2). Methyl carnosate inhibited hydroperoxide formation better at 150 μM than carnosic acid at 150 and 300 μM. Carnosic acid and methyl carnosate inhibited hydroperoxide formation more effectively than α-tocopherol at the same concentrations.

Decomposition of Hydroperoxides. The order of inhibition of hexanal formation was similar to that of hydroperoxide formation (Figure 3). Methyl carnosate inhibited hexanal formation more effectively than carnosic acid and α-tocopherol at both 150 and 300 μM (Figure 3b and Table 2). At the same concentration, carnosic acid was a better inhibitor of hexanal formation than α-tocopherol.

Oxidative Stability of Antioxidants at 60 °C. *In Bulk Corn Oil Triglycerides.* During oxidation of bulk corn oil at 60 °C, carnosic acid disappeared more rapidly than methyl carnosate and α-tocopherol (Figure 4a). Only 20−22% carnosic acid remained unoxidized after 12 h, and none was detected after 2 days for 150 μM and after 3 days for 300 μM. Of the original methyl carnosate, only 42−44% remained after 1 day, and essentially all disappeared after 4 days at 150 μM and after 5 days at 300 μM. α-Tocopherol at 300 μM was depleted gradually and disappeared after 4 days. However, the oxidations were retarded even when carnosic acid and methyl carnosate were depleted (Figures 2a and 4a). In contrast, although α-tocopherol was depleted more slowly at 300 μM, it inhibited hydroperoxide formation less effectively than carnosic acid and methyl carnosate between 1 and 4 days.

In 1% Tween 20 Micelle Solution. The emulsion systems used contained Tween 20 micelles and the Tween 20 interfaces between oil and water phases (Huang et al., 1996). To determine how the oxidative stabilities of these antioxidants were related to their antioxidant activities at the oil−water interfaces and in Tween 20 micelles, their oxidative stabilities in Tween 20 micelles were measured. In Tween 20 micelle solution as in bulk corn oil, carnosic acid also had the lowest stability followed by methyl carnosate and α-tocopherol at 300 μM, and it disappeared after 1 day (Figure 4b). Methyl carnosate also disappeared rapidly, and only about 2% remained after 2 days. α-Tocopherol was depleted about 50% after 4 days, and about 18% remained after 14 days.

Partition Study. The partition coefficients of methyl carnosate and carnosic acid between the oil and water phases were determined at ambient temperature (around 25 °C). The concentrations of carnosic acid and methyl carnosate in the oil phase were 96.2 ± 0.4% ($n = 3$) and 91.6 ± 0.8% ($n = 3$) after partitioning between corn oil and the same weight of deionized water. The partition

Table 1. Inhibition of Hydroperoxide and Hexanal Formation by Carnosic Acid, Methyl Carnosate, and α-Tocopherol in Bulk Corn Oil Triglycerides (Percent Mean Inhibition ± SD)[a,b]

	hydroperoxides			hexanal		
sample	day 4	day 6	day 8	day 4	day 6	day 8
control	0.0 ± 0.2[d]	0.0 ± 0.3[e]	0.0 ± 0.1[g]	0.0 ± 2.0[e]	0.0 ± 2.7[d]	0.0 ± 6.0[e]
+ carnosic acid						
150 μM	95.6 ± 0.2[a]	93.8 ± 0.1[b]	81.8 ± 0.1[d]	73.6 ± 4.5[ab]	77.3 ± 0.3[a]	71.5 ± 1.0[b]
300 μM	94.4 ± 0.3[a]	94.2 ± 0.1[b]	87.5 ± 0.1[c]	78.0 ± 0.4[a]	60.8 ± 0.9[b]	56.9 ± 3.0[c]
+ methyl carnosate						
150 μM	94.5 ± 0.2[a]	95.4 ± 0.1[a]	89.4 ± 0.1[b]	60.8 ± 2.3[c]	72.6 ± 8.0[a]	85.9 ± 0.4[a]
300 μM	95.5 ± 0.1[a]	96.5 ± 0.1[a]	95.4 ± 0.1[a]	60.4 ± 1.0[c]	74.9 ± 0.8[a]	82.9 ± 0.1[a]
+ α-tocopherol						
150 μM	65.8 ± 0.2[c]	29.0 ± 0.1[d]	15.6 ± 0.4[f]	43.5 ± 1.0[d]	32.7 ± 3.0[c]	30.6 ± 2.2[d]
300 μM	81.5 ± 0.3[b]	79.1 ± 0.1[c]	43.9 ± 0.4[e]	72.2 ± 1.5[b]	75.3 ± 0.7[a]	62.8 ± 0.1[c]

[a] Percent inhibition = $[(C - S)/C] \times 100$, where C = hydroperoxide or hexanal formed in control and S = hydroperoxide or hexanal formed in sample. Negative values represent prooxidant activity. SD, standard deviation; $n = 2$. [b] Values within each column followed by the same superscript letter are not significantly different ($p < 0.05$).

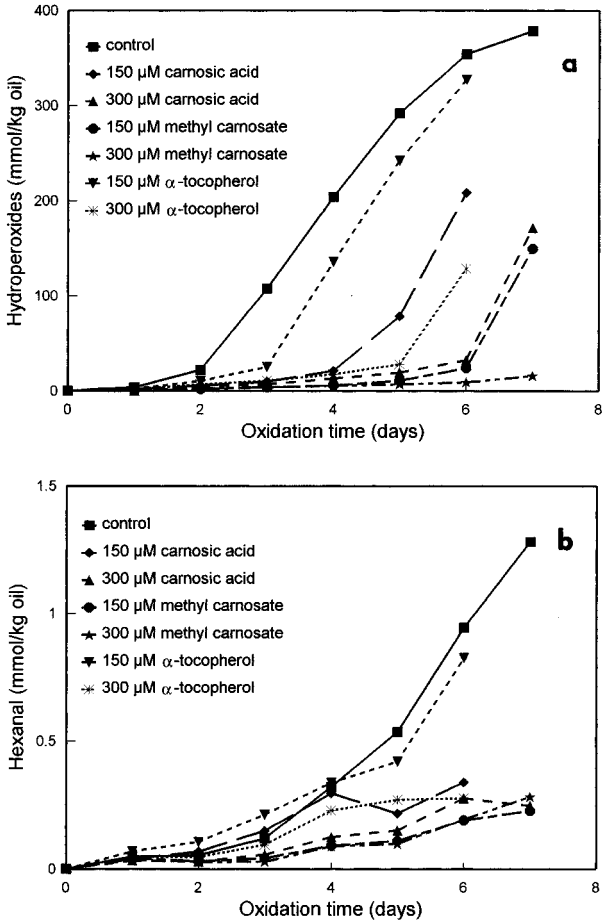

Figure 3. Effects of carnosic acid, methyl carnosate, and α-tocopherol on oxidative stability of emulsified corn oil triglycerides at 60 °C: (a) hydroperoxide formation and (b) hexanal formation.

coefficients were 23.4 ± 2.3 for methyl carnosate and 10.2 ± 1.1 for carnosic acid. These results show that carnosic acid is more polar than methyl carnosate.

DISCUSSION

This study compared the slightly hydrophilic carnosic acid and its more lipophilic methyl ester to determine the effect of different physical states of lipid systems on their antioxidant activities. The oxidative stabilities of both antioxidants in different systems were also measured to determine how their depletion during oxidation was related to their antioxidant activities.

Carnosic acid and methyl carnosate were more effective antioxidants than α-tocopherol in bulk and emulsion systems. The higher antioxidant activities of both diterpenes compared to α-tocopherol were probably due to the *o*-dihydroxyl groups in their aromatic ring, while α-tocopherol only has one phenolic hydroxyl group. Methyl carnosate was more effective than carnosic acid in bulk and emulsified corn oil, but the difference was greater in the emulsion system. We reported previously that the hydrophilic antioxidants ascorbic acid and Trolox were more active than their lipophilic analogues ascorbyl palmitate and α-tocopherol in bulk oils (Frankel et al., 1994). The greater effectiveness of both hydrophilic antioxidants may be explained by their affinity toward the oil−air interfaces where they could more efficiently protect lipids against oxidation. Although the hydrophilic carnosic acid was expected to be more concentrated at the air−oil interfaces than the more lipophilic methyl carnosate in the bulk oil system, according to their partition coefficients, carnosic acid was less stable than methyl carnosate in bulk corn oil. However, the difference in antioxidant activities between carnosic acid and methyl carnosate was not as marked as that between α-tocopherol and Trolox observed previously in bulk corn oil (Frankel et al., 1994). Carnosic acid is less polar than Trolox, according to their partition coefficients (Huang et al., 1996). Methyl carnosate is less lipophilic than α-tocopherol, based upon the mobilities in the solvents used in the HPLC method. The difference between the polarities of carnosic acid and methyl carnosate is smaller than that between the polarities of Trolox and α-tocopherol, which may partially explain the small difference between the antioxidant effectiveness of carnosic acid and methyl carnosate in bulk corn oil. In addition, the carboxyl group of carnosic acid may interact with the phenolic hydroxyl group in their proximity via intrahydrogen bonding, resulting in deactivation of the hydrogen-donating ability of this phenolic hydroxyl group, thus lowering the antioxidant activity of carnosic acid.

In emulsions, methyl carnosate was more effective than carnosic acid as an inhibitor of hydroperoxide formation and decomposition. This result is in agreement with those of Frankel et al. (1994) with α-tocopherol and ascorbyl palmitate and their analogues Trolox and ascorbic acid. More methyl carnosate was expected to be located at the oil−water interfaces and in the oil phase and to better protect lipids against oxidation than carnosic acid which partitioned into the water phase. In another study (Hopia et al., 1996), we found that carnosic acid was a better antioxidant than carnosol (a less polar lactone derivative of carnosic acid) in bulk methyl linoleate, but the reverse trend was

Table 2. Inhibition of Hydroperoxide and Hexanal Formation by Carnosic Acid, Methyl Carnosate, and α-Tocopherol in 10% Corn Oil-in-Water Emulsions (Percent Mean Inhibition ± SD)[a,b]

	hydroperoxides		hexanal	
sample	day 3	day 6	day 3	day 6
control	0.0 ± 0.8[e]	0.0 ± 0.3[g]	0.0 ± 8.4[e]	0.0 ± 2.7[e]
+ carnosic acid				
150 μM	90.9 ± 0.1[c]	41.1 ± 0.2[e]	−25.2 ± 1.6[f]	64.1 ± 0.5[c]
300 μM	93.6 ± 0.1[b]	90.9 ± 0.1[c]	53.3 ± 0.6[c]	70.8 ± 0.1[b]
+ methyl carnosate				
150 μM	96.5 ± 0.2[a]	93.3 ± 0.1[b]	65.1 ± 0.9[b]	79.8 ± 0.4[a]
300 μM	96.6 ± 0.2[a]	97.4 ± 0.1[a]	76.5 ± 0.1[a]	79.4 ± 0.1[a]
+ α-tocopherol				
150 μM	76.7 ± 0.3[d]	7.5 ± 0.6[f]	−78.2 ± 1.1[g]	12.5 ± 0.7[d]
300 μM	90.0 ± 0.1[c]	63.7 ± 0.1[d]	20.6 ± 2.2[d]	70.7 ± 1.0[b]

[a] Percent inhibition = $[(C - S)/C] \times 100$, where C = hydroperoxide or hexanal formed in control and S = hydroperoxide or hexanal formed in sample. Negative values represent prooxidant activity. SD, standard deviation; $n = 2$. [b] Values within each column followed by the same superscript letter are not significantly different ($p < 0.05$).

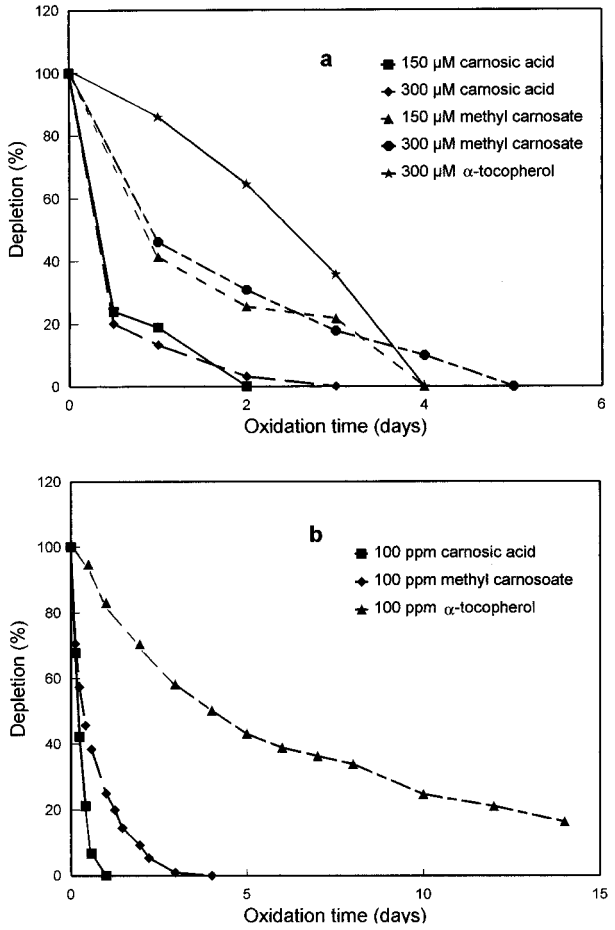

Figure 4. Oxidative stability of carnosic acid, methyl carnosate, and α-tocopherol at 60 °C in (a) bulk corn oil triglycerides and (b) 1% Tween 20 micelle solution.

observed in bulk linoleic acid. Therefore, the type and polarity of the lipid system used as substrate significantly affect the order of antioxidant activity of these rosemary compounds.

The depletion of both diterpene compounds did not correspond to their inhibition of hydroperoxide formation in bulk corn oil, especially carnosic acid. Carnosic acid was relatively unstable in bulk oils and Tween 20 micelles. Methyl carnosate was more stable than carnosic acid but less stable than α-tocopherol. Hence, in emulsions carnosic acid and its methyl ester were expected to be less stable than α-tocopherol, whether they were located in the oil phase, in Tween 20 micelles, or at the oil−water interface. The oxidation in the presence of either carnosic acid or methyl carnosate was still retarded after their complete depletion in bulk and emulsified corn oil triglyceride. Therefore, the oxidation products of carnosic acid and methyl carnosate may act as antioxidants by retaining their ability to retard lipid oxidation.

Carnosol and other γ- and δ-lactones like rosmanol were identified as oxidation products of carnosic acid (Schwarz and Ternes, 1992b; Cuvelier et al., 1994; Geoffroy et al., 1994). These compounds were found to act as antioxidants (Nakatani and Inatani, 1981, 1984; Chen et al., 1992; Frankel et al., 1996), but their antioxidant activities were lower than those of carnosic acid and methyl carnosate (Cuvelier et al., 1994). These oxidation products of carnosic acid are more lipophilic than carnosic acid.

The oxidation products of carnosic acid and methyl carnosate produced during oxidation need to be further identified to improve our understanding of their antioxidant mechanisms. The partition of these oxidation products also needs to be measured to clarify the relationship between their phase distribution and antioxidant activities. In addition, the other decomposition products of hydroperoxides need to be determined to investigate the effects of carnosic acid and methyl carnosate on hydroperoxide decomposition.

ABBREVIATIONS USED

PUFA, polyunsaturated fatty acid; Tween 20, polyoxyethylene sorbitan monolaurate; GC, gas chromatography; HPLC, high-performance liquid chromatography.

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