Effect of Different Lipid Systems on Antioxidant Activity of Rosemary Constituents Carnosol and Carnosic Acid with and without α-Tocopherol

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The effect of oxidizing lipid substrate on the antioxidant activity of carnosol and carnosic acid were evaluated in bulk and emulsified systems. In bulk methyl linoleate, carnosic acid was a better antioxidant than carnosol on the basis of conjugated diene hydroperoxide formation, and both were more active than α-tocopherol. However, in linoleic acid carnosol was more active than carnosic acid. In bulk corn oil triglycerides, α-tocopherol exhibited the most antioxidant activity followed by carnosic acid and carnosol. In all emulsified systems, α-tocopherol consistently exhibited more antioxidant activity than carnosol and carnosic acid. In mixtures, carnosol decreased and carnosic acid increased the oxidative stability of α-tocopherol in corn oil. During oxidation carnosic acid and carnosol are converted to unknown compounds which exhibit antioxidant activity. The type and polarity of the lipid system used as a model substrate significantly affect the antioxidant activity of carnosol and carnosic acid.

Keywords: *Antioxidants; a-tocopherol; carnosol; carnosic acid; methyl linoleate; linoleic acid; corn oil; oil; emulsion; synergism*

INTRODUCTION

Many herbs and spices are known to exhibit antioxidant activity in food lipids (Chipault et al., 1952; Bracco et al., 1981; Löliger, 1983). Commercially available rosemary extracts exhibit potent antioxidant activity (Chang et al., 1977), and are widely used in the food industry. In a study of 16 compounds isolated from rosemary, including flavones, diterpenes, steroids, and triterpenes, Bracco et al. (1981) concluded that the antioxidant activity of rosemary extracts is primarily related to two phenolic diterpenes: carnosic acid and carnosol. This conclusion was confirmed by others (Schwartz et al., 1992; Chen et al., 1992). Several other antioxidative diterpenes such as rosmanol, epirosmanol, and isorosmanol (Nakatani and Inatani, 1984), rosmaridiphenol (Houlihan et al., 1984), and rosmariquinone (Houlihan et al., 1985) have also been reported to contribute to the antioxidant activity of rosemary extracts. In fresh rosemary leaves carnosic acid is the major phenolic diterpene. The level of carnosol is approximately 10% of the carnosic acid content, and the other diterpenes are found in minor amounts (Wenkert et al., 1965). During the extraction of rosemary extracts carnosic acid is partially converted either into carnosol or into other diterpenes (Wenkert et al., 1965; Schwartz and Ternes, 1992a).

The application of rosemary extracts in foods has resulted in a variety of systems being tested and in great variability in the results depending on the test model being used. The model lipid system appears to affect the apparent antioxidant activity of the rosemary diterpenes. For example carnosol was a more active antioxidant in lard when measured by the active oxygen method (AOM) than in ethanolic solution of linoleic acid at 40 °C (Inatani et al., 1983). Systematic studies on the effect of an oxidizing lipid system on the activity of rosemary diterpenes have not been reported. Rosemary extracts are now being applied to various foods such as sausages (Barbut et al., 1985) or beef steaks (Stoick et al., 1991), and it is important to know the antioxidant activity of rosemary diterpenes in various food systems.

Carnosol and carnosic acid are effective peroxyl radical scavengers (Aruoma et al., 1992). However, little is reported about the interactions of phenolic diterpenes and other antioxidants such as tocopherols, which are present in most food lipid systems. Wada and Fang (1992) observed a strong synergistic effect between rosemary extract (0.02%) and α-tocopherol (0.05%) in sardine oil at 30 °C and in frozen-crushed fish meat models. The active compounds of rosemary extract were not identified, but the authors suggested that rosemary extract functions as a hydrogen atom donor regenerating the α-tocopheroxyl radical to α-tocopherol.

In most studies of antioxidant activity, oxidation has been viewed as a single step process. Since lipid oxidation is a complex multistep process (Huang et al., 1994), it is important to study the ability of antioxidants to inhibit the various steps of the oxidation process. The formation of hydroperoxides and their decomposition are discrete steps which are affected quite differently by various types of antioxidants.

The aim of this work was to study the effect of the colloidal state of the oxidizing lipid system on the activities of rosemary diterpenes carnosol and carnosic acid and their interaction with α-tocopherol. Corn oil triglycerides, methyl linoleate, and linoleic acid were investigated as model lipids, in bulk form and in aqueous dispersions. Using these systems, the antioxidant activity of carnosol and carnosic acid was compared with that of α-tocopherol. Antioxidant activity was monitored by measuring the formation of conjugated

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diene hydroperoxides. The decomposition of hydroperoxides was monitored by measuring hexanal, the major volatile product of linoleic acid hydroperoxides. To further understand the mechanisms of the action of rosemary diterpenes in inhibiting oxidation and particularly their interaction with α-tocopherol, the concentration of antioxidants was followed by high-performance liquid chromatography (HPLC) during the course of oxidation.

MATERIALS AND METHODS

Materials and Reagents. Methyl linoleate and linoleic acid were obtained from Nu-Chek-Prep, Inc. (Elysian, MN), and the tocopherol-free corn oil triglycerides, from Eastman Chemical Co. (Rochester, NY). The initial hydroperoxide content of test lipids was less than 5 mmol/kg of oil. α-Tocopherol and Tween 20 (polyoxylene sorbitan monolaurate) were purchased from Sigma Chemicals (St. Louis, MO). Carnosol and carnosic acid were kindly donated by Dr. R. Aeschbach (Nestlé Research Center, Lausanne, Switzerland).

Preparation of Oil and Emulsion Samples. Samples of bulk lipids (2.5 g) and emulsions (2.0 g) were prepared for each oxidation experiment with or without added carnosol or carnosic acid at 150 μM (50 ppm) or 300 μM (100 ppm) levels, and α-tocopherol at 300 μM (130 ppm) was used as reference. Antioxidants were freshly prepared in methanol solutions, and samples were purged with nitrogen to remove methanol.

Emulsions were prepared in distilled water as described by Frankel et al. (1994). Corn oil triglycerides, methyl linoleate, or linoleic acid with or without added antioxidants were emulsified with 1% Tween 20 (w/w) in an ice bath by sonicating the solution for 6 min with a sonicator (Model W-10, Heat Systems, Ultrasonics Inc., New York). The particle sizes of the emulsions were determined by a Microtrac ultrafine particle analyzer (Leeds & Northrup, North Wales, PA). The average particle size was 0.12−0.21 μm for fresh samples of corn oil triglyceride emulsion, 0.13−0.18 μm for methyl linoleate emulsions, and 0.15−0.24 μm for linoleic acid emulsions.

Oxidation Experiments. Bulk oil samples were oxidized in screw-capped vials (11.1 mL) and emulsion samples in screw-capped 50-mL Erlenmeyer flasks in the dark at 37 °C or at 60 °C with continuous shaking. Each oxidation experiment was done in duplicate. Oxidation of the samples was followed periodically by analyzing conjugated diene and hexanal contents. All analyses were carried out in duplicate.

Conjugated dienes in bulk oils were determined by diluting the weighed oil sample with isooctane and measuring the absorbance at 234 nm. Conjugated dienes in oil-in-water emulsions were determined by diluting the weighed sample with methanol and measuring the absorbance at 234 nm. Results were calculated as millimoles methyl linoleate hydroperoxides per kilogram of oil, using a molar absorptivity of 26 000 (Chan and Levett, 1977).

Hexanal content was measured by static headspace gas chromatography (GC) analysis in bulk oil samples (Frankel et al., 1994) and in emulsion samples (Huang et al., 1994). Hexanal contents were determined by using known amounts of hexanal standard as a reference (Frankel et al., 1989), and the results were calculated as millimoles of hexanal per kilogram of oil.

The α-tocopherol contents of the oxidizing oils were measured by normal phase HPLC analysis with a fluorescence detector (Handelman et al., 1985). The detection limit of the tocopherol analysis was 3 μg/g.

Carnosol and carnosic acid contents were analyzed by solid phase extraction using C-18 cartridges (No. 1210−2028, Varian, Habor City, CA) followed by HPLC analysis. Cartridges were conditioned with 4 mL of acetonitrile. An oil sample containing antioxidants was dissolved in 1 mM isopropyl alcohol and diluted with 2 mL of acetonitrile. Carnosol and carnosic acid were eluted with 2 mL of acetonitrile and then evaporated under nitrogen, the residue was dissolved in 250 μL of acetonitrile and stabilized with citric acid monohydrate (Merck & Co., Rahway, N.J.), and erythorbic acid (Pfizer Co., New York, N.Y.) or ascorbic acid (Sigma) was added to acetonitrile (50 μg/mL). The HPLC was performed with a Hewlett-Packard liquid chromatograph HP 1090 with the software chemstation HP79994 A using a column of LC-18 DB Supelcosil (Supelco, Belfonte, PA) with a particle size of 5 μm. Carnosol and carnosic acid were monitored by UV detection at 230 nm. Gradient elution was as follows: 0−0.30 min, 40% methanol/water/2 M citric acid (50:50:1) and 60% methanol/2 M citric acid (100:1); 0.30−7.00 min, 33% methanol/water/2 M citric acid (50:50:1) and 67% methanol/2 M citric acid (100:1); 7.00−15.00 min, 10% methanol/water/2 M citric acid (50:50:1) and 90% methanol/2 M citric acid (100:1). Between runs the column was conditioned by eluting for 5 min 40% methanol/water/2 M citric acid (50:50:1) and 60% methanol/2 M citric acid (100:1). The solvent flow rate was 0.3 mL/min.

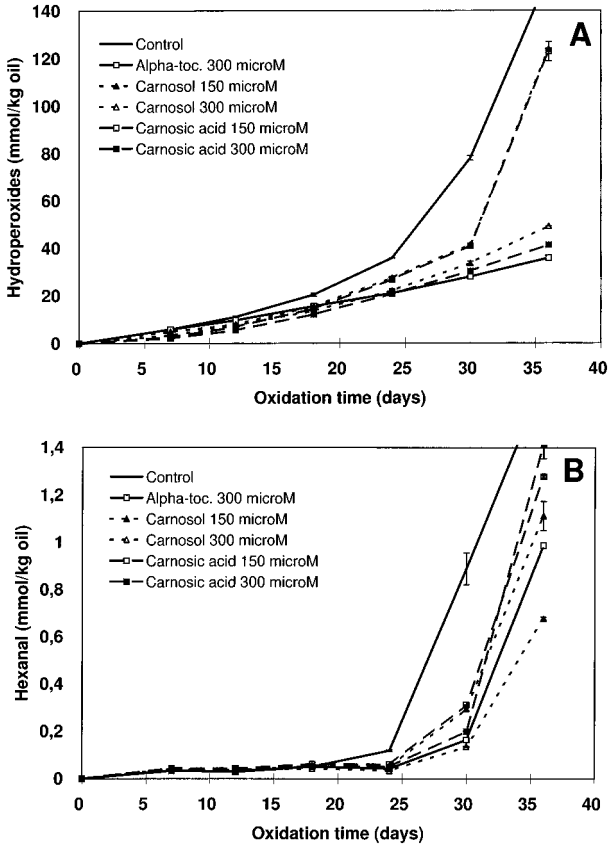

Figure 1. Antioxidant activity of carnosol, carnosic acid, and α-tocopherol in corn oil triglycerides at 37 °C: (A) hydroperoxide formation; (B) hexanal formation.

Statistical Analyses. Significant differences between the samples were calculated by analysis of the variance (ANOVA). One-way analysis of variance was calculated on the two duplicate measurements of both hydroperoxides and hexanal using a significance level of $p < 0.05$.

RESULTS

Bulk Corn Oil. In bulk oil at 37 °C the relative antioxidant activity of the rosemary compounds was dependent on the time of oxidation (Figure 1A). Thus, on the basis of conjugated dienes after 12 days, the order of activity at 300 μM was carnosic acid > carnosol > α-tocopherol, and after 36 days of oxidation, the order was α-tocopherol > carnosic acid > carnosol. At earlier stages of oxidation, α-tocopherol was less active than carnosol and carnosic acid but more active at later oxidation stages. The differences in activity between α-tocopherol, carnosol, and carnosic acid were small but statistically significant ($p < 0.05$). At 150 μM, carnosol and carnosic acid showed no difference in activity after

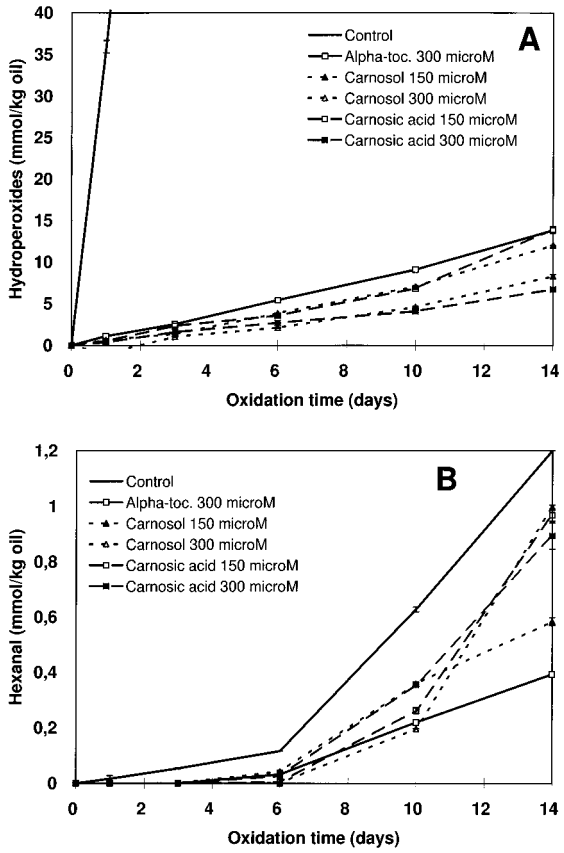

Figure 2. Antioxidant activity of carnosol, carnosic acid, and α-tocopherol in methyl linoleate at 37 °C: (A) hydroperoxide formation; (B) hexanal formation.

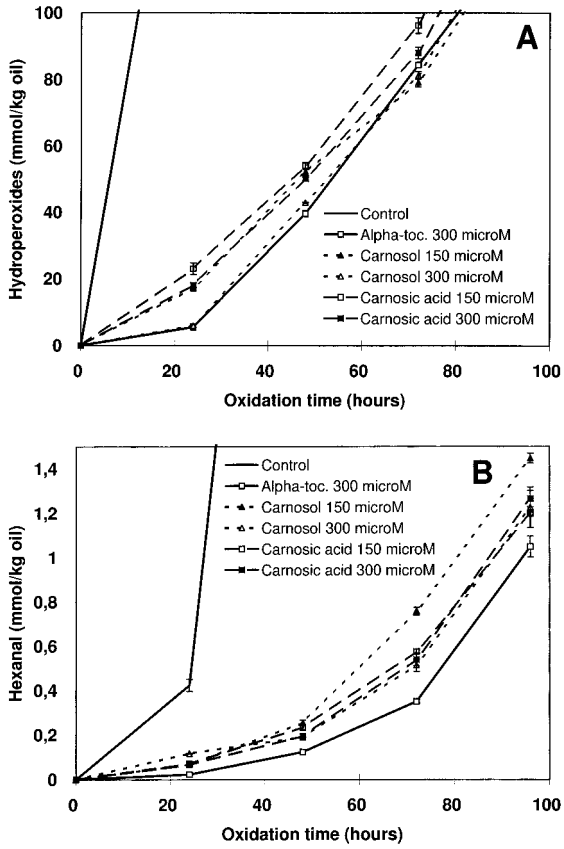

Figure 3. Antioxidant activity of carnosol, carnosic acid, and α-tocopherol in linoleic acid at 37 °C: (A) hydroperoxide formation; (B) hexanal formation.

36 days. In the presence of all antioxidants, hexanal formation was low during the first 24 days of oxidation, but increased rapidly in all samples after 30 days of oxidation. On the basis of hexanal formation after 30 days, the order of activity at 300 μM was carnosol > α-tocopherol > carnosic acid (Figure 1B).

Bulk Methyl Linoleate. Both carnosol and carnosic acid were very effective antioxidants in bulk methyl linoleate (Figure 2A). These diterpenes were more active antioxidants at 300 μM than equal molar concentration of α-tocopherol. On the basis of conjugated diene formation, the activity after 14 days of oxidation at 300 μM decreased in the order carnosic acid > carnosol > α-tocopherol. However, on the basis of hexanal formation, the order of activity at 300 μM was α-tocopherol > carnosic acid > carnosol, but at 150 μM carnosol exhibited better antioxidant activity than carnosic acid (Figure 2B).

Bulk Linoleic Acid. The relative antioxidant activities of carnosol and carnosic acid with respect to α-tocopherol were different in bulk linoleic acid compared to bulk methyl linoleate (Figure 3A). On the basis of conjugated diene formation, the order of antioxidant activity after 48 h at 300 μM was α-tocopherol > carnosol > carnosic acid, and after 72 h the order was carnosol > α-tocopherol > carnosic acid. On the basis of hexanal measurement, α-tocopherol was the most effective antioxidant and the order of activity after 72 h of oxidation was α-tocopherol > carnosol ∼ carnosic acid (Figure 3B).

Corn Oil Emulsion. When dispersed in water as surfactant coated emulsion particles, corn oil oxidized more rapidly than as bulk oil. In the corn oil emulsion, carnosol and carnosic acid were markedly less effective antioxidants than in bulk oils (Figure 4A) and α-tocopherol was much more active than carnosol and carnosic acid. On the basis of conjugated diene formation, the order of antioxidant activity after 16 days of oxidation at 37 °C was α-tocopherol > carnosic acid ≈ carnosol. However, after 21 days of oxidation only α-tocopherol at 300 μM and carnosic acid at 150 μM showed antioxidant activity, and carnosol and carnosic acid at 300 μM had no antioxidant activity. In contrast to their lack of inhibition of conjugated diene formation, both carnosol and carnosic acid inhibited hexanal formation but were significantly less active antioxidants than α-tocopherol (Figure 4B).

Methyl Linoleate Emulsion. In methyl linoleate emulsion, carnosol and carnosic acid had the same antioxidant properties as in triglyceride emulsion and α-tocopherol was the most effective antioxidant (Figure 5). On the basis of conjugated diene formation, the order of antioxidant activity after 14 days of oxidation at 300 μM was α-tocopherol > carnosic acid > carnosol (Figure 5A). On the basis of hexanal formation, the order of antioxidant activity was α-tocopherol ≃ carnosic acid > carnosol (Figure 5B).

Linoleic Acid Emulsion. As in emulsified corn oil triglyceride and methyl linoleate, α-tocopherol was the most effective antioxidant in emulsified linoleic acid (Figure 6). Carnosol showed higher antioxidant activity than carnosic acid in emulsified linoleic acid as in bulk linoleic acid. On the basis of conjugated diene formation, the order of antioxidant activity after 24 h of oxidation at the 300 μM level was α-tocopherol ≈ carnosol > carnosic acid, and after 48 h of oxidation the order was α-tocopherol > carnosol ≈ carnosic acid (Figure 6A). On the basis of hexanal formation after 48 h of oxidation, α-tocopherol and carnosol were the

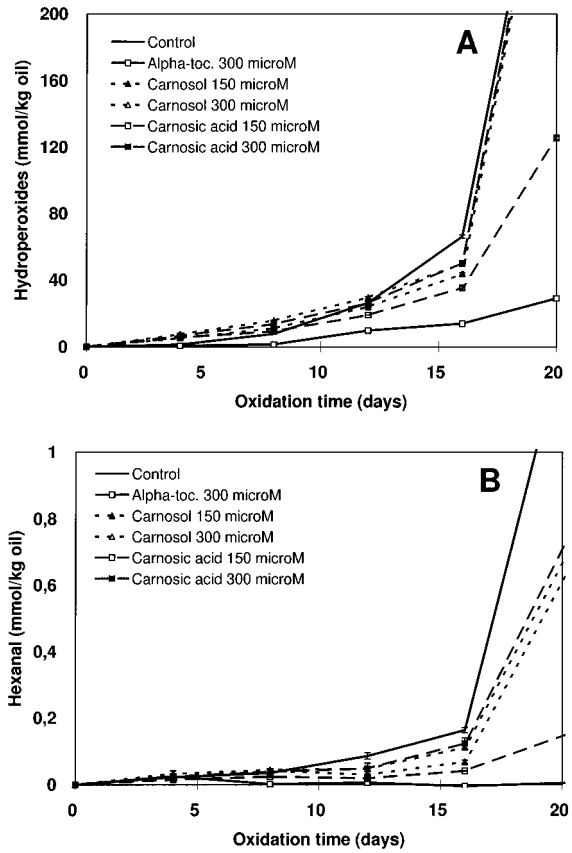

Figure 4. Antioxidant activity of carnosol, carnosic acid, and α-tocopherol in an oil-in-water emulsion of corn oil triglycerides at 37 °C: (A) hydroperoxide formation; (B) hexanal formation.

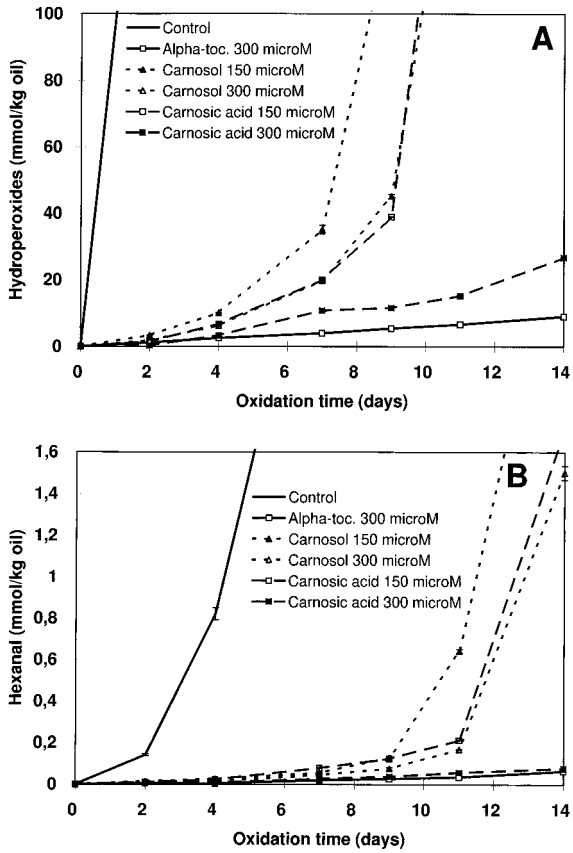

Figure 5. Antioxidant activity of carnosol, carnosic acid, and α-tocopherol in an oil-in-water emulsion of methyl linoleate at 37 °C; (A) hydroperoxide formation; (B) hexanal formation.

most effective antioxidants and carnosic acid had lower antioxidant activity (Figure 6B).

Interaction of Carnosol and α-Tocopherol. The activity of mixtures of carnosol and carnosic acid with α-tocopherol was studied in bulk corn oil at 60 °C. Carnosol and α-tocopherol had no effect or a slightly negative effect toward the oxidative stability of corn oil. Pure carnosol at 300 μM was a more active antioxidant than the mixtures of carnosol and α-tocopherol, either at 150 μM each or at 300 μM each (Figure 7A).

α-Tocopherol decreased the oxidative stability of carnosol. Thus, pure carnosol at 300 μM was consumed in 5 days, whereas in the presence of α-tocopherol carnosol was consumed in 4 days of oxidation (Figure 7B). Similarly, carnosol decreased slightly the oxidative stability of α-tocopherol. The concentrations of α-tocopherol were consistently lower in the presence than in the absence of carnosol (Figure 7C). Thus, the combination of carnosol and α-tocopherol decreased the oxidative stability of bulk corn oil, and carnosol decreased the stability of α-tocopherol. After 2 days of oxidation only 20% of carnosol remained in the sample. However, despite the rapid consumption of carnosol, the oxidation of the corn oil sample was still efficiently inhibited.

Interaction of Carnosic Acid and α-Tocopherol. At 60 °C, pure carnosic acid at 150 and 300 μM was more effective in inhibiting hydroperoxide formation than α-tocopherol at equal molar concentration (Figure 8A). Mixtures of carnosic acid and α-tocopherol, both at 300 μM and at 150 μM each, were less active in inhibiting hydroperoxide formation than pure carnosic acid at 300 μM (Figure 8A). Thus, in corn oil at 60 °C the combination of carnosic acid and α-tocopherol had no synergistic effect in preventing hydroperoxide formation compared to pure carnosic acid. At 300 μM pure carnosic acid disappeared after 3 days and after 2 days in the presence of α-tocopherol (Figure 8B). On the other hand, the oxidative stability of α-tocopherol increased in the presence of carnosic acid. Pure α-tocopherol decomposed after 5 days, but in the presence of carnosic acid it disappeared after 7 days of oxidation (Figure 8C). Thus, although no synergism between carnosic acid and α-tocopherol was observed, in contrast to carnosol, carnosic acid increased the stability of α-tocopherol. During oxidation, there was no evidence that carnosic acid was converted into carnosol.

As observed with carnosol, carnosic acid continued to show antioxidant activity after it was completely consumed in the corn oil sample during oxidation. After 2 days of oxidation only 10% of carnosic acid was left in the sample, and after 3 days of oxidation it was completely consumed (Figure 8B). Yet, the oxidation of corn oil was effectively retarded even after 5 days of oxidation (Figure 8A).

DISCUSSION

In bulk lipids the rosemary compounds carnosol and carnosic acid usually had similar antioxidant activity at 37 °C as α-tocopherol at equal molar concentrations, whereas in emulsified lipids α-tocopherol was consistently more active than carnosol and carnosic acid. In bulk corn oil at 300 μM, α-tocopherol was the most active antioxidant followed by carnosic acid and carnosol, whereas in corn oil emulsion α-tocopherol was markedly more active than carnosic acid, and carnosol had no antioxidant activity. In bulk methyl linoleate, carnosic acid was the most active antioxidant, followed by carnosol and α-tocopherol, whereas in methyl li-

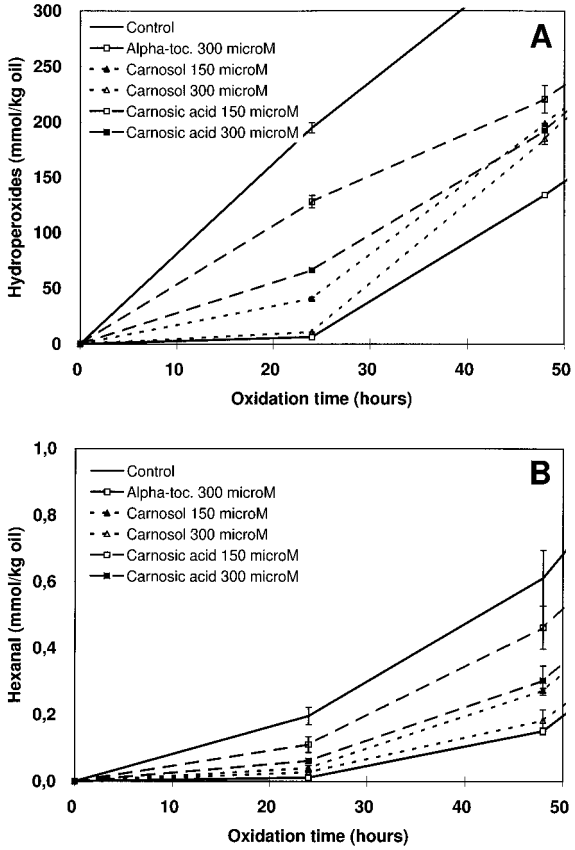

Figure 6. Antioxidant activity of carnosol, carnosic acid, and α-tocopherol in an oil-in-water emulsion of linoleic acid at 37 °C; (A) hydroperoxide formation; (B) hexanal formation.

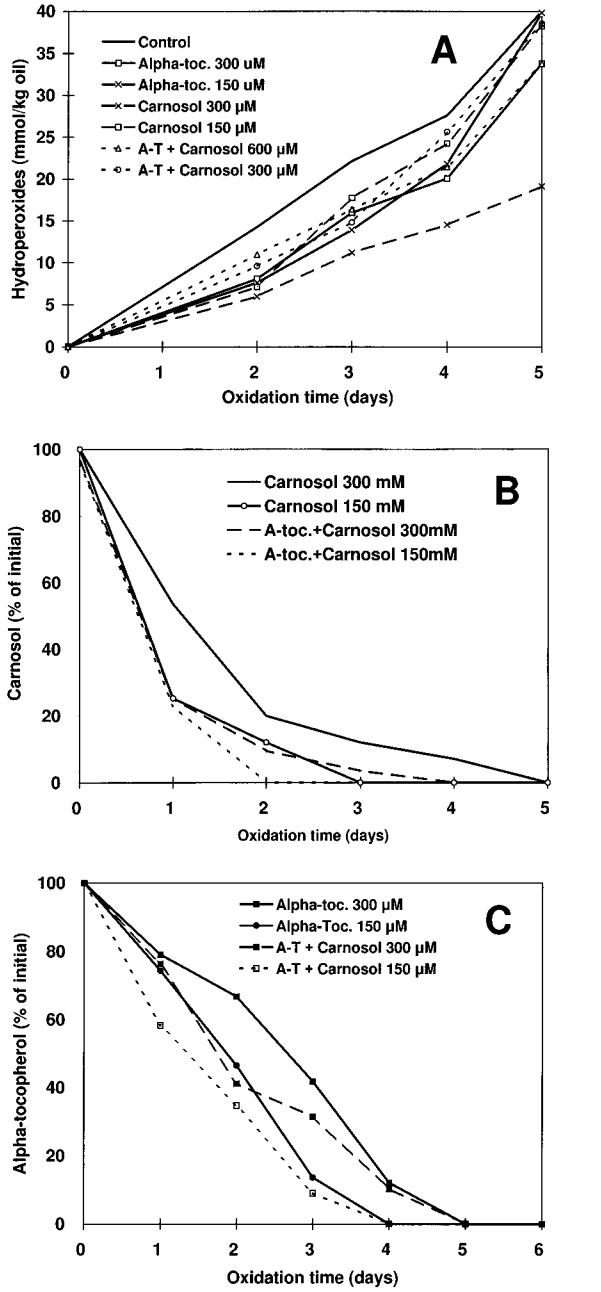

Figure 7. Antioxidant activity and stability of mixtures of carnosol and α-tocopherol in corn oil at 60 °C: (A) hydroperoxide formation; (B) consumption of carnosol; (C) consumption of α-tocopherol.

noleate emulsion, α-tocopherol was most effective followed by carnosic acid and carnosol. In contrast to the nonpolar lipids, where carnosic acid was more effective than carnosol, the reverse trend was observed in the more polar linoleic acid systems. Both in bulk and in emulsified linoleic acid, carnosol and α-tocopherol were more active antioxidants than carnosic acid.

These results may be explained in part by the differences in physical states of the lipid substrates. Linoleic acid is an amphiphilic lipid substrate and an ionizable acid with a pK_a value of approximately 5, and its physical and chemical characteristics vary markedly compared to the triglycerides. The free acid group of linoleic acid can interact with the carboxyl group of carnosic acid and thus affect its antioxidant activity. Furthermore, in emulsions linoleic acid moves freely between three states in aqueous dispersion: as emulsion particles, as mixed micelles, and as free molecules in solution. In contrast, nonpolar lipids remain located within the oil droplets of an emulsion. Also antioxidants are distributed between the water and oil phases of an emulsion in different proportions, depending on their hydrophilic or hydrophobic characteristics. Therefore, the interaction of antioxidants in bulk or dispersed linoleic acid differs from that of triglycerides. Since free fatty acids are rare in food systems and the vast majority of fatty acids are esterified as either triglycerides or phospholipids, the antioxidant activities observed with linoleic acid as lipid substrate may be largely irrelevant to most food or biological systems.

Lipophilic antioxidants are more active than their hydrophilic derivatives in corn oil-in-water emulsions, and the reverse is true in bulk corn oil, as was shown by comparing the activities of α-tocopherol *vs* Trolox and ascorbic acid *vs* ascorbyl palmitate (Frankel et al., 1994). Because of its free carboxylic group, carnosic acid is assumed to be more hydrophilic than carnosol. Also, on the basis of their solubility and chromatographic properties, carnosol and carnosic acid are both more polar than α-tocopherol (Schwarz and Ternes, 1992b) and can be assumed to be more hydrophilic than α-tocopherol. These more hydrophilic diterpenes may be less active in oil-in-water emulsions than in the bulk oils because they would not be as concentrated in the oil−water interphase as the lipophilic α-tocopherol.

Carnosic acid is usually reported to be a more active antioxidant than carnosol (Aruoma et al., 1992; Chen et al., 1992; Nakatani and Inatani, 1984). Relative radical scavenging activity of carnosic acid is higher than that of carnosol (Aruoma et al., 1992). Also, when evaluated in lard at 100 °C (Chen et al., 1992) carnosic acid was reported to be a more active antioxidant than

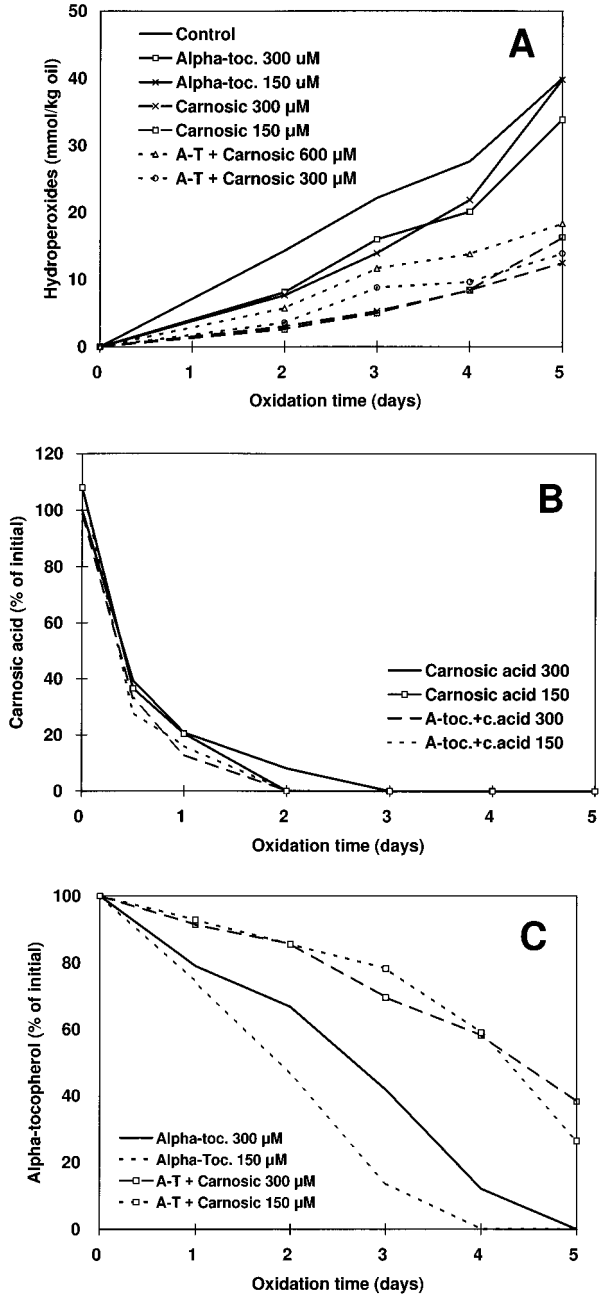

Figure 8. Antioxidant activity and stability of mixtures of carnosis acid and α-tocopherol in corn oil at 60 °C: (A) hydroperoxide formation; (B) consumption of carnosic acid; (C) consumption of α-tocopherol.

carnosol. Rosemary diterpenes were reported to have markedly higher antioxidant activity than many commonly used phenolic antioxidants. Under the conditions of the AOM test, rosmanol and carnosol were markedly more active antioxidants than α-tocopherol (Nakatani and Inatani, 1984), and under conditions of the Rancimat method, carnosol and carnosic acid were more active than BHA or BHT. In this study, the activities of rosemary antioxidants were found to be temperature dependent. At 37 °C (Figures 1−3), carnosol and carnosic acid were as active antioxidants as α-tocopherol at equal molar concentration, whereas at 60 °C carnosic acid was markedly more active than α-tocopherol (Figures 7 and 8). The high temperatures of the AOM (98 °C) and Rancimat (110 °C) tests may therefore account for the high antioxidant activity of carnosic acid in those studies.

The activity of carnosol and carnosic acid in preventing the decomposition of hydroperoxides has not previously been reported. In this work similar activities were observed for carnosol, carnosic acid, and α-tocopherol in inhibiting both hexanal and conjugated diene formation. α-Tocopherol was most active in inhibiting hexanal formation in bulk methyl linoleate, in bulk linoleic acid, and in corn oil emulsion. In bulk corn oil, carnosol was more active than α-tocopherol. In methyl linoleate emulsion, carnosic acid was as active as α-tocopherol in inhibiting hexanal formation, while, in linoleic acid emulsion, carnosol was as active as α-tocopherol. However, further studies on the effect of the diterpenes in inhibiting hydroperoxide decomposition are needed, especially in more complex food model systems.

Mixtures of carnosol or carnosic acid with α-tocopherol did not improve the oxidative stability of corn oil compared to pure carnosic acid. Mixtures of carnosol and α-tocopherol decreased the oxidative stability of corn oil compared to pure carnosol. Nevertheless, carnosic acid exhibited a significant protective effect toward α-tocopherol during oxidation of the corn oil. A possible mechanism for this effect may be similar to that of ascorbic acid and tocopherols (Tappel et al., 1961) (eq 1), where TocH = α-tocopherol, Toc$^{\bullet}$ = tocopheroxyl

$$\mathrm{Toc}^{\bullet} + \mathrm{AH} \rightarrow \mathrm{TocH} + \mathrm{A}^{\bullet} \qquad (1)$$

radical, AH = ascorbic acid, and A$^{\bullet}$ = ascorbyl radical. This mechanism, in which ascorbic acid reduces tocopheroxyl radical to active tocopherol is generally accepted to be responsible for the synergistic antioxidant properties of mixtures of ascorbic acid and tocopherols. Ascorbic acid has lower oxidation potential (0.28 V) than α-tocopherol (0.48 V), and thus ascorbic acid can reduce α-tocopherol radical into α-tocopherol (Simic, 1991). However, the reducing capacity of carnosic acid or other phenolic compounds from rosemary has not been reported in the literature.

The low halfway potential reported for carnosic acid compared to carnosol and other phenolic diterpenes from rosemary (Schwarz and Ternes, 1992b) indicates a higher oxidizability for carnosic acid. Therefore, carnosic acid may also protect α-tocopherol from oxidation by a sparing effect.

Although mixtures of carnosic acid and α-tocopherol showed no synergism in this study, synergism was previously reported between rosemary extract and α-tocopherol in sardine oil at 30 °C (Wada and Fang, 1992) and a protective effect of rosemary extract toward tocopherols in rapeseed oil under frying conditions (Gordon and Kourimská, 1995). The results of our present study suggest that the protective effect of carnosic acid on the oxidative stability of α-tocopherol may explain the reported synergism between rosemary extract and tocopherols.

In the present study we observed for the first time that carnosol had negative effects in combination with α-tocopherol. We observed that carnosol decreased the oxidative stability of α-tocopherol, and the combination of carnosol and α-tocopherol were actually antagonistic on the oxidative stability of corn oil. In fresh rosemary leaves carnosic acid is the major phenolic diterpene present (Wenkert et al., 1965), and during extraction and storage in methanol it is converted to carnosol (Wenkert et al., 1965; Schwarz and Ternes, 1992a). In most food systems where rosemary antioxidants are used, tocopherols are also present. The results of this study suggest that carnosol present in rosemary extracts

actually has a negative effect on the oxidative stability in systems containing tocopherols.

In corn oil at 60 °C, rosemary antioxidants disappeared faster than α-tocopherol. After 1 day of incubation the initial concentrations left in corn oil were 80% α-tocopherol, 55% carnosol, and 40% carnosic acid. α-Tocopherol disappeared after 5 days, carnosol after 4 days, and carnosic acid after 3 days of oxidation. Although carnosic acid and carnosol disappeared most rapidly in oxidizing corn oil, their antioxidant activities remained high over the test period of 5 days. This result indicates that carnosic acid and carnosol are converted into other compounds, which retain high antioxidant activity. On the basis of an ESR study of carnosic acid in the presence of oxidized oleic acid, Geoffroy et al. (1994) suggested that carnosic acid is converted into carnosol. They also suggested the formation of a ketophenoxy radical. In this study, no evidence was observed for the conversion of carnosic acid into carnosol during oxidation. Further studies to identify these conversion products are needed to improve our understanding of the antioxidant mechanism of rosemary compounds.

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LITERATURE CITED

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