Antioxidant Activity of a Rosemary Extract and Its Constituents, Carnosic Acid, Carnosol, and Rosmarinic Acid, in Bulk Oil and Oil-in-Water Emulsion

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This study was aimed at evaluating the antioxidant activity of a commercial rosemary extract and the active constituents carnosol, carnosic acid, and rosmarinic acid, in inhibiting the formation and decomposition of hydroperoxides in tocopherol-stripped corn oil and in the corresponding corn oil-in-water emulsions. In bulk corn oil, the rosemary extract, carnosic acid, rosmarinic acid, and α-tocopherol were significantly more active than carnosol. In contrast, in corn oil-in-water emulsion, the rosemary compounds were less active than in bulk oil, and the rosemary extract, carnosic acid, carnosol, and α-tocopherol were more active than rosmarinic acid. Similar results were obtained in corn oil-in-water phosphate buffer emulsion at pH 5, but α-tocopherol was less active. Carnosol and carnosic acid were much more active antioxidants in corn oil-in-water emulsions buffered at pH 4 and 5 than at pH 7. The decreased antioxidant activity of the polar hydrophilic rosemary compounds in the emulsion system may be explained by their interfacial partitioning into the water, thus becoming less protective than in the bulk oil system. The effect of pH may be related to the stability of the rosemary antioxidants.

Keywords: *Antioxidants; rosemary extracts; corn oil; hydroperoxides; hexanal; bulk oil; emulsion*

INTRODUCTION

Rosemary extracts contain a large number of compounds including carnosic acid, carnosol, and rosmarinic acid (Figure 1) and provide a major source of natural antioxidants used commercially at present in foods (Löliger, 1983). Carnosol and carnosic acid are the most important active components of rosemary extracts (Aeschbach et al., 1990; Löliger, 1991). There is a large body of literature on the activity of natural antioxidants and stability evaluations of different unsaturated food lipids. However, the literature on the antioxidant activity of commercial rosemary extracts and compounds is difficult to interpret because of the diverse testing systems, methods, and questionable conditions of oxidation used (Frankel, 1993). The antioxidant activity of carnosol was demonstrated in lard oxidized at 60 °C (Wu et al., 1982) and at 98 °C under AOM conditions (Nakatani et al., 1984) by determining the increase in peroxide value. On the other hand, carnosol was tested in an emulsion of linoleic acid in phosphate buffer at pH 7 oxidized at room temperature by determining TBA-reactive compounds (Inatani et al., 1983). The antioxidant activity of carnosic acid was tested in lard and peanut oil oxidized at 100 °C and at 140 °C using the Rancimat based on conductivity measurements (Schuler, 1990) and in lard oxidized at 110 °C using the Rancimat based on induction period measurements (Chen et al., 1992).

Conditions of accelerated oxidation used by many investigators to test rosemary compounds have been too drastic and the methods employed to measure lipid oxidation have been unspecific and not sufficiently sensitive to be relevant to flavor and oxidative deterioration in food systems. The use of the automated Rancimat instrument is particularly questionable because it is based on the conductivity of volatile organic acids produced at elevated temperatures and requires relatively high amounts of oxidation corresponding to levels of rancidity that are far in excess of normal (Frankel, 1993). The results of antioxidant tests carried out at temperatures of 100 °C and above are further

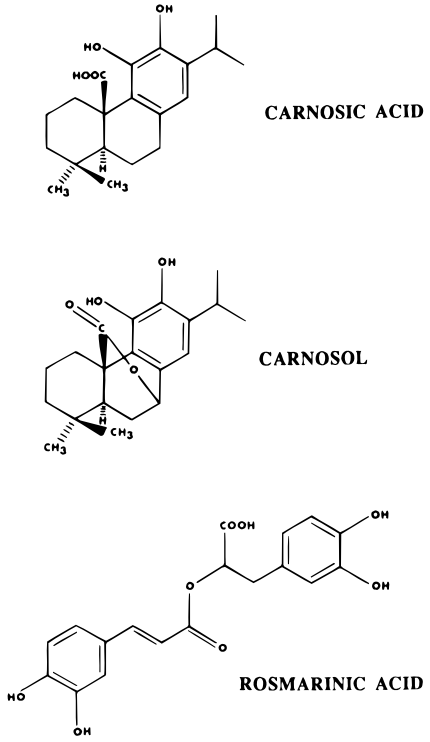

Figure 1. Structures of carnosic acid, carnosol, and rosmarinic acid.

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Table 1. Inhibition of Hydroperoxide and Hexanal Formation by a Rosemary Extract, Rosmarinic Acid, Carnosic Acid, Carnosol, and α-Tocopherol in Stripped Corn Oil and Emulsified Corn Oil Oxidized at 60 °C[a]

	hydroperoxide (%)		hexanal (%)	
sample	bulk oil, 6 days	emulsion, 4 days	bulk oil, 6 days	emulsion, 4 days
rosmarinic acid, 30 ppm	37.8 ± 0.3d	16.1 ± 0.8c	48.9 ± 1.4c	22.1 ± 2.2d
rosmarinic acid, 50 ppm	67.9 ± 0.01bc	−10.8 ± 7.8d	78.4 ± 1.1b	−53.2 ± 0.3e
carnosic acid, 50 ppm	70.1 ± 2.7b	36.5 ± 0.6b	28.8 ± 3.0d	83.2 ± 0.1b
carnosol, 50 ppm	6.3 ± 0.03e	42.1 ± 1.0b	−19.6 ± 0.8f	84.4 ± 0.4b
α-tocopherol, 100 ppm	68.0 ± 1.2bc	90.1 ± 0.5a	39.6 ± 2.0cd	98.4 ± 0.4a
rosemary extract, 250 ppm	63.9 ± 3.7c	10.5 ± 4.5c	−3.2 ± 11e	35.3 ± 7.4c
rosemary extract, 500 ppm	81.3 ± 1.2a	11.6 ± 9.1c	97.8 ± 0.9a	28.1 ± 8.3cd

[a] Inhibition ± standard deviation. % inhibition = [(control − sample)/control] × 100. Mean values within each column followed by the same letters are not significantly different at $P < 0.05$.

complicated with the phenolic diterpenes of rosemary extracts, which were shown to be thermally unstable (Schwarz et al., 1992). Several degradation products were reported to be formed from the phenolic diterpenes of rosemary extracts between 100 and 170 °C and under steaming conditions at 200 °C; some of these thermal degradation products were active as antioxidants (Schwarz et al., 1992).

Natural antioxidants have been particularly difficult to evaluate in oils and emulsions because of the complex interfacial phenomena affecting the partition of the antioxidants in multiphase food systems. Thus, the lipophilic antioxidants α-tocopherol and ascorbyl palmitate were found to be more effective in an oil-in-water emulsion system than in bulk oil, while the opposite trend was found for the hydrophilic antioxidants Trolox, a carboxylic acid derivative of α-tocopherol, and ascorbic acid (Frankel et al., 1994).

In evaluations of antioxidants, it is important to distinguish between their effects at different stages of lipid oxidation. Thus, although α-tocopherol was shown to have prooxidant activity at high levels on the basis of hydroperoxide formation, as measured by conjugated diene formation, it was an effective antioxidant in inhibiting hydroperoxide decomposition, as measured by hexanal formation, a major volatile decomposition product of linoleate hydroperoxides (Frankel et al., 1994). In contrast to hydroperoxide formation, the effectiveness of both α- and γ-tocopherols to inhibit hexanal formation was improved with increasing concentrations and oxidation time at 60 °C (Huang et al., 1994). The relative properties of natural antioxidants in inhibiting *both* hydroperoxide formation and their decomposition are properties that have received little attention in the literature, yet the decomposition of hydroperoxides and the resulting formation of volatile compounds have significant impact on the flavor deterioration of food lipids.

In this study we used corn oil stripped of natural tocopherols, in bulk or in oil-in-water emulsions, to evaluate the antioxidant activities of a commercial rosemary extract and its components, carnosic acid, carnosol, and rosmarinic acid, using α-tocopherol as reference. We also determined the effect of pH on antioxidant activity in stripped corn oil-in-water emulsion systems. The antioxidant effectiveness of the rosemary compounds was evaluated at different stages of oxidation by measuring both hydroperoxide formation, on the basis of conjugated dienes, and hydroperoxide decomposition, on the basis of hexanal formation.

MATERIALS AND METHODS

Materials. Corn oil stripped of tocopherols was obtained commercially (Eastman Kodak Co., Rochester, NY). Corn oil samples were found to be free of tocopherols by HPLC, and the peroxide values were less than 5. Fatty acid composition determined by gas chromatography (GC) of the methyl esters was the same (10.5% 16:0, 2.1% 18:0, 25.5% 18:1, 60.8% 18:2, and 1.1% 18:3) as reported previously (Frankel et al., 1994). One commercial rosemary extract (FIS S.A., Châtel-St.Denis, Switzerland) was analyzed by HPLC (Aruoma et al., 1992) to contain 10.3% carnosic acid and 4.4% carnosol. The concentration of rosmarinic acid was not detectable. Carnosol and carnosic acid were obtained by purification of rosemary extracts (Aeschbach et al., 1990). The purified carnosol and carnosic acid were better than 98% pure. Rosmarinic acid was purchased commercially (Carl Roth GmbH, Karlsruhe, Germany).

Preparation of Oil and Emulsion Samples. Stripped corn oils without added antioxidants and oils containing rosemary antioxidants were made up to a concentration of 10% by weight with deionized water in a 50-mL Erlenmeyer flask and emulsified with 1% Tween 20. Emulsification was carried out in an ice bath for 6 min with a sonicator set at high power (Model W-10, Heat Systems, Ultrasonics, Inc., New York). The initial pH of the emulsions ranged between 4.8 and 5.0 and was not affected by the addition of the rosemary antioxidants at the concentrations used. The particle size of emulsions was determined with a Microtrac particle analyzer (Leeds & Northrup, North Wales, PA). Average particle size in fresh samples of emulsions ranged from 0.1 to 0.25 μm. In the experiments designed to test the effect of pH, the emulsions were prepared with 0.025 M phosphate buffer and the pH was adjusted with phosphoric acid. Oxidation is assumed to be initiated by trace metals, and therefore no metal chelator was used to inactivate any metal contaminants present in the phosphate buffer.

Oxidation. Oil samples (5 g) weighed into screw-capped 25-mL Erlenmeyer flasks and 10% oil emulsion samples (30 mL) in 50-mL flasks were oxidized at 60 °C in a shaker oven (Lab-Line Instrument, Inc., Melrose Park, IL). Oxidative stability was evaluated by analyzing samples periodically for conjugated dienes by spectrophotometry and for hexanal by headspace GC. The data on conjugated dienes are expressed as hydroperoxides because we found with pure hydroperoxides that conjugated hydroxydienes are only formed in minor amounts (Hopia et al., unpublished results). All analyses were done in duplicate. The methods used to measure hydroperoxide formation as conjugated dienes and hydroperoxide decomposition as hexanal and the statistical analyses were the same as described previously (Frankel et al., 1994; Huang et al., 1994).

RESULTS

Bulk Corn Oil. On the basis of hydroperoxide formation, the best antioxidant activity was obtained with 50 ppm of rosmarinic acid, 50 ppm of carnosic acid, and 100 ppm of α-tocopherol. Rosmarinic acid was less effective at 30 ppm, and 50 ppm of carnosol had only very little antioxidant activity (Figure 2a; Table 1). The rosemary extract at 250 ppm showed an antioxidant activity similar to that of its main active component, carnosic acid, at 50 ppm; at 500 ppm, it showed the best antioxidant activity (Table 1). On the basis of hexanal

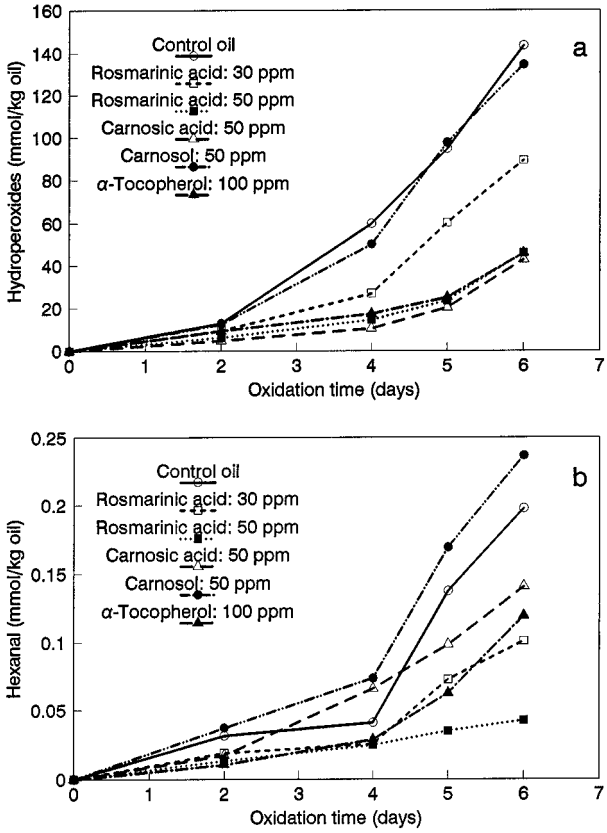

Figure 2. Effect of rosmarinic acid, carnosic acid, carnosol, and α-tocopherol on the oxidative stability of stripped corn oil at 60 °C: (a) hydroperoxide formation; (b) hexanal formation.

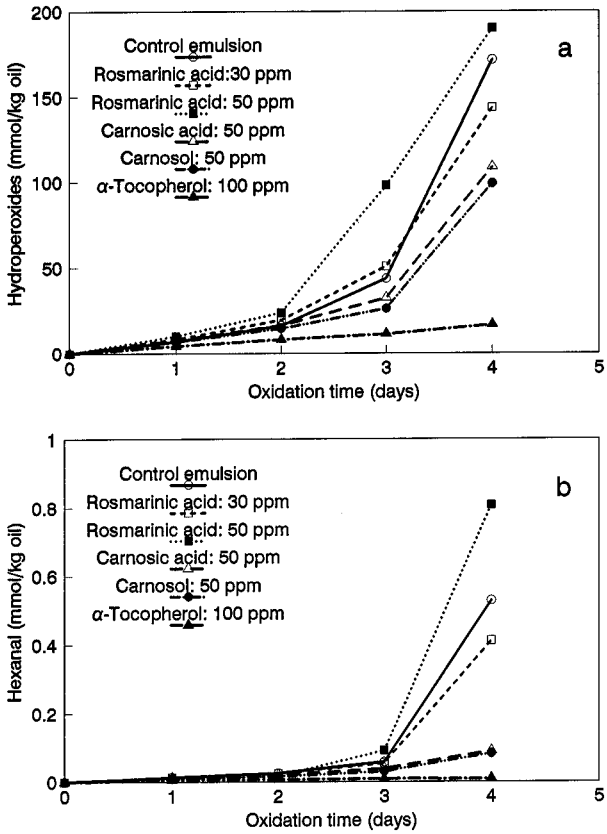

Figure 3. Effect of rosmarinic acid, carnosic acid, carnosol, and α-tocopherol on the oxidative stability of stripped corn oil emulsion at 60 °C: (a) hydroperoxide formation; (b) hexanal formation.

formation by headspace GC, rosmarinic acid had better antioxidant activity at 50 ppm than at 30 ppm, followed by 100 ppm of α-tocopherol and 50 of ppm carnosic acid (Figure 2b, Table 1). Carnosol exhibited prooxidant activity at 50 ppm. Although the rosemary extract had no activity at 250 ppm, it showed the best hexanal inhibition at 500 ppm (Table 1).

Corn Oil-in-Water Emulsion. The trends in antioxidant activities were significantly different in oil-in-water emulsion than in the bulk oil system. Thus, in stripped corn oil emulsified with Tween 20, α-tocopherol was the most active in inhibiting hydroperoxide formation, followed by carnosol and carnosic acid (Figure 3a). Carnosic acid and carnosol were not significantly different in antioxidant activity (Table 1). On the other hand, rosmarinic acid had only slight antioxidant activity at 30 ppm and behaved as a prooxidant at 50 ppm (Figure 3a; Table 1). Similarly, the rosemary extract had low antioxidant activity at 250 and 500 ppm (Table 1). Similar trends in antioxidant activities were observed on the basis of hexanal measurements (Figure 3b, Table 1).

Effect of pH in Corn Oil-in-Water Emulsions. The pH exerted a remarkably strong effect on the antioxidant activity of rosemary compounds in stripped corn oil-in-water emulsion prepared in phospate buffer. When the corn oil-in-water emulsions were prepared with a phosphate buffer at pH 5.0 (0.025 M), carnosic acid and carnosol showed the best performance on the basis of hydroperoxide formation and were more active than α-tocopherol. On the other hand, rosmarinic acid had very little antioxidant activity at both concentrations tested (Figure 4a; Table 2). On the basis of hexanal formation, smaller differences between these antioxidants were observed. Carnosic acid was again the best antioxidant followed by carnosol and α-tocopherol. Rosmarinic acid had no antioxidant activity at 30 ppm and was prooxidant at 50 ppm. α-Tocopherol had significantly lower antioxidant activity in the buffered emulsion at pH 5 than in the unbuffered emulsion (Figures 3 and 4; Tables 1 and 2).

The antioxidant activities of carnosol and carnosic acid were also compared in phosphate-buffered emulsions at pH 4 and 7. On the basis of hydroperoxide formation, carnosic acid and carnosol were both much more active as antioxidants at pH 4 and 5 than at pH 7 (Table 2). Although the control emulsion oxidized much more rapidly at pH 4 than at pH 5 and 7, the inhibition of hydroperoxide formation for carnosic acid and carnosol was calculated to be 92 and 94% at pH 4 and 90 and 88% at pH 5, respectively. In contrast, at pH 7 carnosol had no antioxidant activity, while carnosic acid had a calculated inhibition of only 6.5% (Table 2).

On the basis of hexanal formation, the activities of carnosic acid and carnosol were also significantly greater at pH 4 and 5 than at pH 7, and the calculated inhibition for carnosic acid and carnosol was 80% for both at pH 4 and 44 and 33% at pH 5 compared to 28 and 12%, respectively, at pH 7 (Table 2). This difference observed between hydroperoxide and hexanal formation could be due to a smaller degree of hydroperoxide decomposition at pH 4 than at pH 5 and 7.

DISCUSSION

In a study of the oxidation of a lecithin liposome model catalyzed by hematin, Porter et al. (1989) advanced the so-called "polar paradox" to describe the observation that polar antioxidants are more effective in nonpolar

Table 2. Efffect of pH on Inhibition of Hydroperoxide and Hexanal Formation by Rosmarinic Acid, Carnosic Acid, Carnosol, and α-Tocopherol in Emulsified Corn Oil Oxidized at 60 °C for 4 Days[a]

	hydroperoxide (%)			hexanal (%)		
sample	pH 4	pH 5	pH 7	pH 4	pH 5	pH 7
rosmarinic acid, 30 ppm	nd[b]	11.4 ± 1.6d	nd	nd	3.2 ± 1.8d	nd
rosmarinic acid, 50 ppm	nd	17.7 ± 4.9c	nd	nd	−9.8 ± 2.8e	nd
carnosic acid, 50 ppm	92.3 ± 0.1a	89.9 ± 0.6a	6.5 ± 2.3a	79.6 ± 0.1a	43.9 ± 0.6a	28.0 ± 0.4a
carnosol, 50 ppm	94.0 ± 0.2a	88.1 ± 1.5a	−3.9 ± 1.0b	78.9 ± 1.0b	33.0 ± 0.9b	11.5 ± 2.3b
α-tocopherol, 100 ppm	nd	80.0 ± 1.8b	nd	nd	13.7 ± 2.7c	nd

[a] See footnote *a* in Table 1. [b] nd, not determined.

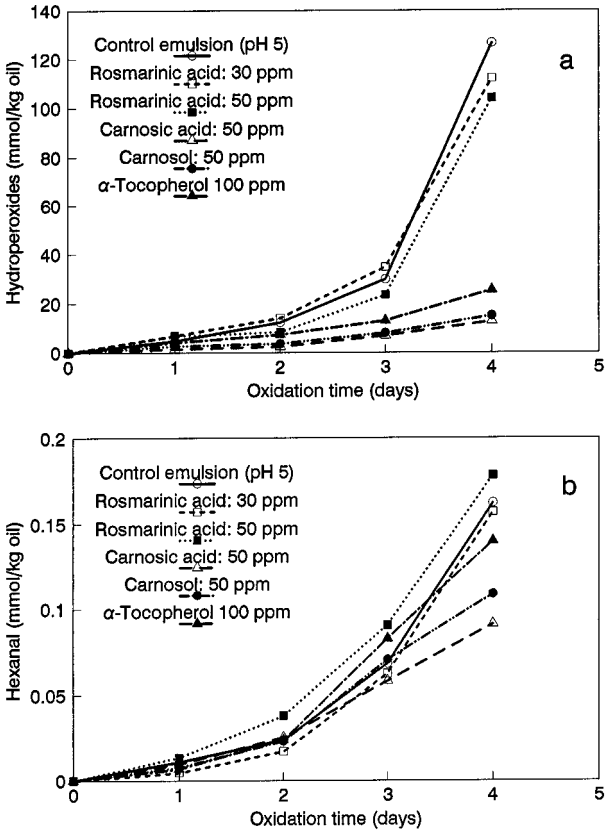

Figure 4. Effect of rosmarinic acid, carnosic acid, carnosol, and α-tocopherol on the oxidative stability of stripped corn oil emulsion in phosphate buffer (pH 5.0) at 60 °C: (a) hydroperoxide formation; (b) hexanal formation.

lipids, whereas nonpolar antioxidants are more active in polar lipid emulsions. To explain this paradox, a mechanism was previously postulated on the basis of the interfacial properties of different antioxidants (Frankel et al., 1994). Accordingly, the hydrophilic antioxidants (Trolox and ascorbic acid) were proposed to be more active in bulk oil by being oriented in the air−oil interface to provide better protection than the lipophilic antioxidants (α-tocopherol and ascorbyl palmitate), which remain in solution in the oil phase. In the emulsion system, the surface active lipophilic antioxidants are more protective, by being oriented in the oil−water interface, than the hydrophilic antioxidants, which remain in solution in the water phase.

In the present study, rosmarinic acid, carnosic acid, and the rosemary extract were significantly more active in bulk corn oil than in the corresponding corn oil emulsion, while carnosol and α-tocopherol performed better in the emulsion. Similar results were obtained in the corn oil emulsion buffered at pH 5, but α-tocopherol was less active. These results can be explained on the basis of the interfacial properties of the antioxidants by assuming that in the emulsion systems used in this study the more polar rosmarinic acid and carnosic acid are more favorably partitioned into the water phase and less in the oil phase, thus becoming less protective. This trend is in agreement with our previous results showing that the polar hydrophilic antioxidants Trolox and ascorbic acid were much less protective in emulsion than in bulk systems (Frankel et al., 1994).

A significant pH effect was observed on the antioxidant activity of carnosic acid and carnosol when tested in corn oil emulsion. At pH 4 and 5 these antioxidants were highly active, whereas at pH 7 they had no antioxidant activity on the basis of both hydroperoxide and hexanal formation. This strong pH effect may indicate that the partition of both carnosic acid and carnosol favors either the oil phase or the oil-water interface at lower pH. At the lower pH carnosol and carnosic acid may also be more stable, and thus their protective effects may last longer during oxidation. Another possible cause is that these antioxidants may have better reducing capacity at lower pH values.

The classical mechanism of inhibited lipid oxidation does not predict the changes in antioxidant activities between solutions and emulsion systems. The questions of why the highly polar rosemary compounds were more active in bulk oil than in oil-in-water emulsion systems and why carnosol and carnosic acid performed better in oil-in-water emulsions at lower pH are fundamental questions that need to be investigated to better understand the antioxidant mechanism of polar natural antioxidants. Partition studies to determine the distribution of antioxidants in the different phases (oil, water, and interface) of emulsions may elucidate the pH effect. A better understanding of the association and driving forces of antioxidants in multiphase systems may provide a better basis to predict the activity of natural antioxidants in multicomponent food and biological systems.

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