Antioxidant Activities of Rosemary and Sage Extracts and Vitamin E in a Model Meat System

Jon W. Wong, Kelly Hashimoto, and Takayuki Shibamoto*

Department of Environmental Toxicology, University of California, Davis, California 95616

Thiobarbituric acid (TBA) assay and capillary gas chromatography (GC) were used to assess the effects of storage and the potential antioxidants, vitamin E and rosemary and sage extracts, on lipid peroxidation of a cooked beef homogenate. Increased levels of malondialdehyde (MDA) or TBA-reactive substances (TBARS) were observed in samples during a 5-day period. TBARS levels ranged from 2.0 to 3.9 times higher than GC-analyzed MDA levels, indicating the presence of other reactants in the TBA assay. Despite elevated levels, TBARS formation is similar to the actual MDA formation. Addition of vitamin E at 25–100 μg/g in the homogenate showed a concentration dependence in inhibiting lipid peroxidation. Samples containing either herbal extract (30 μg/g) showed effective inhibition since MDA levels of treated samples were 62 and 53% of the control. Improved antioxidative activity was not observed when the antioxidants were mixed together in equal amounts.

Keywords: *Antioxidant; lipid peroxidation; malondialdehyde analysis*

INTRODUCTION

During production, processing, distribution, and storage, food undergoes deterioration from chemical and microbial processes. Typically, oxidative deterioration of meat and meat products results from degradative reactions of fats in the raw meat and a "warmed over flavor" in processed meats, a result of the cooking, slicing, deboning, or freezing processes. In either case, the mechanism of rancidity involves the peroxidation of unsaturated lipids, often the phospholipids (Roozen, 1987). Spanier et al. (1992) and Love (1983) attribute various initiation mechanisms responsible for lipid oxidation in foods such as the catalytic effect of free and bound iron, enzymatic processes, and changes in ground state molecular oxygen.

The rate and extent of oxidative deterioration can be reduced by various means such as curing to preserve the meat tissue, vacuum packaging to remove the oxygen source, or adding antioxidants to scavenge the oxidants. The use of synthetic antioxidants such as BHT and BHA has been effective because of their low cost, high stability, and effectivenes. However, their use in food has been decreasing because of a possible mutagenicity and consumers' rejection of synthetic food additives (Namiki, 1990). Vitamin E (α-tocopherol) is an effective antioxidant for lipid-containing foods but has limited usage (Fang and Wada, 1993).

Recently, the food industry has been using other natural antioxidants from plant material (Kanner et al., 1994). The use of naturally occurring antioxidants extracted from plants and vegetables has been widely studied (Rhee, 1987; Shahidi et al., 1992). Extracts from two herb plants, rosemary and sage, have been shown to have strong antioxidative characteristics in foods and food model systems (Cuvelier et al., 1994). Rosemary extracts have been studied extensively for their antioxidative activity as well as for industrial and commercial uses. Sage has been shown recently to contain six major compounds also found in rosemary (Cuvelier et al., 1994). These plant extracts, when combined with α-tocopherol, are reported to be synergists that enhance and improve the antioxidant activity in certain food systems (Wada and Fang, 1992).

Various methods are available for quantitation of lipid peroxidation products in biological and food systems (Hoyland and Taylor, 1991; Pryor and Godber, 1991). A common method used for quantitating malondialdehyde (MDA), a major lipid peroxidation product, is the spectrophotometric detection of thiobarbituric acid reactive substances (TBARS) from the thiobarbituric acid (TBA) assay. However, various oxidized lipid products such as 2-alkenals, 2,4-alkadienals, and 4-hydroxyalkenals can also react in this procedure (Kosugi et al., 1987; Esterbauer et al., 1991; Raharjo et al., 1993). Other nonlipid materials such as sugars, amino acids, urea, biliverdin, glyoxal, and furfuraldehyde may also react with TBA to form complexes that absorb at 530–535 nm, the same wavelength range in which the TBA–MDA adduct is measured (Kosugi et al., 1987; Hoyland and Taylor, 1991). To distinguish MDA from other sample components, GC and HPLC methods have been utilized with or without derivatization of MDA (Bird et al., 1983; Wong et al., 1987; Beljean-Leymarie and Bruna, 1988).

The objectives of this work are to (1) assess and compare antioxidative activities of sage and rosemary extracts and D,L-α-tocopherol (vitamin E) and (2) compare results of the TBA assay with those obtained from MDA derivatization/GC-NPD analysis in cooked meat systems undergoing lipid peroxidation at storage conditions. This study uses *N*-methylhydrazine derivatization of malondialdehyde to 1-methylpyrazole (1-MP), extraction of 1-MP using solid phase extraction, and subsequent gas chromatographic analysis with nitrogen–phosphorus detection (GC-NPD) (Wong et al., 1994).

MATERIALS AND METHODS

Chemicals and Reagents. 2-Methylpyrazine (99% purity), malonaldehyde bis(diethyl acetal) (98% purity), and vitamin E (D,L-α-tocopherol; 97% purity) were obtained from Aldrich Chemical Co. (Milwaukee, WI). *N*-Methylhydrazine (98% purity) was obtained from Fluka Chemical Co. (Buchs, Switzerland). (CAUTION: *N*-methylhydrazine is highly cor-

* Author to whom correspondence should be addressed [telephone (916) 752-4523; fax (916) 752-3394; e-mail Internet tshibamoto@ucdavis.edu].

0021-8561/95/1443-2707$09.00/0 © 1995 American Chemical Society

rosive, a toxic mutagen and carcinogen, and should be handled with care in a chemical fume hood.) Butylated hydroxytoluene (BHT) and thiobarbituric acid were purchased from Sigma Chemical Co. (St. Louis, MO). Solid phase extraction cartridges (6 cm^3, C_{18} octadecyl bonded phase) were purchased from Varian Sample Preparation Products (Harbor City, CA). Reagent grade dichloromethane (J. T. Baker Inc., Phillipsburg, NJ) and pesticide grade methanol (Fisher Scientific, Fair Lawn, NJ) were used for extraction and preparation of the extracts and standards. 1-Methylpyrazole (1-MP) was synthesized according to the method of Umano et al. (1988). All other chemicals, reagents, and solvents were of analytical grade. The oil-soluble rosemary and sage extracts, HERBALOX (Type O) and HERBALOX-Sage (Type O) were gifts from Kalsec Co. (Kalamazoo, MI).

Gas Chromatographic Analysis. A Hewlett-Packard (HP) 5890 gas chromatograph equipped with a 3396 Series II integrator, a nitrogen–phosphorus detector, and a 30 m × 0.25 mm i.d. DB-Wax bonded phase fused silica capillary column of phase thickness 0.25 μm (J&W Scientific, Folsom, CA) was used for analysis. The corresponding gas flow rates were 30, 101, and 3.6 mL/min for nitrogen, air, and hydrogen gases, respectively. The linear velocity of the helium carrier gas was 40 cm/s, and an injector split ratio of 20:1 was used. The oven temperature was held at 60 °C for a 2-min hold, programmed to 90 °C at 4 °C/min and to 180 °C at 10 °C/min, and held at 180 °C for 10 min. The injector and detector temperatures were 250 and 275 °C, respectively.

GC–MS Analysis. Mass spectral (MS) identification of the 1-methylpyrazole derivative was carried out on a VG-Trio 2 mass spectrometer with a VG11-250 computer data system interfaced to an HP 5890 GC. An MS ionization voltage of 70 eV and an ion source temperature of 250 °C were used. Chromatographic conditions were as described above; however, a splitless injection was employed.

Calibration Plot for 1-Methylpyrazole Analysis. 1-Methylpyrazole (1-MP) calibration standards (0.5, 1, 2, 5, 10, 25, 50, 75, and 100 nmol/mL) were prepared in dichloromethane. A 250-μL aliquot of the internal standard, 2-methylpyrazine (2-MP, 50 μg/mL) was added to 5 mL of the standard 1-MP solution. Each standard was injected onto the GC as 2-μL injections using the GC conditions described above. The integrated peak areas were determined, and the ratio of analyte (1-MP) to internal standard (2-MP) was calculated.

Sample Preparation. Ground beef (approximately 30% fat) was purchased from a local supermarket. A mixture of 100 g of ground beef and 67 g of distilled and deionized water with 30 mg (dissolved in ethanol) of the antioxidant was thoroughly blended in a blender (Waring Blendor 5011G Model 5011, Waring Products Division, Dynamics Corp. of America, New Hartford, CT) at the high setting at room temperature. Control samples contained only beef, water, and ethanol. The meat and water mixture was cooked at 225 °F in an electric frying pan until all of the meat turned to a brown color. The meat and juices were collected and weighed, and water was added to form a ca. 60% added meat mixture. This mixture was blended until a smooth homogenate was formed. The samples were transferred to amber jars and stored in a 5 °C cold room over a period of 5 days.

GC Analysis. A 1.0-g portion of the ground beef homogenate was weighed, and 50 μL of 0.4% BHT in ethanol was added to each sample. A 1% NaCl (3 mL) solution was added to the homogenate and stirred to form a fine slurry. *N*-Methylhydrazine (50 μL) was added, and the mixture was covered and mixed for 15 min. Samples were treated with 1 mL of 6 N HCl, and contents were stirred at room temperature for 10 min followed by heating at 50–55 °C for 15 min in a water bath and cooling to room temperature in an ice bath. An additional 50 μL of *N*-methylhydrazine was added, the pH of the sample was adjusted to 7–8 with NaOH, and the mixture was covered and allowed to react, with stirring, for an additional 30 min at room temperature. Solids were removed following centrifugation at 4400*g* for 10 min (10–15 °C). The resulting pellet was resuspended in 3 mL of 1% NaCl and recentrifuged using the same conditions. Supernatants were combined and extracted using 6-cm^3 C_{18} solid phase extraction (SPE) cartridges and dichloromethane as the extracting solvent. The SPE cartridges were conditioned with 3 column volumes each of dichloromethane, methanol, deionized water, and 1% NaCl solution. The dichloromethane extract was adjusted to 5 mL and spiked with 250 μL of 2-methylpyrazine (50 μg/mL internal standard). For spike recoveries of MDA, the triplicate homogenate samples containing 100 μg/g vitamin E were spiked with 1 mL of an aqueous MDA standard (100 nmol/mL MDA) into 1 mL of 1% NaCl and 100 μL BHT (0.2%). The spiked samples were then analyzed as before, and the background values in unspiked samples were subtracted from spiked samples to give the corrected peak area ratios.

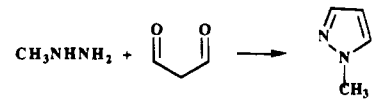

Figure 1. Reaction of *N*-methylhydrazine (NMH) with malondialdehyde to form 1-methylpyrazole (1-MP).

TBA Assay. The meat samples were prepared as described in the preceding section and analyzed using the thiobarbituric acid (TBA) assay procedures of Uchiyama and Mihara (1978) with some modifications. To the meat sample (0.5 g) were added 50 μL of a 4% BHT (dissolved in ethanol), 3 mL of 1% phosphoric acid, and 1 mL of a 0.8% TBA solution dissolved in deionized water. Samples were stirred and heated in a boiling water bath for 15 min and were immediately cooled in an ice bath. To the samples was added 5 mL of 1-butanol, and the mixture was vortexed rigorously to extract the pink adduct from the aqueous phase. The samples were left undisturbed for approximately 15–30 min. The butanol layer was removed and was measured using a Hewlett-Packard 8452A diode array UV spectrophotometer. The difference spectrum of 535 − 520 nm was used to quantitate the TBA–MDA adduct. A calibration curve was prepared using an MDA standard, reacting with the TBA/phosphoric acid solution, and measuring the difference of the absorbance spectrum of 535 − 520 nm. The stock solution was prepared by dissolving 220 mg of the malondialdehyde [bis(diethyl acetal)] in a 1% solution of H_2SO_4 to a final volume of 100 mL. The solution was mixed for 3 h at room temperature and stored overnight at 5 °C. The working solution was prepared by adding 1 mL of the stock solution to a final volume of 100 mL of 1% H_2SO_4, and the MDA concentration was checked by measuring the UV absorbance at 245 nm and using the extinction coefficient of 13 700 (Esterbauer et al., 1984). The working solution was diluted to the appropriate conditions and reacted with the TBA/phosphoric acid solution to form the standard curve. The amount of MDA is determined by nanomoles of thiobarbituric acid reactive substances (TBARS).

RESULTS AND DISCUSSION

Capillary gas chromatography with nitrogen–phosphorus detection has been a selective and sensitive method for the analysis of 1-MP that results from a reaction of MDA with *N*-methylhydrazine in a variety of biological, food, and model systems (Umano et al., 1988; Ichinose et al., 1989; Ebeler et al., 1994; Wong et al., 1994). The detection limit was previously reported as 8.9 pg of 1-MP, equivalent to 7.8 pg of MDA (Ichinose et al., 1989). In this study, the limit of quantitation was 44.5 pg of 1-MP, equivalent to 36.0 pg of MDA. The derivatization of MDA with NMH to 1-MP is shown in Figure 1. Recovery of spiked MDA (100 nmol) was 72.2 ± 5.1% (mean ± standard deviation, $n = 3$) from the ground beef homogenates.

Parts A and B of Figure 2 show the amount of MDA formed at each day for up to 5 days at storage conditions of 5 °C using GC analysis and TBA assay, respectively. These results show that storage of the samples for up to 5 days increases the MDA and TBARS levels; the

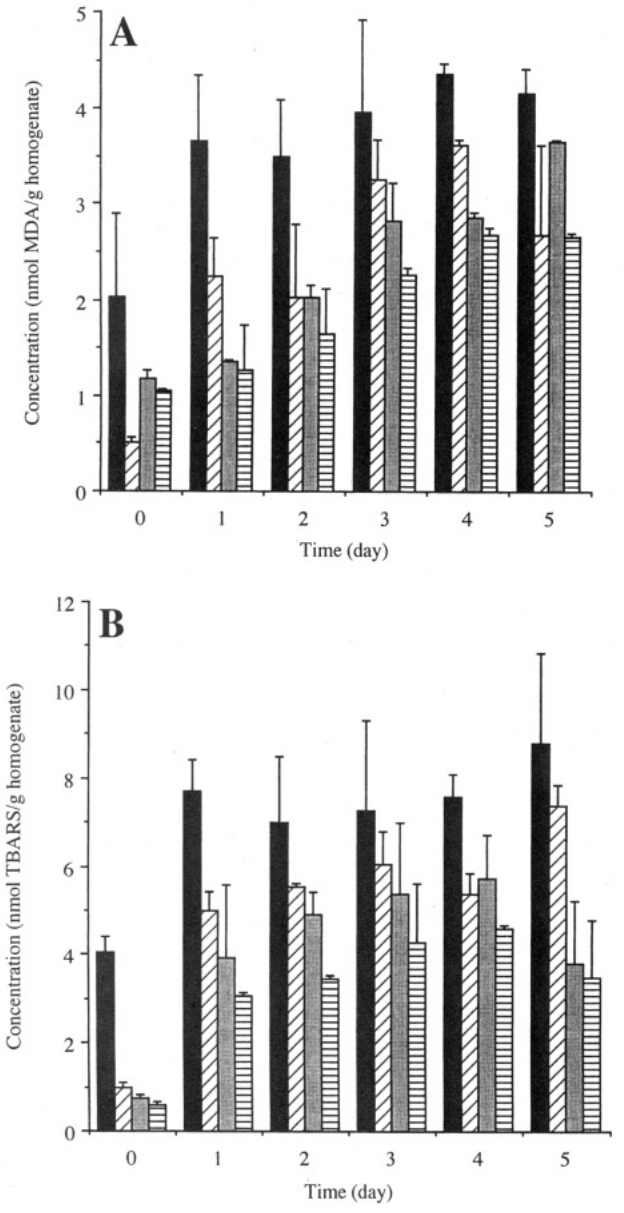

Figure 2. Production of (A) MDA and (B) TBARS from homogenized ground beef samples treated with various concentrations of vitamin E. Shown are mean ±SD ($n = 3$). MDA was analyzed as the NMH derivative, 1-MP. Experimental conditions are given in the text. Vitamin E concentrations: (solid bar) control; (slashed bar) 25 μg/g; (lightly shaded bar) 50 μg/g; (striped bar) 100 μg/g.

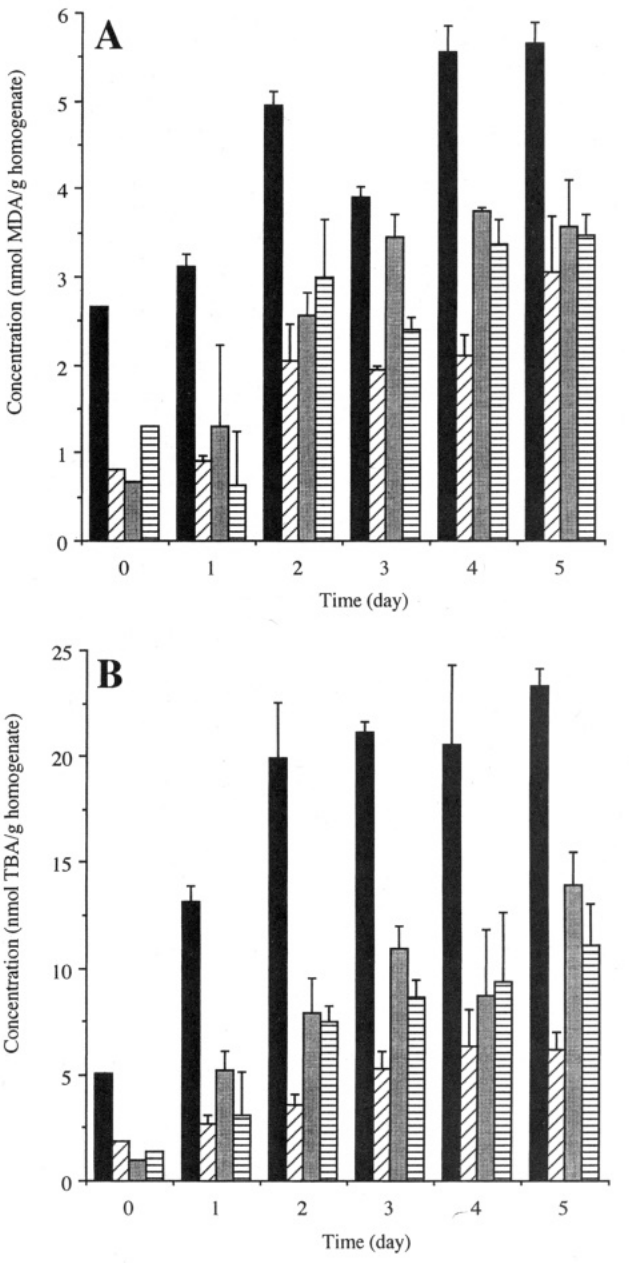

Figure 3. Production of (A) MDA and (B) TBARS from homogenized ground beef samples treated with antioxidants at 30 μg/g. Shown are mean ±SD ($n = 3$ except for day 0, where $n = 1$): (solid bar) control; (slashed bar) vitamin E; (lightly shaded bar) rosemary extract; (striped bar) sage extract.

TBARS values are significantly higher than the amount of MDA formed as determined by GC analysis, and vitamin E is an effective concentration-dependent antioxidant (25−100 μg/g) in the inhibitory effect of lipid peroxidation in the cooked meat system.

Parts A and B of Figure 3 show the comparison of MDA levels using GC and TBA analysis of ground meat mixtures containing rosemary and sage extracts or vitamin E at the same antioxidant concentration (30 μg/g) under storage at 5 °C for up to 5 days. These results follow trends similar to those presented in Figure 2, revealing an increase of TBARS and MDA levels. In addition, Figure 3B shows higher TBARS levels in comparison to Figure 2B, and there was effective inhibition against lipid peroxidation by the two plant extracts.

Parts A and B of Figure 4 reveal that when either plant antioxidant was mixed with vitamin E in equal quantities (15 μg/g each), the antioxidant mixture did not exhibit any enhanced antioxidative activity due to synergistic effects when compared to vitamin E alone. However, an equal mixture (15 μg/g each) of the rosemary and sage extracts was also shown to be effective in inhibiting lipid peroxidation in the meat system.

The increase of MDA and TBARS levels from Figure 2 over the 5-day refrigeration period is consistent with the results presented by Stoick et al. (1991), in which they evaluated the oxidation of restructured beef steaks over a period of 6 days at 4 °C. The increase of MDA or TBARS results from reactions of lipids in the meat, resulting in the accumulation of lipid secondary oxidation products such as MDA. However, the higher TBARS levels in comparison to MDA are attributed to an increased production of secondary oxidation products due to excessive conditions employed and the presence of interfering substances during spectrophotometric analysis by the TBA assay. Raharjo et al. (1994) have

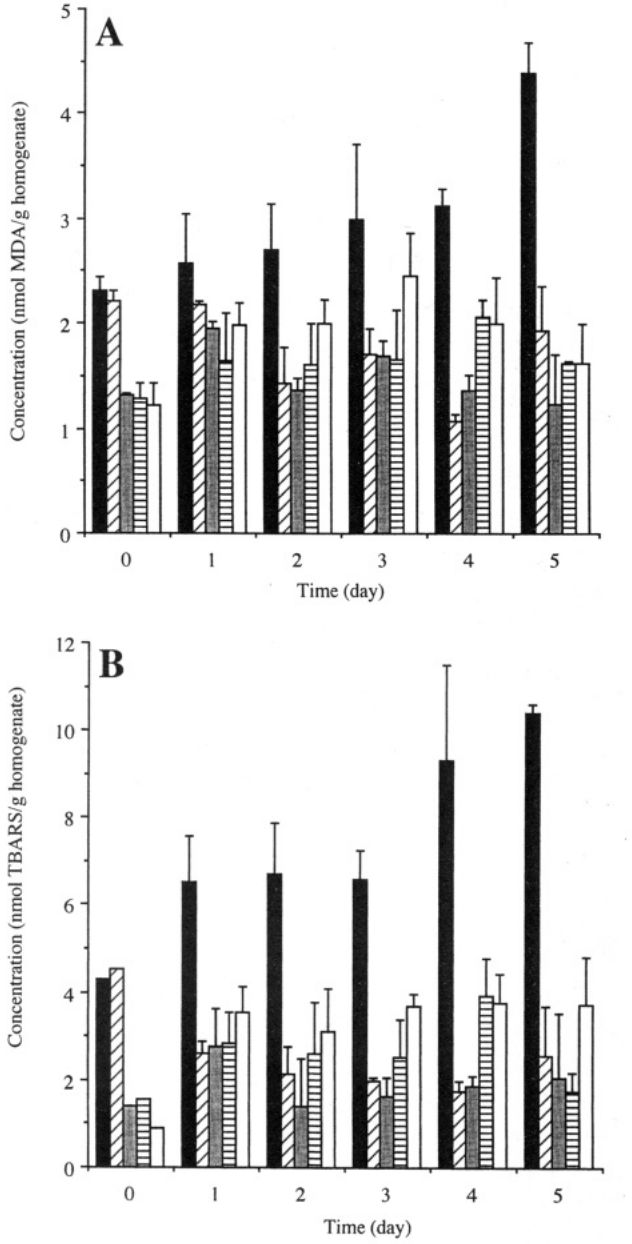

Figure 4. Production of (A) MDA and (B) TBARS from homogenized ground beef samples treated with mixed antioxidant systems at 30 μg/g. Shown are mean ±SD ($n = 3$ except day 0, where $n = 1$); (solid bar) control; (slashed bar) vitamin E; (lightly shaded bar) rosemary/vitamin E; (striped bar) sage/vitamin E; (open bar) rosemary/sage.

shown that the standard TBA assay forms excessive TBARS values in ground cooked beef. The TBARS values from Figures 2, 3, and 4 averaged 2.0, 3.9, and 2.4 times higher than the MDA values, respectively. Figure 3B also indicates overestimated values with the TBA assay. These results show that the TBARS values in Figure 3B averaged 2.8 times higher (from day 3 to day 5) than the levels shown in Figure 2B. Dispersion and dissolution of lipid particles, fats, and proteins from tissue samples have been shown to increase the turbidity of the extraction solutions that result in artificially high TBARS values (Shahidi and Hong, 1991). In contrast, the MDA levels shown in Figure 3A averaged 1.2 times higher (from day 3 to day 5) than the levels in Figure 2A, which may indicate variations of the tissue and fat contents of the different ground beef samples used in the two sets of experiments for determining lipid peroxidation.

In contrast, the NMH derivatization/GC analysis is specific for MDA, the sample preparation takes place under milder conditions, and the method used hydrolyzes MDA that may be bound to proteins (Wong et al., 1994). In this study, the control meat samples from Figure 2 from the TBA and GC analyses (from day 0 to day 5) showed the highest TBARS (4.07−8.81 nmol/g) and MDA (2.03−4.17 nmol/g) contents. Correlation of TBARS values or other lipid peroxidation products such as hexanal with sensory acceptance has been widely used in the comparison of flavor acceptability of meats with lipid oxidation; thus, a precise method for assessing specific lipid oxidation products is required and preferred (Shahidi et al., 1987; Spanier et al., 1992). From Figure 2, the amount of MDA generally parallels the corresponding TBARS for the control and treated samples but the results for MDA content are more reliable because of its specificity. Therefore, the derivatization/GC analysis can be useful in providing accurate correlation of MDA to the sensory tests.

The antioxidative activity of vitamin E is concentration dependent. The meat samples treated with vitamin E at 25, 50, and 100 μg/g had average MDA levels of 70, 61, and 53% of the untreated control, respectively (based on the average of treated samples for each day in comparison to the control for each day for days 1−5). Average TBA values were 76, 63, and 50% of the control levels, respectively, and are consistent with those of Dugan (1980), who indicated a concentration and temperature dependence on the activity and effectiveness of α-tocopherol in inhibiting lipid peroxidation in food systems. The levels of antioxidant used at these conditions were excessive enough to offset any losses during the cooking or storage conditions.

In general, the controls on day 0 had higher TBARS or MDA levels than the antioxidant-treated meat samples. Since cooking can initiate the development of warmed-over flavor, the addition of antioxidants prior to cooking is important in inhibiting lipid peroxidation during the cooking process. Meat samples treated with vitamin E, rosemary, and sage at 30 μg/g had average MDA levels of 42, 62, and 53% of the untreated control, respectively, while the average TBA values were 24, 39, and 47% (Figure 3). All three antioxidants were effective as antioxidants, although the TBA assay and the GC results indicate that vitamin E is more effective than either plant extract. These results agree with other studies that showed the antioxidative effectiveness of these plant extracts, with rosemary being a more effective antioxidant in a pork fat system than the synthetic compounds BHA and BHT (Dugan, 1980).

Commercial rosemary and sage extracts contain the compounds carnosol, rosmanol, isorosmanol, and epirosmanol (Namiki, 1990), phenolic primary antioxidants that react with lipid or hydroxy radicals and convert them into stable products (Gordon, 1990). Although these major components have been identified, the antioxidative mechanism of rosemary and sage extracts has not been determined. The major antioxidants in these extracts may be protected by other substances such as flavonoids, found in smaller amounts, which provide thermal stability and enhanced antioxidative activity (Cuvelier et al., 1994).

The vitamin E, vitamin E/rosemary, vitamin E/sage, and rosemary/sage meat systems at a total concentration of 30 μg/g had average MDA levels of 55, 51, 56, and 67% of the untreated control, respectively, while the average TBA levels of the treated samples showed

similar trends at 29, 26, 36, and 47% of the control (Figure 4). These results indicate that while the two vitamin E/plant extract mixtures were comparable to vitamin E alone, enhanced inhibition against lipid peroxidation was not observed. Although the rosemary/sage system showed an inhibitory effect, no enhancement was observed. Antioxidant synergism in food systems has been studied in the recycling of α-tocopherol and ascorbic acid. Sharma and Buettner (1993) proposed that these two antioxidants work synergistically to protect lipids from peroxidation by regenerating ascorbic acid or recycling the α-tocopheroxyl radical to tocopherol by the one-electron oxidation of ascorbic acid to ascorbyl radical. The components in rosemary may also be used as a substitute for ascorbic acid to enhance the antioxidative activity. Fang and Wada (1992) reported that α-tocopherol was a more effective antioxidant than rosemary extract in sardine dark muscle and showed that a mixture of α-tocopherol and rosemary had a synergistic antioxidant effect in frozen-crushed bonito meat during storage at 5 °C. Our work, however, agrees more with the findings of Stoick et al. (1991) and Liu et al. (1992), who found no significant differences between rosemary oleoresin mixed with sodium tripolyphosphate in restructured beef and pork steaks and steak samples mixed with sodium tripolyphosphate alone. Although their work did not study the effects of rosemary extract alone, the antioxidative activity was attributed to the larger proportion of sodium tripolyphosphate.

Food systems, especially muscle tissue, are very complex in the number and type of chemicals in the mixture, and a combination of these compounds may behave differently from the individual components. To further understand the antioxidant and oxidation mechanisms in cooked meat systems, parallel studies are required to characterize the levels of total lipids and the fatty acid composition of triacylglycerols and phospholipids, antioxidants, and oxidized products (carbonyls, alkanes, acids, and hydroperoxides). Studies have shown phospholipids in uncooked frozen meat are more susceptible to oxidation than triglycerols and cholesterol esters because of their high content of polyunsaturated fatty acids (Pikul et al., 1984; Pikul and Kummerow, 1990). Additional information can also be achieved by monitoring specific proteins that are modified by oxidation products or reactions during storage or by proteins that can enhance the oxidation of lipids such as the catalytic activation of heme proteins (Rehbein et al., 1990; Pellett et al., 1994; Love, 1983; Rhee et al., 1987). The results presented here in comparison to other studies of meat systems suggest that the differences in antioxidant types and amounts, chemical components, and meat tissue composition are important in observing effective activities of antioxidants.

LITERATURE CITED

Beljean-Leymarie, M.; Bruna, E. Subpicogram determination of malondialdehyde by gas–liquid chromatography with nitrogen phosphorus detector. I. Standardization. *Anal. Biochem.* **1988**, *173*, 174–184.

Bird, R. P.; Hung, S. S. O.; Hadley, M.; Draper, H. H. Determination of malonaldehyde in biological materials by high-pressure liquid chromatography. *Anal. Biochem.* **1983**, *128*, 240–244.

Cuvelier, M.; Berset, C.; Richard, H. Antioxidant constituents in sage *(Salvia officinalis)*. *J. Agric. Food Chem.* **1994**, *42* (3), 665–669.

Dugan, L. R. Natural antioxidants. In *Autoxidation in Food and Biological Systems*; Simic, M. G., Karel, M., Eds.; Plenum Press: New York, 1980.

Ebeler, S. E.; Hinrichs, S. H.; Clifford, A. J.; Shibamoto, T. Volatile carbonyl levels in tissues of transgenic mice with nerve sheath tumors. *J. Chromatogr., B: Biomed. Appl.* **1994**, *654* (1), 9–18.

Esterbauer, H.; Lang, J.; Zadravec, S.; Slater, T. F. Detection of malonaldehyde by high-performance liquid chromatography. *Methods Enzymol.* **1984**, *105*, 319–328.

Esterbauer, H.; Schaur, R. J.; Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biol. Med.* **1991**, *11* (1), 81–128.

Fang, X.; Wada, S. Enhancing the antioxidant effect of α-tocopherol with rosemary in inhibiting catalyzed oxidation caused by Fe^{2+} and hemoprotein. *Food Res. Int.* **1993**, *26*, 405–411.

Gordon, M. H. The mechanism of antioxidant action *in vitro*. In *Food Antioxidants;* Hudson, B. J. F., Ed.; Elsevier Science Publishing: New York, 1990.

Hoyland, D. V.; Taylor, A. J. A review of the methodology of the 2-thiobarbituric acid test. *Food Chem.* **1991**, *40* (3), 271–291.

Ichinose, T.; Miller, M. G.; Shibamoto, T. Gas chromatographic analysis of free and bound malonaldehyde in rat liver homogenates. *Lipids* **1989**, *24* (10), 895–898.

Kanner, J.; Frankel, E.; Granit, R.; German, B.; Kinsella, J. E. Natural antioxidants in grapes and wines. *J. Agric. Food Chem.* **1994**, *42*, 64–69.

Kosugi, H.; Kato, T.; Kikugawa, K. Formation of yellow, orange, and red pigments of alk-2-enals with 2-thiobarbituric acid. *Anal. Biochem.* **1987**, *165* (2), 456–464.

Liu, H. F.; Booren, A. M.; Gray, J. I.; Crackel, R. L. Antioxidant efficacy of oleoresin rosemary and sodium tripolyphosphate in restructured pork steaks. *J. Food Sci.* **1992**, *57* (4), 803–806.

Love, J. D. The role of heme iron in the oxidation of lipids in red meats. *Food Technol.* **1983**, *37* (7), 117–120, 129.

Namiki, M. Antioxidants/antimutagens in food. *Crit. Rev. Food Sci. Nutr.* **1990**, *29* (4), 273–300.

Pellett, L. J.; Andersen, H. J.; Chen, H.; Tappel, A. L. β-Carotene alters vitamin E protection against heme protein oxidation and lipid peroxidation in chicken liver slices. *J. Nutr. Biochem.* **1994**, *5* (10), 479–484.

Pikul, J.; Kummerow, F. A. Relative role of individual phospholipids on thiobarbituric acid reactive substances formation in chicken meat, skin and swine aorta. *J. Food Sci.* **1990**, *55* (5), 1243–1248, 1254.

Pikul, J.; Leszczynski, D. E.; Kummerow, F. A. Relative role of phospholipids, triacylgycerols, and cholesterol esters on malonaldehyde formation in fat extracted from chicken meat. *J. Food Sci.* **1984**, *49*, 704-708.

Pryor, W. A.; Godber, S. S. Noninvasive measures of oxidative stress status in humans. *Free Radical Biol. Med.* **1991**, *11* (1), 81–128.

Raharjo, S.; Sofos, J. N.; Schmidt, G. R. Solid-phase acid extraction improves thiobarbituric acid method to determine lipid oxidation. *J. Food Sci.* **1993**, *58* (4), 921–924, 932.

Rehbein, H.; Orlick, B. Comparison of the contribution of formaldehyde and lipid oxidation products to protein denaturation and texture deterioration during frozen storage of minced ice-fish fillets. *Int. J. Refrig.* **1990**, *13* (5), 336–341.

Rhee, K. S. Natural antioxidants for meat products. In *Warmed-over Flavor of Meat;* St. Angelo, A. J., Bailey, M. E., Eds.; Academic Press: Orlando, FL, 1987; pp 267–289.

Rhee, K. S.; Ziprin, Y. A.; Ordonez, G. Catalysis of lipid oxidation in raw and cooked beef by metmyoglobin–H_2O_2, nonheme iron, and enzyme systems. *J. Agric. Food Chem.* **1987**, *35*, 1013–1017.

Roozen, J. P. Effects of types I, II and III antioxidants on phospholipid oxidation in a meat model for warmed over flavour. *Food Chem.* **1987**, *24*, 167–185.

Shahidi, F.; Hong, C. Evaluation of malonaldehyde as a marker of oxidative rancidity in meat products. *J. Food Biochem.* **1991**, *15*, 97–105.

Shahidi, F.; Yun, J.; Rubin, L. J.; Wood, D. F. The hexanal content as an indicator of oxidative stability and flavour acceptability in cooked ground pork. *Can. Inst. Food Sci. Technol. J.* **1987**, *20* (2), 104–106.

Shahidi, F.; Wanasundara, P. K. J. P. D.; Hong, C. Antioxidant activity of phenolic compounds in meat model systems. In *Phenolic Compounds in Food and Their Effects on Health I: Analysis, Occurrence, and Chemistry;* Ho, C. T., Lee, C. Y., Huang, M. T., Eds.; ACS Symposium Series 506; American Chemical Society: Washington, DC, 1992.

Sharma, M. K.; Buettner, G. R. Interaction of vitamin C and vitamin E during free radical stress in plasma—an ESR study. *Free Radical Biol. Med.* **1993**, *14* (6), 649–653.

Spanier, A. M.; St. Angelo, A. J.; Shaffer, G. P. Response of beef flavor to oxygen depletion and an antioxidant chelator mixture. *J. Agric. Food Chem.* **1992**, *40* (9), 1656–1662.

Stoick, S. M.; Gray, J. I.; Booren, A. M.; Buckley, D. J. Oxidative stability of restructured beef steaks processed with oleoresin rosemary, tertiary butylhydroquinone, and sodium tripolyphosphate. *J. Food Sci.* **1991**, *56* (3), 597–600.

Uchiyama, M.; Mihara, M. Determination of malonaldehyde precursor in tissues by thiobabituric acid test. *Anal. Biochem.* **1978**, *86*, 271–278.

Umano, K.; Dennis, K. J.; Shibamoto, T. Analysis of free malonaldehyde in photoirradiated corn oil and beef fat via a pyrazole derivative. *Lipids* **1988**, *23* (8), 811–814.

Wada, S.; Fang, X. The synergestic antioxidant effect of rosemary extract and α-tocopherol in sardine oil model system and frozen-crushed fish meat. *J. Food Process. Preserv.* **1992**, *16*, 263–274.

Wong, J. W.; Ebeler, S. E.; Isseroff, R. R.; Shibamoto, T. Analysis of malondialdehyde in biological samples by capillary gas chromatography. *Anal. Biochem.* **1994**, *220* (1), 73–84.

Wong, S. H.; Knight, J. A.; Hopfer, S. M.; Zaharia, O.; Leach, C. N.; Suderman, F. W. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clin. Chem.* **1987**, *33* (2, Part 1), 214–220.

Received for review September 28, 1994. Revised manuscript received June 14, 1995. Accepted July 5, 1995.[⊗]

JF940549F

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1995.