Antioxidative Activity of Volatile Heterocyclic Compounds

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Several volatile heterocyclic compounds formed in Maillard reactions were evaluated for antioxidative activity. Antioxidative activity was measured by the oxidation of heptanal to heptanoic acid or by malonaldehyde formation. Alkylthiophenes, 2-thiophenethiol, 2-methyl-3-furanthiol, and furfuryl mercaptan inhibited heptanal oxidation for up to 30 days. The degree of unsaturation in the heterocyclic ring, as well as the substituent type, had variable effects on the antioxidative capacity of these compounds. The *tert*-butyl hydroperoxide induced oxidation of tocopherol-stripped corn oil, as measured by malondialdehyde formation, was also inhibited in the presence of these heterocyclic compounds. Reactions of the above compounds with *tert*-butyl hydroperoxide and *m*-chloroperoxybenzoic acid resulted in the formation and identification of various oxidized products. The products of these reactions suggested that these heterocyclic Maillard reaction products had antioxidative activity.

INTRODUCTION

Volatile compounds formed by the Maillard reaction have played a major role in the flavor and aroma of various foods and beverages such as cooked meats, vegetables, and coffee (Whitfield, 1992). Cysteine, glutathione, and thiamine have been most commonly used as a source of sulfur in model Maillard reaction systems to characterize the volatile products (Yeo and Shibamoto, 1991; Zhang and Ho, 1991).

The antioxidative activity of Maillard reaction products (MRPs) was first observed by Franzke and Iwainsky (1954), who reported the oxidative stability of margarine following the addition of products from the reaction of glycine and glucose. Later, the formation of antioxidative MRPs from model systems was extensively studied (Park and Kim, 1983; Lingnert and Ericksson, 1980). Volatile MRPs prepared by heating a glucose–glycine solution were found to slow the oxidative degradation of soybean oil (Elizalde et al., 1991, 1992). Recently, headspace extracts of heated peanut oil/cysteine and peanut oil/methionine reportedly possessed antioxidative activity. Typical extracts from these reactions are composed primarily of sulfur-containing heterocyclic compounds (Macku and Shibamoto, 1991a).

Macku and Shibamoto (1991b) identified 1-methylpyrrole as an antioxidant in the headspace of a heated corn oil/glycine model reaction system. Similarly, Eiserich et al. (1992) showed that thiazoles, oxazoles, and furanones formed in an L-cysteine/D-glucose Maillard model system possessed antioxidative activity. These recent studies clearly showed the potential of volatile MRPs to inhibit the oxidative degradation of lipid-rich foods.

In the present study, various volatile heterocyclic compounds were evaluated for antioxidative activity.

MATERIALS AND METHODS

Materials. Heptanal, nonadecane, 2-methyl-3-furanthiol, furfuryl mercaptan, thiophene, 2-acetylthiophene, 2-methylthiophene, 2-ethylthiophene, and *tert*-butyl hydroperoxide (*t*-BuOOH) were purchased from Aldrich Chemical Co. (Milwaukee, WI). 2-Butylthiophene and 2-thiophenethiol were purchased from Lancaster (Windham, NH). α-Tocopherol and *m*-chloroperoxybenzoic acid (*m*-CPBA) were obtained from Sigma Chemical Co. (St. Louis, MO). *N*-Methylhydrazine (NMH) was purchased from Fluka Chemical Co. (Ronkonkoma, NY). Standard 1-methylpyrazole (1-MP) was synthesized by a method previously reported (Umano et al., 1988). Tocopherol-stripped corn oil contained less than 10 IU of tocopherol/kg of oil and was purchased from ICN Biomedicals (Costa Mesa, CA).

Measurement of Antioxidative Activity. The antioxidative activity of the Maillard reaction products was evaluated according to the method developed previously (Eiserich et al., 1992). A standard solution (1 mM) of 2-alkylthiophenes, 2-thiophenethiol, 2-methyl-3-furanthiol, or furfuryl mercaptan was added to dichloromethane solutions of heptanal (5 μg/μL). Nonadecane (80 ng/μL) was added as a gas chromatographic internal standard to each solution, and the resulting solution was brought to a 5-mL final volume with dichloromethane. The solution was transferred to a small vial and stored at room temperature. The headspace of each vial was purged with air every 2 days. Controls containing only heptanal, the internal standard, and dichloromethane were prepared for each experiment. The experimental vials and controls were periodically analyzed.

A Hewlett-Packard (HP) Model 5890 gas chromatograph (GC) equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm i.d. DB-Wax bonded-phase fused silica capillary column (J&W Scientific, Folsom, CA) was used to quantitate heptanoic acid formed from heptanal upon oxidation. The injector and detector temperatures were 220 and 250 °C, respectively. The oven temperature was programmed from 110 to 180 °C at 6 °C/min. The linear velocity of helium carrier gas was 27.0 cm/s with a split ratio of 1:30.

Antioxidant Effects on the Oxidation of Tocopherol-Stripped Corn Oil. 2-Butylthiophene, 2-methyl-3-furanthiol, and α-tocopherol (1 mM in ethanol) were added to separate 5-mL aliquots of tocopherol-stripped corn oil in glass test tubes sealed with Teflon-lined caps. An ethanol solution of *t*-BuOOH (0.1 mM) was added, and the tubes were then incubated in a water bath at 50 °C with stirring. Control oil samples were oxidized without antioxidant in the same manner but with ethanol. The amount of ethanol never exceeded 0.5% of the total solution volume.

Determination of MA in Oxidized Oil Samples. Oil samples were analyzed for malonaldehyde (MA) by a method similar to that used by Niyati-Shirkhodaee and Shibamoto (1992). Prior to GC analysis, 100-μL aliquots of each oil sample were mixed with 4 mL of a hexane solution containing 15 μL of NMH to derivatize MA to 1-MP. The reaction mixture was stirred with a magnetic stirrer for 1 h at room temperature. Acetylacetone (50 μL) was then added to remove excess unreacted NMH. The samples were brought to a final volume of 5 mL in a volumetric flask, and then 2-methylpyrazine (15 nmol/mL) was added as a gas chromatographic internal standard. A HP Model 5890 GC equipped with a 30 m × 0.25 mm i.d. bonded-phase DB-Wax capillary column (J&W Scientific) and a nitrogen–phosphorus detector (NPD) was used for quantitation of 1-MP.

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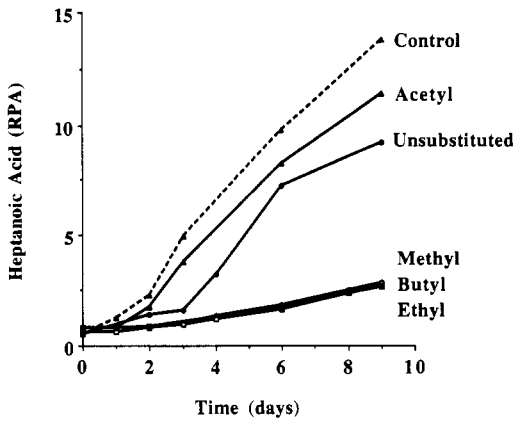

Figure 1. Antioxidative activity of various thiophene derivatives at concentrations of 1 mM. The relative peak area (RPA) is equal to the GC peak area of heptanoic acid divided by the GC peak area of the internal standard.

The oven temperature was held at 60 °C for 2 min and then programmed to 190 °C at 3 °C/min. Injector and detector temperatures were 250 °C. The linear velocity of helium carrier gas was 30 cm/s with a split ratio of 1:25.

Preparation of Authentic Oxidized Products. Authentic oxidized products from 2-butylthiophene, 2-methyl-3-furanthiol, 2-thiophenethiol, or furfuryl mercaptan were prepared by reaction with *m*-CPBA (3 mM) and *t*-BuOOH (3 mM). Each chemical was reacted separately with equal molar concentrations in 5 mL of dichloromethane. The reactions were conducted in sealed glass vials at 25 and 50 °C for *m*-CPBA and *t*-BuOOH, respectively. The reactions were allowed to proceed for 12 (*m*-CPBA) and 24 h (*t*-BuOOH). The reaction products were subsequently analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

Identification of *m*-CPBA- and *t*-BuOOH-Induced Oxidation Products. The oxidation reaction products of the above-listed heterocyclic compounds were analyzed by a HP Model 5890 gas chromatograph equipped with a 30 m × 0.25 mm i.d. DB-1 bonded-phase fused silica capillary column (J&W Scientific) and a flame photometric detector (FPD). The injector and detector temperatures were 250 °C. The GC oven temperature was programmed from 80 to 250 °C at 5 °C/min. The linear velocity of helium carrier gas was 26.5 cm/s with a split ratio of 1:25.

Mass spectral (MS) identification of the oxidation products was conducted on a HP Model 5971 series mass selective detector (MSD) interfaced to a HP Model 5890 GC and equipped with NIST/EPA/NIH mass spectral database (G1033 A #AAD). Mass spectra were obtained by electron impact ionization at 70 eV and a source temperature of 250 °C. The capillary column and GC conditions were as described above.

RESULTS AND DISCUSSION

The oxidation of aldehydes to the corresponding carboxylic acids has been successfuly adapted to evaluate the antioxidative activity of various compounds produced in Maillard reaction model systems (Eiserich et al., 1992; Macku and Shibamoto, 1991b). Figure 1 shows the antioxidative activities of various thiophene derivatives at 1 mM concentrations. Compared to the control, all of the thiophene derivatives inhibited the formation of heptanoic acid from heptanal in a dichloromethane solution. Unsubstituted thiophene was slightly effective, and substitution with a methyl, ethyl, or butyl group at the 2-position greatly increased its antioxidative activity. The length of the alkyl substituent had no observable effects on the activity of these compounds. These alkyl groups are effective electron-donating substituents and increase the π-electron excessive character of carbons in the heteroaromatic ring. On the other hand, substituting the thiophene with an acetyl group, an electron-withdrawing substituent, decreased the antioxidative activity as compared to that of the unsubstituted thiophene.

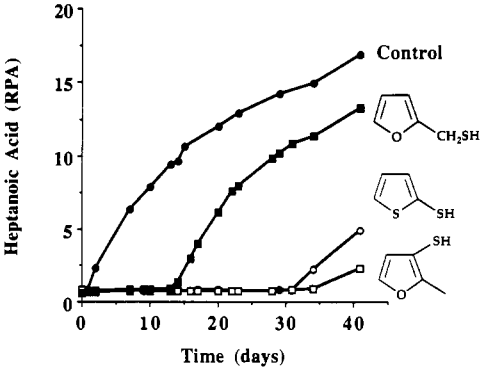

Figure 2. Antioxidative activity of furfuryl mercaptan, 2-thiophenethiol, and 2-methyl-3-furanthiol at concentrations of 1 mM. RPA is as defined in Figure 1.

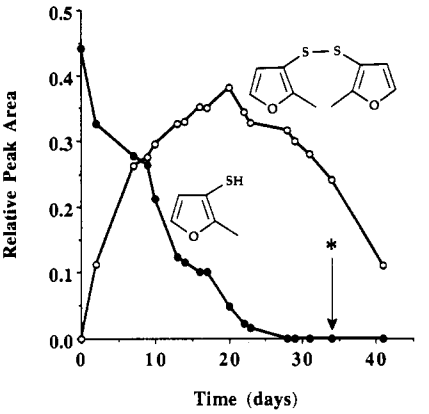

Figure 3. Relative concentrations of 2-methyl-3-furanthiol and its corresponding disulfide, 3,3'-dithiobis(2-methylfuran), over time in a dichloromethane solution of heptanal containing 2-methyl-3-furanthiol at 1 mM. The asterisk indicates the time at which heptanoic acid formation began. RPA is as defined in Figure 1.

The antioxidative activities of thiol compounds are shown in Figure 2. 2-Methyl-3-furanthiol and 2-thiophenethiol inhibited heptanal oxidation for approximately 34 and 30 days, respectively. Furfuryl mercaptan showed much lower activity than either thiol tested above, inhibiting heptanal oxidation for only 13 days. Thiol compounds such as cysteine and glutathione are free-radical scavengers and thus antioxidants in biological systems (Murphy et al., 1992). Taylor and Richardson (1980) have shown that heat treatment of milk increases its oxidative stability, which is correlated directly with increasing "reactive" sulfhydryl content. 2-Methyl-3-furanthiol, 2-thiophenethiol, and furfuryl mercaptan were chosen for study because of their appearance and reported importance in cooked meat flavor and aroma. For example, 2-methyl-3-furanthiol was recently identified in cooked beef aroma (MacLeod and Ames, 1986) and in a heated yeast extract composition (Ames and MacLeod, 1985).

Figure 3 shows the consumption of 2-methyl-3-furanthiol and the formation of the corresponding disulfide, bis(2-methyl-3-furyl) disulfide. The asterisk in the graph indicates the point at which heptanoic acid formation began, corresponding closely with the complete consumption of 2-methyl-3-furanthiol. The disulfide appears to reach a maximum concentration at approximately 20 days and then decreases immediately, presumably due to further oxidation on the sulfur atoms.

Figure 4 shows malonaldehyde (MA) formation in the tocopherol-stripped corn oil with or without antioxidants.

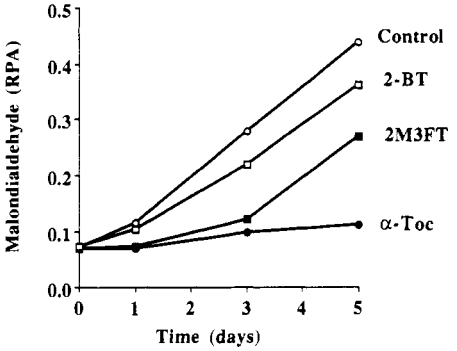

Figure 4. Inhibition of MA formation in tocopherol-stripped corn oil with 2-butylthiophene (2-BT), 2-methyl-3-furanthiol (2M3FT), and α-tocopherol (α-Toc). The RPA of MA was determined by dividing the peak area of 1-MP by the peak area of the internal standard.

Table 1. Oxidized Products from 2-Methyl-3-furanthiol and 2-Thiophenethiol and Their Mass Spectral Data

oxidized product	spectral data
from 2-methyl-3-furanthiol	
3,3′-dithiobis(2-methylfuran)	M^+ = 226 (58), 155 (14), 113 (100), 85 (12), 69 (10), 51 (11), 43 (22)
2-methyl-3-thiofuran 2-methylthiophene 3-sulfone	M^+ = 258 (28), 210 (5), 194 (5), 145 (28), 129 (70), 113 (100), 97 (16), 43 (30)
from 2-thiophenethiol	
2,2′-dithiobis(thiophene)	M^+ – 230 (37), 166 (7), 115 (100), 71 (80), 45 (14)
2-thiothiophene thienyl sulfone	M^+ = 262 (23), 198 (10), 147 (33), 131 (70), 115 (100), 71 (91), 57 (18), 45 (28)

Tocopherol-stripped corn oil was used to compare the activity of the candidate compounds with that of endogenous α-tocopherol. *t*-BuOOH was added to the oil to accelerate lipid peroxidation. α-Tocopherol almost completely inhibited the formation of MA over the 5-day period, whereas 2-butylthiophene only slightly inhibited MA formation. 2-Methyl-3-furanthiol, on the other hand, suppressed the formation of MA for approximately 3 days. The results validated previous experimental results and provide a practical application of these antioxidative compounds in a lipid-rich food system. No appreciable flavor change was recognized in the oils with antioxidant, whereas the oils without antioxidant possessed a slightly rancid odor.

To identify oxidized products of some heterocylic compounds, 2-butylthiophene, 2-methyl-3-furanthiol, 2-thiophenethiol, and furfuryl mercaptan were reacted with the oxidant *m*-CPBA or *t*-BuOOH in a dichloromethane solution. Table 1 shows the oxidized products from 2-methyl-3-furanthiol and 2-thiophenethiol along with their mass spectral data. 2-Methyl-3-furanthiol and 2-thiophenethiol produced the corresponding disulfides as well as the thiolsulfonates (sulfones). 2-Butylthiophene did not react with either *m*-CPBA or *t*-BuOOH. Furfuryl mercaptan was readily consumed in the reaction with both oxidants. However, no oxidized products were detected by the GC. Furfuryl mercaptan may have rapidly undergone oxidative reactions leading all the way to the sulfonic acid, which would not be analyzed by GC. Thiophene derivatives may be able to scavenge radicals by adduction to their double bond, as was previously proposed for thiazole and oxazole derivatives (Eiserich et al., 1992). The π-electron excessive character of the thiophene ring can be enhanced by the presence of electron-donating substituents such as methyl, ethyl, or butyl groups. The presence of electron-withdrawing substituents such as the acetyl group, however, decreases the π-electron density of the aromatic ring and hence its ability to scavenge free radicals. The antioxidative activity of thiophene derivatives increased in the former and reduced in the latter case and does not involve the sulfur heteroatom.

The antioxidative mechanism for the aromatic thiol compounds may involve several modes. The nucleophilic thiol group can behave as a one-electron-reducing agent and scavenge peroxyl and alkoxyl radicals. It can also act to decompose hydroperoxides by two-electron reduction and subsequent disulfide formation, as was clearly shown in the present investigation. The five-membered heterocyclic aromatic ring may be able to scavenge reactive radicals. Direct attachment of the thiol group to the aromatic ring appeared to be necessary for strong antioxidative activity. An intermediate aromatic thiyl radical generated upon $H^\bullet$ abstraction would be stabilized by delocalization. However, an aliphatic thiyl radical, as generated from furfuryl mercaptan, would be more reactive and provide only moderate antioxidative activity.

The presence of these sulfur-containing heterocyclic compounds in various foods may, in part, explain and account for the increased oxidative stability of various cooked foods and the antioxidative effects of synthetic meat flavor extracts (Bailey and Um, 1992). The potential use of these compounds to inhibit the oxidation of lipid-rich foods is of interest to the food industry because the safety of synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole has been questioned.

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