Antioxidative Activity of Volatile Browning Reaction Products and Related Compounds in a Hexanal/Hexanoic Acid System

Emad S. Shaker,† M. Atef Ghazy,† and Takayuki Shibamoto*

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

Volatile products prepared from a browning model system consisting of D-glucose/L-cysteine and related heterocyclic compounds were evaluated for antioxidative activity, measured by the oxidation of hexanal to hexanoic acid either in the dark or in sunlight. In the case of the experiments in the dark, the volatile browning reaction mixture inhibited hexanoic acid formation by almost 100% for up to 13 days. Some column chromatographic (CC) fractions of the above reaction mixture exhibited activities comparable to that of whole samples. Imidazole, 2-thioimidazole, and 2-methyl-3-furanthiol exhibited some inhibitory activity. In the case of the experiments in sunlight, the reaction mixture and some CC fractions exhibited satisfactory inhibition, whereas some CC fractions showed prooxidative activity. Mixtures of some heterocyclic compounds behaved either as an antioxidant or as a prooxidant after 75 days.

Keywords: *Antioxidant; browning reaction; heterocyclic compounds*

INTRODUCTION

It is now well established that heat treatment improves the oxidative stability of food products. Examples are the oxidative stability of dairy products (Josephson and Dahle, 1945), various heated cereals (Anderson et al., 1963), and heat-sterilized beef (Zipser and Watts, 1961). More recently numerous studies on antioxidative substances formed by nonenzymatic browning reactions (more generally amino–carbonyl reactions) have been reported (Kato, 1973; Yamaguchi and Fujimaki, 1973). Among browning products obtained from low molecular carbonyl compounds with amino acids, the products from dihydroacetone gave the most effective products (Kawashima et al., 1977). Consequently, products prepared from amino–carbonyl reactions have begun to receive much attention as possible antioxidants for lipid-rich foods (Dworschak and Szabo, 1986).

Recently, volatile compounds, in particular heterocyclic flavor chemicals, have been found to protect against oxidation of lipids (Macku and Shibamoto, 1991; Eiserich and Shibamoto, 1994). The formation of volatile antioxidants that inhibited the oxidative degradation of soybean oil was observed in a heated glucose–glycine solution (Elizalde et al., 1991, 1992). The headspace volatiles collected from corn oil heated with glycine inhibited the oxidative process from aldehyde to carboxylic acid (Macku and Shibamoto, 1991). The antioxidative strength of 1-methylpyrrole found in the above headspace was comparable to that exhibited by α-tocopherol. Several volatile heterocyclic compounds including alkylthiophenes, thiazoles, thiazolidine, and 1,3-dithiolane formed in the browning reaction inhibited heptanal oxidation for various periods of time (Eiserich and Shibamoto, 1994). These recent studies clearly indicated that some heterocyclic flavor chemicals possess antioxidative activity.

In the present study, the inhibitory activity of browning reaction products and related heterocyclic compounds toward the oxidation of hexanal to hexanoic acid was investigated.

MATERIALS AND METHODS

Materials. Hydrogen peroxide, imidazole, 2-methyl-3-furanthiol, and 2-thioimidazole were purchased from Aldrich Chemical Co. (Milwaukee, WI). Hexanal, α-tocopherol, and *n*-tridecane were bought from Sigma Chemical Co. (St. Louis, MO).

Preparation of Volatile Browning Mixtures from a D-Glucose/L-Cysteine Model System. L-Cysteine and D-glucose (0.1 mol) were dissolved in 60 mL of deionized water. The pH of the solution was adjusted to 12 with 6 N NaOH. The solution was then brought to a final volume of 150 mL with deionized water. The solution was refluxed at 100 °C for 10 h. After the reaction mixture was cooled to room temperature, its pH was adjusted to 11.6 with 6 N NaOH to enhance the extraction efficiency of nitrogen-containing heterocyclic compounds. The solution was extracted with 75 mL of dichloromethane using a liquid–liquid continuous extractor for 6 h. The extract was dried over anhydrous sodium sulfate for 12 h. After removal of the sodium sulfate, the dichloromethane extract was concentrated to approximately 1 mL by a rotary flash evaporator.

Column Chromatography. The browning reaction mixture was transferred to a 15 cm × 1 cm i.d. glass column packed with silica gel (Kieselgel 60, E. Merck, Darmstadt, Germany). The sample was developed with hexane and ethyl acetate (30-mL aliquots) into seven fractions. The solvent was removed from each fraction by vacuum distillation, and the column chromatographic (CC) fractions were stored at −5 °C for subsequent experiments.

Measurement of Antioxidative Activity. The antioxidative activity of the samples was evaluated according to the method developed previously (Macku and Shibamoto, 1991). Imidazole (2 μg), 2-methyl-3-furanthiol (2 μg), 2-thioimidazole (2 μg), and mixtures of imidazole with the other two compounds in various ratios (total 2 μg), or each CC fraction (100 μL) were added to a dichloromethane solution of heptanal (50 μL/mL). A solution containing α-tocopherol (2, 5, or 25 μg/mL) or BHT (2, 5, or 25 μg/mL) instead of a heterocyclic compound or a CC fraction was prepared as a sample of a known antioxidant. A sample containing only heptanal and dichloromethane was prepared for each experiment as a control. *n*-Tridecane (4 μL/mL) was added as a gas chromatographic internal standard to each solution, and the resulting solution was brought to a 10-mL final volume with dichloromethane.

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

† Present address: Faculty of Agriculture, University of Minia, Minia, Egypt.

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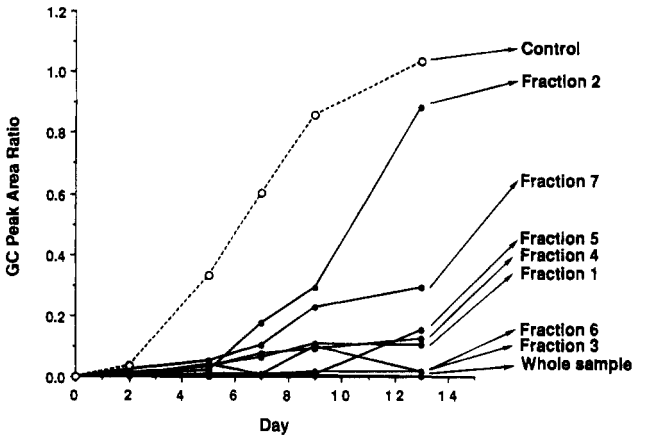

Figure 1. Antioxidative effect of a browning reaction mixture and its CC fractions prepared from a D-glucose/L-cysteine model system in the dark.

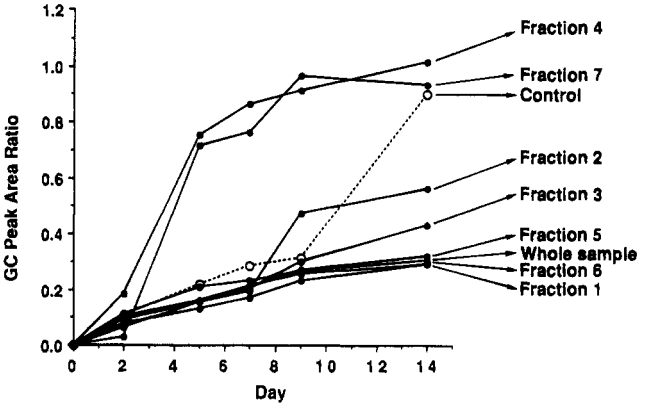

Figure 2. Antioxidative effect of a browning reaction mixture and its CC fractions prepared from a D-glucose/L-cysteine model system in sunlight.

Each solution was transferred to three small vials in equal amounts, and then oxidation was initiated with 12 μL of 30% (w) H_2O_2 solution. Sample vials for the experiments in the dark were wrapped with aluminum foil and allowed to stand at room temperature. Sample vials for the sunlight experiments were exposed to the sunlight through a glass window of a building at the University of California, Davis, during May and June, 1994, at room temperature (23 ± 1 °C). The days during the sunlight experiments were clear and sunny. Thus, the samples were exposed to naturally varying levels of sunlight. The experimental vials and controls were periodically analyzed for hexanoic acid by gas chromatography (GC). The relative amount of hexanoic acid formed was determined using a relative GC peak area that was calculated by dividing the peak area of hexanoic acid by the peak area of the internal standard (*n*-tridecane).

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 hexanoic acid formed from hexanal upon oxidation. The injector and detector temperatures were 220 and 250 °C, respectively. The oven temperature was held at 60 °C for 4 min and then programmed to 180 °C at 10 °C/min, which was further held for 30 min. The linear velocity of the helium carrier gas was 30 cm/s with a split ratio of 1:42. Gas chromatographic peaks were integrated by a Spectra-Physics Model 4290 integrator.

RESULTS AND DISCUSSION

Figures 1 and 2 show the results obtained from the browning reaction mixture and their CC fractions tested in the dark and in sunlight, respectively. Values are the average of three duplicate samples. In the case of the experiments in the dark, the volatile browning reaction mixture (whole sample) prepared from D-glucose/L-cysteine inhibited hexanoic acid formation by almost 100% for up to 13 days. Fractions 3 and 6 exhibited antioxidative activities comparable to that of the whole samples. Fractions 1, 4, and 5 showed slightly less antioxidative activity than did the whole sample. It is interesting that fractionation suppressed the inhibitory activity. This phenomenon has been observed in the case of natural antioxidants found in young green barley leaves (Osawa et al., 1992). In the experiments in sunlight, four samples, including the whole samples, exhibited satisfactory inhibition. On the other hand, fractions 4 and 7 showed prooxidative activity, suggesting the formation of volatile prooxidants in the browning reaction mixture. Sunlight (mainly UV light) produces many reactive oxygen species such as superoxide, singlet oxygen, and a hydroxyl radical. Therefore, it is very difficult to determine phenomena

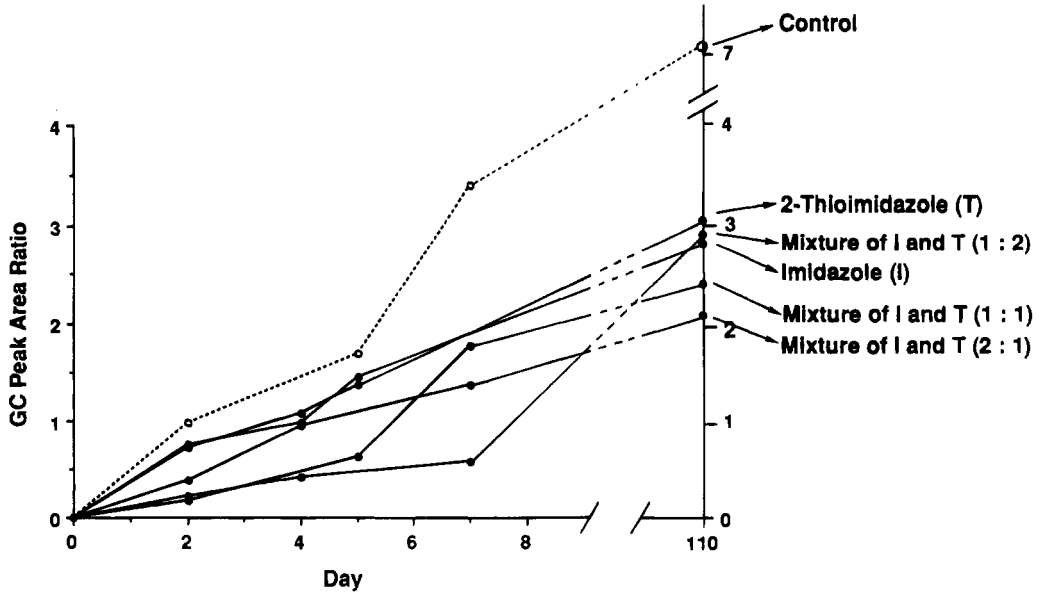

Figure 3. Antioxidative effect of imidazole, 2-thioimidazole, and their mixtures in the dark.

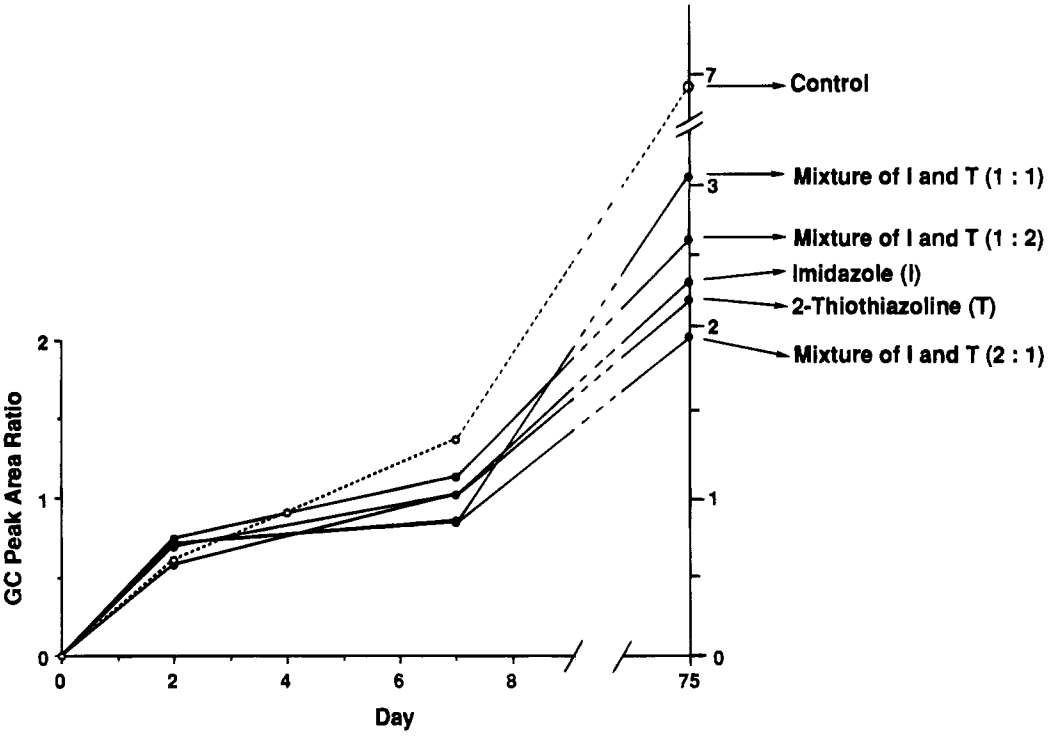

Figure 4. Antioxidative effect of imidazole, 2-thiothiazoline, and their mixtures in the dark.

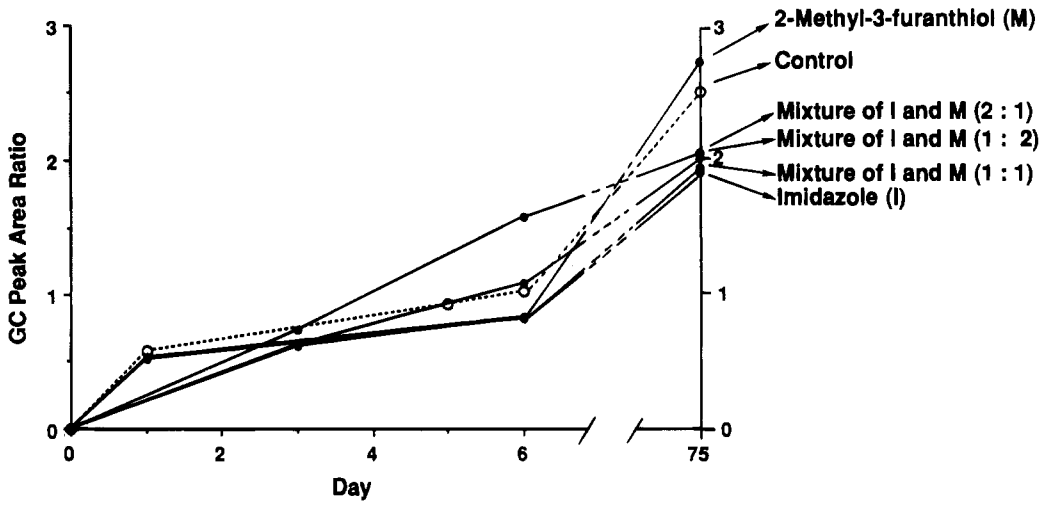

Figure 5. Antioxidative effect of imidazole, 2-methyl-3-furanthiol, and their mixtures in the dark.

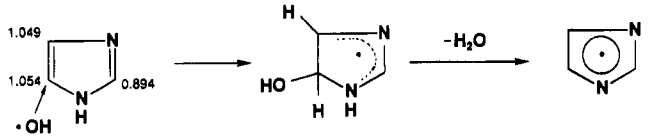

Figure 6. Reaction mechanism of imidazole and a hydroxyl radical. The numbers are electron density at the carbon atoms.

occurring in a sample exposed to sunlight because many reactions might be involved compared to a sample in the dark.

It is well-known that an organic solvent extract of browning reaction mixtures consists mainly of heterocyclic compounds including furans, thiophenes, pyrroles, pyrazines, thiazoles, oxazoles, and imidazoles (Shibamoto, 1983; Sheldon et al., 1986). Although it is not classified as a flavor chemical, imidazole was examined because it is one of the major volatile compounds formed from browning reactions (Shibamoto, 1983). Imidazoles are the only volatile browning heterocyclic compound soluble in water. Generally, a browning model reaction is conducted in an aqueous solution and the products are subsequently extracted with an organic solvent. Imidazoles are not easily extracted from an aqueous solution because of their high water solubility. Therefore, there have been virtually no reports on isolation of imidazoles from browning model systems. However, when a liquid–liquid continuous extractor was used, many alkylimidazoles were recovered from a browning reaction mixture. For example, imidazoles comprised 4.89% of the total heterocyclic compounds formed in a L-rhamnose/ammonia browning model system (Shibamoto and Bernhard, 1978). Because an amino–carbonyl reaction produces numerous heterocyclic compounds, complex interactions among those chemicals must be occurring.

Figures 3–5 show the results of the inhibitory activity of imidazole, 2-thioimidazole, 2-thiothiazoline, 2-methyl-3-furanthiol, and their mixtures from the experiments in the dark. Values are the average of three duplicate samples. Generally, the results suggested that these three compounds have some inhibitory activity toward

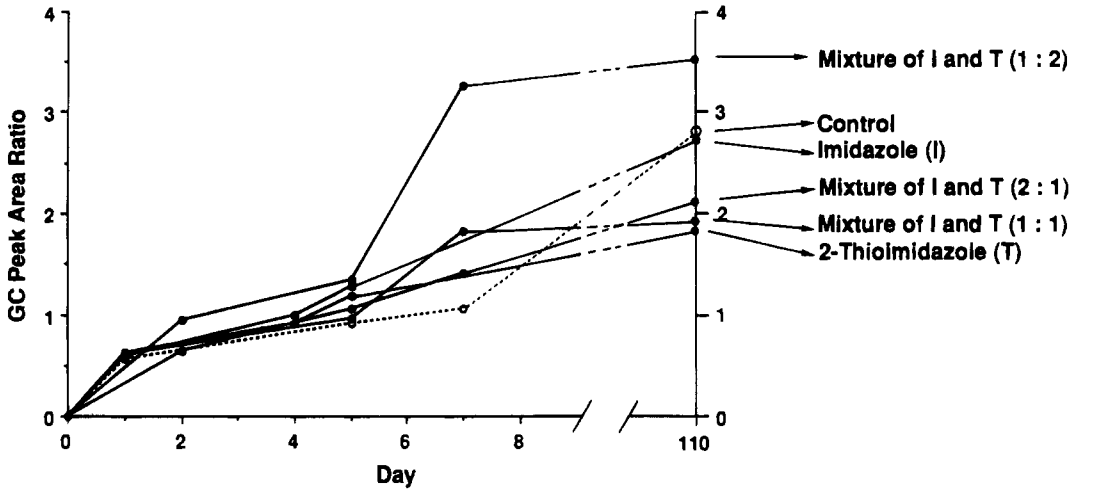

Figure 7. Antioxidative effect of imidazole, 2-thioimidazole, and their mixtures in sunlight.

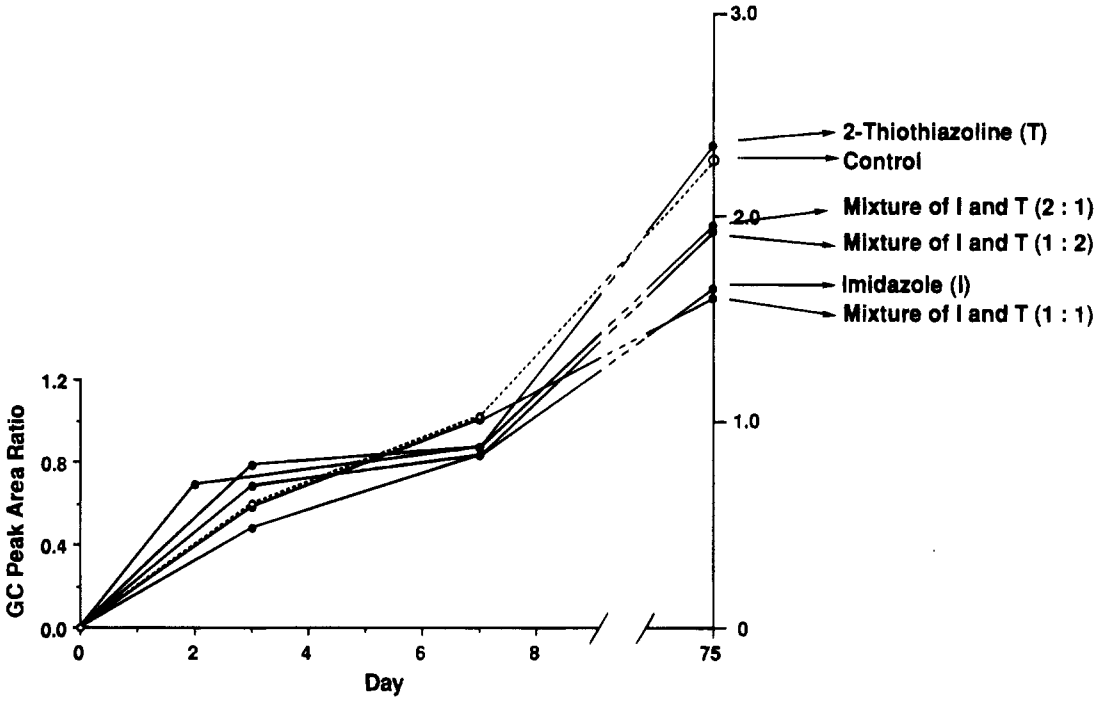

Figure 8. Antioxidative effect of imidazole, 2-thiothiazoline, and their mixtures in sunlight.

the aldehyde/acid conversion reaction in the dark. The thiol compounds reportedly scavenged free radicals in biological systems (Murphy et al., 1992). Several thiol compounds, including 2-methyl-3-furanthiol, showed inhibitory activity toward the aldehyde/acid conversion reaction in a previous study (Eiserich and Shibamoto, 1994). For example, 2-methyl-3-furanthiol inhibited oxidation of heptanal for up to 30 days and then began to lose its activity. In the present study, 2-methyl-3-furanthiol showed activity consistent with the previous study during the first week (Figure 5). However, after 75 days, the amount of hexanoic acid in the sample with 2-methyl-3-furanthiol was slightly greater than that of hexanoic acid in the control sample. It is difficult to explain these phenomena. After consumption of a reactive sulfhydryl group, certain prooxidants might be formed in the testing solution with 2-methyl-3-furanthiol.

The five-membered heterocyclic aromatic ring may be able to scavenge reactive radicals such as a hydroxyl radical (Samuni and Neta, 1973). As shown in Figure 6, the carbon 5 in an imidazole ring possesses the highest electron density (Mahanti, 1977) and consequently may be able to absorb a hydroxyl radical to form a 5-hydroxyimidazoline radical (Dogan et al., 1990).

The mixtures of imidazole and 2-thioimidazole or 2-thiothiazoline showed a slight synergetic effect. The 2 (imidazole) to 1 (2-thioimidazole or 2-thiothiazoline) ratio exhibited the greatest effect (Figures 3 and 4). On the other hand, when 2-methyl-3-furanthiol was mixed with imidazole, the inhibitory effect was suppressed (Figure 5).

Figures 7–9 show the results of the experiments in sunlight. Values are the average of three duplicate samples. When samples were exposed to direct sunlight, hexanoic acid formation increased steadily in all samples. 2-Thioimidazole inhibited hexanoic acid formation by nearly 30% over 110 days (Figure 7). On the other hand, the amount of hexanoic acid in the sample with a mixture of imidazole and 2-thioimidazole (1:2) was 3 times that in the control sample (Figure 7). No appreciable difference in inhibitory activity was observed between the results from a mixture of imidazole

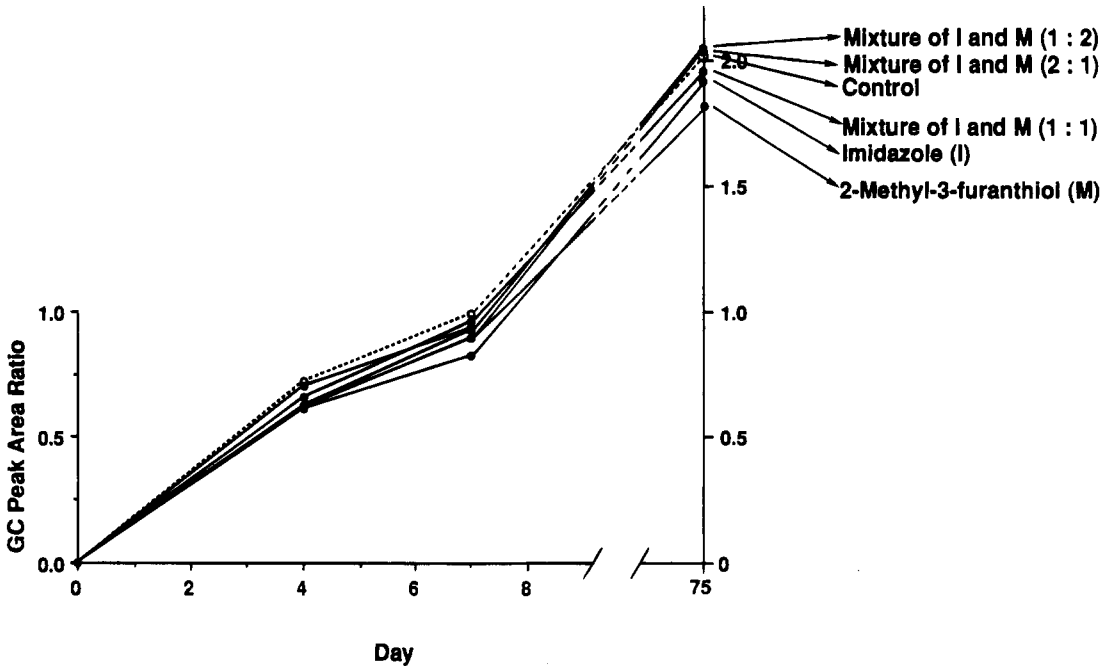

Figure 9. Antioxidative effect of imidazole, 2-methyl-3-furanthiol, and their mixtures in sunlight.

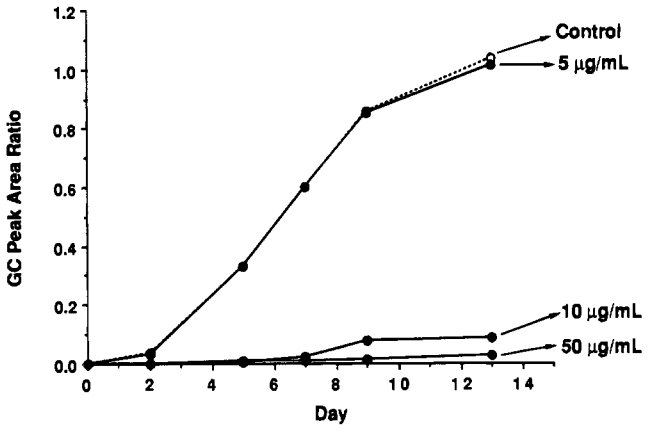

Figure 10. Antioxidative effect of α-tocopherol in the dark.

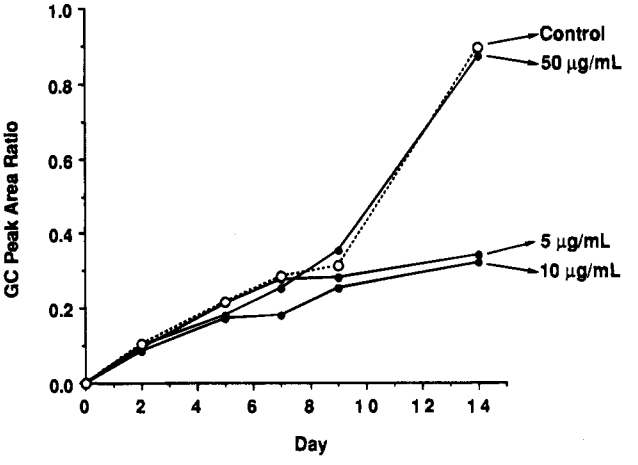

Figure 11. Antioxidative effect of α-tocopherol in sunlight.

and 2-thiothiazoline or 2-methyl-3-furanthiol and the results from the individual compounds (Figures 8 and 9). 2-Methyl-3-furanthiol showed slightly higher inhibitory effects than imidazole (Figure 9). The solutions of some samples exposed to the sunlight changed from clear to yellow after several weeks, suggesting that some polymerization occurred in those samples. This phenomenon was not observed in the case of the samples kept in the dark.

As mentioned above, a five-membered aromatic ring can absorb a hydroxyl radical. The thiol group can scavenge a one-electron-reducing agent such as a peroxyl or an alkoxyl radical and can also decompose hydroperoxides via two-electron reduction. This may be why 2-thioimidazole (Figure 7) and 2-methyl-3-furanthiol (Figure 9), which have an aromatic ring in addition a thiol group, exhibited greater antioxidative activity than did 2-thiothiazoline (Figure 8), which does not have an aromatic ring.

Figures 10 and 11 show the results of the experiments with α-tocopherol in the dark and in sunlight, respectively. Values are the average of three duplicate samples. At a dose of 50 μg/mL, α-tocopherol inhibited hexanoic acid formation by almost 100% over 7 days in the dark. On the other hand, α-tocopherol did not show any inhibitory activity at a dose of 50 μg/mL in the experiment with sunlight. Also, in a previous study, α-tocopherol did not exhibit antioxidative activity toward lipids oxidized by UV light in the higher doses (Nishiyama et al., 1994). In contrast to α-tocopherol, BHT inhibited hexanoic acid formation by nearly 99% over 75 days at a level of 5 μg/mL both in the experiment in dark and in the experiment with sunlight. At levels of 10 and 50 μg/mL, BHT inhibited hexanoic acid formation by almost 100% over 75 days.

Many heterocyclic flavor compounds have been isolated and identified from numerous foods such as cooked meat (Shibamoto, 1980) and beverages such as coffee (Shibamoto, 1991). These compounds have received much attention as chemicals that possess a toasted or roasted flavor, and they have been widely used as flavoring ingredients for many years. The antioxidative activity of the heterocyclic compounds exhibited in the present study is not as strong as that of known antioxidants such as α-tocopherol or BHT. However, these heterocyclic compounds are present in heat-treated foods and beverages in tremendous quantities. The total activity of these compounds may, therefore, have a significant effect. The potential use of these compounds to inhibit the oxidation of food products must be investigated further.

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