Antioxidative Activities of Heterocyclic Compounds Formed in Brewed Coffee

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Typical volatile heterocyclic compounds found in brewed coffee extracts—pyrroles, furans, thiophenes, and thiazoles—were examined for antioxidative activity, which was determined by measuring the oxidative conversion of hexanal to hexanoic acid using gas chromatography. 2-Acetylpyrrole, 1-methylpyrrole, and pyrrole inhibited hexanal oxidation by 98, 87, and 78%, respectively, at a concentration of 500 μg/mL over a period of 30 days. 2-Methylfuran, which inhibited hexanal oxidation by 90% at all concentrations tested (500, 200, and 100 μg/mL) for a 30-day period, exhibited the greatest activity among furans tested. Similarly, 2-methylthiophene, which inhibited hexanal oxidation by almost 100% at a concentration of 500 μg/mL over 30 days, exhibited the greatest activity among the thiophenes tested. In general, thiazoles were ineffective antioxidants at all concentrations tested. However, 4,5-dimethylthiazole was able to inhibit hexanal oxidation by 50% at the highest level tested (500 μg/mL). 2-Acetylpyrrole, 2-methylfuran, and 2-methylthiophene at concentrations of 500, 200, and 100 μg/mL and furan at a concentration of 500 μg/mL exhibited antioxidative activities comparable to that of the synthetic antioxidant butylated hydroxytoluene at a concentration of 50 μg/mL.

Keywords: *Antioxidants; coffee aroma; heterocyclic compounds*

INTRODUCTION

Coffee is one of the most popular beverages in the world. Its unique flavor has been intensively studied since the beginning of this century. To date, the number of volatile chemicals identified in coffee has reached almost 1000 (Shibamoto, 1992). The coffee constituents that have received the most attention from flavor chemists are the heterocyclic compounds because of their characteristic roasted or toasted flavors. There are almost 350 heterocyclic compounds including thiophenes, thiazoles, oxazoles, pyrroles, pyrazines, imidazoles, and furans that have been identified in coffee (Flament and Chevalier, 1988). Until recently, aroma chemicals have been investigated from the viewpoint of flavor and fragrance chemistry. However, some medicinal activities of aroma chemicals have been discovered (Hoffmann, 1987; Tisserand, 1988).

Recently, volatile compounds obtained from a glucose/cysteine browning model system were reported to possess certain antioxidative activities (Shaker et al., 1995). Also, column chromatographic fractions prepared from a dichloromethane extract of a glucose/cysteine browning model system demonstrated the ability to inhibit the oxidative transformation of hexanal to hexanoic acid. Additionally, several nitrogen- and/or sulfur-containing heterocyclic compounds, which are major flavor compounds formed by the Maillard reaction (Shibamoto, 1983), exhibited antioxidative activity in two separate testing systems (Eiserich and Shibamoto, 1994). For example, alkylthiophenes, 2-thiophenethiol, 2-methyl-3-furanthiol, furfuryl mercaptan, 2-thiothiazoline, and imidazole—which are all found in coffee—inhibited hexanal oxidation for up to 30 days and also exhibited antioxidative activities measured in lipid peroxidation systems and by the thyrosyl radical scavenging assay (Eiserich et al., 1995).

In comparison, higher molecular weight substances, such as melanoidins, are thought to be the major antioxidative products formed by the browning reactions (Yamaguchi, 1986). For example, the addition of sugar and/or amino acids to baked foods, such as cookies, enhances the browning reaction and subsequently increases the stability against oxidative rancidity (Elizalde et al., 1991, 1992). In the case of coffee, roasted coffee brews have higher antioxidative activity than crude coffee brews, which contained higher concentrations of known polyphenolic antioxidants (Nicoli et al., 1997; Daglia et al., 2000). This suggests that compounds other than polyphenols are responsible for the antioxidative activity of the roasted coffee brews.

In the present study, typical heterocyclic volatile compounds—including pyrroles, furans, thiopenes, and thiazoles—found in brewed coffee were examined for antioxidative activity.

EXPERIMENTAL PROCEDURES

Materials. Butylated hydroxytoluene (BHT) was bought from Sigma Chemical Co. (St. Louis, MO). Reagent grade hexanal, pyrrole, 1-methylpyrrole (MP), 2-acetylpyrrole, furan, 2-methylfuran, 5-(hydroxymethyl)furfural (5-HMF), 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), thiophene, 2-methylthiophene, 2-acetylthiophene, thiazole, 4-methylthiazole, and

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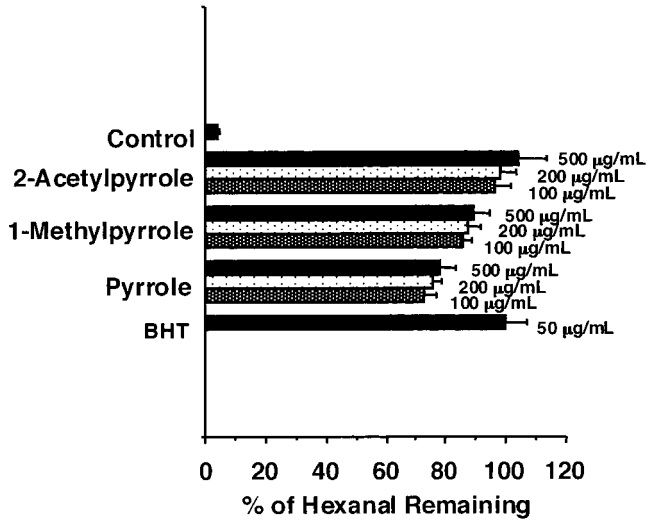

Figure 1. Inhibitory effect of pyrroles toward hexanal oxidation.

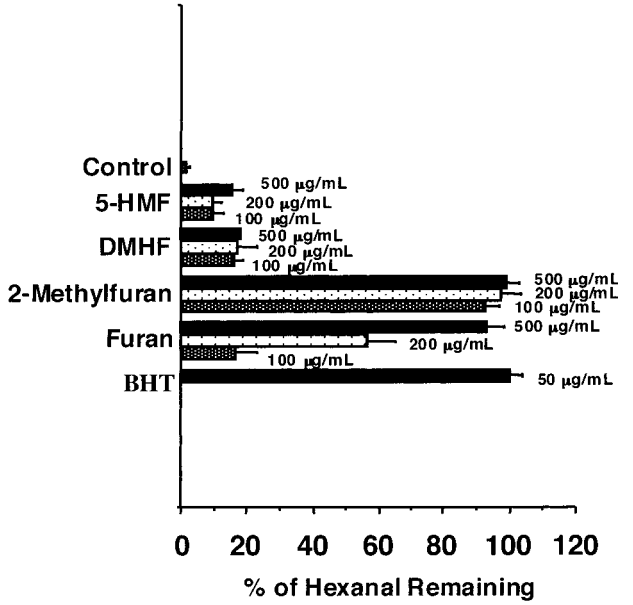

Figure 2. Inhibitory effect of furans toward hexanal oxidation.

4,5-dimethylthiazole were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Antioxidative Test of Heterocyclic Compounds Found in Coffee Extracts. Antioxidative activities of the samples were tested using their inhibitory effect toward the oxidative conversion of hexanal to hexanoic acid (Macku and Shibamoto, 1991). A sample (100, 200, or 500 μg/mL) of each of the test compounds was dissolved into a 2-mL dichloromethane solution of hexanal (1 mg/mL) containing 0.1 mg/mL of undecane as a gas chromatographic (GC) internal standard. A sample containing no testing sample was prepared as a control. The oxidation of the sample solution was initiated by heating at 60 °C in a sealed vial for 10 min. Following this, air was bubbled through samples for 3 s daily, at room temperature, for the first 10 days. The amount of hexanal was determined after 30 days by GC. BHT was also examined for its antioxidative activity using the same testing method in each experiment in order to compare its activity with those of the tested chemicals.

Quantitative Analysis of Hexanal. The GC internal standard method was used (Ettre, 1967). A Hewlett-Packard (HP) model 5890 GC equipped with a 30 m × 0.25 mm i.d. DB-1 bonded-phase fused silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID) was used to monitor the relative amounts of hexanal in the samples. The injector and detector temperatures were 300 and 280 °C, respectively. The oven temperature was programmed from 40 °C to 105 °C at 4 °C/min.

RESULTS AND DISCUSSION

The aldehyde/carboxylic acid test is a fast and simple method to assess the antioxidative properties of chemicals or a group of chemicals. This method is based on the autoxidation of aldehydes to carboxylic acids with active oxygen species such as a hydroxy radical (Horner, 1961). Fatty aldehydes are converted readily to a corresponding fatty acid in an oxygen-rich dichloromethane solution through a radical-type reaction (Nonhebel et al., 1979). This method has been validated using typical antioxidants such as BHT, α-tocopherol, and caffeine (Singhara et al., 1998).

Baseline resolution of hexanal was obtained by the column used. There were no peaks that interfered with the quantitative analysis of hexanal.

Figure 1 shows the inhibitory effect of pyrroles toward hexanal oxidation. Values are reported as the mean ± standard deviation ($n = 3$). All pyrroles tested exhibited concentration-dependent activity. 2-Acetylpyrrole, 1-methylpyrrole, and pyrrole inhibited hexanal oxidation by 98, 87, and 78%, respectively, at a concentration of 500 μg/mL over 30 days. In an earlier study, the antioxidative activity of 1-methylpyrrole was shown to be comparable to that of α-tocopherol (Shaker et al., 1995).

Pyrroles have not received as much attention as flavor components or other heterocyclic compounds such as pyrazines and thiazoles, even though the number of derivatives found in foods and beverages is almost the same as that of pyrazines (Shibamoto, 1983).

Figure 2 shows the inhibitory effect of furans toward hexanal oxidation. Values are reported as the mean ± standard deviation ($n = 3$). All furans tested exhibited concentration-dependent activity, which was especially pronounced with furan. For example, furan inhibited hexanal oxidation by 16, 57, and 94% at levels of 100, 200, and 500 μg/mL over 30 days, respectively. Among the furans tested, 2-methylfuran exhibited the greatest antioxidant activity. It inhibited hexanal oxidation over 90% at all levels for 30 days. Addition of a methyl group to the furan greatly enhances the antioxidative activity of a furan ring. This may be due to the electron-donating nature of the methyl group. Addition of a methyl group increases the electron density at certain carbon atoms in the furan ring, which subsequently increases its ability to scavenge free radicals (Eiserich and Shibamoto, 1994; Eiserich et al., 1995). In contrast, addition of an electron-withdrawing group, such as a formyl group (5-HMF) or a carbonyl group (DMHF), decreased the antioxidative activity of the furan ring. For example, 5-HMF inhibited hexanal oxidation by only 15% at 500 μg/mL over 30 days. However, it inhibited hexanal oxidation over 90% at the same concentration over 10 days (Singhara et al., 1998). Even though the antioxidative activity of DMHF was not as high as that of either furan or methylfuran in the present test system, it did inhibit (by 22%) the formation of malonaldehyde in equine plasma oxidized with Fenton's reagent at a concentration of 100 nM (Miyake and Shibamoto, 1998).

Many furans, such as 5-HMF, are sugar degradation products (Hodge, 1967). Furans are the most abundant volatile chemicals in roasted coffee, and some of them

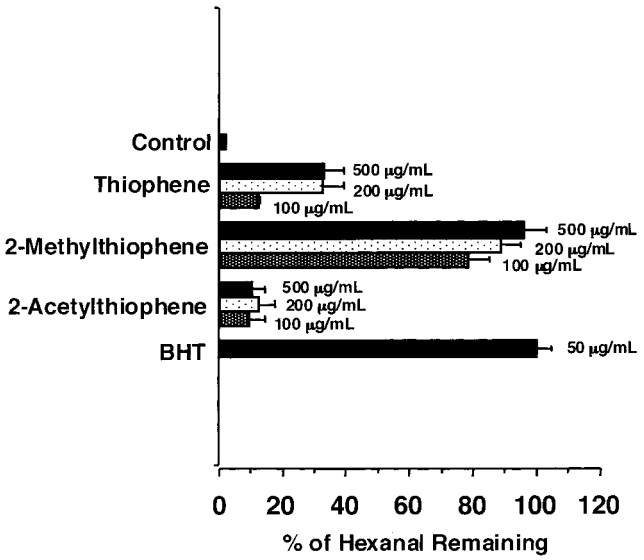

Figure 3. Inhibitory effect of thiophenes toward hexanal oxidation.

possess a characteristic caramel-like flavor (Flament and Chevalier, 1988).

Figure 3 shows the inhibitory effect of thiophenes toward hexanal oxidation. Values are reported as the mean ± standard deviation ($n = 3$). Similar to furan, 2-methylthiophene exhibited the greatest activity among the thiophenes tested. These data demonstrate the reliability of the hypothesis that an electron-donating group, such as a methyl group, increases the radical scavenging activity and that an electron-withdrawing group, such as an acetyl group, decreases the radical scavenging activity (Samuni and Neta, 1973; Mahanti, 1977). For example, 2-methylthiophene inhibited hexanal oxidation by almost 100% at 500 μg/mL, whereas 2-acetylthiophene inhibited hexanal formation by only 10% at the same concentration. Addition of a methyl group to a thiophene ring increased the activity by 70%, whereas the addition of an acetyl group to a thiophene ring decreased the activity by 20% at 500 μg/mL. Acetylpyrrole, however, exhibited a discrepancy to this hypothesis, and further investigation is necessary to clarify this point.

Many thiophenes are also found in coffee aroma. Their presence in coffee may be unique among beverages because there have been virtually no reports of thiophenes in other beverages such as cocoa and tea (Shibamoto, 1992).

Figure 4 shows the inhibitory effect of thiazoles toward hexanal oxidation. Values are reported as the mean ± standard deviation ($n = 3$). The unsubstituted thiazole did not have appreciable inhibitory activity toward hexanal oxidation. The addition of one methyl group (4-methylthiazole) did not increase its antioxidant activity significantly, whereas the addition of two methyl groups (4,5-dimethylthiazole) considerably increased the antioxidative activity of the thiazole. At the highest concentration tested (500 μg/mL), the unsubstituted thiazole inhibited hexanal oxidation by only 10%, whereas 4,5-dimethylthiazole inhibited hexanal oxidation by 50%. In general, at all concentrations tested, thiazoles exhibited low antioxidant activity.

Thiazoles are known to possess a cooked meatlike flavor and are used in the formulation of imitation meat flavors. Many thiazoles are also reported in brewed coffee (Vizthum and Werkhoff, 1974).

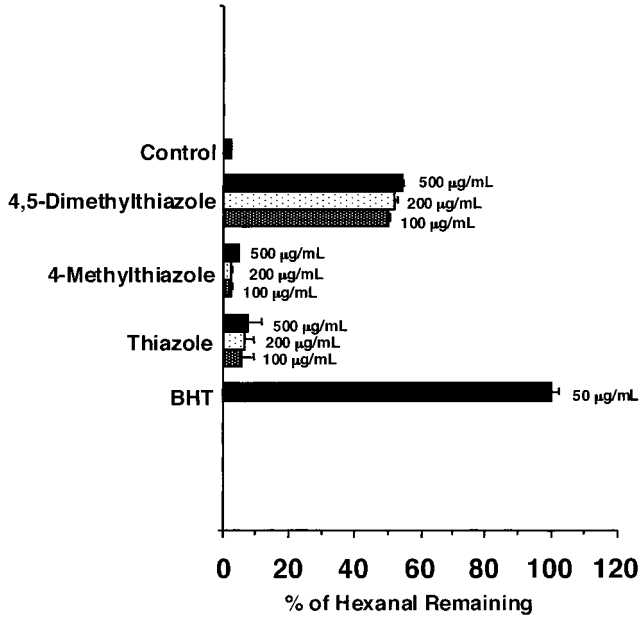

Figure 4. Inhibitory effect of thiazoles toward hexanal oxidation.

Antioxidative activity in brewed coffee extracts, containing >1000 volatile chemicals, including nearly 400 heterocyclic compounds (Flament and Chevalier, 1988; Shibamoto, 1992), has been reported (Singhara et al., 1998). Many of these heterocyclic volatile compounds—including pyrroles, furans, thiophenes, and thiazoles—give cooked foods their characteristic roasted or toasted flavors (Shibamoto, 1983). For example, they have been identified in many cooked or processed foods, such as cooked meat, brewed coffee, and processed cocoa (Shibamoto, 1992). Since the antioxidative activity of these heterocyclic flavor compounds has been reported (Eiserich and Shibamoto, 1994; Shaker et al., 1995), these chemicals have begun to receive much attention as biologically active constituents in foods. The results from the present study indicate that several of the heterocyclic compounds present in coffee possess antioxidative activity, although this activity is not as strong as that of the synthetic antioxidant BHT. However, because tremendous numbers of these heterocyclic compounds are present in cooked foods and beverages, their combined activity might be comparable to those of known antioxidants.

It is nearly impossible to test the possible synergism among the >1000 compounds identified in brewed coffee. However, the present study suggests that the antioxidative activities of brewed coffee are due in part to the contributions of volatile heterocyclic compounds. The presence of various heterocyclic compounds may explain the improvement of food stability. Moreover, ingestion of these heterocyclic compounds may help to prevent in vivo oxidative damage such as lipid peroxidation, which is associated with many diseases including cancer, arteriosclerosis, aging, diabetes, and immune deficiency.

The levels of chemicals tested in the present study are considerably higher than levels present in actual brewed coffee. Levels of heterocyclic flavor compounds found in brewed coffee range from micrograms to milligrams per kilogram. However, it is important to know the antioxidative activities of chemicals first in order to investigate the possible presence of antioxidants in brewed coffee. Once activity is demonstrated, the next

step is to investigate the activity at the more relevant low levels shown above. Therefore, investigation of the antioxidative activity of the chemicals at the levels of micrograms to milligrams per kilogram is in order.

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