JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antioxidative Activity of Heterocyclic Compounds Found in Coffee Volatiles Produced by Maillard Reaction

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Typical heterocyclic compounds substituted with various functional groups found in Maillard reaction products were examined for antioxidant activity. Pyrroles exhibited the greatest antioxidant activity among all heterocyclic compounds tested. All pyrroles inhibited hexanal oxidation by almost 100% at a concentration of 50 μg/mL over 40 days. Addition of formyl and acetyl groups to a pyrrole ring enhanced antioxidative activity remarkably. Pyrrole-2-carboxaldehyde, 2-acetylpyrrole, 1-methyl-2-pyrrolecarboxaldehyde, and 2-acetyl-1-methylpyrrole inhibited hexanal oxidation by >80% at 10 μg/mL. Unsubstituted furan exhibited the greatest antioxidant activity among furans tested. Addition of all functional groups used in this study to furan decreased antioxidative activity. The antioxidant activity of thiophene increased with the addition of methyl and ethyl groups, but the addition of formyl or acetyl groups to thiophene decreased antioxidant activity. Thiazoles and pyrazines were ineffective antioxidants at all concentrations tested. Reaction of all heterocyclic compounds with hydrogen peroxide resulted in the formation of various oxidized products.

KEYWORDS: Antioxidants; heterocyclic compounds; pyrroles; furans; thiophenes

INTRODUCTION

Many heterocyclic compounds have been reported in various processed foods and beverages. For example, >300 heterocyclic compounds, including pyrroles, oxazoles, furans, thiazoles, thiophenes, imidazoles, and pyrazines, were identified and quantified in brewed coffee (*1*−*4*). These heterocyclic compounds are known as chemical products of the Maillard reaction (*5*). They contribute toasted or roasted flavors to the heat-treated foods and beverages (*6*). Until recently, these heterocyclic compounds have been investigated from the viewpoint of flavor chemistry. However, some medicinal activities, such as antioxidant activity, have lately been discovered in volatile heterocyclic compounds (*7*). Volatile compounds obtained from a glucose/cysteine browning model system were reported to possess certain antioxidative activities (*8*). Also, column chromatographic fractions prepared from a dichloromethane extract of a glucose/cysteine browning model system inhibited the oxidation from hexanal to hexanoic acid. Additionally, several nitrogen- and/or sulfur-containing heterocyclic compounds, which are major flavor compounds formed by the Maillard reaction (*6*), exhibited antioxidative activity in two different testing systems (*9*). Characteristic volatile heterocyclic compounds found in brewed coffee extracts—pyrroles, furans, thiophenes, and thiazoles—exhibited certain levels of antioxidative activity (*10*).

In the present study, heterocyclic compounds derived from the Maillard reaction were examined for antioxidative activity.

MATERIALS AND METHODS

Chemicals. Hydrogen peroxide was obtained from Fisher Scientific Co., Ltd. (Fair Lawn, NJ). Hexanal, undecane, pyrrole, 1-methylpyrrole, 2-ethylpyrrole, pyrrole-2-carboxaldehyde, 2-acetylpyrrole, 1-methyl-2-pyrrolecarboxaldehyde, 2-acetyl-1-methylpyrrole, furan, 2-methylfuran, 2-ethylfuran, 2-furaldehyde, 2-acetylfuran, thiazole, 2-thiazolecarboxaldehyde, 2-acetylthiazole, thiophene, 2-methylthiophene, 2-ethylthiophene, 2-thiophenecarboxaldehyde, 2-acetylthiophene, pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2-acetylpyrazine, maleimide, *N*-methylsuccinimide, maleic anhydride, 2(5*H*)-furanone, and 2(5*H*)-thiophenone were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Antioxidative Effects of Heterocyclic Compounds. Antioxidative activities of the samples were tested using their inhibitory effect toward the oxidative conversion of hexanal to hexanoic acid according to previously published methods (*11*, *12*). Each sample (5, 10, 20, 50, 100, 200, and 500 μg/mL) was added to a 2-mL dichloromethane solution of hexanal (3 mg/mL) containing 0.2 mg/mL undecane as a 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. The headspace of each vial was purged with pure air for 2 s every 24 h for the first 10 days. The decrease in hexanal was monitored by gas chromatography (GC) at 5-day intervals. Each experiment was repeated three times.

Quantitative Analysis of Hexanal. The GC internal standard method was used (*13*). A Hewlett-Packard (HP) model 6890 GC equipped with

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10.1021/jf025616h CCC: $22.00
Published on Web 08/06/2002

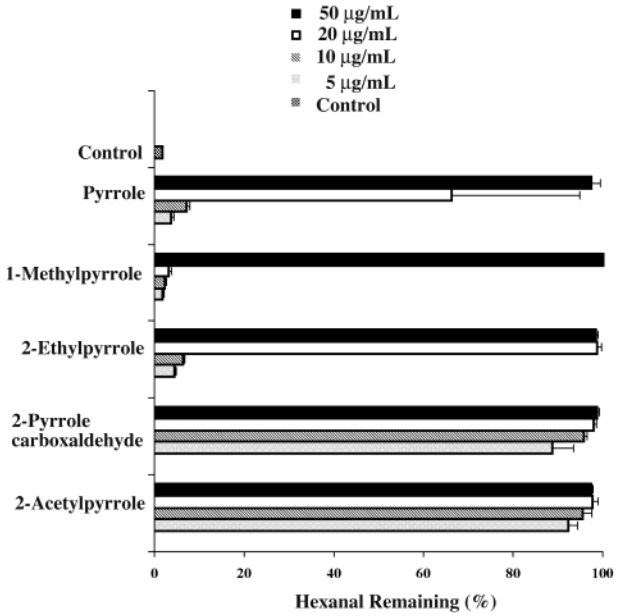

Figure 1. Inhibitory effects of pyrroles toward hexanal oxidation at the end of a 40-day storage period.

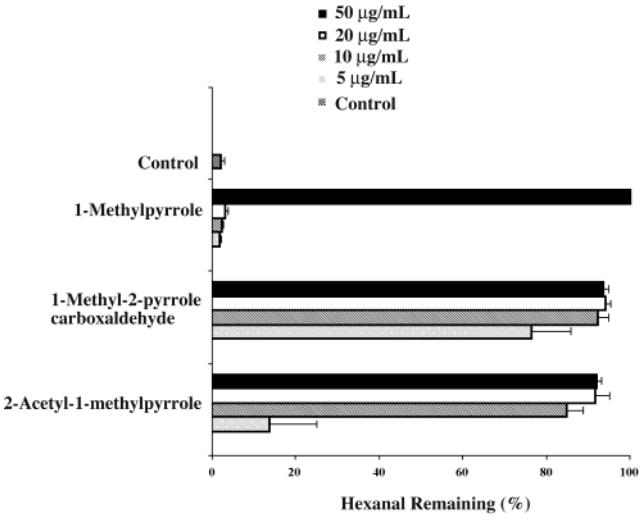

Figure 2. Inhibitory effects of 1-methylpyrroles, 1-methyl-2-pyrrolecarboxaldehyde, and 2-acetyl-1-methylpyrrole toward hexanal oxidation at the end of a 40-day storage period.

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 linear velocity of the helium carrier gas was 34 cm/s. The injector and detector temperatures were 300 and 280 °C, respectively. The oven temperature was programmed from 40 to 180 °C at 8 °C/min.

Reaction of Tested Heterocyclic Compounds and Hydrogen Peroxide. Hydrogen peroxide (30%, 200 μL) was added to a dichloromethane solution containing each heterocyclic compound (5 mg/mL). The solutions were stored in a vial at room temperature. After verification of peaks of oxidized products by GC, the reaction mixture was analyzed by gas chromatography−mass spectrometry (GC-MS). GC-MS was conducted on an HP model 6890 GC interfaced to an HP 5791 series mass selective detector at an MS ionization voltage of 70 eV. The column and GC conditions were as described above.

RESULTS AND DISCUSSION

Figure 1 shows the antioxidative activity of pyrroles toward hexanal oxidation. All pyrroles tested exhibited dose-dependent

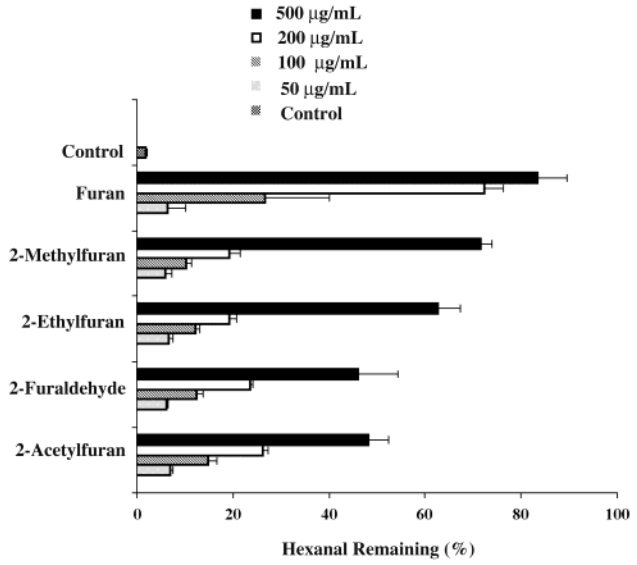

Figure 3. Inhibitory effects of furans toward hexanal oxidation at the end of a 40-day storage period.

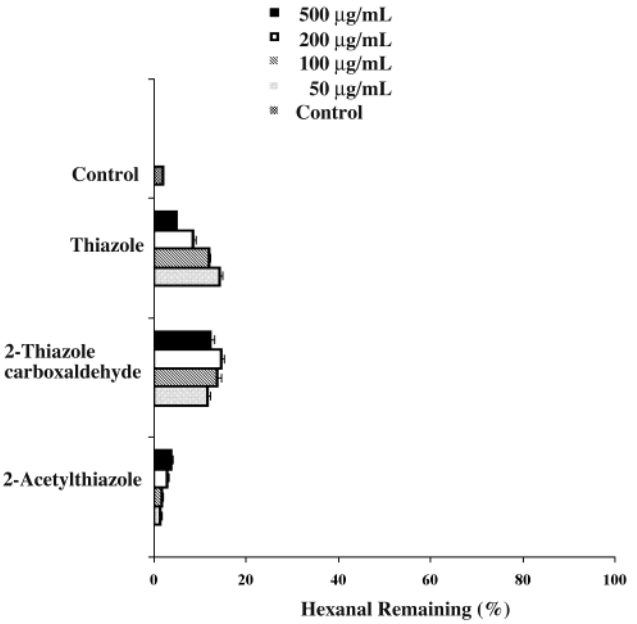

Figure 4. Inhibitory effects of thiazoles toward hexanal oxidation at the end of a 40-day storage period.

activity and inhibited hexanal oxidation by almost 100% at a level of 50 μg/mL over 40 days. However, pyrrole and 1-methylpyrrole inhibited hexanal oxidation by only 66 and 3% at 20 μg/mL, respectively. 2-Ethylpyrrole inhibited hexanal oxidation by almost 100% at a level of 20 μg/mL, but the inhibition was quite low at 10 and 5 μg/mL. On the other hand, pyrrole-2-carboxaldehyde and 2-acetylpyrrole exhibited potent antioxidative activity at all concentrations. The antioxidative activities of pyrroles substituted with electron-withdrawing groups, such as formyl and acetyl, were higher than those of pyrroles substituted with electron-donating groups, such as methyl and ethyl. According to previous studies (*7*, *9*), the addition of electron-donating substituents to a heterocyclic ring increased radical scavenging activity as a result of increased electron density at carbon atoms in the heterocyclic ring. In contrast, the presence of electron-withdrawing substituents decreases electron density around the heterocyclic ring, hence decreasing its ability to scavenge free radicals. However, results

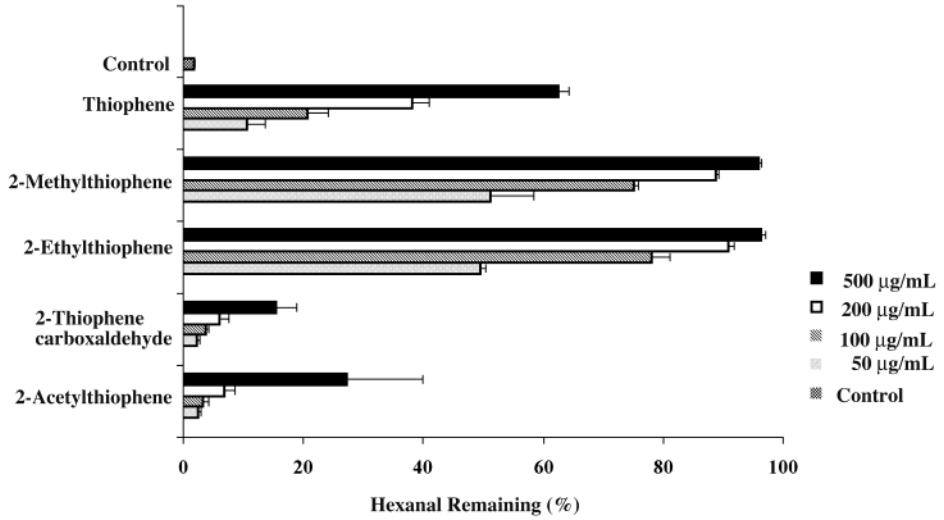

Figure 5. Inhibitory effects of thiophenes toward hexanal oxidation at the end of a 40-day storage period.

from the present study differ from results reported in previous studies (*7*−*9*).

The effects of substituted formyl and acetyl groups on pyrrole, 1-methylpyrrole, 1-methyl-2-pyrrolecarlboxaldehyde, and 2-acetyl-1-methylpyrrole were examined on the basis of their antioxidative activities toward hexanal oxidation (**Figure 2**). 1-Methyl-2-pyrrolecarboxaldehyde and 2-acetyl-1-methylpyrrole exhibited stronger antioxidative activities compared to 1-methylpyrrole. In the present study, addition of electron-withdrawing groups enhanced antioxidative activity. Acetylpyrrole also exhibited strong antioxidative activity in the previous study (*10*). Further investigation is necessary to clarify the effects of substitutions to the pyrrole ring on their antioxidative activity.

Figure 3 displays the inhibitory effect of furans toward hexanal oxidation. All furans exhibited dose-dependent activity. Among tested furans, furan exhibited the greatest antioxidant activity. It inhibited hexanal oxidation by 80% at 500 μg/mL over 40 days. Addition of electron-donating groups, such as methyl or ethyl, slightly decreased antioxidant activity. Addition of electron-withdrawing groups, such as formyl or acetyl groups, also decreased antioxidant activity. In the case of furans, addition of functional groups resulted in a negative effect on antioxidant activity.

Figure 4 shows the antioxidative activities of thiazoles. All thiazoles lacked inhibitory activity toward hexanal oxidation. Addition of electron-withdrawing groups to the thiazole did not significantly affect antioxidant activity.

Figure 5 exhibits the antioxidative activities of thiophenes. 2-Methylthiophene and 2-ethylthiophene exhibited stronger activities than that of thiophene. However, the antioxidative activities of 2-thiophenecarboxaldehyde and 2-acetylthiophene significantly decreased compared to those of thiophene, 2-methylthiophene, and 2-ethylthiophene. This result corresponds with the hypothesis that an electron-donating group increases radical scavenging activity and an electron-withdrawing group decreases radical scavenging activity (*14*, *15*).

Figure 6 illustrates the antioxidative activities of pyrazines. All pyrazines lacked antioxidative activity. The addition of electron-donating groups to pyrazine did not enhance antioxidant activity, whereas the addition of acetyl groups to pyrazine decreased antioxidative activity.

The present study demonstrates that the effects of various functional groups on heterocyclic rings toward antioxidative activity are different. This result suggests that only the electron

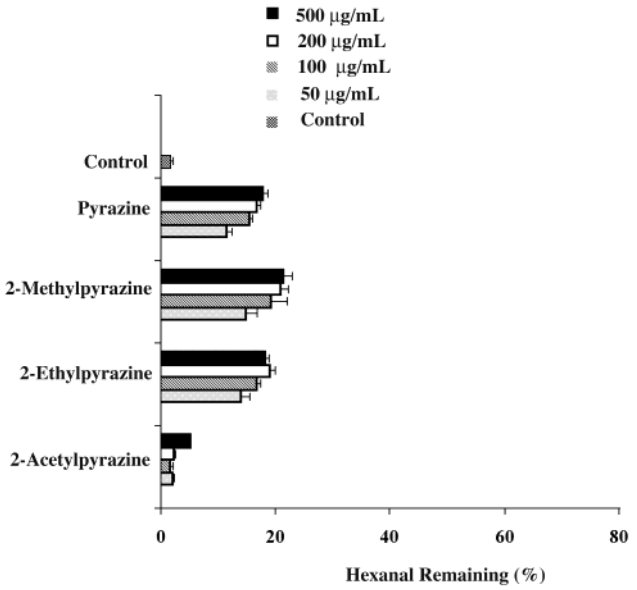

Figure 6. Inhibitory effects of pyrazines toward hexanal oxidation at the end of a 40-day storage period.

density of carbon atoms on a heterocyclic ring may not determine the strength of antioxidative activity. Other properties of the compounds, such as polarity, may also be involved in their antioxidative activity. Thus, further investigation is necessary to clarify this point.

The aldehyde/carboxylic acid test used in the present study 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 (*16*). Fatty aldehydes are converted readily to a corresponding fatty acid in an oxygen-rich dichloromethane solution with a hydroxy radical (*17*). Therefore, heterocyclic compounds tested were reacted with H_2O_2 in dichloromethane solution in order to elucidate mechanisms of antioxidant activity in this system. Hydrogen peroxide was added as an $^{\bullet}$OH source because hydroxy radicals present in the above testing solution were not sufficient to produce detectable levels of an $^{\bullet}$OH adduct of heterocyclic compounds.

Table 1 shows the oxidized products from the heterocyclic compounds. Pyrrole, 2-ethylpyrrole, pyrrole-2-carboxaldehyde, and 2-acetylpyrrole produced 1*H*-pyrrole-2, 5-dione in which

Table 1. Oxidized Products from Heterocyclic Compounds Reacted with Hydrogen Peroxide

heterocyclic compound	oxidized product	RT[a]	MS[b]
pyrrole groups			
pyrrole	1*H*-pyrrole-2,5-dione (maleimide)	+	+
1-methylpyrrole	1-methyl-2,5-pyrrolidinedione (*N*-methylsuccinimide)	+	+
	1,5-dihydro-1-methyl-2*H*-pyrrole-2-one		+
2-ethylpyrrole	1*H*-pyrrole-2,5-dione (maleimide)	+	+
pyrrole-2-carboxaldehyde	1*H*-pyrrole-2,5-dione (maleimide)	+	+
2-acetylpyrrole	1*H*-pyrrole-2,5-dione (maleimide)	+	+
2-acetyl-1-methylpyrrole	ND[c]		
1-methyl-2-pyrrolecarboxaldehyde	1-methyl-2,5-pyrrolidinedione (*N*-methylsuccinimide)	+	+
	1,5-dihydro-1-methyl-2*H*-pyrrole-2-one		+
furan groups			
furan	ND		
2-methylfuran	5-methyl-2(3*H*)-furanone		+
	2,5-furandione (maleic anhydride)	+	+
2-ethylfuran	5-ethyl-2(5*H*)-furanone		+
	2,5-furandione (maleic anhydride)	+	+
2-furaldehyde	2(5*H*)-furanone	+	+
	2(3*H*)-furanone		+
2-acetylfuran	ND		
thiazole groups			
thiazole	ND		
2-thiazolecarboxaldehyde	ND		
2-acetylthiazole	ND		
thiophene groups			
thiophene	ND		
2-methylthiophene	5-methyl-2(5*H*)-thiophenone		+
2-ethylthiophene	2-acetylthiophene	+	+
2-thiophenecarboxaldehyde	2(5*H*)-thiophenone	+	+
2-acetylthiophene	ND		
pyrazine groups			
pyrazine	ND		
2-methylpyrazine	2-methyl-1-oxidepyrazine		+
	2-methyl-4-oxidepyrazine		+
2-ethylpyrazine	ND		
2-acetylpyrazine	ND		

[a] Identification performed by comparing retention times with those of authentic compounds. [b] Identification performed by comparing the mass spectra with those of authentic compounds. [c] ND, not detected.

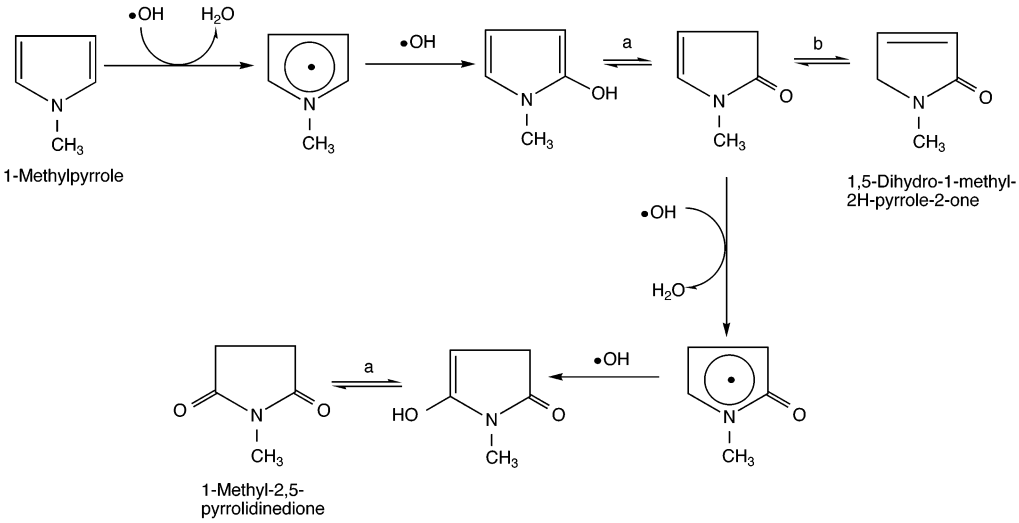

Figure 7. Hypothesized mechanism of hydroxyl radical abstraction by 1-methylpyrrole: step a, keto–enol tautomerization; step b, stabilization by conjugation.

oxygen atoms combined at the 2- and 5-positions of the pyrrole ring. However, 1-methylpyrrole produced 1-methyl-2,5-pyrrolidinedione. Unlike 1*H*-pyrrole-2,5-dione, 1-methyl-2,5-pyrrolidinedione does not possess a double bond in the heterocyclic ring.

Figure 7 shows the hypothesized formation mechanism of 1-methyl-2,5-pyrrolidinedione from 1-methylpyrrole and hydrogen peroxide. Carbon 2 in a pyrrolidyl radical absorbs a hydroxyl radical to form 2-hydroxy-1-methylpyrrole (*18*). Then, the first keto−enol tautomerization occurs, and 1-methyl-1,3-dihydropyrrole-2-one is produced. 1-Methyl-1,3-dihydropyrrole-2-one can then be converted to 1,5-dihydo-1-methyl-2*H*-pyrrole-2-one, which was identified as an oxidized product (**Table 1**) because of stabilization by conjugation. Conversion of 1-methyl-1,3-dihydropyrrole-2-one to 1,5-dihydo-1-methyl-2*H*-pyrrole-2-one has already been investigated with pyrrole (*19*). In the next step, carbon 5 in a pyrrole ring absorbs a hydroxyl radical via a 1-methyl dehydro-2-pyrrolidone radical to form 5-hydroxy-

1-methyl-1,3-dihydropyrrole-2-one. The second keto−enol tautomerization steps occur, and 1-methyl-2,5-pyrrolidinedione is finally produced. Similar to 1-methyl-2,5-pyrrolidinedione formation, production of 2,5-pyrrolidinedione has been previously reported in pyrrole oxidation (*20*). However, in that study a mechanism for 1*H*-pyrrole-2,5-dione formation from pyrrole was not proposed. As shown in **Table 1**, furans produced their oxidized products except furan and 2-acetylfuran. No oxidized products from furan and 2-acetylfuran were detected by GC-MS. 2-Methylthiophene and 2-thiophenecarboxaldehyde produced 5-methyl-2(5*H*)-thiophenone and 2(5*H*)-thiophenone as their respective oxidized products. In the case of 2-ethylthiophene, the ethyl group on the thiophene ring was oxidized to an acetyl group. This result demonstrates that the hydroxyl radical directly attacked the ethyl group on the thiophene. Most of the thiazoles and pyrazines, except for 2-methylpyrazine, did not produce oxidized products. This result may indicate that thiazoles and pyrazines are unable to scavenge hydroxyl radicals.

The results from the present study indicate that some of the heterocyclic compounds present in coffee possess antioxidative activity, although this activity is not as strong as that of the synthetic antioxidant butylated hydroxytoluene (BHT). However, because tremendous numbers of these heterocyclic compounds are present in coffee, their combined activity might be comparable to those of known antioxidants.

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 their 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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Received for review April 23, 2002. Revised manuscript received July 6, 2002. Accepted July 6, 2002.

JF025616H