JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Degradation of the Coffee Flavor Compound Furfuryl Mercaptan in Model Fenton-type Reaction Systems

IMRE BLANK,*,† EDERLINDA C. PASCUAL,‡ STÉPHANIE DEVAUD,† LAURENT B. FAY,† RICHARD H. STADLER,† CHAHAN YERETZIAN,† AND BERNARD A. GOODMAN*,‡

Nestec Ltd., Nestlé Research Center, Vers-chez-les-Blanc, P.O. Box 44, CH-1000 Lausanne 26, Switzerland, and Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA United Kingdom

The stability of the coffee flavor compound furfuryl mercaptan has been investigated in aqueous solutions under Fenton-type reaction conditions. The impact of hydrogen peroxide, iron, ascorbic acid, and ethylenediaminetetraacetic acid was studied in various combinations of reagents and temperature. Furfuryl mercaptan reacts readily under Fenton-type reaction conditions, leading to up to 90% degradation within 1 h at 37 °C. The losses were lower when one or more of the reagents was omitted or the temperature decreased to 22 °C. Volatile reaction products identified were mainly dimers of furfuryl mercaptan, difurfuryl disulfide being the major compound. In addition, a large number of nonvolatile compounds was observed with molecular masses in the range of 92−510 Da. The formation of hydroxyl and carbon-centered radicals was indicated by electron paramagnetic resonance spectra using α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone or 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-*N*-oxide as spin traps. Whereas •OH was generated by Fenton-type reactions, the C-centered radical is probably a secondary product of the reaction of •OH with various organic molecules, the reaction with furfuryl mercaptan appearing to be the most important. No evidence for S-centered radicals was seen in the spin-trapping experiments, but a sulfur-containing radical was detected when measurements were made at 77 K in the absence of spin traps.

KEYWORDS: Coffee; furfuryl mercaptan; thiols; Fenton chemistry; mass spectrometry; electron paramagnetic resonance spectroscopy

INTRODUCTION

Furfuryl mercaptan (**2**) has been reported to be a volatile constituent of many foods and beverages (*1*), particularly when thermal treatment is involved in their production. In coffee, it was first described by Reichstein and Staudinger (*2*), and more recently **2** has been suggested to be an important odorant of coffee (*3*). Its sensory relevance, evidenced by various groups (*4*, *5*), is due to the "roasty, coffee-like aroma note" and low odor thresholds of 0.01 ng/L in air (*6*) and 0.01 μg/kg in water (*7*).

The concentration of **2** in roasted and ground coffee is typically in the range of 1−2 mg/kg (*8*), but only about one-third of this is detected in coffee brews (*9*). This might be due to low extractability during the preparation of coffee beverages or the consequence of sensitivity to oxidative processes. In addition to atmospheric oxygen, oxidative processes in beverages may be initiated by hydrogen peroxide (H_2O_2), which is a common constituent of liquid coffee (*10*, *11*). In the presence of transition metals in low oxidation states, H_2O_2 produces hydroxyl radicals (•OH) via the Fenton reaction (*12*):

$$H_2O_2 + Fe(II) \rightarrow {}^{\bullet}OH + OH^- + Fe(III)$$

In coffee solutions, •OH is to some extent scavenged by caffeine with the formation of 8-oxocaffeine (*13*). However, OH radicals are extremely reactive entities, able to extract hydrogen atoms from a wide range of organic molecules. They may, therefore, also attack odor-active thiols, such as furfuryl mercaptan, methyl mercaptan, 3-methyl-2-buten-1-thiol, 2-methyl-3-furanthiol, and 3-methyl-3-mercaptobutyl formate (*14*), which could potentially lead to alteration of the coffee aroma.

This study has investigated the stability of furfuryl mercaptan in model systems under Fenton-type reaction conditions. Experiments were conducted with the objectives of (i) determining the losses of **2**, (ii) identifying the major volatile degradation products, (iii) characterizing the free radicals produced in these reactions, and (iv) studying the mechanisms involved in the oxidative/radical-induced degradation of **2**. Chromatographic and mass spectrometric techniques were used to quantify the levels

* Authors to whom correspondence should be addressed [I.B. (principal corresponding author) telephone +41 21 7858607, fax +41 21 7858554, e-mail imre.blank@rdls.nestle.com; B.G. (EPR spectroscopy) telephone +44 1382 568532, fax +44 1382 562426, e-mail bgoodm@scri.sari.ac.uk].
† Nestec Ltd.
‡ Scottish Crop Research Institute.

10.1021/jf011329m CCC: $22.00
Published on Web 03/03/2002

Table 1. Experimental Design To Study the Effect of Fenton Reagents on the Degradation of Furfuryl Mercaptan in Aqueous Model Systems[a]

	samples							
reagent	1	2	3	4	5	6	7	8
furfuryl mercaptan	100	100	100	100	100	100	100	100
$FeCl_3$	10		10	10	10		10	
H_2O_2	10	10		10	10			
EDTA	10	10	10		10			
ascorbic acid	50	50	50	50		50	50	
phosphate buffer	800	800	800	800	800	800	800	800
water	20	30	30	30	70	50	40	100

[a] Values represent volumes expressed in μL. The total volume of each sample was 1 mL.

of **2** in model solutions under conditions of oxidative stress and to identify products generated (*15*). Electron paramagnetic resonance (EPR) spectroscopy was used to detect free radical species, mainly by the chemical spin-trapping approach, in which the reaction of spin traps (e.g., nitrones) with unstable free radicals generates new more stable radicals (nitroxides), which can then be characterized (*16*).

MATERIALS AND METHODS

Chemicals. The purity of all chemicals was at least of analytical grade. Furfuryl mercaptan (**2**) was purchased from Aldrich (Buchs, Switzerland) or Sigma (Dorset, U.K.). Benzyl mercaptan, cuprous chloride (CuCl), difurfuryl monosulfide (**5**), difurfuryl disulfide (**7**), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, and manganese(II) chloride ($MnCl_2$) were from Aldrich. Ascorbic acid was from Aldrich or FSA Laboratory Supplies (Loughborough, U.K.). Tripotassium phosphate (K_3PO_4), ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), and α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (4-POBN) were from Sigma. Diethyl ether (Et_2O), ethanol (EtOH), H_2O_2, ethylenediaminetetraacetic acid (EDTA, disodium salt), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), dipotassium hydrogen phosphate (K_2HPO_4), sodium dodecyl sulfate (SDS), and anhydrous sodium sulfate (Na_2SO_4) were from Merck (Darmstadt, Germany) or Sigma. 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline-*N*-oxide (DEPMPO) was from Calbiochem−Novabiochem (Beeston, Nottinghamshire, U.K.).

Sample Preparation. *(i) Loss of Furfuryl Mercaptan (2) from Solutions at Room Temperature.* Aqueous solutions of **2** (0.427 mmol/150 mL) and either $FeCl_3$ (0.043 mmol/mL) or H_2O_2 (0.043 mmol/mL) were mixed to obtain 10:1 ratios by volume of **2**/$FeCl_3$ and **2**/H_2O_2. The samples (pH 5.1) were stored at room temperature (22 °C) for 8 days, and the loss of **2** was monitored as a function of time. Aliquots (5 mL) were taken after 2, 5, 24, 48, 120, and 192 h. Benzyl mercaptan (0.5 mL) was added as internal standard and the pH adjusted to 4.0 with aqueous HCl (0.1 M). Neutral compounds were extracted with Et_2O (5 mL), and **2** was quantified by gas chromatography (GC).

(ii) Loss of Furfuryl Mercaptan from Fenton-type Model Systems. The degradation of **2** was investigated in a systematic study for a series of eight model Fenton-type reaction systems (**Table 1**) at pH 5.5, which is close to the pH of coffee beverages. Sample 1 (complete Fenton model) contained all of the reagents for a Fenton reaction. EDTA was used to ensure complete solubilization of Fe(III), and ascorbic acid was used for the reduction of Fe(III) to Fe(II), which initiated decomposition of H_2O_2 to yield OH radicals. In samples 2−5 one of the reagents was omitted, whereas samples 6 and 7 had more than one component of the Fenton reaction system missing. The control sample 8 was a buffered aqueous solution of **2**.

The aqueous solutions described in **Table 1** were freshly prepared before use, that is, ascorbic acid (20 mM), EDTA (25 mM), H_2O_2 (1.5%), $FeCl_3 \cdot 6H_2O$ (10 mM), K_2HPO_4 (20 mM, pH 5.5), and **2** (33.3 mM in 3.3% aqueous SDS), the latter being added as the last reagent to the mixtures. Samples were stirred vigorously for 5 s and incubated for 1 h at 37 °C, after which time EtOH (0.1 mL) was added to terminate the Fenton reaction. Chemical analyses were performed after the pH had been adjusted to pH 3.5 and neutral compounds extracted with Et_2O (1 mL). The organic phase was centrifuged (5−15 min, 3500 rpm) and analyzed by GC coupled with a flame ionization detector (FID) and/or a mass spectrometer (MS). All reactions were performed at least in duplicate and samples injected twice into the GC.

(iii) Roles of Components of the Model Fenton-type Reaction System. The composition of the Fenton model systems described above (**Table 1**) was modified to study the roles of selected constituents. For example, ascorbic acid was replaced by 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone and $FeCl_3$ by $MnCl_2$ or CuCl. In some other model reactions, the concentrations of the reactants and reaction time, as well as the reaction temperature, were varied. All reactions, however, were performed at physiological (37 °C) and/or room (20−22 °C) temperature.

(iv) Volatile and Nonvolatile Reaction Products. A simplified model reaction was carried out for the characterization of volatile and nonvolatile compounds by mass spectrometric analysis. The model reaction was composed of **2**, H_2O_2, and $FeCl_3$ in the ratio of 10:1:1 on a molar basis. After 2 h at room temperature, neutral compounds were extracted with Et_2O (2 × 50 mL). The combined etheral phases were dried over anhydrous Na_2SO_4 and concentrated to 20 mL. Volatile compounds were separated by distillation in high vacuum (3×10^{-5} mbar) and collected in two glass traps cooled with liquid nitrogen (*17*). The distillate was concentrated to 1 mL using a Vigreux column (50 × 1 cm) for MS analysis of volatile compounds. The residue was taken for analysis of nonvolatile degradation products of **2**.

(v) Kinetic Studies. Experiments were performed at 37 °C on samples analogous to sample 1 in **Table 1**, except that 5 times higher volumes were used to obtain five data points from the same preparation. EtOH (0.1 mL) was added to 1 mL aliquots after 5, 10, 20, 40, or 120 min to terminate the reaction. The cleanup procedure was as described in section *ii* above. The concentrations of **2**, **5**, **7**, and **8** were determined by GC-MS.

(vi) Spin Trapping of Unstable Free Radicals. Solutions for EPR measurements at room temperature (20 ± 2 °C) and 37 °C were similar to samples 1, 4, and 5 (**Table 1**), except that a spin trap solution (100 mM concentration in the final solution for 4-POBN or 5.3 mM for DEPMPO) was included and the H_2O_2 was added last in order that the Fenton-type reaction was initiated only after all of the other reagents were present. Samples were taken at intervals during the 37 °C incubation and transferred rapidly to a quartz flat cell (Wilmad WG812Q, Fluorochem, Old Glossop, Derbyshire, U.K.) for the EPR measurements, which were made at room temperature. Spectra from incubations at room temperature were made on samples in flat cells, which remained in the EPR cavity for the duration of the experiment, as were additional measurements using DEPMPO as spin trap with solutions from which EDTA or ascorbic acid were omitted. Further samples were also prepared with DEPMPO as spin trap and were analogous to samples 1 and 5, but without **2**.

(vii) Low-Temperature EPR Measurements. For low-temperature (77 K) EPR measurements, a solution was prepared with just $FeSO_4 \cdot 7H_2O$ (0.4 mM), H_2O_2 (38 mM), and **2** (87 mM). Samples were taken at intervals after mixing the reagents at room temperature, transferred to a 4 mm i.d. quartz tube, and frozen immediately in liquid nitrogen. For EPR measurements the tubes were transferred to a quartz "Finger Dewar" (Wilmad WG816B) filled with liquid nitrogen, which was inserted into the microwave cavity.

(viii) γ-Irradiation of Furfuryl Mercaptan. Samples of **2** and 10% **2** in water were placed in 3 mm i.d. quartz EPR tubes and frozen in liquid nitrogen. They were maintained at that temperature during and after irradiation with 0.6 MeV γ-rays (received dosage ∼ 100 Gy) from a ^{137}Cs source of ∼63 TBq in a radiation facility in the School of Biology at the University of St. Andrews.

Capillary GC. A Hewlett-Packard gas chromatograph (HP-5890) equipped with an autosampler (HP-7673A) and cold on-column injector was used. Samples were analyzed on an OV-1701 fused silica capillary column, 30 m × 0.25 mm i.d., film thickness 0.25 μm (J&W Scientific, Folsom, CA). The column pressure was 80 kPa using helium as carrier gas. The effluent was split 1:1 to a flame ionization detector (FID) and a flame photometric detector (FPD). For the oven temperature program see ref *6*.

Quantification of Volatile Compounds. This was performed by GC-FID on a DB-5 capillary column (J&W Scientific) using benzyl

Table 2. Degradation of Furfuryl Mercaptan (**2**) in Aqueous Model Systems Containing Fenton Reagents as a Function of Time[a]

time (h)	**2** (control)	**2**/$FeCl_3$[b] (10:1)	**2**/H_2O_2[b] (10:1)
0	100	100	100
5	99	96	93
24	88	84	74
48	83	77	65
120	80	75	62
192	80	74	62

[a] Amounts of **2** are related to its concentration at $t = 0$ h (2.85 μmol/mL) and expressed in percent. [b] See (*i*) under Sample Preparation for more details.

mercaptan as internal standard (49.4 mg/100 mL Et_2O, 0.5 mL). It was added to the solutions after quenching with EtOH. The pH was adjusted rapidly to 3.5, and the cleanup was performed as described in section *ii* under Sample Preparation above. The response factors were determined in model solutions with known amounts of benzyl mercaptan and the compounds to be quantified, that is, 1.57 (**2**), 1.49 (**5**), and 1.66 (**7**). The response factor for **8** was set at 1.7.

Mass Spectrometry. *(i) Capillary GC-MS.* Electron impact (EI) and positive chemical ionization (PCI) mass spectra were obtained on a Finnigan MAT 8430 mass spectrometer (Finnigan MAT, San Jose, CA) at 70 and 150 eV, respectively. Ammonia was used as reagent gas for PCI. Volatile compounds were sampled via a cold on-column injector (HP-5890 GC) using the conditions described above. Relative abundances of the ions are given in percent.

(ii) Electrospray Ionization (ESI)-MS. Nonvolatile compounds were investigated by ESI-MS using a Finnigan TSQ 700 triple-quadrupole mass spectrometer equipped with an electrospray ionization source. This worked with a voltage of 4.5 kV and a transfer capillary heated at 150 °C. Argon was used as a sheath gas at a pressure of 40 psi. The samples were introduced by continuous infusion at 5 μL/min using a Harvard model 22 syringe pump. Data acquisition was performed on a DEC station 2100 running under Ultrix 4.2A (Digital Equipment) using the Finnigan software package ICIS2, version 7.0. Mass spectra were acquired in positive mode by scanning from *m*/*z* 20 to 1000 in 1 s.

EPR Spectroscopy. EPR measurements were made at X-band frequencies (~9.5 GHz) using a Bruker ESP300E (Bruker U.K. Ltd., Banner Lane, Coventry, U.K.) computer-controlled spectrometer incorporating an ER4103TM cylindrical cavity. Microwave generation was by means of a klystron (ER041MR), and the frequency was measured with a built-in frequency counter. All spectra were collected in 1024 data points using a modulation frequency of 100 kHz. A microwave power of 10 mW and modulation amplitude of 0.1 mT were used for fluid solution measurements at room temperature. The respective values for low temperature (77 K) spectra were 0.5 mW and 0.5 mT.

As is conventional in EPR spectroscopy, spectra were recorded as first derivatives of the microwave absorption and displayed as functions of absorption versus magnetic field at a constant microwave frequency. In a small number of instances, second-derivative spectra were also recorded to enhance resolution of the hyperfine structure from overlapping components. In most cases, spectral interpretations were confirmed by simulation using the Bruker Simfonia software package. However, measurements of variations in intensity as a function of time are based on the maxima and minima of the first peak of first-derivative spectra after smoothing by a double application of a 15-point polynomial function.

RESULTS AND DISCUSSION

Stability of Furfuryl Mercaptan in Aqueous Solutions. To provide background information on the stability of **2**, its concentration in aqueous solutions was monitored by GC-FID at room temperature over a time period of 8 days in the presence of either Fe(III) or H_2O_2. Compound **2** is relatively stable, and only 12% was lost from an aqueous solution after 24 h (**Table 2**). The rate of loss was increased in the presence of either Fe(III) or H_2O_2, but >60% remained after 8 days of incubation.

Table 3. Effect of Fenton Reagents on the Decomposition of Furfuryl Mercaptan (**2**) in Aqueous Model Systems

	concn of **2**[b] (mg/50 mL)		loss of **2** (%)	
model system[a]	37 °C[c]	22 °C[d]	37 °C	22 °C
1: complete Fenton system	2.4 ± 0.1	14.5 ± 0.8	89	20
2: no $FeCl_3$	17.6 ± 0.3	18.4 ± 0.6	19	<3
3: no H_2O_2	14.0 ± 0.5	16.7 ± 0.2	35	8
4: no EDTA	5.2 ± 0.1	5.3 ± 0.2	76	71
5: no ascorbic acid	9.2 ± 0.1	17.8 ± 1.0	57	<3
6: no $FeCl_3$, EDTA, H_2O_2	18.3 ± 0.8	18.7 ± 0.5	15	<3
7: no EDTA, H_2O_2	17.7 ± 0.7	17.8 ± 0.8	18	<3
8: reference sample	21.2 ± 0.4	18.5 ± 0.4	<3	<3

[a] For more details see **Table 1** and (*ii*) under Sample Preparation. [b] Mean values with standard deviations were obtained using benzyl mercaptan as internal standard. The variation coefficients were 1–6% with 3.5% in average. [c] Initial concentration of **2** was 21.6 mg/50 mL (3.8 mM). [d] Initial concentration of **2** was 18.2 mg/50 mL (3.2 mM).

Table 4. Effect of Selected Reagents on the Decomposition of Furfuryl Mercaptan (**2**) in Aqueous Fenton Model Systems at 37 °C

model system	concn of **2**[a] (mg/25 mL)	loss of **2** (%)
1: complete Fenton system	5.0 ± 0.1	90
2a: with $MnCl_2$[b]	43.8 ± 1.4	15
2b: with CuCl[c]	38.5 ± 0.8	25
5a: with 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone[d]	22.0 ± 0.8	57
8: control sample	51.2 ± 3.2	<3

[a] Initial concentration of **2** was 51.2 mg/25 mL (18 mM). Quantification is relative to benzyl mercaptan as internal standard, added to the sample after quenching with ethanol. For more details see (*iii*) under Sample Preparation. [b] $FeCl_3$ was replaced by the same concentration of $MnCl_2$ (10 mM). [c] $FeCl_3$ was replaced by the same concentration of CuCl (10 mM). [d] Ascorbic acid was replaced by the same concentration of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (20 mM).

Loss of Furfuryl Mercaptan under Fenton-type Reaction Conditions. Although **2** on its own is rather stable in aqueous solutions, it is rapidly decomposed in the presence of H_2O_2 and transition metals, as shown in **Table 3**. The concentration of **2** remaining after a 1-h incubation at 37 °C was strongly dependent on the composition of the solution. Apart from the results for sample 2, these data are in good agreement with those published in ref *15*. The previously reported value of 45% for the loss of **2** in sample 2 might be explained by trace impurities of transition metals in the water used. These can promote the reaction of H_2O_2 in the presence of reducing agents and, thus, initiate decomposition of **2**. Light also affects the stability of H_2O_2 and can lead to the generation of $^{\bullet}OH$.

The degradation of **2** is strongly temperature dependent (**Table 3**). At room temperature, only ~20% of **2** was decomposed in sample 1 after a reaction time of 1 h, in contrast to ~90% at 37 °C. Successive repetitions of the experiment led to similar results, that is, losses of 80−90% of **2** at 37 °C and of 10−20% at room temperature. Negligible losses of **2** were observed in samples 2 and 5−8, and only a small loss (5−10%) was observed in sample 3. In contrast, when EDTA was omitted from the reaction mixture (sample 4), ~70% of **2** was lost (i.e., comparable to that observed at 37 °C).

Replacing Fe(III) by either Mn(II) or Cu(I) in reactions at 37 °C decreased the loss of **2** from 90 to 15−25% (**Table 4**). Changing the transition metal from Fe to Mn or Cu has also been reported to decrease the level of oxidation of caffeine to 8-oxocaffeine under Fenton reaction conditions (*13*). These results suggest, therefore, that Fe(III)/Fe(II) is more effective than the other metal ions in cleaving H_2O_2, probably because of easier redox cycling between the two oxidation states.

Table 5. Mass Spectrometric Data of Volatile Degradation Products of Furfuryl Mercaptan Found in Various Fenton Reaction Systems[a]

no.	volatile compound	retention index[b]	mass spectrometric fragment ions[c] (*m/z*, relative intensity)
1	2-furfural	970	96 (M^+, 70), 96 (65), 67 (5), 42 (5), 40 (10), 39 (100), 38 (35), 37 (25), 29 (35)
2	2-furfuryl mercaptan	1000	114 (M^+, 50), 81 (100), 53 (65), 52 (10), 51 (10), 50 (10), 45 (10), 39 (5), 27 (15)
3	bifurfuryl	1260	162 (M^+, 35), 81 (90), 53 (25), 51 (5), 39 (10), 28 (100), 27 (30)
4	unknown[d]	1530	194 (M^+, 25), 113 (10), 85 (10), 81 (100), 53 (25), 45 (5), 43 (5), 27 (5)
5	difurfuryl monosulfide	1605	194 (M^+, 25), 126 (10), 113 (20), 85 (5), 81 (100), 53 (30), 51 (10), 45 (15), 27 (15)
6	unknown[d]	1825	212 (M^+, 1), 194 (30), 161 (5), 113 (35), 100 (10), 81 (100), 53 (20), 45 (10), 43 (30), 27 (10)
7	difurfuryl disulfide	1860	226 (M^+, 25), 161 (5), 85 (5), 81 (100), 53 (45), 51 (10), 45 (10), 27 (20)
8	difurfuryl trisulfide	2125	258 (M^+, 3), 193 (2), 161 (5), 113 (3), 85 (5), 81 (100), 53 (25), 51 (5), 45 (10), 27 (10)
9	unknown[d]	2380	384 (M^+, 1), 225 (10), 193 (40), 161 (10), 62 (5), 81 (100), 53 (15), 45 (5), 27 (5)

[a] Volatile compounds were isolated from the complete Fenton reaction model [sample 1, (*ii*) under Sample Preparation] except bifurfuryl (**3**), which was identified in the simplified reaction sample as described under (*iv*) of Sample Preparation. [b] Linear retention indices were calculated on OV-1701 capillary columns. [c] Mass spectrometry was performed using the electron impact (EI) and chemical ionization (CI, ammonia) technique. [d] Unknowns are sulfur-containing volatile compounds.

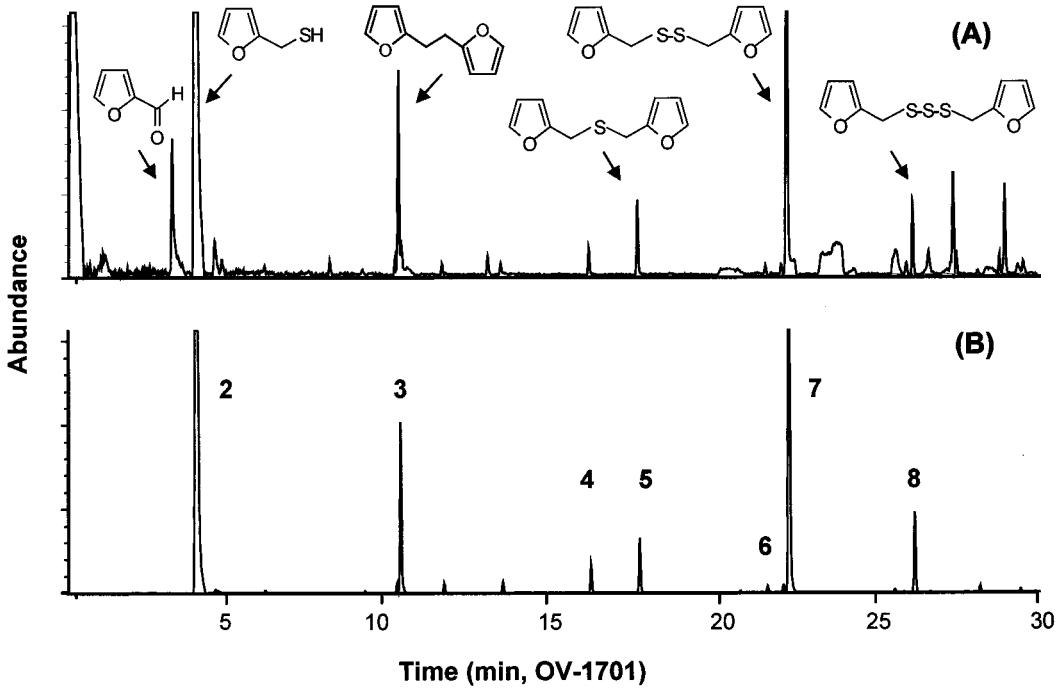

Figure 1. GC-MS identification of volatile compounds detected in a sample containing furfuryl mercaptan, hydrogen peroxide, and iron(III) ions in the molar ratio of 10:1:1: (A) total ion current; (B) extract thereof showing only the trace of *m/z* 81. Numbering corresponds to that in **Table 5**.

Table 6. Formation of Volatile Degradation Products of Furfuryl Mercaptan under Fenton Conditions[a]

model system[b]	difurfuryl monosulfide (**5**)	difurfuryl disulfide (**7**)	difurfuryl trisulfide (**8**)
1: complete Fenton system	12	54	7
2: no $FeCl_3$	+[c]	9	nd[d]
3: no H_2O_2	nd[d]	15	+[c]
4: no EDTA	+[c]	51	6
5: no ascorbic acid	nd[d]	80	8
6: no $FeCl_3$, EDTA, H_2O_2	nd[d]	3	nd[d]

[a] Concentrations are in μg/mL using benzyl mercaptan as internal standard. The variation coefficients were <10%. [b] For experimental details see **Table 1** and (*ii*) under Sample Preparation. [c] Only trace amounts were detected (<1 μg/mL). [d] Compound was not detected.

Replacing ascorbic acid with the cyclic enoloxo compound 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone in the Fenton reaction mixture led to ~50−60% loss of **2** after a 1-h incubation at 37 °C (**Table 4**), a result which is comparable to that obtained with ascorbic acid. The amounts of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone in roasted and ground coffee are relatively high (~100 mg/kg), and these are fully recovered in the coffee brews (*8*, *9*). 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone may, therefore, represent an important component for the redox cycling of the Fe during oxidative processes in liquid coffees.

Volatile Degradation Products of Furfuryl Mercaptan. As reported previously (*15*), most of the volatile degradation products detected in sample 1 contain sulfur. Compound **7** was the major degradation product of **2** followed by **5** and **8**. Three minor peaks detected by GC-FPD and GC-MS remain unknown (**4**, **6**, and **9**). Their structures are most likely related to **2**, because they all have a major fragment ion with *m/z* 81 (**Table 5**), which is characteristic of the furfuryl moiety.

The distribution of volatile degradation products of **2** is influenced by the composition of the Fenton-type model systems. Compound **5** was found preferentially in sample 1, whereas **8** was detected at similar levels in samples 1, 4, and 5 (**Table 6**). Compound **7** was the most abundant volatile degradation product of **2** in all samples, indicating that it is very readily formed. The amounts varied from 3 to 80 μg/mL, the highest amounts (50−80 μg/mL) being in samples 1, 4, and 5, for which the losses of **2** were greatest.

In a simplified model system, **2**, H_2O_2, and $FeCl_3$ in the molar ratio 10:1:1 was reacted for 2 h at room temperature to study the degradation products of **2**. In the volatile fraction of this

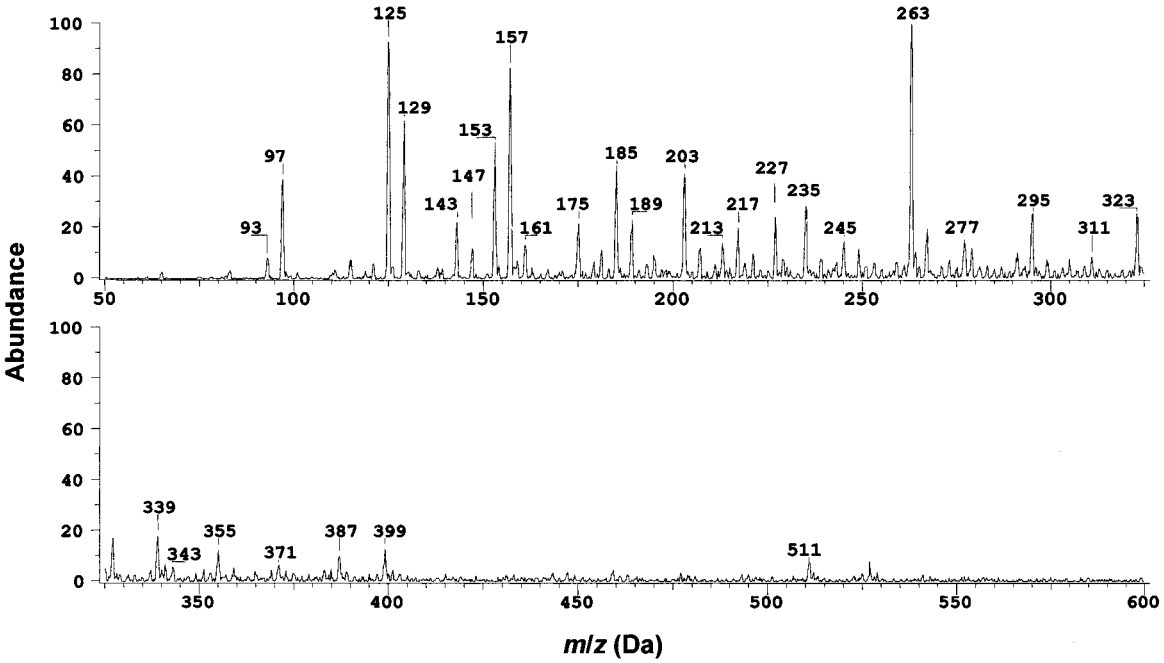

Figure 2. ESI-MS of a sample containing furfuryl mercaptan, hydrogen peroxide, and $FeCl_3$ in the molar ratio of 10:1:1.

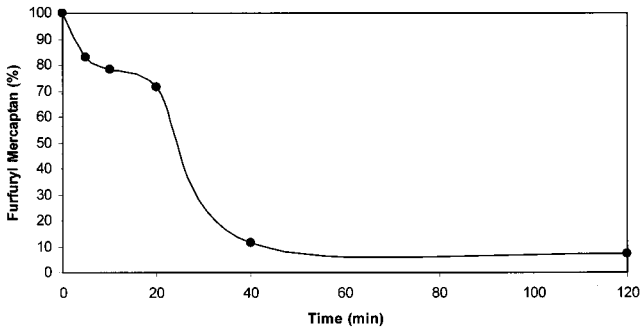

Figure 3. Degradation of furfuryl mercaptan under Fenton-type reaction conditions over a time period of 2 h.

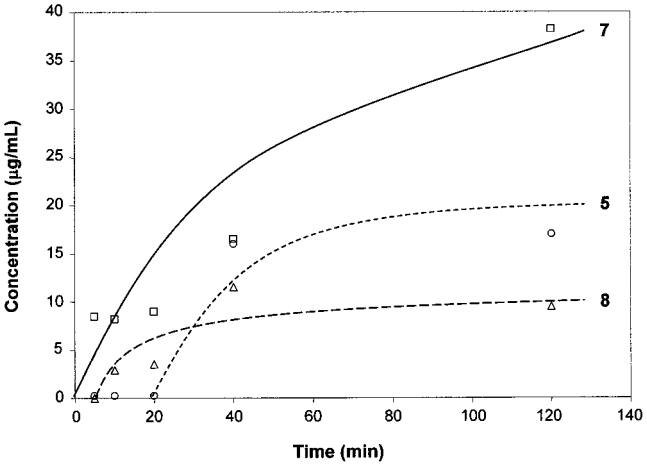

Figure 4. General tendency in the formation of difurfuryl monosulfide (○, **5**), difurfuryl disulfide (□, **7**), and difurfuryl trisulfide (Δ, **8**) over time as degradation products of furfuryl mercaptan under Fenton conditions.

sample, the fragment ion at *m*/*z* 81 (**Figure 1**) revealed bifurfuryl (**3**), which might be formed by dimerization of two furfuryl radicals, in addition to **5**, **7**, and **8**. An unknown compound (**4**) at RI = 1530, showing MS data similar to those of **5** (**Table 5**), was also detected. This experiment thus confirms that H_2O_2 and $FeCl_3$ are capable of initiating the Fenton reaction in the presence of **2** and suggests that **2** can act as a reducing agent to generate Fe(II).

Compounds **3**, **5**, and **7**, which have been identified as decomposition products of **2** in the present experiments, are known volatile components of roasted coffee (*18*−*20*). The aroma notes of **5** and **7** are described as burnt, sulfury, roasty, and rubbery, but they lack the characteristic coffee-type aroma of **2**. When their low odor thresholds are considered, for example, 0.0004 ng/L in air for **7** (*21*), it is likely that the loss of **2** and concomitant formation of various difurfuryl sulfides and other furfuryl-based moieties will lead to an imbalance in coffee aroma during storage or under conditions of oxidative stress.

Nonvolatile Degradation Products of Furfuryl Mercaptan. Quantitation of the total concentrations of volatiles detected by GC techniques showed that ∼40−50% of furfuryl mercaptan equivalents were missing from samples 1, 4, and 5 at 37 °C, for which losses of **2** were particularly high. Consequently, an appreciable fraction of the products of **2** degradation in the presence of Fenton reagents is thought to occur as nonvolatile substances (*15*). These nonvolatile compounds might be ionic low molecular weight compounds or polymeric materials that cannot be analyzed by GC-hyphenated techniques. Therefore, direct inlet MS was applied using electrospray ionization in an attempt to characterize the residue of the simplified Fenton reaction sample obtained by vacuum distillation. As shown in **Figure 2**, a large number of compounds with mass range from M = 92 to 510 Da were found, with the majority being in the range of M = 124−262 Da. Unfortunately, these data do not allow conclusions to be drawn about the chemical nature and structure of the compounds, but they do indicate the chemical complexity of the nonvolatile fraction obtained from a simple Fenton reaction system. Some ions also suggest the presence of volatile compounds, such as furfural (*m*/*z* 97), **2** (*m*/*z* 115), **5** (*m*/*z* 195), unknown **6** (*m*/*z* 213), and **7** (*m*/*z* 227), as minor constituents.

Changes in Volatile Compounds over Time. To get an insight into the dynamics of degradation of **2**, a series of measurements on sample 1 at 37 °C were performed to determine the concentration of the principal volatile compounds, that is, **2**, **5**, **7**, and **8**, over a time period of 2 h. The initial

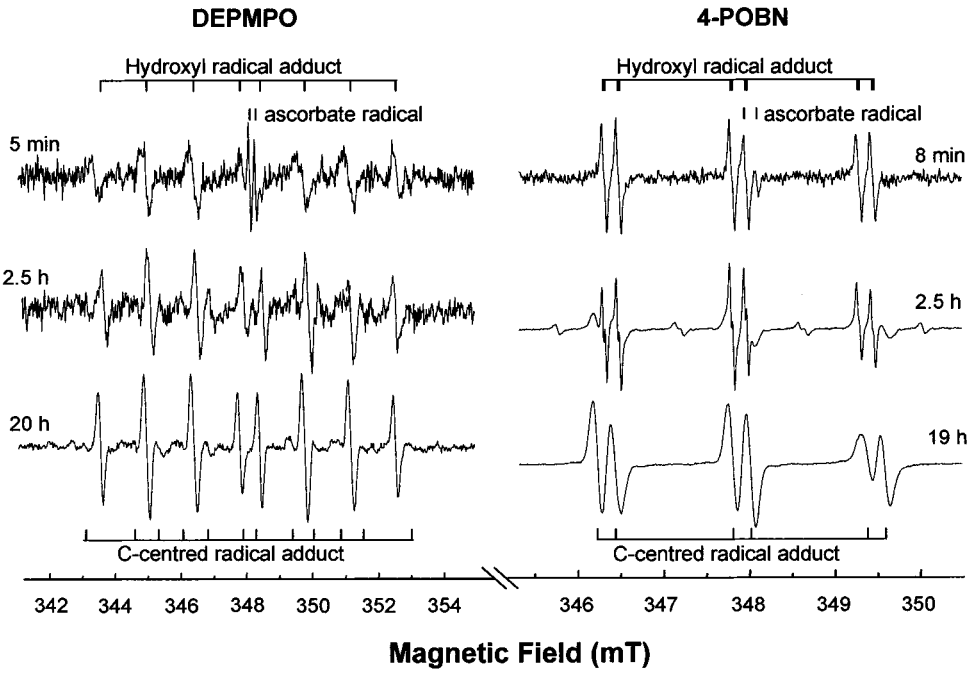

Figure 5. Representative EPR spectra obtained during incubation of model solution 1 with the spin trap DEPMPO or 4-POBN at room temperature (~20 °C).

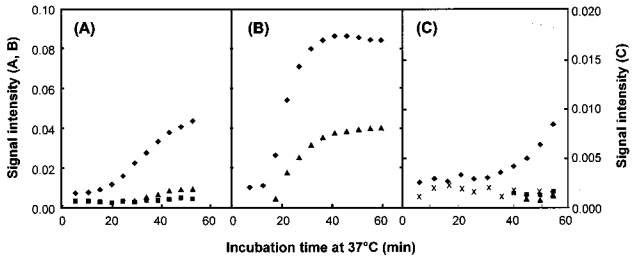

Figure 6. Variation of the intensities of the EPR signals from •OH (◆) and C-centered radical (■) adducts along with those of the unidentified adduct with high- and low-field peaks separated by 11.6 mT (▲) and 10.4 mT (×) as a function of incubation time with DEPMPO at 37 °C of (A) sample 1, (B) sample 1 minus furfuryl mercaptan, and (C) sample 4, which contained no EDTA.

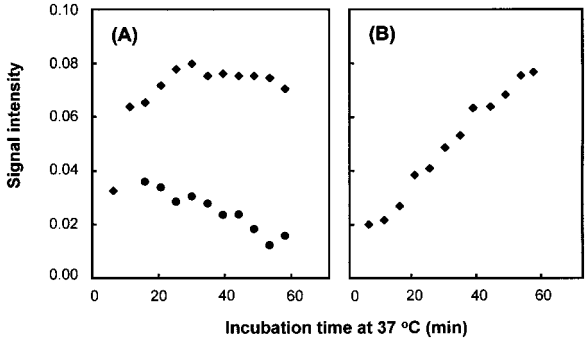

Figure 7. Variation of the intensities of the EPR signals from the •OH adduct (◆), along with that of the unidentified adduct with high- and low-field peaks separated by 12.2 mT (●), as a function of incubation time with DEPMPO at 37 °C of (A) sample 5, which contained no ascorbic acid, and (B) sample 5, but without furfuryl mercaptan.

phase of furfuryl mercaptan decomposition was fast but reached a plateau after ~10−15 min of incubation when ~25% of **2** was lost (**Figure 3**). Rapid decomposition set in again after ~20 min, and only ~10% of **2** remained after a reaction period of 40 min.

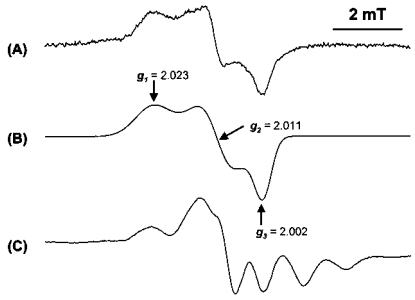

Figure 8. (A) EPR spectrum at 77 K from a rapidly frozen solution of furfuryl mercaptan in a simple model Fenton reaction system; (B) a simulation of spectrum (A) with *g* values of 2.002, 2.011, and 2.023 and a Gaussian line shape; (C) spectrum obtained at 77 K from furfuryl mercaptan that had received 100 Gy of γ-radiation from a ^{137}Cs source.

The concomitant formation of **5**, **7**, and **8** over time is shown in **Figure 4**. Compound **7** was detected as early as the 5 min sample. It was the major product generated, the concentration of which increased throughout the reaction period. In the 10-min sample, **8** was found as a second degradation product. Its concentration, however, increased less rapidly than that of **7**, and it reached a plateau after ~40 min. Compound **5** was not detected in the first 20 min, but its concentration increased rapidly over the next 20 min. These data suggest a complex series of reactions leading to the formation of these difurfuryl derivatives and are consistent with the types of events expected for free radical mediated processes.

The data presented in this work clearly demonstrate the crucial role of H_2O_2 and iron in the degradation of **2**. Reductive cleavage of the O−O bond in H_2O_2 is catalyzed by Fe(II) with the generation of •OH, which is a powerful oxidant. •OH readily reacts with organic compounds, including thiols, and initiates formation of volatile and nonvolatile degradation products with **2**. In addition to •OH, the formation of metal-based oxidizing species, such as Fe(II)OOH and iron(IV)−oxo complexes (*13*, *22*), has also been reported to occur during the Fenton reaction.

EPR Spectroscopy of Spin Trap Adducts. The products determined by GC-MS analysis of sample 1 suggest that both

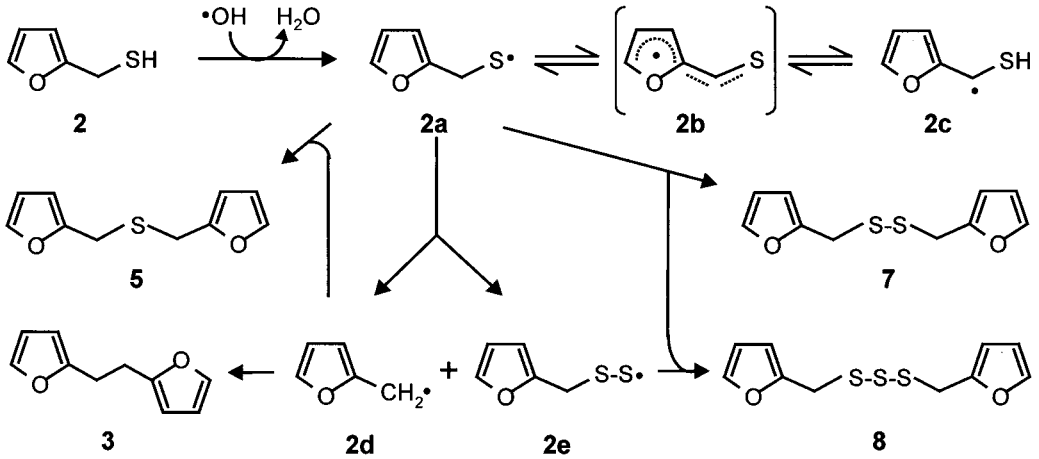

Figure 9. Hypothetical mechanism showing the degradation of furfuryl mercaptan (**2**) under Fenton conditions and the formation of volatile reaction products identified in this study, that is, bifurfuryl (**3**), difurfuryl monosulfide (**5**), difurfuryl disulfide (**7**), and difurfuryl trisulfide (**8**).

C- and S-centered radicals are involved as intermediates in the degradation of **2**. Consequently, experiments using EPR spectroscopy have been performed in an attempt to provide information on free radical species generated in the reaction mixtures.

When solutions equivalent to sample 1 were incubated with DEPMPO at room temperature, the spectra obtained over a 20-h period (**Figure 5**) were all dominated by the •OH adduct with hyperfine splittings $a(^{31}P) = 4.72$ mT, $a(^{14}N) = 1.38$ mT, and $a(^{1}H) = 1.40$ mT (*23*). The ascorbate radical ($a_H = 0.18$ mT) (*24*) was seen in the early stages of incubation but decreased in intensity with time and was not detectable after ~1 h. This radical may, however, contribute to various redox processes in the reaction mixtures, and the role of ascorbic acid may not be limited to reduction of Fe(III).

There were small amounts of a component from C-centered radical adducts [$a(^{31}P) = 4.91$ mT, $a(^{14}N) = 1.47$ mT, and $a(^{1}H) = 2.13$ mT] (*23*) in spectra from ~6 h, but it was never more than a minor fraction of the total signal. A further unidentified signal, with outermost peaks separated by 11.6 mT, is also present in these spectra. It is related to the Fenton reaction, as it is also seen when DEPMPO is added to a simple Fenton system (unpublished results), and its spectrum can be analyzed in terms of the approximate hyperfine splittings, $a(^{31}P) = 4.55$ mT, $a(^{14}N) = 1.38$ mT, and $a(^{1}H) = 1.64$ mT, along with either an additional doublet splitting with $a = 2.67$ mT or a triplet with $a = 1.34$ mT. If the latter assignment is correct, this could represent an adduct of an oxidized spin trap molecule, analogous to that seen with 4-POBN (*25*).

Results obtained at room temperature with 4-POBN as spin trap (**Figure 5**) were qualitatively similar to those described above for DEPMPO. In the early stages of incubation, the OH radical adduct [$a(^{14}N) = 1.50$ mT and $a(^{1}H) = 0.16$ mT] (*26*) was the dominant species, and small amounts of the ascorbate radical were seen, although it is partly obscured by the spectrum of the adduct. The signal from C-centered radical adducts [$a(^{14}N) = 1.56$ mT and $a(^{1}H) = 0.26$ mT] (*26*), however, grew progressively with time and dominated the spectra after ~15 h. This result indicates that appreciable quantities of C-centered radical adducts were generated during longer incubation periods. Small amounts of a signal with $a(^{14}N) = 1.42$ mT and $a(^{1}H) = 1.46$ mT were observed from ~1 h. This reached a maximum around 3−4 h and then subsequently decreased in intensity. The parameters are similar to those of the *tert*-butyl hydronitroxide radical (*27*), which is thought to be a breakdown product of 4-POBN radical adducts.

When **2** was excluded from the complete Fenton reaction system, the •OH adduct signal increased more rapidly than with the original solution. After 1 h of incubation in the presence of DEPMPO, its intensity was approximately twice that which was observed when **2** was present (**Figure 6A**,**B**). This approximately 2:1 ratio for the •OH adduct signal intensity for solutions without and with **2** is in line with the 92:59 ratio that would be predicted on the basis of the combined molarities of **2**, DEPMPO, EDTA, and ascorbic acid, if •OH showed no reaction selectivity among them. The result does indicate, however, that the buffer is not reactive in these solutions. The intensity of the unknown radical adduct also increased more rapidly and its contribution to the overall EPR signal was appreciably higher in the absence of **2**, thus adding further support to it being formed as a result of a reaction between DEPMPO and Fenton reaction products.

Compared to the results described above, removal of EDTA from the reaction mixture resulted in a major reduction in the rate of generation of the •OH adduct signal in the presence of DEPMPO (**Figure 6C**). Only very weak signals were observed from the C-centered radical adduct and the unidentified adduct with outer peak separations of 11.6 mT. This result is consistent with a reduction in the rate of generation of •OH. It illustrates the important role played by EDTA in maintaining a sufficient concentration of Fe(III) for effective redox cycling and competing with hydrolysis reactions, which lead to precipitation of Fe(III) oxyhydroxide species (*28*). In addition to the components described above, the EPR spectra contained a further weak signal with outer peak separations of 10.4 mT. This adduct has not yet been identified, and its intensity showed little variation.

The solution from which ascorbic acid was absent showed an initial rapid increase in •OH adduct signal, but this reached a maximum after a few minutes (**Figure 7A**). This provides further evidence to support the suggestion that **2** can reduce Fe(III) to Fe(II) for the Fenton reaction. This hypothesis is supported by the observation that the rate of production of the EPR signal was much slower in a similar solution without **2** (**Figure 7B**), although it did eventually achieve a similar intensity. However, the relatively short duration of the •OH burst suggests that **2** oxidation products are not readily recycled back to **2**. An additional spectrum with outer peak separations of 12.2 mT from another unidentified adduct was observed with the solution containing **2**. The intensity of this signal decreased progressively.

In addition to reactions initiated by •OH from the Fenton reaction system, it is possible that radicals could be generated

from reactions between other components in the reaction mixtures. For example, the reaction between **2** and Fe(III) could produce Fe(II) and furfuryl mercaptan-derived radicals. These radicals and the ascorbate radical, which is also formed as a result of reaction with Fe(III), may then participate in further reactions and could be the sources of one or more of the unidentified radical adducts described above. Such reactions could be more important in coffee brews than in the model system because of the greater abundance of potentially reactive species.

Identification of the Presence of S-Containing Radicals. There was no direct evidence for any S-centered radical species in the spin-trapping experiments, despite the GC-MS results suggesting that such radicals are intermediates in the formation of some of the reaction products identified. However, when higher concentrations of reagents were used and the reaction mixture was frozen in liquid nitrogen to stabilize any radical(s) that might be generated, an anisotropic radical signal was observed at 77 K (**Figure 8A**) with g values of 2.002, 2.011, and 2.023 (**Figure 8B**). The radical had moderate stability, with a half-life of a few minutes, and is probably an intermediate in the decomposition of **2**. It does not appear to be reactive with either of the spin traps used in the present investigation.

There are several studies in the literature of S-containing radicals with parameters similar to those observed here, but there is no universal agreement on their identities. γ-Irradiation of thioglycolic acid, methionine, or acetylmethionine yields a radical with g values virtually identical to those from furfuryl thiol in the present work. On the basis of ENDOR studies on single crystals of thioglycolic acid, it has been argued that this signal corresponds to radicals of the type $(R_2S)^{\bullet+}$ (*29*). However, it has also been assigned to dimeric cationic radicals of the type $(R_2S-SR_2)^{\bullet+}$ (*30*, *31*). The g values are also close to those of sulfinyl radicals $(R_2SO)^{\bullet+}$ (*32*, *33*) and the radical $(RSSH)^{\bullet-}$ (*34*). A number of possible radicals can, however, be eliminated on the basis of EPR data, including general species such as $(RS)^{\bullet}$, $(RS)^{\bullet+}$, $(RSS)^{\bullet}$, and $RSSR^{\bullet-}$.

When frozen **2** was exposed to γ-irradiation at 77 K, a complex spectrum was obtained (**Figure 8C**). One of the components bears a close resemblance to that in **Figure 8A**, suggesting that they may correspond to the same radical. The fact that a completely different spectrum (not shown) was observed when a 10% solution of **2** was irradiated provides further evidence that a dimeric species derived from **2** has parameters similar to those in **Figure 8**. However, mercaptans readily react with peroxyl radicals to generate sulfinyl radicals (*33*), which might also be generated in our model system. Indeed, sulfinyl radicals have been observed as a result of reaction of *tert*-butyl mercaptan in the $Ti(III)/H_2O_2$ Fenton reaction system (*35*). Unfortunately, the EPR spectra do not discriminate between the sulfinyl and dimeric cationic radicals, so it is not possible to identify their relative levels in the present system.

Formation Mechanism. On the basis of the results obtained in the present study, a hypothetical mechanism is proposed for the degradation of **2** under Fenton-type reaction conditions explaining the formation of volatile degradation products (**Figure 9**). The initial phase involves attack by OH radicals and the abstraction of an H atom. This leads to the generation of new free radical species, which have not yet been fully characterized. Evidence has been found for the formation of C-centered radicals derived from **2** using EPR spectroscopy and spin-trapping techniques, but the presence of S-centered radicals was neither confirmed nor excluded by these measurements. Ab initio molecular calculations, however, indicate similar stability levels for the mononuclear S- and C-centered radicals derived from **2** (W. Andreoni, IBM Rüschlikon, personal communication), **2a** and **2c**, respectively, and it is conceiveable that the spin traps used in the present work show a preference for the C-centered form.

The formation of **7** after the addition of **2** to the Fenton reaction system was very rapid, and this is the major furfuryl-based moiety identified in the volatile fraction of the reaction mixture. Production of an S-centered free radical was also rapid. The observation that dimerization of thiyl radicals (**2a**) is an early event in the decomposition process of **2** (**Figure 4**) adds support to the tentative identification of this radical as a dimeric species, although it is also likely that sulfinyl radicals will be present in this system. Disproportionation of **2a** to the radicals **2d** and **2e** and their subsequent reactions might represent a route to the other volatiles, the formation of which is delayed relative to the formation of **7**. Other free radicals are also formed in the reaction medium (e.g., from ascorbate), and these may interfere with the main reaction scheme. Such radical formation may explain the observation that the highest level of **7** was found in sample 5, in which ascorbic acid was absent (**Table 6**).

Furfurylsulfenic acid (MW = 130 Da) was not detected in the mass spectrometric measurements, although the identification of **7** and **8** as reaction products indicates that its formation is probable. As shown in **Figure 2**, there is some evidence for the higher oxidation product furfurylsulfinic acid (MW = 146 Da), but not for furfurylsulfonic acid (MW = 162 Da). In similar reaction systems, that is, ascorbate- and transition-metal-mediated oxidation of methanethiol, methanesulfenic acid (CH_3-SOH) has been proposed as an intermediate in the formation of dimethyl disulfide and dimethyl trisulfide (*36*). However, its existence could not be substantiated, possibly because of the high reactivity of CH_3SOH (*37*). It is known that sulfenic acids easily convert to thiosulfinate esters due to their dual electrophilic/nucleophilic character (*38*).

In conclusion, the processes by which furfuryl mercaptan is degraded in Fenton-type reaction systems are complex. Evidence has been found to support reaction pathways involving both C- and S-centered free radical intermediates. These lead to the formation of a large number of volatile and nonvolatile products, among which are the odor-active compounds difurfuryl monosulfide, difurfuryl disulfide, and difurfuryl trisulfide. However, the differences in the nature of the aroma notes ascribed to **2** and those of the volatile compounds described above indicate that $^{\bullet}$OH-initiated degradation of **2** is likely to lead to a distortion of the aroma in oxidized coffee brews. The existence of Fenton chemistry may, therefore, represent a significant factor that affects the aroma composition of coffee beverages. The practical significance of other oxidation products has not been considered in this work.

ACKNOWLEDGMENT

We are grateful to S. Metairon for expert technical assistance.

LITERATURE CITED

(1) Nijssen, L. M.; Visscher, C. A.; Maarse, H.; Willemsens, L. C.; Boelens, M. H. *Volatile Compounds in Foods. Qualitative and Quantitative Data*, 7th ed.; TNO Nutrition and Food Research Institute: Zeist, The Netherlands, 1996; pp 72.1−72.23.

(2) Reichstein, T.; Saudinger, H. A new or improved method of producing artificial coffee oil. Patent Appl. UK 260,960, Feb 22, 1928.

(3) Tressl, R. Formation of flavor components in roasted coffee. In *Thermal Generation of Aromas*; Parliment, T. H., McGorrin, R. J., Ho, C. T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp 285−301.

(4) Holscher, W.; Vitzthum, O. G.; Steinhart, H. Identification and sensorial evaluation of aroma-impact compounds in roasted Colombian coffee. *Cafe Cacao The* **1990**, *34*, 205−212.
(5) Blank, I.; Sen., A.; Grosch, W. Aroma impact compounds of Arabica and Robusta coffee. Qualitative and quantitative investigations. In *ASIC, Proceedings of the 14th International Scientific Colloquium on Coffee*, San Francisco, CA, July 14−17, 1991; ASIC: Paris, France, 1991; pp 117−129.
(6) Blank, I.; Sen, A.; Grosch, W. Potent odorants of roasted powder and brew of Arabica coffee. *Z. Lebensm. Unters. Forsch.* **1992**, *195*, 239−245.
(7) Schieberle, P.; Hofmann, T. Investigation of the influence of manufacturing parameters on the aroma performance of aromatic compounds in cysteine/carbohydrate reaction mixtures. *Lebensmittelchemie* **1996**, *50*, 105−108 (in German); *Chem. Abstr.* **1996**, *125*, 326725v.
(8) Semmelroch, P.; Laskawy, G.; Blank, I.; Grosch, W. Determination of potent odorants in roasted coffee by stable isotope dilution assay. *Flavour Fragrance J.* **1995**, *10*, 1−7.
(9) Semmelroch, P.; Grosch, W. Studies on character impact odorants of coffee brews. *J. Agric. Food Chem.* **1996**, *44*, 537−543.
(10) Nagao, M.; Fujita, Y.; Wakabayashi, H.; Nukaya, T.; Kosuge, T.; Sugimura, T. Mutagens in coffee and other beverages. *Environ. Health Perspect.* **1986,** *67*, 89−91.
(11) Stadler, R. H.; Turesky, R. J.; Müller, O.; Markovic, J.; Leong-Morgenthaler, P.-M. The inhibitory effects of coffee on radical-mediated oxidation and mutagenicity. *Mutat. Res.* **1994**, *308*, 177−190.
(12) Korycka-Dahl, N. B.; Richardson, T. Activated oxygen species and oxidation of food constituents. *CRC Crit. Rev. Food Sci. Nutr.* **1978**, *10*, 209−241.
(13) Stadler, R. H.; Fay, L. B. Antioxidative reactions of caffeine: Formation of 8-oxocaffeine (1,3,7-trimethyluric acid) in coffee subjected to oxidative stress. *J. Agric. Food Chem.* **1995**, *43*, 1332−1338.
(14) Grosch, W. Flavour of coffee. A review. *Food* **1998**, *42*, 344−350.
(15) Blank, I.; Pascual, E. C.; Fay, L. B.; Stadler, R. H.; Goodman, B. A.; Yeretzian, C. Degradation of furfuryl mercaptan in Fenton-type model systems. In *Caffeinated Beverages. Health Benefits, Physiological Effects, and Chemistry*; Parliment, T. H., Ho, C.-T., Schieberle, P., Eds.; ACS Symposium Series 754; American Chemical Society: Washington, DC, 2000; pp 230−240.
(16) Pascual, E. C.; Blank, I.; Goodman, B. A.; Yeretzian, C. The detection and characterization of free radicals generated during the decomposition of solutions of the coffee flavour compound furfuryl mercaptan. In *ASIC, Proceedings of the 18th International Scientific Colloquium on Coffee*, Helsinki, Aug 2−6, 1999; ASIC: Paris, France, 1999; pp 50−57.
(17) Sen, A.; Laskawy, G.; Schieberle, P.; Grosch, W. Quantitative determination of β-damascenone in foods using a stable isotope dilution assay. *J. Agric. Food Chem.* **1991**, *39*, 757−759.
(18) Gautschi, F.; Winter, M.; Flament, Y.; Willhalm, B.; Stoll, M. New developments in coffee aroma research. *J. Agric. Food Chem.* **1967**, *15*, 15−23.
(19) Stoll, M.; Winter, M.; Gautschi, F.; Flament, I.; Willhalm, B. Research on aromas. XIII. Coffee aroma. *Helv. Chim. Acta* **1967**, *50*, 628−694 (in French); *Chem. Abstr.* **1967**, *66*, 94855v.
(20) Tressl, R.; Silwar, R. Investigation of sulfur-containing components in roasted coffee. *J. Agric. Food Chem.* **1981**, *29*, 1078−1082.
(21) Gasser, U.; Grosch, W. Aroma extract dilution analysis of commercial meat flavorings. *Z. Lebensm. Unters. Forsch.* **1990**, *190*, 511 (in German); *Chem. Abstr.* **1990**, *113*, 229885s.
(22) Wink, D. A.; Nims, R. W.; Saavedra, J. E.; Utermahlen, W. E.; Ford, P. C. The Fenton oxidation mechanism: Reactivities of biologically relevant substrates with two oxidizing intermediates differ from those predicted for the hydroxyl radical. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6604−6608.
(23) Fréjaville, C.; Karoui, H.; Tuccio, B.; Le Moigne, F.; Culcasi, M.; Pietri, S.; Lauricella, R.; Tordo, P. 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline *N*-oxide: A new efficient phosphorylated nitrone for the in vitro and in vivo spin trapping of oxygen-centered radicals. *J. Med. Chem.* **1995**, *38*, 258−265.
(24) Liu, Y.; Liu, Z.; Chen, P.; Wu, L. Generation of radical cations—A facile generation of radical cations via the action of an oxoammonium trifluoroacetate. *Sci. Sinica (B)* **1988**, *31*, 1062−1072.
(25) McCormick, M. L.; Buettner, G. R.; Britigan, B. E. The spin trap α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone stimulates peroxidase-mediated oxidation of deferoxamine. *J. Biol. Chem.* **1995**, *270*, 29265−29269.
(26) Buettner, G. R. Spin trapping: ESR parameters of spin adducts. *Free Radical Biol. Med.* **1987**, *3*, 259−303.
(27) Hensley, K.; Aksenova, M.; Carney, J. M.; Harris, M.; Butterfield, D. A. Amyloid β-peptide spin trapping II: Evidence for decomposition of the PBN spin adduct. *NeuroReport* **1995**, *6*, 493−496.
(28) Stucki, J. W., Goodman, B. A., Schwertmann, U., Eds. *Iron in Soils and Clay Minerals*; D. Reidel Publishing: Dordrecht, The Netherlands, 1988.
(29) Box, H. C.; Budzinski, E. E. Electron spin resonance detection of the radicals RS•, RO• and $R_1-S-R_2^{\bullet+}$ in irradiated solids. *J. Chem. Soc., Perkin Trans. 2* **1976**, 553−555.
(30) Gilbert, B. C.; Hodgeman, D. K. C.; Norman, R. O. C. Electron spin resonance studies. Part XXXVIII. Evidence for the formation of dimeric radical-cations, $R_2S-SR_2^{+\bullet}$, in the one-electron oxidation of sulphides. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1748−1752.
(31) Petersen, R. L.; Nelson, D. J.; Symons, M. C. R. Unstable Intermediates. Part 179. Electron spin resonance studies of radicals formed in irradiated organic sulphides and disulphides. *J. Chem. Soc., Perkin Trans. 2* **1978**, 225−231.
(32) Symons, M. C. R.; Janes, R. Radical cations of trialkylphosphine oxides, trialkylphosphates, hexamethylphosphoramide, dimethyl sulphoxide and various sulphones, sulphites and sulphates. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 383−399.
(33) Swarts, S. G.; Becker, D.; DeBolt, S.; Sevilla, M. D. Electron spin resonance investigation of the structure and formation of sulfinyl radicals; reaction of peroxyl radicals with thiols. *J. Phys. Chem.* **1989**, *93*, 155−161.
(34) Franzi, R.; Geoffroy, M.; Reddy, M. V. V. S.; Weber, J. Theoretical and single-crystal ESR study of a (RSSH)•− species. *J. Phys. Chem.* **1987**, *91*, 3187−3190.
(35) Gilbert, B. C.; Laue, H. A. H.; Norman, R. O. C.; Sealy, R. C. Electron spin resonance studies. Part XLVI. Oxidation of thiols and disulphides in aqueous solution: formation of RS•, RSO•, $RSO_2^{\bullet}$, $RSSR^{-\bullet}$, and carbon radicals. *J. Chem. Soc., Perkin Trans. 2* **1974**, 892−900.
(36) Chin, H.-W.; Lindsay, R. C. Ascorbate and transition-metal mediation of methanethiol oxidation to dimethyl disulfide and dimethyl trisulfide. *Food Chem.* **1994**, *49*, 387−392.
(37) Penn, R. E.; Block, E.; Revelle, L. K. Methanesulfenic acid. *J. Am. Chem. Soc.* **1978**, *100*, 3622−3623.
(38) Block, E.; O'Connor, J. The chemistry of alkyl thiosulfinate esters. VII. Mechanistic studies and synthetic applications. *J. Am. Chem. Soc.* **1974**, *96*, 3929−3944.

Received for review October 5, 2001. Revised manuscript received January 11, 2002. Accepted January 14, 2002. We are grateful to the Scottish Executive Rural Affairs Department for funding for B.A.G. and the EPR facilities.

JF011329M