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Model Studies on the Oxidative Stability of Odor-Active Thiols Occurring in Food Flavors

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Dilute ethereal solutions of mercaptoacetone (MA), 2-mercapto-3-butanone (MB), 3-mercapto-2-pentanone (MP), 2-methyl-3-furanthiol (MFT), and 2-furfurylthiol (FFT), either alone or in pairs, were stored at 6 °C. During a period of 10 days the oxidation of the thiols to the corresponding disulfides or mixed disulfides was followed by high-resolution gas chromatography/mass spectrometry. In diethyl ether MFT showed the highest oxidation rate, which decreased in the order MFT ≫ MA > MB = MP = FFT. After 1 day, mixed disulfides (e.g. MFT–FFT, FFT–MB) were detectable when pairs of the five thiols dissolved in diethyl ether were stored at 6 °C. MFT oxidized more slowly in dichloromethane than in diethyl ether, and it was stable in pentane. However, a heat treatment increased the oxidation rate of MFT dissolved in dichloromethane or pentane.

Keywords: *2-Methyl-3-furanthiol; 2-furfurylthiol; disulfide formation*

INTRODUCTION

The thiols shown in Figure 1 are formed by the Maillard reaction of cysteine and might contribute to the flavors of heated foods and beverages, due to their low odor thresholds (Whitfield et al., 1992). Actually, 2-methyl-3-furanthiol (MFT) and 2-furfurylthiol (FFT) have been detected as character impact odorants of roasted coffee (Tressl, 1981, 1989, 1990; Tressl et al., 1983; Holscher et al., 1990; Blank et al., 1992), cooked meat (Gasser and Grosch, 1988, 1990a; Farmer and Patterson, 1991; Grosch and Zeiler-Hilgart, 1992), and wheat bread (Baltes and Song, 1994).

It is well-known that thiols oxidize easily to disulfides. For this reason it is not surprising that the 15 disulfides or mixed disulfides (Table 1) resulting from the oxidation of the 5 thiols listed in Figure 1 have been identified in commercial meat flavorings (Gasser and Grosch, 1990b; Ruther and Baltes, 1994a,b) as well as in model Maillard reaction systems (Tressl et al., 1983, 1990; Hartmann et al., 1984; Reineccius and Liardon, 1985; Silwar and Tressl, 1989; Farmer and Mottram, 1990; Güntert et al., 1990, 1992a,b; Werkhoff et al., 1990; Zhang and Wo, 1991; Farmer and Patterson, 1991; Whitfield et al., 1992; Mottram and Whitfield, 1994; Hofmann and Schieberle, 1995). FFT–FFT and MFT–MFT (nomenclature in Table 1) were found in cooked meat (Gasser and Grosch, 1988, 1990a; Farmer and Patterson, 1991; Grosch and Zeiler-Hilgart, 1992), and FFT–FFT was found in roasted coffee (Tressl, 1981) as well as in wheat bread (Baltes and Song, 1994). The contribution of MFT–MFT to the flavor of roasted coffee (Blank et al., 1992) and black tea (Guth and Grosch, 1993) has been suggested on the basis of aroma extract dilution analysis.

Furthermore, it has been revealed recently (Eiserich and Shibamoto, 1994) that the rapid oxidation of MFT to its disulfide and to other products contributes to the antioxidative activity of Maillard reaction products.

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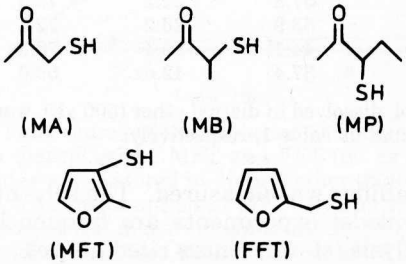

Figure 1. Chemical structures of the five thiols used in the model experiments: mercaptoacetone (MA), 2-mercapto-3-butanone (MB), 3-mercapto-2-pentanone (MP), 2-methyl-3-furanthiol (MFT), and 2-furfurylthiol (FFT).

Table 1. Nomenclature of the Disulfides and Mixed Disulfides

disulfides	
MA–MA	bis(2-oxopropyl) disulfide
MB–MB	bis(3-oxo-2-butyl) disulfide
MP–MP	bis(2-oxo-3-pentyl) disulfide
MFT–MFT	bis(2-methyl-3-furyl) disulfide
FFT–FFT	bis(2-furfuryl) disulfide
mixed disulfides	
MA–MB	2-oxopropyl 3-oxo-2-butyl disulfide
MA–MP	2-oxopropyl 2-oxo-3-pentyl disulfide
MP–MB	2-oxo-3-pentyl 3-oxo-2-butyl disulfide
MFT–MA	2-methyl-3-furyl 2-oxo-propyl disulfide
MFT–MB	2-methyl-3-furyl 3-oxo-2-butyl disulfide
MFT–MP	2-methyl-3-furyl 2-oxo-3-pentyl disulfide
FFT–MA	2-furfuryl 2-oxo-propyl disulfide
FFT–MB	2-furfuryl 3-oxo-2-butyl disulfide
FFT–MP	2-furfuryl 2-oxo-3-pentyl disulfide
FFT–MFT	2-furfuryl 2-methyl-3-furyl disulfide

In the studies mentioned above, the volatiles have been extracted with a solvent either in the course of simultaneous distillation and extraction or from an adsorbent in the course of dynamic headspace analysis. On the basis of the sensitivity of the thiols toward oxidation, the question arises whether the disulfides identified might be partially formed during storage and/or preparation of concentrated extracts for GC/MS analysis.

To check this source of artifact formation, the thiols shown in Figure 1 were dissolved in diethyl ether; MFT was also dissolved in dichloromethane and *n*-pentane, and their oxidation to the corresponding disulfides or

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Table 2. Mass Spectral Data (MS-EI) and Retention Indices (RI) of Disulfides and Mixed Disulfides

compound[a]	retention indices FFAP	retention indices SE-54	m/z (relative abundance in %) mol ion	predominant fragment ions
MA–MA	2347	1405	178 (10)	43 (100), 57 (9), 135 (6), 121 (4), 90 (3)
MB–MB	2204	1460	206 (25)	43 (100), 61 (28), 59 (24), 72 (20), 71 (19), 103 (19), 104 (18), 163 (16), 119 (14)
MP–MP	2247	1594	234 (25)	43 (100), 85 (37), 41 (30), 57 (24), 73 (24), 55 (21), 71 (18), 105 (13)
MFT–MFT	2100	1526	226 (100)	113 (95), 114 (40), 51 (25), 85 (18), 45 (18), 61 (16), 72 (16)
FFT–FFT	2465	1673	226 (20)	81 (100), 53 (80), 45 (20), 82 (20), 51 (15), 85 (12)
MA–MB	2280	1436	192 (11)	43 (100), 71 (9), 192 (9), 149 (8), 57 (8), 121 (2)
MA–MP	2308	1506	206 (20)	43 (100), 73 (26), 72 (16), 57 (14), 74 (12), 163 (11), 55 (11), 133 (10), 105 (10)
MP–MB	2227	1532	220 (64)	43 (100), 73 (56), 71 (42), 41 (41), 59 (35), 177 (32), 85 (32), 75 (30), 45 (24)
MFT–MA	2200	1462	202 (29)	43 (100), 113 (65), 114 (31), 44 (22), 51 (10), 81 (8), 144 (5)
MFT–MB	2127	1491	216 (50)	43 (100), 113 (74), 114 (52), 59 (52), 85 (24), 173 (20), 51 (20), 86 (18)
MFT–MP	2145	1561	230 (61)	43 (100), 113 (90), 114 (50), 45 (32), 145 (29), 73 (24), 85 (21), 187 (19)
FFT–MA	2400	1540	202 (18)	53 (100), 81 (92), 43 (79), 45 (37), 82 (33), 51 (19), 85 (16)
FFT–MB	2352	1577	216 (5)	81 (100), 43 (61), 53 (48), 45 (19), 82 (15), 59 (12), 85 (9)
FFT–MP	2385	1649	230 (4)	81 (100), 43 (22), 53 (19), 82 (8), 45 (8)
MFT–FFT	2323	1624	226 (19)	81 (100), 113 (18), 53 (14), 45 (11), 114 (10), 51 (9), 43 (6)

[a] For abbreviations see Table 1.

Table 3. Oxidation of Thiols to the Corresponding Disulfides[a]

storage period (days at 6 °C)	amount in μg MFT	MFT–MFT	MA	MA–MA	MB	MB–MB	MP	MP–MP	FFT	FFT–FFT
0	80.1	0	79.3	0	80.0	0	80.2	0	79.9	0
1	63.4	16.7	73.9	5.4	79.1	1.7	78.6	1.6	78.2	1.7
2	57.8	22.2	73.4	5.9	78.4	2.4	77.9	2.3	77.7	2.2
3	53.9	26.2	72.5	6.7	77.7	3.0	77.5	2.7	77.3	2.6
6	44.1	35.1	70.3	8.9	76.3	4.4	76.4	3.6	76.3	3.6
10	37.4	42.6	69.0	10.2	75.0	5.8	75.7	4.5	75.5	4.2

[a] The thiol, dissolved in diethyl ether (500 μL), was stored at 6 °C. The thiols and disulfides are abbreviated as reported in the legend of Figure 1 and in Table 1, respectively.

mixed disulfides was measured. The solvents selected for these model experiments are frequently used in flavor analysis (cf. references cited above).

EXPERIMENTAL PROCEDURES

Chemicals. The following compounds were obtained commercially: 2-acetylpyrrole, 2-furfurylthiol, and 2-methyl-3-furanthiol (Aldrich, Steinheim, Germany).

Synthesis. The following thiols were synthesized as reported in the literature given: 1-mercapto-2-propanone and 3-mercapto-2-butanone (Hofmann and Schieberle, 1995); 3-mercapto-2-pentanone (Asinger et al., 1964).

Immediately before the start of a model experiment, each thiol was freed from disulfides: the thiol (ca. 150 μg) was dissolved in the corresponding solvent (either diethyl ether, dichloromethane, or *n*-pentane; 20 mL each) and extracted with an aqueous solution of sodium hydroxide (10 mL, 1 mol/L). The aqueous phase was then acidified to pH 3 with hydrochloric acid (1 mol/L), and the thiol was isolated by extraction with the respective solvent (total volume 15 mL). The ice-cooled solution of the thiol was concentrated to 10 mL by evaporating the solvent with a stream of nitrogen. The amount of each thiol was determined gas chromatographically with 2-acetylpyrrole as an internal standard.

Disulfides. The corresponding thiol (0.2 mmol) was dissolved in diethyl ether (15 mL) and, after addition of an aqueous solution of copper(II) sulfate (3 mol/L; 15 mL), shaken for 35 min at room temperature. After addition of ammonium acetate (2 g) and shaking, the ethereal layer was separated, washed three times with brine (total volume 60 mL), and then dried over Na_2SO_4. The mixed disulfides were synthesized by oxidation of a mixture of the corresponding two thiols (Hofmann and Schieberle, 1995). MS-EI data of the disulfides and mixed disulfides are summarized in Table 2.

Storage of the Thiols. Solutions (500 μL each) containing the thiols in the amounts given in Tables 3–5 and 2-acetylpyrrole (25 μg) as an internal standard were pipetted into brown glass vials. After the vials were sealed with a septum, they were either stored at 6 °C in a refrigerator or refluxed for 1 h.

Table 4. Oxidation of MFT to MFT–MFT during Storage in Different Solvents[a]

expt	solvent	days of storage 0	1	2	9
		MFT (μg)			
1	diethyl ether	81.5	69.7	59.5	40.4
2	diethyl ether[b]	81.5	66.0	59.3	40.6
3	dichloromethane	80.5	78.1	77.5	75.4
4	*n*-pentane	79.6	78.9	78.5	77.7
		MFT–MFT (μg)			
1	diethyl ether	0.0	21.8	27.0	40.7
2	diethyl ether[b]	0.0	15.3	21.9	40.3
3	dichloromethane	0.0	2.9	3.4	5.0
4	*n*-pentane	0.0	0.8	1.1	1.7

[a] MFT, dissolved in the respective solvent (500 μL), was stored at 6 °C. [b] Butylhydroxyanisole (22 μg) was added prior to storage.

At the times given in Tables 3–5 samples of 1.0 μL were drawn with a syringe and then analyzed by HRGC/MS.

High-Resolution Capillary Gas Chromatography (HRGC)/Mass Spectrometry (MS). HRGC was performed with a Fisons gas chromatograph (Type 5300) by using the following capillaries: FFAP (30 m × 0.32 mm; fused silica capillary, free fatty acid phase, 0.25 μm; J&W Scientific, Fisons Instruments, Mainz, Germany) and SE-54 (30 m × 0.32 mm; fused silica capillary, DB-5, 0.25 μm, J&W Scientific, Fisons Instruments). The samples (1.0 μL) were applied by the on-column injection technique at 40 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 60 °C (FFAP) or 50 °C (SE-54), held isothermally for 5 min, then raised at 6 °C/min to 230 °C, and held for 15 min. Linear retention indices (RI) of the disulfides on the capillaries FFAP and SE-54 were calculated from the retention times of *n*-alkanes (cf. Table 2). MS analysis was performed with an INCOS XL (Finnigan, Bremen, Germany). Mass spectra were generated in the electron impact mode (MS-EI) at 70 eV.

RESULTS AND DISCUSSION

In a first set of experiments, each of the five thiols shown in Figure 1 was dissolved in diethyl ether and

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Table 5. Oxidation of Pairs of Thiols to Their Disulfides and Mixed Disulfides, Respectively

expt	reaction system[a]	amount (μg) after 1 day of storage at 6 °C		
		thiol	disulfide	mixed disulfide
1	MFT plus FFT	MFT (64.3), FFT (78.5)	MFT−MFT (14.9), FFT−FFT (0.5)	MFT−FFT (1.9)
2	MFT plus MA	MFT (68.9), MA (74.3)	MFT−MFT (8.9), MA−MA (3.4)	MFT−MA (4.5)
3	MFT plus MB	MFT (64.9), MB (78.5)	MFT−MFT (14.2), MB−MB (0.6)	MFT−MB (1.2)
4	MFT plus MP	MFT (64,6), MP (78.7)	MFT−MFT (14.5), MP−MP (0.5)	MFT−MP (2.0)
5	FFT plus MA	FFT (78.8), MA (74.6)	FFT−FFT (0.5), MA−MA (4.8)	FFA−MA (1.2)
6	FFT plus MB	FFT (78.7), MB (78.8)	FFT−FFT (0.8), MB−MB (0.8)	FFT−MB (0.8)
7	FFT plus MP	FFT (78.8), MP (78.9)	FFT−FFT (0.8), MP−MP (0.8)	FFT−MP (0.8)
8	MA plus MB	MA (74.2), MB (78.4)	MA−MA (4.8), MB−MB (0.6)	MA−MB (2.0)
9	MA plus MP	MA (74.5), MP (79.3)	MA−MA (4.9), MP−MP (0.5)	MA−MP (1.3)
10	MB plus MP	MB (78.8), MP (78.9)	MB−MB (0.8), MP−MP (0.7)	MB−MP (0.7)

[a] Pairs of thiols (80 μg each) were dissolved in diethyl ether (500 μL). The abbreviations of the thiols and disulfides refer to Figure 1 and Table 1, respectively.

then stored at 6 °C. The decrease of each thiol and the increase of the corresponding disulfide were measured over a period of 10 days.

After 10 days, a portion of each thiol had been oxidized to the correspondig disulfide (Table 3), which was identified by comparison of the MS-EI with that obtained for the synthesized reference compound.

According to the data in Table 3, MFT oxidized very rapidly: 20% of the thiol was converted into MFT−MFT after 1 day of storage and more than 50% after 10 days of storage. Compared to MFT, the four other thiols were more stable. After 10 days of storage (Table 3), their losses amounted to not more than 5 (MP) or 13% (MA).

The high oxidation rate is in agreement with the high antioxidative activity of MFT (Eiserich and Shibamoto, 1994). This activity is based on the easy abstraction of a hydrogen atom from the thiol group of MFT with the intermediate formation of a thiyl radical being stabilized by electron delocalization. As this stabilization is lacking in FFT, MA, MB, and MP, the formation of a thiyl radical is less favorable and consequently these thiols oxidize much more slowly than MFT.

A comparison of experiments 1 and 2 in Table 4 indicates that the oxidation of MFT dissolved in diethyl ether was not inhibited by the antioxidant BHT.

The influence of the solvent on the oxidation of MFT at 6 °C was studied in further experiments (Table 4). Compared to diethyl ether, storage in dichloromethane significantly lowered the oxidation rate during the storage period of 9 days. While in diethyl ether about 50% of the MFT had been oxidized to the disulfide, the decrease of MFT in dichloromethane amounted only to 6% (Table 4). In *n*-pentane MFT was most stable as no significant oxidation was observed. After that, solutions of MFT in dichloromethane and pentane were heated for 1 h under the conditions used for the simultaneous distillation and extraction according to the procedure of Nickerson and Likens (1966). The results indicated that MFT is oxidized under these conditions. In dichloromethane as well as in *n*-pentane 7.5 or 2.1 μg of MFT−MFT was formed from 80 μg of MFT.

In a third set of experiments pairs of different thiols were dissolved in diethyl ether in a weight ratio of 1:1. After storage at 6 °C, the mixed disulfides, formed by the oxidation of the two thiols present in each reaction system, were identified by comparison of the mass spectral data with those of the reference compounds (Table 2). As shown in Table 5, the mixed disulfides were present in the reaction systems in addition to the disulfides that had already been detected in the first set of experiments (Table 3).

The consumption of the thiols and the formation of the corresponding disulfides and mixed disulfides were

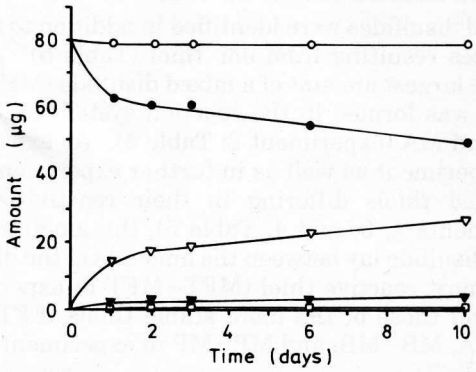

Figure 2. Time course of the oxidation of a mixture of MFT and FFT in diethyl ether. MFT and FFT (80 μg each)—free from disulfides—were stored in diethyl ether (500 μL) at 6 °C for 10 days. The consumption of MFT (●) and FFT (○) and the production of the mixed disulfide MFT−FFT (▼) and of the corresponding disulfides MFT−MFT (▽) and FFT−FFT (□) were determined.

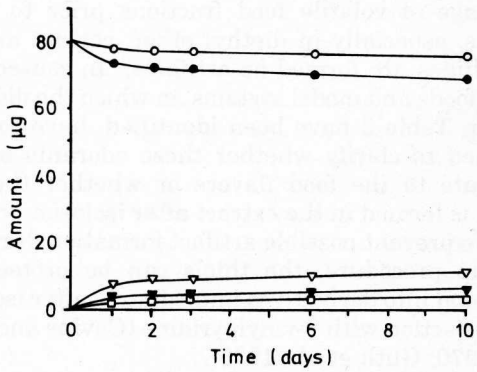

Figure 3. Time course of the oxidation of a mixture of MFT and MA in diethyl ether MFT and MA (80 μg each)—free from disulfides—were dissolved in diethyl ether (500 μL) and then stored at 6 °C for 10 days. The consumption of MFT (●) and MA (○) and the production of the mixed disulfide MFT−MA (▼) and of the corresponding disulfides MFT−MFT (▽) and MA−MA (□) were determined.

quantified during the storage period of 10 days in the reaction systems MFT plus FFT (Figure 2) and MFT plus MA (Figure 3). In both mixtures the unstable MFT was oxidized to MFT−MFT. However, in the presence of the stable FFT the rate was higher than in the presence of the more reactive MA, which to some extent protected MFT like an antioxidant. The time courses shown in Figures 2 and 3 confirm the results of the first set of experiments (Table 3), indicating that the oxidation rate of the thiols was highest at the first day.

In Table 5 the concentrations of the disulfides, formed after 1 day of storage in the 10 reaction systems investigated, are compared with the amounts of the

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unreacted thiols left. In experiments 1–4 (Table 5) the most reactive thiol, MFT, was combined with each of the resting thiols shown in Figure 1. In the presence of the relatively stable thiols FFT, MB, and MP (experiments 1, 3, and 4) the consumption of MFT at 1 day was of the same order of magnitude as in the reaction system containing MFT alone (Table 3). However, in the presence of the more reactive MA (experiment 2; Table 5) MFT was partially protected because only 14% of the MFT was oxidized to the disulfides MFT–MFT and MFT–MA.

The consumption of MA, which was next reactive compared to MFT, amounted to 6% (experiments 2, 5, 8, and 9), while the more stable thiols FFT, MB, and MP were oxidized to only 2% (Table 5).

Mixed disulfides were identified in addition to the two disulfides resulting from one thiol (Table 5). After 1 day, the largest amount of a mixed disulfide (MFT–MA; 4.5 μg) was formed in the reaction system containing MFT and MA (experiment 2; Table 5). As expected in this experiment as well as in further experiments that contained thiols differing in their reactivities (e.g. experiments 1, 3, and 4; Table 5), the amount of the mixed disulfide lay between the amounts of the disulfide of the most reactive thiol (MFT–MFT in experiments 1–4) and those of the more stable thiols (FFT–FFT, MA–MA, MB–MB, and MP–MP in experiments 1–4).

CONCLUSION

The results clearly demonstrate that during handling and storage in organic solvents oxidation of thiols may generate significant amounts of the corresponding disulfides. The data imply that during concentration and/or storage of volatile food fractions prior to HRGC analysis, especially in diethyl ether, certain amounts of disulfides are formed as artifacts. In consequence, heated foods and model systems, in which the disulfides listed in Table 2 have been identified, have to be reexamined to clarify whether these odorants actually contribute to the food flavors or whether the main portion is formed in the extract after isolation from the food. To prevent possible artifact formation during the work up procedure, the thiols can be protected by conversion into derivatives immediately after isolation, e.g. by reaction with 4-vinylpyridine (Cavins and Friedman, 1970; Guth et al., 1995).

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