

Interaction of Thiol and Disulfide Flavor Compounds with Food Components

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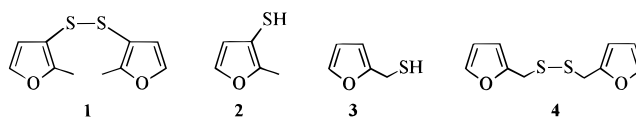
When a commercial savory flavoring containing thiol and disulfide flavor components was heated at 100 °C in aqueous solution with egg albumin, considerable changes in the relative concentration of the sulfur compounds were observed. Apparent redox interactions with the protein caused disulfide reduction and formation of the corresponding thiol. Heating bis(2-furanylmethyl) disulfide or bis(2-methyl-3-furanyl) disulfide with albumin in aqueous solution resulted in over 100-fold decreases in disulfide concentrations with a large proportion of the disulfides converted to the corresponding thiols. It is suggested that this was caused by interchange of thiol and disulfide groups with sulfhydryl and disulfide groups of proteins. This was substantiated by observing the effect in water or maltodextrin solutions where no loss of disulfide was observed. In systems containing casein, which has a very low proportion of sulfhydryl groups, only a small amount of disulfide reduction occurred.

Keywords: Savory flavorings; flavor interaction; protein; sulfhydryl–disulfide interchanges

INTRODUCTION

A large number of different sulfur-containing volatile compounds have been found in foods. Most have very low odor threshold values and are important in determining the aroma characteristics of foods. Thiazoles, thiophenes, trithiolanes, trithianes, and other heterocyclic sulfur compounds are important classes of flavor compounds in cooked foods. The meaty and savory characteristics of cooked meat are of considerable interest to the flavor chemist. Furanthiols and thiophenethiols have been shown to have meatlike aromas at low concentrations, especially when the thiol substituent is present in the 3-position on the furan ring (Evers et al., 1976). A number of patents have been taken out for the use of these thiols and their disulfides in meatlike flavorings (Evers, 1970; van den Ouweland and Peer, 1972). These compounds have been found in heated model systems containing cysteine and ribose (Farmer and Mottram, 1990; Hoffman and Schieberle, 1995), in the products of the reaction between 4-hydroxy-5-methyl-3(2*H*)-furanone and hydrogen sulfide or cysteine (van den Ouweland and Peer, 1975; Mottram and Whitfield, 1994), and in the thermal degradation of thiamin (van der Linde et al., 1979; Werkhoff et al., 1990). Bis(2-methyl-3-furanyl) disulfide (**1**) has an exceptionally low odor threshold of 2×10^{-5} $\mu\text{g}/\text{kg}$ and was isolated from thermally degraded thiamin by Buttery et al. (1984). More recently it has been found in cooked meat, together with the corresponding thiol, 2-methyl-3-furanthiol (**2**), and a number of related sulfides (Gasser and Grosch, 1988; Werkhoff et al., 1993; Madruga and Mottram, 1995). Isomers of this thiol and disulfide, namely 2-furanmethanethiol (**3**) and bis(2-furanylmethyl) disulfide (**4**), have been isolated from a number of cooked foods, including meat, and are believed to be particularly important in roast coffee aroma.

These are widely used as components of flavorings for soups, savory products, and meat substitutes, where



they are either added to the flavorings as nature-identical chemicals or as components of reaction-product flavorings. The thiols are easily oxidized to the corresponding disulfides, and mixtures of different thiols readily form mixed disulfides (Mottram et al., 1995; Hoffman et al., 1996). It has also been shown that, in boiling aqueous solution, disulfides were hydrolyzed to thiols, which were detected using 4-vinylpyridine as a thiol-trapping agent (Guth et al., 1995). In the absence of such a reagent free thiols were not detected because they reoxidize to the disulfide, but in a mixture of deuterated and unlabeled disulfides mixed disulfides were obtained. Such changes could result in modification of the sensory properties of foods containing thiols and disulfides. In proteins reduction–oxidation (redox) reactions involving interchange of sulfhydryl and disulfide groups, within the protein or with external thiol groups, are well-known (Jocelyn, 1972). This raises the possibility of thiol and disulfide flavor compounds interacting with proteins in foods and causing changes in their relative concentrations. This paper reports on such redox changes in thiol and disulfide flavor compounds which have been shown to occur in the presence of food proteins.

EXPERIMENTAL PROCEDURES

Materials. Spray-dried chicken egg albumin was obtained from Nive, Nunspeet, Netherlands, purified casein powder was from Sigma Chemical Co., and maltodextrin, with average dextrose equivalent (DE) of 12, was from Roquette, Tunbridge Wells, UK. Compounds **1–3** were purchased from Aldrich Chemical Co., and **4** was obtained as a gift from a flavor company. Samples of a savory flavoring (powdered solid) was obtained from a commercial source, but no information about the composition or mode of preparation was available.

Preparation of Flavored Substrate. Albumin (50 g) or maltodextrin were dissolved in 750 mL of distilled water and

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Table 1. Approximate Quantities ($\mu\text{g}/10\text{ g}$ of Flavoring) of Selected Sulfur Compounds and Terpenes Recovered from Heated Aqueous Mixtures of Savory Flavoring with Egg Albumin or Maltodextrin

compound	flavor alone	albumin + flavor	maltodextrin + flavor
α -pinene	6	9	7
β -pinene	10	25	12
limonene	17	19	18
1,8-cineole	74	86	88
linalool	14	16	14
camphor	54	58	55
borneol	9	10	10
2-furanmethanethiol	76	570	48
dipropyl disulfide	51	— ^a	56
2-[(methylthio)methyl]furan	6	6	4
dipropyl trisulfide	59	—	64
bis(2-methyl-3-furanyl) disulfide	23	—	—
bis(2-furanylmethyl) disulfide	1212	20	1200

^a —, not found.

mixed with 2.5 g of commercial flavoring. The mixtures were stirred gently for 2 h. Other mixtures containing albumin, casein, or maltodextrin with the disulfides **1** and **4** were also prepared by dissolving each substrate (50 g) separately in 750 mL of distilled water and adding ethanolic solutions of each of the disulfides (500 μg , contained in 0.5 mL of ethanol). Other systems were prepared containing the substrates and a mixture of thiol **3** (40 μg) and disulfide **4** (500 μg). These mixtures were also stirred for 2 h.

Simultaneous Distillation Extraction (SDE). Volatiles were extracted from the aqueous mixtures using simultaneous steam distillation solvent extraction in a modified Likens–Nickerson apparatus. Extractions were carried out for 2 h using a mixture of redistilled *n*-pentane (27 mL) and diethyl ether (3 mL). After extraction, an internal standard (130 μg of 1,2-dichlorobenzene in 0.1 mL of *n*-hexane) was added to the solvent extract which was dried and concentrated to about 0.5 mL by distillation in a Kuderna–Danish apparatus. Samples of the flavoring (2.5 g) in 750 mL of distilled water were also extracted by SDE. In addition, the efficiency of recovery of the SDE method for the disulfides was examined using aqueous solutions containing the thiols and disulfides in concentrations similar to those used in the substrate systems.

Gas Chromatography–Mass Spectrometry (GC–MS).

A split/splitless injection was used to introduce 1 μL aliquots of each extract onto a fused silica capillary column installed in a Hewlett-Packard HP5890 gas chromatograph coupled to a HP5972 mass-selective detector. The column was a 50 m \times 0.32 mm (i.d.) fused silica capillary column coated with BPX5 at 0.5 μm film thickness (SGE Ltd.). Helium at 1.6 mL/min (35 cm/s) was used as a carrier gas. The oven was initially held at 50 $^{\circ}\text{C}$ for 2 min, and then programmed at 4 $^{\circ}\text{C}/\text{min}$ to 250 $^{\circ}\text{C}$. The injector temperature was 250 $^{\circ}\text{C}$, and the interface of the GC to the MS was maintained at 280 $^{\circ}\text{C}$. The MS was operated in the electron impact mode with an

ionization energy of 70 eV and a scan rate of 1.9 scans/s over the mass range 33–400 amu. A solution containing C₆–C₂₂ *n*-alkanes was also analyzed to allow calculation of linear retention indices (LRI) for each sample component. Volatiles from the systems containing commercial flavorings were identified by comparison of mass spectra and LRI with those obtained from GC–MS analyses of the authentic compounds, or by comparison of mass spectra with those in the NIST/EPA/MSDC spectral collection or in published literature. Quantities of the thiols **2** and **3** and the disulfides **1** and **4** in the extracts were determined by comparison of the peak areas in the total ion chromatogram with areas of the compounds in analyses of standard solutions, using 1,2-dichlorobenzene as internal standard. Approximate quantities of other components of the flavorings were estimated by comparison of total ion chromatogram (TIC) peak areas with that for the 1,2-dichlorobenzene standard.

RESULTS AND DISCUSSION

The flavorings contained complex mixtures of aroma compounds, including a large number of terpenes and several sulfur compounds. Some of the major compounds in these classes are given in Table 1. Heating egg albumin with the flavoring in aqueous solution during SDE extraction had little effect on the recovery of the terpenes; however major differences in sulfur compounds were found. Most decreased significantly in concentration and some could not be detected in the albumin-containing systems. The sulfur compounds were mainly thiols and disulfides, and the large losses appeared to be due to binding on the protein. When maltodextrin was heated with the flavoring, these losses were not found. Particularly interesting were the amounts of bis(2-furanylmethyl) disulfide (**4**) and 2-furanmethanethiol (**3**) recovered from the albumin system. The concentration of the disulfide decreased by almost 100-fold but a considerable *increase* in the amounts of the thiol was found, which was attributed to reduction of the disulfide in the presence of protein. No such change was observed with the maltodextrin system.

Known quantities of the disulfides **1** and **4** were mixed separately with egg albumin, casein or maltodextrin and were subsequently extracted using SDE. An effect similar to that shown with the commercial flavoring was observed with a marked loss of the disulfides in the presence of protein and a large increase in the level of the corresponding thiol (Table 2). The recovery of the disulfide **4** from water was almost 100%, and a similar recovery was obtained for the maltodextrin system, although the recovery of **1** was lower. It is interesting to note that when the thiol **3**, together with its disulfide, was added to the maltodextrin or water-only systems, most was lost either on mixing or during the extraction.

Table 2. Quantities of Disulfides and Thiols Recovered from Aqueous Systems Containing Protein or Carbohydrate Substrates^a

compound	quantity added (μg)	quantity recovered (μg)			
		egg albumin	casein	malto-dextrin	water only
Experiment 1					
bis(2-furanylmethyl) disulfide	500	3 (1)	377 (63)	492 (10)	476 (23)
2-furanmethanethiol	1 ^b	127 (14)	12 (4)	1 (1)	1 (1)
Experiment 2					
bis(2-furanylmethyl) disulfide	500	11 (6)	393 (23)	566 (17)	548 (4)
2-furanmethanethiol	40	158 (14)	16 (1)	1 (0)	6 (1)
Experiment 3					
bis(2-methyl-3-furanyl) disulfide	500	6 (2)	272 (21)	378 (24)	— ^c
2-methyl-3-furanthiol	1 ^b	226 (54)	67 (8)	1 (1)	— ^c

^a Each value is the mean of triplicate determinations, and standard deviations are shown in parentheses. ^b The disulfides contained very small amounts of the corresponding thiols as impurities. ^c Not determined.

However, the amount of disulfide increased compared with the systems which did not contain the thiol, indicating that oxidation had occurred.

In the systems containing protein there was no evidence of thiol oxidation but the disulfides were reduced to the corresponding thiol (Table 2). However, all the lost disulfides were not recovered as thiols, indicating that interaction had occurred with the protein. The disulfide losses and conversion to thiol were considerably greater in the systems containing egg albumin than in those with casein. The most likely explanation for these observations is interaction between the thiol and disulfide flavor compounds with sulfhydryl groups and disulfide bridges, from cysteine and cystine amino acid units in the protein. This appears to involve reduction of the flavor disulfide as well as the formation of disulfide links between protein and thiol with the associated loss of flavor compound from the volatile extract. Albumin contains a much greater proportion of these -SH groups than casein, which explains the different behaviors of the two proteins toward the flavor compounds. Such sulfhydryl-disulfide interchange reactions are well-known in protein chemistry (Jocelyn, 1972). An example of such interchanges is seen in bread-making where reduction-oxidation (redox) reactions in the flour proteins can occur, and sulfhydryl-disulfide interchanges with glutathione are important in relation to dough rheology and bread-making (Grosch, 1986; Chen and Schofield, 1995).

The odor threshold values of the thiols may differ from those of the corresponding disulfides, e.g., the odor threshold value for the disulfide **1** is reported as 2×10^{-5} $\mu\text{g}/\text{kg}$ while that of the corresponding thiol **2** is 5×10^{-3} $\mu\text{g}/\text{kg}$. The aroma characteristics of these compounds may also change with concentration. In general, at low concentrations approaching the odor threshold values, the compounds have pleasant savory or roasted aromas but at higher concentration they become more sulfurous and unpleasant (Arctander, 1969; Tressl and Silwar, 1981; Fors, 1983). Redox-induced changes in the relative concentrations of these thiols and disulfides due to protein-sulfhydryl interchanges could result in significant changes in the sensory properties of the flavoring when added to a food product.

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