Quantification of 2-Methyl-3-furanthiol, 2-Furfurylthiol, 3-Mercapto-2-pentanone, and 2-Mercapto-3-pentanone in Heated Meat

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A stable isotope dilution assay was developed for quantification of the potent odorants 2-methyl-3-furanthiol (MFT), 2-furfurylthiol (FFT), 2-mercapto-3-pentanone (2M3P), and 3-mercapto-2-pentanone (3M2P) in heated meat. The volatiles of meat were extracted with dichloromethane, which was spiked with definite amounts of stable isotopomers of MFT, FFT, and 3M2P. The analytes and their labeled standards were enriched by reaction with *p*-hydroxymercuribenzoic acid, and after liberation, the thiols were assayed by dynamic headspace gas chromatography in combination with mass spectrometry. The following amounts (micrograms per kilogram) were found in meat samples boiled for 45 min: (beef) MFT, 7−28, FFT, 13−42, 3M2P, 55−73, 2M3P, 20−44; (pork) MFT, 6−9, FFT, 8−10, 3M2P, 66−117, 2M3P, 11−14; (lamb) MFT, 5−11, FFT, 9−14, 3M2P, 30, 2M3P, 10. Chicken contained per kilogram 4.5 μg of MFT, 2.4 μg of FFT, 100 μg of 3M2P, and 13 μg of 2M3P after a boiling period of 60 min.

Keywords: *Isotope dilution assay; 2-methyl-3-furanthiol; 2-furfurylthiol; 3-mercapto-2-pentanone; 2-mercapto-3-pentanone; beef; chicken; pork; lamb; flavor*

INTRODUCTION

2-Methyl-3-furanthiol (MFT), 2-furfurylthiol (FFT), and a mixture consisting of 2-mercapto-3-pentanone (2M3P) and 3-mercapto-2-pentanone (3M2P) are formed by the reaction of cysteine and ribose (Farmer et al., 1989; Farmer and Mottram, 1990). Furthermore, MFT, 3M2P, and 2M3P were also detected as breakdown products of thiamin (Van der Linde et al., 1979; Güntert et al., 1992). As these precursors are ubiquitous in animals and plants, the four thiols have been detected in various heated foods (Table 1).

The thiols may contribute to the flavor of these foods, as their odor threshold values in water are low: 0.007 μg/kg for MFT, 0.01 μg/kg for FFT, and 0.7 μg/kg for 3M2P (Schieberle and Hofmann, 1996).

As demonstrated for stewed beef juice (Guth and Grosch, 1994), coffee (Semmelroch et al., 1995), and boiled chicken (Kerler and Grosch, 1997), FFT can be accurately quantified by an isotope dilution assay (IDA) using 2-[α-$^2H_2$]furfurylthiol (d-FFT) as internal standard. In contrast to FFT, MFT oxidizes rapidly to the corresponding disulfide (Hofmann et al., 1996). This reaction and other degradation processes may lead to such great losses that neither MFT nor its labeled internal standard 2-[$^2H_3$]methyl-3-furanthiol (d-MFT) was detected in extracts obtained from heated meat samples (unpublished results). Although MFT smells like boiled meat (Evers et al., 1976), the contribution of this odorant to the flavor of cooked meat from different animal species is uncertain, as no quantitative data are available.

The aim of this study was to improve the sensitivity of the stable isotope dilution assay so far that MFT can be quantified in samples of heated meat. To reach this goal, the volatiles of cooked meat were extracted with a solvent that was spiked with definite amounts of d-MFT, and then the thiols were trapped according to the method of Darriet et al. (1995) by a reaction with *p*-hydroxymercuribenzoic acid (HMBA). MFT and d-MFT were liberated from their HMBA derivatives by the addition of cysteine, isolated by a dynamic headspace procedure, and finally analyzed by high-resolution gas chromatography (HRGC) in combination with mass spectrometry (MS).

Table 1. Detection of MFT, FFT, and 3(2)-Mercapto-2(3)-pentanone (MP) in Heated Food

food sample	thiol	ref[a]
beef	MFT	Gasser and Grosch (1988)
	MFT, FFT	Farmer and Patterson (1991)
	FFT, MP	Guth and Grosch (1993)
pork	FFT	Mottram (1985)
	MFT, FFT	Gasser and Grosch (1991)
chicken	FFT	Noleau and Toulemonde (1986)
	MFT, FFT, MP	Gasser and Grosch (1990)
tuna fish	MFT	Withycombe and Mussinan (1988)
roasted coffee	FFT	Reichstein and Staudinger (1955)
	MFT, FFT	Tressl and Silwar (1981)
roasted sesame	FFT	Schieberle (1993)
popcorn	FFT	Schieberle (1991)
yeast extract	FFT	Golovnya et al. (1983)
	MFT, FFT	Ames and MacLeod (1985)
	FFT, MP	Münch et al. (1997)
wheat bread	FFT	Baltes and Song (1994)

[a] Authors who had detected first the thiol are quoted.

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Table 2. Selected Ions and Calibration Factors for Mass Chromatography of MFT, FFT, 3M2P, and 2M3P

compound	selected ion (*m*/*z*)	internal standard	selected ion (*m*/*z*)	calibration factor
MFT	115	d-MFT	118	1.19
FFT	81	d-FFT	83	0.99
3M2P	119	d-3M2P	121	0.81
2M3P	119	d-3M2P	121	0.81

Table 3. Concentrations of MFT, FFT, 3M2P, and 2M3P in Different Meat Samples

sample	heat treatment[a] (min)	concentration[b] MFT	FFT	3M2P	2M3P
beef					
top round I	bld (45)	6.7	13	73	33
top round II	bld (45)	16	42	65	35
forerib I	bld (45)	18	30	68	44
forerib II	bld (45)	25	32	69	33
forerib III	bld (45)	28	33	55	20
forerib III[c]	st, rht (45)	16	14	22	6.4
broth III[d]		2.6	2.2	9.1	2.4
pork					
chuck	bld (45)	5.9	7.6	117	14
shoulder[e]	bld (45)	9.1	9.5	66	11
ham[e]		1.6	4.4	5.4	<0.5
lamb					
neck[f]	bld (45)	4.8	9.4	31	10
shoulder[e]	bld (45)	11	14	30	11
chicken					
stewing, breast	bld (30)	1.1	0.4	23	1.6
broiler, breast	bld (30)	2.0	1.0	35	2.4
broiler, breast	bld (60)	4.5	2.4	100	13
broiler, breast	rst (60)	0.4	0.1	31	<0.5
broiler, skin	rst (60)	4.1	1.9	27	3.4

[a] bld, boiled; st, stored; rht, reheated; rst, roasted. [b] Values in micrograms per kilogram of the material after heat treatment. The data are mean values of at least duplicates. [c] Forerib III after refrigerated storage and reheating. [d] After forerib III was boiled, the broth was separated and cooled to 10 °C in an ice bath, and the congealed fat was removed by filtration. [e] Visible fat was removed before analysis. [f] Neck with bones.

Besides MFT, the thiols FFT, 2M3P, and 3M2P were derivatized by HMBA. Therefore, after addition of the labeled internal standards, d-FFT and 3-mercapto-2-[4,5-$^2H_2$]pentanone (d-3M2P, internal standard for 3M2P and 2M3P, cf. Table 2) to the meat extracts, these thiols were included into the analytical procedure that was applied to heated meat samples from different animals.

EXPERIMENTAL PROCEDURES

Meat. Beef (top round, forerib), pork (shoulder, chuck), lamb (shoulder, neck with bones), stewing fowl, broiler fowl, and ham from Parma (Italy) were purchased from a local market. Meat from beef, pork, and lamb was trimmed of all excess fat, and samples (400 g) in water (300 mL) were boiled under pressure (8×10^4 Pa, 116 °C) for 45 min. Half of the freshly cooked beef (forerib III in Table 3) was analyzed immediately. The other half was packed in a polyethylene bag, which was sealed and stored for 48 h at 4 °C. Subsequently, the sample was reheated in the polyethylene bag in a water bath at 70 °C for 45 min. During this time the sample, in which an off-flavor was formed, reached a temperature of 57 °C. Whole stewing fowl and whole broiler (1.5 kg each, without offals) were boiled under the same conditions for the time given in Table 3. Broiler fowl (1.5 kg, without offals) was roasted in a frying pan of Pyrex glass ($32 \times 10 \times 22$ cm) containing coconut oil (15 g) for 1 h in an oven having a temperature of 180 °C.

Chemicals. The following compounds were obtained commercially: MFT, FFT (98%), 2,3-pentanedione (97%), and the sodium salt of HMBA were from Aldrich, Steinheim, Germany. L-Cysteine was from Merck, Darmstadt, Germany, and sodium hydrogen sulfide monohydrate was from Fluka, Buchs, Switzerland.

To obtain the HMBA reagent (Darriet et al., 1995), the sodium salt of HMBA (90 mg) was dissolved in NaOH (10 mL, 50 mmol/L) and, after addition of 20 mL of phosphate buffer (pH 8.5, Na_2HPO_4/NaH_2PO_4, 1:15 mol/L, 95:5, v/v,), the final volume of 100 mL was adjusted with distilled water.

Syntheses. *A mixture of 3M2P and 2M3P* was prepared according to the indications of Mottram et al. (1995). Sodium hydrogen sulfide monohydrate (0.74 g, 10 mmol) was added to 30 mL of an aqueous solution of 2,3-pentanedione (0.5 g, 5 mmol). After acidification with one drop of aqueous HCl (4 mol/L), the flask containing the reaction mixture was sealed and then placed for 2 h in a boiling water bath. The mixture was cooled to room temperature and extracted with dichloromethane (3×20 mL). The combined organic layers were extracted with aqueous sodium hydroxide (1 mol/L, 3×30 mL). After adjustment of the pH to 3 with aqueous HCl (3 mol/L), the thiols were extracted with dichloromethane (3×30 mL). The organic layer was washed with a saturated aqueous solution of NaCl and finally dried over anhydrous Na_2SO_4. The retention indices (RI) of 3M2P (896) and 2M3P (903) on capillary DB-5 and the MS-EI agreed with the corresponding data reported by Mottram et al. (1995). Quantitative measurements indicated that 3M2P and 2M3P were formed in a ratio of 1:8.

3-Mercapto-2-pentanone (3M2P), which was free from the 2M3P isomer, was synthesized according to the method of Asinger et al. (1964). The MS-EI of 3M2P agreed with that reported by Sen and Grosch (1991).

2-[$^2H_3$]Methyl-3-furanthiol (d-MFT), 2-[α-$^2H_2$]furfurylthiol (d-FFT), and 3-mercapto-2-[4,5-$^2H_2$]pentanone (d-3M2P) were prepared and purified according to the methods of Sen and Grosch (1991). The MS-EI of the labeled thiols agreed with those reported earlier (Sen and Grosch, 1991) and indicated that the standards were not contaminated with unlabeled material.

HRGC/MS Analysis. The purity of the synthesized compounds was checked by HRGC in combination with the MS system MAT 95 S (Finnigan, Bremen, Germany). HRGC was performed using a DB-5 capillary column (30 m × 0.32 mm, 0.25 μm film thickness) supplied from J&W Scientific, Folsom, CA. HRGC/MS analyses were performed using the conditions reported earlier (Münch et al., 1997).

Concentrations of Compounds. MFT (0.5 g) was dissolved in dichloromethane (30 mL) and extracted with an aqueous solution of sodium hydroxide (3×10 mL, 1 mol/L). The aqueous phase was then acidified to pH 3 with aqueous HCl (3 mol/L), and MFT was extracted with dichloromethane (3×10 mL). The extract was washed with a saturated aqueous solution of NaCl (2×10 mL) and dried over anhydrous Na_2SO_4. The amount of MFT was determined gas chromatographically with FFT as internal standard, using a calibration factor of 1.00. The concentration of d-FFT was gas chromatographically determined as described by Semmelroch et al. (1995). The concentrations of d-MFT, 2M3P, 3M2P, and d-3M2P were determined by HRGC using the internal standards FFT for d-MFT and 2,3-pentanedione for the resting thiols. The calibration factor was set to 1.00 for each compound, and the purity of FFT and 2,3-pentanedione was considered by the calculation of the results.

IDA of MFT, FFT, 2M3P, and 3M2P. *Isolation of the Thiols from Meat Samples.* After the heat treatment, the following amounts of hot material were immediately analyzed: (beef) top round, 100 g, forerib, 50 g; (pork, lamb) 100 g each; and (chicken) breast, 200 g; and (skin of roasted chicken) 40 g. Parma ham without visible fat (100 g) was used for IDA. Each sample was frozen in liquid nitrogen, mixed with anhydrous Na_2SO_4 (25 g), and ground in a Waring blender. The powdered material was suspended in dichloromethane (300 mL), which was spiked with the internal standard substances d-MFT, d-FFT, and d-3M2P. The amounts of the standards varied between the half-concentration and 3-fold concentration of the thiols to be determined. After

homogenization of the suspension with an Ultra-Turrax (Janke and Kunkel, Oberstaufen, Germany, 2 × 1 min), the solvent was filtered off. The residue was resuspended in dichloromethane (100 mL) and filtered after homogenization for 30 s. The filtrates were combined and concentrated in vacuo to 150 mL.

For separation of the thiols the organic extract was magnetically stirred three times (20 min each) with a freshly prepared HMBA reagent (1 × 40 mL, 2 × 30 mL) in the dark and under a nitrogen atmosphere. Between each extraction step the aqueous layer was separated and finally pooled. The combined aqueous phases were concentrated in vacuo using a Rotavapor (Büchi, Eislingen, Germany) at a water bath temperature of 40 °C to a volume of 10 mL and then poured into a headspace purge vessel (volume = 25 mL, Chrompack, Frankfurt, Germany). After the addition of cysteine (600 mg) to liberate the thiols, the vessel was closed and the solution magnetically stirred for 5 min. Then the vessel was then connected to the purge and trap apparatus.

Beef Broth. Broth obtained from forerib was cooled to ~10 °C in an ice bath, and the congealed fat was removed by filtration. Subsequently, the broth (100 g) was saturated with NaCl, spiked with the internal standard substances, and extracted with dichloromethane (3 × 100 mL). The organic extracts were combined and treated as described above for meat.

Analysis by HRGC/MS. The vessel containing the liberated thiols was placed in the purge and trap system (TCT/PTI 4001, Chrompack, Frankfurt, Germany), which was connected with a gas chromatograph (CP 9001, Chrompack). The purge and trap system operated in the PTI mode. The gas chromatograph was equipped with a CP-Sil 8 CB fused silica capillary (25 m × 0.32 mm, film thickness = 1.2 μm, Chrompack). The exit of the capillary was coupled with the mass spectrometer Incos XL (Finnigan, Bremen, Germany). The flow of the carrier gas helium was 2 mL/min and, after the start of the HRGC run, the temperature of the oven was held at 35 °C for 1 min and then raised at a rate of 6 °C/min to 250 °C, which was held for 5 min. Mass spectra in the chemical ionization mode were generated at 115 eV with methane as reagent gas. The calibration factors (cf. Table 2) and quantitative data were calculated as reported by Sen et al. (1991).

The volatile thiols were purged with helium (flow = 20 mL/min) for 25 min. During this period, the sample was magnetically stirred and held in a water bath at 40 °C. The rising volatiles and water vapor passed a condenser, which was cooled to −10 °C by a water/diethylene glycol mixture (1 + 1, v/v) to remove the water.

The carrier gas passed through an empty glass tube in the desorption heating block of the purge and trap facility, which was held at 250 °C. The volatiles were then cryofocused on the trap (20 cm × 0.53 mm fused silica capillary coated with CP-Sil 8 CB, film thickness = 5 μm), which was cooled with liquid nitrogen at −110 °C.

The trap was flash heated to 200 °C, which was held for 1 min, and the volatiles were flushed by the helium flow into the gas chromatograph. After each HRGC/MS run, the purge and trap system was automatically cleaned for 10 min (cleanup flow = 50 mL/min helium, cleanup temperature = 275 °C).

Odor Threshold Values. The odor threshold values of 3M2P and 2M3P in air were determined by gas chromatography−olfactometry using (*E*)-2-decenal as internal standard (Ullrich and Grosch, 1987; Blank et al., 1989).

RESULTS AND DISCUSSION

During the cleanup procedure for IDA the reaction with HMBA provided a fraction of thiols, of which the total ion gas chromatogram is shown in Figure 1a. To differentiate between the unlabeled odorants (from boiled beef, forerib) and the deuterated internal standards, mass chromatograms were recorded for the ions that were selected for quantification as shown in Table 2. The mass chromatograms obtained are displayed in Figure 1b−g. The concentrations of the thiols were calculated from the areas of the analytes and their standards using the calibration factors listed in Table 2.

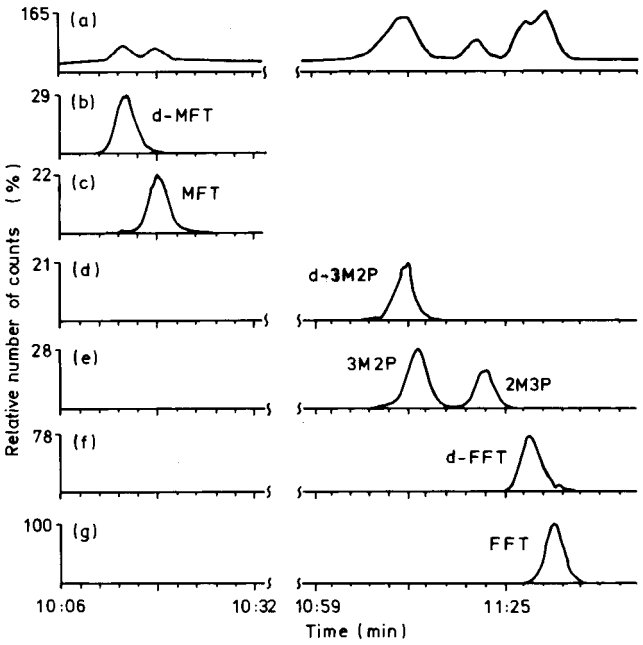

Figure 1. HRGC/MS chromatograms of the sulfur compounds from boiled beef (forerib) obtained after trapping with HMBA, liberation from the derivatives by addition of cysteine, and purge and trap enrichment: (a) total ion chromatogram, (b−g) mass chromatograms of the ions given in parentheses: d-MFT (118), MFT (115), d-3M2P (121), 3M2P and 2M3P (119), d-FFT (83), and FFT (81). The number of counts was set to 100% for FFT.

Table 4. Recovery of MFT, FFT, and 3M2P from a Model Mixture

	amount[a] (μg)		
compound	added	measured	recovery (%)
MFT	1.40	1.36	97
FFT	2.53	2.42	96
3M2P	7.70	7.90	103

[a] The amounts of unlabeled thiols listed in the table were dissolved in dichloromethane (150 mL). After addition of their isotopomers, d-MFT (1.18 μg), d-FFT (2.21 μg), and d-3M2P (6.25 μg), the model mixture was treated as reported.

A first model experiment was carried out to check the efficiency of the derivatization and cleanup procedures. The results in Table 4 indicate that the recovery of the analytes was in the range of 100%. This suggested that no deuterium/protium exchange, which would hinder the IDA, has taken place during derivatization and quantification of the thiols. The results suggested that losses of the analytes during the cleanup procedure were completely corrected by the labeled standards as their chemical properties agreed with those of the thiols to be quantified. Small isotope effects, which led to a partial separation of the analyte and its standard during HRGC (Figure 1), did not affect the accuracy of the IDA.

A second model experiment was performed to check the recovery of the thiols when added to a meat sample. Therefore, after boiling and cooling, beef samples with and without additions of the four thiols were analyzed. The results in Table 5 indicate that the recovery values for the thiols were between 83 and 97%. This suggests that the sensitivity of the method is sufficient to measure the low levels of the four thiols occurring in

Table 5. Recoveries of MFT, FFT, 3M2P, and 2M3P When Added to Beef

compound	amount[a,b] (μg) A	B	C	D	recovery (%)
MFT	1.02	1.63	2.65	2.20	83
FFT	1.25	1.67	2.92	2.83	97
3M2P	2.23	5.57	7.70	6.45	84
2M3P	1.14	3.00	4.14	3.84	93

[a] The amounts of the four odorants were quantified in the beef sample (forerib) as reported under IDA of MFT, FFT, 2M3P, and 3M2P. The following amounts of the standards were used: d-MFT (1.53 μg), d-FFT (1.70 μg), and d-3M2P (4.80 μg). The results are mean values of duplicates. [b] A, Odorants occurring in boiled in beef (50 g) before additions; B, model mixture added to boiled beef (50 g) after cooling; C, calculated sum of A and B; D, amount measured.

meat. In comparison to the first model experiment, the recoveries of MFT and 3M2P were somewhat lower. Possibly, small fractions of these thiols were lost by oxidation or by reactions with meat components before the meat sample came into contact with the solvent used for extraction.

Meat samples obtained from different animal species were heated, and the amounts of MFT, FFT, 3M2P, and 2M3P formed were quantified. The results are summarized in Table 3. A comparison of the data showed that the highest concentrations of MFT are formed in boiled beef, in particular in the forerib, which on an average contained 24 μg/kg. Pork and lamb provided significantly lower amounts, ranging from 5 to 11 μg/kg. In cured ham prepared from pork traces of 1.6 μg/kg were detectable. The MFT concentration reached only 4.5 μg/kg in chicken meat, although the boiling period was extended to 60 min. Shorter boiling of 30 min led to the formation of only 1−2 μg/kg. An experiment in which the skin and breast meat of roasted chicken were separately analyzed indicated that more MFT was produced in the former.

The highest concentration of FFT was found in beef meat, amounting nearly to 30 μg/kg in boiled forerib. FFT was significantly higher than MFT in the top round samples from beef as well as in the neck from lamb, whereas in boiled chicken its concentration was only half as large as that of MFT. In the other meat samples the formation of FFT went parallel to that of MFT.

In the fraction of mercaptopentanones the 3M2P isomer was the major component. On average, the concentrations of 3M2P amounted to 66 μg/kg in boiled beef. This value was also found in the boiled shoulder from pork, but the 3M2P level in the chuck, 117 μg/kg, was much higher. Lamb contained nearly half (30 μg/kg) as much 3M2P as beef. This concentration was also formed in chicken during boiling for 30 min and increased to 100 μg/kg when this process was extended to 60 min.

Different proportions of 3M2P and 2M3P were formed in the cooked meat samples. On an average, the ratio of 3M2P to 2M3P was 2:1 in beef, 7:1 in pork, 3:1 in lamb, and 11:1 in chicken. A study of the sensory properties indicated that 3M2P smelled sulfurous and catty and 2M3P sulfurous and garlic- and fried onion-like. The odor thresholds in air of 3M2P and 2M3P were 0.15 and 0.60 ng/L, respectively. On the basis of the higher concentration in the meat samples and the lower odor threshold, we suggest that 3M2P is more important for the flavor of heated meat than 2M3P.

In the case of the forerib, sample III, the four thiols were also determined in the broth from which the fat was removed. The results in Table 3 indicate that the concentrations, in particular of MFT and FFT, were very small. The low value of FFT corresponded to that reported by Guth and Grosch (1994) for stewed beef juice.

A cardboard-like, metallic off-flavor is formed after refrigerated storage and reheating of cooked beef (Love, 1988). This loss in flavor quality was also perceivable when boiled forerib, sample III, was refrigerated and reheated. According to the results in Table 3, the concentrations of the four thiols were lowered by 43−68% after this treatment. In addition to odorants formed by lipid peroxidation, for example, hexanal (St. Angelo et al., 1987), this decrease in the concentration of potent odor-active thiols might contribute to the formation of the off-flavor.

CONCLUSION

The developed stable IDA are suitable to quantify precisely MFT, FFT, 3M2P, and 2M3P in heated meat samples and in broth.

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