Thermal Degradation of *S*-Methylcysteine and Its Sulfoxide—Important Flavor Precursors of *Brassica* and *Allium* Vegetables

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Two naturally occurring S-containing amino acids, *S*-methyl-L-cysteine sulfoxide and its biochemical precursor *S*-methyl-L-cysteine, were heated in closed model systems at different temperatures (from 80 to 200 °C) in the presence of variable amounts of water (0−98%) for 1−60 min. The arising volatile compounds were extracted with diethyl ether, analyzed, and identified by means of GC and GC/MS. It was found that thermally generated breakdown products of either *S*-methyl-L-cysteine or particularly *S*-methyl-L-cysteine sulfoxide can significantly contribute to the typical aroma of culinary processed *Brassica* and *Allium* vegetables. Nevertheless, degradation pathways of these closely related amino acids seem to be quite different. Dimethyl disulfide was identified as the predominant volatile compound generated by thermal degradation of both *S*-methylcysteine and its sulfoxide. Dimethyl trisulfide, dimethyl thiosulfinate, dimethyl thiosulfonate, and alkyl- and alkylthio-substituted pyridines were identified as minor volatile breakdown products arising from *S*-methylcysteine sulfoxide. Formation of dimethyl trisulfide, the major off-flavor principle of overcooked *Brassica* vegetables, was investigated in detail.

Keywords: *S-Methylcysteine; S-methylcysteine sulfoxide; MCSO; sulfur volatiles; flavor precursor; dimethyl disulfide; dimethyl trisulfide; Allium; Brassica*

INTRODUCTION

Important secondary metabolites of many vegetables are nonprotein sulfur-containing amino acids *S*-alk(en)-yl-L-cysteines and their sulfoxides. The most widely distributed among all *S*-alk(en)ylcysteine derivatives in plants appear to be *S*-methyl-L-cysteine and *S*-methyl-L-cysteine sulfoxide (MCSO, sometimes called methiin). These occur in all members of genus *Allium*, at least in two genera of *Brassicaceae* family (*Brassica* and *Raphanus*) and sporadically in the families *Compositae*, *Leguminosae* (genera *Phaseolus* and *Vigna*), and *Umbelliferae* in concentrations ranging between 0.2 and 4% of dry matter (Benevenga et al., 1989; Whitaker, 1976).

The characteristic flavor of these vegetables is generated partly due to the enzymatic degradation of these amino acids when their cellular tissue is disrupted by cutting, slicing, or chopping. In 1963, Mazelis initially characterized an enzyme present in *Brassica* that is released upon disruption of the plant tissue and catalyzed breakdown of *S*-alk(en)yl-L-cysteine sulfoxides, including methyl derivative, into pyruvate, ammonia, and the corresponding alk(en)ylsulfenic acid (Mazelis, 1963). This enzyme, subsequently termed cystine lyase (EC 4.4.1.8), is closely related to alliinase (EC 4.4.1.4) present in *Allium* vegetables, except that it is also able to cleave L-cystine and *S*-alkylcysteines (Hamamoto and Mazelis, 1986). However, culinary processing causes thermal denaturation of these enzymes, and thus a certain amount of the aroma precursors remains unreleased. These can subsequently themselves participate in developing the characteristic flavor of processed vegetables (Yu et al., 1994a−e; Ho et al., 1995; Kubec et al., 1997). Moreover, along with *S*-methylcysteine and its sulfoxide a considerable amount of both γ-glutamyl-*S*-methyl-L-cysteine and γ-glutamyl-*S*-methyl-L-cysteine sulfoxide was also found in the cellular tissue (about 0.1% of fresh weight) (Whitaker, 1976; Lawson et al., 1991). In that these dipeptides are not cleaved by above-mentioned enzymes, it can be assumed that they also significantly participate in flavor generation of thermally processed vegetables.

To the best of our knowledge, hitherto were published only two studies dealing with the thermal decomposition of MCSO in detail (Dateo et al., 1957; Ostermayer and Tarbell, 1960). In both, authors studied degradation of MCSO in boiling hydrochloric acid. They identified pyruvic acid, ammonia, dimethyl disulfide, and methyl methanethiosulfonate as the major breakdown products. Alanine, *S*-methyl-L-cysteine, and an unknown carbonyl compound were formed in small amounts. Nevertheless, during the previous 40 years any new or more detailed information about the thermal degradation of these important amino acids has not been published.

In the present study, the volatiles formed from thermally degraded *S*-methyl-L-cysteine sulfoxide and *S*-methyl-L-cysteine were identified and quantified to understand the formation mechanisms of volatile compounds generated during vegetable processing.

EXPERIMENTAL PROCEDURES

Synthesis of *S*-Methylcysteine and *S*-Methylcysteine Sulfoxide. *S*-Methylcysteine was synthesized by alkylation of L-cysteine with methyl iodide according to the procedure of Theodoropoulos (1959). (±)-*S*-Methyl-L-cysteine sulfoxide was

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10.1021/jf980379x CCC: $15.00
Published on Web 09/12/1998

Table 1. Stability of *S*-Methylcysteine Sulfoxide and Influence of Time, Temperature, and Water Content on Volatiles Formation

	time of heating[a] (min)						
	1	2	4	8	15	30	60
undecomp MCSO (%)	99.9	99.8	86.3	74.2	28.6	8.3	1.1
MeCys (mol %)[b]	nd[c]	nd	3.0	4.2	10.1	6.3	6.2
total volatiles (mg/g)[d]	0.12	0.55	4.0	14.6	55.6	70.9	85.2
conversion of sulfur (%)[e]	0.03	0.12	0.9	3.6	15.4	20.5	25.5

	temperature of heating[f] (°C)						
	80	100	120	140	160	180	200
undecomp MCSO (%)	73.8	61.6	1.1	0.9	0.3	0.1	tr
MeCys (mol %)[b]	nd	2.5	6.2	4.5	0.5	tr[g]	tr
total volatiles (mg/g)[d]	1.7	17.2	85.2	127.7	143.8	151.2	133.9
conversion of sulfur (%)[e]	0.4	5.0	25.5	39.0	41.5	41.8	35.2

	water content[h] (%)								
	0	5	10	20	40	80	90	95	98
undecomp MCSO (%)	22.0	14.6	1.1	0.5	1.0	6.0	7.6	8.5	14.8
MeCys (mol %)[b]	tr	0.8	6.2	6.2	3.2	1.9	0.7	0.2	tr
total volatiles (mg/g)[d]	0.86	23.2	85.2	109.4	110.7	106.4	96.0	47.9	18.4
conversion of sulfur (%)[e]	0.3	6.8	25.5	33.4	33.9	31.4	27.2	12.9	4.8

[a] At 120 °C and 10% water. [b] Expressed as moles of MeCys formed per mole of MCSO. [c] nd, not detected. [d] Amount of volatiles formed from 1 g of MCSO. [e] Expressed as moles of sulfur bound in volatiles per mole of MCSO. [f] For 60 min and 10% water. [g] tr, traces (<0.1%). [h] At 120 °C for 60 min.

obtained by oxidation of *S*-methylcysteine with hydrogen peroxide. Structures of synthesized amino acids were confirmed by ^{1}H NMR, ^{13}C NMR, and IR spectroscopy. Their purity (>99%) was checked by means of HPLC.

Synthesis of 2-(Methylthio)acetaldehyde (3-Thiobutanal). Chloroacetaldehyde (10 mL of 45% aqueous solution, 0.068 M, Fluka) was added dropwise to 50 mL of methanolic solution of sodium methanethiolate (0.068 M, Fluka) with external cooling and stirring. After 2 h the reaction mixture was extracted with 100 mL of diethyl ether, and an ether fraction was dried over sodium sulfate and evaporated. Purity of the obtained product was about 70% (based on the GC analysis).

Synthesis of Methyl Methanethiosulfinate. This compound was synthesized by oxidation of dimethyl disulfide with peracetic acid according to the method of Moore and O'Connor (1966).

Thermal Decomposition of Amino Acids. Amino acid (50 mg) was placed in a 5 mL glass tube, water was added, and the tube was sealed. Following equilibration for 24 h, the tube was heated in an oven at temperatures in the range of 80−200 °C, then cooled in the freezer to −18 °C, and crushed under water (total volume of 2.45 mL). The resulting solution was immediately extracted with 5 mL of diethyl ether. The extract obtained was dried using anhydrous sodium sulfate and analyzed by GLC without any further treatment.

Gas Chromatographic Analysis. A Hewlett-Packard 5890 chromatograph equipped with a flame ionization detector and a HP-5 or HP−INNOWax fused silica capillary column (30 m × 0.25 mm i.d.; film thickness of 25 μm; Hewlett-Packard) was used. The operating conditions were as follows: injector temperature, 180 °C, detector temperature, 250 °C; nitrogen carrier gas flow rate of 2 mL/min; and temperature program (for HP-5 column), 40 °C (3 min), raised at 4 °C to 240 °C (10 min), and (for HP−INNOWax column), 40 °C (3 min), raised at 4 °C to 190 °C (10 min); 1 μL of sample was injected, using a split ratio of 1:5. The amount of volatiles was estimated by computing the areas against that of the internal standard (2,6-di-*tert*-butyl-4-hydroxytoluene, BHT). The response factor of all compounds to the FID was assumed to be the same.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis. GC/MS analyses were carried out using a Hewlett-Packard G1800A chromatograph. The operating conditions were the same as described above, with the exception of a helium carrier gas flow rate of 0.6 mL/min. Mass spectra were obtained by EI ionization at 70 eV over the range of 15−300 mass units, with an ion source temperature of 250 °C.

Aroma Extract Dilution Analysis (AEDA). For AEDA experiments the effluent of the HP-5 column was split with a ratio of 1:1 to the FID and the sniffing port (SGE International, Australia) with addition of humidified air. The analyzed samples were diluted stepwise from 1:2 up to 1:16 with diethyl ether. Odor description and aroma thresholds were evaluated by three trained assessors.

High Performance Liquid Chromatographic Analysis (HPLC). Amino acids in the pyrolysates were detected as the *o*-phthaldialdehyde (OPA) derivatives using slightly modified method of Marks et al. (1992). Three parallel determinations were done.

Thin-Layer Chromatographic Analysis (TLC). Aqueous extracts of pyrolysates were analyzed on silica gel plates using either *n*-propanol/water (7:3 v/v) or *n*-butanol/acetic acid/water (4:1:1 v/v/v) as mobile phase. Spots of amino acids were visualized by spraying the plates with ninhydrin reagent (0.2% ethanolic solution).

Detection of Hydrogen Sulfide, Methanethiol, and Volatile Carbonyls. Amino acid (2 g) dissolved in 50 mL of water was heated under reflux in a 250 mL three-neck round-bottom flask at 180 °C for 1 h and arising volatiles were immediately purged with nitrogen stream (25 mL/min) into the train of 10 mL traps consisted of (a) 30% aqueous solution of lead acetate, (b) 4% aqueous solution of mercuric cyanide, and (c) saturated solution of 2,4-dinitrophenylhydrazine in 1 M sulfurous acid (Dateo et al., 1957). Alternatively, to detect methanethiol and acetaldehyde, 0.5 mL of the headspace gas was directly injected into GC using a 1 mL gastight syringe.

RESULTS AND DISCUSSION

Degradation of *S*-Methyl-L-cysteine Sulfoxide (MCSO). *S*-Methylcysteine sulfoxide appeared to be a rather thermolabile compound compared with commonly occurring protein amino acids. Its stability and volatiles formation as influenced by time of heating, temperature, and water content are summarized in Table 1. As can be seen, the rate of its degradation was strongly dependent not only on temperature and time of heating but also on water content in the reaction system. MCSO broke down almost completely during 1 h heating at temperature of 120 °C in the presence of

Table 2. Volatile Compounds Generated from *S*-Methylcysteine Sulfoxide at Different Temperatures[a]

		Kovats index		mg/g (°C)						
no.	compound identified	HP-5	Wax	80	100	120	140	160	180	200
1	methanol		<1000	0.21	0.24	0.36	0.68	1.09	1.22	0.32
2	pyridine		1186				0.10	0.31	0.37	0.53
3	dimethyl disulfide	746	1082	0.49	11.18	66.18	95.99	102.52	103.22	83.93
4	2-methylpyridine	816	1219	0.05	0.08	0.67	1.53	2.31	2.71	3.34
5	ethyl methyl disulfide	838	1151					0.04	0.13	0.34
6	3-methylpyridine	864	1292				0.17	0.42	0.55	0.86
7	4-methylpyridine	864	1298				0.27	0.51	0.64	1.20
8	2,4-dithiapentane	889	1289		tr[b]	0.06	0.08	0.12	0.12	0.08
9	2,4-dimethylpyridine	932	1324				0.19	0.55	1.10	1.18
10	3-ethylpyridine	959	1401				0.35	0.87	1.20	1.36
11	dimethyl trisulfide	970	1381	0.06	1.00	8.93	11.00	11.81	14.57	15.24
12	dimethyl thiosulfinate	979		0.11	0.15					
13	2-methyl-5-ethylpyridine	1023	1409		tr	0.76	2.64	4.64	6.31	6.56
14	2-methyl-5-ethenylpyridine	1040	1509		tr	0.33	0.79	0.82	0.63	0.48
15	dimethyl thiosulfonate	1068	1998	0.73	3.26					
16	3-(methylthio)pyridine	1152	1803			0.21	0.93	1.78	2.08	2.28
17	5-acetyl-2-methylpyridine	1189	1876				0.06	0.08	0.13	0.29
18	methyl (methylthio)methyl disulfide	1211	1666				0.08	0.07	0.04	0.02
19	methyl (methylthio)pyridine	1214	1818	tr	0.72	5.45	8.04	9.81	10.49	10.17
20	methyl (methylthio)pyridine	1214	1852				0.12	0.14	0.13	0.13
21	methyl (methylthio)pyridine	1259	1901			0.18	0.51	0.70	0.72	0.78
22	unknown1 ($C_6H_{14}S_3$)	1355	2038		0.18	0.26	tr			
23	dimethyl (or ethyl)-5-[(methylthio)methyl]pyridine	1358	1960				0.75	1.58	1.78	2.02
24	2,3,5-trithiahexane-5-oxide	1395			0.10					
25	ethyl methyl (methylthio)pyridine	1424	1979			0.13	0.21	0.30	0.39	0.44
26	ethyl methyl (methylthio)pyridine	1430				0.16	0.21	0.26	0.26	0.28
27	2,3,5-trithiahexane-5,5-dioxide	1447			0.13					
28	dimethyl (or ethyl) [(methylthio)methyl]pyridine	1448	2233				0.27	0.57	0.54	0.55
29	bis(methylthio)pyridine	1565				0.13	0.41	0.45	0.42	0.38
30	2,3,6,7-tetrathiaoctane	1589					0.65	0.95	0.38	0.12
31	methyl bis(methylthio)pyridine	1627	2029		0.14	0.99	1.19	0.64	0.65	0.66
32	methyl bis(methylthio)pyridine	1677					0.38	0.37	0.28	0.24
33	unknown2 ($C_7H_{16}S_4$)	1695			tr	tr				
34	2,5-dithiahexane		1479				0.06	0.12	0.12	0.14

[a] Heated for 1 h in the presence of 10% water. [b] tr, traces (<0.02 mg/g).

10−40% of water. When it was heated dry or conversely in more diluted samples, it degraded somewhat slower. It decomposed to form partly volatiles, partly nonvolatile compounds; among them *S*-methylcysteine, alanine, and pyruvic acid were positively identified by means of HPLC and TLC. *S*-Methylcysteine is likely formed through the reduction of MCSO by dimethyl thiosulfinate, dimethyl disulfide, or methyl mercaptan, while alanine might be generated via amination of pyruvic acid or via a reductive cleavage of sulfoxide group as proposed by Ostermayer and Tarbell (1960). On the other hand, neither cysteine nor any other OPA- or ninhydrin-positive compounds were detected. However, effort was focused mainly on the formation of volatile compounds; therefore, we have not studied arising nonvolatile breakdown products in detail.

Influence of Temperature. A total of 34 compounds generated from *S*-methylcysteine sulfoxide were identified as listed in Table 2. Among them were present 24 sulfur-containing volatiles. Additionally, three other compounds were positively detected using headspace analyses, namely acetaldehyde, methanethiol, and dimethyl sulfide. These compounds could not be quantified because of their overlapping by solvent peak. As can be seen, dimethyl disulfide (**3**) was generated as the predominant volatile compound. With a single exception it represented more than 60% of total volatiles. This corresponds well with the results of Yu et al. (1995), who found dimethyl disulfide as the dominant volatile compound arising from thermally degraded methionine sulfoxide, the nearest homologue of MCSO. Dimethyl trisulfide (**11**), the second most abundant volatile, was formed in considerably smaller scale and its content increased with temperature. However, quite surprising was the absence of detectable amounts of dimethyl tetrasulfide. Significant amounts of various pyridines (both alkyl- and alkylthio-substituted) were also identified in the model systems, in particular at higher temperatures. These compounds are proposed to be mainly generated from the interactions of ammonia, one of the thermal degradation product of MCSO, and aldehydes. These pyridines identified are considered to be responsible for the pyridine-like odor detected in the model systems. On the other hand, none of the pyrazines were detected under any conditions. As can be seen in Table 1, into volatiles was incorporated 25.5−41.8% of the starting amount of sulfur bound in MCSO (at temperatures between 120 and 200 °C). The fate of the remaining sulfur is still questionable. Substantial amounts of sulfur were undoubtedly bound in hydrogen sulfide, dimethyl sulfide, methyl mercaptan (all were positively identified), sulfur dioxide, and elementar sulfur as well as in various pigments and other macromolecules.

Influence of Time of Heating. The amount of dimethyl disulfide, dimethyl trisulfide, and most of the pyridines increased with time. No surprise that the total amount of volatiles arising increased with time as well (see Table 1). On the contrary, the content of dimethyl thiosulfinate and dimethyl thiosulfonate reached the maximum in 8 and 15 min, respectively, and decreased with prolonged time of heating (not shown).

Influence of Water Content. Generally ignored reaction parameter, namely water content, considerably influenced not only the degradation rate but also breakdown products structure, as shown in Table 3. The

Table 3. Volatile Compounds Generated from *S*-Methylcysteine Sulfoxide at Different Water Content[a]

		mg/g (%)								
no.	compound identified	0	5	10	20	40	80	90	95	98
1	methanol	0.06	0.24	0.36	0.42	0.45	0.47	0.57	1.43	1.16
3	dimethyl disulfide	0.57	12.95	66.18	92.46	94.06	86.62	66.60	17.26	2.59
4	2-methylpyridine		0.43	0.67	0.58	0.35	0.7	0.61	0.37	0.29
8	2,4-dithiapentane		tr[b]	0.06	0.06	0.06	0.10	0.05		
11	dimethyl trisulfide	0.16	6.60	8.93	7.00	5.94	2.50	2.62	1.50	1.16
12	dimethyl thiosulfinate			0.19	0.28	0.37	1.03	2.24	16.40	9.76
13	2-methyl-5-ethylpyridine	tr	0.42	0.76	0.71	0.78	2.58	3.44	1.27	
14	2-methyl-5-ethenylpyridine		0.20	0.33	0.29	0.21	0.28	0.30	0.12	
15	dimethyl thiosulfonate			0.22	0.35	0.74	2.33	12.39	6.45	2.70
16	3-(methylthio)pyridine			0.21	tr					
19	methyl (methylthio)pyridine	0.07	2.02	5.45	4.92	3.81	3.64	3.23	0.72	
21	methyl (methylthio)pyridine		tr	0.18	0.15	0.14	0.30	0.23		
22	unknown1 ($C_6H_{14}S_3$)		0.11	0.26	1.13	2.38	3.73	2.44	0.12	
24	2,3,5-trithiahexane-5-oxide							0.29	1.52	0.58
25	ethyl methyl (methylthio)pyridine		tr	0.13	0.10	0.15	0.15	0.12		
26	ethyl methyl (methylthio)pyridine		tr	0.16	0.13	tr	tr			
27	2,3,5-trithiahexane-5,5-dioxide							0.37	0.73	0.14
29	bis(methylthio)pyridine			0.13	tr	tr				
31	methyl bis(methylthio)pyridine		0.27	0.99	0.68	0.52	0.51	0.38	tr	
33	unknown2 ($C_7H_{16}S_4$)			tr	0.11	0.69	1.41	0.11		

[a] Heated at 120 °C for 1 h. [b] tr, traces (<0.02 mg/g).

Table 4. Aroma Extract Dilution Analysis of MCSO Pyrolysate[a]

no.	compound	FD[b]	odor description
3	dimethyl disulfide	4	cauliflower, cabbage-like
11	dimethyl trisulfide	16	spoiled, cooked cabbage-like
12	dimethyl thiosulfinate	4	cabbage, cauliflowerlike
15	dimethyl thiosulfonate	8	cooked cabbage-like
19	methyl (methylthio)pyridine	2	ammonia, pyridine-like

[a] Heated at 120 °C for 1 h, 90% water. [b] FD - flavor dilution factor.

maximum amount of volatiles arose in the presence of 20−80% of water and decreased greatly both at higher and lower water amounts (see Table 1). Dimethyl disulfide was identified as the predominant volatile in most of samples. Nevertheless, the content of thermolabile dimethyl thiosulfinate and its derivatives, namely dimethyl thiosulfonate (**15**) and 2,3,5-trithiahexane oxides (**24**, **27**), considerably increased with water amount probably due to the stabilization effect of water. Such conditions can be expected especially during cooking, while on frying, baking, roasting, etc. water content greatly decreases (especially in the outer layers of foods), and the formation of unpleasant smelling dimethyl disulfide, trisulfide, and pyridines will prevail.

Proposed Volatiles Formation Pathways. It seems to be indisputable that the first step involved in the volatiles formation is a cleavage of MCSO into methanesulfenic acid and α-aminoacrylic acid as proposed by Ostermayer and Tarbell (1960). The latter compound can spontaneously hydrolyze giving ammonia and pyruvic acid, which might further decarboxylate to acetaldehyde. The self-condensation of methanesulfenic acid leads to the formation of dimethyl thiosulfinate, the key breakdown product. This can easily decompose into dimethyl disulfide and dimethyl thiosulfonate or alternatively might form 2,3,5-trithiahexane oxides (**24**, **27**). The latter mechanism, first reported by Block and O'Connor (1973), is favored in the presence of enhanced water amount. The pathway leading to dimethyl trisulfide formation seems to be more complicated. No mechanism is generally accepted, but a few have been proposed. Maruyama (1970) predicted its generation through the reaction of two molecules of methanesulfenic acid with hydrogen sulfide, while Boelens et al. (1971) speculated that it could be a product of dimethyl disulfide interaction with elementar sulfur. Chin and Lindsay (1994a) and Nedjma and Hoffmann (1996) found that dimethyl trisulfide can arise from methyl mercaptan in the presence of metal ions or ascorbic acid. The formation of trisulfides via decomposition of disulfides is also very well-known. Recently, Chin and Lindsay (1994b,c) proposed a pathway based on the interaction of both dimethyl thiosulfinate and thiosulfonate with hydrogen sulfide. Therefore, we carried out a series of experiments to resolve this controversy. It was found that decomposition of dimethyl disulfide took place only in the small degree at temperatures below 200 °C. Also the mechanism of Boelens et al. appears to be inaccurate, since the generation of adequate amounts of elementar sulfur is highly improbable, especially in the early stages of MCSO degradation as well as at low temperatures. Furthermore, the negligible amounts of dimethyl trisulfide arising from *S*-methylcysteine (see Table 5) indicate that the key compound(s) involved in its formation is (are) related to methanesulfenic acid and/or its derivatives. On the basis of our findings, we believe that the self-degradation of dimethyl thiosulfinate is the most important pathway leading to the dimethyl trisulfide generation. This decomposition can be strongly accelerated by the interaction with hydrogen sulfide as predicted by Chin and Lindsay (1994b,c). However, it cannot be excluded even the parallel reaction of hydrogen sulfide with methanesulfenic acid and/or dimethyl thiosulfonate. The proposed formation of volatiles from thermally degraded MCSO is schematically shown in Figure 1.

Sensory Properties. Color formation (usually dark brown) during the thermal decomposition of MCSO was very intensive, indicating that besides the formation of volatile compounds some high molecular weight nonvolatile polymers and pigments should also be generated. The resulting odor of degraded MCSO samples could be generally described as unpleasant, spoilt, or overcooked cabbage-like with strong ammonia and pyridine-like notes. At higher temperatures burnt, sulfury, and biting odors prevailed. The contribution of the individual components to the overall aroma as determined by aroma extract dilution analysis (Grosch,

Table 5. Volatile Compounds Generated from *S*-Methylcysteine at Different Temperatures[a]

		Kovats index		mg/g (°C)						
no.	compound identified	HP-5	Wax	80	100	120	140	160	180	200
3	dimethyl disulfide	746	1082	tr[b]	0.06	0.15	0.23	0.49	19.27	28.11
4	2-methylpyridine	816	1219						0.05	0.36
8	2,4-dithiapentane	889	1289						0.06	0.03
11	dimethyl trisulfide	970	1381						0.08	0.11
13	2-methyl-5-ethylpyridine	1023	1409						0.19	0.26
16	3-(methylthio)pyridine	1152	1803						0.16	0.40
18	methyl (methylthio)methyl disulfide	1211	1666						0.22	0.32
19	methyl (methylthio)pyridine	1214	1818						0.94	1.87
20	methyl (methylthio)pyridine	1214	1852						0.17	0.35
21	methyl (methylthio)pyridine	1259	1901						0.23	0.74
25	ethyl methyl (methylthio)pyridine	1424	1979						0.20	1.25
26	ethyl methyl (methylthio)pyridine	1430							tr	0.18
29	bis(methylthio)pyridine	1565								0.82
31	methyl bis(methylthio)pyridine	1627	2029						tr	2.19
34	2,5-dithiahexane		1479						0.18	0.27
35	2-(methylthio)acetaldehyde		1270						0.05	0.07
36	2-(methylthio)ethanol		1537						0.21	0.59
	total			tr	0.06	0.15	0.23	0.49	22.01	37.92
	conversion of sulfur (%)[c]			tr	0.02	0.04	0.07	0.14	5.88	9.27
	undecomposed MeCys (%)			96.3	84.6	62.1	47.4	38.0	31.2	15.5

[a] Heated for 1 h in the presence of 10% water. [b] tr, traces (<0.02 mg/g). [c] Expressed as moles of sulfur bound in volatiles per mole of MeCys.

Table 6. Mass Spectra of Newly Tentatively Identified, Unknown, and Unusual Compounds Arising from Thermally Degraded *S*-Methylcysteine and *S*-Methylcysteine Sulfoxide

no.	compound	MS, *m/e* (rel intensity)
12	dimethyl thiosulfinate	110 (50, M^+), 95 (29), 94 (14), 79 (10), 64 (100), 63 (42), 48 (18), 47 (73), 46 (44), 45 (77)
15	dimethyl thiosulfonate	126 (80, M^+), 111 (7), 95 (2), 81 (100), 79 (62), 65 (14), 64 (37), 63 (67), 47 (79), 46 (27), 45 (49)
19	methyl (methylthio)pyridine	139 (100, M^+), 124 (66), 106 (2), 97 (15), 92 (7), 80 (18), 65 (6), 53 (12), 45 (8), 39 (9)
20	methyl (methylthio)pyridine	139 (100, M^+), 124 (47), 106 (25), 97 (22), 93 (35), 80 (19), 65 (15), 51 (12), 45 (11), 39 (52)
21	methyl (methylthio)pyridine	139 (100, M^+), 124 (28), 106 (7), 97 (18), 92 (19), 80 (11), 65 (11), 53 (16), 45 (12), 39 (16)
22	unknown1 ($C_6H_{14}S_3$)	182 (0.5, M^+), 155 (14), 153 (100), 137 (9), 135 (92), 120 (8), 107 (18), 91 (69), 76 (6), 59 (17), 47 (11), 45 (30)
23	dimethyl (or ethyl)-5-[(methylthio)methyl]pyridine	167 (17, M^+), 152 (2), 136 (2), 121 (10), 120 (100), 104 (2), 92 (6), 91 (7), 77 (9), 65 (5), 51 (4), 39 (4)
24	2,3,5-trithiahexane-5-oxide	156 (2, M^+), 140 (2), 124 (0.5), 108 (1), 95 (11), 94 (36), 93 (100), 79 (20), 78 (9), 64 (11), 63 (22), 61 (19), 47 (24), 46 (41), 45 (85)
25	ethyl methyl (methylthio)pyridine	167 (100, M^+), 166 (12), 152 (68), 134 (13), 120 (12), 106 (13), 91 (9), 77 (15), 65 (11), 51 (9), 45 (7), 39 (10)
26	ethyl methyl (methylthio)pyridine	167 (100, M^+), 166 (30), 152 (45), 139 (8), 120 (8), 111 (17), 104 (7), 91 (8), 77 (12), 65 (9), 51 (7), 45 (9), 39 (10)
27	2,3,5-trithiahexane-5,5-dioxide	172 (12, M^+), 157 (0.5), 142 (3), 125 (0.5), 111 (0.5), 95 (9), 93 (100), 79 (8), 78 (7), 63 (4), 47 (10), 46 (12), 45 (39)
28	dimethyl (or ethyl) [(methylthio)methyl]pyridine	167 (33, M^+), 120 (6), 119 (16), 107 (7), 106 (30), 91 (5), 77 (14), 65 (6), 63 (7), 61 (100), 51 (6), 39 (8)
29	bis(methylthio)pyridine	173 (10, $M^+ + 2$), 171 (100, M^+), 155 (4), 141 (14), 138 (18), 125 (10), 123 (10), 114 (11), 112 (7), 97 (5), 92 (6), 85 (13), 82 (13), 69 (9), 45 (13)
30	2,3,6,7-tetrathiaoctane	188 (3, $M^+ + 2$), 186 (22, M^+), 171 (0.5), 154 (1), 139 (3), 107 (17), 106 (72), 95 (6), 81 (14), 79 (100), 64 (8), 61 (12), 60 (20), 45 (19)
31	methyl bis(methylthio)pyridine	187 (10, $M^+ + 2$), 185 (100, M^+), 170 (32), 155 (7), 152 (8), 139 (29), 138 (8), 124 (14), 122 (12), 114 (8), 95 (6), 85 (14), 69 (9), 51 (4), 45 (10)
32	methyl bis(methylthio)pyridine	187 (9, $M^+ + 2$), 185 (100, M^+), 170 (13), 155 (8), 152 (12), 139 (7), 138 (8), 136 (9), 124 (8), 106 (5), 92 (8), 69 (6), 51 (6), 45 (10)
33	unknown2 ($C_7H_{16}S_4$)	228 (0.1, M^+), 181 (19), 155 (14), 153 (100), 138 (5), 106 (8), 91 (54), 76 (4), 59 (4), 47 (6), 45 (10)
35	2-(methylthio)acetaldehyde	92 (3, $M^+ + 2$), 90 (45, M^+), 61 (100), 47 (16), 46 (13), 45 (24)

1993; Etiévant et al., 1994) is shown in Table 4. As can be seen, among 17 volatiles identified in the evaluated sample (see Table 3) only a few of them significantly influenced the resultant odor (apart from extremely volatile compounds, such as ammonia, methyl mercaptan, and hydrogen sulfide, which could not be analyzed using AEDA). Dimethyl trisulfide was identified as the most important sensory-impact compound, whereas dimethyl disulfide, the entirely dominant volatile, played only a minor role. This is in good agreement with the finding of Maruyama (1970), who identified dimethyl trisulfide as the major aroma component of cooked *Brassica* vegetables despite it was not as abundant as dimethyl disulfide.

Degradation of *S*-Methyl-L-cysteine (MeCys). This amino acid seemed to be essentially more stable in comparison with MCSO. As shown in Table 5, significant amounts of volatiles were formed at temper-

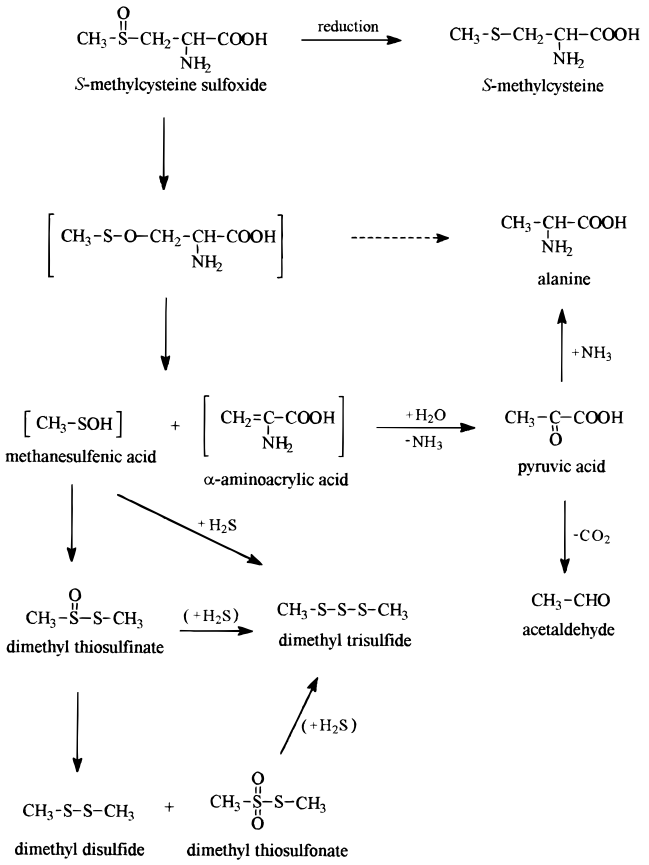

Figure 1. Thermal degradation of *S*-methylcysteine sulfoxide (simplified).

atures beyond 180 °C. The differences in the stability and yields of volatile compounds between MCSO and MeCys could result from different degradation pathways of these closely related amino acids. While the decomposition of MCSO presumably begins in the very reactive sulfoxide region, the molecule of *S*-methylcysteine misses any such extremely thermolabile moiety. Thus, decarboxylation and deamination are probably the most important mechanisms involved in the thermal breakdown of MeCys at temperatures lower than 160 °C. Beyond this temperature a homolytic cleavage of the labile C−S bond can occur, resulting in the formation of methylthio radicals. These can consequently combine to give dimethyl disulfide as the major volatile compound and together with (methylthio)methyl radicals also some other components, such as 2,4-dithiapentane (**8**), methyl (methylthio)methyl disulfide (**18**), and 2,5-dithiahexane (**34**). 2-(Methylthio)acetaldehyde (**35**) was found in small amounts as a result of the Strecker degradation of MeCys. This interesting aldehyde, the lower homologue of methional, was first reported by Buttery et al. (1971) in tomato volatiles. It smells similar to methional and resembles cooked potatoes with a slight earthy odor. However, in our hands it appeared to be quite unstable and during a few days polymerized to form dark brown pigments. It is reasonable to assume that most of the arising 2-(methylthio)-acetaldehyde readily reacted with ammonia and other amino compounds giving pyridines and various polymers. Hydrogen sulfide was generated from MeCys at high temperatures forming a precipitate in the lead acetate trap. In agreement with the study of Gruenwedel and Patnaik (1971), no precipitate was observed in the mercuric cyanide trap, indicating the absence of volatile mercaptans. GC analysis of the headspace gas confirmed the absence of both methyl mercaptan and acetaldehyde as well. Anyway, the formation of methyl mercaptan could greatly increase in the presence of other food components (e.g. pyridoxal or some metal ions). The formation of neither cysteine, alanine, nor any other OPA- or ninhydrin-positive compounds was observed under any conditions.

Authenticity of Identified Compounds. Mass spectra of newly tentatively identified, unknown, and some unusual compounds arising from thermally degraded *S*-methylcysteine and its sulfoxide are listed in Table 6. Although volatiles identified in this study were generated at relatively high temperatures, it could not be excluded that some of the listed components are artifacts produced during GC analyses. It is very well-known that many sulfur volatiles (in particular these containing the sulfoxide group, such as thiosulfinates and their derivatives) are extremely labile compounds, which easily break down at elevated temperature. Furthermore, Block et al. (1992) reported that thiosulfinates did not survive gas chromatographic analysis when a polar stationary phase was used. It corresponds with our findings, since we were not able to detect dimethyl thiosulfinate (**12**), 2,3,5-trithiahexane-5-oxide (**24**), and 2,3,5-trithiahexane-5,5-dioxide (**27**) using a HP−INNOWax column due to their complete decomposition. We also paid sharp attention to the injector temperature. Therefore, three different temperatures were investigated, namely 140, 180, and 220 °C. It was found that 93% of dimethyl thiosulfinate survived the injector temperature of 180 °C, whereas at a temperature of 220 °C only 18% of the initial thiosulfinate amount did not decompose (as against the temperature of 140 °C). Anyway, despite a slight dimethyl thiosulfinate breakdown, we used an injector temperature of 180 °C to avoid uncomplete volatilization of the sample.

CONCLUSIONS

In summary, nonvolatile amino acid precursors undoubtedly play a significant role in developing the characteristic flavor of processed *Brassica* and *Allium* vegetables. Moreover, it seems that *S*-methylcysteine sulfoxide decomposes both thermally and enzymatically in the same way, giving the identical primary breakdown products (dimethyl thiosulfinate, pyruvic acid, and ammonia). Amounts of volatiles formed are strongly dependent on temperature and time of heating as well as on water content. However, needless to emphasize, in real food systems we can expect their decomposition to be much more complicated. It is necessary to take into account many additional factors, in particular various pH values, the presence of sugars, lipids, prooxidants (ascorbic acid, metal ions), and other *S*-alk(en)ylcysteine derivatives or glucosinolates (in *Allium* or *Brassica* vegetables, respectively).

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Received for review April 14, 1998. Revised manuscript received July 8, 1998. Accepted July 9, 1998. This work was supported in part by the Research Grant 509/94/0318 from the Grant Agency of the Czech Republic.

JF980379X