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Volatile Components of the Thermal Degradation of Cystine in Water

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Thermal degradation of cystine in aqueous solutions was studied by using a closed model system. The degradations were carried out at pH's of 5.5 and 2.3 and at 160 °C, representing roasting temperature. Forty-two volatile compounds were identified at pH 5.5 and twenty-three at pH 2.3. Three novel sulfides were found in the pH 5.5 volatile mixture: ethyl 1-(ethylthio)ethyl disulfide and the corresponding tri- and tetrasulfides. Only the disulfide was found at pH 2.3. The organoleptic character of the disulfide was sulfury, roasted, and oniony. A mechanism for the formation of these sulfides is proposed. Fifty-five percent of the volatile yield at pH 2.3 were 1,2,4-trithiolanes. Mechanisms for the formation of secondary products are discussed.

INTRODUCTION

Sulfur-containing amino acids are generally recognized as very important precursors of food flavors (Hurrell, 1982; Ching, 1979). Thermal degradation of amino acids produces the corresponding amines via degradation (Lien and Nawar, 1974; de Rijke et al., 1981). Sulfur-containing amino acids also form additional breakdown products which are highly reactive and can generate various types of heterocyclic compounds (Fujimaki et al., 1969; Boelens et al., 1974; Sakaguchi and Shibamoto, 1978).

Obata and Tanaka (1965) studied the photolysis of cystine and cysteine and observed that hydrogen sulfide, ammonia, carbon dioxide, and acetaldehyde were evolved. Fujimaki et al. (1969) pyrolyzed cystine and cysteine separately at 270–300 °C at reduced pressure under nitrogen. They identified several highly volatile compounds including ethylamine, mercaptoethylamine, hydrogen sulfide, ammonia, acetaldehyde, and 2-methylthiazolidine by using GC and classic derivatization methods. They also proposed the mechanism of the formations of those volatiles. Later, Kato et al. (1973) in a similar pyrolysis study identified additional compounds including thiazoles, pyridines, and several thiophenes. Ledl (1976) heated cystine/cysteine in soybean oil at 200 °C and formed various heterocycles including 1,2-dithiane, 3,5-dimethyl-1,2,4-trithiolane, and 3-methyl-1,2,4-trithiane.

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Boelens et al. (1975) studied the degradation of cystine/cysteine in terms of the primary products and secondary products. They postulated a mechanism for the interaction between acetaldehyde and hydrogen sulfide which formed 2,4,6-trimethyl-1,3,5-trithiane, 2,4,6-trimethyl-1,3,5-dithiazine, 3,5-dimethyl-1,2,4-trithiolane, and various sulfides.

The volatile products of thermal degradation at 160 °C of aqueous cystine at pH 5.5 and pH 2.3 in a closed system were identified in this study. Mechanisms for the formation of the novel compounds generated are also presented.

EXPERIMENTAL SECTION

Sample Preparation by Parr Bomb, A Closed Model System. A mixture of 0.05 mol L-cystine (Ajinomoto Co., Tokyo, Japan) and 500 g of distilled water was placed in a 2-L Parr Bomb (Parr Instruments Co., Moline, IL) equipped with a magnetic stirrer, an internal cooling coil, and a temperature controller. The pH of the mixture was measured as 5.5. The reaction mixture was heated to 160 °C for $^1/_2$ h and then allowed to cool to room temperature. Another sample was prepared in a similar fashion, however, the initial pH was adjusted to 2.3 with 1% HCl.

Isolation of the Volatiles. The reaction mass (200 g) was diluted with 200 mL of distilled water and steam distilled under a vacuum (10–12 mmHg) at 15–18 °C for 1 h and 45 min. Approximately 500–550 mL of distillate was obtained. The total distillate was saturated with sodium chloride and extracted, in a 1-L separatory funnel, with 100 mL of methylene chloride three times. The combined extracts were washed twice with 50% NaCl saturated water, dried over anhydrous magnesium sulfate, and then filtered through Whatman no. 1 filter paper. After filtration, the extract was concentrated to about 5 mL with a Kurdena-Danish apparatus fitted with a Vi-
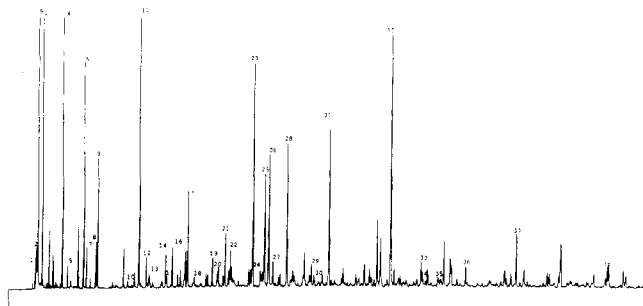
Figure 1. The GC profile of the volatiles from the degradation of cystine at pH 5.5 (OV-1 column). Column: OV-1 fused silica capillary (50 m × 32 mm). Temperature: 50–225 °C programmed at 2 °C/min. Detector: flame ionization.

greaux distillation column and then slowly concentrated further under a stream of nitrogen to about 100–300 mg. The percentage of volatiles in the vial was calculated by using a GC area normalization method; in turn, the total quantity of volatiles in the reaction mass was estimated.

Gas Chromatographic Analysis. Samples were analyzed on a Hewlett-Packard (H/P) gas chromatograph (GC) equipped with a flame-ionization detector (FID) and two fused silica capillary columns (Carbowax 20M and OV-1, 50 m × 0.32 mm each) programmed from 50 to 225 °C at 2 °C/min with a carrier gas (H) flow rate of 27 cm/s.

Isolation of the Individual Components by GC Trapping. The individual novel components were isolated from a GC unit, Varian 3700/FID, employing a wide bore glass capillary column (30 m × 0.62 mm, OV-1) under the similar chromatographic conditions as previously described.

Identification. Identification of most components was based on gas chromatography–mass spectrometry (GC–MS) and the retention index (t_R) information. Novel compounds were spectroscopically identified by interpretation of nuclear magnetic resonance (NMR) and mass spectra.

(1) GC–MS Analysis. Samples were analyzed by GC–MS on a Kratos MS-50L mass spectrometer/Kratos DS-55 data system by using an EI of 70 eV and a Varian 3700 GC unit equipped with the same fused silica capillary columns and operated under the same conditions as described previously.

(2) Retention Index. Retention indices (t_R values) of the individual components were calculated by using the system developed by van den Dool and Kratz (1963). A comparison between the t_R values of known chemicals and those GC peaks from both polar and nonpolar columns provides the confirmatory identification from GC–MS.

(3) NMR Analysis. Proton NMR spectra were determined at 100 MHz in CFCl$_3$ on a Varian XL-100 spectrometer modified with ^{19}F internal lock by using the microsampling technique of Ledig and Jacobs (1978). Chemical shifts are expressed in parts per million with tetramethylsilane as an internal standard.

Organoleptic Evaluation. The individual novel compounds trapped from the GC were diluted in an appropriate amount of water and evaluated by Dr. Manfred Vock, Chief Flavorist of IFF.

RESULTS AND DISCUSSION

Figures 1 and 2 show the GC profiles of the volatiles from the degradation of aqueous cystine at pH 5.5 from OV-1 and Carbowax 20M columns, respectively. Table I lists the components identified along with the peak identification numbers, quantitative data, and the mass spectrum references. The components unidentified are not
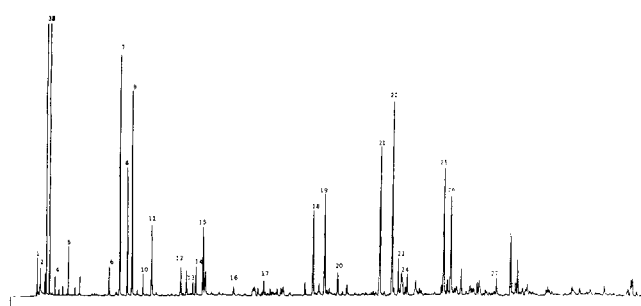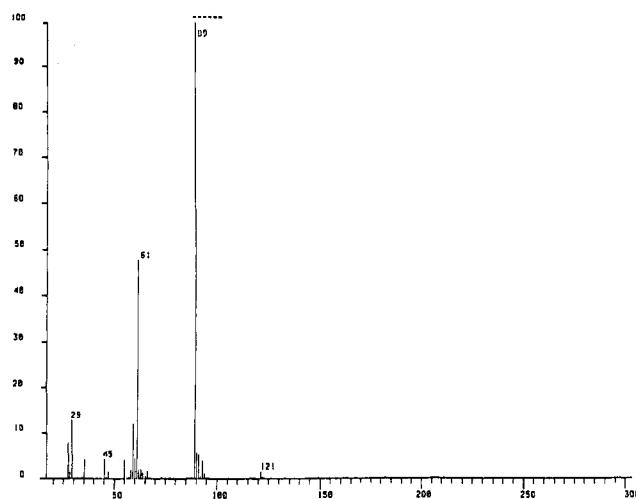

Figure 2. The GC profile of the volatiles from the degradation of cystine at pH 5.5 (CWX column). Column: Carobwax 20M, fused silica capillary (50 m × 0.32 mm). Temperature: 50–225 °C programmed at 2 °C/min. Detector: flame ionization.

Figure 3. Mass spectrum of peak no. 32 from OV-1 column.
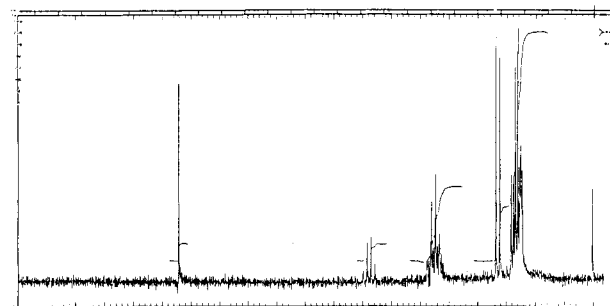

Figure 4. Proton NMR spectrum of peak no. 32 from OV-1 column.

numbered for their peaks in the chromatograms. Except the novel compounds, all of the components were identified by GC–MS. It is apparent that most of the components identified are sulfur containing, of which thiazoles, sulfides, thiolanes, and thianes are dominating.

Of particular interest are the identifications of the three novel sulfides. From this sample, GC–MS analysis revealed three mass spectra with very similar fragmentation patterns which corresponded to peaks no. 32, 37, and 38 from the OV-1 column (Figure 1). Figure 3 shows the mass spectrum of peak no. 32. Major peaks are at m/e 89 and m/e 61, which contain one sulfur, indicating common fragments of $C_4H_9S^+$ and $C_2H_5S^+$, respectively. No apparent molecular ion peak was present. The proton NMR spectrum of peak no. 32 (Figure 4) shows the following signals: a quartet at 3.86 δ (1 H, J = 7 Hz), a complex multiplet at 2.55–2.9 δ (4 H), a doublet at 1.65 δ (3 H, J = 7 Hz), and two triplets at 1.30 and 1.34 δ (3 H each, J = 7 Hz). The signals at 3.81 and 1.65 δ indicate an
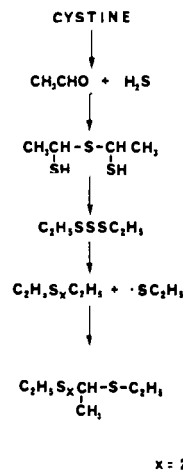
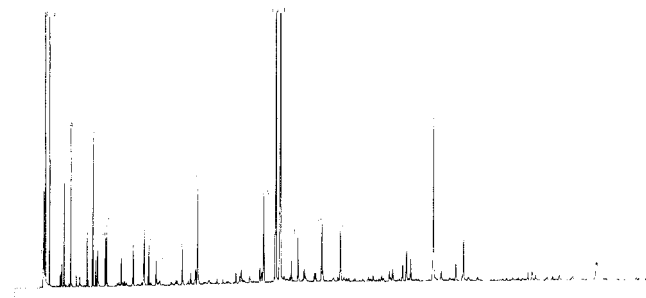**Figure 5.** Formation mechanism proposed for the three novel sulfides.

Figure 6. The GC profile of the volatiles from the degradation of cystine at pH 2.3 (OV-1 column). Column: OV-1, fused silica capillary (50 m × 0.32 mm). Temperature: 50–225 °C programmed at 2 °C/min. Detector: flame ionization.

Figure 7. The GC profile of the volatiles from the degradation of cystine at pH 2.3 (CWX column). Column: Carbowax 20M, fused silica capillary (50 m × 0.32 mm). Temperature: 50–225 °C programmed at 2 °C/min. Detector: flame ionization.

Figure 8. Formation mechanism proposed for some sulfur-containing compounds.

$XCHCH_3Y$ subunit where X and Y are electronegative groups. The remaining signals describe two slightly different ethyl groups. The non-first-order appearance of the spectrum suggested the presence of a chiral center. Therefore, the structure is likely to be $C_2H_5XCHCH_3YC_2H_5$. Expected fragments $C_2H_5SCHCH_3^+$ and $C_2H_5S^+$ would account for m/e 89 and 61, respectively. In addition, a small peak at m/e 182 appeared to be the perfect candidate for the molecular ion. Consequently, the structure has been identified as $C_2H_5SCHCH_3SSC_2H_5$ or ethyl 1-(ethylthio)ethyl disulfide. In the same manner, peak no. 37 was identified as the corresponding trisulfide or ethyl 1-(ethylthio)ethyl trisulfide. Logically the third compound, peak no. 38 was identified as the corresponding tetrasulfide or ethyl 1-(ethylthio)ethyl tetrasulfide, though no molecular ion peak appeared.

The mechanism proposed for the formation of these three compounds is presented in Figure 5. Acetaldehyde and hydrogen sulfide which are the primary degradation products from cystine form a mixture of different sulfides through a series of reactions including the disproportionations. A radical reaction could then take place to generate these sulfides as described in Figure 5.

In a sensory evaluation, the ethyl 1-(ethylthio)ethyl disulfide from the GC trap was described as sulfury, roasted, and oniony.

Figures 6 and 7 represent the GC profile of the volatiles from the degradation of cystine under the same condition as the previous experiment except at pH 2.3. Table I also includes the components identified from this experiment. From this table, it is revealed that the 3,5-dimethyl-1,2,4-trithiolanes and 3,6-dimethyl-1,2,4,5-tetrathiane are formed more readily at pH 2.3 than at pH 5.5, and ethyl
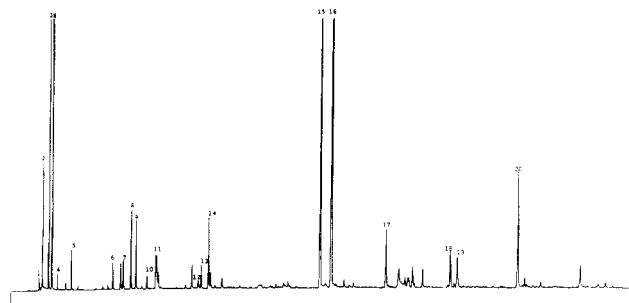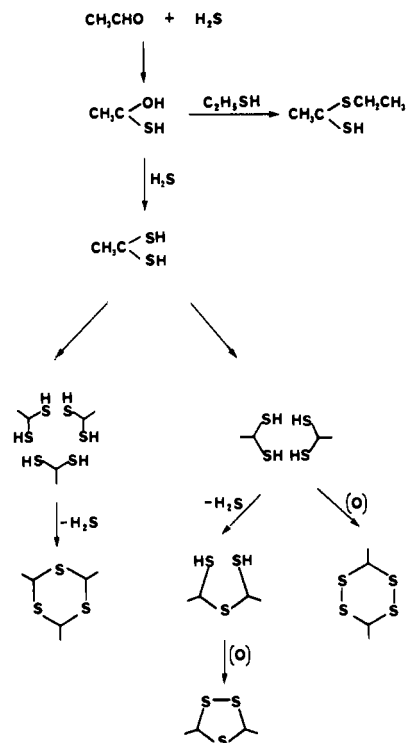
disulfide and the novel disulfide are formed more readily at pH 5.5.

Comparison of the yield and aroma from the degradations of cystine at pH 2.3 and at pH 5.5 is summarized in Table II. The yields obtained at the two pH values were almost the same. One of the aroma characters of the volatiles prepared at pH 2.3 was roasted, which could be due to the presence of the 1,2,4-trithiolanes at such a high level (over 55%).

Figure 8 shows some of the sulfur-containing products including 3,5-dimethyl-1,2,4-trithiolane, 2,4,6-trimethyl-1,3,5-trithiane, ethyl 1-(mercapto)ethyl sulfide, and 3,6-dimethyl-1,2,4-tetrathiane; these compounds may be also formed by the reaction of acetaldehyde and hydrogen sulfide as previously reported in literature (Obata and Tanaka, 1965; Boelens et al., 1975).

This study provided a thorough information of the degradation of cystine in aqueous solution at roasting temperature. Changes in the pH of the aqueous solution alter the volatile profile of the reaction mixture. Forty-two volatile compounds were identified at pH 5.5 and twenty-three at pH 2.3. Most components were sulfur containing and no pyrazines were found. This information

Thermal Degradation of Cystine

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Table I. Volatile Components Identified from the Degradation of Cystine[a]

compd ID	pH 5.5			pH 2.3			MS ref or *m/e* (rel intens)
	OV-1	CWX	GC area %	OV-1	CWX	GC area %	
			Thiazoles/Isothiazoles				
thiazole	4	9	5.6	4	9	2.3	Ten Noever de Brauw et al. (1983)
2-methylthiazole	6	8	3.4	6	8	2.6	Ten Noever de Brauw et al. (1983)
5-methylthiazole	9	11	2.1	9	11	1.3	Ten Noever de Brauw et al. (1983)
2-methyl-4-ethylthiazole	14	12	T				Ten Noever de Brauw et al. (1983)
2,4,5-trimethylthiazole		13	T		12	T	Ten Noever de brauw et al. (1983)
2-*n*-propylthiazole	16	14	T	12	13	1.0	Ledl and Severin (1973)
2-acetylthiazole		20	T				Ten Noever de Brauw et al. (1983)
2-thiazolyl ethyl ketone	23	21	4.9	14	17	2.8	112 (100), 113 (88), 57 (77), 29 (43), 85 (25), 58 (23)
3-methylisothiazole	7	10	T	7	10	T	Poite et al. (1969)
5-ethylthiazole	12		T	11		T	Ten Noever de Brauw et al. (1983)
5-acetylthiazole	19		T				112 (100), 127 (48), 43 (26), 84 (24), 57 (15), 45 (13)
2-methyl-5-ethylthiazole	17	15	2.1	13	14	3.1	Ten Noever de Brauw et al. (1983)
			Thiophenes				
2-acetylthiophene	22	23	1.1				Ten Noever de Brauw et al. (1983)
3-acetylthiophene	21	24	1.1				Ten Noever de Brauw et al. (1983)
thienothiophene	29		T	18		1.7	Gautschi et al. (1967)
thienothiophene	30		T				Gautschi et al. (1967)
			Sulfides				
ethyl disulfide	11	7	6.8	10	7	1.0	Ten Noever de Brauw et al. (1983)
ethyl trisulfide	27	17	T				Ledl (1976)
ethyl 1-(ethylthio)ethyl disulfide	32	22	6.0	20		T	89 (100), 61 (48), 29 (13), 59 (12)
ethyl 1-(ethylthio)ethyl trisulfide	37	29	1.1				89 (100), 61 (42), 29 (16), 59 (16)
ethyl butyl disulfide	24		T				94 (100), 29 (73), 150 (72), 41 (58), 57 (52), 66 (35)
ethyl 1-(ethylthio)ethyl tetrasulfide	38		T				89 (100), 61 (34), 29 (11), 59 (18)
ethyl 1-mercaptoethyl sulfide	10		T				Boelens et al. (1974)
			Thiolanes/Thianes/Thiolactones				
3,5-dimethyl-1,2,4-trithiolane	25	18	3.0	15	15	24.1	Ten Noever de Brauw et al. (1983)
3,5-dimethyl-1,2,4-trithiolane	26	19	3.6	16	16	21.6	Ten Noever de Brauw et al. (1983)
2-methyl-1,3-dithiolane		16	T				Ledl and Severin (1973)
3-methyl-1,2,4-trithiane	31	25	4.3	19	18	1.6	Kleipool and Tas (1974)
3,6-dimethyl-1,2,4,5-tetrathiane	33	28	T	21	20	5.1	Nixon et al. (1979)
γ-thiobutyrolactone	15		T				102 (100), 55 (68), 42 (49), 41 (41), 45 (20), 46 (19)
3-methyl-1,2-dithiolane	18		T				55 (100), 120 (33), 29 (12), 41 (11), 64 (10)
2,4,6-trimethyl-1,3,5-trithiane	34		T				Ten Noever de Brauw et al. (1983)
4-methyl-1,2-dithiole-3-thione	35		T				Pedersen and Moller (1972)
5-methyl-1,2-dithiole-3-thione	36		T				Pedersen and Moller (1972)
			Aldehyde/Ketones				
acetaldehyde	1	1	T	1	1	T	Ten Noever de Brauw et al. (1983)
acetone	2	2	1.1	2	2	2.0	Ten Noever de Brauw et al. (1983)
methyl ethyl ketone	3	3	6.1	3	3	7.3	Ten Noever de Brauw et al. (1983)
2-pentanone		4	T		4	T	Ten Noever de Brauw et al. (1983)
3-hexanone		5	T				Ten Noever de Brauw et al. (1983)
3-methylcyclopentanone	8	6	T	8	6	T	Stenhagen et al. (1974)
2,3-pentanedione					5	T	Ten Noever de Brauw et al. (1983)
			Pyrroles				
2-acetylpyrrole	20	27	T				Vernin and Petitjean (1982)
pyrrole	5		T	5		T	Ten Noever de Brauw et al. (1983)
N-acetylpyrrole	13		T				Vernin and Petitjean (1982)
			Unknown				
unknown 1, M_r 141	28	26		17	19	1.4	
methylene chloride (solvent)	S	S		S	S		

[a] T = trace, less than 1%.

Table II. Comparison of Yield and Aroma from the Degradation of Cystine in Water at pH 2.3 and at pH 5.5

	pH 2.3	pH 5.5
yield, mg	147	145
aroma	heated, roasted, oniony, biting	oniony, vegetable, cabbage, garlic

is valuable to understand and to manipulate the reaction of cystine with other chemicals such as with sugars in the Maillard reaction (Maillard, 1912; Hodge, 1953) or with α-dicarbonyls in the Strecker degradations (Schonberg et al., 1948); especially, it is important for the investigations of the formation mechanism of the flavor compound when cystine is involved in the reaction.

methyl-5-ethylthiazole, 19961-52-5; 2-acetylthiophene, 88-15-3; 3-acetylthiophene, 1468-83-3; ethyl disulfide, 110-81-6; ethyl trisulfide, 3600-24-6; ethyl 1-(ethylthio)ethyl disulfide, 94944-48-6; ethyl 1-(ethylthio)ethyl trisulfide, 94944-49-7; ethyl butyl disulfide, 63986-03-8; ethyl 1-(ethylthio)ethyl tetrasulfide, 94944-50-0; ethyl 1-mercaptoethyl sulfide, 31331-54-1; 3,5-dimethyl-1,2,4-trithiolane, 23654-92-4; 2-methyl-1,3-dithiolane, 5616-51-3; 3-methyl-1,2,4-trithiane, 43040-01-3; 3,6-dimethyl-1,2,4,5-tetrathiane, 67411-27-2; γ-thiobutyrolactone, 1003-10-7; 3-methyl-1,2-dithiolane, 55487-20-2; 2,4,6-trimethyl-1,3,5-trithiane, 2765-04-0; 4-methyl-1,2-dithiole-3-thione, 3354-41-4; 5-methyl-1,2-dithiole-3-thione, 3354-40-3; acetaldehyde, 75-07-0; acetone, 67-64-1; methyl ethyl ketone, 78-93-3; 2-pentanone, 107-87-9; 3-hexanone, 589-38-8; 3-methylcyclopentanone, 1757-42-2; 2,3-pentanedione, 600-14-6; 2-acetylpyrrole, 1072-83-9; pyrrole, 109-97-7; *N*-acetylpyrrole, 609-41-6.

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pH Effect on the Volatile Components in the Thermal Degradation of Cysteine

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The pH effect on the volatile components in the thermal degradation of cysteine was studied at pH's 2.2, 5.1, and 7.1, representing the pH values below, around, and above the isoelectric point of cysteine, with a Parr bomb model system. A vigorous degradation occurred at pH 5.1 and a very mild one at pH 7.1. At pH 2.2 the major components formed are 1,2,3-trithia-5-cycloheptene and 2-thiophenethiol. The trithiacycloheptene compound was evaluated as roasted onion and roasted meat odor and was found for the first time in a model system. The mechanism of formation of these two major compounds is proposed. At pH's 5.1 and 7.1, 3,5-dimethyl-1,2,4-trithiolanes possessing a roasted odor are the major components.

INTRODUCTION

Cysteine and other sulfur-containing amino acids are considered important contributions to the formation of various food flavors, especially meat flavor (Hurrell, 1982;

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Ching, 1979; Fujimaki et al., 1969; Kato et al., 1973). Thermal degradation of cysteine was reported by several researchers. Obata and Tanaka (1965) photolyzed cysteine in aqueous medium and found several primary degradation volatiles such as H_2S, NH_3, CO_2, and acetaldehyde. Fujimaki et al. (1969) and Kato et al. (1973) pyrolyzed cysteine at 270–300 °C with no medium and identified additional volatile compounds including amines, thiazolidine, thiazoles, pyridines, and thiophenes. Ledl and Severin (1973) and Ledl (1976) heated cysteine in tributyrin at 150 °C and in soybean oil at 200 °C, respectively. They identified many volatile heterocycles including various types of sulfur-containing compounds. Patterson et al. (1976) pyrolyzed cysteine at higher temperature (850 °C) with no medium and found tht most volatile compounds