

Influence of Cysteine Oxidation on Thermal Formation of Maillard Aromas

Chao-Ying Tai and Chi-Tang Ho*

Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903

Cysteic acid and cysteinesulfinic acid, which are oxidized forms of reduced cysteine, were heated separately with D-glucose in an aqueous system of pH 6 at 160 °C for 2 h. The reaction products were extracted and analyzed. Among the identified compounds, pyrazines and furans are the most predominant compounds and no or only trace amounts of sulfur-containing compounds were identified in these two reaction systems. In the same reaction condition, the reduced cysteine and D-glucose produced mainly sulfur-containing compounds; no pyrazines were found in this system. The reaction mass of cysteic acid and D-glucose possessed smoky, nutty, and yeasty aromas, while cysteinesulfinic acid with D-glucose generated a strong coffee note. The reduced cysteine with glucose produced onion and garlic, as well as slightly meaty and sulfury, flavors.

Keywords: *Cysteine; cysteic acid; cysteinesulfinic acid; oxidation; Maillard aromas*

INTRODUCTION

Cysteine is an important precursor for many sulfur-containing aroma compounds identified in meat and other foods. These sulfur-containing compounds are well recognized as major contributors to meat flavors (MacLeod, 1986). During the cooking of meat, hydrogen sulfide is liberated from the cysteine residues of the meat proteins or peptides and participates in the subsequent thermal reactions to generate a number of sulfur-containing flavor compounds.

The oxidation of amino acids in the food system is a common event due to the existence of the naturally generated or intentionally added oxidizing agents. The oxidizing agents such as hydrogen peroxide are used to control microorganisms and improve the color of many foods. Benzoyl peroxide and potassium bromate have been used in bread dough to improve the baking quality. Lipid hydroperoxides are not a food additive, although they can be naturally generated during food processing and storage. The oxidation of the amino acid residues in protein is known to adversely affect the nutritional value of foods (Rasekh et al., 1972). The oxidation of protein, peptide, or free amino acids is expected to affect the generation of flavor compounds. Volatile compounds generated from the thermal reaction of methionine and its oxidation product, methionine oxide, with glucose have been reported (Yu and Ho, 1995). The formation of methional was found to be more favorable from the thermal reaction of methionine with glucose, whereas the formation of dimethyl disulfide and dimethyl trisulfide was found to be more favorable from the reaction of methionine sulfoxide with glucose (Yu and Ho, 1995).

Cysteine is more susceptible to oxidizing agents than most of the amino acids due to its chemical nature. In a weak oxidizing environment, cysteine forms cystine, which is a disulfide-bound cysteine dimer. In the presence of the stronger oxidants, cysteine can be oxidized into monomeric oxidation products such as cysteinesulfinic acid and cysteic acid (Little and O'Brien,

1967). Although the thermal reaction of the reducing sugars with cysteine has been studied, it is also interesting to know the influence of the oxidation on their thermal Maillard aroma generation.

EXPERIMENTAL PROCEDURES

Thermal Reactions. L-Cysteine or L-cysteic acid (0.024 mol; Sigma, St. Louis, MO), with an equal amount of α -D-glucose, was dissolved in 50 mL of distilled water. The solution was adjusted to pH 6.0 and sealed in a 120 mL bomb reactor. The bomb was heated at 160 °C in an oven for 2 h. In the case of the cysteinesulfinic acid and glucose reaction system, 0.002 mol of each reactant was used in this reaction. After reaction, the bomb was immersed into an ice-water bath to bring the temperature down and terminate the reaction.

Isolation of the Volatiles. The reaction mixture was mixed with 1 mL of internal standard (tridecane, 100 ppm) and extracted using methylene chloride (50 mL \times 5 times). The extract was dehydrated by anhydrous sodium sulfate and filtered. The filtrate was concentrated to 5 mL by a Kuderna-Danish concentrator. Finally, the extract was concentrated to 1 mL under a nitrogen flow.

Gas Chromatographic Analysis. The volatile compounds isolated from the thermal reaction systems were analyzed by a Varian 3400 gas chromatograph (GC) equipped with a fused silica capillary column (60 m \times 0.25 mm i.d.; 1 μ m thickness, DB-1, J&W Scientific Inc.) and a flame ionization detector. For each sample, 1 μ L was injected into the GC with a split ratio of 25:1. The GC was run with an injector temperature of 270 °C, a detector temperature of 300 °C, and a helium carrier flow rate of 1 mL/min. The column temperature was programmed as follows: from 40 to 280 °C at 2 °C/min increasing rate.

GC/Mass Spectrometry Analysis. The concentrated isolates from different reaction systems were analyzed by GC/mass spectrometry (GC/MS), using a Varian 3400 GC coupled to a Finnigan MAT 8230 high-resolution, double-focusing magnetic sector mass spectrometer equipped with a direct split interface. Mass spectra were obtained by electron ionization at 70 eV and a source temperature of 250 °C.

Identification of the Volatile Compounds. The identification of the volatile compounds was based on GC/MS analysis. The compound from the isolate was identified by comparing the mass spectral data with those of authentic compounds available in the NBS computer library or previous publications (Zhang and Ho, 1991; Zhang et al., 1988).

* Author to whom correspondence should be addressed [fax (908) 932-8004; e-mail ho@aesop.rutgers.edu].

RESULTS AND DISCUSSION

Odor Descriptions. The odor generated from the cysteine/glucose system was described by two expert flavorists as a roasted, meaty, soy sauce-like flavor. Cysteine without glucose produced strong onion, garlic, and slightly meaty and sulfury odors. Cysteic acid and glucose generated smoky, nutty, and yeasty notes, while cysteic acid alone produced a slightly sulfury smell. Cysteinesulfinic acid and glucose produced a strong coffee aroma as well as smoky and roasted flavor notes.

Reduced Cysteine with Glucose Reaction System. The Maillard reaction between cysteine and glucose has been well-studied (Tressl et al., 1989; Zhang and Ho, 1991). Under the current experimental conditions, a total of 38 compounds were identified in the reaction of cysteine and glucose. The majority of compounds identified in this reaction were sulfur-containing compounds including thiophenes, polysulfides, and thiazoles (Table 1).

Thiophenes are widely distributed in foods such as vegetables, cooked meat, nut products, coffee, and tea (Maga, 1992). Early studies indicated that thermally induced reactions are a prerequisite for the formation of thiophenes in foods. Many thiophenes were isolated from meat-based products (Nonaka et al., 1967; Wilson et al., 1973; Werkhoff et al., 1993); this revealed that thiophenes are a substantial contribution to the overall meaty flavor.

Thiophenes are always one of the group of major products in the cysteine-involved reactions (Mulders, 1973; Zhang and Ho, 1991; Umamo et al., 1995). The formation pathway of thiophene through the Maillard reaction has been suggested to occur at the early stages of the reaction (Shibamoto, 1989).

Polysulfides are another major group of product identified in this system. 1,1-Ethanedithiol has recently been reported as a naturally occurring flavor component of pork (Werkhoff et al., 1993). 1,1-Ethanedithiol shows sulfury, rubbery, cheesy, oniony, and slightly meaty flavor notes (Werkhoff et al., 1993). This compound has been proposed as a precursor for the formation of other polysulfides such as 3,5-dimethyl-1,2,4-trithiolane, 3-methyl-1,2,4-trithiane, 3,6-dimethyl-1,2,4,5-tetrathiane, and 4,6-dimethyl-1,2,3,5-tetrathiane, which are also present in the current reaction system (Whitfield and Mottram, 1996). Most of these polysulfides have been reported as flavor components of meat. 3,5-Dimethyl-1,2,4-trithiolanes, the most predominant compounds in this study, are an important meaty flavor component and are characterized by their meaty, roasty, and onion-like flavor (Chang, 1968). 3-Methyl-1,2,4-trithiane, a naturally occurring constituent of pork (Werkhoff et al., 1993) which is characterized by its low threshold and high potency, has been used as a meat flavoring (Ohloff and Flament, 1978). 3,6-Dimethyl-1,2,4,5-tetrathiane is a well-known constituent of boiled mutton flavor (Nixon et al., 1979), which has also been identified in pork and chicken (Werkhoff et al., 1993). The primary reactants for these polysulfides are hydrogen sulfide and aldehydes (Boelens et al., 1974).

Thiazoles have been considered as one of the main constituents widely used to impart imitation meat flavors (Takken et al., 1976). These compounds were found in cooked beef and in many model reactions that have the potential to produce meat aromas. To date, the exact formation mechanism is not clear; however, the most acceptable pathway was proposed by Takken et al. (1976). In a sugar with cysteine reaction system,

the precursors include α -dicarbonyl compounds, which are generated from the sugar degradation; hydrogen sulfide, aldehyde, and ammonia are provided by cysteine. The relatively small amount of thiazoles and the large quantity of thiophenes and polysulfides generated may indicate that the ammonia released from cysteine is at a much slower rate than that of hydrogen sulfide.

Cysteinesulfinic Acid and Glucose Reaction System. A total of 24 compounds were identified in this reaction (Table 1). When cysteine is in this oxidized state, the sulfur-containing products completely disappear. At the same time, pyrazines are produced dramatically in tremendous amount. Pyrazines and furans become the main products instead of the sulfur-containing compounds. Pyrazines have a roasty, toasty, or nutty flavor note (Maga, 1992). This family is formed primarily through the Strecker degradation, which is a substantial pathway of the Maillard reaction scheme. The only two carbonyls identified are 1-hydroxy-2-propanone and acetoin. Both compounds are important precursors of pyrazines.

The explanation for the loss of sulfur-containing products is due to the lack of hydrogen sulfide, which is the major precursor for the sulfur-containing compounds. Cysteinesulfinic acid does not possess the thiol group as in the reduced cysteine, but a relatively less reactive sulfite group is in its side chain. This change makes the sulfur source not readily available. The absence of the sulfur source, on the other hand, allows the slowly released ammonia to react with the precursor of pyrazines and promote the formation of pyrazines.

An enormous amount of 5-(hydroxymethyl)-2-furfural, a typical glucose degradation product, was generated, indicating that 3-deoxyhexosone, the precursor of 5-(hydroxymethyl)-2-furfural, is a more favorable glucose degradation product in this system. 5-(Hydroxymethyl)-2-furfural was absent in the cysteine/glucose system.

Cysteic Acid and Glucose Reaction System. There were 29 compounds identified in this reaction system (Table 1). Similar to the results of the cysteine-sulfinic acid and glucose reaction system, none of the sulfur-containing compounds were found. Again, pyrazines and furans were the major products.

1-Hydroxy-2-propanone, the precursor of 2,5-dimethylpyrazine and 2,3-dimethylpyrazine, was the most predominant product of this reaction. Acetoin, which is another precursor of pyrazines, was also present in large quantities.

From a comparison of the amounts of different types of pyrazines identified in the cysteic acid/glucose system to those of the cysteinesulfinic acid/glucose system, it is clear that the degree of oxidation on cysteine significantly affects the profile of pyrazines generated. 3-Ethyl-2,5-dimethylpyrazine, which has a strong roasted, nutty odor and is the major character-impact compound in peanut butter (Joo and Ho, 1997), dominated in the cysteinesulfinic acid/glucose system; however, the amount of this pyrazine was significantly reduced in the cysteic acid/glucose system.

The degree of oxidation also had a profound effect on other compounds generated. For example, the ratio of 1-hydroxy-2-propanone/acetoin in the cysteinesulfinic acid/glucose system is approximately 3:1, while in the system of cysteic acid/glucose, this ratio was changed to 1:5. This difference has a significant impact on their total sensory quality.

Table 1. Volatile Compounds Generated from Thermal Reaction of Glucose with Cysteine, Cysteinesulfinic Acid, or Cysteic Acid

compound	amount, ppm		
	CG ^a	CSFAG ^b	CAG ^c
thiophenes			
thiophene	1.50		
2-methylthiophene	1.91		
2-ethylthiophene	0.61		
2-acetylthiophene	7.78		
2,3-dimethylthiophene	0.54		
2,5-dimethylthiophene	0.74		
thieno(3,2- <i>b</i>)thiophene	2.56		
tetrahydrothiophen-3-one	1.06		
2-methyltetrahydrothiophen-3-one	1.52		
5-methyltetrahydrothiophen-3-one	0.33		
5-methyl-2-thiophenecarboxylic acid	1.45		
2-butanoylthiophene	0.41		
thiazoles			
2-methylthiazole	0.97		
2,5-dimethylthiazole	0.11		
2,4,5-trimethylthiazole	1.92		
polysulfides			
1,1-ethanedithiol	0.66		
2-methyl-1,3-dithiolane	0.85		
3,5-dimethyl-1,2,4-trithiolane	11.42		
3-methyl-1,2,4-trithiane	9.94		
3,6-dimethyl-1,2,4,5-tetrathiane	2.60		
4,6-dimethyl-1,2,3,5-tetrathiane	2.61		
4,7-dimethyl-1,2,3,5,6-pentathiepane	3.62		
pyrazines			
pyrazine		5.90	4.49
methylpyrazine		9.84	15.69
2,3-dimethylpyrazine		1.57	2.12
2,5-dimethylpyrazine		22.90	30.59
ethylpyrazine		4.12	1.40
vinylpyrazine		0.36	
trimethylpyrazine		11.01	
2-ethyl-5-methylpyrazine		6.95	8.88
2-ethyl-6-methylpyrazine		4.80	2.00
2-vinyl-5-methylpyrazine			0.67
2-vinyl-6-methylpyrazine			0.22
2-ethyl-3,5-dimethylpyrazine		3.04	1.43
3-ethyl-2,5-dimethylpyrazine		27.38	2.04
2-methyl-5-isopropylpyrazine			0.62
2,3-diethyl-5-methylpyrazine		2.38	
3,5-diethyl-2-methylpyrazine		1.48	
2,5-dimethyl-3-propylpyrazine			1.61
quinoxaline			0.49
pyrroles			
2-acetylpyrrole		0.77	5.04
5-methyl-2-formylpyrrole			0.61
furans			
2-ethylfuran	0.66		
2,5-dimethylfuran		0.17	
2,5-dimethyl-3(2 <i>H</i>)-furanone		2.59	
2-acetylfuran	7.18	1.36	3.26
2-furfural		0.82	0.98
5-methyl-2-furfural			8.63
5-(hydroxymethyl)-2-furfural		18.66	36.26
2-furanmethanol	12.44	1.34	4.41
1-(2-furyl)-1,2-propanedione	0.61		
2,3-dihydro-5-methylfuran	26.86		
carbonyls			
2-butanone	6.28		0.85
1-hydroxy-2-propanone	1.88	28.02	5.08
acetoin	0.77	8.43	26.32
1-methoxy-2-propanone			0.12
3-methyl-2-butanone	0.94		
3-hexanone	1.00		
2-mercapto-3-butanone	1.54		
2-methylcyclopentanone	0.31		
3-methyl-2-cyclopenten-1-one	1.27		0.22
2-methyl-3-heptanone	1.84		
3-methyl-2,4-pentanedione	1.03		
cycлотene	2.41		0.05
2,3-dihydro-3,5-dihydroxy-6-methyl-4 <i>H</i> -pyran-4-one		5.49	1.71
miscellaneous			
acetic acid	0.67	4.11	0.74

^a Cysteine/glucose reaction system. ^b Cysteinesulfinic acid/glucose reaction system. ^c Cysteic acid/glucose reaction system.

Conclusions. The influence of cysteine oxidation on the subsequent thermal flavor formation is absolutely significant. From the odor description, we can notice the change. Cysteine/glucose, or cysteine alone, produced meaty, soy sauce-like, garlic, or onion type aromas. These flavor notes are characteristic to the sulfur-containing volatile compounds, which are the main products in the reduced cysteine reaction system. In the oxidized cysteine/glucose reaction model, the final reaction mixture was described as smoky, roasty, or yeasty smelling, which are the flavor notes of pyrazines. The odor descriptions of the three reaction systems are well correlated to the identified reaction products. As summarized in Table 1, sulfur-containing compounds exist almost only in the cysteine/glucose system; thus, the final reaction mass was dominated by the aroma of the sulfur-containing compounds. On the other hand, the deficiency of sulfur-containing compounds as well as the prevalence of pyrazines can notably affect the final smell of the oxidized cysteine/glucose reaction mixture.

Cysteine oxidation occurs commonly in food systems. How to minimize the loss of the cysteine-derived flavor compounds and maintain the authentic flavor of foods are challenges to food scientists.

LITERATURE CITED

- Boelens, M.; Van der Lind, L. M.; De Valois, P. J.; Van Dort, H. M.; Takken, H. C. Organic sulfur compounds from fatty aldehydes, hydrogen sulfide, thiols, and ammonia as flavor constituents. *J. Agric. Food Chem.* **1974**, *22*, 1071–1076.
- Chang, S. S.; Hirai, C.; Reddy, B. R.; Herz, K. O.; Kato, A., Sipma, G. Isolation and identification of 2,4,5-trimethyl-3-oxazoline and 2,5-dimethyl-1,2,4-trithiolane in the volatile flavor compounds of boiled beef. *Chem. Ind.* **1968**, 1639–1641.
- Joo, K.; Ho, C.-T. Quantitative analysis of alkylpyrazines in regular- and low-fat peanut butter preparations. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 171–173.
- Little, C.; O'Brien, P. J. Products of oxidation of a protein thiol group after reaction with various oxidizing agents. *Arch. Biochem. Biophys.* **1967**, *122*, 406–410.
- MacLeod, G. The scientific and technological basis of meat flavors. In *Developments in Food Flavors*; Birch, G. G., Lindey, M. G., Eds.; Elsevier Applied Science: London, 1986; pp 191–223.
- Maga, J. A. Pyrazines update. *Food Rev. Int.* **1992**, *8*, 479–558.
- Mulders, E. J. Volatile compounds from the non-enzymatic browning reaction of the cysteine/cystine-ribose system. *Z. Lebensm.-Unters. Forsch.* **1973**, *152*, 193–201.
- Nixon, L. N.; Wong, E.; Johnson, C. B.; Birch, E. J. Nonacidic constituents of volatiles from cooked mutton. *J. Agric. Food Chem.* **1979**, *27*, 355–359.
- Nonaka, M.; Black, D. R.; Pippen, E. L. Gas chromatographic and mass spectral analyses of Cooked chicken meat volatiles. *J. Agric. Food Chem.* **1967**, *15*, 713–717.

- Ohloff, G.; Flament, I. Heterocyclic constituents of meat aroma. *Heterocycles* **1978**, *11*, 663–695.
- Rasekh, J.; Stillings, B. R.; Sidwell, V. Effect of hydrogen peroxide on the color, composition and nutritive quality of FPC (fish protein concentrate). *J. Food Sci.* **1972**, *37*, 423–425.
- Shibamoto, T. Volatile flavor chemicals formed by the Maillard reaction. In *Thermal Generation of Aromas*; Parliment, T. H., McGorin, R. J., Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp 134–142.
- Takken, H. J.; Van der Linde, L. M.; de Valois, P. J.; van Dort, H. M.; Boelens, M. Reaction products of -dicarbonyl compounds, aldehydes, hydrogen sulfide and ammonia. In *Phenolic, Sulfur and Nitrogen Compounds in Food Flavors*; Charalambous, G., Katz, I., Eds.; ACS Symposium Series 26; American Chemical Society: Washington, DC, 1976; pp 114–121.
- Tressl, R.; Helak, B.; Martin, N.; Kersten, E. Formation of amino acid specific Maillard products and their contribution to thermally generated aromas. In *Thermal Generation of Aromas*; Parliment, T. H., McGorin, R. J., Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp 156–171.
- Umamo, K.; Hagi, Y.; Nakahara, K.; Shyoji, A.; Shibamoto, Y. Volatile chemicals formed in the headspace of a heated D-glucose/L-cysteine Maillard reaction model system. *J. Agric. Food Chem.* **1995**, *43*, 2212–2218.
- Werkhoff, P.; Brunig, J.; Emberger, R.; Guntert, M.; Hopp, R. Flavor chemistry of meat volatiles. New results on flavor components from beef, pork, and chicken. In *Recent Developments in Flavor and Fragrance Chemistry*; Hopp, R., Mori, K., Eds.; VCH: Weinheim, 1993; pp 183–213.
- Whitfield, F. B.; Mottram, D. S. Maillard lipid interactions in low moisture systems. In *The Contribution of Low- and Nonvolatile Materials to the Flavor of Foods*; Pickenhagen, W., Ho, C.-T., Spanier, A., Eds.; Allured Publishing: Carol Stream, IL, 1996; pp 149–185.
- Wilson, R. A.; Mussinan, C. J.; Katz, I.; Sanderson, A. Isolation and identification of some sulfur chemicals present in pressure-cooked beef. *J. Agric. Food Chem.* **1973**, *21*, 873–876.
- Yu, T. H.; Ho, C.-T. Volatile Compounds Generated from Thermal Reactions of Methionine and Methionine Sulfoxide with or without Glucose. *J. Agric. Food Chem.* **1995**, *43*, 1641–1646.
- Zhang, Y.; Ho, C.-T. Comparison of the volatile compounds formed from the thermal reaction of glucose with cysteine and glutathione. *J. Agric. Food Chem.* **1991**, *39*, 760–763.
- Zhang, Y.; Chien, M.; Ho, C.-T. Comparison of the volatile compounds obtained from thermal degradation of cysteine and glutathione in water. *J. Agric. Food Chem.* **1988**, *36*, 992–996.

Received for review April 10, 1997. Revised manuscript received June 16, 1997. Accepted June 16, 1997.®

JF970297T

® Abstract published in *Advance ACS Abstracts*, August 1, 1997.