

Comparison of the Volatile Compounds Formed from the Thermal Reaction of Glucose with Cysteine and Glutathione

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Sulfur-containing amino acid, cysteine, and tripeptide, glutathione (γ -Glu-Cys-Gly), were reacted with glucose in an aqueous medium. Each reaction solution was adjusted to pH 7.5 and heated for 1 h at 180 °C. A roasted sesame and cooked-rice-like aroma with strong sulfur notes was observed for both reaction masses. A total of 62 compounds including 7 furans, 8 carbonyls, 10 thiazoles, 19 thiophenes, 10 pyrazines, and 6 cyclic polysulfides were identified by GC and GC/MS. Most of them can be accounted for by well-known chemical reaction mechanisms. In the reaction system of glucose and cysteine, more sugar-amino acid interaction products were formed, and the major product was 3,5-dimethyl-1,2,4-trithiolane. In contrast, for the reaction of glucose and glutathione, carbonyls and furans dominated quantitatively, and 5-methylfurfural was found as the most abundant product. They were derived from the thermal degradation of glucose. The degradation of cysteine alone produced the greatest amount of volatile compounds compared with the interaction of either 2,4-decadienal or glucose. However, when glutathione reacted with 2,4-decadienal, the volatile production increased dramatically and decreased again when reacting with glucose.

INTRODUCTION

Sulfur-containing amino acids, such as cysteine and cystine, are indispensable components for generating meat-like aromas through reaction with reducing sugars (MacLeod, 1986). In 1971, Kitada et al. patented their method to produce a meat-like flavor by heating glucose with cysteine. Almost at the same time, Kato et al. (1973) reacted cysteine with glucose at various temperatures, and a Japanese rice cracker with sesame-like aroma was obtained at the reaction temperature of 160 °C. Ledl and Severin (1973) reported that a roast-meat-like aroma was observed when cysteine was reacted with xylose. Mulders (1973) carried out an experiment to study the non-enzymatic browning reaction of a cystine/cysteine-ribose system. Although a great deal of attention has been given to Maillard reactions by model systems due to their importance in the development of food flavor, little is known about the reactivity differences between amino acid and peptides, in particular, the difference of cysteine in its free form and in the form of glutathione, toward the formation of Maillard reaction products, especially the sulfur-containing heterocyclic compounds.

It is known from our previous study (Zhang et al., 1988) that the release of hydrogen sulfide was much faster than that of ammonia during the thermal degradation of glutathione (γ -Glu-Cys-Gly) in an aqueous solution. However, the release of both hydrogen sulfide and ammonia from cysteine was fast and produced 4 times as many volatiles as glutathione under the same conditions (Zhang et al., 1988). On the other hand, when cysteine and glutathione reacted with 2,4-decadienal, respectively, the yields of volatile generation became almost identical. This phenomenon was attributed to the fact that carbonyls, such as 2,4-decadienal and its retroaldolization products, catalyzed the ammonia release from glutathione via the formation of the Schiff base, and a novel formation mechanism of 2-pentylpyridine was proposed from this intermediate (Zhang and Ho, 1989).

The present study reports the volatile compounds generated from the reaction of glucose with cysteine and glutathione.

EXPERIMENTAL PROCEDURES

Sample Preparation. A total of 900 mg (0.005 mol) of D-(+)-glucose (anhydrous, reagent grade, Aldrich Chemical Co., Milwaukee, WI) was mixed with 600 mg (0.005 mol) of cysteine (reagent grade, Sigma Chemical Co., St. Louis, MO) or 1500 mg (0.005 mol) of glutathione (reduced form, reagent grade, Sigma), respectively. The mixture was dissolved in 100 mL of distilled water, and the solution was adjusted to pH 7.5 with 1 N HCl or 10% NaOH. The mixture was transferred into a 0.3-L Hoke SS-DOT sample cylinder, and the cylinder was sealed and heated at 180 °C in an oil bath for 1 h. The reaction mass then was simultaneously solvent-extracted and steam-distilled by using diethyl ether with a Likens-Nickerson apparatus. Internal standards for the quantification were added before distillation and extraction. A portion of 0.5 mL of tridecane solution in dichloromethane (1.886 mg/mL) was added to each sample as an internal standard. The distillates or extracts were dried over anhydrous sodium sulfate and concentrated with a Kuderna-Danish apparatus to a final volume of 0.5 mL.

Volatile Separation by Gas Chromatography. A Varian 3400 gas chromatograph equipped with an FID and a nonpolar fused silica capillary column [60 m \times 0.25 mm (i.d.), 0.25- μ m thickness, DB-1; J&W] was used to analyze the volatile compounds isolated from the thermal reaction systems. For each sample, 0.2 μ L was injected with a split ratio 100:1. 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 temperature program included an initial column temperature of 10 °C, a temperature increase of 2 °C/min from 40 to 220 °C, and a 10-min isothermal period at the final column temperature.

Quantitative determination was accomplished by internal standards. The quantity of each component was finally converted into milligrams of volatiles generated by either 1 mol of cysteine or 1 mol of glutathione. Linear retention indices for the volatile compounds were calculated by using *n*-paraffin standards (C₆-C₂₆, Alltech Associates) as references (Majlat et al., 1974).

GC/MS Analysis. The concentrated samples were analyzed by GC/MS using a Varian 3400 gas chromatograph coupled to a Finnigan MAT 8230 high-resolution mass spectrometer, using the same GC program as for the separation. Mass spectra were obtained by electron ionization at 70 eV and a source temperature of 250 °C. The filament emission current was 1 mA, and spectra were recorded on a Finnigan MAT SS 300 data system.

Table I. Volatile Compounds Formed from Thermal Interaction of Glucose and Cysteine or Glutathione

compd	MW	Ik (DB-1)	amount, mg/mol		I.D.
			CG ^d	GG ^e	
carbonyls					
diacetyl	86	561	4.83	1.83	a
2-butanone	72	572	8.05	4.34	a
ethyl acetate	88	599	8.37	15.56	a
2,3-pentanedione	100	668	4.40	3.60	a
acetoin	88	680	4.14	0.82	a
3-penten-2-one	84	719	1.29	0.31	a
2,4-pentanedione	100	760	0.44	nd	a
2-hydroxy-3-pentanone	102	787	t	nd	a
total carbonyls			31.52	26.46	
furans					
2-methyl-4,5-dihydro-3(2H)-furanone	100	775	7.43	9.26	a
furfural	96	802	3.28	9.95	a
furfuryl alcohol	98	835	1.31	0.88	a
2-acetylfuran	110	882	10.35	23.12	a
5-methylfurfural	110	933	63.86	111.01	a
5-methyl-2-acetylfuran	124	1019	nd	2.13	a
1-(2-furyl)-1,2-propanedione	138	1034	2.18	2.61	a
total furans			88.41	158.96	
thiazoles					
thiazole	85	707	20.02	9.53	a, b
2-methylthiazole	99	783	6.03	1.29	a
4-methylthiazole	99	793	5.87	2.57	a, b
5-methylisothiazole	99	820	2.77	1.09	a
5-methylthiazole	99	825	2.42	nd	a, b
2,5-dimethylthiazole	113	860	4.72	nd	a
trimethylthiazole	127	976	4.64	0.45	a
2-acetylthiazole	127	989	73.77	21.68	a
4-methyl-2-acetylthiazole	141	1085	0.97	0.88	a
2-(1-propanonyl)thiazole	141	1094	4.69	0.84	a
total thiazoles			125.90	38.33	
thiophenes					
2-methylthiophene	98	758	18.67	21.71	a, b
2-ethylthiophene	112	844	1.08	1.69	a
2,5-dimethylthiophene	112	853	0.96	0.61	a
2,3-dimethylthiophene	112	877	3.20	0.81	a
4,5-dihydro-3(2H)-thiophenone	102	913	47.86	19.43	a
5-methyltetrahydrothiophen-3-one	116	948	3.78	1.52	a
2-methyltetrahydrothiophen-3-one	116	956	63.86	9.74	a
2-formylthiophene	112	961	3.74	4.40	a, b
3-formylthiophene	112	968	13.08	9.95	a, b
2-acetylthiophene	126	1052	52.12	11.20	a, c
methylformylthiophene	126	1054	7.91	2.16	a, c
3-acetylthiophene	126	1061	58.52	24.31	a, c
5-methyl-2-formylthiophene	126	1089	26.68	11.49	a, b
3-methyl-2-formylthiophene	126	1092	26.68	5.20	a, b
5-methyl-2-acetylthiophene	140	1130	19.22	8.85	a, c
3-methyl-2-acetylthiophene	140	1137	11.63	3.34	a, c
2-(1-propionyl)thiophene	140	1153	6.07	1.31	a
3-methyl-2-(2-oxopropyl)thiophene	154	1192	32.92	2.59	a
5-methylthieno[2,3-d]thiophene	154	1280	2.80	1.45	a
total thiophenes			400.78	141.76	
pyrazines					
methylpyrazine	94	799	44.50	13.09	a
2,5-dimethylpyrazine	108	889	17.98	8.19	a
2-ethylpyrazine	108	892	9.21	1.06	a
2,3-dimethylpyrazine	108	896	9.74	2.52	a
2-ethyl-5-methylpyrazine	122	976	2.32	nd	a
trimethylpyrazine	122	978	5.87	3.91	a
2-ethyl-6-methylpyrazine	122	981	8.76	nd	a
3,5-dimethyl-2-ethylpyrazine	136	1067	15.40	2.86	a
2-ethyl-5,6-dimethylpyrazine	136	1068	3.85	t	a
5,7-dihydro-5H-cyclopentapyrazine	120	1084	t	nd	a
total pyrazines			117.63	31.63	
cyclic polysulfides					
2-methyl-1,3-dithiolane	120	971	t	nd	a
anti-3,5-dimethyl-1,2,4-trithiolane	152	1101	86.22	1.49	a
syn-3,5-dimethyl-1,2,4-trithiolane	152	1107	100.94	4.40	a
3-acetyl-1,2-dithiolane	148	1201	3.35	nd	a
anti-4,6-dimethyl-1,2,3,5-tetrathiane	184	1368	11.44	0.70	a
syn-4,6-dimethyl-1,2,3,5-tetrathiane	184	1389	2.69	0.38	a
total cyclic polysulfides			204.64	6.97	
miscellaneous					
trimethyloxazole	111	828	3.87	nd	a
4H-tetrahydrothiopyran-4-one	116	1017	4.92	0.44	a

^a Identifications were done by searching with computer or following published sources: Stoll et al. (1967), Pittet et al. (1974), Heller and Milne (1980), MSDC (1983), Ten Noever de Brauw et al. (1983), Shu et al. (1986), Zhang et al. (1988), Werkhoff et al. (1990). ^b Identification confirmed by GC retention index. ^c Tentative identification for the analogous isomers. ^d CG, glucose with cysteine. ^e GG, glucose with glutathione.

Table II. Quantitation of Classified Compounds in Different Model Systems* (Milligrams per Mole)

compd class	Cys	Glut.	CD	GD	CG	GG
carbonyls			735.9	399.5	31.5	26.5
furans			19.2	15.9	88.4	159.0
thiazoles	488.2	72.6	30.0	50.1	125.9	38.3
thiophenes	47.8	2.5	219.0	339.6	400.8	141.8
pyridines	2.1		501.1	1219.0		
pyrroles	23.7					
pyrazines					117.6	31.6
poly-S compds	3220.8	740.7	1373.1	537.0	204.6	7.0
total	3782.6	815.8	2879.6	2561.1	968.8	404.2

* Model reaction system: Cys, cysteine alone (Zhang et al., 1988); Glut., glutathione alone (Zhang et al., 1988); CD, cysteine reacted with 2,4-decadienal (Zhang and Ho, 1989); GD, glutathione with 2,4-decadienal (Zhang and Ho, 1989); CG, cysteine with glucose; GG, glutathione with glucose; reaction pH value 7.5; time, 1 h; temperature, 180 °C.

RESULTS AND DISCUSSION

Qualitative examination by GC/MS of volatile compounds generated from the reaction of cysteine with glucose (CG) and of glutathione with glucose (GG) demonstrated that 62 volatiles were identified. Their identification, retention indices, and quantitation data are listed in Table I according to their chemical classification. The identification was accomplished by comparing the mass spectra with those of authentic compounds available in either the computer library (NBS) or published literature (Stoll et al., 1967; Pittet et al., 1974; Heller and Milne, 1980; MSDC, 1983; Ten Noever de Brauw et al., 1983; Shu et al., 1986; Zhang et al., 1988; Werkhoff et al., 1990).

It is well-known that various carbonyls and furans are produced from sugar caramelization or degradation (Hodge, 1967). Furans that do not contain sulfur are usually nutty, fruity, and caramel-like in odor and have no meaty note. Although sulfur-containing amino acid and peptide were used in this study, no sulfur-containing furans were detected. 5-Methylfurfural and 2-acetylfuran were the major furans for both CG and GG model systems. They were clearly derived from the thermal degradation and caramelization of glucose. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF) has been reported to be the major volatile constituent in the reaction mixture of cysteine and glucose (Tressl et al., 1989), but it was not detected in the present study. This is not surprising, because it is known that DMHF is unstable at high temperatures. When it reacted with cysteine at three different temperatures from 100 to 200 °C for 0.5 h, it was diminished from 30% to a trace amount (Shu, 1984).

Pyrazines have been well characterized as volatiles contributing to the roasted and smoky aroma in amino-carbonyl reactions (Shibamoto, 1979). They have been found in a wide range of food systems, such as roasted coffee (Tressl, 1989), cocoa (Dietrich et al., 1964), peanuts (Mason and Johnson, 1966), roasted barley (Wang et al., 1969), heated beef fats (Watanabe and Sato, 1971), and cooked pork liver (Mussinan and Walradt, 1974). A total of 10 pyrazines were identified in this study. The fact that no pyrazines were detected when cysteine and glutathione reacted with 2,4-decadienal suggests that the α -dicarbonyl-initiated Strecker degradation is of importance for the formation of pyrazines (Table II) (Zhang and Ho, 1989).

Of the 10 thiazoles identified in CG and GG model systems, 6 have been reported in the thermal degradation of cysteine and glutathione alone (Zhang et al., 1988). According to the mechanism proposed by Mulders (1973), 2-acetylthiazole, the most abundant thiazole identified,

should be formed from the reaction of Strecker aldehyde of cysteine with pyruvaldehyde, a degradation product of glucose. The formation of 2-(1-propanonyl)thiazole followed the same mechanism as 2-acetylthiazole, and 4-methyl-2-acetylthiazole also must be formed from the interaction of amino acid and sugar. There is little known about the mechanism of the formation of thiophenes. Thiophene itself was observed in the thermal degradation of cysteine and glutathione, and many thiophenes, especially the long-chain, alkyl-substituted thiophenes, were formed when they reacted with 2,4-decadienal (Zhang and Ho, 1989). In the present study, thiophenes became the most dominant volatiles. This indicated that the formation of thiophenes could be catalyzed by the Maillard browning reaction.

Cyclic polysulfides, such as 3,5-dimethyl-1,2,4-trithiolane, are the components frequently associated with meat-like aromas. They are formed from the interaction of aldehydes, hydrogen sulfide, and ammonia (Boelens et al., 1974). Although they are the major volatile compounds formed by cysteine and glutathione degradation, in the presence of glucose, their formation was significantly decreased. In the GG system, a very limited number of cyclic polysulfides were detected, and this may be due to the lack of aldehydes available for the reaction with hydrogen sulfide.

Roasted sesame and cooked-rice-like aromas with stronger sulfur notes were observed in the system of cysteine/glucose (CG) compared with glutathione/glucose (GG). Although about the same amount of carbonyls were produced in these two systems, twice as many furans were detected in the GG system. On the other hand, about 3 times the amount of thiazoles and thiophenes, 4 times the amount of pyrazines, and 30 times the amount of polysulfur-containing compounds were produced in the CG system than in the GG.

As shown in Table II, the degradation of cysteine alone produced the greatest amount of volatile compounds compared with the interaction of either 2,4-decadienal or glucose. Glutathione was less reactive than cysteine in the reaction with carbonyl compounds. The quantitation also shows that, in the presence of carbonyls, the formation of thiophene was promoted but the generation of other sulfur-containing compounds was depressed, especially in the GG model system. The amount of other sulfur-containing compounds was in the order Cys > CD > CG and Glut. > GD > GG. The lower yield of polysulfides and thiadines when cysteine and glutathione reacted with glucose may be the reason that sesame and cooked-rice-like aromas were generated instead of fresh garlic flavor and a bloody and burnt beef aroma which was observed in the interaction of cysteine or glutathione with 2,4-decadienal (Zhang and Ho, 1989).

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LITERATURE CITED

- Boelens, M.; van der Linde, L. M.; de Valois, P. J.; van Dort, H. M.; Takken, H. J. Organic sulfur compounds from fatty aldehydes, hydrogen sulfide, thiols and ammonia as flavor constituents. *J. Agric. Food Chem.* **1974**, *22*, 1071-1076.
- Dietrich, P.; Lederer, E.; Winter, M.; Stoll, M. *Helv. Chim. Acta* **1964**, *47*, 1581-1590.

- Heller, S. R.; Milne, G. W. A. *EPA/NIH Mass Spectral Data Base*; U.S. Department of Commerce: Washington, DC, 1980.
- Hodge, J. E. Chemistry of browning reactions in model systems. *J. Agric. Food Chem.* **1953**, *1*, 928-942.
- Kato, S.; Kurata, T.; Kujimaki, M. Volatile compounds produced by the reaction of L-cysteine or L-cystine with carbonyl compounds. *Agric. Biol. Chem.* **1973**, *37*, 539-544.
- Kitada, N.; Shimazaki, H.; Komata, Y. Meat-like flavor, U.S. Patent, 3620772, 1971.
- Ledl, F.; Severin, T. Sulfur compounds from cysteine and xylose. *Z. Lebensm.-Unters. Forsch.* **1974**, *154*, 29-31.
- MacLeod, G. The scientific and technological basis of meat flavors. In *Developments in Food Flavours*; Birch, G. G., Lindley, M. G., Eds.; Elsevier Applied Science: London, 1986; pp 191-223.
- Majlat, P.; Erdos, Z.; Takacs, J. Calculation and application of retention indices in programmed temperature gas chromatography. *J. Chromatogr.* **1974**, *91*, 89-103.
- Mason, M. E.; Johnson, B.; Hamming, M. Flavor components of roasted peanuts, some low molecular weight pyrazines and a pyrrole. *J. Agric. Food Chem.* **1966**, *14*, 454-460.
- MSDC, *Eight Peak Index of Mass Spectra*; The Royal Society of Chemistry: London, 1984.
- Mulders, E. J. Volatile compounds from the non-enzymic browning reaction of the cysteine/cystine-ribose system. *Z. Lebensm.-Unters. Forsch.* **1973**, *152*, 193-201.
- Mussinán, C. J.; Walradt, J. P. Volatile constituents of pressure cooked pork liver. *J. Agric. Food Chem.* **1974**, *22*, 827-831.
- Pittet, A. O.; Muralidhara, R.; Walradt, J. P.; Kinlin, T. The synthesis and properties of alkylated five- and six-membered alicyclic pyrazines. *J. Agric. Food Chem.* **1974**, *22*, 273-279.
- Shibamoto, T.; Akiyama, T.; Sakaguchi, M.; Enomoto, Y.; Masuda, H. A study of pyrazine formation. *J. Agric. Food Chem.* **1979**, *2*, 1027-1031.
- Shu, C.-K. Study of the Reaction between Cysteine and 2,5-Dimethyl-4-hydroxy-3(2H)-Furanone. Ph.D. Dissertation, Rutgers University, New Brunswick, NJ, 1984.
- Shu, C.-K.; Hagedorn, M. L.; Ho, C.-T. Two novel thiophenes identified from the reaction between cysteine and 2,5-dimethyl-4-hydroxy-3(2H)-furanone. *J. Agric. Food Chem.* **1986**, *34*, 344-346.
- Stoll, M.; Winter, M.; Gaustchi, F.; Flament, I.; Willhalm, B. *Helv. Chim. Acta* **1967**, *50*, 628-694.
- Ten Noever de Brauw, M. C.; Bouwman, J.; Tas, A. C.; La Vos, G. F. *Compilation of Mass Spectra of Volatile Components in Foods*; Central Institute for Nutrition and Food Research: Zeist, The Netherlands, 1983.
- Tressl, R. Formation of flavor components in roasted coffee. 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 285-301.
- Tressl, R.; Helak, B.; Martain, 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.
- Wang, P. S.; Kato, H.; Fujimaki, M. Studies on flavor components of roasted barley. Part III. The major volatile basic compounds. *Agric. Biol. Chem.* **1969**, *33*, 1775-1781.
- Watanabe, K.; Sato, Y. Gas chromatographic and mass spectral analysis of heated beef flavor compounds of beef fats. *Agric. Biol. Chem.* **1971**, *35*, 756-763.
- Werkhoff, P.; Bruning, J.; Emberger, R.; Guntert, M.; Kopsel, M.; Kuhn, W.; Surburg, H. Isolation and characterization of volatile sulfur-containing meat flavor components in model systems. *J. Agric. Food Chem.* **1990**, *38*, 777-791.
- Zhang, Y.; Ho, C.-T. Volatile compounds formed from the thermal interaction of 2,4-decadienal with cysteine and glutathione. *J. Agric. Food Chem.* **1989**, *37*, 1016-1020.
- 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.

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