Microwave-Induced Volatiles of the Maillard Model System under Different pH Conditions

Helen C. H. Yeo and Takayuki Shibamoto*

Department of Environmental Toxicology, University of California, Davis, California 95616

The volatile heterocyclic compounds produced from a D-glucose/L-cysteine browning model system by microwave irradiation were isolated and identified by gas chromatography and mass spectrometry. Microwave samples were prepared under different pHs. As the pH increased, the total volatiles generated also increased. A total of 22 compounds were identified. The major compounds formed at pH 2, 5, and 7 were furans, pyrroles, and thiophenes. At pH 9, the major compounds formed were oxazoles, pyrazines, thiazoles, pyranones, and furanones. The samples at pH 2, 5, and 7 had a pungent and sulfurous odor, whereas at pH 9 a nutty, meaty, and roasted aroma. 2-Methylthiazolidine was detected for the first time in a sugar/amino acid model system. The formation mechanism of 2-methylthiazolidine is proposed on the basis of the formation of a hemimercaptal intermediate at different pHs.

Microwave-cooked foods often suffer from a lack of browning and preferable flavors. These shortcomings have led to a slow acceptance of microwave cooking despite its several advantages, especially simplicity. It is expected that in 1990 the percentage of U.S. households owning at least one microwave oven exceeds 80% (Shaath and Azzo, 1989).

In contrast to the great number of papers on the production of flavor compounds caused by thermal heating in the Maillard system, there are virtually no papers on those produced by microwave irradiation. Few papers have been published on the production of flavor compounds by microwave irradiation in real systems such as boiled beef (MacLeod and Coppock, 1976), baked cake (Whorton and Reineccius, 1989), and popcorn (Walradt et al., 1970). A recent paper on the influence of microwave heating on flavors produced by a model system of amino acid and diacetyl demonstrated that the loss of volatile acids varied widely depending on the composition of the microwave medium, such as water and salt concentration (Steinke et al., 1989).

Previous studies have demonstrated that pH played an important role in the production of heterocyclic flavor compounds in the Maillard model system (Leahy and Reineccius, 1989; Shibamoto and Bernhard, 1977; Shibamoto, 1980, 1983). In the present study, therefore, the heterocyclic flavor compounds produced from the L-cysteine/D-glucose Maillard model system upon microwave irradiation under various pH conditions were isolated and identified to investigate a role of pH in flavor formation.

EXPERIMENTAL PROCEDURES

Materials. L-Cysteine and D-glucose were purchased from Aldrich Chemical Co. (Milwaukee, WI); dichloromethane was purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). All authentic chemicals were purchased from reliable commercial sources.

Sample Preparation. *Microwave Sample.* L-Cysteine (0.05 mol) and D-glucose (0.05 mol) were dissolved in approximately 30 mL of deionized water. Each solution was then adjusted to the desired pH (2, 5, 7, 9) with either 6 N NaOH or 6 N HCl, and the final volume was brought to 50 mL with deionized water. The group of solutions was then irradiated at the high setting of a 700-W microwave oven for 15 min. This irradiation time was chosen because it coincided with the onset of sample browning. Before 15 min, no browning would occur, but after that time, the sample would begin to char. At 4-min intervals, the irradiation was interrupted and the samples were rotated 90° to ensure uniform irradiation. The uniformity of the sample was confirmed by placing identical samples in different starting positions, and irradiating the sample for the same time period. No significant variations in the total yield of volatiles were observed.

After microwave irradiation, each brown mass formed was dissolved in 100 mL of deionized water, and the resulting solution was adjusted to pH 8 with 6 N NaOH to enhance the extraction of nitrogen-containing heterocyclic compounds. The microwave products were extracted with 50 mL of dichloromethane with a liquid–liquid continuous extractor for 6 h, and the extract was dried over anhydrous sodium sulfate for 12 h. The sodium sulfate was then removed by filtration, and the filtrate was concentrated to slightly in excess of 1 mL by fractional distillation with a Vigrex column. The solution was then transferred into a concentration tube and its volume reduced to exactly 1 mL under a purified nitrogen stream. Undecane (0.5 mL) was then added as a gas chromatographic internal standard, and the solution was furhter reduced to 0.5 mL under a nitrogen stream.

All data obtained in this study were collected in duplicate.

Identification of Products Formed in the L-Cysteine/D-Glucose Model System. The samples prepared by the procedure described above were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Identification of the chromatographic peaks of the samples was made by comparing their mass spectra and gas chromatographic retention indices to those of authentic compounds.

Instruments. A Quasar Model MQ 7796 AW, 700-W, "Easy-Matic Cooking" microwave oven was used for the irradiation of the Maillard model mixtures.

A Hewlett-Packard (HP) Model 8452A diode array spectrophotometer with HP 89510 UV/vis software was used to measure the degree of browning at 420 nm.

A HP Model 5890 gas chromatograph equipped with a flame ionization detector (FID) and a 60 m × 0.25 mm i.d. DB-Wax bonded-phase fused silica capillary column (J&W Scientific, Folsom, CA) was used for routine analyses. Peak areas were integrated by using a Spectra Physics Chromjet integrator. The GC oven was held at 60 °C for 4 min and programmed at 4 °C/min to a final temperature of 180 °C, which was held for 30 min. The temperatures of the injector and the detector were 240 and 250 °C, respectively. The helium carrier gas flow rate was 27.8 cm/s, with a split ratio of 1:45.

A HP Model 5890 GC interfaced to a VG Trio II mass spectrometer with a VG 11-250 computer data system was used for MS identification on the GC components. The ionization voltage was 70 eV, and the ion source temperature was 150 °C. The column and oven conditions for GC/MS were as described for the HP 5890 GC/FID analysis.

0021-8561/91/1439-0370$02.50/0

Table I. Products Identified in the L-Cysteine/D-Glucose Model System upon Microwave Irradiation

products	Kovats index (DB-Wax)	GC peak area ratio[a] pH 2	pH 5	pH 7	pH 9
oxazole					
4,5-dimethyloxazole	1152	*b*	*b*	*b*	13.44
trimethyloxazole	1206	*b*	0.27	1.08	1.68
pyridine					
2-methylpyridine	1234	*b*	0.11	008	0.54
thiazole					
unsubstituted	1242	0.22	0.12	0.23	1.26
2,5-dimethylthiazole	1354	*b*	*b*	*b*	5.06
2-methylthiazolidine	1415	1.85	0.56	0.69	*b*
pyrazine					
2-methylpyrazine	1263	*b*	*b*	*b*	4.26
2,5-dimethylpyrazine	1316	*b*	*b*	*b*	5.40
2,6-dimethylpyrazine	1319	*b*	*b*	*b*	3.84
2-ethylpyrazine	1323	*b*	*b*	*b*	3.81
2,3-dimethylpyrazine	1335	*b*	*b*	*b*	4.94
2-ethyl-6-methylpyrazine	1363	*b*	*b*	*b*	3.27
trimethylpyrazine	1391	*b*	*b*	*b*	10.75
tetramethylpyrazine	1457	*b*	*b*	*b*	2.92
furan					
furfurylmercaptan	1402	*b*	0.18	0.22	*b*
2-acetylfuran	1483	5.69	31.19	41.49	5.46
2-furanmethanol	1573	*b*	0.11	0.26	*b*
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	2039	3.39	1.04	2.81	8.12
thiophene					
2-thiophenethiol	1530	0.75	0 .71	0.70	*b*
3-thiophenethiol	1555	0.04	0.32	0.47	*b*
pyrrole					
2-acetylpyrrole	1952	2.79	3.60	5.64	3.88
pyran					
2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	2266	0.92	4.31	13.01	76.10

[a] GC peak area of product/GC peak area of internal standard; values are means of duplicates. [b] Not detected.

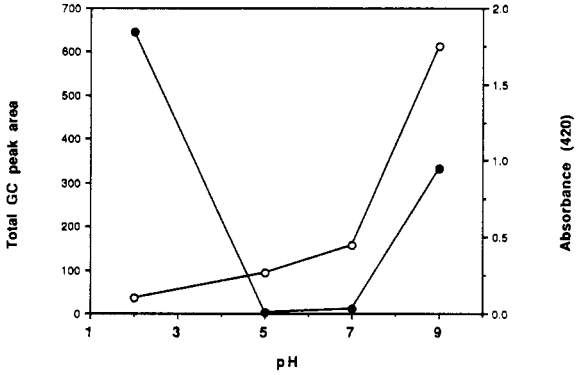

Figure 1. Effects of pH on the total GC peak area of volatiles (○) and the degree of browning (●) in the L-cysteine/D-glucose model system upon microwave irradiation.

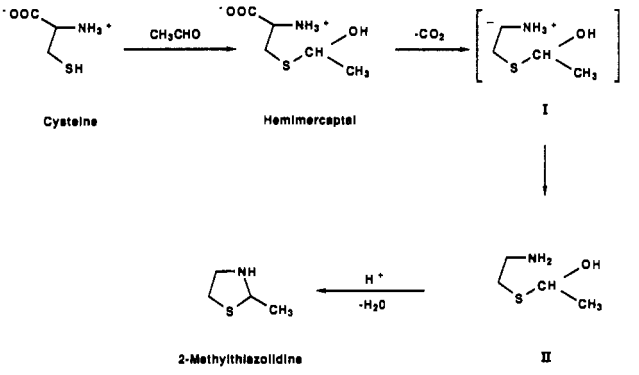

Figure 2. Possible formation mechanism of 2-methylthiazolidine.

RESULTS AND DISCUSSION

Numerous studies (Sheldon et al., 1986; Kato et al., 1973) have demonstrated that the production of flavors in the L-cysteine/D-glucose Maillard system involves complex mechanisms. Many papers examined the thermal activation (Scanlan et al., 1973) or the photochemical activation (Sheldon et al., 1988; Sheldon and Shibamoto, 1988) of this model system, but virtually none investigated the effects of microwave irradiation.

Figure 1 shows the effects of pH on the total GC peak area of volatiles and the degree of browning in microwave samples. The peak areas of the volatiles extracted were calculated and normalized to the peak area of the internal standard added. As pH increases, the total GC peak area of volatiles also increases. The total GC peak area of volatiles generated at pH 9 is 12-fold greater than that at pH 2. The degree of browning of the resulting samples, however, followed a different pattern. The sample at pH 2 had the darkest brown color, whereas the sample at pH 5 had a very light yellow color. This suggests that the degree of browning does not necessarily match the amount of preferable volatiles produced. The higher amount of total volatiles formed at pH 9 also indicates that the production of volatiles in the Maillard system upon microwave irradiation is catalyzed by base.

Table I shows the volatile chemicals produced by microwave irradiation under different pH conditions (pH 2, 5, 7, and 9). Of the four pH samples studied, only the sample at pH 9 produced a distinct nutty, roasted, and meaty flavor, in addition to a strong popcorn flavor. This sample also gave an intrinsic pungent, raw, and burnt aroma. The major compounds identified in this sample were oxazoles, pyridines, thiazoles, and pyrazines. 4,5-Dimethyloxazoles reportedly possessed characteristic green- and vegetable-like flavor (Lee et al., 1981); 2-methylpyridine also had a green note (Pittet and Hruza, 1974). These findings may explain the off-flavors present in the pH 9 sample. The sample also generated significant amounts of thiazole and 2,5-dimethylthiazole which generated meaty and roasted flavors, a finding previously reported

by Baltes (1979). The eight pyrazines formed in the pH 9 sample constituted the largest number of compounds formed in any class. A roasted peanut flavor was associated with 2-methylpyrazine (Pittet and Hruza, 1974), and a sweet and toasted corn aroma was associated with 2,6-dimethylpyrazine (Boyko et al., 1978), which explains the popcorn and nutty flavors in the pH 9 sample.

The samples at pH 5 and 7 gave a strong, pungent, and sulfurous odor characteristic of rotten eggs. The GC profiles of these two samples were very similar. The major compounds formed were 2-acetylfuran, 2-acetylpyrrole, 3-thiophenethiol, and furfurylmercaptan. In food systems, 2-acetylfuran gives a pleasant and ketonic odor (Hodge, 1967). The flavor characteristics of 2-acetylpyrrole are not mentioned in the literature, but they are generally thought to contribute to the undesirable flavor in cooked meat (Fors, 1983). 3-Thiophenethiol and furfurylmercaptan formed at these two pHs are sulfur-containing heterocyclic compounds, which may contribute to the intense pungent characteristics found in these samples. In contrast to the pH 9 sample, which had eight pyrazines, these samples at pH 5 and 7 did not yield any detectable pyrazine. The lack of desirable flavors found in microwave heating may be due to the absence of these pyrazines.

The sample at pH 2 generated the fewest volatiles but produced the most intense brown color. Like the samples at pH 5 and 9, this sample also gave a pungent odor and lacked the favorable roasted and nutty attributes. A major compound formed was 2-methylthiazolidine. Thiazolidines generally produce a characteristic popcorn flavor. The amount of 2-methylthiazolidine identified in the microwave sample decreased with increasing pH. This compound has not been previously reported in any thermal systems. From these pH studies, it appears that the formation of 2-methylthiazolidine is pH dependent via a hemimercaptal intermediate.

The possible mechanism of the formation of 2-methylthiazolidine is shown in Figure 2. At pH 2, 5, and 7, cysteine exists predominantly in the zwitterionic form. The amino group (pK_a = 10.7) is protonated, and therefore it cannot undergo nucleophilic attack. However, the mercapto group (pK_a = 8.3) on cysteine can act as a nucleophile and can attack the carbonyl group on acetaldehyde to form a hemimercaptal. This is followed by decarboxylation to form an intermediate, I, which readily isomerizes to form II via intramolecular hydrogen transfer, freeing the amino group. A water molecule is subsequently eliminated to form 2-methylthiazolidine.

Under basic conditions (pH 9), the mercapto group on cysteine is deprotonated, giving a nucleophilic thiolate anion. However, the hemimercaptal intermediate mentioned above is not formed because the hydroxyl group would react with acetaldehyde more readily than with the thiolate anion. This explains the absence of 2-methylthiazolidine in the pH 9 sample.

The yields of the heterocyclic compounds like 2-acetylpyrrole, 3-thiophenethiol, furfurylmercaptan, and 2-acetylfuran were found to be higher at pH 5 and 7 than at pH 2 and 9. These compounds contain four or five carbon units, indicating that their precursors are derived from glucose, as cysteine has only two carbon units. In contrast, pyrazines and oxazoles, which contain two carbon units, are formed readily at pH 9, thus allowing the base-catalyzed fragmentation of glucose through a reverse aldol reaction into a two-carbon unit, which in turn reacts with ammonia to form an α-aminocarbonyl intermediate (Shibamoto and Bernhard, 1977).

LITERATURE CITED

Baltes, W. Röstaromen. *Dtsch. Lebensm. Rundsch.* **1979**, *75*, 2–7.

Boyko, A.; Morgon, M.; Libbey, L. Porous Polymer Trapping for GC/MS Analysis of Vegetable Flavors. In *Analysis of Food and Beverages; Headspace Techniques*; Charalambous, G., Ed.; Academic Press: New York, 1978; pp 57–79.

Fors, S. *The Maillard Reaction in Foods and Nutrition*; Waller, G. R., Feather, M. S., Eds.; ACS Symposium Series 215; American Chemical Society: Washington, DC, 1983; pp 185.285.

Hodge, J. Origin of Flavor in Foods: nonenzymatic browning reactions. In *Chemistry and Physiology of Flavors*; Schultz, H. W., Day, E. A., Libbey, L. M., Eds.; AVI Publishing: Westport, CT, 1967; pp 465–491.

Kato, S.; Kurata, T.; Fujimaki, M. Volatile Compounds Produced by the Reaction of L-cysteine or L-cystine with Carbonyl Compounds. *Agric. Biol. Chem.* **1973**, *37*, 539–544.

Leahy, M. M.; Reineccius, G. A. Kinetics of the formation of alkylpyrazines—Effects of pH and water activity. In *Thermal Generation of Aromas*; Parliment, T. H., McGorrin, R. J., Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp 196–208.

Lee, M.-H.; Ho, C.-T.; Chang, S. Thiazoles, Oxazoles, and Oxazolines Identified in the Volatile Flavor of Roasted Peanuts. *J. Agric. Food Chem.* **1981**, *29*, 684–686.

MacLeod, G.; Coppock, B. M. Volatile Flavor Components of Beef Boiled Conventionally and by Microwave Radiation. *J. Agric. Food Chem.* **1976**, *24*, 835–843.

Pittet, A. O.; Hruza, D. E. Comparative Study of Flavor Properties of Thiazole Derivatives. *J. Agric. Food Chem.* **1974**, *22*, 264–269.

Scanlan, R. A.; Kayser, S. G.; Libbey, L. M.; Morgan, M. E. Identification of Volatile Compounds from Heated L-Cysteine.HCl/D-Glucose. *J. Agric. Food Chem.* **1973**, *21*, 673–675.

Shaath, N. A.; Azzo, N. R. The Delta T Theory. In *Thermal Generation of Aromas*; Parliment, T. H., McGorrin, R. J., Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp 512–518.

Sheldon, S. A.; Shibamoto, T. Volatile Compounds Produced in L-cysteine/D-glucose Model System by Sunlight Irradiation. *J. Food Sci.* **1988**, *53*, 196–198.

Sheldon, S. A.; Russell, G. F.; Shibamoto, T. Photochemical and Thermal Activation of Model Maillard Reaction Systems; Amino-Carbonyl Reaction in Food and Biological Systems. In *Proceedings of the 3rd International Symposium on the Maillard Reaction*; Fujimaki, M., Namiki, M., Kato, H., Eds.; Elsevier: New York, 1986; pp 145–154.

Sheldon, S. A.; Jones, A. D.; Shibamoto, T. Photochemical Products of a Cysteine/D-Glucose Browning Model System. *J. Agric. Food Chem.* **1988**, *36*, 604–606.

Shibamoto, T. Heterocyclic compounds found in cooked meats. *J. Agric. Food Chem.* **1980**, *28*, 237–243.

Shibamoto, T. Heterocyclic compounds in browning and browning/nitrite model systems: Occurrence, formation mechanisms, flavor characteristics and mutagenic activity. In *Instrumental analyis of foods, recent progress*; Charalambous, G., Inglett, G., Eds.; Academic Press: New York, 1983; Vol. 1, pp 229–278.

Shibamoto, T.; Bernhard, R. A. Investigation of Pyrazine Formation Pathways in Glucose-Ammonia Model Systems. *Agric. Biol. Chem.* **1977**, *41*, 143–153.

Steinke, J. A.; Frick, C. M.; Gallagher, J. A.; Strassburger, K. J. Influence of Microwave Heating on Flavor. In *Thermal Generating of Aromas*; Parliment, T. H., McGorrin, R. J., Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp 519–252.

Walradt, J. P.; Lindsay, R. C.; Libbey, L. M. Popcorn Flavor: Identification of Volatile Compounds. *Agric. Food Chem.* **1970**, *18*, 926–928.

Whorton, C.; Reineccius, G. A. Flavor Development in a Microwaved Versus a Conventional Baked Cake. In *Thermal Generation of Aromas*; Parliment, T. H., McGorrin, R. J., Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp 526–532.

Received for review April 10, 1990. Revised manuscript received July 6, 1990. Accepted July 30, 1990.

Registry No. D-Glucose, 50-99-7; L-cysteine, 52-90-4; 2-methylthiazolidine, 24050-16-6; 4,5-dimethyloxazole, 20662-83-3; trimethyloxazole, 20662-84-4; 2-methylpyridine, 109-06-8; thiazole, 288-47-1; 2,5-dimethylthiazole, 4175-66-0; 2-methylpyrazine, 109-08-0; 2,5-dimethylpyrazine, 123-32-0; 2,6-dimethylpyrazine, 108-50-9; 2-ethylpyrazine, 13925-00-3; 2,3-dimethylpyrazine, 5910-89-4; 2-ethyl-6-methylpyrazine, 13925-03-6; trimethylpyrazine, 14667-55-1; tetramethylpyrazine, 1124-11-4; furfurylmercaptan, 98-02-2; 2-acetylfuran, 1192-62-7; 2-furanmethanol, 98-00-0; 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 3658-77-3; 2-thiophenethiol, 7774-74-5; 3-thiophenethiol, 7774-73-4; 2-acetylpyrrole, 1072-83-9; 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one, 28564-83-2.