Volatile Compounds Generated in Serine−Monosaccharide Model Systems

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The model Maillard reactions of serine−ribose (S−R) and serine−glucose (S−G) were studied. The reactions were conducted in a pH 8 aqueous solution at 160 °C for 2 h. Three times more volatiles were generated in the S−R system than in the S−G system, and in total, 37 compounds were identified in the two reaction systems. Some novel pyrazines were formed in both reaction solutions. In the serine−ribose system, the identified pyrazines were 2-(2-furfuryl)pyrazine, 2-(2-furfuryl)-5(and 6)-methylpyrazine, and 2-(2-furfuryl)-3,5-dimethylpyrazine. In the serine−glucose system, 2-[5-(hydroxymethyl)-2-furfuryl]-3,5-dimethylpyrazine was the only identified bicyclic pyrazine compound. To confirm the formation of these pyrazine compounds, they were prepared in a model reaction involving acetol, ammonium acetate, and corresponding aldehydes. In such model reaction systems, we also prepared 2-(2-thienylmethyl)-3,5(and 3,6)-dimethylpyrazine and 2-(2-pyrrylmethyl)-3,5(and 3,6)-dimethylpyrazine. Mass spectra of some compounds were reported for the first time.

Keywords: *Serine; glucose; ribose; Maillard; volatile; flavor; roasty; pyrazine; furfuryl; acetol; aldehyde*

INTRODUCTION

Serine is a ubiquitous amino acid in nature, and is classified as a nonessential amino acid. There have been few studies on serine-containing model systems as compared to other amino acids. In an early work on pyrolysis of serine, pyrazines, pyrroles, 2,5-diketo-3,6-dimethylpiperazine, and appreciative amounts of paraldehyde were identified (Kato et al., 1970). Baltes and Bochmann have published a series of papers on the generation of volatiles from serine and threonine with sucrose under coffee roasting conditions (Baltes and Bochmann, 1987a−d). They have identified approximately 350 compounds, including alkyl-, alkenyl-, and acyl-substituted furans, pyrroles, pyrazines, pyridines, oxazoles, and other compounds. Many of these volatile compounds exist in roasted coffee. Baltes and Bochmann have also found that almost all of these compounds, except furans and oxazoles, can be observed when serine and threonine are heated without sucrose, demonstrating the role of pyrolysis in volatile generation. Reese and Baltes have studied the model reactions involving serine and fructose, glucose, diacetyl, or cyclotene in an aqueous solution (Reese and Baltes, 1992). Their results show that the generation of pyrazines, pyridines, and carbonyl compounds increases with rising temperature, while the generation of furans, furanones, and pyranones decreases.

Higher temperatures and weakly basic conditions are favorable conditions for Maillard reaction. In this study, we compared the volatile generation between serine−ribose and serine−glucose model systems. Experiments were performed at 160 °C with pH 8 aqueous solutions. Some new volatile compounds were identified. To confirm the formation of the newly identified furfuryl-substituted pyrazines, we have prepared them in model reactions involving acetol, ammonium acetate, and corresponding aldehydes. Two isomers of these pyrazine compounds were observed in these model reactions.

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MATERIALS AND METHODS

Materials. Serine, ribose, glucose, acetol, ammonium acetate, furfural, 5-(hydroxymethyl)-2-furfural, thiophene-2-carboxyaldehyde, and pyrrole-2-carboxyaldehyde were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Model Reaction of Serine with Monosaccharide. Equimolar amounts (10 mmol) of serine and ribose or glucose were dissolved in 50 mL of distilled water, and the pH of the solution was adjusted to 8.0 using 1 M NaOH. The solution was then sealed in a 300 mL Hoke stainless steel vessel and heated at 160 °C for 2 h in an incubating oven. After it was cooled to room temperature, the heated mixture was transferred in a beaker, and the pH was adjusted from approximately 4.5 to 8.0. The solution was extracted with 3 × 30 mL of CH_2Cl_2 after spiking with tridecane as the internal standard. The organic phase was dried over anhydrous sodium sulfate and concentrated to approximately 0.5 mL under a gentle stream of nitrogen gas. The concentrated sample was ready for GC and GC−MS analysis.

Pyrazine Preparation in Acetol/Ammonium Acetate/Aldehyde Reactions. Equimolar amounts (5 mmol) of acetol, ammonium acetate, and aldehyde [2-furfural, 5-(hydroxymethyl)-2-furfural, thiophene-2-carboxyaldehyde, or pyrrole-2-carboxyaldehyde] were dissolved in 50 mL of distilled water, and the reaction solution was sealed in a reaction bomb and heated in an oven at 100 °C for 4 h. After the solution was cooled to room temperature, the pH value of the heated mixture was adjusted to pH 11 using a 1 M NaOH solution, except in the reaction mixture containing 5-(hydroxymethyl)-2-furfural where the pH was adjusted to pH 8. The extraction and concentration procedures were the same as described above.

Gas Chromatography (GC) and Gas Chromatography−Mass Spectrometry (GC−MS) Analysis. The gas chromatography was performed on a Varian model 3400

S0021-8561(97)00934-5 CCC: $15.00
Published on Web 04/03/1998

Table 1. Identified Volatile Compounds in Serine−Ribose (S−R) and Serine−Glucose (S−G) Systems

compound	RI[a]	concentration (mg/mol)[b] S−R	S−G	reference	*m/z* (relative intensity)
2,3-butanedione	<600	nd[c]	1.0	Lib.[d]	
2-butanone	<600	nd	3.5	Lib.	
2-methylfuran	<600	2.5	nd	Lib.	
1-hydroxy-2-propanone	631	39.5	45.0	Lib.	
3-hydroxy-2-butanone	680	11.5	9.0	Lib.	
pyrazine	709	40.5	69.0	Lib.	
dihydro-2-methyl-3(2*H*)-furanone	780	nd	4.0	Lib.	
methylpyrazine	806	17.5	20.0	Lib.	
furfural	820	808	9.0	Lib.	
1-(acetyloxy)-2-propanone	836	2.5	nd	Lib.	
2-furanmethanol	842	76.0	4.5	Lib.	
2(5*H*)-furanone	871	19.5	nd	Lib.	
2-acetylfuran	891	17.5	4.0	Lib.	
2,5-dimethylpyrazine	896	14.5	23.5	Lib.	
ethylpyrazine	899	nd	4.5	Lib.	
2,3-dimethylpyrazine	902	nd	1.5	Lib.	
1-(2-furyl)propanone	931	7.5	nd	Lib. and Salter et al. (1988)	124 (38), 82 (36), 81 (96), 53 (66), 43 (100), 39 (10)
5-methyl-2-furfural	939	nd	11.0	Lib.	
2-ethyl-6-methylpyrazine	988	nd	2.5	Lib.	
1*H*-pyrrole-2-carboxyaldehyde	989	3.5	nd	Lib.	
trimethylpyrazine	992	4.5	nd	Lib.	
2-acetylpyrazine	998	trace	3.0	Lib.	
2-hydroxy-3-methyl-2-cyclopenten-1-one	1006	2.5	22.0	Lib.	
1-(2-furyl)-1,2-propanedione	1035	5.5	nd		138 (18), 95 (100), 67 (10), 43 (50), 39 (38)
3,5-dimethylcyclopentene-1,2-dione	1040	5.5	nd	Lib.	
1-(2-furyl)butan-3-one	1048	4.0	nd	Lib. and Salter et al. (1988)	
benzoxazole	1067	21.5	nd	Lib.	
2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	1131	nd	17.0	Lib.	
1-(2-furfuryl)-1*H*-pyrrole	1163	6.0	nd	Lib.	147 (54), 117 (5), 81 (100), 53 (40)
5-hydroxymethyl-2-furfural	1200	nd	99.0	Lib.	
2-(2-furfuryl)pyrazine	1279	10.5	nd	Baltes et al. (1987c)	161 (10), 160 (93), 132 (9), 131 (100), 104 (13), 81 (48), 53 (29), 39 (17)
2-(2-furfuryl)-5-methylpyrazine	1346	3.5	nd	Baltes et al. (1987c)	175 (13), 174 (89), 159 (5), 145 (100), 131 (10), 104 (10), 81 (19), 67 (6), 53 (11), 42 (16), 9 (14)
2-(2-furfuryl)-6-methylpyrazine	1361	4.5	nd	Baltes et al. (1987c)	175 (13), 174 (98), 145 (100), 104 (8), 81 (33), 66 (10), 53 (13), 51 (15), 39 (31)
1-(2-furfuryl)pyrrole-2-carboxyaldehyde	1374	43.0	nd	Baltes et al. (1987b)	175 (34), 147 (8), 146 (6), 81 (100), 53 (32), 39 (16)
1-(2-furfuryl)pyrrole-2-carboxyaldehyde	1394	15.5	nd	Baltes et al. (1987b)	175 (43), 146 (8), 81 (100), 53 (32), 39 (12)
2-(2-furfuryl)-3,5-dimethylpyrazine	1417	6.0	nd	Baltes et al. (1987c)	189 (16), 188 (100), 160 (13), 159 (98), 145 (12), 107 (5), 81 (21), 65 (6), 42 (20), 39 (27)
2-[5-(hydroxymethyl)furfuryl]-3,5-dimethylpyrazine	1753	nd	1.0		219 (18), 218 (100), 217 (17), 201 (72), 187 (35), 173 (13), 159 (32), 111 (33), 59 (15), 39 (23)

[a] Retention index, calculated according to the retention time of *n*-alkanes on a DB-1 column. [b] Milligrams of volatile per mole of serine. [c] Not detected. [d] Wiley mass spectra library.

instrument equipped with a flame ionization detector (FID) and a nonpolar fused silica capillary column [DB-1, 60 m × 0.32 mm (inside diameter), 1.0 μm film thickness, J&W Scientific]. The column temperature was programmed from 40 to 260 °C at a rate of 2 °C/min. The injector and detector temperatures were maintained at 270 and 300 °C, respectively. The flow rate of the helium carrier gas was 1 mL/min. The volume of the injected sample was 1 μL, and the split ratio was 25:1. GC−MS analysis was performed using an HP model 5790 chromatograph coupled with an HP 5970A mass-selective detector. The capillary column and temperature program were the same as those for the GC analysis. Mass spectra were obtained by electron ionization at 70 eV and mass scanning from 33 to 300. Compound quantification was based on the GC−FID data, and compound identification was based on mass spectra obtained from the GC−MS.

RESULTS AND DISCUSSION

Roasty and caramel flavor was obtained in both the S−R and S−G reactions. The composition of the isolated volatiles in both reaction mixtures is listed in

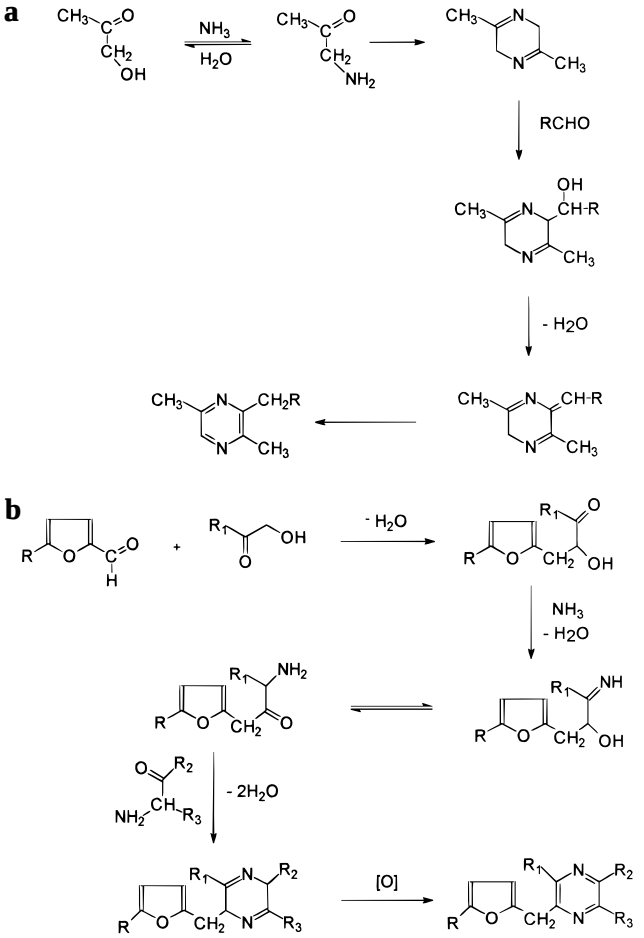

Figure 1. Mechanism of substituted pyrazine formation. (a) Pathway of substituted pyrazine formation in the model reaction involving acetol, ammonium acetate, and aldehydes (Chiu et al., 1990). (b) Modified pathway of 2-(2-furfuryl)-pyrazine formation in the serine-containing model system proposed by Reese and Baltes (1992).

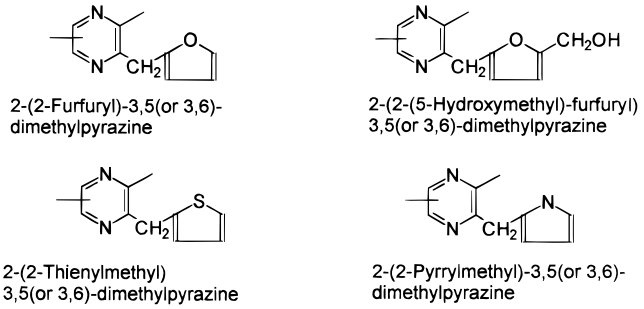

Figure 2. Chemical structure of bicyclic pyrazines prepared from the acetol-containing model reaction.

Table 1. The compounds are listed according to their elution order. In total, there were 1600 and 520 (milligrams per mole of serine) volatiles that were generated in the S−R and S−G systems, respectively, and 37 compounds were identified in the two reactions. The identified compounds included furans, furanones, pyrazines, pyrroles, and others. Due to the weak basic extraction condition we utilized, the acidic components did not show up in the chromatogram.

In the S−R system, the largest identified compounds were 2-furfural, 2-furanmethanol, 1-(2-furfuryl)pyrrole-2-carboxyaldehyde, pyrazine, 1-hydroxy-2-propanone (acetol), benzoxazole, 2(5*H*)-furanone, methylpyrazine, 2-acetylfuran, 2,5-dimethylpyrazine, and 3-hydroxy-2-butanone (acetoin), in decreasing order; the largest identified compounds in the S−G system were 5-(hydroxymethyl)-2-furfural, pyrazine, 1-hydroxy-2-propanone (acetol), 2,5-dimethylpyrazine, 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene), methylpyrazine, 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one, 5-methyl-2-furfural, 3-hydroxy-2-butanone (acetoin), and 2-furfural in decreasing order.

Quantitative data show that more total volatiles, furfuryl-substituted pyrazines, and pyrrole compounds were produced in a serine−ribose system [1600/24.5/68.0 (milligrams per mole of serine)] than in a serine−glucose system [520/1.0/0 (milligrams per mole of serine)], but there was no big difference in the total number of pyrazine compounds generated between these two model systems. A total of 101.5 (milligrams per mole of serine) and 125.0 (milligrams per mole of serine) pyrazines were identified in the serine−ribose and serine−glucose systems, respectively. Our results indicate that pentose sugar ribose is more active than hexose sugar glucose in a sugar−amino acid reaction.

Furfural is a well-known product derived from ribose. It is interesting to note that some furan-derived compounds with more than five carbon atoms are identified in the S−R system, such as 1-(2-furyl)propanone, 1-(2-furyl)-1,2-propandione, and 1-(2-furyl)butan-3-one. The

Table 2. Mass Spectra of Some Bicyclic Pyrazines

compound	RI[a]	*m/z* (relative intensity)
2-(2-furfuryl)-3,5-dimethylpyrazine	1417	189 (9), 188 (95), 160 (15), 159 (100), 145 (22), 133 (8), 118 (6), 107 (5), 91 (12), 81 (28), 71 (30), 65 (7), 53 (18), 42 (26), 39 (36)
2-(2-furfuryl)-3,6-dimethylpyrazine	1429	189 (10), 188 (76), 160 (12), 159 (100), 145 (6), 133 (5), 118 (7), 107 (4), 91 (8), 81 (18), 66 (8), 53 (14), 42 (17), 39 (22)
2-[5-(hydroxymethyl)furfuryl]-3,5-dimethylpyrazine	1753	219 (19), 218 (100), 217 (12), 201 (85), 173 (10), 159 (51), 111 (14), 107 (10), 65 (19), 42 (27), 39 (28)
2-[5-(hydroxymethyl)furfuryl]-3,6-dimethylpyrazine	1767	219 (12), 218 (100), 217 (11), 201 (66), 173 (11), 159 (52), 111 (5), 65 (5), 42 (11), 39 (20)
2-(2-thienylmethyl)-3,5-dimethylpyrazine	1603	206 (4), 205 (21), 204 (100), 203 (32), 189 (18), 171 (21), 159 (20), 97 (46), 53 (4), 39 (23)
2-(2-thienylmethyl)-3,6-dimethylpyrazine	1617	206 (7), 205 (14), 204 (100), 203 (31), 189 (20), 171 (21), 159 (44), 97 (33), 53 (5), 39 (17)
2-(2-pyrrylmethyl)-3,5-dimethylpyrazine	1596	188 (13), 187 (100), 186 (35), 172 (20), 159 (17), 145 (12), 80 (62), 53 (14), 39 (13)
2-(2-pyrrylmethyl)-3,6-dimethylpyrazine	1606	188 (7), 187 (100), 186 (35), 172 (25), 159 (31), 145 (20), 80 (51), 53 (17), 39 (23)

[a] Retention index, calculated according to the retention time of *n*-alkanes on a DB-1 column.

identification and occurrence of some of these compounds have been described in the paper of Salter et al. (1988).

Pyrroles are very important aroma compounds in food. Two pathways of pyrrole formation in the Maillard reaction have been defined (Baltes and Bochmann, 1987b): one is the reaction of furans with amino acids or amines, and the other is the reaction between 3-deoxyglycosone and amino acids. In the study of Baltes and Bochmann (1987b), the pyrolysis of serine and threonine contributed significantly to the pyrrole formation, in that nearly all alkylpyrroles and *N*-pyrroloalkanols were generated when the two amino acids were heated alone (Baltes and Bochmann, 1987b). In our study, five pyrroles were observed in the S−R system, including 1-(2-furfuryl)-1*H*-pyrrole and 1-(2-furfuryl)pyrrole-2-carboxylaldehyde. These two compounds were also reported in the roasted mixture of serine, threonine with surcrose (Baltes and Bochmann, 1987b), and heat-treated cereals (Shen et al., 1995). It has been suggested that they contribute to the aroma of popcorn (Shen et al., 1995).

Pyrazines are one of the most important groups among the identified volatiles in S−R and S−G systems. These compounds are widely distributed in food systems, especially when foods are processed at high temperatures under dry conditions. A series of extensive reviews on pyrazines in food have been published (Maga, 1982, 1992).

In terms of the individual pyrazine compounds in the S−R and S−G systems, trimethylpyrazine, 2-(2-furfuryl)pyrazine, 2-(2-furfuryl)-5(and 6)-methylpyrazine, and 2-(2-furfuryl)-3,5-dimethylpyrazine were only identified in the serine−ribose system, while ethylpyrazine, 2-ethyl-6-methylpyrazine, 2-acetylpyrazine, and 2-[5-(hydroxymethyl)-2-furfuryl]-3,5-dimethylpyrazine existed exclusively in the serine−glucose reaction solution. Some of furfuryl-substituted pyrazines were tentatively identified before, and the mass degradation pattern of furfurylpyrazine was also described (Baltes et al., 1987c; Reese et al., 1992). The structure difference of furfuryl-substituted pyrazines between the two systems may be explained by the large amount of furfural derived from ribose and the large amount of 5-(hydroxymethyl)-2-furfural derived from glucose.

The α-aminocarbonyls, which can be formed from the reactions between dicarbonyl compounds and amino acids or amines during Strecker degradation, were generally considered to be the precursors of pyrazine compounds. An alternative pyrazine formation pathway is also recognized in pyrolysis of such β-hydroxy amino acids as serine and threonine. These α-aminocarbonyls may react with each other to generate pyrazines during thermal processing, without involving reducing sugar (Baltes et al., 1987c).

Whichever pathway the formation of pyrazines takes, dihydropyrazines are the reasonable intermediates of substituted pyrazine compounds. These intermediates can react with aldehydes or ketones to generate various substituted pyrazines. Some methods based on dihydropyrazine for synthesizing substituted pyrazines were previously reported. An earlier paper reported the synthesis of various 5-substituted 2,3-dimethylpyrazines from 2,3-dimethyl-5,6-dihydropyrazines and various aldehydes and ketones in the presence of a metal alkoxide solution (Masuda et al., 1980). In the work ofChiu et al. (1990), they have synthesized some long-chain alkyl-substituted pyrazine at 100 °C for 4 h using a model system containing acetol, ammonium acetate, and long-chain aldehydes such as pentenal and hexanal, which are lipid oxidation products. Figure 1 demonstrates the reaction mechanism in this model and also shows the modified mechanism of 2-(2-furfuryl)pyrazine formation proposed by Reese and Baltes (1992).

To confirm the formation of furfuryl-substituted pyrazines in our study, we prepared them using the acetol-containing model systems with 2-furfural or 5-(hydroxymethyl)-2-furfural. Two isomers were observed in these model reactions, and their mass spectra showed a similar degradation pattern. Considering the steric hindrance difference of these compounds, the bicyclic substituted 3,5-dimethylpyrazine should be eluted earlier than its 3,6-dimethylpyrazine counterparts. Using this model reaction, 2-(2-thienylmethyl)-3,5(and 3,6)-dimethylpyrazine and 2-(2-pyrrylmethyl)-3,5(and 3,6)-dimethylpyrazine were also prepared when thiophene-2-carboxyaldehyde or pyrrole-2-carboxyaldehyde was applied. The mass spectral data are presented in Table 2. Even though these two bicyclic pyrazines were not among the compounds identified in the S−R or S−G systems, including their mass spectra here can be useful in identifying them in other models or real food systems.

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Received for review November 3, 1997. Revised manuscript received January 28, 1998. Accepted February 4, 1998.

JF970934F