Comparison of Volatile Generation in Serine/Threonine/Glutamine−Ribose/Glucose/Fructose Model Systems

Jianhong Chen and Chi-Tang Ho*

Department of Food Science, Cook College, Rutgers, The State University of New Jersey, 65 Dudley Road, New Brunswick, New Jersey 08901-8520

Thermal generation of volatiles in nine model reactions was studied and compared. Each of the model systems contained one amino acid and one monosaccharide. The amino acid was serine, threonine, or glutamine, and the monosaccharide was ribose, glucose, or fructose. More unsubstituted pyrazine was generated in serine−sugar systems than threonine−sugar systems. The formation of several furfuryl-substituted pyrazines and pyrroles was observed in some of the studied systems. Total pyrazines were generated more in glutamine-containing systems than in serine- and threonine-containing systems, and the reverse was true for generation of furfuryl-substituted compounds. Acetylpyrazine was generated in serine/threonine/glutamine−glucose and serine/glutamine−fructose systems.

Keywords: *Serine; threonine; glutamine; ribose; glucose; fructose; Maillard reaction; flavor*

INTRODUCTION

Serine and threonine are β-hydroxy α-amino acids, which are specific precursors in nitrogen-containing heterocyclic compound generation. The formation of several pyrazines was observed when serine or threonine was pyrolyzed alone (Wang and Odell, 1973). Baltes and Bochmann have published a series of papers on the generation of volatiles from serine, threonine, and sucrose under coffee-roasting conditions (Baltes and Bochmann, 1987a,b,c,d). In their experiment, serine, threonine, and sucrose were mixed thoroughly with 10% water and 10% sand, and the roasting was carried out by heating the mixture at 20 °C/min to a final temperature of 225 °C. Approximately 350 compounds have been identified, and they included alkyl-, alkenyl-, and acyl-substituted furans, pyrroles, pyrazines, pyridines, oxazoles, and other compounds. Almost all of these compounds, except furans and oxazoles, were observed when serine and threonine were heated without sucrose, demonstrating the role of pyrolysis of amino acids on volatiles generation. Many of these identified volatile compounds exist in roasted coffee. Reese and Baltes studied the model reactions involving serine and fructose, glucose, diacetyl, or cyclotene in an aqueous solution (Reese and Baltes, 1992). Their results showed that the generation of pyrazines, pyridines, and carbonyl compounds increased, while furans, furanones, and pyranones decreased with rising temperature.

Ammonia is an important precursor for nitrogen-containing flavor compounds. It can be released from amino acids, peptides, and proteins by deamination and/or deamidation processes. It is known that glutamine can undergo both processes, release ammonia easier than other amino acids during thermal reactions (Sohn and Ho, 1995), and serve as a good ammonia provider. Several papers have reported volatile, especially pyrazine, generation in glutamine-containing systems (Koehler et al., 1969; Koehler and Odell, 1970; Huang et al., 1995; Yoo and Ho, 1997; Chun and Ho, 1997).

High temperature and weak basicity are favorable conditions for Maillard reaction (Ellis, 1959). In this study, we compared the volatile generation among nine model systems, which included one amino acid (serine/threonine/glutamine) and one monosaccharide (ribose/glucose/fructose). Experiments were performed at 160 °C in pH 8 aqueous solutions. The structural effect of amino acid and sugar on flavor compound generation was discussed.

MATERIALS AND METHODS

Materials. Serine, threonine, glutamine hydrochloride, ribose, glucose, and fructose were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Model Reaction of Amino Acid with Monosaccharide. Equimolar amounts (10 mmol) of amino acid (serine, threonine, or glutamine) and monosaccharide (ribose, glucose, or fructose) 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 being 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 an 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 subjected to GC and GC/MS analysis.

Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS) Analysis. Gas chromatography was performed on a Varian model 3400 equipped with a flame ionization detector (FID) and a nonpolar fused silica capillary column (DB-1, 60 m × 0.32 mm (i.d.), 1.0 μm film thickness, J&W Scientific). The column temperature was programmed to rise 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

* Corresponding author [fax (732) 932-8004; e-mail ho@aesop.rutgers.edu].

10.1021/jf980771a CCC: $18.00
Published on Web 01/14/1999

Table 1. Identified Volatile Compounds in the Nine Systems

compound[a]	RI[b]	concn (mg/mol)[c]								
		S−R[d]	S−G[d]	S−F[d]	T−R[d]	T−G[d]	T−F[d]	G−R[d]	G−G[d]	G−F[d]
2,3-butanedione	<600		1.0	1.4	1.4	1.4	1.3	4.0	5.0	5.0
2-butanone	<600		3.5	2.0		3.4	2.0	2.5		3.0
2-methylfuran	<600	2.5			7.0		1.0	1.5		
1-hydroxy-2-propanone	632	40	45	72	2.7×10^2	1.5×10^2	1.5×10^2	1.3×10^2	1.1×10^2	1.2×10^2
3-methyl-2-butanone	638						3.1			
2,3-pentanedione	668				4.1	2.7	2.8		5.0	8.5
3-hydroxy-2-butanone	682	12	9	8.9	13	7.7	8.8	35		5.5
pyrazine	701	41	69	36	1.4	5.7		38	1.1×10^2	17
(*E*)-3-penten-2-one	712				1.8					
2-methyl-2-butenal	722				4.3	2.2	1.2			
1-hydroxy-2-butanone	739					1.9				
4-methyl-2,3-pentanedione	763				1.4					
dihydro-2-methyl-3(2*H*)-furanone	780		4.0	1.5		4.0	7.8		16	26
methylpyrazine	804	18	20	28		13	9.0	2.0×10^2	5.5×10^2	5.7×10^2
furfural	817	8.1×10^2	9.0	9.5	2.6×10^3	7.6	7.9	1.5×10^3	70	1.1×10^2
1-(acetyloxy)-2-propanone	838	2.5		4.2	7.4		5.2	7.5		
2-furanmethanol	839	76	4.5	9.7	17	5.7	11	18	23	34
dihydro-2(3*H*)-furanone	871							84	22	24
2(5*H*)-furanone	876	20	4.5		6.8					
2-acetylfuran	890	18	4.0	4.5	7.4	2.5	1.0	39	8.5	15
2,5-dimethylpyrazine	895	15	24	1.1×10^2	80	22	1.5×10^2	30	73	2.2×10^2
2,6-dimethylpyrazine	898		4.5	0.7	6.8	2.0	1.0	4.0	9.0	3.0
2,3-dimethylpyrazine	902		1.5	0.8	19	3.2	0.9	7.5	10	7.0
vinylpyrazine	912			0.9					9.0	8.0
2,5-dimethyl-3(2*H*)-furanone	924				20	3.1				
1-(2-furanyl)-1-propanone	931	7.5			trace			1.0		
5-methyl-2-furfural	937		11	0.9	2.2	15	1.2			8.0
1*H*-pyrrole-2-carboxaldehyde	983	3.5			2.4			1.1×10^2		3.5
trimethylpyrazine	986	4.5		13	2.7	5.0	26			13
2-ethyl-6-methylpyrazine	988		2.5							
acetylpyrazine	1000	trace	3.0	1.8		1.9		trace	1.0	3.5
2-vinyl-6-methylpyrazine	1002								3.0	7.0
2-hydroxy-3-methyl-2-cyclopenten-1-one	1014	2.5	22	6.5	2.5	12	9.5		6.5	9.5
1-(2-furyl)-1,2-propanedione	1035	5.5								
2-pyrrolidione	1045							12		
2,5-dimethyl-4-hydroxy-3(2*H*)-furanone	1049			2.7	8.3	1.1	2.6			28
1-(2-furyl)-butan-3-one	1059	4.0			1.7			1.5		
benzoxazole	1067	22								
1-(2-furyl)-2-hydroxyethanone	1070			2.3	2.7		6.7			55
3-ethyl-2,5-dimethylpyrazine	1072			0.8		0.6	3.5			
2-methyl-5-propylpyrazine	1078				0.2					
2(1*H*)-pyridinone	1094				5.8			14		
2,3-dihydro-3,5-dimethyl-6-methyl-4*H*-pyran-4-one	1133		17	15		7.0			37	68
1-methyl-2-acetylpyrrole	1144				2.6					
1-(2-Furfuryl)-1H-pyrrole	1155	6.0			1.3					
2-methylfuro[2,3-*c*]pyridine	1188				9.2					
5-methyl-2(1*H*)-pyridinone	1191				8.2					
1*H*-pyrrolo[2,3-*b*]pyridine	1192							9.5		
5-(hydroxymethyl)-2-furfural	1200		99	85		1.2×10^2	67		4.6×10^2	9.6×10^2
2,3-dihydroxyacetophenone	1255				3.5					
2-(2-furfuryl)pyrazine	1279	11						2.5		
3(4)-methylpyrrolo(1,2-*a*)pyrazine	1317							3.0		
2-(2-furfuryl)-5-methylpyrazine	1346	3.5			2.3			3.5		
2-(2-furfuryl)-6-methylpyrazine	1361	4.5			4.9			1.5		
1-(2-furfuryl)pyrrole-2-carboxaldehyde	1374	43			2.2×10^2			3.5		
1-(3-furfuryl)pyrrole-2-carboxaldehyde	1399	16			22					
3-(2-furfuryl)-2,5-dimethylpyrazine	1409	6.0			11					
3-(2-furfuryl)-2,6-dimethylpyrazine	1418				2.5					
1,3-dimethylpyrrolo(1,2-*a*)pyrazine	1420						1.1			
2-(2-(5-(hydroxymethyl)furfuryl))-2,5-dimethylpyrazine	1702		1.0	6.4		0.9	5.3			

[a] Identification refers to the Wiley mass library and one of our previous papers (Chen and Ho, 1998). [b] Retention index, calculated according to the retention time of *n*-alkanes on a DB-1 column. [c] mg of volatile/mol of amino acid. [d] S−R, S−G, S−F, T−R, T−G, T−F, G−R, G−G, and G−F represented the nine systems, which were serine−ribose, serine−glucose, serine−fructose, threonine−ribose, threonine−glucose, threonine−fructose, glutamine−ribose, glutamine−glucose, and glutamine−fructose, respectively.

was 1 μL, and the split ratio was 25:1. GC/MS analysis was performed using an HP model 5790 GC coupled with an HP 5970A mass-selective detector. The capillary column and temperature program were the same as the conditions for the GC analysis. Mass spectra were obtained by electron ionization at 70 eV and mass scan from 33 to 300. Compound quantification was based on GC/FID data, and identification was based on mass spectra obtained from the GC/MS.

RESULTS AND DISCUSSION

The composition of the isolated volatiles in the nine reaction mixtures is listed in Table 1. The compounds are listed according to their elution order. The abbreviations of S−R, S−G, S−F, T−R, T−G, T−F, G−R, G−G, and G−F in the following text represented the nine systems, which were serine−ribose, serine−glucose,

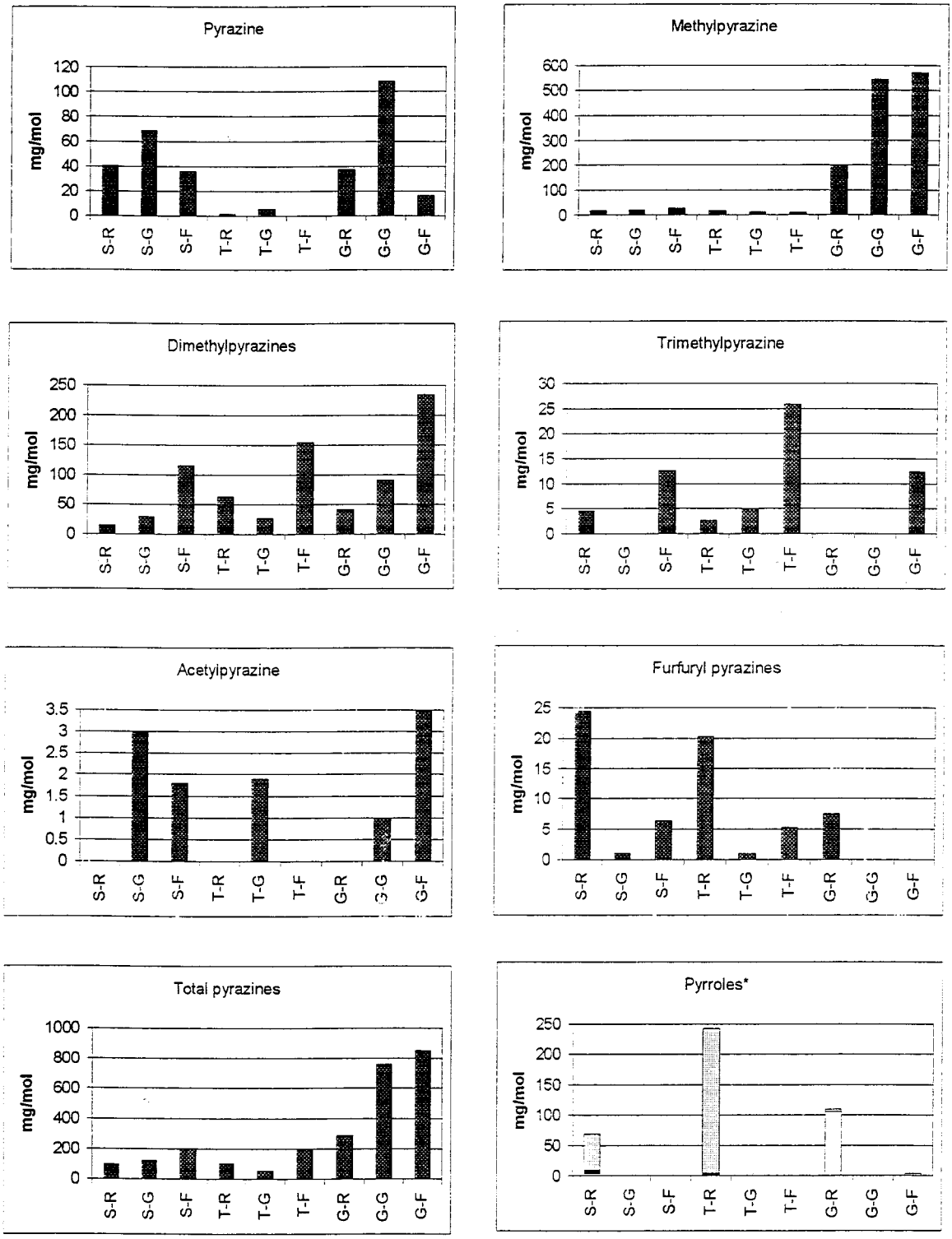

Figure 1. Comparison of nitrogen-containing heterocyclic compounds generation in the nine studied systems. Blank bar indicates 1*H*-pyrrole-2-carboxaldehyde, gray bar indicates 1-(2-furfuryl)-1*H*-pyrrole and dark bar indicates 1-(2-furfuryl)-1*H*-pyrrole-2-carboxaldehyde. Note: The abbreviations of S−R, S−G, S−F, T−R, T−G, T−F, G−R, G−G, and G−F represented the nine systems, which were serine−ribose, serine−glucose, serine−fructose, threonine−ribose, threonine−glucose, threonine−fructose, glutamine−ribose, glutamine−glucose, and glutamine−fructose, respectively.

serine−fructose, threonine−ribose, threonine−glucose, threonine−fructose, glutamine−ribose, glutamine−glucose, and glutamine−fructose, respectively. A total of 60 compounds were identified in these reactions. They can be classified as furans, furanones, pyrazines, pyrroles, and others.

The reactions of S−R and S−G have been discussed in our previous paper (Chen and Ho, 1998). In this work they were compared with seven other reaction systems to study the effect of reactant structure on volatile generation. In Figure 1, the formation of some nitrogen-containing compounds is compared; they are unsubstituted pyrazine, acetylpyrazine, furfuryl-substituted pyrazines, total pyrazines, and pyrroles.

Pyrazine is one of the most important groups among the identified volatiles in the nine studied systems. They

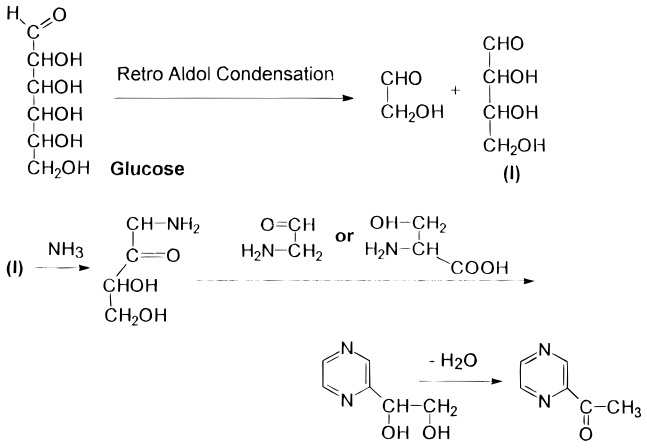

Figure 2. Suggested mechanism of acetylpyrazine formation.

are widely distributed in food systems, especially foods processed at high temperatures and low water environment. Extensive reviews on pyrazines in food have been published (Maga, 1982, 1992).

There are several precursors or pathways for pyrazine compounds generation. The α-amino carbonyls, which can be formed from the reactions between dicarbonyl compounds and amino acids during Strecker degradation, are generally considered to be the precursors of pyrazines. During Maillard reactions and thermal degradation of sugars, some active intermediates such as acetol (1-hydroxy-2-propanone) and acetoin (3-hydroxy-2-butanone) can be produced. These intermediates react with ammonia to generate α-amino ketones and then form pyrazines. According to this pathway, more pyrazine generation is expected in the systems involving amino acids such as glutamine, which release ammonia easily during thermal reactions (Sohn and Ho, 1995). An alternative pyrazine formation pathway is also recognized in pyrolysis of such *β*-hydroxy amino acids as serine and threonine. These α-amino carbonyls may react with each other to generate pyrazines during thermal processing without reducing sugar (Wang and Odell, 1973; Baltes and Bochmann, 1987c).

As shown in Figure 1, it is obvious that more unsubstituted pyrazine was generated in serine−monosaccharide systems than in threonine−monosaccharide systems. Previous study on thermal reactions of some amino−hydroxy compounds also showed similar results (Wang and Odell, 1973). The chemical structure difference in the methyl group between serine and threonine may be used to explain this phenomenon.

Another interesting result is the difference in acetylpyrazine generation among some of the studied systems. Acetylpyrazine was generated in glucose- and fructose-containing systems (serine/threonine/glutamine−glucose and serine/glutamine−fructose systems), but not in ribose-containing systems. On the basis of this result, the formation mechanism of acetylpyrazine was proposed in Figure 2. The retro-aldol condensation products from monosaccharides were supposed to be important intermediates for acetylpyrazine formation. Acetylpyrazine is one of the α-acetyl-*N*-heterocyclic compounds, which are important flavor compounds due to their desirable roasty note and low thresholds in food. One extensive review on this class of compounds was published recently (Kerler et al., 1997). Acetylpyrazine was described as a nutty and popcornlike flavor (Fors, 1983) and was identified in bread volatiles (Schieberle and Grosch, 1987).

Several furfuryl-substituted pyrazines were formed in some of the studied systems. The identification of those compounds was reported in one of our previous papers (Chen and Ho, 1998). As shown in Table 1, there was big difference in such furfuryl-substituted pyrazines generation among those model reactions: 2-(2-furfuryl)-pyrazine was formed in S−R and G−R systems; 2-(2-furfuryl)-5 (and 6)-methylpyrazine were observed in S−R, T−R, and G−R reactions; 3-(2-furfuryl)-2,5 (and 2,6)-dimethylpyrazine were produced only in S−R and T−R systems, and T−R was more productive in furfuryl dimethylpyrazine generation; and 2-(2-(5-hydroxymethyl)-furfuryl)-2,5-dimethyl-pyrazine was the characteristic compound in six-carbon sugar model reactions (S−G, S−F, T−G, and T−F).

For furfuryl-substituted pyrazine formation, as shown in Figure 1, it was obvious that the systems containing a *β*-hydroxy α-amino acid such as serine or threonine with ribose were more productive than the other seven systems. The reason for this phenomenon was still unclear.

The structure difference of furfuryl-substituted pyrazines between the five-carbon and six-carbon systems may be explained by the difference in the large amount of aldehydes formed from monosaccharides: furfural derived from ribose and 5-hydroxymethyl-2-furfural derived from glucose and fructose.

Figure 1 also indicates that more total pyrazine was generated in glutamine-containing systems than in serine- and threonine-containing systems. The reverse was true for generation of furfuryl-substituted pyrazines. It is known that glutamine and asparagine release more free ammonia during thermal reactions than other amino acids (Sohn and Ho, 1995). Our results indicated that ammonia can be an important precursor of pyrazine and methyl-substituted pyrazines but not the determining factor for furfuryl-substituted pyrazine formation.

The formation of several pyrroles was observed in this study. They included 1-*H*-pyrrole-2-carboxaldehyde in S−R, T−R, and G−R systems and 1-(2-furfuryl)-1*H*-pyrrole and 1-(2-furfuryl)pyrrole-2-carboxaldehyde in S−R and T−R systems. Even though G−R generated the largest amounts of 1-H-pyrrole-2-carboxaldehyde, no furfuryl-substituted pyrroles were observed in this model reaction. It seems that ribose and *β*-hydroxy amino acid were good precursors for such furfuryl-substituted pyrrole formation. Those furfuryl-substituted pyrroles were previously found in coffee (Reichstein and Staudinger, 1926; Gianturco et al. 1964) and in heat-treated cereals, and they may contribute to the aroma of popcorn (Walradt et al., 1970; Shen and Hosenney, 1995). These *N*-2-furfurylpyrrole compounds were also reported in some model reactions such as roasted mixture of serine, threonine with sucrose (Baltes and Bochmann, 1987b), and the reaction mixture of tryptophan with glucose or xylose under the conditions of coffee roasting (Baltes and Knoch, 1993).

Pyrroles are very important aroma compounds in food. Two general 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 with amino acids. Other pathways may also exist for pyrrole compound generation, such as pyrolysis of amino acids. When serine and threonine are pyrolyzed alone, a lot of alkylpyrroles and *N*-

pyrroloalkanols can be generated (Baltes and Bochmann, 1987b).

The formation mechanism of furfurylpyrroles was proposed before (Baltes and Knoch, 1993). They were suggested as the reaction products between 2-(aminomethyl)furan with furfural or 2-methylfurfural. This mechanism can be used to explain the formation of 1-alkyl-2-pyrrolecarboxaldehydes in other studied systems (Kato, 1966; 1967; Shibamoto, 1977; Shibamoto and Bernhard, 1978), but it cannot be extended to explain the result in this study. The reason for more *N*-2-furfurylpyrrole generation in serine−ribose and threonine−ribose systems needs to be further investigated.

LITERATURE CITED

Baltes, W.; Bochmann, G. Model reactions on roast aroma formation. 1. Reaction of serine and threonine with sucrose under the conditions of coffee roasting and identification of new coffee aroma compounds. *J. Agric. Food Chem.* **1987a**, *35*, 340−346.

Baltes, W.; Bochmann, G. Model reactions on roast aroma formation. III. Mass spectrometric identification of pyrroles from the reaction of serine and threonine with sucrose under the conditions of coffee roasting. *Z. Lebensm. Unters. Forsch.* **1987b**, *184*, 478−484.

Baltes, W.; Bochmann, G. Model reactions on roast aroma formation. IV. Mass spectrometric identification of pyrazines from the reaction of serine and threonine with sucrose under the conditions of coffee roasting. *Z. Lebensm. Unters. Forsch.* **1987c**, *184*, 485−493.

Baltes, W.; Bochmann, G. Model reactions on roast aroma formation. V. Mass spectrometric identification of pyridines, oxazoles, and carbocyclic compounds from the reaction of serine and threonine with sucrose under the conditions of coffee roasting. *Z. Lebensm. Unters. Forsch.* **1987d**, *185*, 5−9.

Baltes, W.; Knoch, E. Model reactions on roast aroma formation, XIII. The formation of some uncommon *N*-heterocyclic compounds and furans after roasting of tryptophan with reducing sugars and sugar degradation products. *Food Chem.* **1993**, *46*, 343−349.

Chen, J.; Ho, C.-T. Volatile compounds generation in serine−monosaccharide systems. *J. Agric. Food Chem.* **1998**, *46*, 1518−1522.

Chun, H. K.; Ho, C.-T. Volatile nitrogen-containing compounds generated from Maillard reactions under simulated deep-fat frying conditions. *J. Food Lipids* **1997**, *4*, 239−244.

Ellis, G. P. The Maillard reaction. In *Advances in Carbohydrate Chemistry*; Wolfrom, M. L., Ed.; Academic Press: New York, 1959; pp 63−134.

Fors, S. Sensory properties of volatile Maillard reaction products and related compounds. In *The Maillard Reaction in Foods and Nutrition*; Waller, G. R. Feather, M. S., Eds.; American Chemical Society: Washington, DC, 1983; pp 185−286.

Gianturco, M. A.; Giammarino, A. S.; Friedel, P. Volatile constituents in coffee: furanic and pyrrolic compounds. *Tetrahedron* **1964**, *20*, 2951−2961.

Huang, H. I.; Hartman, T. G.; Ho, C.-T. Relative reactivities of amino acids in pyrazine formation. *J. Agric. Food Chem.* **1995**, *43*, 179−184.

Kato, H. Formation of *N*-alkylpyrrole-2-carboxaldehydes in the browning reaction of D-xylose with alkylamines. *Agric. Biol. Chem.* **1966**, *30*, 822−823.

Kato, H. Formation of substituted pyrrole-2-aldehydes by reaction of aldose with alkylamines. *Agric. Biol. Chem.* **1967**, *31*, 1086−1090.

Kerler, J.; van der Ven, J. G. M.; Weenen, H. α-Acetyl-N-heterocycles in the Maillard reaction. *Food Rev. Int.* **1997**, *13*, 553−575.

Koehler, P. E.; Mason, M. E.; Newell, J. A. Formation of pyrazine compounds in sugar−amino acid model systems. *J. Agric. Food Chem.* **1969**, *17*, 393−396.

Koehler, P. E.; Odell, G. V. Factors affecting the formation of pyrazine compounds in sugar−amine reactions. *J. Agric. Food Chem.* **1970**, *18*, 895−898.

Maga, J. A. Pyrazine update. *Food Rev. Int.* **1992**, 8, 479−558.

Maga, J. A. Pyrazines in foods: an update. *Crit. Rev. Food Sci. Nutr.* **1982**, *1*, 1−48.

Reese, G.; Baltes, W. Model reactions on roast aroma formation. XI. Heating of serine with selected sugars and sugar degradation products in an autoclave. *Z. Lebensm. Unters. Forsch.* **1992**, *194*, 417−421.

Reichstein, T.; Staudinger, H. A new or improved method of producing artificial coffee oil. Br. Patent 260.960, 1926.

Schieberle, P.; Grosch, W. Quantitative analysis of aroma compounds in wheat and rye bread crusts using a stable isotope dilution assay. *J. Agric. Food Chem.* **1987**, *35*, 3001−3003.

Shen, G.; Hosenney, R. C. Comparison of aroma extracts of heat-treated cereals. *Lebensm.-Wiss. Technol.* **1995**, *28*, 208−212.

Shibamoto, T. Formation of sulfur- and nitrogen-containing compounds from the reaction of furfural with hydrogen sulfide and ammonia. *J. Agric. Food Chem.* **1977**, *25*, 206−208.

Shibamoto, T.; Bernhard, R. A. Formation of heterocyclic compounds from the reaction of L-rhamnose with ammonia. *J. Agric. Food Chem.* **1978**, *26*, 183−187.

Sohn, M. G.; Ho, C.-T. Ammonia generation during thermal degradation of amino acids. *J. Agric. Food Chem.* **1995**, *43*, 3001−3003

Walradt, J. P.; Lindsay, R. C.; Libbey, L. M. Popcorn flavor: identification of volatile compounds. *J. Agric. Food Chem.* **1970**, *16*, 926−928.

Wang, P.-S.; Odell, G. V. Formation of pyrazines from thermal treatment of some amino−hydroxy compounds. *J. Agric. Food Chem.* **1973,** *21*, 868−870.

Yoo, S. S.; Ho, C.-T. Pyrazine generation from the Maillard reaction of mixed amino acids in model systems. *Perfum. Flavor.* **1997**, *22*, 49−52.

Received for review July 16, 1998. Accepted November 23, 1998.

JF980771A