Generation of Flavor Compounds by the Reaction of 2-Deoxyglucose with Selected Amino Acids

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The volatile compounds generated by the reaction of 2-deoxyglucose with glutamine, glutamic acid, asparagine, and aspartic acid were studied. The nitrogen-containing compounds identified included pyrazines, pyridines, and pyrroles. Non-nitrogen-containing volatiles included aldehydes, ketones, cyclic compounds, furans, and aromatic compounds. As in the case with glucose, higher pHs were favorable to the formation of pyrazines in the reaction systems containing 2-deoxyglucose. Asparagine, glutamine, and asparatic acid produced similar amounts of pyrazines at pH 8.0. In general, lower pHs favor the generation of non-nitrogen-containing compounds. The reaction of 2-deoxyglucose with the selected amino acids produced only about 1% of the amount of pyrazines generated in the systems containing glucose under the same reaction conditions. The results indicated that the 2-hydroxy group on the glucose molecule is essential for the effective generation of flavor compounds.

Keywords: *2-Deoxyglucose; flavor compounds; Maillard reaction; pyrazines*

INTRODUCTION

Many reactions occur in an amino-reducing sugar reaction system. Amadori pathway (via the formation of Amadori compound) has been considered to be the important intermediate for the generation of flavor compounds. However, it is well-known that the aldose−ketose transformation via the formation of the *N*-glycoside in the Maillard reaction is analogous to the Lobry−de-Bruyn−van-Ekenstein transposition even though the reactions catalyzed by amines do not require as high a temperature as those catalyzed by an acid or base do (Mauron, 1981). The important intermediates for the generation of flavor compounds such as 1-deoxyglucosone and 3-deoxyglucosone are commonly generated by both pathways. In addition, other reactions, e.g., fragmentation, at alkaline pH will directly generate many pyrazine precursors such as glycolaldehyde, glyceraldehyde, and pyruvaldehyde (Kort, 1970). The acid/base-catalyzed sugar degradation should, therefore, contribute to the formation of flavor compounds as does the amine-catalyzed sugar degradation (via Amadori pathways) in an amine-reducing sugar reaction system. This would be true especially when a reaction system is practically designed for the generation of flavor compounds, where the preferred temperature and pH are favorable to the acid/base catalysis. However, the importance of Amadori pathways and non-Amadori pathways to the generation of flavor compounds has not been evaluated in an amine-reducing sugar reaction system. It would be interesting to determine how much contribution Amadori pathways or non-Amadori pathways can make to the generation of flavor compounds in an amine-reducing sugar reaction system.

It is difficult to study the contribution of non-Amadori pathways to the generation of volatiles or the browning in an amino-reducing sugar reaction system because the reaction of an amine with an aldose will inevitably render the formation of Amadori compounds. 2-Deoxyglucose has been used to study the importance of Amadori compounds in Maillard browning (Hodge, 1953). It was found that, in most cases, Amadori compounds are essential for Maillard browning. In the present work, we studied the reaction of 2-deoxyglucose with amino acids with the intention of determining the contribution of non-Amadori pathways to the generation of flavor compounds even though the reaction of 2-deoxyglucose cannot represent all of the non-Amadori pathways. 2-Deoxyglucose was selected because it cannot form Amadori compounds with amino acids. Amino acids used in this study were glutamine, glutamic acid, asparagine, and aspartic acid. These amino acids were used because the effect of both deamination and deamidation on the formation of flavor compounds could be considered. Deamination and deamidation of these amino acids (Sohn and Ho, 1995) and their effect on the generation of flavor compounds in amino acid/glucose systems have been studied by Sohn (1996).

EXPERIMENTAL PROCEDURES

Materials. 2-Deoxyglucose, glucose, glutamine, glutamic acid, asparagine, and aspartic acid were purchased from Sigma Chemicals (St. Louis, MO).

Sample Preparation. 2-Deoxyglucose (0.01 mol) and selected amino acids (0.015 mol) were dissolved in 50 mL of distilled water, and the pH of the solution was adjusted with 1 N NaOH to 5.0 or 8.0 for different samples. The reaction solution was then transferred into a 150 mL Hoke stainless steel cylinder. The cylinder was capped and incubated in an oven at 180 °C for 2 h. The cylinder was put into a water tank at room temperature immediately after the reaction finished. The temperature cooled to room temperature within 1 min. The pH of the reaction solution was readjusted to 8.0 prior to extraction of volatile compounds. An internal standard (C_{13}) along with 50 mL of methylene chloride was put into the reactor, and the reactor was recapped. The reactor was well shaken, and the mixture was poured into a separatory funnel (150 mL). The methylene chloride portion was collected after

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Table 1. Pyrazines Generated in the Reaction Systems Containing 2-Deoxyglucose and Selected Amino Acids at pH 5.0 and 8.0

	amount (μg/g 2-deoxyglucose)							
	pH = 5.0				pH = 8.0			
pyrazines	Asn	Asp	Gln	Glu	Asn	Asp	Gln	Glu
pyrazine	5.14	2.66	2.89	0.24	6.19	6.49	4.18	1.75
methylpyrazine	4.29	3.17	3.13	0.38	9.58	7.79	8.95	1.89
2,6-dimethylpyrazine	4.08		0.30		1.71	1.17	0.54	0.12
ethylpyrazine	0.10	0.36	0.79		4.05	2.15	4.92	0.30
2,3-dimethylpyrazine	0.86	0.05	0.27		1.05	0.69	0.65	0.11
vinylpyrazine			0.62		0.16	0.11	2.97	
2-ethyl-6-methylpyrazine	0.32	0.16	0.25		1.17	0.59	0.41	
2-ethyl-5-methylpyrazine	0.08	0.25			0.45	0.70		
2-ethyl-3-methylpyrazine	0.17	0.06	0.19		0.88	0.65	0.38	0.87
3-ethyl-2,5-dimethylpyrazine	0.56	1.04	0.37		1.01	1.37	0.26	
2-ethyl-3,5-dimethylpyrazine	0.69	0.42	0.24		1.50	0.41	0.71	
isopropenylpyrazine			0.12				0.30	
total amount	16.29	8.17	9.17	0.62	27.76	22.13	24.27	5.05

it was completely separated from the aqueous solution (reaction mixture). Extraction of flavor compounds was repeated three times. The methylene chloride portion (around 200 mL) was then concentrated in a Kuderna-Danish evaporator at 55−60 °C. The concentrate within 5 mL was purged with N_2 gas and finally around 0.5 mL concentrate was collected and subjected to GC and GC−MS analysis. Glucose was also used to react with the same group of amino acids under the same reaction conditions. Reaction of 2-deoxyglucose with or without glutamine or glutamic acid was also studied under the condition without the pH adjustment. The solutions of 2-deoxyglucose alone, 2-deoxyglusose with aspartic, and 2-deoxyglucose with asparagine have the pH values of 6.0, 2.2, and 4.0, respectively.

Gas Chromatography−Mass Spectrometry (GC−MS) Analysis. The analysis of volatiles of all the samples were performed with a Varian 3400 gas chromatograph. A nonpolar fused silica capillary column, DB-1 (60 m × 0.32 mm i.d., 1 mm thickness, J&W Scientific Co.), was used to separate the volatiles. The initial temperature was 40 °C, the final temperature 280 °C, the injector temperature 270 °C, and the detector temperature 300 °C. The initial hold time was 2 min and the final hold time 40 min. The temperature gradient was 2 °C/min. The split ratio ranged from 10 to 50 depending on the volatile concentration of samples. The inject volume of samples was 1 μL. The standards for determining the retention indices were C_5−C_{25} *n*-paraffin. A Finnagan MAT 8320 high-resolution, double-focusing magnetic sector mass spectrometer was coupled with a Varian gas chromatograph and used for MS analyses. Electron ionization was set at 70 eV.

RESULTS AND DISCUSSION

Pyrazine Formation. Pyrazine is an important group of volatiles commonly generated by the reaction of amino acids with reducing sugars. In the present work, a total of 12 pyrazines were identified. Among the identified pyrazines, unsubstituted pyrazine, methylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-3-methylpyrazine, and 2-ethyl-3,5-dimethylpyrazine have been also detected in a reaction system containing 2-deoxyglucose and ammonium hydroxide (Shibamoto and Bernhard, 1977). The effects of amino acids and pH on the generation of pyrazines were summarized in Table 1. The most abundant pyrazines generated in all the reaction systems studied were the unsubstituted pyrazine and methylpyrazine. This is true at both low and high pHs. This was observed also in the reaction system of glucose and glutamine (Hwang, 1995). Ethylpyrazine was also abundantly generated at pH 8.0. As in the case with glucose, the pyrazine generation by the reaction of 2-deoxyglucose and amino acid was affected by the pH of the reaction solution. pH 8.0 is more favorable than pH 5.0 for the generation of pyrazines. This is understandable because higher pHs facilitate the fragmentation of sugar and generate small active fragments (pyrazine precursors). However, the fragments with two and three carbons (dicarbonyl or α-hydroxy carbonyl) were not detected in the present reaction systems. This may be due to their higher reactivity with ammonia or the amino group. In addition, higher pHs decrease the protonation of nitrogen atom in the amino group or ammonia and increase the effective concentration of amino group or ammonia. Some pyrazines were only produced at pH 8.0. The effect of pH on the generation of pyrazines was related with the amino acids used. With glutamic acid at pH 5.0, only the unsubstituted pyrazine and methylpyrazine were produced. However, the effect of pH on the generation of pyrazines in the reaction systems containing asparagine, aspartic acid, and glutamine was more quantitative than qualitative.

Table 2. Pyrazines Generated by the Reaction of Glucose with the Selected Amino Acid at pH 8.0

		amount (μg/g glucose)			
pyrazine	RI[a]	Asn	Asp	Gln	Glu
pyrazine	723	585	196	366	171
methylpyrazine	800	1692		2041	380
2,6-dimethylpyrazine	890	1066	1224	395	1259
ethylpyrazine	894	169	150	85	18
2,3-dimethylpyrazine	899	87	74	57	14
vinylpyrazine	906	22	25		
2-ethyl-6-methylpyrazine	977	150	283	38	295
2-ethyl-5-methylpyrazine	983	78	225	27	
2-ethyl-3-methylpyrazine	985	150	373	11	
3-ethyl-2,5-dimethylpyrazine	1061	166	909	69	32
2-ethyl-3,5-dimethylpyrazine	1072	7	6	23	20
isopropenylpyrazine	1083	11		6	17
total amount		4182	3464	3121	2209

[a] RI: retention indices.

With regard to the effect of amino acids, asparagine generated more pyrazines than aspartic acid, glutamine, and glutamic acid. The reaction with glutamic acid generated only 5.1 μg/g 2-deoxyglucose and the reactions with other three amino acids more than 20 μg/g 2-deoxyglucose at pH 8.0. According to Sohn (1996), only 1.3% of glutamic acid and more than 50% of asparagine, aspartic acid, and glutamine were deaminated at pH 8.0. In other words, the free ammonia was important for the formation of pyrazines in the present reaction systems. The three amino acids other than glutamic

Table 3. Volatile Compounds Other than Pyrazines Generated by the Degradation of 2-Deoxyglucose or the Reaction of 2-Deoxyglucose (Dog) with Asparagine or Aspartic Acid

		amount (μg/g 2-deoxyglucose)		
compd	RI[a]	Asp + Dog (pH = 2.2)	Dog (pH = 6.0)	Asn + Dog (pH = 4.0)
Aldehydes and Ketones				
acetaldehyde	574			0.05
2-butanone	617	4.78	2.47	0.88
2-butenal	648	0.49		
3-methyl-2-butanone	659	0.44		
3-methyl-3-buten-2-one	671	0.12		
2,3-butanedione	677			0.12
1-hydroxy-2-pentanone	695	0.12		
3-hydroxy-2-butanone	703	1.19		
3-butene-1,2-diol	729	0.38		
1,2-butanediol	751	2.38		
3-pentanol	876	0.15		
Cyclic Compounds				
cyclopentanone	747	0.10		0.08
2,5-dimethyl-2,5-cyclohexadiene-1,4-dione	776	0.05		
2-cyclopenten-1-one	781			0.68
cyclohexanone	858	0.09		
2-methyl-2-cyclopenten-1-one	881			0.74
3-methyl-2-cyclopenten-1-one	935	0.88		0.23
2-hydroxycyclohexanone	955	1.91		0.30
3,5,5-trimethyl-2-cyclopenten-1-one	1000	0.19		
2,3-dimethylcyclopent-2-en-1-one	1001			0.13
Furans				
2-methylfuran	776	0.56		
2-furfural	783		0.15	
2-ethylfuran	825	0.05	0.41	
2-acetylfuran	885	6.77		3.13
2-(methoxymethyl)furan	917			0.27
1-(2-furanyl)-1,2-ethanediol	1099			0.05
tetrahydro-5-methyl-2-furanmethanol	1075	0.11		
2,4-(3*H*,5*H*)-furandione	833		0.05	
2(3*H*)-furanone	914		0.12	
Aromatic Compounds				
benzofuran	968		0.04	
phenol	973	0.95		
acetophenone	1027			0.09
2-methylphenol	1038	0.16		
4-hydroxyacetophenone	1093	0.26		0.28
total non-nitrogen-containing compounds		26.19	4.87	7.12
3-methyl-2*H*-1-benzopyran-2-one	1437		0.10	
1-phenyl-1-penten-3-one	1704		0.86	
3-methyl-2*H*-1-benzopyran-2-one	1728		0.64	
4,6-dimethyl-3(2*H*)-benzofuranone	1770			0.09
N-Containing Compounds Other than Pyrazines				
pyridine	733			0.02
3-ethylpyridine	939			0.08
2-acetylpyrrole	1035	1.34		0.80
3-acetyl-2,4-dimethylpyrrole	1298	0.13		
1-methyl-1*H*-pyrrole-2-carboxaldehyde	1320			0.05

[a] RI: retention indices.

acid generated similar amounts of pyrazines, probably because the amino acids were in excess compared to the amount of 2-deoxyglucose.

To consider the importance of the 2-hydroxy group on the glucose molecule for the generation of pyrazines, the same reaction conditions were applied to the reaction of glucose with the selected amino acids. These results are presented in Table 2. It was found that the pyrazines generated by the reaction of 2-deoxyglucose and amino acids were also generated by the reaction of glucose and the same group of amino acids (Sohn, 1996). These pyrazines accounted for more than 50% of the total amounts of pyrazines generated in the reaction systems with glucose and the same group of amino acids used in the present study. In other words, 2-deoxyglucose can produce the most common pyrazines generated by the reaction of glucose and the selected amino acids. However, as seen in Table 1, the total amounts of pyrazines generated were in the range 0.62−27.76 μg/g 2-deoxyglucose. In comparison, this accounted for only approximately 1% of the amount of pyrazines generated by the reaction of glucose and the selected amino acids. These results indicated that the 2-hydroxy group of glucose is important to the generation of pyrazines.

Formation of Other Volatile Compounds. Other volatile compounds detected in the present reaction systems are shown in Table 3. The non-nitrogen-containing compounds include mainly aliphatic aldehydes and ketones, cyclic ketones, furan derivatives, and aromatic compounds. Besides pyrazines, other minor nitrogen compounds include pyridines and pyrroles. Their formation may be influenced by the pH and/or the activity of specific amino acids. Pyridines were favor-

ably generated at higher pHs and pyrroles generated at lower pHs. 2-Acetylpyrrole was abundantly generated. 2-Acetylpyrrole was also detected by Nyhammra et al. (1983) in the reaction of 2-deoxyglucose with ammonium acetate.

With regard to non-nitrogen-containing compounds, furans were generally found in all the samples. The common furan derivatives identified were 2-methylfuran, 2-furfural, 2-ethylfuran, 2-(methoxymethyl)furan, and 1-(2-furanyl)-1,2-ethanediol. Low pHs were favorable for the formation of furans. However, specific furans were generated only specific pHs. Among the furans identified, 1-(2-furanyl)-1,2-ethanediol was detected in the degradation of glycol (Feather and Harris, 1973). According to Feather and Harris (1973), 2-deoxyglucose is an intermediate for the formation of this furan derivative from glycol.

Carbocyclic compounds are another group of compounds identified in this study. These compounds are common in the pyrolysate of carbohydrate even though not all the compounds may be present in the pyrolysate of a specific sugar. Some of the five-membered carbocyclic compounds identified in this study may be formed by dehydration and cyclization reactions or a combination of carbonyl fragments. Some phenolic compounds were also detected in this study. Phenols are commonly generated by the degradation of carbohydrates or in the reaction of amino acid with reducing sugar (Baltes and Bochmann, 1987). It is probable that the phenolic compounds were formed by the fragmentation of sugar and a combination of reactive carbonyl compounds.

Conclusion. 2-Deoxyglucose can react with amino acids and generate the common pyrazines generated by the glucose and the same amino acids. The amounts of pyrazines generated by the reaction of 2-deoxyglucose and the selected amino acids was only approximately 1% of the amount of pyrazines generated by the reaction of glucose and the same selected amino acids. The 2-hydroxy group on the glucose molecule is important in the formation of pyrazines.

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