Relative Reactivities of Amino Acids in Pyrazine Formation

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The contributions of ^{15}N-labeled glycine and tested amino acids (glutamine, glutamic acid, asparagine, aspartic acid, lysine, arginine, phenylalanine, and isoleucine) to pyrazine formation were investigated. A total of 56 pyrazines were identified in the present studies. Some specific high molecular weight pyrazines were found in the reaction systems containing phenylalanine and isoleucine. In the presence of glycine, glutamine and glutamic acid showed the least contributions, whereas asparagine had the highest contribution to pyrazine formation. By comparing the total yields of pyrazines generated from different reaction mixtures, it was found that the reaction mixture containing lysine had the highest, while the reaction mixture containing arginine possessed the lowest. The results also implied that lysine was able to increase the reactivity of glycine; however, arginine could decrease the capability of glycine to produce pyrazines. The variety and quantity of the pyrazine formation depended on the reactivity and type of amino acid used.

Keywords: *Pyrazines; amino acid reactivities; model Maillard reaction*

INTRODUCTION

The Maillard or nonenzymatic browning reaction has generated much interest over the past 50 years. The Maillard reaction mainly involves the reaction of free amino groups of amino acids and reducing sugars. The principal chemistry of this reaction in food was reviewed by Hodge in 1953. One of the major attributes of the Maillard reaction is flavor production. The aromas in most thermally processed foods, such as bread, cereal products, roasted peanuts, and roasted coffee, are largely due to the Maillard reaction. Among these Maillard-type flavors, the heterocyclic compounds with desirable aromas and low odor thresholds make the most significant contribution. These heterocyclic compounds include furans, thiazoles, thiophenes, oxazoles, pyrroles, pyridines, and pyrazines. The nitrogen sources of these heterocyclic compounds come from amino acids. Thus, the nature of the nitrogen source has a profound effect on both the kinds and amounts of flavors formed.

The effects of different amino acids on the Maillard reaction have been widely studied. In 1979, Piloty and Baltes investigated the degradation rates of amino acids with α-dicarbonyl compounds and found that the basic and hydroxy amino acids reacted strongly, while the acidic and nonpolar amino acids had the least reactivity. Fry and Stegink in 1982 also concluded that proline and amino acids with hydrophobic side chains reacted more slowly than other amino acids, while tryptophan and amino acids with aliphatic hydroxy side chains reacted most rapidly. In addition, Ashoor and Zent in 1984 examined the reactivities of amino acids in the Maillard reaction by comparing their color intensities. They observed that lysine, glycine, tryptophan, and tyrosine gave the most intense browning, followed by proline, leucine, isoleucine, alanine, hydroxyproline, phenylalanine, methionine, valine, and the amide-containing amino acids glutamine and asparagine. Histidine, threonine, aspartic acid, arginine, glutamic acid, and cysteine were the lowest browning-producing amino acids. The effect of amino sources on the formation of flavors has also been reported. Koehler et al. in 1969 investigated the distribution of pyrazine compounds produced by heating glucose with various nitrogen sources such as asparagine, glutamine, glutamic acid, aspartic acid, and ammonium chloride and found that ammonium chloride yielded mostly pyrazine and only traces of alkylated pyrazines, while the amino acids gave mostly alkylated pyrazines with very small amounts of pyrazine. Shigematsu et al. (1972) also observed that the nature of the nitrogen source had influences on both the quantity and type of pyrazines.

According to previous publications, they all emphasized the reaction between a single amino acid and sugar. Since there is more than one amino acid in foods, competition between amino acids to generate flavors can occur. Far less information is available on the formation of volatile components from the interaction of two different amino acids and one sugar in the same reaction medium. To investigate this competition, a ^{15}N isotope labeled glycine was chosen as a reference, and another tested amino acid (glutamine, glutamic acid, asparagine, aspartic acid, lysine, arginine, phenylalanine, or isoleucine) was added to compete with glycine in production of flavors. Because of the complexity of the reactions, only pyrazines are discussed in the present paper.

EXPERIMENTAL PROCEDURES

Materials. L-Glycine, L-glutamine, L-glutamic acid, L-asparagine, L-lysine, L-arginine, L-phenylalanine, L-isoleucine, and wheat starch were purchased from the Sigma Chemical Co. (St. Louis, MO). Glucose and deuterated toluene (Toluene-d_8), the internal standard, were obtained from Aldrich Chemical Co. (Milwaukee, WI). Glycine-α-*amine*-^{15}N was purchased from Isotec, Inc. (Miamisburg, OH), with a stated purity of 99%. Tenax-TA (2,6-diphenyl-*p*-phenylene oxide) adsorbent (60–80 mesh) was obtained from Alltech Associates (Deerfield, IL). Carbotrap (activated graphitized carbon) adsorbent (20–40 mesh), C_5–C_{25} *n*-paraffin standard, and silanized glass wool were purchased from Supelco, Inc. (Bellefonte, PA).

Volatile Generation and Isolation. Twenty grams of wheat starch, as well as an equal mole (2.66 μmol of each) of glucose, L-glycine-α-*amine*-^{15}N, and the tested amino acid (L-

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glutamine, L-glutamic acid, L-asparagine, L-aspartic acid, L-lysine, L-arginine, L-phenylalanine, or L-isoleucine) were mixed with 150 mL of deionized water and adjusted to pH 7 by using hydrochloric acid or sodium hydroxide. After being freeze-dried, the solid mixture was placed in the upper level of a desiccator; a Pyrex dish containing 20 mL of deionized water was placed in the lower level to adjust the moisture content of the samples back to 12–14%. The moisture content of the samples was measured according to the AOAC air oven method (AOAC, 1986). The samples were further transferred into a reaction vessel and heated at 180 °C for 1 h.

The heated samples (2 g of each) were packed in the center of glass tubes, and the silanized glass wool was placed at the two ends of the tubes; 1 μL of 1.001 mg/mL deuterated toluene was spiked into the tubes as the internal standard. The tubes were further sealed in a Scientific Instrument Services (SIS) solid sample purge-and-trap apparatus (Ringoes, NJ), and the volatiles were purged with nitrogen at a flow rate of 40 mL/min to silanized glass-lined stainless steel desorption tubes (4.0 mm i.d. × 10 cm length). The desorption tubes were from SIS and consisted of 3-cm bed volume of Tenax-TA adsorbent and 3-cm bed volume of Carbotrap adsorbent. This volatile isolation was carried out at 80 °C for 1 h.

Volatile Analysis by Gas Chromatography–Mass Spectrometry (GC–MS). The volatile analysis was conducted according to the same method as described by Hwang et al. (1993). Linear retention indices for the volatiles were determined through the use of a C_5–C_{25} *n*-paraffin standard, according to the method of Majlat et al. (1974). All mass spectra obtained were identified by utilizing an on-line computer library (NIST) or published literature.

Calculations for the Relative Contribution of ^{14}N Nitrogen and ^{15}N Nitrogen to Pyrazine Formation. Each pyrazine may have three different molecular weights, denoted W_1, W_2, and W_3. W_1, W_2, and W_3 represent two ^{14}N nitrogen atoms in the pyrazine ring, one ^{14}N and one ^{15}N nitrogen atoms in the pyrazine ring, and two ^{15}N nitrogen atoms in the pyrazine ring, respectively. The simultaneous equations below are used to solve the contributions of each component (W_1, W_2, and W_3) present in a mixture. The detailed explanation was reported previously (Hwang et al., 1993).

$$M_{exp} = M_n W_1 + (M-1)_n W_2$$

$$(M+1)_{exp} = (M+1)_n W_1 + M_n + (M-1)_n W_3$$

$$(M+2)_{exp} = (M+1)_n W_2 + M_n W_3$$

$(M-1)_n$, M_n, and $(M+1)_n$ are the experimental relative abundances of the ion peaks of the pyrazines from the reaction of nonlabeled glycine, the tested amino acid, and glucose. M_{exp}, $(M+1)_{exp}$, and $(M+2)_{exp}$ are the experimental abundances of the ion peaks of the pyrazines generated from the reaction of glycine-^{15}N, the tested amino acid, and glucose. The experimental data are provided as supplementary material.

After the relative contributions of the three different compounds (W_1, W_2, and W_3) were calculated, the percent contributions from glycine and the tested amino acid could be determined by using the following equations. As mentioned above, W_2 contains one ^{14}N nitrogen atom and one ^{15}N nitrogen atom in the pyrazine ring; therefore, half of the nitrogen of the component W_2 is from the tested amino acid. The component W_1 contains two ^{14}N nitrogen atoms, both coming from the tested amino acid. A similar equation is also shown to calculate the percentage of the contribution of labeled glycine.

$$\text{percentage contribution of tested amino acid} = [(W_1 + {}^1/_2 W_2)/(W_1 + W_2 + W_3)] \times 100\%$$

$$\text{percentage contribution of labeled glycine} = [(W_3 + {}^1/_2 W_2)/(W_1 + W_2 + W_3)] \times 100\%$$

RESULTS AND DISCUSSION

Among flavor compounds in foods, pyrazines are the most important class produced in the Maillard reaction. Hence, the present paper has concentrated on the relative contributions of amino acids to pyrazine formations. A total of 56 pyrazines were identified (Table 1). Also, they have been reported in a large number of cooked, roasted, and toasted foods, for example, roasted barley, cocoa, roasted peanuts, potato chips, green peas, coffee, and roast beef drippings (Maga, 1992). As we know, pyrazines are nitrogen-containing heterocyclics and their formation is a quite complicated process. There is apparently more than one pathway whereby different compounds can form pyrazines in the sugar–amine reaction system and in natural food products (Koehler and Gdell, 1970). The most direct route for their formation results from the interaction of α-dicarbonyls and amines through Strecker degradation. In general, pyrazines possess roasted and green odors with low threshold values. Furthermore, the yields of methyl-, 2,3-dimethyl-, 2,5(6)-dimethyl-, and trimethylpyrazine are the highest in the reaction system (Table 1), which suggests that the two-, three-, and four-carbon fragments are abundant in the system. These carbon fragments could be formed from retro-aldol condensation which is catalyzed by amines (Shibamoto and Bernhard, 1977). Two amino acids were chosen for this study. As a result, the high sugar fragments were catalyzed by both amino groups of amino acids.

Most pyrazines are present regardless of amino acid type. However, some higher molecular weight pyrazines were found to be amino acid specific (Table 1). From the data, we found 2 specific products of phenylalanine and 10 particular compounds of isoleucine. Apparently, 2-(2′-phenylethyl)-5(6)-methylpyrazine and 2-(2′-phenylethyl)-3,5(6)-dimethylpyrazine are characteristic products generated from phenylalanine because both of them contain phenyl residues. The identification of these two pyrazines is confirmed by comparing their mass spectra to the ones published by Kunert-Kirchhoff and Baltes (1990). Possible formation pathways have also been discussed by Kunert-Kirchhoff and Baltes (1990). First, phenylacetaldehyde, a Strecker aldehyde of phenylalanine, reacts with a hydroxyaldehyde to form phenylhydroxy ketone through aldol condensation. This phenylhydroxy ketone, like other α-diketones, can condense with the amino group of amino acid to yield a phenylamino ketone. Condensation of the phenylamino ketone and other amino ketones produces phenyl-substituted pyrazines. In addition, phenylacetaldehyde possesses a very strong hyacinth-like flavor (Wiseblatt and Zoumut, 1963) and has been reported as a significant contributor to the flavor of rye bread crust (Schieberle and Grosch, 1989). This may imply that these two phenylpyrazines are potential aromas in rye bread.

2-Methylbutanal, a Strecker aldehyde of isoleucine, was also found to be involved in the formation of pyrazines. All 10 particular pyrazines from isoleucine possessed a 2-methylbutyl group substituted on the pyrazine rings. There are two possible ways for 2-methylbutanal to be incorporated into pyrazine rings. One is that 2-methylbutanal condenses with carbonyl compounds that are from the Maillard reaction to form α-diketones or α-hydroxyketo components by aldol condensation. These long-chain α-diketones or α-hydroxyketo components further yield amino ketones through Strecker degradation. Any two amino ketones condensed together leads to the formation of pyrazines. Another way is that 2-methylbutanal directly reacts with the unstable dihydropyrazine intermediate and follows with a dehydration reaction to form pyrazines. Moreover, only the reaction mixture containing isoleucine has a roasted cocoa-like flavor within all the reaction mixtures tested in the present study. 3-Meth-

Table 1. Pyrazines Identified in the Reaction of Glucose, Glycine-α-*amine*-^{15}N, and Tested Amino Acids

compound	yield (μg/g of glucose)								
	ctrl[a]	Gln[a]	Lys[a]	Asn[a]	Phe[a]	Glu[a]	Asp[a]	Ile[a]	Arg[a]
pyrazine	6.71	41.99	99.94	78.85	31.65	75.09	25.91	8.43	11.49
methylpyrazine	146.77	279.85	1253.43	226.53	366.44	253.66	147.92	148.95	61.69
2,5(6)-dimethylpyrazine	132.31	115.47	162.71	260.33	571.07	249.77	112.30	140.69	99.47
2,3-dimethylpyrazine	45.68	60.66	274.89	79.64	162.51	81.29	61.86	43.28	33.68
ethylpyrazine	–[b]	–	–	14.25	–	–	–	–	16.59
vinylpyrazine	1.23	12.19	10.04	9.48	9.51	2.79	6.04	2.95	0.89
2-ethyl-6-methylpyrazine	39.01	49.23	66.58	84.83	93.15	80.54	49.00	22.08	8.29
2-ethyl-5-methylpyrazine	–	17.92	66.58	14.25	103.96	125.49	30.10	22.08	8.29
trimethylpyrazine	133.57	–	281.27	148.48	207.91	41.49	41.56	105.54	73.43
propylpyrazine	–	14.81	12.69	9.18	7.52	–	8.73	–	2.64
isopropylpyrazine	–	–	–	0.96	–	–	1.39	–	–
2-vinyl-5-methylpyrazine	5.74	23.29	20.63	25.44	10.04	12.60	14.79	6.81	2.03
2-vinyl-6-methylpyrazine	2.79	9.59	12.69	9.73	7.52	7.27	11.05	5.50	1.16
2,6-diethylpyrazine	–	–	–	–	–	–	40.81	–	–
3-ethyl-2,5-dimethylpyrazine	10.40	34.54	28.79	53.75	39.29	36.76	20.40	20.91	13.25
2-ethyl-3,5-dimethylpyrazine	–	23.33	–	–	–	79.41	63.49	33.38	4.11
2-methyl-6-propylpyrazine	–	8.53	–	–	–	8.67	1.66	–	–
tetramethylpyrazine	61.21	17.70	80.36	71.28	85.62	–	28.33	33.38	38.81
dimethylvinylpyrazine	–	8.53	–	11.66	–	–	19.12	–	2.14
2-methyl-6-(1-propenyl)pyrazine	3.89	4.33	–	0.96	–	2.23	1.66	6.37	2.14
2-methyl-5-(1-propenyl)pyrazine	1.64	11.89	–	5.06	–	3.42	2.73	–	2.29
3,5-diethyl-2-methylpyrazine	7.68	17.72	24.46	83.60	10.12	40.48	33.41	59.37	23.13
2,5-diethyl-3-methylpyrazine	–	10.91	33.22	8.63	3.98	9.70	19.43	6.82	5.19
2,3-diethyl-5-methylpyrazine	–	10.27	7.89	7.29	11.29	4.85	41.55	4.63	2.29
2,5-dimethyl-3-propylpyrazine	–	7.70	–	–	–	2.07	–	–	–
2-(2-methylbutyl)pyrazine	–	–	–	–	–	–	–	27.76	–
2,6-diethyl-3,5-dimethylpyrazine	–	5.06	–	8.54	–	3.48	18.71	–	2.68
2,5-diethyl-3,6-dimethylpyrazine	–	–	–	2.39	–	–	20.71	–	–
2,3-dimethyl-5-(1-methylpropyl)pyrazine	–	1.71	–	2.32	–	–	10.72	–	–
5-methyl-2-(2-methylbutyl)pyrazine	–	–	–	–	–	–	–	51.99	–
6-methyl-2-(2-methylbutyl)pyrazine	–	–	–	–	–	–	–	39.87	–
3-methyl-2-(2-methylbutyl)pyrazine	–	–	–	–	–	–	–	36.27	–
ethyl-2-(2-methylbutyl)pyrazine	–	–	–	–	–	–	–	53.08	–
dimethyl-2-(2-methylbutyl)pyrazine	–	–	–	–	–	–	–	29.90	–
dimethyl-2-(2-methylbutyl)pyrazine	–	–	–	–	–	–	–	29.90	–
ethyl-2-(2-methylbutyl)pyrazine	–	–	–	–	–	–	–	37.93	–
C_8-alkylpyrazine	–	–	–	–	–	–	–	50.01	–
C_8-alkylpyrazine	–	–	–	–	–	–	–	53.41	–
C_9-alkylpyrazine	–	–	–	–	–	–	–	19.75	–
5-methyl-5*H*-cyclopentapyrazine	–	10.91	15.74	–	–	–	–	–	–
5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine	–	7.51	28.48	17.29	18.45	9.38	14.19	13.60	11.62
2-ethyl-6,7-dihydro-5*H*-cyclopentapyrazine	–	–	–	3.23	–	–	6.89	6.37	1.82
5,7-dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine	–	17.86	22.74	21.83	–	10.26	12.09	21.39	19.02
2,6-dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine	–	4.22	–	2.39	–	–	6.16	–	1.32
2-ethyl-5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine	–	4.93	–	5.19	–	1.43	–	–	–
2-(2′-furanyl)pyrazine	–	21.18	30.5	14.02	4.26	1.81	4.15	–	10.94
2-(2′-furanyl)-6-methylpyrazine	–	24.79	18.34	17.35	6.01	1.87	3.89	–	12.96
2-(2′-furanyl)-5-methylpyrazine	–	24.79	18.34	8.68	6.01	1.87	2.82	–	8.64
2-(2′-furanyl)-5,6-dimethylpyrazine	–	11.16	8.43	5.58	1.75	2.09	2.89	–	5.73
2-(2′-furanyl)-methylethylpyrazine	–	2.01	–	–	–	–	4.82	–	2.34
2-(2′-phenylethyl)-5(6)-methylpyrazine	–	–	–	–	4.85	–	–	–	–
2-(2′-phenylethyl)-3,5(6)-dimethylpyrazine	–	–	–	–	20.47	–	–	–	–
6-methyl-2-acetylpyrazine	–	18.99	8.47	14.15	4.86	15.27	15.14	–	5.87
5-methyl-2-acetylpyrazine	–	14.19	8.47	13.29	4.86	7.14	10.10	–	1.38
3,5(6)-dimethyl-2-acetylpyrazine	–	5.04	–	–	–	5.22	1.78	–	–
3,5,6-trimethyl-2-acetylpyrazine	–	3.50	–	7.29	–	8.02	5.68	27.61	3.11
totals	598.63	958.30	2595.68	1347.72	1793.10	1185.42	923.98	1170.01	500.43

[a] ctrl contained glycine only; Gln contained labeled glycine and glutamine; Lys contained labeled glycine and lysine; Asn contained labeled glycine and asparagine; Phe contained labeled glycine and phenylalanine; Glu contained labeled glycine and glutamic acid; Asp contained labeled glycine and aspartic acid; Ile contained labeled glycine and isoleucine; Arg contained labeled glycine and arginine. [b] Not observed.

yl-2-(2-methylbutyl)pyrazine, 6-methyl-2-(2-methylbutyl)pyrazine, 5,6-dimethyl-2-(2-methylbutyl)pyrazine, 3,5-dimethyl-2-(2-methylbutyl)pyrazine, and 3,6-dimethyl-2-(2-methylbutyl)pyrazine have also been identified in roasted cocoa (Vitzthum et al., 1975; van der Wal et al., 1971). Therefore, these 2-methylbutyl-substituted pyrazines are possibly responsible for the unique roasted cocoa flavors.

The yields and relative contributions of tested amino acids to pyrazine formation are shown in Figure 1. In the presence of glycine, glutamine and glutamic acid are the smallest contributors, while asparagine is the highest contributor to pyrazine formation among these tested amino acids (glutamine, glutamic acid, asparagine, aspartic acid, lysine, arginine, phenylalanine, and isoleucine). In addition, the yields of pyrazines from glycine in the reaction systems containing tested amino acids are compared to the one in the reaction system containing glycine only. The figure shows that glycine has the highest yield in the reaction mixture containing lysine and has the least yield in the reaction mixture containing arginine. It appears that lysine acts as a synergist to increase the reactivity of other amino acids (glycine in this case) in forming pyrazines; however,

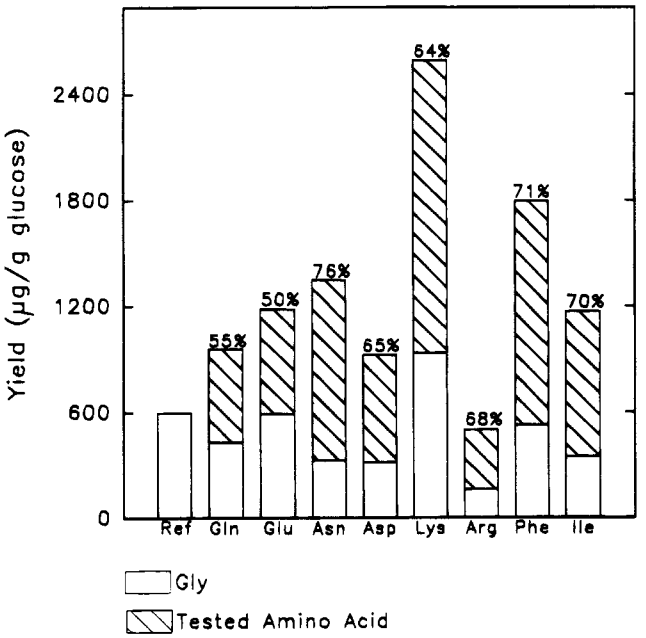

Figure 1. Total yields and relative contributions of all pyrazines generated from the reaction systems containing tested amino acids and glycine. Ref contained glycine only; Gln contained labeled glycine and glutamine; Glu contained labeled glycine and glutamic acid; Asn contained labeled glycine and asparagine; Asp contained labeled glycine and aspartic acid; Lys contained labeled glycine and lysine; Arg contained labeled glycine and arginine; Phe contained labeled glycine and phenylalanine; Ile contained labeled glycine and isoleucine. The numbers on the tops of columns show the percent contributions of tested amino acids.

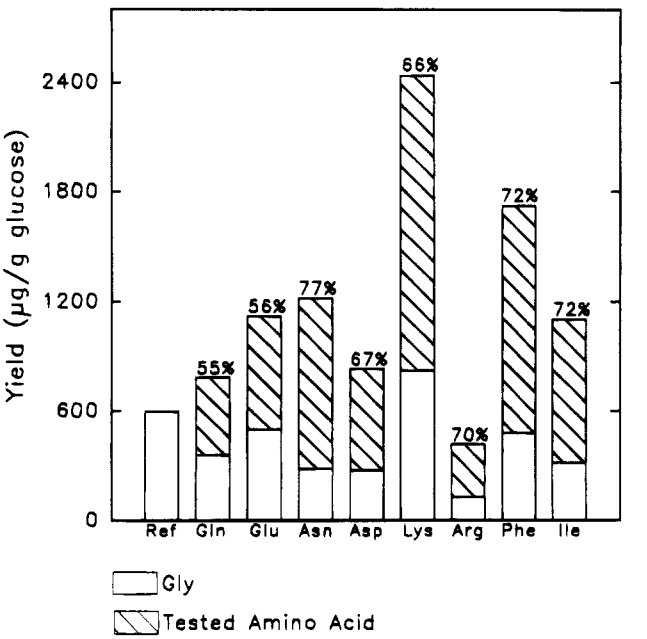

Figure 2. Total yields and relative contributions of alkylpyrazines generated from the reaction systems containing tested amino acids and glycine. Details are given in the Figure 1 caption.

arginine acts like an inhibitor to depress the ability of other amino acids in pyrazine generation. Generally speaking, the variety and quantity of the pyrazine formation depend on the reactivity and type of amino acid used.

The figure also shows that the yield of pyrazines from the reaction mixture containing lysine is the highest, whereas the amount of pyrazines from the reaction mixture containing arginine is the lowest. This is expected because arginine consists of a strong basic δ-guanidino group, which is actually unreactive, and only very small fractions of the nonionized form are present at normal pH values (Creighton, 1984). On the other hand, lysine is known to be the most reactive amino acid in the Maillard reaction. Its nonionized amino groups, which are potent nucleophiles, can easily catalyze sugar fragmentation and are involved in the Strecker degradation. However, the yields of pyrazines from the reaction mixtures containing asparagine and glutamine, which consist of labile amide side chains, were not as large as we expected. It is found that the amount of pyrazines in the asparagine reaction system was higher than the one in the glutamine reaction system. It was also reported that the rates for deamidation of asparagine were faster than those for deamidation of glutamine (Wright, 1991). This difference may be due to the greater distance from the α-amino group to the side-chain amide group of glutamine compared with that of asparagine. Therefore, this greater distance in the glutamine molecule results in not only a slower deamidation rate but also a lower yield in the production of pyrazines. However, the quantity of pyrazines in the aspartic acid reaction system is less than the one in the glutamic acid reaction system. In other words, this extra methylene group of the side chain in the glutamic acid molecule makes it easy for the α-amino group to transfer to α-diketones during Strecker degradation. Surprisingly, the yield of pyrazines from the reaction mixture containing phenylalanine is the second highest. These results further explain why phenylacetaldehyde is an important aroma in baked bread even though its precursor, phenylalanine, is relatively low in the total amino acid content of wheat (Wrigley and Bietz, 1988).

These pyrazines are further divided into three groups that include alkylpyrazines, bicyclic pyrazines, and acetylpyrazines. It has been well recognized that the alkylpyrazines significantly contribute to the flavors of heat-treated foods (Fors and Olofsson, 1985). Twenty-eight alkylpyrazines were detected in this study. In general, monosubstituted-pyrazines and substituted methylpyrazines possess nutty and/or roasted notes, while most higher alkyl-substituted pyrazines have fatty and/or waxy odors (Masuda and Mihara, 1988). However, they all tend to possess a green odor. Furthermore, the odor threshold values of alkylpyrazines decrease with the increasing number of substituents within a homologous series. Substituents in the 2,3-position result in higher threshold values as compared with substituents in the 2,5- or 2,6-position (Fors and Olofsson, 1985). The types and yields of alkylpyrazines are dominant compared to those of acetylpyrazines and bicyclic pyrazines. The yields and the contributions of tested amino acids to the formation of alkylpyrazines are shown in Figure 2. The results are quite similar to the ones in the formation of pyrazines.

The bicyclic pyrazines identified in the present study include cyclopentapyrazines (either saturated or unsaturated), furanylpyrazines, and phenylpyrazines. It was reported that bicyclic pyrazines, such as furanyl- and dihydrocyclopentapyrazines, mostly require temperatures over 150 °C for their formation, whereas monocyclic pyrazines are formed at around 120 °C (Baltes et al., 1989). In this study, there were six cyclopentapyrazines identified. In general, cyclotene (also called 2-hydroxy-3-methyl-2-cyclopenten-1-one) is a precursor of cyclopentapyrazines and has a burnt sugar type of odor (Ohloff and Flament, 1979). The first step of

Table 2. Mass Spectra of Furanylpyrazines and Acetylpyrazines Tentatively Identified in the Reaction Systems Containing Labeled Glycine, Tested Amino Acid, and Glucose

compound	MW	RI[a]	MS, m/z (relative intensity)
2-(2′-furanyl)pyrazine	146	1476	146 (100), 93 (43), 39 (38), 63 (24), 64 (18), 53 (16), 52 (16), 118 (16), 117 (12)
2-(2′-furanyl)-6-methylpyrazine	160	1728	160 (100), 92 (51), 39 (44), 93 (19), 63 (28), 64 (22), 131 (15), 132 (12), 53 (11)
2-(2′-furanyl)-5-methylpyrazine	160	1732	160 (100), 39 (52), 92 (49), 63 (28), 64 (22), 93 (23), 131 (15), 53 (15), 132 (14)
2-(2′-furanyl)-5,6-dimethylpyrazine	174	2024	92 (100), 174 (84), 63 (30), 39 (22), 64 (21), 93 (10), 53 (9), 52 (8), 145 (7)
2-(2′-furanyl)-methylethylpyrazine	188	2214	188 (100), 92 (47), 63 (33), 64 (25), 39 (21), 159 (21), 160 (18), 173 (10), 52 (9)
6-methyl-2-acetylpyrazine	136	1141	43 (100), 94 (72), 52 (53), 136 (51), 93 (46), 66 (41), 67 (28), 108 (28), 53 (25)
5-methyl-2-acetylpyrazine	136	1149	43 (100), 136 (96), 94 (69), 93 (62), 108 (40), 67 (28), 66 (27), 52 (25), 53 (25)
3,5(6)-dimethyl-2-acetylpyrazine	150	1328	43 (89), 150 (70), 107 (70), 108 (47), 53 (30), 122 (23), 80 (22), 52 (19), 66 (10)
3,5,6-trimethyl-2-acetylpyrazine	164	1587	164 (100), 43 (75), 121 (68), 122 (37), 53 (36), 136 (25), 80 (21), 94 (16), 67 (16)

[a] Calculated retention indices with n-paraffins (C_5–C_{25}) as references on a DB-1 column.

forming cyclopentapyrazines is to produce cyclotene by degrading 5-hydroxy-5,6-dihydromaltol, which is an important intermediate of 1-deoxyosone (Baltes and Bochmann, 1987). This cyclotene like other α-diketones can react with amines or amino acids to form cyclotene imine which, by further reactions, yields cyclopentapyrazines. The formation pathway for 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazines also was proposed by Shibamoto et al. in 1979. In addition, the alkyl substituted dihydrocyclopentapyrazines were identified in the aromas of fried beef, roasted nuts, cocoa, and coffee (Maga, 1992). With the exception of 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine, which has a sweet, crust-like odor, most of the cyclopentapyrazines possess an earthy, baked potato-like flavor (Maga, 1992). The furanylpyrazines investigated in this study include 2-(2′-furanyl)-pyrazine, 2-(2′-furanyl)-6-methylpyrazine, 2-(2′-furanyl)-5-methylpyrazine, 2-(2′-furanyl)-5,6-dimethylpyrazine, and 2-(2′-furanyl)-methylethylpyrazine. Since these furanylpyrazines were tentatively identified, their mass spectra are listed in Table 2. 2-(2-Hydroxyacetyl)furan is possibly the precursor of furanylpyrazine and is the decomposition product of the 4-deoxyosone which is degraded from glucose through 2,3-enolization. 2-(2-Hydroxyacetyl)furan possesses the properties of α-diketones and is able to react with amines or amino acids via Strecker degradation to produce furanylpyrazines. Since these furanylpyrazines were the first to be investigated in the present study, their sensory properties are still unclear. The discussion of the formation pathways and odor characters for phenylpyrazines has been mentioned above.

The yields of bicyclic pyrazines are quite low if they are compared to the yields of alkylpyrazines (Figure 3). The bicyclic pyrazines are still important flavor substances due to their unique structures and possibly uncommon sensory properties. As mentioned above, the yields of pyrazines from the reaction mixtures containing asparagine and glutamine were lower than we expected. However, the yields of bicyclic pyrazines in the reaction system containing glutamine, asparagine, and lysine were the largest of the three. This means that those amino acids containing nitrogen atoms in their side chains, except arginine, did have an influence on pyrazine generation. This effect was not significantly observed in the formation of alkylpyrazines or pyrazines. It is probably because the α-amino group of amino acids is the one most likely to participate in pyrazine formation, and when they are involved in the reaction, their primary choice is alkylpyrazines. Thus, the contribution of side-chain nitrogen is revealed only in the generation of bicyclic pyrazines. The other reason may simply be that the yield of alkylpyrazine is high enough to cover the contribution of side chains.

Acetylpyrazines found in this study were 6-methyl-2-acetylpyrazine, 5-methyl-2-acetylpyrazine, 3,5(6)-dimethyl-2-acetylpyrazine, and 3,5,6-trimethyl-2-acetylpyrazine. These acetylpyrazines were also tentatively identified, and their mass spectra are listed in Table 2. The detailed formation pathways for acetylpyrazines are not quite understood. It is assumed that acetylpyrazines are formed through a 1-deoxyosone type of intermediate which may undergo a series of processes, such as keto–enol tautomerism and retro-aldol condensation, and lead to production of acetylpyrazines. Generally, acetylpyrazines tend to possess biscuit- or cracker-like odors (Yong et al., 1989). It was also reported that 5-methyl-2-acetylpyrazine and 6-methyl-2-acetylpyrazine had a popcorn-like flavor (Pittet and Hruza, 1974). The yield of acetylpyrazines was very low in comparison with that of bicyclic pyrazines and alkylpyrazines (Figure 4). The amount of acetylpyrazines seemed to be much lower in the reaction mixture consisting of lysine, arginine, and phenylalanine than in the reaction mixture containing glutamine, glutamic acid, asparagine, aspartic acid, and isoleucine. It appears that the formation of acetylpyrazines is quite difficult, especially for those amino acids having bulky side chains like lysine, arginine, and phenylalanine.

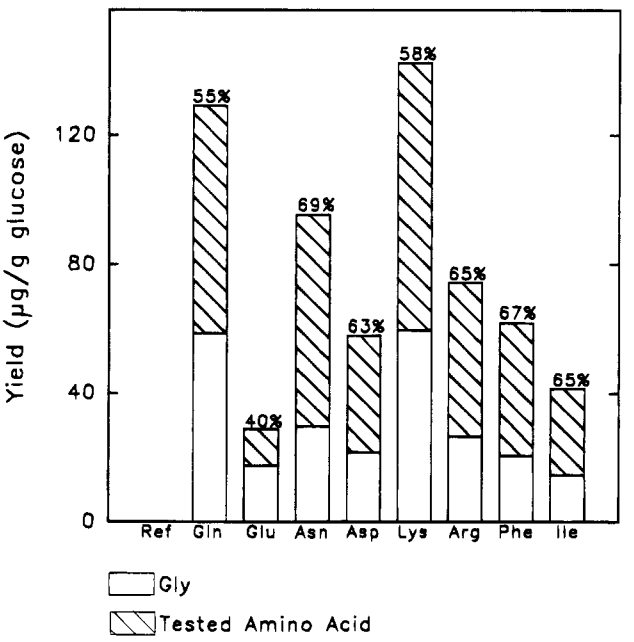

Figure 3. Total yields and relative contributions of bicyclic pyrazines generated from the reaction systems containing tested amino acids and glycine. Details are given in the Figure 1 caption.

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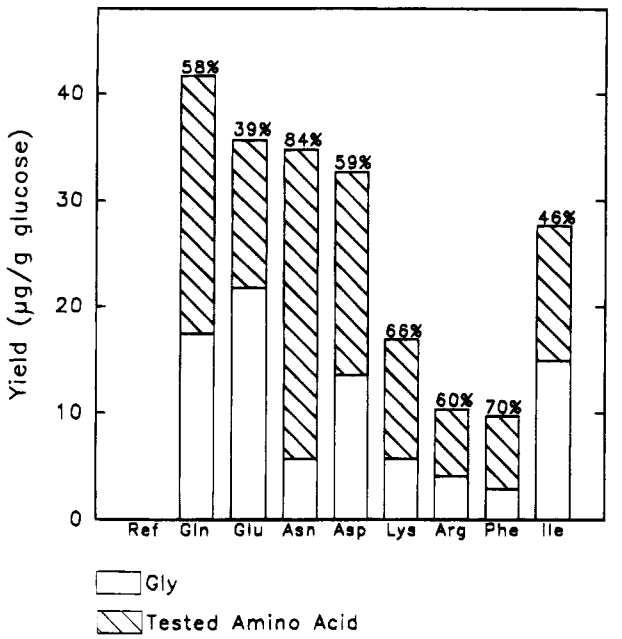

Figure 4. Total yields and relative contributions of acetylpyrazines generated from the reaction systems containing tested amino acids and glycine. Details are given in the Figure 1 caption.

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Supplementary Material Available: Experimental data from the reaction of glycine, the tested amino acid, and glucose (13 pages). Ordering information is given on any current masthead page.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, 1986.

Ashoor, S. H.; Zent, J. B. Maillard browning of common amino acids and sugars. *J. Food Sci.* **1984**, *49*, 1206–1207.

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.* **1987**, *184*, 478–484.

Baltes, W.; Kunert-Kirchhoff, J.; Reese, G. Model reactions on generation of thermal aroma compounds. In *Thermal Generation of Aromas;* Parliment, T. H., McGorrin, R. J., Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemistry Society: Washington, DC, 1989; pp143–155.

Creighton, T. E. Chemical nature of polypeptides. In *Proteins: Structures and Molecular Principles;* Freeman: New York, 1984; pp 10–14.

Fors, S. M.; Olofsson, B. K. Alkylpyrazines, volatiles formed in the Maillard reaction. I. Determination of odor detection thresholds and odor intensity functions by dynamic olfactometry. *Chemical Senses* **1985**, *10*, 287–296.

Fry, L. K.; Stegink, L. D. Formation of Maillard reaction products in parenteral alimentation solutions. *J. Nutr.* **1982**, *112*, 1631–1637.

Hodge, J. E. Dehydrated foods: Chemistry of browning reactions in model systems. *J. Agric. Food Chem.* **1953**, *1*, 928–943.

Hwang, H. I.; Hartman, T. G.; Rosen, R. T.; Ho, C.-T. Formation of pyrazines from the Maillard reaction of glucose and glutamine-*amide*-^{15}N. *J. Agric. Food Chem.* **1993**, *41*, 2112–2115.

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

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.

Kunert-Kirchhoff, J.; Baltes, W. Model reactions on roast aroma formation. VII. Specific products of phenylalanine after cooking L-phenylalanine with D-glucose in a laboratory autoclave. *Z. Lebensm. Unters. Forsch.* **1990**, *190*, 9–13.

Maga, J. A. Pyrazines update. *Food Rev. Int.* **1992**, *8*, 479–558.

Majlat, P.; Erdos, Z.; Takacs, J. Calculation and application of retention indices in programmed temperature gas chromatography. *J. Chromatogr.* **1974**, *91*, 89–103.

Masuda, H.; Mihara, S. Olfactive properties of alkylpyrazines and 3-substituted 2-alkylpyrazines. *J. Agric. Food Chem.* **1988**, *36*, 584–587.

Ohloff, G.; Flament, I. Heterocyclic constituents of meat aroma. *Heterocycles* **1978**, *11*, 663.

Piloty, M.; Baltes, W. Investigations on the reaction of amino acids wityh α-dicarbonyl compounds. I. Reactivity of amino acids in the reaction with α-dicarbonyl compounds. *Z. Lebensm. Unters. Forsch.* **1979**, *168*, 368–373.

Pittet, A. O.; Hruza, D. E. Comparative study of flavor properties of thiazole derivatives. *J. Agric. Food Chem.* **1974**, *22*, 264–269.

Schieberle, P.; Grosch, W. Break flavor. In *Thermal Generation of Aromas;* Parliment, T. H., McGorrin, R. J., Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemistry Society: Washington, DC, 1989; pp 258–267.

Shibamoto, T.; Bernhard, R. A. Investigation of pyrazine formation pathways in sugar-ammonia model systems. *J. Agric. Food Chem.* **1977**, *25*, 609–614.

Shibamoto, T.; Akiyama, T; Sakaguchi, M.; Enomoto, Y.; Masuda, H. A study of pyrazine formation. *J. Agric. Food Chem.* **1979**, *27*, 1027–1031.

Shigematsu, H.; Kurata, T.; Kato, H.; Fujimaki, M. Volatile compounds formed on roasting DL-α-alanine with D-glucose. *Agric. Biol. Chem.* **1972**, *36*, 1631–1637.

van der Wal, B.; Kettenes, D. K.; Stoffelsma, J.; Sipma, G.; Semper, A. Th. J. New volatile components of roasted cocoa. *J. Agric. Food Chem.* **1971**, *19*, 276–280.

Vitzthum, O. G.; Werkhoff, P.; Hubert, P. Volatile components of roasted cocoa: Basic fraction. *J. Food Sci.* **1975**, *40*, 911–916.

Wiseblatt, L.; Zoumut, H. F. Isolation, origin, and synthesis of a bread flavor constituents. *Cereal Chem.* **1963**, *40*, 162–169.

Wright, T. H. Nonenzymatic deamidation of asparaginyl and glutaminyl residues in proteins. *CRC Crit. Rev. Biochem. Mol. Biol.* **1991**, *26*, 1–52.

Wrigley, C. W.; Bietz, J. A. Protein and amino acids. In *Wheat: Chemistry and Technology;* Pomeranz, A., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1988.

Yong, L. F. M.; Acree, T. E.; Lavin, E. H.; Butts, R. M. Aroma chemistry of crackers. In *Thermal Generation of Aromas;* Parliment, T. H., McGorrin, R. J., Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemistry Society: Washington, DC, 1989; pp 276–284.

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