# Formation of Pyrazines from the Maillard Reaction of Glucose and Lysine-α-*amine*-$^{15}N$

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The contribution of α- and ε-amino nitrogen atoms to pyrazine formation in the reaction of labeled lysine with glucose was investigated. The labeled lysine, which contained a $^{15}N$ isotope labeled at the α-amino group and a $^{14}N$ at the ε-amino group, was reacted with glucose at different pHs and temperatures in a dry system. A similar reaction mixture was also studied in an aqueous system at 180 °C and pH 8.5. Pyrazine, methylpyrazine, ethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, vinylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-6-methylpyrazine, 2-vinyl-5-methylpyrazine, and trimethylpyrazine were identified in the dry system. All pyrazines identified in the dry system were also found in the aqueous system. Two additional pyrazines, three pyridines, and one pyrrole were also observed in the aqueous system. Both the α- and ε-amino groups of lysine were involved in pyrazine generation. The nitrogen atoms from α-amino groups of lysine react more readily with dicarbonyls to form pyrazines than the nitrogen atoms from ε-amino groups. The amounts of pyrazines significantly decreased as the reaction temperature decreased. Neither the reaction temperature nor the pH of the reaction mixture had any effect on the contribution of the two different amino groups to pyrazine formation.

## INTRODUCTION

Pyrazines are nitrogen-containing heterocyclic compounds and are one of the most important roast aromas in a large number of cooked, roasted, and toasted foods (Maga, 1992). The most direct route for pyrazine formation results from the interaction of α-dicarbonyls and amines through Strecker degradation. Basically, sugars are the principal sources of α-dicarbonyls, while amines come from α-amino groups of amino acids (Koehler et al., 1969). However, most α-amino groups of amino acids are not available and form peptide bonds in food systems. Therefore, the participation of the side chains of amino acids in pyrazine formation becomes more important, especially those side chains containing nitrogen atoms such as glutamine, asparagine, and lysine. In a recent study, we (Hwang et al., 1993) have shown that ammonia from the side chains of glutamine can contribute to pyrazine formation. It is also known that both α- and ε-amino groups of lysine can catalyze the fragmentation and dehydration of sugar molecules during the Maillard reaction. This results in an interest to study the highly reactive side chains of lysine involved in pyrazine formation. In addition, it was reported that at higher temperatures amino acids can undergo reactions other than traditional Strecker degradation (de Rijke et al., 1981). The degradation involves decarboxylation and/or deamination to generate a series of compounds including ammonia, amines, carbon dioxide, and the Strecker aldehyde. These compounds can serve as reactants to form pyrazines. This seems to suggest that temperature might influence the formation pathways between amino groups of lysine and α-dicarbonyls leading to pyrazine generation.

The object of this study was to investigate the participation of α- and ε-amino groups of lysine in pyrazine formation by using a stable isotope $^{15}N$ labeled at the α-amino group and a $^{14}N$ at the ε-amino group. The effects of pH and temperature on the relative contributions of α- and ε-amino groups to pyrazine generation were also studied.

## EXPERIMENTAL PROCEDURES

**Materials.** D/L-Lysine 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). L-Lysine-α-*amine*-$^{15}N$ was purchased from Isotec, Inc. (Miamisburg, OH) and has 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 from the Dry System.** Fifty grams of wheat starch, 500 mg of glucose, and 100 mg of D/L-lysine or L-lysine-α-*amine*-$^{15}N$ were mixed with 500 mL of deionized water and adjusted to a desired pH (7 or 8.5). After freeze-drying, 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 closed reaction vessel and heated in a conventional oven at different temperatures (100, 140, and 180 °C) for 1 h.

The heated samples (5 g of each) were packed in the center of glass tubes and the silanized glass wool was placed on the two ends of the tubes. One microliter 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, Ringoes, NJ) solid sample purge-and-trap apparatus, and the volatiles were purged with nitrogen at a flow rate of 40 mL/min into 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 Generation and Isolation from the Aqueous System.** Glucose (500 mg) and 100 mg of D/L-lysine or L-lysine-

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**Table 1. Relative Ion Abundance (Percent) of Alkylpyrazines Generated from Lysine with Glucose in the Aqueous System at pH 8.5[a]**

| compound | $(M-1)_n$[b] | $M_n$[b] | $(M+1)_n$[b] | $M_{exp}$[c] | $(M+1)_{exp}$[c] | $(M+2)_{exp}$[c] |
|---|---|---|---|---|---|---|
| pyrazine | 0.7 | 100.0 | 5.9 | 8.2 | 100.0 | 39.9 |
| methylpyrazine | 1.9 | 86.8 | 5.6 | 3.8 | 88.2 | 100.0 |
| 2,6-dimethylpyrazine | 2.9 | 85.8 | 6.8 | 0.3 | 5.5 | 81.1 |
| ethylpyrazine | 27.4 | 24.3 | 8.4 | 4.3 | 17.3 | 19.3 |
| 2,3-dimethylpyrazine | 0 | 47.0 | 4.6 | 0 | 8.1 | 7.8 |
| vinylpyrazine | 4.9 | 25.5 | 1.1 | 1.7 | 9.6 | 10.3 |
| 2-ethyl-5-methylpyrazine | 100.0 | 66.7 | 6.4 | 1.1 | 38.9 | 25.3 |
| trimethylpyrazine | 11.3 | 67.3 | 4.8 | 0.1 | 9.4 | 43.1 |
| 2-vinyl-5-methylpyrazine | 13.1 | 17.3 | 1.2 | 0.9 | 3.6 | 4.4 |
| 2-vinyl-6-methylpyrazine | 8.0 | 20.3 | 3.5 | 0.4 | 3.9 | 7.2 |
| (*E*)-2-methyl-6-(1-propenyl)pyrazine | 84.1 | 52.3 | 5.2 | 0.6 | 27.5 | 18.8 |

[a] Numbers are the average of five separate determinations. [b] $(M-1)_n$, $M_n$, and $(M+1)_n$ are the relative ion abundances from the reaction of nonlabeled lysine with glucose. [c] $M_{exp}$, $(M+1)_{exp}$, and $(M+2)_{exp}$ are the relative ion abundances from the reation of lysine-α-amine-$^{15}N$ with glucose in the aqueous system.

**Table 2. Relative Ion Abundance (Percent) of Alkylpyrazines Generated from Lysine with Glucose in the Dry System at pH 8.5[a]**

| | 180 °C | | | 140 °C | | | 100 °C | | |
|---|---|---|---|---|---|---|---|---|---|
| compound | $M_{exp}$[b] | $(M+1)_{exp}$[b] | $(M+2)_{exp}$[b] | $M_{exp}$ | $(M+1)_{exp}$ | $(M+2)_{exp}$ | $M_{exp}$ | $(M+1)_{exp}$ | $(M+2)_{exp}$ |
| pyrazine | 16.9 | 100.0 | 49.3 | 17.9 | 100.0 | 47.1 | 13.6 | 100.0 | 25.9 |
| methylpyrazine | 3.2 | 92.0 | 100.0 | 14.1 | 100.0 | 79.6 | 14.8 | 99.7 | 42.1 |
| 2,6-dimethylpyrazine | 4.8 | 31.6 | 77.2 | 2.4 | 21.6 | 73.2 | 2.5 | 7.5 | 30.8 |
| ethylpyrazine | 60.8 | 100.0 | 51.0 | 65.9 | 100.0 | 43.3 | –[c] | – | – |
| 2,3-dimethylpyrazine | 4.1 | 57.1 | 34.0 | 4.9 | 73.7 | 36.5 | – | – | – |
| vinylpyrazine | 9.9 | 49.0 | 36.4 | 19.3 | 79.2 | 51.2 | – | – | – |
| 2-ethyl-5-methylpyrazine | 18.4 | 27.4 | 10.3 | 16.5 | 27.7 | 14.3 | – | – | – |
| 2-ethyl-6-methylpyrazine | 0.3 | 1.6 | 0.9 | – | – | – | – | – | – |
| trimethylpyrazine | 1.1 | 15.5 | 26.1 | 2.5 | 26.3 | 39.7 | – | – | – |
| 2-vinyl-5-methylpyrazine | 0 | 0.4 | 0.3 | 8.6 | 15.4 | 19.9 | – | – | – |

[a] Numbers are the average of five separate determinations. [b] $M_{exp}$, $(M+1)_{exp}$, and $(M+2)_{exp}$ are the relative ion abundances from the reaction of lysine-α-amine-$^{15}N$ with glucose in the dry system. [c] Not observed.

α-amine-$^{15}N$ were dissolved in 100 mL of deionized water and adjusted to pH 8.5 by 1 N NaOH. The samples were transferred to a closed reaction vessel and heated in a conventional oven at 180 °C for 1 h. The pH of the heated mixtures dropped to 3–4 and was titrated back to pH 12 to increase the extraction efficiency of the pyrazines. The heated reaction solution was introduced into a 100-mL Wheaton liquid sample purge-and-trap apparatus obtained from Fisher Scientific Co. (Fair Lawn, NJ). Volatiles were isolated by using nitrogen at a flow rate of 40 mL/min into a desorption tube that consisted of Tenax TA and Carbotrap adsorbents (1:1) at 50 °C for 1 h.

**Volatile Analysis for the Dry System by Gas Chromatography–Mass Spectrometry (GC–MS).** The desorption tubes prepared from the isolation procedure were connected to a SIS Model TD-1 short path thermal desorption unit. This desorption unit was interfaced into a Varian 3400 gas chromatograph coupled with a Finnigan MAT 8320 high-resolution, double-focusing magnetic sector mass spectrometer (TD-GC–MS). The volatiles trapped in the desorption tube were then thermally desorbed into the GC at 220 °C for 5 min. However, the temperature of the GC chamber was maintained at –20 °C by dry ice to cryofocus the volatiles as a narrow band at the head of the capillary column during the desorption process. After the desorption tube was removed from the injection port of the GC, the volatiles were analyzed by GC–MS. The GC was operated with an injector temperature of 250 °C with a split ratio of 10:1 and a helium carrier flow rate of 1.0 mL/min. The GC column was a nonpolar fused silica capillary column [60 m × 0.32 mm i.d., 1-μm thickness, DB-1 (J&W Scientific Co.)] and was temperature-programmed from –20 to 280 °C at a rate of 5 °C/min with a 20-min hold at the upper limit. Volatiles were quantified via peak area ratio calculation to that of the internal standard (deuterated toluene). 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). The mass spectrometer electron ionization was set at 70 eV, and the source temperature was 250 °C with a filament emission current of 1 mA, scanning masses 35–350, and a 0.8-s interscan time. All mass spectra obtained were identified by utilizing an on-line computer library (NIST).

**Volatile Analysis for the Aqueous System by Gas Chromatography–Mass Spectrometry (GC–MS).** The GC–MS operating conditions for the aqueous system studies were the same as described for the dry system studies, except for the program for GC separation. The temperature program of the GC was raised from –20 to 40 °C at a rate of 5 °C/min, from 40 to 150 °C at a rate of 2 °C/min, and then to 280 °C at a rate 10 °C/min with a final hold time of 10 min.

**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 atom in the pyrazine ring, and two $^{15}N$ nitrogen atoms in the pyrazine ring, respectively. The following simultaneous equations were used to solve the contributions of each component ($W_1$, $W_2$, and $W_3$) present in a mixture. The detailed explanation has been reported previously (Hwang et al., 1993).

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

$$(M+1)_{exp} = (M+1)_n W_1 + M_n W_2 + (M-1)_n W_3$$

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

$(M-1)_n$, $M_n$, and $(M+1)_n$ are the experimental relative abundances of the ion peaks of the pyrazines from the reaction of nonlabeled lysine with 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 lysine-$^{15}N$ (labeled at the α-amine nitrogen only) with glucose. The experimental data are shown in Tables 1–3.

After the relative contributions of the three different compounds ($W_1$, $W_2$, and $W_3$) were calculated, the percent contribution from ε-amine nitrogen (from a starting material containing labeled α-amine and unlabeled ε-amine nitrogens) could be determined by using the following equation. 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

**Table 3. Relative Ion Abundance (Percent) of Alkylpyrazines Generated from Lysine with Glucose in the Dry System at pH 7[a]**

| | 180 °C | | | 140 °C | | | 100 °C | | |
|---|---|---|---|---|---|---|---|---|---|
| compound | $M_{exp}$[b] | $(M+1)_{exp}$[b] | $(M+2)_{exp}$[b] | $M_{exp}$ | $(M+1)_{exp}$ | $(M+2)_{exp}$ | $M_{exp}$ | $(M+1)_{exp}$ | $(M+2)_{exp}$ |
| pyrazine | 21.9 | 100.0 | 48.8 | 22.3 | 100.0 | 41.7 | 15.4 | 100.0 | 19.4 |
| methylpyrazine | 2.1 | 90.8 | 100.0 | 11.4 | 100.0 | 96.9 | 3.2 | 21.3 | 9.6 |
| 2,6-dimethylpyrazine | 2.4 | 15.5 | 43.1 | 2.1 | 17.2 | 68.3 | –[c] | – | – |
| ethylpyrazine | 46.2 | 100.0 | 48.6 | 56.9 | 100.0 | 39.9 | – | – | – |
| 2,3-dimethylpyrazine | 0 | 35.9 | 29.4 | 3.4 | 44.7 | 30.5 | – | – | – |
| vinylpyrazine | 5.6 | 26.1 | 21.1 | 14.4 | 74.7 | 57.2 | – | – | – |
| 2-ethyl-5-methylpyrazine | 4.6 | 11.4 | 3.4 | 6.1 | 16.6 | 8.3 | – | – | – |
| trimethylpyrazine | 0 | 12.9 | 20.9 | 1.8 | 27.9 | 36.1 | – | – | – |
| 2-vinyl-5-methylpyrazine | – | – | – | 4.7 | 8.9 | 16.8 | – | – | – |

[a] Numbers are the average of five separate determinations. [b] $M_{exp}$, $(M+1)_{exp}$, and $(M+2)_{exp}$ are the relative ion abundances from the reaction of lysine-$\alpha$-*amine*-$^{15}N$ with glucose in the dry system. [c] Not observed.

component $W_2$ is from the $\epsilon$-amine side chains. The component $W_1$ contains two $^{14}N$ nitrogen atoms, both coming from the $\epsilon$-amine nitrogens.

$$\% \text{ contribution of } \epsilon\text{-amine nitrogen} = [(W_1 + {}^1/_2W_2)/(W_1 + W_2 + W_3)] \times 100\%$$

## RESULTS AND DISCUSSION

The pyrazines that were identified from heating isotope-labeled lysine with glucose in the dry system included pyrazine, methylpyrazine, ethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, vinylpyrazine, 2-ethyl-5-methylpyrazine, trimethylpyrazine, 2-ethyl-6-methylpyrazine, and 2-vinyl-5-methylpyrazine. In the aqueous system, the same types of pyrazines were also found. Furthermore, two additional pyrazines [2-vinyl-6-methylpyrazine and (*E*)-2-methyl-6-(1-propenyl)-pyrazine], three pyridines (pyridine, 3-butylpyridine, and 2-methyl-5-butylpyridine), and 1-methyl-1*H*-pyrrole were observed in the aqueous system.

The relative contributions of $\epsilon$-amino nitrogens to pyrazine formation are shown in Figures 1 and 2. These data clearly demonstrate that both $\alpha$- and $\epsilon$-amino groups of lysine participate in the pyrazine formation. From these figures, it is also shown that less than half of the nitrogen sources came from the $\epsilon$-amino groups of lysine. This implies that nitrogen atoms from the $\epsilon$-amino side chains of lysine were more difficult to react with dicarbonyls than the ones from the $\alpha$-amino groups.

The details of the proposed mechanisms for pyrazine formation are shown in Figure 3. It is assumed that both of the amines react with $\alpha$-dicarbonyls as nucleophiles and bases. However, the Schiff bases from $\alpha$-amino groups produce $\alpha$-aminoketones through Strecker degradation, whereas the Schiff bases from $\epsilon$-amino groups form $\alpha$-aminoketones by an intermolecular rearrangement and a hydration reaction. These $\alpha$-aminoketones can further react with each other to form pyrazines. The data and proposed formation pathways illustrate that the decarboxylation in the Strecker degradation assists $\alpha$-amines to generate $\alpha$-aminoketones. This also explains why the $\alpha$-amino groups of lysine have a greater contribution to pyrazine production. Another interesting phenomenon is that the $\epsilon$-amines have the highest contribution in pyrazine and methylpyrazine as well as the lowest contribution in 2,6-dimethylpyrazine and trimethylpyrazine. Pyrazine and methylpyrazine consist of two two-carbon fragments and one two-carbon and one three-carbon fragment, respectively. 2,6-Dimethylpyrazine and trimethylpyrazine consist of two three-carbon fragments and one three-carbon and one four-carbon fragment, respectively. This explains why long-chain carbon fragments have more difficulty proceeding through the intermolecular rearrangements due to steric hindrance. These carbon fragments tend to be involved in the Strecker degradation rather than the intermolecular rearrangement.

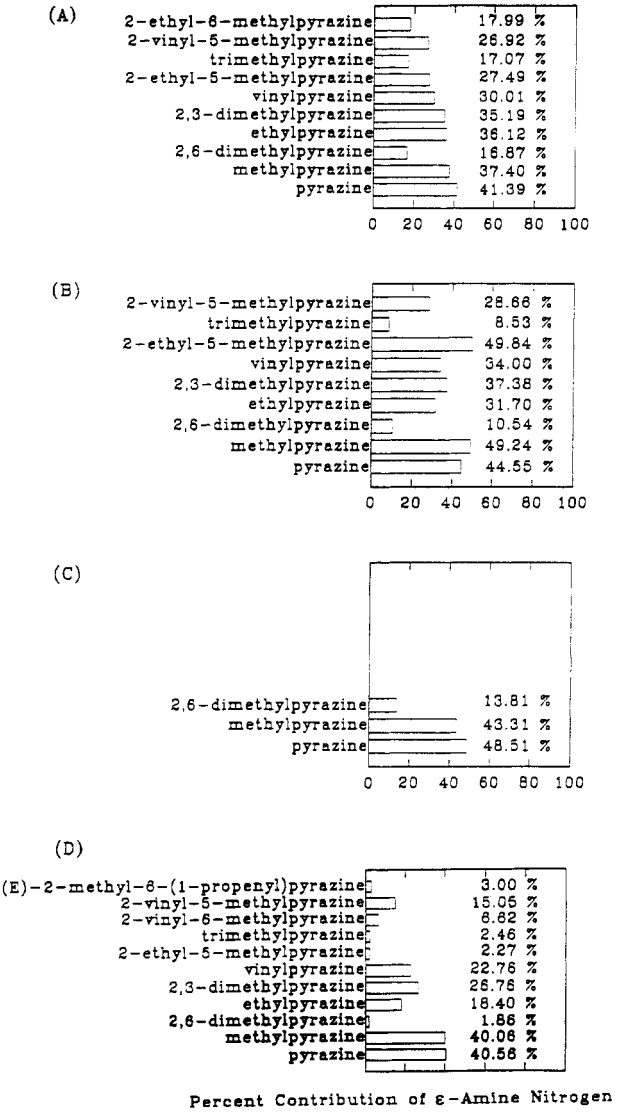


**Figure 1.** Relative contribution of $\epsilon$-amino nitrogen to pyrazine formation in the reaction of labeled lysine with glucose at pH 8.5. (A) Reaction temperature = 180 °C in the dry system; (B) reaction temperature = 140 °C in the dry system; (C) reaction temperature = 100 °C in the dry system; (D) reaction temperature = 180 °C in the aqueous system.

As we mentioned above, both $\alpha$- and $\epsilon$-amino groups of lysine can be involved in pyrazine formation. In food proteins, most available free amino groups are $\epsilon$-amino groups of lysine. Thus, the major amino groups partic-

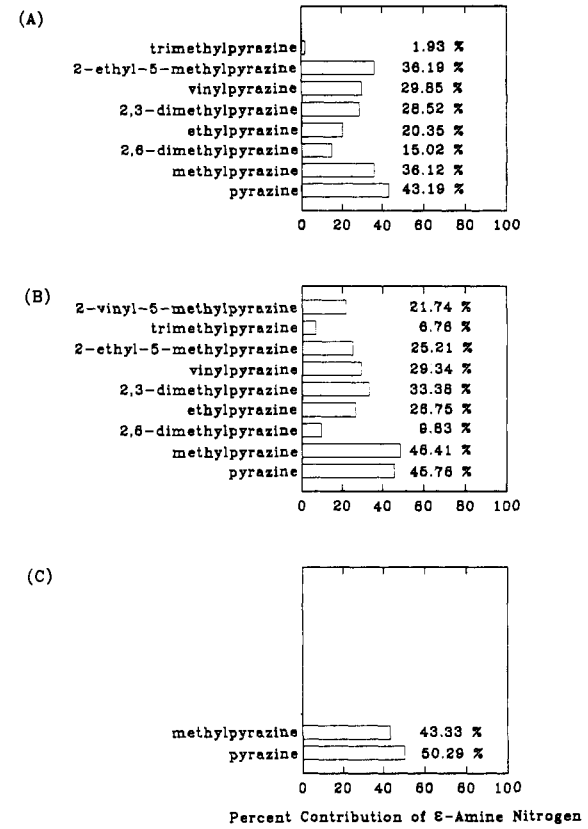


**Figure 2.** Relative contribution of ε-amino nitrogen to pyrazine formation in the reaction of labeled lysine with glucose at pH 7. (A) Reaction temperature = 180 °C in the dry system; (B) reaction temperature = 140 °C in the dry system; (C) reaction temperature = 100 °C in the dry system.

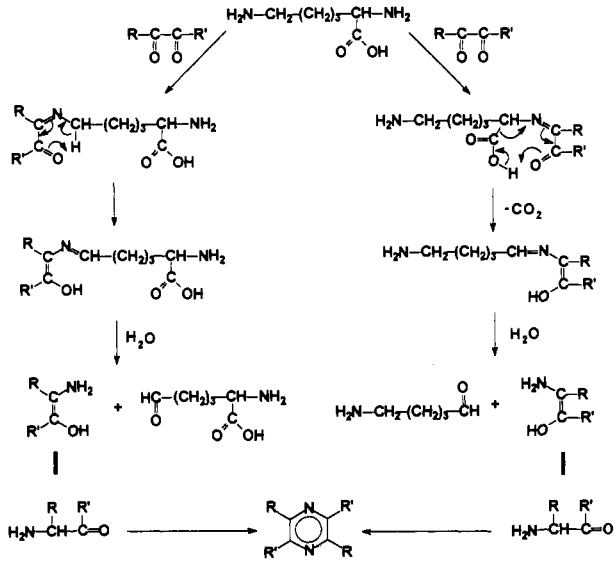

**Figure 3.** Proposed mechanisms for transamination from α- and ε-amino groups of lysine to pyrazines.

ipating in pyrazine generation are from the ε-amino groups of lysine. If the ε-amino groups of lysine react in this way, the essential amino acid is not available anymore. This causes a nutrition loss in food protein during thermal processing. The results also show that ε-amino groups of lysine might potentially react with carbonyl groups or other reactive groups in the protein molecule to induce unusual cross-linkage and result in polymerization of protein. Such types of deamination by amino–carbonyl reaction, different from that of α-amino groups of amino acid via the Strecker degradation reaction, might possibly proceed through the formation pathway as proposed in Figure 3.

**Table 4. Alkylpyrazines Identified in the Reaction of Lysine-α-amine-$^{15}N$ with Glucose at pH 8.5**

| compound | yield (μg/g of glucose) A–180 °C[a] | D–180 °C[a] | D–140 °C[a] | D–100 °C[a] |
|---|---|---|---|---|
| pyrazine | 1.5 | 236.5 | 491.9 | 2.9 |
| methylpyrazine | 2.2 | 1490.9 | 348.8 | 1.5 |
| 2,6-dimethylpyrazine | 2.4 | 204.9 | 188.4 | 0.5 |
| ethylpyrazine | 0.2 | 15.7 | 10.2 | –[b] |
| 2,3-dimethylpyrazine | 0.2 | 33.9 | 22.2 | – |
| vinylpyrazine | 0.2 | 7.1 | 7.7 | – |
| 2-ethyl-5-methylpyrazine | 0.2 | 5.5 | 1.4 | – |
| trimethylpyrazine | 0.4 | 30.2 | 27.7 | – |
| 2-ethyl-6-methylpyrazine | – | 4.8 | – | – |
| 2-vinyl-5-methylpyrazine | 0.1 | 3.2 | 1.9 | – |
| 2-vinyl-6-methylpyrazine | 0.2 | – | – | – |
| (*E*)-2-methyl-6-(1-propenyl)pyrazine | 0.3 | – | – | – |

[a] A–180 °C, sample reacted at 180 °C in the aqueous system; D–180 °C, sample reacted at 180 °C in the dry system; D–140 °C, sample reacted at 140 °C in the dry system; D–100 °C, sample reacted at 100 °C in the dry system. [b] Not observed.

**Table 5. Alkylpyrazines Identified in the Reaction of Lysine-α-amine-$^{15}N$ with Glucose at pH 7**

| compound | yield (μg/g of glucose) 180 °C | 140 °C | 100 °C |
|---|---|---|---|
| pyrazine | 274.9 | 145.9 | 1.9 |
| methylpyrazine | 2010.6 | 184.4 | 0.2 |
| 2,6-dimethylpyrazine | 141.5 | 62.8 | –[a] |
| ethylpyrazine | 24.6 | 5.2 | – |
| 2,3-dimethylpyrazine | 23.7 | 3.8 | – |
| vinylpyrazine | 8.1 | 2.7 | – |
| 2-ethyl-5-methylpyrazine | 6.7 | 1.1 | – |
| trimethylpyrazine | 29.8 | 11.4 | – |
| 2-vinyl-5-methylpyrazine | – | 0.9 | – |

[a] Not observed.

Tables 4 and 5 quantitatively summarize all pyrazines that are identified in the lysine and glucose reaction systems. In this study, we found that the yields of pyrazines from the dry system were 10–400 times higher than those from the aqueous system. Similar data were also reported by Hwang et al. (1993). Again, it showed that the yields of pyrazines were enhanced at the intermediate water level. In addition, we found that the yields of unsubstituted pyrazine, methylpyrazine, and 2,6-dimethylpyrazine were the highest ones in the reaction system (Tables 4 and 5). This suggests that the two- and three-carbon fragments were in abundance in the system. These two- and three-carbon fragments could be formed from retro-aldol condensation which is catalyzed by amines (Shibamoto and Bernhard, 1977). In the present studies, the high sugar fragments, of course, were catalyzed by both amino groups of lysine.

The temperature of the reaction mixture can also affect pyrazine production. We observed that the amounts of pyrazines significantly changed when the reaction temperature decreased. At the reaction temperature, around 100 °C, only pyrazine, methylpyrazine, and 2,6-dimethylpyrazine were found. It has also been reported that pyrazines were generated at temperatures above 100 °C (Koehler et al., 1969). Therefore, we concluded that the long-chain alkyl-substituted pyrazines had a higher activation energy and required a higher temperature, such as 140 °C, to produce.

The effects of pH on the production of pyrazines are temperature-dependent. At higher temperatures (180 °C),

the yields of pyrazines were slightly influenced by pH. At lower temperatures (140 and 100 °C), the amounts of pyrazines were larger in the alkaline reaction system (pH 8.5) than in the neutral system. These results were expected because the base can enhance the yields of pyrazines by increasing the reactivity of the amino groups toward the carbonyl of glucose, increasing the rearrangement and fragmentation of sugars (Koehler and Odell, 1970). However, the reaction temperatures and pHs have no effect on the contribution of two different amines ($\alpha$ or $\epsilon$) to form pyrazines.

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