Pyrazine Generation from the Maillard Reaction of Mixed Amino Acids in Model Systems

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There has been much interest in the Maillard reaction, or non-enzymatic browning reaction, from the flavor chemistry point of view over the past decade. Most processed foods generate their characteristic flavors through the Maillard reaction.[1] Heterocyclic compounds such as pyrazines, pyrroles, pyridines, thiazoles and oxazoles are generated by a series of these reactions and have an important impact on the flavor of many foods. Among the heterocyclic compounds, which commonly possess the sensory properties of roasted, toasted and nutty characters,[2] pyrazines are considered to be fairly representative of the flavor of roasted foods.

Although the mechanism of the Maillard reaction is quite complex, it is generally known that the process is initiated by the condensation reaction between an amine and a reducing sugar.[3] Numerous model system studies have been reported on the formation of pyrazines through the Maillard reaction involving single amino acids and an equal mole of sugar.[4-6] Since real food systems consist of mixtures of different amino acids, the Maillard reaction models based on two or more amino acids have generated a great deal of interest. Recently, the contributions of several amino acids to the reactivity of ^{15}N-labeled glycine to form pyrazines has been investigated in this laboratory.[7] However, pyrazine formation involving the interaction of more than one amino acid has not been well studied.

Pyrazines often are present in food in minor or trace quantities. As a result, the analysis and quantitation of pyrazines in food systems are often difficult. In the present study, we used Kuo et al.'s previously reported selective purge-and-trap method[8] to study the effect of four amino acids, acting singly or in combination, on the generation of pyrazines.

Materials

L-Glutamic acid, L-glutamine, L-lysine, and L-alanine were purchased from Sigma Chemical Co. (St. Louis, Missouri). Glucose and propylene glycol were obtained from Aldrich Chemical Co., Inc. (Milwaukee, Wisconsin). Anhydrous sodium sulfate (10-60 mesh), sodium chloride (A.C.S. grade) and methylene chloride (HPLC grade) were purchased from Fischer Scientific (Fair Lawn, New Jersey). 2-Ethoxy-3-isopropyl pyrazine, used as an internal standard, was obtained from Pyrazine Specialties (Atlanta, Georgia).

Conditions for Volatile Generation

In our model system, each reaction mixture was prepared from 0.2 mole of glucose and 0.2 mole of the selected amino acids (L-glutamic acid, L-glutamine, L-lysine and L-alanine) or 0.2 mole of a combination of these amino acids in 165 ml of propylene glycol. Each amino acid combination, totaling 0.2 mole, was prepared with 0.1 mole, 0.067 mole and 0.05 mole of two, three and four amino acids respectively. These reaction mixtures were then transferred to a Parr reaction vessel (Parr Instrument Co., Moline, Illinois) and heated in the reactor at 155°C for 20 minutes. The speed of the agitator was maintained at 155 rpm, and the internal pressure of the reactor was monitored and not allowed to exceed 120 psi.

Pyrazine Fractionation

The modified apparatus for selective purge-and-trap isolation and fractionation is shown in Figure 1. The temperature of the water bath was maintained at 60°C during pyrazine fractionation. Thirty grams of the reaction mixture from the Parr reactor, 45 g of NaCl (Fischer Scientific, Fair Lawn, New Jersey) and 360 ml of distilled water were placed in the round-bottom flask. 2-Ethoxy-3-isopropyl pyrazine (1.2 mg) was added as an internal standard for quantitation. The suspension was purged using nitrogen gas at a flow rate of 400 ml/minute for 2 hours and trapped in 10 ml of 11.7% (w/v) hydrochloric acid solution. While most of the non-basic volatile constituents pass through the acid solution, pyrazines are retained in the acid trap, owing to their weakly basic character.

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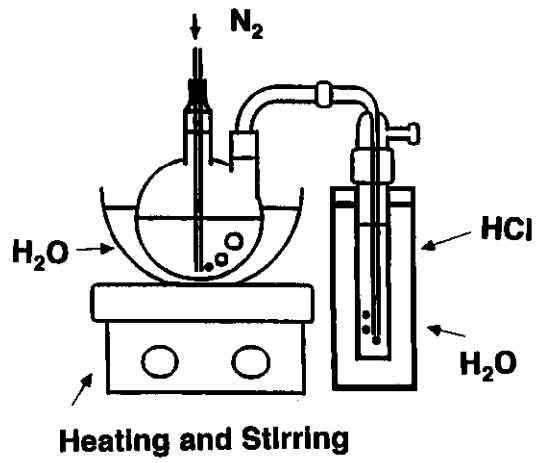

Figure 1. Apparatus for the purge-and-trap isolation and fractionation

After the sample suspension was purged for 2 hours, the acid solution was washed several times with methylene chloride to further remove the remaining non-basic compounds in the acid trap. The pyrazine-containing acid solution was titrated with 30% NaOH solution to pH 13. The alkaline solution was then extracted with methylene chloride in a separatory funnel to isolate the pyrazines, and further concentrated by nitrogen evaporation.

GC Analysis

A Varian 3400 gas chromatograph equipped with a flame ionization detector and a fused silica capillary column (60 m x 0.32 mm [i.d.], df = 1.0 µm, DB-1; J&W Scientific, Folsom, California) was used to analyze the volatiles isolated by the selective purge-and-trap method. The operating conditions were as follows: injector temperature, 270°C; detector temperature, 300°C; flow rate of helium gas, 1 ml/minute. The column temperature was programmed from 40°C to 180°C at the rate of 2°C/minute, and from 180°C to 280°C at 10°C/minute. Quantitative determinations were made without considering response factors and were carried out by using a Varian 4270 integrator. Linear retention indices were calculated against the n-paraffins (C_5-C_{20}) as references.[9]

GC/MS Analysis

Volatiles isolated by the modified purge-and-trap method were analyzed by gas chromatograph/mass spectrometer (GC/MS) using a Varian 3400 gas chromatograph directly coupled with a Finnigan MAT 8230 high resolution mass spectrometer. Mass spectra were obtained by electron ionization at 70 eV. The ion source temperature was 250°C and the filament emission current was 1 mA. All mass spectra generated were recorded and interpreted with the

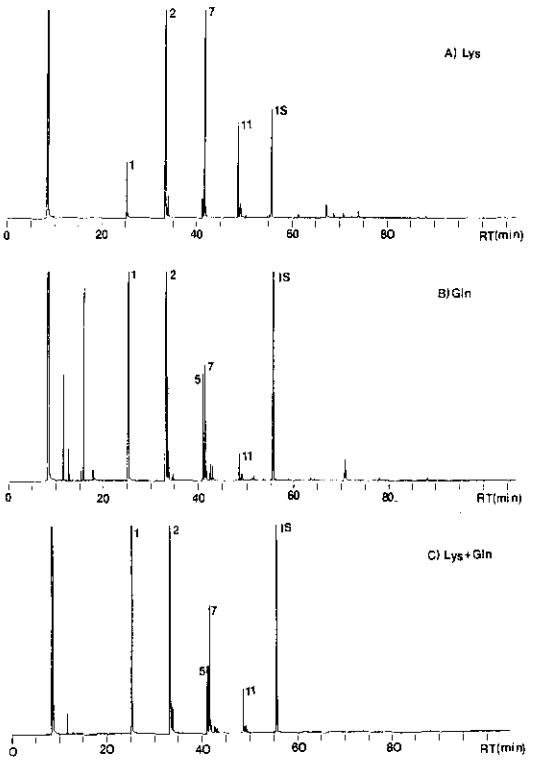

Figure 2. GC profiles of basic fractions generated from glucose with A) lysine, B) glutamine, and C) a lysine/glutamine mixture at 155°C for 20 minutes

Finnigan MAT SS 300 data system. The GC column was the same as described in the previous section. Compound identifications were based on comparisons with published literature or with the computer library (NIST).

Results and Discussion

Heterocyclic compounds identified from the basic fraction of the amino acid-glucose model systems are listed in Table I.

For single amino acid reaction systems, the lysine-containing reaction produced 2.6 to 4 times more total pyrazines than the other amino acids studied. The lysine system also was significantly more abundant in 2,6-dimethylpyrazine (3x to 15x) and trimethylpyrazine (11x to 19x), which may, in part, account for the strong aromatic character of this system. Both the lysine reaction and the alanine reaction produced significantly more 2,5-dimethyl-3-ethylpyrazine and 2-methyl-5-propylpyrazine and less methylpyrazine and ethylpyrazine than the other two amino acids.

Most mixed amino acid systems generated much smaller amounts of pyrazines. Of the 14 pyrazines identified, methylpyrazine was predominant in glutamic acid, 2,6-dimethylpyrazine in lysine and in glutamine, and 2,5-dimethyl-3-ethylpyrazine in model systems containing either

alanine or lysine. In addition to pyrazines, other nitrogen-containing heterocyclic compounds such as pyridines and oxazole were also separated by this selective purge-and-trap method.

Figure 2 shows the GC profiles of basic fractions from the reaction mixtures of glucose with lysine (A), glutamine (B) and a lysine/glutamine mixture (C) under conditions previously described. Again, total pyrazine content was greater for individually reacted amino acids than for the combination of amino acids. Lysine generated the highest yield. The occurrence of 2,6-dimethylpyrazine and 2,5-dimethyl-3-ethylpyrazine in lysine accounted for the roasted, nutty odor characteristics observed for this reaction mixture.

The yield and relative distribution of pyrazine formation for single amino acids are shown in Figure 3. Among the 14 identified pyrazines, methylpyrazine 2,6-dimethylpyrazine, ethylpyrazine, 2-ethyl-5-methylpyrazine and 2,5-dimethyl-3-ethylpyrazine were selected for this comparison. Again, the graph clearly shows that the formation of individual pyrazines varied with the type of amino acids used. For example, the glutamic acid reaction mixture produced a relative abundance of methylpyrazine and ethylpyrazine, but the lysine reaction mixture produced primarily 2,6-dimethylpyrazine and 2-ethyl-5-methylpyrazine. The total pyrazine yield was found to be highest in the reaction mixture containing lysine, followed by those containing glutamine, glutamic acid and alanine.

The amino acids glutamic acid, glutamine, alanine and lysine were selected as a representation of the acidic, neutral and basic amino acid groups. The yields of pyrazines generated from reaction mixtures containing either two, three or four combinations of amino acids were compared with the pyrazine yields from reaction mixtures containing a single amino acid.

Figures 4, 5 and 6 show the effect of combinations of two, three and four amino acids, respectively, on the generation of pyrazines.

To examine the effect of combinations of two different amino acids on the formation of pyrazines, the average total pyrazine yield generated from the reaction containing glutamic acid was added to the average total pyrazine yield generated from the reaction containing glutamine, and that total was compared with the total pyrazine yield generated from the reaction containing a mixture of glutamic acid and glutamine. The results showed that the total pyrazines generated from the reaction mixture containing glutamic acid and glutamine was lower than the total pyrazines from individually reacted amino acids.

Table I. Quantitation of the basic fraction generated from the reaction of amino acid with glucose

Peak	Pyrazines	Quantitation (ppm)													
		E	Q	K	A	E+Q	E+K	E+A	Q+K	Q+A	K+A	E+Q+K	E+Q+A	E+K+A	Q+K+A	E+Q+K+A
1	methylpyrazine	74.19	52.99	17.29	9.92	5.84	23.65	4.20	24.19	t	0.26	20.13	4.36	17.05	12.70	11.78
	trimethyl oxazole	t	t	t	0.16	t	t	t	t	t	t	t	t	t	t	t
	4-methylpyridine	t	t	t	0.81	t	t	t	t	t	t	t	t	t	t	t
2	2,6-dimethylpyrazine	34.90	86.47	258.5	17.42	10.11	84.14	8.04	95.82	9.69	18.26	57.00	11.53	48.67	52.57	36.27
3	ethylpyrazine	9.14	6.28	2.47	2.53	1.37	1.93	1.39	1.90	0.52	0.58	2.02	1.17	1.65	1.51	1.36
4	2,3-dimethylpyrazine	9.95	1.91	6.66	0.86	0.22	1.83	0.19	1.95	t	0.59	1.24	0.19	1.07	1.04	0.67
5	2-ethyl-5-methylpyarzine	4.14	7.06	6.42	15.40	1.52	4.25	2.43	6.35	5.48	9.63	4.39	3.28	7.43	10.84	6.88
6	trimethylpyrazine	5.82	8.79	98.39	5.06	1.44	15.44	2.28	14.38	3.44	12.85	8.15	2.50	10.07	10.42	6.68
7	2-ethyl-3-methylpyrazine	1.88	0.84	3.83	9.22	t	0.68	2.24	0.99	3.31	10.50	0.48	1.89	7.44	6.54	4.48
8	2-methyl-5-vinylpyrazine	0.52	1.01	t	0.14	t	0.64	t	0.47	t	0.16	0.57	0.21	0.39	0.46	0.32
9	2-methyl-6-vinylpyrazine	0.68	t	t	0.22	t	t	0.15	t	0.15	0.28	0.18	0.09	0.44	0.13	0.13
	5-ethyl-2-methylpyridine	t	t	t	2.42	t	t	t	t	t	t	t	t	t	t	t
10	isopropenylpyrazine	t	t	t	1.40	t	t	t	t	t	t	t	t	t	t	t
11	2,5-dimethyl-3-ethylpyrazine	0.61	1.94	31.94	32.80	t	3.05	7.30	4.43	31.45	111.99	2.10	9.12	53.73	56.34	29.61
12	2-methyl-5-propylpyrazine	0.84	0.52	3.25	2.63	t	0.84	0.96	t	3.12	8.39	0.52	0.14	0.97	5.66	0.49
13	2-methyl-5-(1-propenyl)-pyrazine	0.50	0.17	0.79	3.22	t	0.17	0.78	0.20	1.94	t	0.18	0.73	2.17	t	2.48
14	2,3-diethyl-5-methylpyrazine	t	t	t	t	t	t	0.14	t	4.15	t	t	1.41	3.99	t	t
	Total	143.17	167.98	429.54	104.21	20.50	136.62	30.10	150.68	63.25	174.00	96.96	36.62	155.07	158.42	101.25

E = glutamic acid; Q = glutamine; K = lysine; A = alanine; t = trace amount

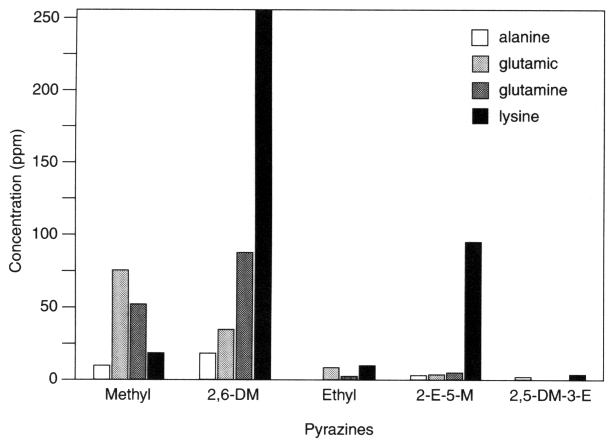

Figure 3. The pyrazine yield and relative distribution generated from the reaction mixtures containing individual amino acids (alanine, glutamic acid, glutamine, lysine) and glucose

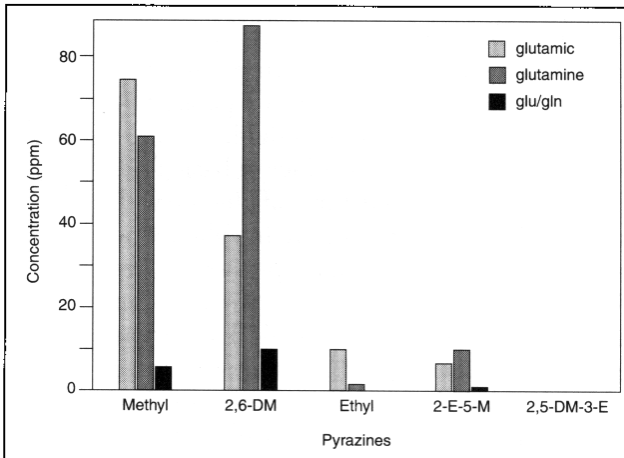

Figure 4. The pyrazine yield and the effect of combinations of two different amino acids, glutamic acid and glutamine

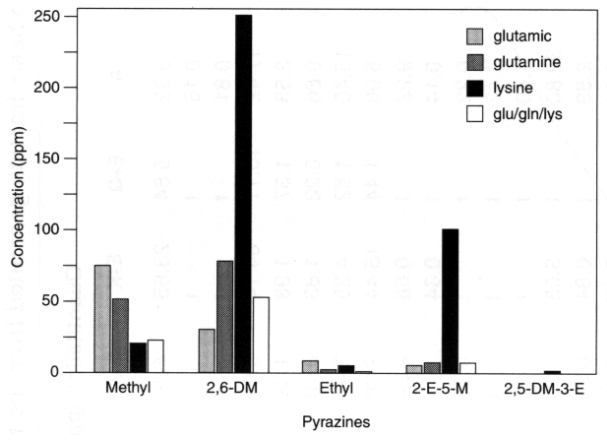

Figure 5. The pyrazine yield and the effect of combinations of three different amino acids, glutamic acid, glutamine and lysine

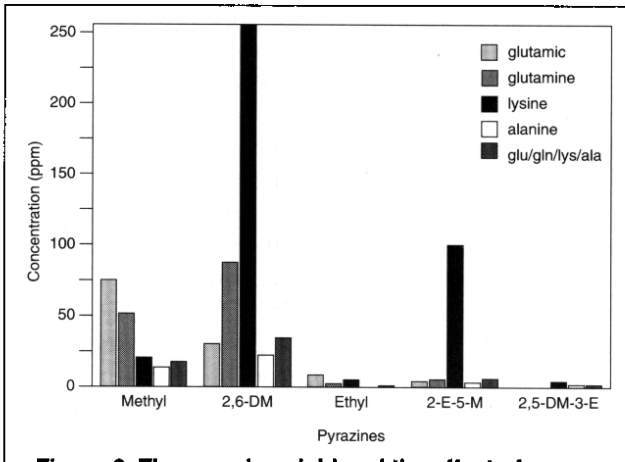

Figure 6. The pyrazine yield and the effect of combinations of four different amino acids, glutamic acid, glutamine, lysine and alanine

Other reactions with two, three and four combinations of amino acids gave similar results to that observed for the glutamic acid/glutamine combination.

Individual pyrazine formation also demonstrates this trend, with higher yields observed for the singly reacted amino acids. Some exceptions were observed, however, for the higher molecular weight pyrazines. For the two amino acid combination systems, the lysine-alanine mixture resulted in 3.5- and 3-fold increases in the yield of 2,5-dimethyl-3-ethylpyrazine and 2-methyl-5-propylpyrazine respectively. Similarly, the glutamine-alanine mixture generated a higher yield of 2,3-diethyl-5-methylpyrazine. This compound has been described as nutty and meaty with a roasted hazelnut-like aroma.

These findings indicate that reactions with combinations of amino acids generally yield lower pyrazine concentrations than reactions with single amino acids. This result implies that amino acids act as suppressors that decrease the reactivity of other amino acids when mixed and reacted with glucose in propylene glycol.

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