two Z or two E double bonds. In addition, the chemical shift of C-13 (δ 20.6) of compound B coincided with that of the *all-Z* isomer, while the chemical shift of C-4 (δ 26.8) coincided with that of the *all-E* isomer. Conclusively, compound B was determined as the 5*E*,8*Z*,11*Z* isomer of compound A, i.e. (5*E*,8*Z*,11*Z*)-5,8,11-tetradecatrien-2-one.

The partial structure and configuration of compound A are same as those of linolenic acid, which could be a precursor of these ketones. However, if a methyl ketone is derived from a natural fatty acid, it is natural that an odd-numbered carbon ketone should be produced (Toda et al., 1982). Therefore, there might be some other metabolic pathway that can produce even-numbered methyl ketones. Compound B had an E double bound in its structure; however, it retained two Z configurations, and no other E isomers could be found through this study. We assume that compound B was originally present in raw shrimps and was not isomerized from compound A during the heating process. Through these discussions and results, we are interested in the occurrence of the ketones in crustaceans and in their contribution to the characteristic seafood flavor. Studies on the identification and formation of these new ketones are continuing.

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LITERATURE CITED

Gunstone, F. D.; Pollard, M. R.; Scrimgeour, C. M.; Vedanayagam, H. S. *Chem. Phys. Lipids* **1977**, *18*, 115.

Kubota, K.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1980a**, *44*, 2677.

Kubota, K.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1980b**, *44*, 2753.

Kubota, K.; Kobayashi, A.; Yamanishi, T. *Nippon Nogeikagaku Kaishi* **1982**, *56*, 1049.

Nickerson, G. B.; Likens, S. T. *J. Chromatogr.* **1966**, *21*, 1.

Silverstein, R. M., Bassler, G. C., Morrill, T. C., Eds. *Spectrometric Identification of Organic Compounds*, 4th ed.; Wiley: New York, 1981.

Toda, H.; Yamaguchi, K.; Shibamoto, T. *J. Agric. Food Chem.* **1982**, *30*, 81.

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Effect of Nitrogen Source on Pyrazine Formation

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The role of the nitrogen source in pyrazine formation in model systems containing glucose and base was examined. The distribution of pyrazines formed in the reactions containing ammonium hydroxide, ammonium formate, ammonium acetate, glycine, and monosodium glutamate depends strongly on the nature of the nitrogen source. Pyrazines were identified by mass spectrometry and by means of Kovat's indices on polar and nonpolar fused silica capillary columns. A novel mechanism for a Strecker degradation and cleavage of glutamate of acetaldehyde and 2-hydroxyacetate is proposed.

The pyrazines have been recognized as important flavor constituents of a large number of cooked, roasted, and toasted foods (Maga, 1982). The latter discovery of naturally occurring pyrazines in a variety of biological systems further illustrates their ubiquity, remarkable physiological activity, and potency (Murray et al., 1970; Attygalle and Morgan, 1984).

The production of pyrazines from the reaction of carbohydrates and amine compounds has been studied extensively over the past several years. Hodge et al. (1972) proposed that amino acids and carbohydrates were important precursors for pyrazines formed during the nonenzymatic browning reaction. Koehler and Odell (1970) and Rizzi (1972) obtained pyrazines as products of lipid autoxidation. Ferretti et al. (1970) obtained pyrazines from the reaction of lactose with casein, while Wang and Odell (1973) demonstrated pyrazine formation from amino hydroxy compounds. Velisek et al. (1976) reported pyrazine formation from the reaction of glyoxal and glycine, and Davidek et al. (1977) reported dehydro-L-ascorbic acid reacted with ammonia or glycine to produce pyrazines.

Formation pathways for pyrazines and other heterocyclic compounds such as quinoxalines, imidazoles, and pyridines have been proposed by numerous researchers (Rizzi, 1972; Walradt et al., 1971; Shibamoto and Bernhard, 1977a,b, 1978). By far the most detailed and extensive formation pathways of alkylpyrazines have been proposed by Shibamoto and Bernhard (1977a,b): sugars react with amines with the formation of α-amino carbonyl intermediates, which condense to produce pyrazine compounds.

There is still much speculation as to the way in which the nitrogen atoms are incorporated into the pyrazine molecule. Free ammonia formed as a result of the decomposition of the amino acids may combine with sugars to form pyrazines. Another hypothesis suggests that nitrogen still bound to the amino acid may react with sugars to form pyrazines. Newell et al. (1967) reported that the same pyrazines formed regardless of the amino acid utilized. van Praag et al. (1968) reacted various amino acids with fructose and reached the same conclusion. Both groups assumed that free ammonia was the primary intermediate in pyrazine formation, thus resulting in the same series of pyrazines for all of the amino acids. Wilkins and Lin (1970) also assumed that free ammonia was nec-

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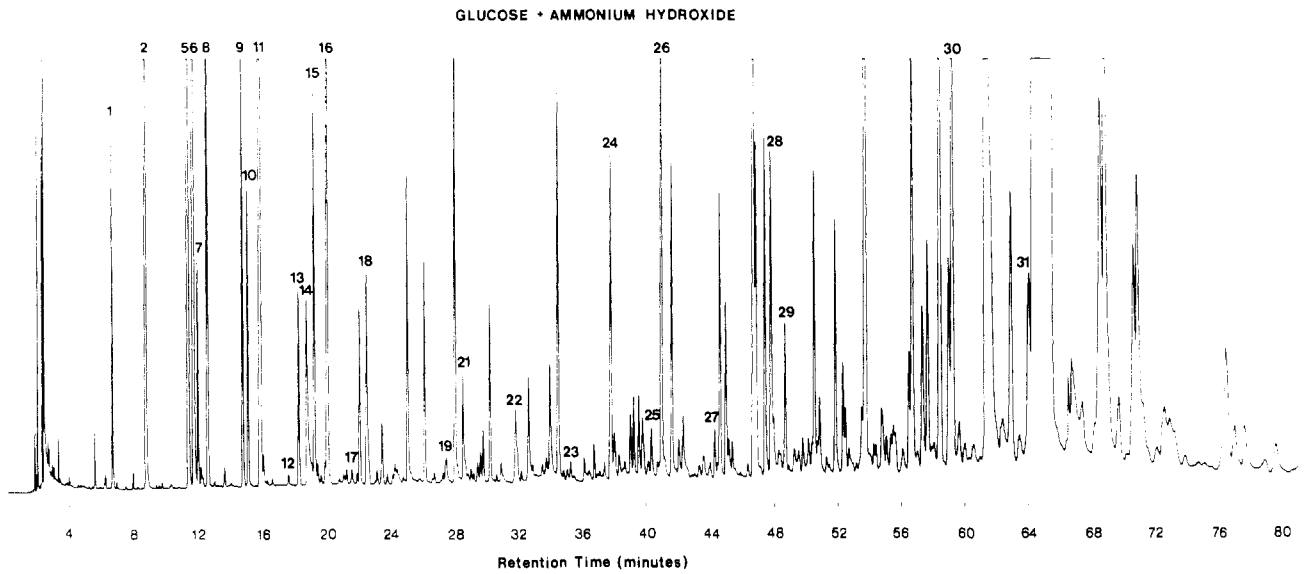

Figure 1. Chromatogram of 0.4 μL of an extract of glucose–NaOH–ammonium hydroxide reaction, split ratio 1:100, 30 m × 0.25 mm fused silica capillary column bonded and cross-linked with DB-Wax+. See Table I for peak identification.

essary for pyrazine formation in soy products. Conversely, Koehler et al. (1969) reported that systems of glucose–amino acid and glucose–ammonium chloride produced different pyrazine products. They concluded that the nitrogen was still bound to the amino acid upon condensation with the sugar. They proposed that variations in pyrazine distribution were due to the different rates at which various amines react with sugar fragments and the ease of nucleophilic attack of the amino acid on the sugar. As a result of this contrast in theories, there is still much speculation as to the mechanism of nitrogen incorporation into the pyrazine molecule.

The purpose of the present study was to investigate the nature of these seemingly contradictory reports and to determine whether the kind of the nitrogen source influences the formation of pyrazines in model systems. Glucose was used as the primary carbon source and ammonium hydroxide, ammonium formate, ammonium acetate, glycine, and monosodium glutamate were used as five sources of nitrogen to determine how the nitrogen source influenced the formation of the products. The present investigations utilized improved chromatographic techniques such as fused silica capillary columns, which enhance resolution, identification, and quantitation. This capability was not available at the time when most of the studies described above were made.

EXPERIMENTAL SECTION

The amino acids, ammonium salts, and solvents were all reagent grade, and the authentic reference compounds were obtained from commercial sources. All reference compounds were examined for purity by gas chromatography. Those found to contain unacceptable amounts of impurities were further purified by recrystallization or by preparative gas chromatography.

Reaction mixtures consisted of 0.1 mol of D-glucose, 0.1 mol of sodium hydroxide, and 0.8 mol of the nitrogen source in 100 mL of deionized water. The reaction solution temperature was maintained at 120 °C for 4 h. Reaction conditions were selected to maximize yield of the pyrazines (Shibamoto and Bernhard, 1976). The method of analysis has been described previously (Shibamoto and Bernhard, 1978). The pH of all reaction mixtures was measured before reaction and on completion of the reaction period. After the reaction period and after cooling, the pH dropped approximately 3 pH units for all samples.

Qualitative and quantitative analyses of reaction mixtures were accomplished with use of a Hewlett-Packard 5711A gas chromatograph modified for use with capillary columns and fitted with a FID. A 30 m × 0.25 mm (i.d.) fused silica capillary column bonded and cross-linked with DB-Wax+ was temperature programmed as follows: 70 °C for 8 min, 70–160 °C at 2 °C/min, isothermal hold at 160 °C. Carrier gas velocity was 30 cm/s He. A Varian Vista 6000 gas chromatograph with a FID was fitted with a 60 m × 0.32 mm (i.d.) fused silica capillary column bonded and cross-linked with DB-1701 and temperature programmed as follows: 50 °C for 45 min, 50–250 °C at 5 °C/min, isothermal hold at 250 °C. Carrier gas velocity was 30 cm/s He.

A Shimadzu C-R3A digital integrator was used to determine retention times and peak areas. Measurement of FID relative response factors and quantitative analyses were made by the procedures of McNair and Bonelli (1968).

A Hewlett-Packard 5792A gas chromatograph coupled with a VG analytical ZAB-HS-2F mass spectrometer with a VG 11/250 data system was used for mass spectral identification of components. Operating conditions: ionizing voltage, 70 eV; source temperature, 180 °C; accelerating voltage, 8000 eV; filament trap current, 100 μA. The gas chromatograph was operated with the DB-Wax+ column with the operating parameters described above. Unknowns were identified by comparison of mass spectra and Kovat's indices with those of authentic samples using polar (DB-Wax+) and relatively nonpolar (DB-1701) columns (Table I). Compounds identified by only a single method of identification have been labeled tentative.

RESULTS AND DISCUSSION

The compounds isolated from the reaction mixtures are listed in Table I in order of their elution from the DB-Wax+ column. A typical gas chromatogram is shown in Figure 1.

Mass balance calculations of weight of extract relative to weight of glucose reveal that reactions utilizing amino acids as the nitrogen source produced the best yields of reaction products: 14.99% from glycine and 15.64% from monosodium glutamate. Ammonium formate and ammonium acetate gave yields of 8.65% and 7.66%, respectively; this was less than the yield of 10.38% from ammonium hydroxide.

Table I. Kovat's Indices and Response Factors

compound	Kovat's indices I_{DBWax+}	I_{DB1701}	FID rel resp factor, f	indent method[a]
pyrazine	1215	822	1.000	I, I*, MS
2-methylpyrazine	1266	908	1.164	I, I*, MS
acetoin	1286	844	0.622	I, I*, MS
acetol	1300	809	0.454	I, I*, MS
2,5-dimethylpyrazine	1320	988	1.177	I, I*, MS
2,6-dimethylpyrazine	1326	991	1.271	I, I*, MS
2-ethylpyrazine	1330	994	1.177	I, I*, MS
2,3-dimethylpyrazine	1341	1000	1.203	I, I*, MS
2-ethyl-6-methylpyrazine	1381	1071	1.339	I, I*, MS
2-ethyl-5-methylpyrazine	1386	1072	1.247	I, I*, MS
2,3,5-trimethylpyrazine	1400	1075	1.305	I, I*, MS
2-ethyl-3-methylpyrazine	1400	1076	1.284	nd
2-vinylpyrazine	1429	1017	1.157	I, I*
2-ethyl-3,6-dimethylpyrazine	1439	1168	1.501	I, I*, MS
2,3-diethylpyrazine	1449	1170	1.408	nd
2,5-diethylpyrazine	1449			nd
2-ethyl-3,5-dimethylpyrazine	1455	1172	1.357	nd
tetramethylpyrazine	1468	1174	1.378	I, I*, MS
2,3-diethyl-5-methylpyrazine	1488	1219	1.384	I, I*, MS
pyrrole	1509	915	1.259	I, I*
2-acetylpyridine	1587	1161	1.217	I, I*, MS
1,2-propanediol	1594	940	1.419	I, I*
5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine	1605	1233	1.327	I, I*, MS
furfuryl alcohol	1661	1021	0.950	I*, MS
5,6,7,8-tetrahydroquinoxaline	1717	1307	1.293	I, I*
acetamide	1764	*b*	0.587	I, MS
propionamide	1807	*b*	0.970	I, MS
1-furfurylpyrrole	1817	1312	1.224	I, I*
quinoxaline	1879	1327	1.411	I, I*
2-methylquinoxaline	1941	1421	1.398	I, I*, MS
2-acetylpyrrole	1959	1251	1.342	I, I*, MS
2-methylimidazole	2146	*b*	1.166	I, tentative
imidazole	2200	*b*	3.079	I, tentative
4-methylimidazole	2211	*b*	1.065	I, tentative

[a] Key: I = Kovat's index on DB-WAX+; I* = Kovat's index on DB-1701; MS = mass spectrum; nd = not detected. [b] Did not elute from DB-1701 column.

In the present study, 32 compounds were identified including 19 pyrazine derivatives, 9 other nitrogen-containing compounds, and 4 oxygen-containing compounds.

Comparison of the relative weight percent of the 32 compounds produced by the five reactions examined is shown in Table II. Components are grouped into pyrazines and non-pyrazines, with peak numbers corresponding to the retention times on the DB-Wax+ column. All compounds identified in this study have been found in foods or in sugar–amine model systems. The differences between quantitative and qualitative results for some of the nitrogen sources examined in the present study and a previous study using similar reaction conditions (Shibamoto and Bernhard, 1977a) may be due to differences in the method of extraction and separation capabilities of the gas chromatographic columns. A continuous extraction method (Shibamoto and Bernhard, 1978) recovered a greater amount of the less volatile compounds, and the improved resolution afforded by the newer fused silica capillary columns enhanced identification.

In general, reactions using ammonium salts as a nitrogen source were very similar in the production of the pyrazines with the exception of 2,6-diethylpyrazine. In regard to the other nitrogen compounds, the ammonium acetate reaction produced a greater amount of acetamide than did the ammonium formate reaction. The ammonium salts also differed in the relative weight percent of the imidazoles produced. The ammonium hydroxide reaction produced different pyrazine and other product profiles than did the ammonium salts, and the amino acid reactions were different from the reactions of the ammonium salts and ammonium hydroxide and from each other.

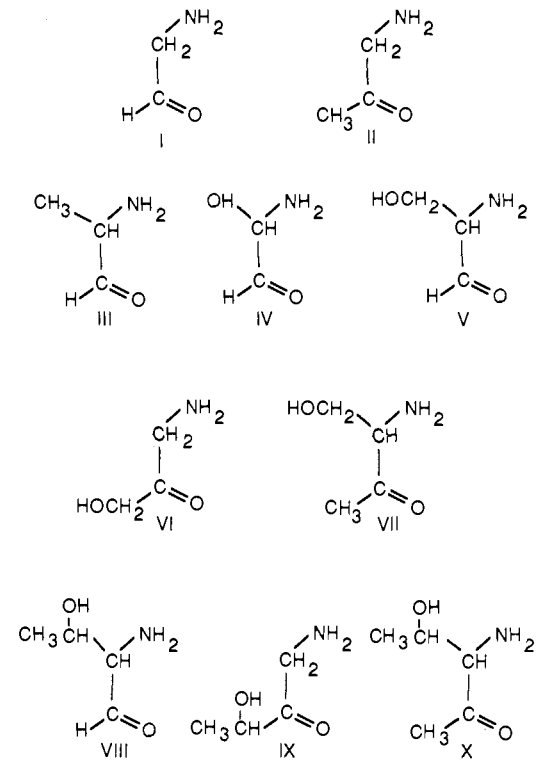

Figure 2. Shibamoto and Bernhard (1977a) proposed α-amino carbonyl fragments.

Although various mechanisms have been proposed, Shibamoto and Bernhard (1977a) proposed the most detailed series of mechanisms for pyrazine formation, with pathways and reactions showing how α-amino carbonyl intermediates (fragments) are formed from glucose (Figure 2). Their mechanisms can be used to explain the formation of pyrazine, 2-methylpyrazine, 2,5- and 2,6-dimethylpyrazine, trimethylpyrazine, 2-ethylpyrazine, 2-ethyl-5-methyl- and 2-ethyl-6-methylpyrazine, and 2-ethyl-3,5-dimethyl- and 2-ethyl-3,6-dimethylpyrazine. Details explaining the formation of cyclopentapyrazines and quinoxalines await further elucidation. Walradt et al. (1971) and Shibamoto and Bernhard (1978) proposed a mechanism for a cyclopentapyrazine formation from 2-hydroxy-3-methyl-2-cyclopenten-1-one, a product of carbohydrate degradation. Shibamoto and Bernhard (1978) also proposed a mechanism for the formation of imidazoles. Acetoin, acetol, and furfuryl alcohol identified in the present study are well-known products of carbohydrate fragmentation and dehydration produced during the nonenzymatic browning reaction (Hodge, 1967; Ferretti and Flanagan, 1973). The acetylpyrrole and 3-acetylpyridine, also identified in the present study, have been found in model systems and cooked foods (Umano and Shibamoto, 1984; Buttery et al., 1975).

The mechanisms proposed by Shibamoto and Bernhard (1977a,b) did not explain the formation of tetramethyl-, 2,3-diethyl-, 2,5-diethyl-, 2,6-diethyl-, and 2,3-diethyl-5-methylpyrazines. At present we are unable to suggest a reasonable mechanism for their formation.

It is not readily evident why the kinds and amounts of products were formed in the reactions using the various nitrogen sources. Shibamoto and Bernhard (1977a) proposed that the large amounts of certain pyrazine compounds might be a consequence of the relative abundance of fragments and/or formation rates. This may explain the very large amounts of 2-methylpyrazine and 2,6-dimethylpyrazine identified in the reactions containing ammonium hydroxide and the ammonium salts. The nitrogen

source utilized in the reactions may influence the formation and reaction of fragments and thus explain the differences in the production of the imidazoles, 2,5-dimethyl-, trimethyl-, and tetramethylpyrazines in the ammonium salt reactions, and 2-methyl- and 2,6-dimethylpyrazines in the amino acid reactions.

By comparison to the ammonium acetate, the ammonium formate reaction did produce large amounts of 4-methylimidazole. This increased production may be due to the well-known ability of formate to participate in redox reactions (Maxon, 1939; March, 1985) and produce acetaldehyde from the carbohydrate. Acetaldehyde in conjunction with formaldehyde may form acetol (Tambawala and Weiss, 1972). The Amadori product of acetol, fragment III, may condense with formate to produce 4-methylimidazole (Shibamoto and Bernhard, 1978).

In the ammonium acetate reaction, the extremely large amount of acetamide can be formed by the direct thermal conversion of ammonium acetate to acetamide (Noller, 1957). Most of the nitrogen may be involved in the formation of acetamide and thus be unavailable for the production of some of the pyrazine and imidazole compounds.

The large increase in the amount of 2,6-diethylpyrazine in the ammonium acetate reaction is difficult to explain. Ammonium acetate may directly or indirectly influence 2-amino carbonyl fragment production and sugar fragmentation or affect the rates of these reactions. Further investigation is required to ascertain why such a large amount of 2,6-diethylpyrazine is produced by this reaction.

In the reactions containing amino acids, the additional reaction of the Strecker degradation of the amino acid to an aldehyde with the loss of ammonia and carbon dioxide must also be considered (Schonberg and Moubasher, 1952). In the reaction utilizing glycine as the nitrogen source, the Strecker degradation of glycine produced formaldehyde (Hodge, 1967). This source of formaldehyde may account for some of the products formed in the reaction. The glycine–glucose model system produced a larger amount of 2,6-dimethylpyrazine than did the reaction utilizing monosodium glutamate. This may occur because of the formation of formaldehyde as described above. 2,6-Dimethylpyrazine may be produced from fragments II and V and/or III and VI (Shibamoto and Bernhard, 1977a). Fragment V may be formed by the reaction of formaldehyde and fragment I, or the autocatalytic condensation of three molecules of formaldehyde may result in the production of dihydroxyacetone (Tambawala and Weiss, 1972). Fragment I may itself be produced from the condensation of two molecules of formaldehyde to form glycolaldehyde followed by amination and an Amadori rearrangement. Rearrangement of dihydroxyacetone to glyceraldehyde and reaction with ammonia can produce fragment VI. Since acetol is present in the glycine reaction, it too may undergo amination and an Amadori reaction to produce fragments II and III. The production of these fragments from the degradation of glycine and glucose may explain the large amounts of 2,6-dimethylpyrazine formed.

Extremely large amounts of trimethylpyrazine were produced in the reaction containing glycine. Trimethylpyrazine may be formed by a number of fragment-pair condensations, e.g., II and VII, III and V, or VII and VI with the Amadori product of acetoin (Shibamoto and Bernhard, 1977a). Marked quantities of acetoin were detected in this reaction, which may, in part, account for the large amount of trimethylpyrazine formed. Another factor may be the formaldehyde produced from glycine. Formaldehyde may condense with fragment III to produce

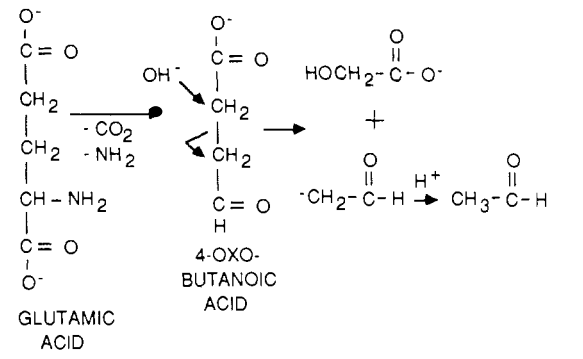

Figure 3. Proposed Strecker degradation of monosodium glutamate and degradation to acetaldehyde.

fragment VII. Fragment VII may further react with fragment II to form trimethylpyrazine. Fragment V may form from fragment I and formaldehyde. Fragment V can then condense with fragment III also to increase the quantity of trimethylpyrazine in the glycine reaction.

2-Ethyl-3,5-dimethylpyrazine was produced in greater amounts in the glycine reaction than in the reaction containing monosodium glutamate. This pyrazine can be produced by the condensation of fragments III and X (Shibamoto and Bernhard, 1977a). An aldol condensation of fragment II with acetaldehyde will produce fragment X. Fragments II and III can form from acetol as noted previously.

The large percentage of tetramethylpyrazine in the glycine reaction may also be explained by the increased production of fragment VII and acetoin since this pyrazine can be formed by the condensation of fragment VII and the Amadori product of acetoin.

The Strecker degradation of glycine to formaldehyde may play a role in the amounts of the imidazoles produced. The mechanisms proposed for the formation of imidazole involve the condensation of the aminated product of fragment I and formate (Shibamoto and Bernhard, 1978). Through the Cannizzaro reaction, formaldehyde may react to produce formic acid and methanol (Tambawala and Weiss, 1972). Since formic acid can be easily produced in this reaction, its presence may account for the high percentage of imidazole. In a similar mechanism, 4-methylimidazole should form from the condensation of fragment III, ammonia, and formate (Shibamoto and Bernhard, 1978). The lower amount of the 4-methylimidazole as compared to the amount of imidazole may be due to less abundant fragment III, which requires dehydration for its formation (Shibamoto and Bernhard, 1977a). 2-Methylimidazole could form by a similar mechanism involving fragment I, ammonia, and acetic acid. The large amounts of 2-methylimidazole may be due to the abundant sources of fragment I (Shibamoto and Bernhard, 1977a).

In the model system containing monosodium glutamate as the nitrogen source, the glutamate may undergo Strecker degradation with aldoses resulting in the decarboxylated and deaminated product, 4-oxobutanoic acid. Attack by a hydroxyl ion on the carbon atom α to the carboxyl group and subsequent cleavage of the molecule could form a very stable acetyl carbanion and 2-hydroxyacetate. Protonation of the carbanion would produce acetaldehyde, which readily oxidizes to acetic acid (Figure 3). Rizzi (1987) has suggested an alternate mechanism in which a base-catalyzed aldol condensation takes place beginning with deprotonation of the carbon α to the aldehyde group. The formation of acetaldehyde and acetic acid may be important precursors for some of the major products in the glutamate reaction.

Table II. Relative Weight Percents of Identified Compounds in the Glucose–NaOH–Nitrogen Source Reaction Mixtures

compound	peak no.[b]	rel wt % of identified compounds[a] ammonium hydroxide	ammonium formate	ammonium acetate	glycine	glutamate
pyrazine	1	1.137 (0.198)[c]	0.161 (0.044)	0.155 (0.024)	0.016 (0.007)	0.593 (0.228)
2-methylpyrazine	2	14.789 (1.457)	17.101 (3.638)	14.392 (2.249)	0.784 (0.219)	1.753 (0.328)
2,5-dimethylpyrazine	5	12.923 (0.974)	3.194 (0.261)	3.749 (0.241)	6.922 (0.741)	18.035 (2.064)
2,6-dimethylpyrazine	6	17.367 (1.189)	13.262 (0.759)	13.964 (1.615)	3.053 (0.277)	0.421 (0.195)
2-ethylpyrazine	7	0.813 (0.182)	0.828 (0.189)	0.711 (0.155)	0.071 (0.013)	0.043 (0.014)
2,3-diethylpyrazine	8	3.946 (0.413)	1.783 (0.302)	1.743 (0.287)	1.492 (0.178)	0.29 (0.148)
2-ethyl-6-methylpyrazine	9	1.347 (0.143)	0.491 (0.032)	0.611 (0.025)	0.349 (0.032)	0.086 (0.047)
2-ethyl-5-methylpyrazine	10	1.004 (0.095)	0.293 (0.026)	0.398 (0.030)	1.656 (0.058)	1.558 (0.240)
trimethylpyrazine	11	15.055 (1.073)	1.119 (0.168)	1.469 (0.108)	28.591 (0.770)	2.744 (0.801)
2-vinylpyrazine	12	0.042 (0.004)	0.219 (0.056)	0.09 (0.029)	0.01 (0.006)	0.142 (0.035)
2-ethyl-3,6-dimethylpyrazine	13	0.555 (0.063)	0.065 (0.012)	0.106 (0.017)	0.324 (0.020)	0.545 (0.222)
2,6-diethylpyrazine	14	1.193 (0.307)	0.592 (0.172)	8.135 (0.292)	0.274 (0.182)	0.267 (0.130)
2-ethyl-3,5-dimethylpyrazine	15	1.413 (0.112)	0.079 (0.008)	nd	4.122 (0.180)	0.292 (0.078)
tetramethylpyrazine	16	3.494 (0.316)	nd	0.043 (0.006)	7.137 (0.751)	0.103 (0.018)
2,3-diethyl-5-methylpyrazine	17	0.047 (0.011)	0.041 (0.017)	nd	0.059 (0.011)	2.496 (0.549)
5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine	21	0.423 (0.071)	0.143 (0.029)	0.25 (0.022)	0.028 (0.009)	3.287 (0.773)
5,6,7,8-tetrahydroquinoxaline	23	0.102 (0.016)	nd	nd	0.044 (0.009)	0.045 (0.016)
quinoxaline	27	0.408 (0.164)	0.14 (0.051)	0.072 (0.037)	0.045 (0.011)	0.054 (0.010)
2-methylquinoxaline	28	2.316 (0.728)	1.905 (0.242)	1.093 (0.219)	2.073 (0.540)	0.27 (0.087)
acetoin	3	nd[d]	nd	nd	2.663 (1.111)	0.721 (0.074)
acetol	4	nd	nd	nd	3.368 (1.847)	21.065 (4.4)
pyrrole	18	0.932 (0.116)	0.07 (0.027)	0.243 (0.027)	1.075 (0.120)	0.078 (0.031)
2-acetylpyridine	19	0.076 (0.036)	0.039 (0.008)	0.043 (0.005)	0.409 (0.081)	0.029 (0.013)
furfuryl alcohol	22	0.52 (0.150)	4.247 (0.443)	0.768 (0.031)	0.192 (0.062)	1.479 (0.175)
acetamide	24	5.422 (1.178)	7.207 (0.600)	44.629 (1.927)	0.839 (0.117)	0.613 (0.055)
propionamide	25	0.489 (0.144)	0.319 (0.063)	0.228 (0.116)	0.232 (0.053)	0.125 (0.057)
1-furfurylpyrrole	26	4.704 (0.579)	0.27 (0.122)	0.073 (0.050)	1.082 (0.114)	2.832 (0.518)
2-acetylpyrrole	29	0.936 (0.271)	3.126 (0.228)	1.387 (0.331)	0.233 (0.063)	0.511 (0.113)
2-methylimidazole	30	7.089 (1.608)	4.327 (1.039)	1.583 (0.321)	19.645 (1.446)	43.178 (6.303)
imidazole	31	1.761 (0.637)	nd	0.16 (0.069)	10.706 (1.271)	0.18 (0.108)
4-methylimidazole	32	nd	38.659 (3.156)	7.461 (1.753)	2.523 (0.509)	0.941 (0.263)
1,2-propanediol	20	nd	nd	nd	nd	0.027 (0.007)

[a] Mean of four replicates. [b] In order of elution on DB-WAX+. [c] Standard deviation in parentheses. [d] nd = not detected.

The formation of 2,5-dimethylpyrazine may be influenced by acetaldehyde produced from glutamate. 2,5-Dimethylpyrazine can be formed by the condensation of fragments III and V and/or of II and VI. As noted previously, fragment III may arise from the Amadori product of acetol, which in turn may come from the condensation of formaldehyde and acetaldehyde. From the mechanism described above, glutamate can be a good source of acetaldehyde, which may ultimately influence the production of fragment III in the reaction.

Given the present state of knowledge of the mechanism of pyrazine formation in model systems, it is difficult to propose additional plausible explanations for the increased formation of 2,3-diethyl-5-methylpyrazine and 5-methyl-

6,7-dihydro-5*H*-cyclopentapyrazine in the glutamate reaction. Shibamoto and Bernhard (1978) have proposed that the precursor of 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine is 2-hydroxyy-3-methyl-2-cyclopenten-1-one. While the latter compound is a common fragment derived from carbohydrates, its formation from glutamate is not obvious.

The large amount of 2-methylimidazole produced is quite probably due to the production of acetaldehyde from glutamate. 2-Methylimidazole may form from the condensation of the diamine form of fragment I and acetate (Shibamoto and Bernhard, 1978). As previously mentioned, the Strecker degradation product of glutamate may further degrade to acetaldehyde, which readily oxidizes to acetate. Since 2-methylimidazole is the only imidazole derivative requiring acetate for its formation, this could explain the large amounts of this component in the glutamate reaction and the reason why so little imidazole and 4-methylimidazole was formed.

The data obtained from these experiments show with great clarity that the nature of the nitrogen source has a profound effect on both the kinds and amounts of pyrazines formed and also affects the formation of other components related to the nonenzymatic browning reaction in model systems. These findings further support the hypothesis of Koehler et al. (1969) and Koehler and Odell (1970) that the nitrogen source does effect pyrazine formation in model systems.

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Registry No. NH_4OH, 1336-21-6; NH_4O_2CH, 540-69-2; NH_4OAc, 631-61-8; $H_2NCH_2CO_2H$, 56-40-6; AcH, 75-07-0; $HOCH_2CO_2H$, 79-14-1; $HOCH_2CH(OH)Me$, 57-55-6; $AcNH_2$, 60-35-5; $EtCONH_2$, 79-05-0; monosodium glutamate, 142-47-2; pyrazine, 290-37-9; 2-methylpyrazine, 109-08-0; acetoin, 513-86-0; acetol, 116-09-6; 2,5-dimethylpyrazine, 123-32-0; 2,6-dimethylpyrazine, 108-50-9; 2-ethylpyrazine, 13925-00-3; 2,3-dimethylpyrazine, 5910-89-4; 2-ethyl-6-methylpyrazine, 13925-03-6; 2-ethyl-5-methylpyrazine, 13360-64-0; 2,3,5-trimethylpyrazine, 14667-55-1; 2-ethyl-3-methylpyrazine, 15707-23-0; 2-vinylpyrazine, 4177-16-6; 2-ethyl-3,6-dimethylpyrazine, 13360-65-1; 2,3-diethylpyrazine, 15707-24-1; 2,5-diethylpyrazine, 13238-84-1; 2-ethyl-3,5-dimethylpyrazine, 13925-07-0; tetramethylpyrazine, 1124-11-4; 2,3-diethyl-5-methylpyrazine, 18138-04-0; pyrrole, 109-97-7; 2-acetylpyridine, 1122-62-9; 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine, 23747-48-0; furfuryl alcohol, 98-00-0; 5,6,7,8-tetrahydroquinoxaline, 34413-35-9; 1-furfurylpyrrole, 1438-94-4; quinoxaline, 91-19-0; 2-methylquinoxaline, 7251-61-8; 2-acetylpyrrole, 1072-83-9; 2-methylimidazole, 693-98-1; imidazole, 288-32-4; 4-methylimidazole, 822-36-6.

LITERATURE CITED

Attygalle, A. B.; Morgan, E. D. "Chemicals from the Glands of Ants". *Chem. Soc. Rev.* **1984**, *13*, 245–278.

Budnitskaya, E. V. "Amino Acids as Catalysts in the Aldol Condensation of Acetaldehyde". *Biokhimiya* **1941**, *6*, 146–154.

Buttery, R. G.; Seifert, R. M.; Ling, L. C. "Characterization of Some Volatile Constituents in Dry Red Beans". *J. Agric. Food Chem.* **1975**, *23*, 516–519.

Davidek, J.; Velisek, J.; Zelinkova, Z.; Kubelka, V. "Pyrazines in the Reaction of L-Dehydroascorbic Acid with Ammonia and Glycine". *J. Food Sci.* **1977**, *42*, 277–278.

Ferretti, A.; Flanagan, V. P. "Characterization of Volatile Constituents of an *N*-Formyl-L-lysine-D-lactose Browning System". *J. Agric. Food Chem.* **1973**, *21*, 35–37.

Ferretti, A.; Flanagan, V. P.; Ruth, J. M. "Nonenzymatic Browning in a Lactose–Casein Model System". *J. Agric. Food Chem.* **1970**, *18*, 13–18.

Fischer, E.; Tafel, J. "Synthetische Versuche in der Zuckergruppe". *Chem. Ber.* **1887**, *20*, 2566–2575.

Hayami, J. "Studies on the Decomposition of Simple Sugars. XII. Mechanism of Acetol Formation". *Bull. Chem. Soc. Jpn.* **1961**, *34*, 927–932.

Hodge, J. E. "Origin of Flavor in Foods. Nonenzymatic Browning Reactions". In *Symposium on Foods: Chemistry and Physiology of Flavors*; Schultz, H. W., Day, E. A., Libbey, L. M., Eds.; AVI: Westport, CT, 1967.

Hodge, J. E.; Mills, F. O.; Fisher, B. E. "Compounds of Browned Flavor Derived from Sugar-Amine Reactions". *Cereal Sci. Today* **1972**, *17*, 34–40.

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.

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.

Kuhn, R.; Weygand, F. "Die Amadori-Umlagerung". *Chem. Ber.* **1937**, *69*, 769–772.

Lea, C. H.; Hannan, R. S. "Reaction between Proteins and Reducing Sugars in the Dry State. I. Effect of Activity of Water, pH and of Temperature on the Primary Reaction between Casein and Glucose". *Nature (London)* **1950**, *165*, 438–439.

Lobry de Bruyn, C. A.; Alberda van Ekenstein, W. "Action des Alcalissur les Sucres. II. Transformation Reciproque desuns dans les Autres des Sucres Glucose, Fructose et Mannose". *Recl. Trav. Chim. Pays-Bas* **1895**, *14*, 203–216.

Maga, J. A. "Pyrazines in Foods: An Update". *CRC Crit. Rev. Food Sci. Nutr.* **1982**, *16*, 1–115.

March, J. *Advanced Organic Chemistry*; Wiley-Interscience; New York, 1985.

Maxon, R. N. "Purification of Silver Residues". In *Inorganic Synthesis*; Booth, H. S., Ed.; McGraw-Hill: New York, 1939.

McNair; H. M.; Bonelli, E. J. "Quantitative Analysis". In *Basic Gas Chromatography*; Varian Aerograph: Walnut Creek, CA, 1968.

Murray, K. E.; Shipton, J.; Whitfield, F. B. "2-Methoxypyrazines and the Flavor of Green Peas (*Pisum sativum*)". *Chem. Ind. (London)* **1970**, 897–898.

Newell, J. A.; Mason, M. E.; Matlock, R. S. "Precursors of Typical and Atypical Roasted Peanut Flavor". *J. Agric. Food Chem.* **1967**, *15*, 767–772.

Noller, C. R. *Chemistry of Organic Compounds*; W. B. Saunders: Philadephia, 1957.

Rizzi, G. P. "A Mechanistic Study of Alkylpyrazine Formation in Model Systems". *J. Agric. Food Chem.* **1972**, *20*, 1081–1085.

Rizzi, G. P., private communication, 1987.

Schonberg, A.; Moubasher, R. "The Strecker Degradation of α-Amino Acids". *Chem. Rev.* **1952**, *50*, 261–277.

Shibamoto, T.; Bernhard, R. A. "Effect of Time, Temperature, and Reactant Ratio on Pyrazine Formation in Model Systems". *J. Agric. Food Chem.* **1976**, *24*, 847–852.

Shibamoto, T.; Bernhard, R. A. "Investigation of Pyrazine Formation Pathways in Glucose-Ammonia Model Systems". *Agric. Biol. Chem. (Jpn.)* **1977a**, *41*, 143–153.

Shibamoto, T.; Bernhard, R. A. "Investigation of Pyrazine Formation Pathways in Sugar–Ammonia Model Systems". *J. Agric. Food Chem.* **1977b**, *25*, 609–614.

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.

Tambawala, H.; Weiss, A. H. "Homogeneously Catalyzed Formaldehyde Condensation to Carbohydrates. II. Instabilities and Cannizzaro Effects". *J. Catal.* **1972**, *26*, 388–400.

Umano, K.; Shibamoto, T. "Chemical Studies on Heated Starch/Glycine Model Systems". *Agric. Biol. Chem. (Jpn.)* **1984**, *48*, 1387–1393.

van Pragg, M.; Stein, H. S.; Tibbetts, M. S. "Steam Volatile Aroma Constituents of Roasted Cocoa Beans". *J. Agric. Food Chem.* **1968**, *16*, 1005–1008.

Velisek, J.; Davidek, J.; Cuhrouva, J.; Kubelka, V. "Volatile Heterocyclic Compounds in the Reaction of Glyoxal with Glycine". *J. Agric. Food Chem.* **1976**, *24*, 3–7.

Walradt, J. P.; Pittet, A. O.; Kinlin, T. E.; Muralidhara, R.; Sanderson, A. "Volatile Components of Roasted Peanuts". J. Agric. Food Chem. 1971, 19, 972–979.
Wang, P.; Odell; G. V. "Formation of Pyrazines from Thermal Treatment of Some Amino–Hydroxy Compounds". J. Agric. Food Chem. 1973, 21, 868–870.
Wilkins, W. F.; Lin, F. M. "Volatile Flavor Components of Deep Fat-Fried Soybeans". J. Agric. Food Chem. 1970, 18, 337–339.

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Isomerization and Losses of *trans*-β-Carotene in Sweet Potatoes as Affected by Processing Treatments

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Carotene content was altered during the processing of sweet potatoes. The change was dependent upon the treatment employed: blanching (4.0–11.9% increase), lye peeling and pureeing (10.4% increase), steam injection (8.0% loss), canning (19.7% loss), dehydration (20.5% loss), microwaving (22.7% loss), and baking (31.4% loss). Increases in carotene content were attributed to an enhanced extraction efficiency of heat-treated samples. Heat processing induced the formation of predominately the 13-*cis*-β-carotene isomer, and the quantity formed was related to the severity and length of the heat treatment.

Carotenoids represent the most widespread group of naturally occurring pigments in nature (Simpson and Chichester, 1981). The carotenoids in food are primarily of plant origin and β-carotene, with few exceptions, predominates (Panalaks and Murray, 1970). β-Carotene serves as an important nutritional component in foods, as a major precursor of vitamin A, and provides pleasant yellow-orange colors to foods (Francis, 1969; Simpson and Chichester, 1981; Klaui and Bauernfeind, 1981). Recent interest concerning dietary β-carotene has been prompted by studies implicating the ingestion of foods containing carotenes with decreased incidence of certain cancers (Peto et al., 1981; Menkes et al., 1986).

all-trans-β-Carotene exhibits the greatest vitamin A activity of the carotenoids. β-Carotene is subject to degradative changes during food processing and cooking (Gregory, 1985). In general, oxidation is a major cause of β-carotene destruction (Simpson, 1985) while thermal processing of foods may lead to β-carotene isomerization (Sweeney and Marsh, 1971).

In 1974, Lee and Ammerman noted that, in conventional processing of carotene-rich foods, much of the provitamin A activity of the carotenes can be lost due to conversion of *all-trans*-β-carotene to stereoisomers having lower provitamin A activity. Because of the lowered biological activity of the cis isomers, characterization of the isomeric forms in cooked and processed products would be desirable (National Research Council, 1980). During processing, several researchers have seen a reduction in the percentage of *all-trans*-β-carotene with the concomitant increase in the 13-cis and 9-cis isomers (Panalaks and Murray, 1970; Sweeney and Marsh, 1971; Lee and Ammerman, 1974; Ogunlesi and Lee, 1979; Chandler and Schwartz, 1987).

Sweet potatoes are an excellent model system for quantitatively observing changes in β-carotene during processing because of the high concentration of β-carotene in this tissue and low concentrations of other interfering carotenes. Some investigators have studied losses of β-carotene during the handling (curing and storage) and processing of sweet potatoes (Reddy and Sistrunk, 1980; Walter and Giesbrecht, 1982; Picha, 1985). However, isomerization reactions of β-carotene and losses during food processing and preparation have not been addressed in detail.

The objectives of this study are (1) to quantitatively follow the changes in total β-carotene concentration during various processes for sweet potatoes and (2) to quantitate the isomeric composition of β-carotene as a result of blanching, canning, dehydrating, and cooking.

MATERIALS AND METHODS

all-trans-β-Carotene (type 1) was purchased from Sigma Chemical Co. (St. Louis, MO), and 15-*cis*-β-carotene was donated by Hoffmann-LaRoche (Basel, Switzerland). Chemicals and solvents were reagent grade. Solvents used for high-performance liquid chromatography (HPLC) were filtered (0.45 μm) and degassed. Raw sweet potatoes *Ipomoea batatas* (Jewel variety, US #1, 300–350 g) were obtained during the 1985 growing season from the North Carolina Agricultural Research Experiment Station, harvested during October, cured (30° C, 85% relative humidity) for 7 days, and then stored (Walter and Schadel, 1982) for 9 months until being processed. The Jewel variety roots are yellow-orange in color.

Processing Conditions. Figure 1 represents a schematic of the sweet potato processing procedure.

Puree. Roots (25 kg) were lye peeled (6% NaOH, 5 min, 101 °C), washed, trimmed, and pureed in a Fitzmill comminutor (Fitzpatrick Co., Chicago, IL) fitted with a 0.06-in. mesh screen. Puree was heated to 81 °C (culinary steam injection) to gelatinize the starch and held for 30 min to allow hydrolysis of the starch by the endogenous amylase enzymes. The puree was then heated (100 °C, steam injection) to inactivate the native amylases (Hoover, 1967; Walter et al., 1976).

Canning. Puree (5 kg) was filled into 303 × 406 cans and processed (116 °C, 90 min) in a still retort.

Dehydration. Drum-dried product was prepared on a 12 × 19 in. double-drum dryer (160 °C, 25 rpm) with a contact time of approximately 1.8–2 s.

Strips. Hand-peeled roots (5 kg) were cut into strips of variable length (1.9 cm wide × 0.64 cm thick) with use

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