Effect of Time, Temperature, and Reactant Ratio on Pyrazine Formation in Model Systems

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The factors affecting the formation of pyrazine compounds from a D-glucose and ammonia model system were studied. Changing the reactant ratio, reaction temperature, and reaction time influences total yield, but not the distribution pattern of the products. Addition of hydroxide ion increases total yield of product. Numerous experiments gave knowledge of optimum yield and unique distribution of pyrazine compounds such as unsubstituted pyrazine, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine, and 2,3,5-trimethylpyrazine in the reaction mixtures.

Amine and carbonyl compounds are important precursors of nonenzymatic browning reactions. From such browning reactions, one can obtain not only brown pigments, but also roasted or smoky aromas. These odors are believed to arise mainly from pyrazine compounds (Parliment and Epstein, 1973; Pittet et al., 1972; Polak's Frutal Works, 1971; Seifert et al., 1972). Pyrazines have been found in a wide variety of food products, for example, roasted barley, cocoa, roasted peanuts, potato chips, green peas, coffee, roast beef drippings, etc. (Collins, 1971; Reymond et al., 1971; Walradt et al., 1971; Watanabe and Sato, 1971; Wilkens and Lin, 1970; Murray et al., 1970). More than 70 of these pyrazine compounds have been identified (Manley and Fagerson, 1970; Newell et al., 1967). Typical of these are the alkylpyrazines (Buttery et al., 1971; Flament et al., 1967; Goldman et al., 1967; Mussinan et al., 1973; Rizzi, 1967).

There has been much speculation on the way pyrazine compounds are formed in food. The two nitrogen atoms in a pyrazine ring can be incorporated into the compound in at least two possible ways, one from free ammonia and the other directly from an amino acid or amine nitrogen. Koehler et al. (1969) reported that the two systems, glucose–amino acid and glucose–ammonium chloride, both at elevated temperatures, produced different products. Ammonium chloride yielded predominantly pyrazine and only traces of alkylated pyrazines, while the amino acids gave alkylated pyrazines with very small amounts of pyrazine. They concluded that a condensation between the amino group of the amino acid and the carbonyl group of the sugar occurred as the first step, rather than condensation with ammonia resulting from decomposition of the amino acid. On the other hand, Newell et al. (1967) reported that they obtained essentially the same alkylpyrazines from their sugar–amino acid system, regardless of the nitrogen amino acid source employed. Also, van Praag et al. (1968) reacted neutral amino acids such as glycine, serine, leucine, isoleucine, valine, and alanine with D-fructose and observed the formation of a similar series of pyrazines much as Newell et al. (1967) reported. van Praag assumed that ammonia was an intermediate, and that the composition of the pyrazine mixture did not depend upon the amino acid involved. Koehler et al. (1969) also reported that the primary carbon source for pyrazine formation comes from sugar and the amino acid serves merely as a nitrogen source. Wang and Odell (1973), however, reported that some aminohydroxy compounds themselves formed pyrazines upon heating.

In order to investigate these seemingly contradictory findings concerning pyrazine formation, we decided to simplify the system and use glucose as the primary carbon source and ammonia itself, in the form of ammonium hydroxide, as the nitrogen source. Hough et al. (1952) reported the tentative identification of two pyrazine compounds in a D-glucose–ammonia system after reaction at 37 °C for 2 weeks. In describing some studies of the volatiles from cocoa beans, van Praag et al. (1968) reported preliminary data using a similar system. They found six alkylpyrazines were formed from glucose and ammonia, using a single set of reaction conditions. Our purpose was to amplify this work and determine the effects of time, temperature, and reactant ratio on pyrazine synthesis.

Koehler and Odell (1970) reported the effect of time, temperature, reactant ratio, and acid and base on the formation of methylpyrazine and a mixture of dimethylpyrazines from glucose–asparagine model systems. Pyrazine compound formation appears to depend on the above factors. Thus, a systematic investigation of reaction parameters for controlling the composition of pyrazine products and maximizing the yields in the sugar–amine model system could be a key element to understanding the mechanism of pyrazine formation and consequently the characteristics of smoky or roasted flavors of foods.

EXPERIMENTAL SECTION

D-Glucose and authentic pyrazine compounds were obtained commercially. The purity of the pyrazines was over 98% as determined by gas chromatographic analysis. The authors are grateful to Dr. Ron Buttery of the USDA, Berkeley, Calif., for samples of 2-ethyl-5-methyl-, 2-ethyl-6-methyl-, and 2-ethyl-3,5-dimethylpyrazine.

Reaction of D-Glucose and Ammonia. Varying amounts of ammonium hydroxide solutions and 18 g of D-glucose were mixed in 300-ml round-bottomed flasks for open system reactions and in 100-ml Kjeldahl flasks, which were used as heavy wall ampules, for closed system reactions. Closed systems were used to increase reaction temperature above 100 °C. The volume of each solution was adjusted to obtain 1 M sugar solutions by adding deionized water. Thus, this reaction media simply consisted of ammonia, glucose, and water.

For the open system, a Friedrich condenser was attached to the flask and the other end of the condenser kept open to the atmosphere. Reaction temperature was controlled by use of an oil bath, and the reactions were designed to approximate conditions of the normal cooking of food. For the closed system, the flask containing the reaction mixture was cooled in an ice bath for 30 min; nitrogen gas was bubbled through the solution for 5 min. The neck of the Kjeldahl flask was then flame sealed and the ampule placed in an oven.

Reaction conditions for each run are listed in Table I.

Method of Analysis. Each reaction mixture was extracted with four 50-ml portions of methylene chloride, the

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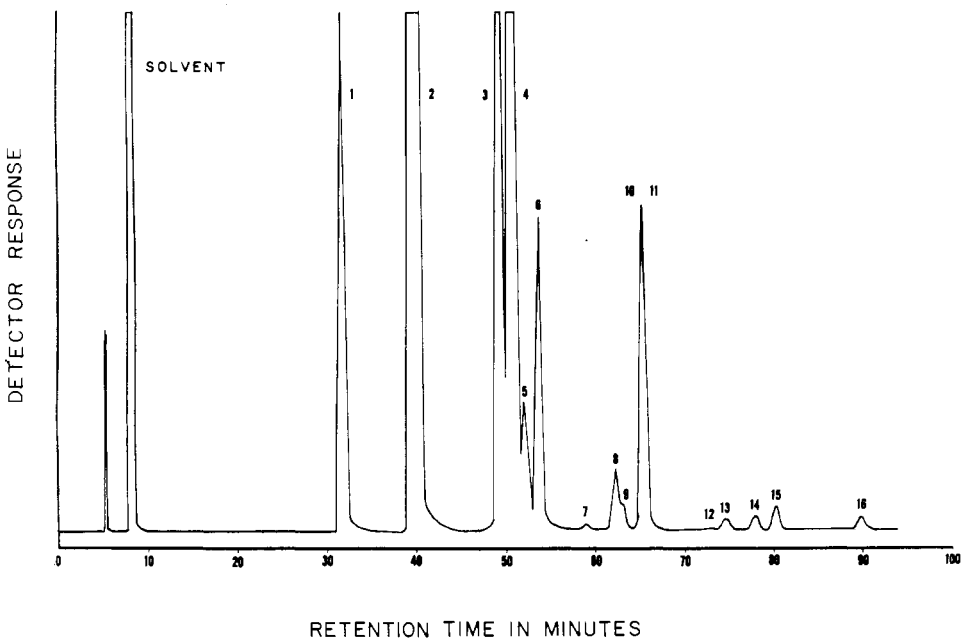

Figure 1. Gas chromatogram of pyrazines formed by reaction of 8 M ammonium hydroxide and 1 M D-glucose at 100 °C for 2 h. For chromatographic conditions see Experimental Section. For peak identification see footnote *a*, Table II.

extract dried over anhydrous sodium sulfate for 12 h, and the solvent removed using a rotary flash evaporator. A brown oily residue remained. This was weighed and analyzed by gas chromatography to determine the qualitative and quantitative distributions of the various alkylpyrazines.

Quantitative tests of the extraction procedure, using authentic pyrazine and 2-methylpyrazine, showed a better than 98% recovery for both compounds.

Quantitative analysis of the alkylpyrazines was accomplished using a Varian Aerograph Series 1200 gas chromatograph fitted with a flame ionization detector and a 6 m × 2.5 mm i.d. stainless steel column packed with 5% Carbowax 20M (+0.25% Igepal CO-880) on acid-washed Chromosorb W 80/100 mesh which was programmed from 70 to 150 °C at 0.5 °C/min (gas flow 30 ml/min of N_2) (see Figure 1). An Infotronics Model CRS-204 automatic digital integrator was used to determine the peak areas. The measurement of the FID relative response factors and quantitative analyses of pyrazines were obtained using the methods described by Ettre and Zlatkis (1967).

A Hewlett-Packard Model 5700 A gas chromatograph equipped with a flame ionization detector and a 110 m × 0.25 mm i.d. glass capillary column coated with Carbowax 20M was programmed from 70 to 170 °C at 1 °C/min (gas flow 0.6 ml/min of N_2; carrier gas velocity, 15 cm/s) for the qualitative analysis of the volatiles.

A Finnigan 3200 combination mass spectrometer–gas chromatograph (Finnigan 9500) equipped with a Finnigan MS data system 6000 was used for mass spectral identification of the gas chromatographic components with the following conditions: beam current, 167 mA; electron multiplier, −2.45 keV; electron energy, 71.6 eV; and ion energy, 3.7 eV. The chromatographic column used in the above instrument was a 100 m × 0.25 mm i.d. glass capillary column coated with Carbowax 20M which was programmed from 70 to 170 °C at 1 °C/min (gas flow, 0.6 ml/min of He; carrier gas velocity, 15 cm/s).

The major gas chromatographic peaks were alkylpyrazines (unsubstituted, 2-methyl-, 2,5-dimethyl-, 2,6-dimethyl-, 2-ethyl, 2,3-dimethyl-, and 2,3,5-trimethylpyrazine). The combined area of the minor peaks of the seven pyrazines (2-ethyl-5-methyl-, 2-ethyl-6-methyl-, 2-ethyl-3-methyl-, 2-vinyl-, 2-ethyl-3,5-dimethyl-, 2-ethyl-3,6-dimethyl-, and ethylvinylpyrazine) comprised less than 0.1% of the total peak area. Thus, these were ignored for routine quantitative analysis.

RESULTS AND DISCUSSION

(1) **Total Yield of Pyrazines.** (a) Effects of reactant ratio and basicity (Table II, experiments 1–20) show that with a reaction time of 2 h at 100 °C the yield of total pyrazines increased as the concentration of ammonia increased. Maximum yield was obtained when the concentration of ammonia reached 8 M, an eightfold excess relative to sugar. Further increases in ammonia concentration appeared to have little effect on the yield of pyrazines.

The solubilities of ammonia in 100 ml of water at 25 and 100 °C are 89.0 and 7.4 g, respectively (Weast, 1972). At room temperature, 25 °C (the reaction mixtures were prepared at this temperature), 100 ml of reaction solution contains 13.6 g (8 M) of ammonia, but on heating to 100 °C, the solution contains only 7.4 g (4.3 M) of ammonia. Therefore, 6.2 g of ammonia must have escaped from the reaction mixture under conditions of reflux when the concentration of ammonia was 8 M. Experiment 17 (13.6 g (8 M) of ammonia used) gave a larger yield than experiments 13 and 14 (6.8 g (4.0 M) and 8.5 g (6.0 M) of ammonia used, respectively).

In view of the above data, it is obvious that 100 ml of H_2O cannot contain the 8 M of ammonia, but also it has been observed that 8 M ammonia solution gave a higher yield of pyrazines. Thus, some ammonia must be used for reaction before the solution temperature reaches its maximum value of 100 °C.

The ammonia in this system should behave in two ways: as a reactant and as a basic catalyst. Increasing the amount of ammonia gave the same effect as the addition of sodium hydroxide (experiments 42 and 33).

Koehler and Odell (1970) reported that the addition of an equal number of equivalents of acid in their sugar–amino acid model systems lowered pyrazine formation to practically zero, while addition of an equal amount of base increased the yield of 2-methylpyrazine tenfold and the yield of dimethylpyrazine fivefold. They concluded that

Table I. Reaction Conditions of Experiments

Expt no.	Concn of ammonia (M)	Reaction temp[a]	Reaction time, h	Replicates	Color of final reaction mixture
1	0.1	100	2	2	Light yellow
2	0.2	100	2	2	Yellow
3	0.3	100	2	1	Dark brown
4	0.4	100	2	1	Dark brown
5	0.5	100	2	1	Dark brown
6	0.6	100	2	2	Dark brown
7	0.7	100	2	2	Dark brown
8	0.8	100	2	2	Dark brown
9	0.9	100	2	1	Dark brown
10	1.0	100	2	3	Dark brown
11	2.0	100	2	2	Dark brown
12	3.0	100	2	2	Dark brown
13	4.0	100	2	1	Dark brown
14	5.0	100	2	3	Dark brown
15	6.0	100	2	1	Dark brown
16	7.0	100	2	1	Dark brown
17	8.0	100	2	8	Dark brown
18	9.0	100	2	1	Dark brown
19	10.0	100	2	1	Dark brown
20	15.0	100	2	2	Dark brown
21	0.8	50	2	1	Light yellow
22	0.8	60	2	1	Light yellow
23	0.8	70	2	1	Light brown
24	0.8	80	2	1	Brown
25	0.8	90	2	1	Dark brown
26	0.8	100	2	3	Dark brown
27[b]	0.8	120	2	3	Dark brown
28	0.8	140	2	1	Dark brown
29	0.8	160	2	1	Dark brown
30	0.8	100	15 min	2	Light yellow
31	0.8	100	30 min	2	Light brown
32	0.8	100	1	1	Dark brown
33	0.8	100	2	8	Dark brown
34	0.8	100	3	2	Dark brown
35	0.8	100	4	2	Dark brown
36	0.8	100	6	1	Dark brown
37	0.8	100	18	3	Dark brown
38	0.8	100	30	1	Dark brown
39	0.8	100	48	1	Dark brown
40	0.8	25	30 days	1	Light yellow
41	0.8	−5	30 days	1	Light yellow
42[c]	0.1	100	2	2	Dark brown
43[c]	8.0	100	2	2	Dark brown
44[c]	8.0	100	18	2	Dark brown

[a] Solution temperature (°C). [b] Experiments 27–29, 44 were run in closed systems. [c] Sodium hydroxide (0.4 g) was added to investigate the influence of base.

this base catalysis is probably due both to increased reactivity of the amino group of the amino acid toward the carbonyl of glucose and to the increased rearrangement and fragmentation of sugars.

On the other hand, the first step of the pyrazine formation from sugar and amine must be condensation of the carbonyl and amino group, as follows (Hodge, 1967; Koehler and Odell, 1970; Wang et al., 1969):

$$>C{=}O + H_2N{-}R \rightarrow >C\begin{matrix}\diagup OH \\ \diagdown NHR\end{matrix} \rightarrow >C{=}NR + H_2O$$

Many carbonyl reactions of this type are known to have their maximum rate of reaction at pH 4–5 (Barrell and Lapworth, 1908; Conant and Bartlett, 1932; Oelander, 1927; Westheimer, 1934). Also Weygand and Bergmann (1947) reported that the Amadori rearrangement was acid catalyzed.

After sugar–amine condensation, fragmentation of sugars occurs through a reverse aldol reaction, enolization and dehydration, all of which are base catalyzed. It is difficult to measure the optimum pH of these reactions since the basicity of the reaction solution decreases as the reaction progresses (Katchalsky and Sharon, 1953):

$$NH_4^+ + OH^- \rightarrow \underset{\downarrow}{NH_3} + H_2O$$
$$\text{to the products}$$

(b) *Effects of Temperature (Table II, Experiments 21–29).* The results from experiments 21, 22, 23, 40, and 41 indicate that the model system produced a very small amount of pyrazines (less than 0.05% relative to sugar used) with a reaction time of 2 h at temperatures below 70 °C. Above 70 °C, but with the same reaction time, the rate of pyrazine formation increased rapidly as the temperature increased. The yield of pyrazines continued to increase as the temperature increased above 70 °C and reached a maximum at 120 °C. Above 120 °C the yield decreased sharply.

From these data it is clear that a temperature of 120 °C gives the optimum yield for pyrazine formation. These data also agree with the fact that most of the alkylpyrazines have been found in foods which were subjected to heat (Buttery et al., 1971; Manley and Fagerson, 1970; Mussinan et al., 1973; Newell et al., 1967; Rizzi, 1967; Walradt et al., 1971). In contrast to these findings, Koehler and Odell (1970) reported that at temperatures below 100 °C essentially no pyrazine compounds (less than 0.1 μmol) were produced. At 100 °C pyrazine formation began, and the yield increased. At temperatures above 150 °C, they reported highly variable yields.

(c) *Effects of Reaction Time (Table II, Experiments 30–41).* The results from experiments 30–39 show that with a solution temperature of 100 °C the production of pyrazines increased with the length of the heating period as expected. Four hours heating gave maximum yield. After 4 h, the yield decreased rapidly as the length of the heating period was increased up to 6 h when the yield began to level off with only minor decreases in yield for periods of up to 48 h.

The reaction mixtures which were heated more than 4 h created brown emulsions in the methylene chloride layer during extraction. Our experiments indicate that the efficiency of recovery of pyrazines from systems in which emulsions occur is considerably less than the 98% figure reported above (see Experimental Section). Therefore, the yields from these mixtures could not be directly compared with yields from mixtures which had been heated less than 4 h.

The reaction mixtures which were treated more than 4 h in closed systems did not create emulsions in the methylene chloride layer during extraction.

The yield of pyrazines from a reaction conducted at 100 °C and 18 h (experiment 44) in a closed system showed a large increase, 5.6 times, over the yield from a similar reaction conducted at 100 °C for 2 h. This increase in yield was possibly due to the efficiency of recovery of the pyrazines, since no emulsions were formed during extraction. It may also be due to the retention of products and ammonia during reactions in the closed system.

Koehler and Odell (1970) reported that the production of pyrazine compounds at 120 °C (solution temperature) increased rapidly as the length of the heating period was increased for periods up to 24 h. Then pyrazine formation began to level off with only minor increases in yield for periods of up to 72 h.

The knowledge gained from the effects of reactant ratio, temperature, and time on the reaction yield will be used for further investigations. The reaction at 100 °C (solution temperature) for 18 h with 0.01 mol of sodium hydroxide

Table II. Quantitative Analyses of Pyrazine Formation Reactions Showing Yields of Individual Compounds and Distribution Pattern

Expt no.	1[a]		2		3		4		5		6		8		9		10		Yield, %[c] rel to sugar used
	%[b]	mg	%	mg	%	mg	%	mg	%	mg	%	mg	%	mg	%	mg	%	mg	
1	6.28	0.3	84.60	3.0	1.69	0.4	6.97	0.4	0.24	0.0	0.45	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.002
2	4.45	0.7	86.18	14.1	1.36	0.2	6.62	1.0	0.33	0.0	1.01	0.1	0.00	0.0	0.00	0.0	0.02	0.0	0.091
3	4.66	1.7	84.16	32.1	1.78	0.6	6.86	2.6	0.36	0.1	1.31	0.5	0.13	0.0	0.00	0.0	0.71	0.2	0.212
4	4.42	2.3	82.27	46.6	2.11	1.1	7.85	4.4	0.42	0.2	1.80	1.0	0.28	0.1	0.00	0.0	0.79	0.4	0.315
5	4.40	3.0	81.74	57.2	2.17	1.5	8.26	5.7	0.40	0.2	1.76	1.2	0.27	0.1	0.01	0.0	0.90	0.6	0.389
6	4.20	3.1	82.31	61.8	2.26	1.9	8.38	6.2	0.60	0.4	1.76	1.2	0.04	0.0	0.02	0.0	0.90	0.6	0.417
7	3.55	2.9	81.99	68.8	2.99	2.5	8.26	6.9	0.34	0.2	1.80	1.5	0.17	0.1	0.12	0.1	0.74	0.6	0.467
8	3.02	2.6	80.93	70.4	3.34	2.9	9.02	7.8	0.31	0.2	2.13	1.8	0.19	0.1	0.01	0.0	1.05	0.9	0.484
9	2.10	1.8	76.73	66.7	5.02	4.3	11.33	9.8	0.40	0.3	2.81	2.4	0.20	0.1	0.01	0.0	1.41	1.2	0.483
10	1.69	1.3	76.32	68.7	5.46	4.9	11.59	10.9	0.40	0.3	2.94	2.6	0.21	0.1	0.01	0.0	1.53	1.3	0.501
11	1.50	1.4	74.32	70.5	5.47	5.1	12.84	12.1	0.50	0.4	3.07	2.9	0.19	0.1	0.09	0.0	1.99	1.8	0.527
12	1.80	2.2	74.09	91.2	5.41	6.6	12.58	15.4	0.45	0.5	2.91	3.5	0.20	0.2	0.00	0.0	1.66	2.0	0.684
13	2.15	2.9	76.51	105.0	5.20	7.1	11.21	15.3	0.40	0.5	2.95	4.0	0.18	0.2	0.01	0.0	1.31	1.7	0.763
14	3.61	5.4	76.95	115.4	4.98	7.4	10.87	16.3	0.35	0.5	2.05	3.0	0.13	0.1	0.03	0.0	1.03	1.5	0.833
15	3.65	5.8	78.08	124.6	4.81	7.6	9.97	15.9	0.36	0.5	1.95	3.1	0.13	0.2	0.03	0.0	1.02	1.6	0.887
16	3.71	6.1	79.33	131.1	4.27	7.0	9.18	15.1	0.34	0.5	1.81	2.9	0.18	0.2	0.15	0.2	1.03	1.7	0.918
17	3.81	6.4	81.65	138.9	4.79	8.1	8.99	15.2	0.36	0.6	2.07	3.5	0.18	0.3	0.17	0.2	0.97	1.6	0.945
18	3.86	6.5	80.43	136.0	3.75	6.3	8.14	13.7	0.32	0.5	1.89	3.1	0.34	0.5	0.19	0.3	1.04	1.7	0.940
19	4.00	6.8	80.22	135.3	4.25	7.1	7.91	13.3	0.35	0.5	1.81	3.0	0.31	0.5	0.17	0.2	0.92	1.5	0.934
20	4.10	6.9	79.49	134.4	4.82	8.1	7.89	13.3	0.35	0.5	1.83	3.0	0.32	0.5	0.18	0.3	1.02	1.7	0.939
21	2.39	0.0	62.28	0.0	21.86	0.0	1.35	0.0	0.00	0.0	0.10	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.001
22	5.10	0.2	87.16	4.4	2.90	0.0	3.60	0.1	0.00	0.0	0.78	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.027
23	0.64	0.0	92.34	7.3	4.06	0.3	2.38	0.1	0.00	0.0	0.14	0.0	0.00	0.0	0.00	0.0	0.37	0.0	0.044
24	1.96	0.5	90.91	25.3	2.60	0.7	3.48	0.9	0.08	0.0	0.88	0.2	0.00	0.0	0.00	0.0	0.05	0.0	0.155
25	3.44	2.3	82.31	56.8	4.45	3.0	8.17	5.6	0.30	0.2	0.94	0.6	0.80	0.0	0.04	0.0	0.30	0.2	0.384
26	3.44	4.4	82.31	106.9	4.45	5.7	8.17	10.6	0.30	0.3	0.94	1.2	0.05	0.0	0.04	0.0	0.30	0.3	0.722
27	3.81	6.4	81.65	138.9	4.79	8.1	8.99	15.2	0.36	0.6	2.07	3.5	0.18	0.3	0.17	0.2	0.97	1.6	0.945
28	4.44	2.8	82.60	53.8	2.88	1.8	7.59	4.9	0.28	0.1	1.34	0.8	0.12	0.0	0.11	0.0	0.60	0.3	0.362
29	3.72	1.0	80.78	22.4	2.81	0.7	7.67	2.1	0.31	0.0	1.43	0.3	0.93	0.2	0.46	0.1	1.84	0.5	0.154
30	1.78	0.0	91.05	2.1	3.30	0.0	2.34	0.0	0.20	0.0	0.77	0.0	0.04	0.0	0.12	0.0	0.35	0.0	0.013
31	3.44	1.0	82.56	25.3	5.42	1.6	4.51	1.3	0.21	0.0	0.59	0.1	0.04	0.0	0.13	0.0	0.05	0.0	0.164
32	3.52	2.7	82.23	64.2	5.25	4.1	6.62	5.1	0.24	0.1	1.36	1.0	0.17	0.1	0.40	0.3	0.17	0.1	0.434
33	3.81	6.4	81.65	138.9	4.79	8.1	8.99	15.2	0.36	0.6	2.07	3.5	0.18	0.3	0.17	0.2	0.97	1.6	0.945
34	2.81	5.8	77.89	162.9	4.36	9.1	10.70	22.3	0.36	0.7	2.37	4.9	0.15	0.3	0.05	0.1	1.27	2.6	1.162
35	3.18	7.1	77.62	175.1	3.82	8.6	10.01	22.5	0.36	0.8	2.08	4.6	1.02	2.3	0.21	0.4	1.66	3.7	1.253
36	4.00	5.3	78.63	105.6	3.36	4.5	9.31	12.5	0.49	0.6	1.59	2.1	0.96	1.2	0.14	0.1	1.46	1.9	0.746
37	3.79	4.6	78.20	95.8	3.27	4.0	9.60	11.7	0.58	0.7	1.75	2.1	0.98	1.2	0.16	0.1	1.62	1.9	0.681
38	3.03	3.9	74.64	97.3	4.27	5.5	12.38	16.1	0.76	0.9	2.15	2.8	0.73	0.9	0.09	0.1	1.92	2.5	0.724
39	2.66	2.7	73.74	75.2	4.46	4.5	12.36	12.6	0.67	0.6	2.26	2.3	1.40	1.4	0.34	0.3	2.07	2.1	0.567
40	63.94	1.2	23.02	0.4	11.41	0.2	1.63	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.007
41	71.89	0.6	15.34	0.1	10.46	0.1	1.76	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.005
42	3.11	0.0	79.16	8.0	4.10	0.4	9.00	0.9	0.36	0.0	1.31	0.0	1.31	0.0	0.00	0.0	0.09	0.0	0.056
43	3.75	7.0	80.91	151.7	3.95	7.4	10.10	18.9	0.36	0.7	2.05	3.8	0.15	0.3	0.10	0.2	0.98	1.8	1.048
44	2.73	26.8	76.93	757 4	3.97	39.1	12.16	119.7	0.57	5.6	1.72	16.9	0.19	1.9	0.08	0.8	1.61	15.9	5.469

[a] Peak number in Figure 1: (1) unsubstituted; (2) 2-methyl-; (3) 2,5-dimethyl-; (4) 2,6-dimethyl-; (5) 2-ethyl-; (6) 2,3-dimethyl-; (7) unknown (*m/e* 96); (8) 2-ethyl-5-methyl-; (9) 2-ethyl-6-methyl-; (10) 1,3,5-trimethylpyrazine; (11) 2-ethyl-3-methyl-; (12) 2-vinyl-; (13) 2-ethyl-3,5-dimethyl-; (14) 2-ethyl-3,6-dimethyl-; (15) unknown (*m/e* 110); (16) ethylvinyl-. Peak 11 comprised less than 0.1% of composite peak 10–11. Peaks 12 through 16 comprised less than 1% by weight of total pyrazines formed; thus, these were not included in the quantitative data. [b] Percent yield of individual pyrazines relative to total pyrazines. [c] Percent yield = (wt of pyrazines obtained)/(wt of glucose used (18 g)) × 100.

in a closed system gave the best yield (5.47% relative to sugar used). For an open system, the reaction at 100 °C for 4 h gave the best yield (1.25% relative to sugar used).

(2) **Distribution of Pyrazines Produced by Various Conditions.** (a) *Reactant Ratio.* The results from experiments 1–20 indicate that the distribution of pyrazines did not change greatly as the reactant ratio changed (Table II, experiments 1–20). 2-Methylpyrazine was always the largest constituent of the total pyrazines (74.06% from experiment 12, 86.19% from 2). The percentage of unsubstituted pyrazine decreased as the concentration of ammonia was increased from 0.1 to 1 M and increased further as the concentration of ammonia was raised from 1 to 10 M. 2,6-Dimethylpyrazine increased when unsubstituted pyrazine decreased and vice versa. The 2,5- and 2,3-dimethylpyrazines increased as the concentration of ammonia was increased from 0.1 to 1 M, but then leveled off. The percentage of 2-ethylpyrazine was always approximately 0.3–0.4% relative to the total pyrazines. It should be pointed out, however, that the greatly differing pyrazine distributions observed with ammonia concentrations lower than 1 M as compared to those observed at higher concentration may be due simply to analytical difficulties inherent with working with very small amounts of material. At 0.1 and 0.5 M ammonia, only a 4 and 16 mg, respectively, total yield of pyrazines was obtained vs. the 100 to 170 mg yields at higher concentrations.

The distribution pattern obtained from the reaction mixture composed of 1 M glucose and 1 M ammonia solution with 0.01 mol of sodium hydroxide was almost the same as the distribution pattern obtained from the reaction mixture composed of 1 M glucose and 1 M ammonia solution without sodium hydroxide. This would appear to indicate that the slight variation of pyrazine distribution caused by different concentrations of ammonia is due to the basicity of the solutions rather than the concentration of ammonia.

The absolute amount of each pyrazine increased as the concentration of ammonia was increased up to 8 M, but then leveled off (Table II, experiments 1–20).

(b) *Temperature.* The results from experiments 21–29, 40, and 41 indicate that significant differences in distribution patterns were observed when the reactions were run at −5 and 25 °C as compared to those at higher temperatures. In reactions at −5 °C, the percentages of unsubstituted pyrazine and 2-methylpyrazine relative to total pyrazines were 71.87 and 15.34%, respectively, vs. 3–6 and 70–90% at reflux temperature. The reaction at 25 °C gave a distribution pattern similar to that of the reaction at −5 °C. These results may be due to a lower activation energy for one or more steps of unsubstituted pyrazine formation as compared to those for formation of the other pyrazines, and from these data, one might conclude that the distribution of pyrazines is dependent upon the reaction temperature if the reaction is run below 50 °C. However, the total yield of pyrazines is very low when a reaction is run below 50 °C (0.0001% relative to sugar used from the 2 h run). Therefore, the data from the reactions run below 50 °C should be considered with caution.

The percentage of 2-methylpyrazine relative to total pyrazines decreased suddenly when the temperature was raised above 80 °C, but leveled off at 100 °C. Then the distribution of pyrazines remained generally constant as the temperature was increased to 160 °C. Small, noticeable changes were also observed in the percentage yield of 2,3-dimethylpyrazine. The percentage of this compound increased rapidly when the temperature was raised above 70 °C, but then leveled off at 100 °C (Table II, experiments 21–29).

(c) *Time (Table II, Experiments 30–41).* The results from experiments 30–39 indicate that the percentage of 2-methylpyrazine decreased rapidly as the reaction time was increased from 15 to 30 min, but then leveled off. This observation may again be due to experimental error owing to the very small total yields of pyrazine obtained for the 15-min run. Also, 2,6-dimethylpyrazine increased slightly as the time increased, and leveled off after 3 h. However, the overall distribution pattern did not change significantly with a change of reaction time. One obvious change was that the yield of larger alkylpyrazines increased slightly as reaction time increased. This is also related to the dehydration reaction of the sugar moiety. We conclude that lengthening the reaction time does not change the distribution pattern significantly except that the yield of larger alkylpyrazines is slightly increased.

Thus, in the model systems examined in this study, the following conclusions can be drawn. (1) Reactant ratio changes total yield but does not change distribution pattern. (2) Ammonia has two functions for pyrazine formation: one is as a reactant and the other is as a basic catalyst. (3) Reaction time and temperature (over 60 °C) influence total yield of pyrazines but not distribution pattern. (4) Temperatures below 50 °C may influence pyrazine distribution patterns. (5) Addition of sodium hydroxide increases total yield up to a certain point.

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Synthesis and Flavor Evaluation of Some Alkylthiophenes. Volatile Components of Onion

William G. Galetto* and Patrick G. Hoffman

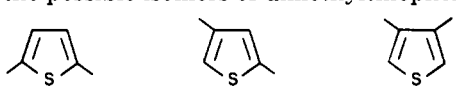
Pure samples of 2,4-, 2,5-, and 3,4-dimethylthiophene have been obtained. These compounds, upon organoleptic evaluation, have been determined not to contribute significantly to the flavor of fried onion when examined alone, in combination, or when added to artificial or natural onion oils. These findings are contrary to those previously reported in the literature. Pure samples of 2-methyl- and 3-methylthiophene, as well as 2,3-dimethylthiophene, have also been purified and evaluated organoleptically.

The complex and characteristic flavor of the onion is readily recognized and enjoyed by people in much of the world. Depending on its state and method of preparation the onion's flavor can be described as either fresh-green, cooked-boiled, or fried and at the same time distinctly that of onion.

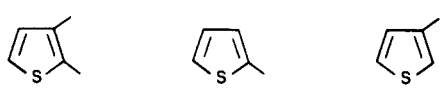
The fresh, raw onion flavor notes are due primarily to the presence of alkyl thiosulfinates (Brodnitz and Pascale, 1973) and alkyl thiosulfonates (Brodnitz et al., 1973; Naarden International, 1974; Boelens et al., 1971). The alkyl di- and trisulfides are primarily responsible for the cooked onion flavor (Boelens et al., 1971) which is characteristic of steam-distilled onion oil. Several recent patents describe the role of these polysulfides in artificial onion oils (Brodnitz and Pascale, 1973; Galetto and Pace, 1973).

Recently, Boelens et al. (1971) in their extensive and detailed analyses of onion oil identified three thiophenes: the 2,5-dimethyl (I), the 2,4-dimethyl (II), and the 3,4-dimethyl (III) isomers. Furthermore, they described the odor of two of these compounds (II and III) as being "distinctly that of fried onion". The same three thiophenes have more recently been found in shallots by Dembele and Dubois (1973). Brodnitz et al. (1969) had previously identified 3,4-dimethylthiophene in onion oil but did not discuss its flavor characteristics.

Our interest in obtaining a better understanding of the components contributing to fried onion flavor has prompted us to purify and organoleptically evaluate all four of the possible isomers of dimethylthiophene (I–IV)



I II III



IV V VI

as well as the two possible methylthiophenes (V and VI).

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MATERIALS AND METHODS

Chemicals and Analytical Equipment. 2-Methylthiophene and 2,5-dimethylthiophene (Aldrich Chemical Co.) and 3-methylthiophene and 3,4-dimethylthiophene (Marstan Chemical Co.) were obtained commercially. All compounds were purified by fractional distillation at atmospheric pressure and were chromatographically and analytically (NMR, MS) pure.

Gas–liquid chromatography (GLC) analyses were performed on a Hewlett-Packard 5702A instrument equipped with a thermal conductivity detector. The column was made of glass (8 ft × 0.25 in. o.d., 0.16 in. i.d.) and packed with 5% Carbowax 20M coated on Chromosorb WAW, 80–100 mesh.

NMR data were obtained on a Varian EM-360 instrument, ir data with a Perkin-Elmer 257 grating spectrophotometer, and MS data on a Du Pont 21-490 mass spectrometer.

2,3-Dimethylthiophene (IV). Using standard procedures, 50 g of 3-methylthiophene-2-carboxaldehyde containing 20% of the 2,4-isomer via GLC was converted to 70 g of the corresponding semicarbazone. Four recrystallizations from methanol yielded white needles free of the 2,4-isomer by NMR analysis, 36 g, mp 219–221 °C.

3-Methylthiophene-2-carboxaldehyde semicarbazone (36 g) was reduced via a modified Wolff–Kishner reaction (Shepard, 1932). It was first mixed with 47.5 g of powdered KOH and then heated over an open flame. The distillate, 20 g (95%), was carefully redistilled to yield a center cut of 5.7 g of chromatographically pure 2,3-dimethylthiophene [bp 140–141 °C; n^{20}D 1.5187; lit. bp 139.5–140.5 °C, n^{20}D 1.5188 (King and Nord, 1949)]. The MS, NMR, and ir spectra were in complete accord with the proposed structure.

2,4-Dimethylthiophene (II). Following the general procedure of Sice (1954a) and Ramanathan and Levine (1962) with the substitution of phenyllithium for butyllithium, 49 g of 3-methylthiophene was first treated with an equivalent amount of phenyllithium in 30% ether in benzene at room temperature and then the mixture was added to a cold solution of dimethylformamide in ether. Distillation of the crude product gave 50.5 g (80%) of a product, bp 67–69 °C (2 mm) which was shown to be a 4:1 mixture of the 2,4- and 2,3-isomeric aldehydes by GLC analysis.

The methylthiophenecarboxaldehyde mixture (50 g) was converted to 66.5 g of its semicarbazone. This derivative