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A Study of the Volatiles Isolated from a D-Glucose–Hydrogen Sulfide–Ammonia Model System

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The compounds produced by heating a D-glucose–hydrogen sulfide–ammonia–water model system were extracted with methylene chloride using a liquid–liquid continuous extractor. Fifty-one compounds were identified by a combination of gas chromatographic and mass spectrometric techniques. The main constituents of the reaction mixtures were thiophenes, furans, pyrazines, and thiazoles. With small amounts of ammonia in the starting mixture, the reaction products consisted mainly of thiophenes and furans and possessed a somewhat sulfury aroma which was also akin to that of rare cooked meat. When the amount of ammonia was increased, however, the reaction mixture contained a large proportion of pyrazines, a smaller proportion of thiazoles, thiophenes, and furans and the mixture possessed a nutty or burnt aroma.

The authors have recently reported on the volatile chemicals generated in a heated D-glucose–hydrogen sulfide–ammonia model system (Shibamoto and Russell, 1976) and identified a number of compounds which have been associated with cooked meat aromas (Persson and von Sydow, 1973; Nonaka et al., 1967). Among the chemicals identified were thiols, sulfides, thiophenes, thiazoles, and furans. Most of the compounds identified in this system have also been found in various food products (Maga and Sizer, 1973; Maga, 1975; Shankaranarayana et al., 1974).

Boelens et al. (1974) studied the reactions between fatty aldehydes, hydrogen sulfide, thiols, and ammonia and subsequently identified sulfides, thiols, and nitrogen- and sulfur-containing heterocyclic compounds in their reaction products. They showed that the reaction of ethanal, hydrogen sulfide, and ammonia produced 2,4,6-trimethyldihydro-1,3,5-dithazine, which has been found in beef broth (Brinkman et al., 1972).

Heterocyclic compounds (thiophenes, furans, thiazoles, and pyrazines) are among the major volatiles which have been identified in cooked meat (Mussinan and Walradt, 1974). For example, Maga (1975) reported the relative percentages of pyrazines and furans found in cooked pork liver volatiles to be 40.96 and 20.90, respectively. Pyrazines are well-known compounds which occur in cooked foods, including meat (Maga and Sizer, 1973). Persson and von Sydow (1973) identified 26 thiophenes and 11 thiazoles in cooked meat.

The precursors and formation pathways of these heterocyclic compounds are not well understood. One reason for this is that the amounts of these compounds formed in foods are very low—usually lower than micrograms per kilogram levels in food (Schutte, 1974); therefore, detection of these compounds has been difficult. This has been especially true when stainless steel columns have been used for gas–liquid chromatographic (GLC) analysis because

sulfur compounds at low levels do not pass through a stainless steel column in sufficient quantities to give a detector response (Withycombe and Walradt, 1975). Shibamoto and Russell (1976) suggested that carbonyls, hydrogen sulfide, and ammonia could be precursors of compounds which are associated with cooked meat aromas.

It is well known to sensory analysts that the relative proportions of volatiles in an aroma complex have a very strong influence on its sensory properties. This appears to be especially true with cooked meat aroma in general and the previously reported model system in particular. It was the purpose of the present study to vary reaction conditions and monitor the products to gain further insight into the role of the reactants in the D-glucose–H₂S–NH₃ model system.

EXPERIMENTAL SECTION

Materials. D-Glucose, ammonium hydroxide, and hydrogen sulfide were obtained commercially. Authentic reference compounds were obtained from reliable commercial sources or were donated by Ogawa & Co., Ltd., Tokyo, Japan.

Reaction of D-Glucose, Hydrogen Sulfide, and Ammonia. Hydrogen sulfide gas was bubbled through aqueous solutions (100 ml) each containing 0.1 mol of D-glucose for 10 min at 0 °C in a Kjeldahl flask. Ammonium hydroxide solutions of 0.10, 0.15, 0.20, and 0.50 mol of NH₃ for experiments 1–4, respectively, were then added to each of the above solutions. The necks of the flasks were flame-sealed and the flasks placed in an oven at 100 °C for 2 h. Volatiles were isolated from each reaction mixture with 200 ml of methylene chloride using a liquid–liquid continuous extractor for 6 h. The qualitative and quantitative analyses of volatiles were conducted following the gas chromatographic–mass spectrometric (GC–MS) methods described previously (Shibamoto and Russell, 1976).

RESULTS AND DISCUSSION

Boelens et al. (1974) reported nine saturated heterocyclic compounds formed from the reaction of fatty aldehydes,

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Table I. Compounds Identified from Heated D-Glucose–Hydrogen Sulfide–Ammonia Model System

Peak no.	Compound	$I,^g$ unknown	$I,^h$ known	Area % of volatiles from each expt[i]				Occurrence in meat	MS ref
				Expt 1	Expt 2	Expt 3	Expt 4		
1	Methanethiol	761	760	0.62	0.41	0.17	0.01	Beef[a]	Stoll et al. (1967)
2	Dimethyl sulfide	818	815	7.45	2.70	2.78	0.37	Beef[a]	Stoll et al. (1967)
3	Diethyl sulfide	899	895	10.44	3.75	2.33	0.15	Chicken[b]	Cornu and Massot (1966)
4	Thiophene	1034	1032	10.19	4.44	1.69	0.09	Beef[c]	API (1948)
5	Methyl isothiocyanate (tentative)	1042		3.56	0.97	0.07	j		IMSD (1969)
6	Unknown	1049		0.40	0.16	0.07	j		
7	2,3-Pentanedione	1051	1046	0.45	0.09	j	j	Beef[c]	van de Ouweland and Peer (1975)
8	2-Methylthiophene	1098	1095	24.92	5.51	0.68	0.06	Beef[c]	Budzikiewicz et al. (1964)
9	3-Methylthiophene	1127	1123	4.44	1.71	0.04	0.06	Beef[c]	Cornu and Massot (1966)
10	Unknown	1163					0.01		
11	2,5-Dimethylthiophene	1167	1165	6.35	2.53	0.23	0.05	Beef[c]	Boelens et al (1971)
12	2-Ethylthiophene	1182	1180	0.28	0.10	0.07	0.04	Beef[d]	Kato et al. (1973)
13	2,4-Dimethylthiophene	1197	1194	0.51	0.15	k	j		Boelens et al. (1971)
14	2,3-Dimethylthiophene (tentative)	1221							
15	Pyrazine	1212	1214	k	0.84	3.14	4.10	Beef[e]	Bondarovich et al. (1967)
16	2-Methylthiazole	1220	1222	k	0.22	0.25	0.02	Beef[d]	Pittet and Hruza (1974)
17	Thiazole	1239	1235	0.28	1.27	0.03	0.75	Beef[d]	Kato et al. (1973)
18	2-Methylpyrazine	1255	1254	k	46.68	60.78	76.15	Beef[e]	Bondarovich et al. (1967)
19	2,4-Dimethylthiazole	1258	1256	k	2.50	2.82	0.40	Beef[d]	
20	5-Methylthiazole	1261	1258	1.95	1.39	0.14	0.03		Kato et al. (1973)
21	4-Methylthiazole	1274	1270	1.10	0.64	0.56	0.03	Beef[d]	Maga (1975)
22	Unknown	1280		k	k	k	j		Pittet and Hruza (1974)
23	2-Ethylthiazole	1319	1319	0.11	0.28	0.14	j		
24	2,5-Dimethylpyrazine	1320	1321	k	1.23	0.96	2.02	Beef[e]	Bondarovich et al. (1967)
25	2,6-Dimethylpyrazine	1326	1325	k	2.53	4.67	7.70	Beef[e]	Bondarovich et al. (1967)
26	2-Ethylpyrazine	1330	1329	k	0.15	0.23	0.37	Beef[e]	Bondarovich et al. (1967)
27	2,3-Dimethylpyrazine	1340	1336	k	0.43	0.71	1.64	Beef[e]	Bondarovich et al. (1967)
28	2-Ethyl-4-methylthiazole (tentative)	1352		k	0.05	0.14	j		
29	2-Ethyl-4,5-dihydrothiophene (tentative)	1362		0.11	0.05	j	j		
30	2,5-Dimethylthiazole	1368	1365	k	0.51	0.35	0.08		Pittet and Hruza (1974)
31	4,5-Dimethylthiazole	1381	1379	k	0.91	0.99	0.03		Pittet and Hruza (1974)
32	Unknown	1389		k	j	k	0.02		
33	2-Ethyl-6-methylpyrazine	1392	1388	k	j	0.65	0.25	Beef[e]	Bondarovich et al. (1967)
34	2,4,5-Trimethylthiazole	1398	1394	0.06	0.83	0.71	0.06		Pittet and Hruza (1974)
35	2-Ethyl-5-methylpyrazine	1398	1396						Bondarovich et al. (1967)
36	2,3,5-Trimethylpyrazine	1401	1400	k	0.49	0.92	0.91		Bondarovich et al. (1967)
37	2-Ethyl-3-methylpyrazine	1408	1406	k	j	0.28	0.03		Bondarovich et al. (1967)
38	2-Vinylpyrazine (tentative)	1415		k	j	j	j		Bondarovich et al. (1967)
39	4-Ethyl-2,5-dimethylthiazole	1417	1419	k	0.40	0.25	j		Buttery et al. (1973)
40	4-Ethyl-5-methylthiazole	1422	1424	k	0.40	0.18	0.03		
41	2,4-Dimethyl-3-thiazoline	1425	1426	k	0.14	0.11	0.06	Beef[f]	Mussinan et al. (1975)
42	2-Methyl-2-thiazoline (tentative)	1435		k	1.17	0.28	1.52		
43	2-Ethyl-4,5-dimethylthiazole	1441	1442	k	0.86	0.74	0.03		Buttery et al. (1973)
44	2-Ethyl-3,6-dimethylpyrazine	1447	1449	k	j	0.04	0.03	Beef[e]	Bondarovich et al. (1967)
45	Furfural	1452	1452	10.67	5.09	1.04	0.01		Stoll et al. (1967)
46	2-Ethyl-3,5-dimethylpyrazine	1460	1460	k	j	j	0.01	Beef[e]	Friedel et al. (1971)

Table I. (*Continued*)

Peak no.	Compound	$I,^g$ un-known	$I,^h$ known	Area % of volatiles from each expt[i]				Occur-rence in meat	MS ref
				Expt 1	Expt 2	Expt 3	Expt 4		
47	2,3,5,6-Tetramethylpyrazine	1472	1471	k	j	0.18	0.07	Beef[e]	Friedel et al. (1971)
48	2-Methyl-6-vinylpyrazine	1489	1489	k	k	j	0.04		Bondarovich et al. (1967)
49	Methyl furfuryl sulfide	1492	1494	0.06	0.04	0.03	0.02		Stoll et al. (1967)
50	2-Methyl-5-vinylpyrazine	1500	1498	k	k	j	j		Bondarovich et al. (1967)
51	2-Acetylfuran	1503	1500	11.18	3.37	1.32	0.02		Stoll et al. (1967)
52	Pyrrole	1505	1504	k	j	0.09	0.02		Budzikiewicz et al. (1949)
53	2-Ethyl-3,5,6-trimethyl-pyrazine	1512	1510	k	j	0.01	0.03		Friedel et al. (1971)
54	(Furyl-3)-1-propanone-2 (tentative)	1522	1529	0.40	0.17	0.17	j		Stoll et al. (1967)
55	2-Thiophenethiol (tentative)	1532		0.51	0.25	0.03	j		Cornu and Massot (1966)
56	2-Hydroxypyridine	1540	1539	k	j	j	0.46		Budzikiewicz et al. (1949)
57	Methyl-2-ethyl furyl sulfide	1545	1543	0.11	0.05	0.05	0.01		
58	Methylthiofuroate	1552	1549	0.17	0.11	0.06	0.03		Stoll et al. (1967)
59	5-Methylfurfural	1560	1559	0.98	0.51	0.09	j		Stoll et al. (1967)
60	Ethyl-2-furyl ketone	1565	1563	0.74	0.47	0.07	0.01		Stoll et al. (1967)
61	Unknown	1570		k	k	k	j		
62	2-Furylmethanethiol	1585	1587	0.06	0.05	0.04	0.03		Stoll et al. (1967)
63	Furfuryl alcohol	1625	1630	k	j	0.01	0.03		Stoll et al. (1967)
64	2-Thiophenemethenethiol (tentative)	1663		0.18	0.07	0.08	0.03		IMSD (1969)
65	2-Acetyl-4-methylthiazole	1665		j	j	4.13	1.62		Kato et al. (1973)
66	Acetamide	1700	1700	k	j	0.81	0.12		Cornu and Massot (1966)
67	2-Acetylthiophene (tentative)	1725		0.28	0.19	0.28	0.20		Mussinan and Walradt (1974)
68	Unknown	1731		k	k	4.03	0.10		

[a] Bender and Ballance (1961). [b] Wilson and Katz (1972). [c] Persson and von Sydow (1973). [d] Wilson et al. (1973). [e] Mussinan et al. (1973). [f] Mussinan et al. (1975). [g] Kovåt's indices of unknown compounds. [h] Kovåt's indices of known compounds. [i] These data are subject to the same experimental limitations as described by Shibamoto and Russell (1976) and are included to show the approximate proportions and the variations which occurred with the changes in reaction parameters of the compounds present. [j] Area percent less than 0.01. [k] Not detected.
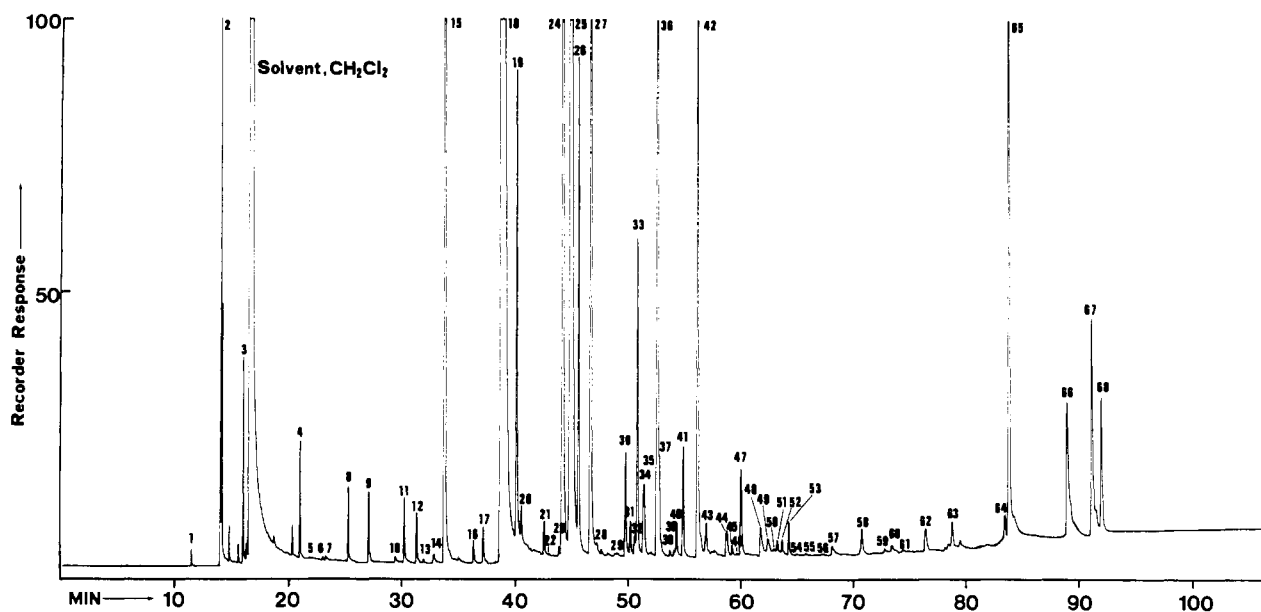

Figure 1. Gas chromatogram of volatiles formed by the reaction of D-glucose (1.0 M), hydrogen sulfide, and ammonia (5.0 M): column, 100 m × 0.25 mm i.d. glass capillary coated with Carbowax 20M, programmed from 70 to 170 °C at 1 °C per min and held. Nitrogen carrier gas flow (μ) was 15 cm/s. See Table I for peak identification.

hydrogen sulfide, thiols, and ammonia. In our model system we have found a large number of unsaturated heterocyclic compounds (thiophenes, pyrazines, furans, and thiazoles) from the reaction of D-glucose, hydrogen sulfide,

and ammonia. We did not, however, identify any saturated cyclic compounds.

Figure 1 shows the flame ionization gas chromatogram of the reaction mixture of experiment 4. Table I shows
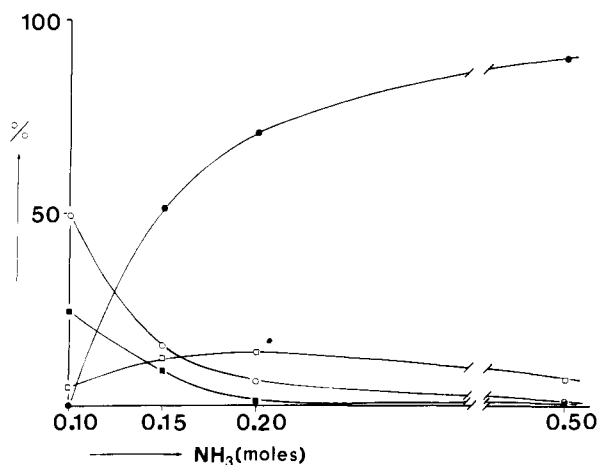
Figure 2. The total chromatograhic area percentages of thiophenes, pyrazines, thiazoles, and furans as a function of the concentration of ammonia in the reaction solution: (o) thiophenes; (•) pyrazines; (□) thiazoles; (■) furans.

the compounds identified in each reaction mixture and their peak area percentage from the gas chromatograms.

Twenty-four of these compounds (sulfides, thiophenes, furans, thiazoles, and pyrazines) have also been found in cooked meat (see Table I). The pyrazines and thiazoles are particularly important for meat flavor because most of these compounds possess cooked meat flavor (Pittet and Hruza, 1974). Some thiophenes contribute to the characteristic odor of cooked meat (Maga, 1975). It is the relative proportion of each compound in the reaction mixture, not the absolute yield of each compound, that is most important in studying flavor variations of such reaction mixtures.

Figure 2 shows the total area percentages of furans, thiophenes, pyrazines, and thiazoles (including thiazolines). It can be seen that the total percentage of compounds which require sugar fragmentation (pyrazines and thiazoles; Shibamoto, 1974) increases and the total percentage of compounds which do not require sugar fragmentation (thiophenes and furans) decreases as the amounts of ammonia in the starting solution increase from 0.1 to 0.2 mol. It is known that the higher the ammonia concentration (higher pH) the faster the sugar fragmentation (Whistler and BeMiller, 1958).

The reaction mixtures obtained from this experiment were not subjected to rigorous sensory panel evaluation; however, it was observed by a trained perfumer that the odors of the reaction mixtures from experiment 1 (0.1 mol of NH_3) to experiment 4 (0.5 mol of NH_3) progressively changed from roasted meatlike to toasted nutlike. This was probably due mainly to the formation of pyrazines, which give a toasted aroma (Pittet and Hruza, 1974). When the amount of ammonia in the starting solutions was increased to over 0.2 mol, the formation of pyrazines became predominant (over 80%). This agrees with the results reported in a previous paper (Shibamoto and Bernhard, 1976). In the process of cooking meat, a longer cooking time or higher cooking temperature generates more ammonia by amino acid degradation. If the ammonia is kept localized and a higher effective concentration is reached, then the production of more pyrazines would be expected. The more toasted flavor noticed in well-done

meat may be developed following the trend observed with this model system.

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