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Isolation and Identification of Some Sulfur Chemicals Present in Two Model Systems Approximating Cooked Meat

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The following two model meat systems were heated: hydrolyzed vegetable protein (HVP)–L-cysteine·HCl–D-xylose–water and L-cysteine·HCl–D-xylose–water. The flavor concentrates were isolated by atmospheric steam distillation, followed by continuous solvent extraction of the distillate. Isolation and identification were accomplished by gas chromatography and coupled gc–mass spectrometry. Identifications were based on I_E values and mass spectra. A total of 24 sulfur compounds were identified in the HVP–L-cysteine·HCl–D-xylose system and 15 were identified in the L-cysteine·HCl–D-xylose system, of which 10 were not present in the former model system.

One of the most challenging problems now confronting the flavor chemist is the successful identification and duplication of cooked meat flavor. The importance of this flavor type for the fabricated foods of today and of the future is clear to all those involved in developing protein foods for affluent as well as developing countries. During the past decade, numerous patents have been granted which encompass the processing of naturally occurring ingredients to produce a meat-like flavor. For instance, U. S. patent no. 3394015 (Giacino, 1968) concerns the processing of thiamine in the presence of cysteine and other amino acids to produce meat flavors. Also, U. S. patent no. 2934437 (Morton *et al.*, 1960) is concerned with the processing of cysteine and other amino acids in the presence of pentose and hexose monosaccharides for the production of meat flavors.

Many workers have speculated as to the importance of sulfur chemicals in meat flavor, but few such speculations have been reported. Brennan and Bernhard (1964) identified hydrogen sulfide and methyl, ethyl, propyl, and butyl mercaptans in the headspace of canned cooked beef. Dimethyl disulfide and dimethyl sulfone were identified by Liebich *et al.* (1972) in boiled beef. In addition, Chang *et al.* (1968) identified 3,5-dimethyl-1,2,4-trithiolan in boiled beef.

It is our opinion that one of the major difficulties in the flavor analysis of cooked beef is that an important part of the meat flavor is due to trace constituents whose identification is made difficult by the large quantities of common lipid-derived aldehydes and ketones formed during the cooking process and present in the flavor isolate. For this reason, we decided to investigate initially a model system in order to give us some insight as to the types of sulfur chemicals present in a nonlipid system.

EXPERIMENTAL SECTION

Two model reaction systems were used throughout this work. In the first system, 3708 g of a carbohydrate-free commercial hydrolyzed vegetable protein, 105.6 g of L-cysteine·HCl (Diamalt A.G.), and 60.0 g of D-xylose (Fisher Scientific) were dissolved in 8076 g of water, refluxed for 4 hr, and then allowed to stand overnight at room temperature. The heated mixture possessed a strong roast odor reminiscent of cooked meat, coffee, and other roasted products. The heated product was atmospherically steam distilled in a 22-l. flask with a Kjeldahl bulb and an ice water cooled condenser. The distillate, 34 l., was collected at a rate of 1 l. per hr in a 5° trap. The distillate was salt saturated and then continuously extracted for 7 hr with 700 ml of distilled diethyl ether in a liquid-liquid extractor. Acidic material was removed by extracting the ether extract with two 0.2-vol of 5% sodium carbonate. The ether extract was dried over anhydrous sodium sulfate and concentrated to approximately 10 ml by careful distillation in a Kuderna-Danish concentrator (Kontes Glass Co., Vineland, N. J.) equipped with a 300-mm × 13 mm i.d. Vigreux reflux column. The concentrate was divided into 17 fractions by small-scale preparative gas chromatography on a 20 ft × ¼ in. o.d. stainless steel column packed with 20% Carbowax 20M on 60–80 mesh AW-DMCS Chromosorb W. The instrument used was an F&M Model 700 gas chromatograph equipped with a thermal conductivity detector. The oven temperature was programmed from 70 to 225° at 2° per min after a 5-min post-injection hold. The temperature of the injector and detector was 230°, and the helium carrier gas flow rate was 80 ml per min. Repetitive 50-μl injections were made and the effluent was collected in 150 mm × 2 mm o.d. glass tubes cooled with crushed Dry Ice.

The second model reaction system consisted of 36.3 g of D-xylose and 181.5 g of L-cysteine·HCl dissolved in 363 g of water. The solution was heated to 121° in a Parr bomb equipped with an agitator and internal cooling coil and held at this temperature for 4 hr. After cooling, the contents were allowed to stand overnight at 4°. The reaction product was extracted with two 300-ml vol of methylene chloride (Matheson, Coleman and Bell, ACS Reagent Grade), and the extract was dried and concentrated in a Kuderna-Danish concentrator as previously described. The concentrate was divided into ten fractions by small-scale preparative gas chromatography on a 10 ft × ¼ in.

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Table I. Sulfur Compounds Identified in the HVP–Xylose–Cysteine Model System

Compound	I_E known	I_E unknown	Natural occurrence
Thiophene	3.84	3.79	Coffee[a]
2-Methylthiophene	4.62	4.68	Chicken[b]
3-Methylthiophene	4.93	4.97	
2,5-Dimethylthiophene	5.30	5.28	
2-Acetylthiophene	11.44	11.16	Coffee[c]
2-Thiophene carboxaldehyde	10.59	10.47	Coffee[c]
5-Methyl-2-thiophenecarboxaldehyde	11.47	11.68	Coffee,[c] popcorn[d]
Tetrahydrothiophen-3-one	9.29	9.33	Coffee,[c] peanuts[e]
2-Methyltetrahydrothiophen-3-one	9.09	9.05	Coffee[c]
3,5-Dimethyl-1,2,4-trithiolan	7.48	7.34[SF-96]	Potato,[f] beef[g]
Furfuryl methyl sulfide	8.59	8.50	Coffee[c]
2-Methyl-5-thiomethylfuran	7.59	7.51	Coffee[c]
Methyl ethyl sulfide	1.00	1.00	Coffee,[c] chicken,[h] potato[i]
Methyl benzyl sulfide	10.29	10.22	
Methional	8.25	8.17	Potato[f]
Methyl thioacetate	4.14	4.17	
Dimethyl disulfide	4.39	4.48	Coffee,[a] chicken,[h] onion[j]
Methyl ethyl disulfide	5.16	5.09	Potato[i]
Diethyl disulfide	5.30	5.31[SF-96]	Chicken[h]
Methyl isopropyl disulfide	5.42	5.43	Potato,[i] cocoa[k]
Ethyl isobutyl disulfide	6.82	6.75[SF-96]	
Methyl benzyl disulfide	13.61	13.53	Cocoa[k]
Isobutyl disulfide	7.94	7.88	
Dimethyl trisulfide	5.91	5.93	Chicken,[l] garlic,[j] onion[m]

[a] Stoffelsma *et al.* (1968). [b] Nonaka *et al.* (1967). [c] Stoll *et al.* (1967). [d] Walradt *et al.* (1970). [e] Walradt *et al.* (1971). [f] Buttery *et al.* (1970). [g] Chang *et al.* (1968). [h] Minor *et al.* (1965). [i] Gumbmann and Burr (1964). [j] Brodnitz *et al.* (1969). [k] van der Wal *et al.* (1971). [l] Swoboda (1970). [m] Brodnitz *et al.* (1971).

o.d. stainless steel column packed with 25% Carbowax 20M on 45–60 mesh AW-DMCS Chromosorb W. The instrument used was a Hewlett-Packard Model 5750 gas chromatograph equipped with a flame ionization detector and a 1:10 effluent splitter. The injector and detector temperatures were 230° and the helium flow rate was 80 ml per min. The oven temperature was programmed from 75 to 225° at 2° per min following a 5-min post-injection hold. Seven area traps and three of single peaks were collected as described above.

Analysis of all traps from both model systems was carried out on a Hitachi model RMU-6E mass spectrometer coupled with a Hewlett-Packard Model 5750 gas chromatograph using a Watson–Biemann helium separator (Watson and Biemann, 1965). The chromatographic columns used included a 50-ft Carbowax Scot column, 25 ft and 50 ft × ⅛ in. o.d. stainless steel Carbowax 20M "Hi-Pak" columns, 200 ft and 500 ft × 0.03 in. i.d. stainless steel open tubular columns coated with Carbowax 20M, and a 500 ft × 0.03 in. i.d. stainless steel open tubular column coated with SF-96.

Identifications were based on the comparison of known and unknown mass spectra and were confirmed wherever possible by I_E values, that is, the retention indices relative to ethyl esters (van den Dool and Kratz, 1963).

For identification purposes, those compounds for which mass spectral data were not available were synthesized: methyl benzyl disulfide, methyl ethyl disulfide, and methyl isopropyl disulfide (Mukaiyama and Takahashi, 1968); ethyl isobutyl disulfide (Milligan and Swan, 1963); cyclopentanethiol (Zinner, 1953); furfuryl methyl sulfide (Obata *et al.*, 1965); 2-methyl-5-thiomethylfuran (Stoll *et al.*, 1967); 1-(thienyl)-2-propanone (Hass *et al.*, 1950); γ-thiovalerolactone (Fries and Mengel, 1912); 3,5-dimethyl-1,2,4-trithiolan (Asinger *et al.*, 1959); and 2-methyltetrahydrothiophene, by a modification of the method of Marvel and Williams (1939).

Table II. Sulfur Compounds Identified in the Xylose–Cysteine Model System

Compound	I_E known	I_E unknown	Natural occurrence
Thiophene*	3.88	3.81	Coffee[a]
2-Methylthiophene*	4.62	4.68	Chicken[b]
3-Methylthiophene*			
2,5-Dimethylthiophene*	5.28	5.28	
2-Ethylthiophene	5.55	5.41	
2,3,5-Trimethylthiophene			
1-(Thienyl)-1-propanone	12.06	12.15	Coffee[c]
1-(Thienyl)-2-propanone			
Thieno[2,3-*b*]thiophene			Coffee[c]
Tetrahydrothiophene	5.00	4.79	
2-Methyltetrahydrothiophene*	4.88	4.93	
2-Methyltetrahydrothiophen-3-one	9.09	9.05	Coffee[c]
γ-Thiovalerolactone	10.09	10.09	
Cyclopentanethiol	5.00	4.98	
Furfuryl mercaptan			Coffee[c]

[a] Stoffelsma *et al.* (1968). [b] Nonaka *et al.* (1967). [c] Stoll *et al.* (1967). * Identified in both model systems.

RESULTS AND DISCUSSION

The cooking and extraction procedures used for the two model systems were necessarily different. Since the first contained hydrolyzed vegetable protein, it could not be directly solvent extracted and a steam distillation was performed prior to extraction. The second model system did not contain hydrolyzed vegetable protein and direct solvent extraction of the reaction product was therefore possible. The second model system was heated in a Parr bomb, since for this system a superior aroma and flavor were obtained in a closed system at a temperature above 100°.

Table I lists the compounds identified in the hydrolyzed vegetable protein–D-xylose–L-cysteine·HCl model system. The I_E values given were obtained on a Carbowax capil-

lary column unless otherwise indicated. The mass spectra of all compounds reported were in complete agreement with those of the known compounds. Many of the sulfur compounds identified in the model systems studied are naturally occurring. Some of the foods in which they occur are reported in Tables I and II. Of the 24 compounds listed in Table I, all but six have been reported as naturally occurring.

The sulfur compounds identified in the D-xylose–L-cysteine·HCl model system are given in Table II, along with their I_E values and natural occurrence. Those compounds designated by an asterisk were identified in both model systems.

SUMMARY

Two model systems approximating the conditions of cooked meats were prepared and analyzed. A total of 34 sulfur compounds were identified. Of these, 21 have been previously reported in natural foods, and the remaining 13 have not been reported as naturally occurring. Eleven of these compounds have been synthesized.

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Identification and Analysis of the Major Acids from Fruit Juices and Wines

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The major organic acids of fruit juices and wines have been investigated using lead precipitation and glc of the trimethylsilyl derivatives. The identity of individual acids was established by glc, thin-layer chromatography, and mass spectral data. The amounts of individual nonvolatile acids were calculated using an internal standard and standard curves. The major differences between juices and wines are the greater overall amounts of acids in the former and the presence of lactic and succinic acids in the latter. A comparison was made between the amounts of acids found by two different nonequivalent methods, glc and titration. In juices the two methods gave similar results but in wines the values were different. The phosphoric acid content of juices was analyzed *via* glc at the same time as major organic acids and the values were found to be comparable with those of a colorimetric method.

The acidic components of fruit juices and wines impart important properties to these foods being prominent in flavor, processing, and preservation. The acidity has also been used as a criterion of adulteration of one fruit with another. Because of these attributes the acids have been extensively studied in the past (Amerine and Cruess, 1960; Hulme, 1970; Tressler and Joslyn, 1961). These studies have employed mainly chemical and enzymatic methods in order to separate, identify, and quantify the acids. In recent years, with the advent of newer and less tedious chromatographic techniques, the acids have again been investigated by numerous workers (Chan *et al.*, 1972; Fernandez-Flores *et al.*, 1970; Martin *et al.*, 1971; Weissberger *et al.*, 1971). These analyses have allowed the major four to seven organic acids to be estimated and the amounts of individual acids to be determined quantitatively. Nevertheless, the identification of the acidic components from various fruit sources has been cursory and sometimes contradictory, often being based only on a single glc peak. In addition, the amounts of acids present in fruit juices and wines have received little attention when compared to the qualitative aspects. The comprehensive nature of the glc technique, whereby all acids are estimat-

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