

- Hirai, C., Herz, K. O., Pokorny, J., Chang, S. S., *J. Food Sci.* **38**, 393 (1973).
- Hodge, J. E., in "Chemistry and Physiology of Flavors", Schultz, H. W., Day, E. A., Libbey, L. M., Ed., Avi Publishing Co., Westport, Conn., 1967, pp 465-491.
- Hornstein, I., Crowe, P. F., *J. Agric. Food Chem.* **8**(6), 494 (1960).
- Hudson, B. J. F., Karis, I. G., *J. Sci. Food Agric.* **25**, 1491 (1974).
- Kolattukudy, P. E., *Lipids* **5**, 259 (1970).
- Liebich, H. M., Douglas, D. R., Zlatkis, A., Muggler-Chavan, F., Donzel, A., *J. Agric. Food Chem.* **20**(1), 96 (1972).
- Likens, S. T., Nickerson, G. B., *Proc. Am. Soc. Brew. Chem.*, **5** (1964).
- MacLeod, A. J., Cave, S. J., *J. Sci. Food Agric.* **26**, 351 (1975).
- MacLeod, A. J., MacLeod, G., *J. Food Sci.* **35**, 744 (1970).
- MacLeod, G., *Food Proc. Ind.*, **27** (March, 1972).
- Merritt, C., Jr., Angelini, P., Bazinet, M. L., McAdoo, D. J., *Adv. Chem. Ser. No. 56*, 225 (1966).
- Mussinan, C. J., Wilson, R. A., Katz, I., *J. Agric. Food Chem.* **21**(5), 871 (1973).
- Patterson, R. L. S., in "Meat", Cole, D. J. A., Lawrie, R. A., Ed., Butterworths, London, 1974, p 359.
- Persson, T., von Sydow, E., *J. Food Sci.* **38**, 377 (1973).
- Proctor, G., Goldblith, S. A., *Adv. Food Res.* **3**, 119 (1951).
- Reineccius, G. A., Keeney, P. G., Weissberger, W. J., *J. Agric. Food Chem.* **20**(2), 202 (1972).
- Reynolds, T. M., in "Symposium on Foods", Schultz, H. W., Cain, R. F., Wrolstad, R. W., Ed., Avi Publishing Co., Westport, Conn., 1969.
- Reynolds, T. M., *Food Technol. Aust.* **22**(11), 610 (1970).
- Rhodes, D. N., in Proceedings of the I.F.S.T. (U.K.) Tenth Anniversary Symposium on Meat and Meat Products, 1974, p 5.
- Rosen, C. G., *Food Technol.*, **36** (July, 1972).
- Roth, H., Rock, S. P., *Baker's Dig.*, **38** (Aug, 1972).
- Self, R., Casey, J. C., Swain, T., *Chem. Ind. (London)*, 863 (1963).
- Sink, J. D., *J. Am. Oil Chem. Soc.* **50**(11), 470 (1973).
- Smith, D. P., *Microwave Energy Applications Newsl.* **5**(4), 2 (1972).
- van Straten, S., de Vrijer, F., Rapport NR. R4030, "Lists of Volatile Compounds in Food", 3rd ed, Centraal Instituut voor Voedingsonderzoek, Zeist, Netherlands, 1973.
- Walradt, J. P., Lindsay, R. C., Libbey, L. M., *J. Agric. Food Chem.* **18**(5), 926 (1970).
- Wang, P. S., Odell, G. V., *J. Agric. Food Chem.* **21**(5), 868 (1973).
- Wasserman, A. E., *J. Agric. Food Chem.* **20**(4), 737 (1972).
- Watanabe, K., Sato, Y., *Agric. Biol. Chem.* **32**(11), 1318 (1968).
- Watanabe, K., Sato, Y., *J. Agric. Food Chem.* **19**(5), 1017 (1971a).
- Watanabe, K., Sato, Y., *Agric. Biol. Chem.* **35**(5), 756 (1971b).
- Watanabe, K., Sato, Y., *J. Agric. Food Chem.* **20**(2), 174 (1972).
- Wilson, R. A., Mussinan, C. J., Katz, I., Sanderson, A., *J. Agric. Food Chem.* **21**(5), 873 (1973).

Received for review December 24, 1975. Accepted March 22, 1976.
This project was financed by the U.K. Meat and Livestock Commission and the University of London Central Research Fund.

Study of Meat Volatiles Associated with Aroma Generated in a D-Glucose-Hydrogen Sulfide-Ammonia Model System

Takayuki Shibamoto and Gerald F. Russell*

The volatile compounds produced by heating a model system of D-glucose-hydrogen sulfide-ammonia were entrained on Porapak Q and subsequently desorbed and transferred to a glass capillary column for separation and identification. Gas chromatographic and mass spectrometric methods were used to identify 34 of the major components. The compounds identified included a thiol, sulfides, thiophenes, thiazoles, and furans. Thiophene and furan derivatives were the major volatile constituents of this reaction mixture which gave roast beef-like aroma.

Over 100 volatile components have been identified in cooked meat extracts. These have included aliphatic aldehydes, esters, ketones, hydrocarbons, and some nitrogen- and sulfur-containing compounds (Nonaka et al., 1967; Persson and von Sydow, 1973). Recently, pyrazine compounds have also been found in cooked meat (Watanabe and Sato, 1971). Some thiazole, oxazole, thiazoline, and oxazoline compounds have been identified in cooked meat and some of these possess a meaty flavor (Mussinan et al., 1975).

Many studies using simulated cooking conditions in model systems have been conducted to investigate meat flavor precursors (Mulders, 1973; Mussinan and Katz, 1973; Kato et al., 1973). Wood (1961) reported that heating an aqueous solution of fresh ox muscle extract with glucose produced a meaty flavor and browning, but neither was observed when the glucose was omitted. Mussinan and Katz (1973) reported the formation of sulfur-containing compounds in model systems consisting of hydrolyzed

vegetable protein (HVP)-L-cysteine-HCl-D-xylose-water and L-cysteine-xylose-water. They found in their reaction mixtures some thiophenes and sulfides which have also been identified in meat. van den Ouweland and Peer (1975) reported the formation of thiophene derivatives from the reaction of a sugar degradation product, 4-hydroxy-5-methyl-3(H)-furanone, and hydrogen sulfide.

Carbonyl compounds (aldehydes, ketones, and diketones) are known to be formed as degradation products of sugars (Nodzu, 1935; Carson, 1953). Carbonyls present in food include glucosamine, dehydroascorbic acid, and pyruvaldehyde. Diketones are also formed from thermal degradation of glucose (Shibamoto, 1974). Takken et al. (1975) obtained heterocyclic compounds such as oxazoles, thiazoles, and thiazolines which had a meaty flavor (Mussinan et al., 1975) from a model system consisting of α -dicarbonyls or aldehydes, hydrogen sulfide, and ammonia. These experiments suggest that compounds which give characteristic meat aroma are formed from the reaction of carbonyl compounds, hydrogen sulfide, and ammonia, and it appears that the compounds associated with characteristic meat aroma include hydrogen sulfide, ammonia, carbonyls, pyrazines, thiophenes, thiazoles,

*Department of Food Science and Technology, University of California, Davis, California 95616.

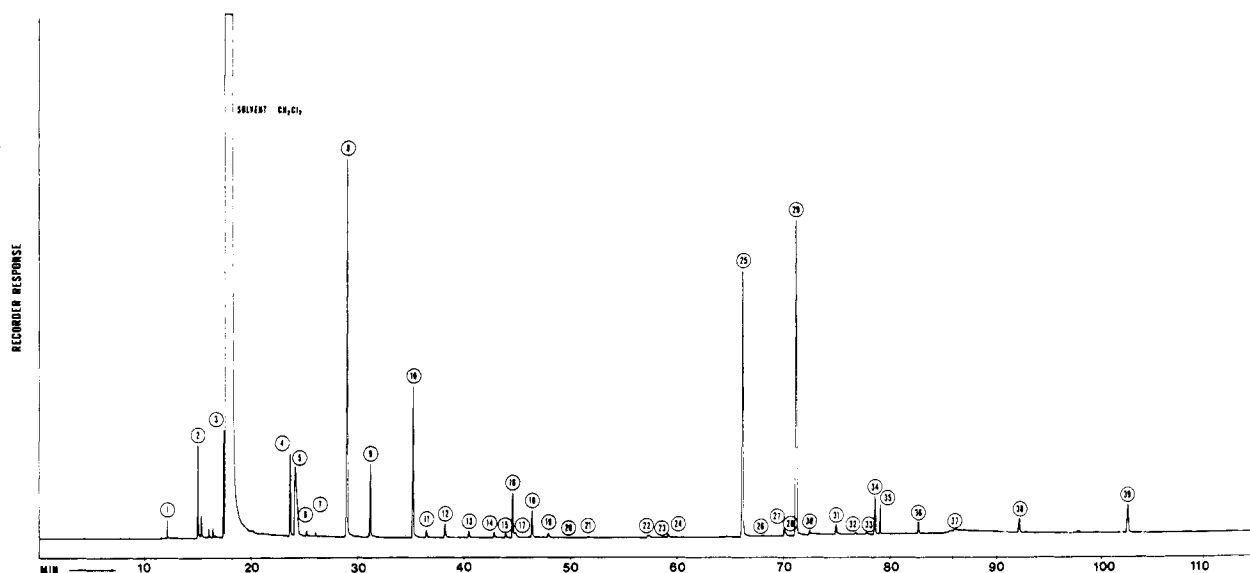


Figure 1. Gas chromatogram of volatiles formed by D-glucose-hydrogen sulfide-ammonia model system. For chromatographic conditions see Experimental Section. For peak identification see Table I.

oxazoles, thiazolines, and oxazolines. The purpose of this study was to isolate and identify the volatile compounds produced in a D-glucose-H₂S-NH₃ model system.

EXPERIMENTAL SECTION

D-Glucose (Mallinckrodt), ammonium hydroxide (Allied Chemical), and hydrogen sulfide (Matheson) were obtained commercially. Authentic samples of sulfides, thiophenes, and furans were obtained from Ogawa & Co., Ltd., Tokyo, Japan.

Sample Preparation. A Kjeldahl flask (100 ml) containing 18 g (0.1 mol) of D-glucose and 0.1 mol (as NH₃) of ammonium hydroxide in 100 ml of deionized water was cooled in an ice bath for 30 min; then hydrogen sulfide gas was bubbled through the solution for 10 min. The neck of the flask was flame sealed and the ampule placed in an oven at 100 °C for 2 h.

The mixture was then transferred to a 500-ml wash bottle. Purified nitrogen gas was introduced into the bottle by means of a glass frit sparger and the volatiles in the reaction mixture were swept into a 5 mm i.d. × 20 cm glass column packed with 80/100 mesh Porapak Q (Waters Associates; previously conditioned at 150 °C for 1 h). The nitrogen flow was maintained at 30 ml/min for 6 h. The column was then disconnected and nitrogen was passed through it in the reverse direction. The column temperature was raised to 100 °C for 10 min while the volatiles were trapped in a 1 mm i.d. glass capillary tube held at -78 °C. The volatiles were transferred from the glass capillary tube to a 10- μ l microsyringe using 5 μ l of methylene chloride and then injected into the analytical glass capillary column.

Gas-Liquid Chromatography/Mass Spectrometry. Identification of volatile constituents of the reaction mixture was made by comparison of their mass spectra and Kováts indices (*I*) (Ettre, 1964) to those of authentic compounds.

A Varian Aerograph series 1200 gas chromatograph equipped with a flame ionization detector (FID) modified for capillary analyses and a 100 m × 0.25 mm i.d. glass capillary column coated with Carbowax 20M (J & W Scientific, Orangevale, Calif.) was used. The column temperature was programmed from 70 to 170 °C at 1 °C/min. The nitrogen carrier gas flow was 0.6 ml/min; $\mu_0 = 15$ cm/s for qualitative and quantitative analyses of the volatiles. The gas chromatograph was fitted with an

all glass injector splitter of our own design to avoid any contact with metal surfaces, and was operated with an injector split ratio which directed 3% of injected volatiles to the capillary column. The injector temperature was 150 °C and the detector temperature was 200 °C.

Peak areas were integrated using an Infotronics CRS-104 digital integrator and are reported in Table I.

A Finnigan Model 3200 combination mass spectrometer-gas chromatograph (Finnigan Model 9500) equipped with a Finnigan Model 6000 MS data system was used for mass spectral identification of the gas chromatographic components under the following conditions: filament current, 167 mA; multiplier voltage, -2.5 kV; electron energy, 70 eV. The gas chromatographic column and oven conditions were as described for the Varian instrument. Other operating temperatures were: injector, 150 °C; separator, 200 °C; transfer line (direct, glass lined steel), 200 °C; analyzer, 50 °C.

RESULTS AND DISCUSSION

Studies of meat flavor have been conducted using many different model systems. The most common systems studied have been cysteine-sugar systems; cysteine as the sulfur and nitrogen source and a sugar as the carbon source (Mussinan and Katz, 1973; Scanlan et al., 1973). Products from these model systems were primarily sulfides, thiophenes, and thiazoles.

The reaction mixtures studied in this report were not subjected to rigorous sensory panel evaluation; however, the resemblance to cooked beef odor was unmistakable. As evaluated by a trained perfumer, the essence desorbed from the Porapak trap had a slightly different odor from the reaction mixture, but it also had the same odor notes of the cooked beef aroma from the reaction mixture.

Figure 1 shows a FID chromatogram of reaction product volatiles. The detector signal was attenuated to show relative response to the eluting compounds. Peak areas shown and also reported in Table I may have been influenced by a nonlinearity in the injector splitter, but are given to indicate approximate proportions of compounds detected.

Table I shows the products obtained from the D-glucose-H₂S-NH₃ model system. Twenty-four compounds, for which Kováts indices of known compounds were available, were identified by agreement with the mass spectra and retention indices of authentic samples; of

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

Peak no. (Fig. 1)	Compounds	Area %	<i>I</i> un- known	<i>I</i> known ^r	Occurrence in foods	MS references
1	Methanethiol	0.62	761	760	Onion, leek, garlic, ^a beef ^b	Stoll et al. (1967)
2	Dimethyl sulfide	7.45	818	815	Onion, garlic, ^a beef ^{b,c}	Stoll et al. (1967)
3	Diethyl sulfide	10.14	899	895	Cabbage ^a	Cornu and Massot (1966)
4	Thiophene	10.19	1035	1032	Coffee ^d	API (1948)
5	Methyl isothiocyan- ate (tentative)	3.56	1046		Cabbage, sprouts, cauliflower ^a	IMSD (1969)
6	Unknown	0.40	1047			
7	2,3-Pentanedione	0.45	1049	1046	Coffee, ^d filberts ^e	van den Ouweland and Peer et al. (1975)
8	2-Methyl- thiophene	24.92	1098	1095	Chicken, ^f beef ^g	Budzikiewicz et al. (1964)
9	3-Methyl- thiophene	4.41	1127	1123	Beef ^f	Cornu and Massot (1966)
10	2,5-Dimethyl- thiophene	6.35	1166	1165	Beef, ^g onion ^h	Boelens et al. (1971)
11	2-Ethylthiophene	0.28	1182	1180	Pressure-cooked beef ⁱ	Kato et al. (1973)
12	2,4-Dimethyl- thiophene	0.51	1197	1194	Onion ^h	Boelens et al. (1971)
13	2,3-Dimethylthio- phene (tentative)	0.28	1218		Beef ^g	
14	Thiazole	0.28	1240	1235	Peanuts, ^j popcorn ^k	Cornu and Massot (1966)
15	2-Formylthio- phene (tentative)	0.23	1246		Coffee, ^l filberts, ^e beef ^g	Stoll et al. (1967)
16	5-Methylthiazole	1.95	1258	1258	(Cysteine- pyruvaldehyde) ^m	Kato et al. (1973)
17	Unknown	0.17	1260			
18	4-Methylthiazole	1.10	1272	1270	Peanuts ^f	Maga (1975)
19	5-Methyl-2-formyl- thiophene (tentative)	0.23	1286		Coffee, ^l popcorn ^k	Stoll et al. (1967)
20	Unknown	0.11	1300			
21	2-Ethylthiazole	0.11	1317	1319	(Cysteine- pyruvaldehyde) ^m	Pittet and Hruza (1974)
22	2-Ethyl-4,5- dihydrothiophene (tentative)	0.11	1368			
23	4,5-Dimethylthiazole (tentative)	0.06	1370		(Thiamine) ⁿ	Pittet and Hruza (1974)
24	Unknown	0.06	1385			
25	Furfural	10.67	1450	1452	Bread, chicory, ^o popcorn ^k	Stoll et al. (1967)
26	Unknown	0.06	1465			
27	Methyl furfuryl sulfide	0.06	1480	1482	Coffee ^c	Stoll et al. (1967)
28	Unknown	0.34	1486			
29	2-Acetylfuran	11.18	1488	1500	Chicory, ^g coffee, ^d popcorn ^k	Stoll et al. (1967)
30	(Furyl-2)-1- propanone-2	0.40	1500	1502	Coffee ^p	Stoll et al. (1967)
31	2-Thiophenethiol (tentative)	0.51	1524			Cornu and Massot (1966)
32	Methyl 2-ethyl furyl sulfide	0.06	1540	1543		
33	Methyl thiofuroate	0.09	1548	1549	Coffee ^q	Stoll et al. (1967)
34	5-Methylfurfural	0.52	1557	1559	Coffee, ^q popcorn, ^k filberts ^e	Stoll et al. (1967)
35	Ethyl 2-furyl ketone	0.39	1560	1563	Coffee ^q	Stoll et al. (1967)
36	2-Furylmethanethiol	0.03	1585	1587	Coffee ^q	Stoll et al. (1967)
37	2-Thiophenemethane- thiol (tentative)	0.21	1639			IMSD (1969)
38	Unknown	0.15	1680			
39	Unknown	0.03	1780			

^a Johnson et al. (1971). ^b Bender and Ballance (1961). ^c Hornstein and Crowe (1960). ^d Stoffelsma et al. (1968).
^e Kinlin et al. (1972). ^f Nonaka et al. (1967). ^g Persson and von Sydow (1973). ^h Boelens et al. (1971). ⁱ Wilson et al.
(1973). ^j Walradt et al. (1971). ^k Walradt et al. (1970). ^l Walter and Weidemann (1968). ^m Obtained only from model
system; Kato et al. (1973). ⁿ Obtained only from model system; Duirvedi et al. (1973). ^o Baker et al. (1953). ^p Reich-
stein and Beitter (1930). ^q Stoll et al. (1967). ^r Obtained using authentic samples.

these, 18 have previously been reported in foods. Four compounds (compounds 22, 31, 32, and 37 in Table I) have not been previously reported in either food or model systems.

Takken et al. (1975) reported products obtained from a similar model system. However, they used α -dicarbonyl compounds and aldehydes instead of glucose. The greatest difference between the results of their study and ours is that our model system produced a large number of thiophene and furan derivatives. Furans, furfural, 2-acetylfuran, and 5-methylfurfural are known as products of sugar caramelization (Schultz et al., 1967). Mussinan and Katz (1973) obtained a number of thiophene derivatives from their HVP-L-cysteine-HCl-D-glucose-water model systems, but they did not find any nitrogen heterocyclic compounds. However, some thiazole derivatives were detected in our reaction mixture. The total yield of nitrogen heterocyclic compounds was very low, however, with total thiazoles estimated to be approximately 2%.

Boelens et al. (1974) also obtained nitrogen heterocyclic compounds (2,4,6-trimethylidihydro-1,3,5-dithiazine and methyltetrahydro-1,3,5-thiadiazine) from the reaction of ethanal, hydrogen sulfide, and ammonia.

It is obvious that ammonia nitrogen was incorporated into the cyclic compounds, and that these compounds can give a characteristic meat aroma to the reaction mixture (Mussinan et al., 1975; Pittet and Hruza, 1974). Some sulfides and thiophene derivatives are well known as compounds which give characteristic flavor to meat (Minor et al., 1965; Nonaka et al., 1967).

Our model system consisting of D-glucose-H₂S-NH₃ produced a large number of compounds which have been found in meat. The role of these compounds in a meat aroma complex is not well understood. However, if the precursors and formation pathways of aroma constituents were known, it would shed more light on the formation of meat flavor. Hydrogen sulfide itself plays a role in meat flavor (Pippen and Mecchi, 1969). Ammonia is formed from amino acids as exemplified by the Strecker degradation (Strecker, 1862). Hydrogen sulfide is formed during the Strecker degradation of cysteine with a diketone (Kobayashi and Fujimaki, 1965). Hydrogen sulfide is also produced in meat from thiamine on heating (Schutte, 1974). While it has been suggested that meat flavor depends upon the quantitative relationship of carbonyls, hydrogen sulfide, and ammonia (Herz and Chang, 1970), we suggest that meat aroma may be dependent upon sugars (carbonyls), hydrogen sulfide, and ammonia as reactants rather than as products.

LITERATURE CITED

- American Petroleum Institute, "Certified Mass Spectra", College Station, Texas A&M University, Chemical Thermodynamics Properties Center, 6 Vol., Research Project 44, 1948.
- Baker, J. C., Parker, H. K., Fortmann, K. L., *Cereal Chem.* **30**, 22 (1953).
- Bender, A. E., Ballance, P. E., *J. Sci. Food Agric.* **12**, 683 (1961).
- Boelens, H., de Valois, P. J., Wobben, H. J., van der Gen, A., *J. Agric. Food Chem.* **19**, 984 (1971).
- Boelens, M., van der Linde, L. M., de Valois, P. J., van Dort, H. M., Takken, H. J., *J. Agric. Food Chem.* **22**, 1071 (1974).
- Budzikiewicz, H., Djerassi, C., Williams, D. H., "Interpretation of Mass Spectra of Organic Compounds", Holden-Day, San Francisco, Calif., 1964, p 231.
- Carson, J. F., *J. Am. Chem. Soc.*, **75**, 4300, 4337 (1953).
- Cornu, A., Massot, R., "Compilation of Mass Spectral Data", Heyden & Son Limited, Presses Universitaires de France, 1966, p 29 B.
- Duirvedi, B. K., Arnold, R. G., Libbey, L. M., *J. Food Sci.* **38**, 450 (1973).
- Ettre, L. S., *Anal. Chem.* **36**, 31A (1964).
- Herz, K. O., Chang, S. S., *Adv. Food Res.* **18**, 45 (1970).
- Hornstein, I., Crowe, P. F., *J. Agric. Food Chem.* **8**, 494 (1960).
- Index of Mass Spectral Data, American Society of Testing and Materials, 1969, p 6.
- Johnson, A. E., Nursten, H. E., Williams, A. A., *Chem. Ind. (London)*, Part I, 556, Part II, 7212 (1971).
- Kato, S., Kurata, T., Fujimaki, M., *Agric. Biol. Chem.* **37**, 539 (1973).
- Kinlin, T. E., Muraledhara, R., Pittet, A. O., Sanderson, A., Walradt, J. P., *J. Agric. Food Chem.* **20**, 1021 (1972).
- Kobayashi, N., Fujimaki, M., *Agric. Biol. Chem.* **29**, 698 (1965).
- Maga, J. A., *Crit. Rev. Food Sci. Nutr.* **6**, 165 (1975).
- Minor, L. J., Pearson, A. M., Dawson, L. E., Schweigert, B. S., *J. Agric. Food Chem.* **13**, 298 (1965).
- Mulders, E. J., *Z. Lebensm.-Unters.-Forsch.* **152**, 193 (1973).
- Mussinan, C. J., Katz, I., *J. Agric. Food Chem.* **21**, 43 (1973).
- Mussinan, C. J., Wilson, R. A., Katz, I., Sanderson, A., Vock, M. H., 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1975, Abstract No. AGFD-22.
- Nodzu, R., *Bull. Chem. Soc. Jpn.* **10**, 122 (1935).
- Nonaka, M., Black, D. R., Pippen, E. L., *J. Agric. Food Chem.* **15**, 713 (1967).
- Persson, T., von Sydow, E., *J. Food Sci.* **38**, 377 (1973).
- Pippen, E. L., Mecchi, E. P., *J. Food Sci.* **34**, 433 (1969).
- Pittet, A. O., Hruza, D. E., *J. Agric. Food Chem.* **22**, 264 (1974).
- Reichstein, T., Beitter, H., *Ber.* **63**, 816 (1930).
- Scanlan, R. A., Kayser, S. G., Libbey, L. M., Morgan, M. E., *J. Agric. Food Chem.* **21**, 675 (1973).
- Schultz, H. W., Day, E. A., Libbey, L. M., "The Chemistry and Physiology of Flavors", Avi Publishing Co., Westport, Conn., 1967, p 472.
- Schutte, L., *Crit. Rev. Food Technol.*, 473 (1974).
- Shibamoto, T., Ph.D. Thesis, University of California, Davis, 1974.
- Stoffelsma, J., Sipma, G., Kettenes, D. S., Pijpker, J., *J. Agric. Food Chem.* **16**, 1000 (1968).
- Stoll, M., Winter, M., Grutsch, F., Flament, I., Willhalm, B., *Helv. Chim. Acta* **50**, 628 (1967).
- Strecker, A., *Ann.* **123**, 363 (1862).
- Takken, H. J., van der Linde, L. M., de Valois, P. J., van Dort, J. M., Boelens, H., 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1975, Abstract No. AGFD-15.
- van den Ouweland, G. A. M., Peer, H. G., *J. Agric. Food Chem.* **23**, 501 (1975).
- Walradt, J. P., Lindsay, R. C., Libbey, L. M., *J. Agric. Food Chem.* **18**, 926 (1970).
- Walradt, J. P., Pittet, A. O., Kinlin, T. E., Muraledhara, R., Sanderson, A., *J. Agric. Food Chem.* **19**, 972 (1971).
- Walter, W., Weidemann, H. L., *Z. Ernährungswiss.* **9**, 123 (1968).
- Watanabe, K., Sato, Y., *J. Agric. Food Chem.* **19**, 1017 (1971).
- Wilson, R. A., Mussinan, C. J., Katz, I., Sanderson, A., *J. Agric. Food Chem.* **21**, 873 (1973).
- Wood, T., *J. Sci. Food Agric.* **12**, 61 (1961).

Received for review December 22, 1975. Accepted March 24, 1976.