Some Alkyl-Substituted Pyrazines and Pyridines in the Flavor Components of Shallow Fried Beef

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The basic compounds were isolated from the total volatiles of shallow fried beef, and analyzed by gas chromatography and the combination of gas chromatography–mass spectrometry. Identification of 11 compounds, nine alkyl-substituted pyrazines, and two alkyl-substituted pyridines was obtained, and four compounds still remained unknown. The identified compounds suggested a possible reaction between sugars and amino acids to produce a specific flavor in beef, and it was proposed that the basic compounds might make important contributions to the typical roasted flavor of beef.

The chemical nature of flavor compounds developed from cooked meat and meat fats has fascinated food chemists for a long time. It has been reported that the difference of flavors in various meats (pork, lamb, beef) might arise from the fats rather than the lean portion of the meat, and the basic meaty flavor in these meats was produced from the lean portion (Hornstein and Crowe, 1960; Macy *et al.*, 1964). The flavor volatiles of cooked lean meat and fats have been reported by several investigators: the common aldehydes, mercaptans, sulfides, and ammonia from the lean portion of beef (Hornstein and Crowe, 1960; Hornstein *et al.*, 1960; Bender and Ballance, 1961; Kramlich and Pearson, 1960; Sanderson *et al.*, 1966; Yueh and Strong, 1960) and several aldehydes, ketones, alcohols, and hydrogen sulfide from beef fats (Hornstein and Crowe, 1960, 1963; Yamato *et al.*, 1970; Pepper and Pearson, 1969) have been identified. Recently, particular ring compounds contributing to beef flavor were isolated (Chang *et al.*, 1968; Tonsbeek *et al.*, 1968).

In our studies, gas chromatography and mass spectrometry were used to identify the flavor compounds from heated beef fats, and an ever-increasing number and variety of volatiles were revealed (Watanabe and Sato, 1971). The present investigation was undertaken as part of a series of flavor studies of meats, and concerned the identification of a number of basic compounds obtained from shallow fried beef and their possible contribution to beef flavor.

EXPERIMENTAL

Material. The lean meat and fats used in the study were veal shoulder (2.0 kg) and depot fats (200 g). The lean meat was trimmed from the bone, and cut into small pieces (thickness: 2–4 mm; width: 3 × 3 cm). Almost all of the fat, including as much of the intermuscular fat as possible, was removed from the lean meat. Samples of lean meat and fats were divided into ten parts, and each one of them (lean meat, 200 g, fats, 20 g) was used as the material for one time in the heat treatment.

Heat Treatment and Separation of Basic Compounds Fraction. The apparatus for the collection of flavor compounds produced by heating was shown in a previous paper (Watanabe and Sato, 1971). The heat treatment was done under the frying condition devised from the Japanese way of cooking, that is, sukiyaki. The fats (20 g) were melted in the pan heated at 120° C and then 200 g of the lean meat was added to the pan. After the addition, the lid was put on the pan. Temperatures of the pan were programmed from 120 to 150° C at a rate of 5° C per min and then were held at 150° C for 1 min. The developed flavor compounds and steam were collected in the cold traps connected with the top of lid. These heat treatments were repeated ten times. The condensate collected in the traps was then melted and extracted with ethyl ether. The ethyl ether extract was treated with 3% HCl, and the resulting aqueous layer containing the hydrochlorides of the basis compounds was concentrated below 50° C *in vacuo* to approximate dryness. To the residue was added 10% NaOH to liberate the free basic compounds, and they were extracted with ethyl ether. The ethyl ether solution was dried over anhydrous Na_2SO_4 and fractionally distilled with a 1 × 30 cm column packed with glass helices to remove the bulk of the ethyl ether. The concentrate was analyzed by gas chromatography and the combination of gas chromatography–mass spectrometry.

Gas Chromatography. The gas chromatographic analysis was performed on a Hitachi Model K-53 gas chromatograph with a flame ionization detector, using a 3 mm × 2 m column containing 10% PEG-20M on 80–100 mesh acid-washed Celite 545 at a helium flow rate of 50 ml per min. The temperatures were programmed from 50 to 170° C at a rate of 2° C per min.

Gas Chromatography–Mass Spectrometry. A combination instrument of gas chromatograph–mass spectrometer, Hitachi Model RMU-6E, was used. The concentrate was analyzed under conditions similar to those used for retention time data. The column effluent was admitted *via* a heated line to a Watson–Biemann helium separator and then to the ion source. Mass spectrometric conditions were as follows: ion accelerating voltage, 1800 V; ionizing voltage, 70 V; total emission, 80 μA; target emission, 72 μA; ion source temperature, 220° C; multiplier, 3 kV; scan rate, 5 sec between *m/e* 25 and 200.

Reference Compounds. Methylpyrazine, 2,5-dimethylpyrazine, and 2,6-dimethylpyrazine were obtained from T. Hasegawa Co., Ltd. Tetramethylpyrazine, 2,6-diethyl-3-methylpyrazine, and 2-ethyl-5,6-dimethylpyrazine were kindly supplied by Victor Krampl and M. A. Gianturco (The Coca-Cola Co.). The other pyrazine compounds were synthesized

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Table I. Basic Compounds in the Flavor Components Obtained from Shallow Fried Beef

Compound	Peak no. (Figure 1)	Relative retention time: Sample	Relative retention time: Reference	Mass spectral peaks, M^+ m/e	Mass spectral reference
Methylpyrazine	1	0.63	0.65	94/94, 67, 39	*d*
2-Ethylpyridine	2	0.66	0.67	107/106, 107, 79	*e*
2,5-Dimethylpyrazine[a]	3	0.72	0.73	108/108, 42, 39	*d*
2,6-Dimethylpyrazine	4	0.74	0.74	108/108, 42, 40	*d*
2,3-Dimethylpyrazine	5	0.76	0.77	108/67, 108, 42	*d*
2-Ethyl-5-methylpyrazine[a]	6	0.87	0.87	122/121, 122, 39	*d*
Trimethylpyrazine[a]	7	0.90	0.91	122/42, 122, 39	*d*
2,5-Dimethyl-3-ethylpyrazine[a]	8	1.00[b]	1.00[c]	136/135, 136, 42	*d*
Tetramethylpyrazine	10	1.06	1.07	136/54, 136, 42	*f*
2,6-Diethyl-3-methylpyrazine	12	1.16	1.17	150/149, 150, 39	*d*
2-Pentylpyridine	13	1.29	1.28	149/93, 106, 120	

[a] Previously reported in beef fat flavor, Watanabe and Sato (1971). [b] Retention time = 28.8 min. [c] Retention time = 28.6 min. Bondarovich *et al.* (1967). [e] Biemann (1962). [f] Flament *et al.* (1967).

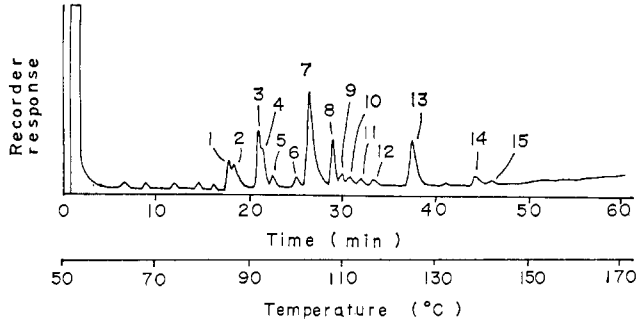

Figure 1. Gas chromatogram of basic compound fraction

by the method of van Praag *et al.* (1968). 2-Ethylpyridine was obtained from commercial source, and 2-pentylpyridine was synthesized by the published method (Benkeser and Holton, 1951).

RESULTS AND DISCUSSION

The gas chromatographic separation of the basic compound fraction is shown in Figure 1. Approximately 15 peaks were evidenced in the chromatogram. Relative retention times were calculated relative to 2,5-dimethyl-3-ethylpyrazine. Those of the flavor components agreed reasonably well with those of the corresponding reference compounds. These gas chromatographic identities were confirmed by mass spectrometry. The identified compounds, of which peaks have numbers assigned in Figure 1, are presented in Table I. Identification of 11 compounds, nine alkyl-substituted pyrazines, and two alkyl-substituted pyridines, was obtained, and four compounds still remained unknown. Components of Peak 9 (M^+ = 136), 11 (M^+ = 150), and 15 (M^+ = 134) were alkyl-substituted pyrazine-like, and compound of Peak 14 (M^+ = 149) was alkyl-substituted pyridine-like.

Pyrazine and alkyl-substituted pyrazines are known to contribute in an important way to the flavor of a variety of roasted or cooked foods. These compounds were isolated from the volatiles of roasted peanut (Mason and Johnson, 1966), potato chips (Deck and Chang, 1965), cacao (Dietrich *et al.*, 1964; Marion *et al.*, 1967; Rizzi, 1967; van Praag *et al.*, 1968), coffee (Goldman *et al.*, 1967; Bondarovich *et al.*, 1967), and barley (Wang *et al.*, 1969). Pyridine and some pyridine compounds were detected in the flavor components of coffee (Goldman *et al.*, 1967), barley (Wang *et al.*, 1969), and lactose–casein system (Ferretti *et al.*, 1970). Pyridine and alkyl-substituted pyridines may also contribute to the flavor of various foods. In the shallow fried beef as well as in the foods described above, it seemed that the basic compound fraction contributed a typical roasted flavor which could be attributed to alkyl-substituted pyrazines and pyridines. Removal of the basic compounds from the total flavor components obtained from the shallow fried beef, that is washing the ethyl ether extract with HCl solution, resulted in a considerable loss of the roasted odor.

From the pathways of the Maillard reaction, it can be assumed that pyrazine compounds were formed in heated sugar–amino acid and sugar–ammonia mixtures (Dawes and Edwards, 1966; Koehler *et al.*, 1969; Koehler and Odell, 1970). Formation of pyridine compounds has not been investigated. It was suggested that they probably were formed in a way analogous to the one leading to formation of pyrazines (Koehler and Odell, 1970). It has gained widespread acceptance that the amino acids and carbohydrates in meat are of extreme importance as flavor and odor precursors, and the products formed by the interaction of these materials during heating undoubtedly contributed to flavor and/or odor of cooked meat. The identified basic compounds might be one of the products formed through a Maillard-type interaction of the precursors in beef.

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LITERATURE CITED

Bender, A. E., Ballance, P. E., *J. Sci. Food Agr.* **12,** 683 (1961).
Benkeser, R. A., Holton, D. S., *J. Amer. Chem. Soc.* **73,** 5861 (1951).
Biemann, M., "Mass Spectrometry," McGraw-Hill, New York, N.Y., 1962, pp 130, 134–135.
Bondarovich, H. A., Friedel, P., Krampl, V., Renner, J. A., Shephard, F. W., Gianturco, M. A., J. AGR. FOOD CHEM. **15,** 1093 (1967).
Chang, S. S., Hirai, C., Reddy, B. R., Herz, K. O., Kato, A., *Chem. Ind. (London)* 1639 (1968).
Dawes, I. W., Edwards, R. A., *Chem. Ind. (London)* 2203 (1966).
Deck, R. E., Chang, S. S., *Chem. Ind. (London)* 1343 (1965).
Dietrich, P., Lederer, E., Winter, M., Stoll, M., *Helv. Chim. Acta* **47,** 1581 (1964).
Ferretti, A., Flanagan, V. P., Ruth, J. M., J. AGR. FOOD CHEM. **18,** 13 (1970).
Flament, I., Willhalm, B., Stoll, M., *Helv. Chim. Acta* **50,** 2233 (1967).
Goldman, I. M., Seibl, J., Flament, I., Gautschi, F., Winter, M., Willhalm, B., Stoll, M., *Helv. Chim. Acta* **50,** 694 (1967).
Hornstein, I., Crowe, P. F., J. AGR. FOOD CHEM. **8,** 494 (1960).
Hornstein, I., Crowe, P. F., J. AGR. FOOD CHEM. **11,** 147 (1963).
Hornstein, I., Crowe, P. F., Sulzbacher, W. L., J. AGR. FOOD CHEM. **8,** 65 (1960).
Koehler, P. E., Odell, G. V., J. AGR. FOOD CHEM. **18,** 895 (1970).
Koehler, P. E., Mason, M. E., Newell, J. A., J. AGR. FOOD CHEM. **17,** 393 (1969).
Kramlich, W. E., Pearson, A. M., *Food Res.* **25,** 712 (1960).
Macy, R. L., Jr., Naumann, H. D., Bailey, M. E., *J. Food Sci.* **29,** 136 (1964).
Mason, M. E., Johnson, B., J. AGR. FOOD CHEM. **14,** 454 (1966).
Marion, J. P., Müggler-Chavan, F., Viani, R., Bricout, J., Reymond, D., Egli, R. H., *Helv. Chim Acta* **50,** 1509 (1967).
Pepper, F. H., Pearson, A. M., *J. Food Sci.* **34,** 10 (1969).
Rizzi, G. P., J. AGR. FOOD CHEM. **15,** 549 (1967).
Sanderson, A., Pearson, A. M., Schweigert, B. S., J. AGR. FOOD CHEM. **14,** 245 (1966).
Tonsbeek, C. H. T., Plancken, A. J., v. d. Weerdhof, T., J. AGR. FOOD CHEM. **16,** 1016 (1968).
van Praagg, M., Stein, H. S., Tibbetts, M. S., J. AGR. FOOD CHEM. **16,** 1005 (1968).
Wang, P-S., Kato, H., Fujimaki, M., *Agr. Biol. Chem.* **33,** 1775 (1969).
Watanabe, K., Sato, Y., *Agr. Biol. Chem.* **35,** 756 (1971).
Yamato, T., Kurata, T., Kato, H., Fujimaki, M., *Agr. Biol. Chem.* **34,** 88 (1970).
Yueh, M. H., Strong, F. M., J. AGR. FOOD CHEM. **8,** 491 (1960).

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