

stituents could be expected as well. However, there are literature reports on the role of "fats," or lipids, in flavor of meat in which involvement of these extraneous factors was not considered. Yamato *et al.* (1970), for instance, state that "although raw beef fat does not possess a meaty aroma, it is produced on heating." These authors heated bovine leaf fat for 5 hr at 150°C. Since "bovine leaf fat" is adipose tissue, the origin of some of the compounds identified as coming from fat may be open to question.

While it is difficult to conclude from the data reported in this paper how the lipids and water-soluble components of adipose tissue interact to produce the characteristic meaty aroma, several mechanisms are suggested, as follows. *Species-specific compounds are formed on heating lipids.* The studies described here and the work of Pippen *et al.* (1969) with raw chicken lipids, however, suggest this does not occur. Pork was identified from its heated lipids, but the aroma produced was not characteristic of pork food products; it was a "piggy" odor, possibly from thermal degradation of unsaturated fatty acids in the lipid. *Interaction between compounds produced on heating both lipid and lean meat.* Pippen and Mecchi (1969) suggested H<sub>2</sub>S produced from amino acids or proteins reacted with carbonyls from thermal degradation of fatty acids to form

odors that contribute to chicken aroma. *Interaction directly between lean and lipids.* Browning reaction may occur directly between amino groups of the lean meat and carbonyl compounds in the lipids to produce odorous compounds. *Fats serve as solvent.* Aroma components, developed by thermal degradation of or reactions occurring in lean meat, may be dissolved in the lipid fraction. Pippen *et al.* (1969) claim this is the role of fat in chicken aroma. Other preformed fat-soluble components may be liberated as heat increases their vapor pressure. The "boar" aroma, described by Patterson (1968) as odor of 5 $\alpha$ -androst-16-ene-3-one, becomes evident when some pork fats are heated.

#### LITERATURE CITED

- Hornstein, I., Crowe, P. F., *J. Gas Chromatogr.* **2**, 128 (1964).  
 Patterson, R. L. S., *J. Sci. Food Agr.* **19**, 31 (1968).  
 Piez, K. A., Morris, L., *Anal. Biochem.* **1**, 187 (1960).  
 Pippen, E. L., Mecchi, E. P., *J. Food Sci.* **34**, 443 (1969).  
 Pippen, E. L., Mecchi, E. P., Nonaka, M., *J. Food Sci.* **34**, 436 (1969).  
 Wasserman, A. E., Gray, N., *J. Food Sci.* **30**, 801 (1965).  
 Wasserman, A. E., Talley, F., *J. Food Sci.* **33**, 219 (1968).  
 Yamato, T., Kurata, T., Kato, H., Fujimaki, M., *Agr. Biol. Chem.* **34**, 88 (1970).

Received for review July 15, 1971. Accepted October 13, 1971.

## Shallow-Fried Beef: Additional Flavor Components

Kenji Watanabe\* and Yasushi Sato

A flavor concentrate of shallow-fried beef was isolated and fractionated into 17 parts by silicic acid column chromatography. Two fractions possessed characteristic flavors, one reminiscent of heated beef odor and the other of sour cream-like odor after the roasted flavor, based on the basic compounds, was removed. The flavor components in these

fractions were isolated in pure state by gas chromatography. Identification was based on gas chromatographic retention times and mass spectra. Several alcohols, methional, 2-acetylfuran, 2-furfuryl methyl ketone, 1-methyl-2-acetylpyrrole, benzothiazole, acetol acetate, and *O*-hydroxyacetophenone were identified.

This series of investigations is ultimately aimed at identifying the volatile components responsible for beef flavor. The first two papers (Watanabe and Sato, 1968a,b) described  $\gamma$ - and  $\delta$ -lactones developed from beef fat. And then the various flavor compounds from heated beef fat were identified and their possible contribution to the heated beef fat flavor was discussed (Watanabe and Sato, 1971a). In a recent report, the heated flavor from the mixture of beef lean meat and fat was investigated, and some alkyl-substituted pyrazines and pyridines which might contribute to the roasted flavors were investigated (Watanabe and Sato, 1971b). This paper deals with the identification of potentially significant flavor compounds developed from the shallow-fried beef. Identification was based on gas chromatographic retention times and mass spectra.

Many literature references about work done elsewhere on beef flavor directly related to the present study have been cited previously (Watanabe and Sato, 1971b).

Laboratory of Food Science & Technology, Animal Products, Faculty of Agriculture, Nagoya University, Nagoya, Japan.

#### EXPERIMENTAL

**Materials and Preparation of Flavor Components.** The lean meat and fat used in this investigation were veal shoulder (20.0 kg) and kidney fat (2.0 kg). These samples were cut into small pieces and heated under the conditions of shallow frying. The procedures for the sample preparation and heating were essentially the same as those reported previously (Watanabe and Sato, 1971b). The flavor concentrate obtained was fractionated by silicic acid column chromatography. This procedure was used in the separation of flavor components obtained from green tea, and their better separations were described (Yamanishi *et al.*, 1970). The 23  $\times$  3 cm column was packed with 90 g of silicic acid (Mallinckrodt, 100 mesh). The column was successively eluted with hexane (250 ml, Fr. 1), hexane (250 ml, Fr. 2), various hexane solutions containing ethyl ether in the concentration described below, 1% (200 ml, Fr. 3), 2% (200 ml, Fr. 4), 3% (200 ml, Fr. 5), 4% (200 ml, Fr. 6), 5% (200 ml, Fr. 7), 6% (200 ml, Fr. 8), 8% (200 ml, Fr. 9), 10% (200 ml, Fr. 10), 20% (200 ml, Fr. 11), 20% (200 ml, Fr. 12), 20% (200 ml, Fr. 13), 30% (200 ml, Fr. 14), and 50% (200 ml, Fr. 15), ethyl ether (200 ml, Fr. 16), and, lastly, methanol (200 ml, Fr. 17). All of the solvents used were

purified by distillation. Each eluted solution was fractionally distilled and examined by the organoleptic method.

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

**Isolation by Gas Chromatography and Identification by Mass Spectrometry.** The gas chromatographic conditions for the fractionation of each flavor component were the same as described above except for the use of the thermal conductivity detector. The effluent from the gas chromatographic column was smelled and collected in the capillary tube cooled with Dry Ice in methanol. When the fractionated compounds were found to be in a mixture, they were rechromatographed with a 3-mm  $\times$  2-m column packed with 10% SE-30 on 60-80 mesh Chromosorb WAW, and collected again as described above. The isolated compounds were subjected to mass spectrometry. For mass spectral analyses, a RMU-6E mass spectrometer was used.

**Reference Compounds.** Several commercially unavailable compounds were synthesized. Methional (Pierson *et al.*, 1948), 2-furfuryl methyl ketone (Newman and Magerlein, 1957), 1-methyl-2-acetylpyrrole (Herz, 1957), and acetol acetate (Ferretti and Flanagan, 1971) were prepared by the published methods. The other reference compounds used were obtained from commercial sources.

## RESULTS AND DISCUSSION

The beef flavor concentrate was highly flavorful, and this flavor represented the original one of shallow-fried beef. Organoleptic tests on the fractions separated by column chromatography indicated that the various different odors resided in the beef flavor concentrate. Fr. 2 and 3: hydrocarbon type odors with a mercaptan-like aroma; Fr. 5, 6, 7, 8, and 9: oily, grassy and butyric acid-like odors; Fr. 11: heated meat-like odor; Fr. 10 and 12: slight heated meat-like odor; Fr. 13: caramel-like odor; Fr. 14: sweet odor; Fr. 16: mixed aroma of roasted and slight sour cream-like odors; Fr. 17: roasted and heated rubber-like odors; Fr.

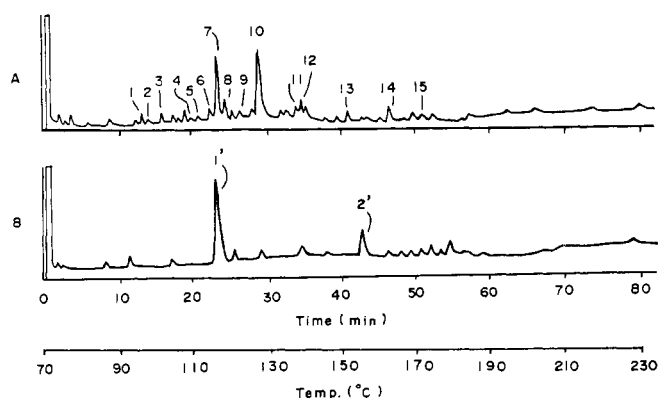


Figure 1. Gas chromatograms of Fr. 11 and Fr. 16'. For conditions see Experimental. (A: Fr. 11; B: Fr. 16')

1, 4, and 15: no special odor. It was estimated from the weight of concentrate in each fraction that the total amounts of concentrates of Fr. 5, 6, 7, 8, and 9 occupied the large part in the beef flavor concentrate. The main components in these fractions were carbonyl compounds, esters, and fatty acids. These compounds might be derived especially from the part of beef fat (Watanabe and Sato, 1971a). A sweet odor (Fr. 14) could be mainly attributed to  $\gamma$ - and  $\delta$ -lactones (Watanabe and Sato, 1968a,b, 1971a) and a roasted odor (Fr. 16 and 17) to basic compounds (Watanabe and Sato, 1971b). A slight sour cream-like odor in Fr. 16 was noticed clearly when the basic compounds were removed from Fr. 16 by washing with HCl solution. The fraction which had no basic compounds of Fr. 16 was named Fr. 16'. The flavor compounds in Fr. 11 and Fr. 16', which only occupied the small part in the beef flavor concentrate, were investigated in the present study because their fractions possessed the characteristic odors. The compounds in Fr. 13 will be reported in the near future.

Figure 1 shows gas chromatograms of Fr. 11 and Fr. 16'. The 14 new compounds identified are listed in Table I, which also shows gas chromatographic and mass spectral data.

In addition to a series of alcohols listed in Table I, *n*-hexanol (peak 3), a mixture of 1-octene-3-ol and *n*-heptanol (peak 7) and *n*-octanol (peak 10), which were identified previously

Table I. Flavor Components in Fr. 11 and Fr. 16'

Peak no. (Fr. 11)	Compound	Retention time (min)		Mass spectral peaks <sup>a</sup> M <sup>+</sup> /m/e	Mass spectral reference
		Sample	Reference		
1	3-Heptanol	13.8	13.9	116/59,69,41	b
2	2-Heptanol	14.8	14.9	116/45,43,55	c
4	4-Octanol	20.2	20.0	130/55,69,73	b
5	3-Octanol	21.2	21.4	130/59,55,83	c
6	Methional	22.8	22.4	104/48,47,104	
8	2-Acetylfuran	25.3	25.0	110/95,110,39	c
9	2-Furfuryl methyl ketone	27.9	27.5	124/81,43,53	c
11	1-Methyl-2-acetylpyrrole	34.5	34.5	123/108,123,53	d
12	1-Nonanol	34.7	34.9	144/41,55,43	b
13	1-Decanol	40.8	40.5	158/41,43,69	b
14	1-Undecanol	46.5	46.8	172/43,41,55	b
15	Benzothiazole	51.2	51.5	135/135,108,69	e
(Fr. 16')					
1'	Acetol acetate	22.7	22.9	116/43,86,116	d
2'	O-Hydroxyacetophenone	42.8	42.5	136/121,136,93	c

<sup>a</sup> The molecular ion peak and the three strongest mass spectral peaks are listed. <sup>b</sup> Friedel *et al.* (1956). <sup>c</sup> Stoll *et al.* (1967). <sup>d</sup> Ferretti and Flanagan (1971). <sup>e</sup> Scanlan *et al.* (1968).

in the flavor concentrate of heated beef fat (Watanabe and Sato, 1971a), could be detected also in this investigation. These alcohols were present as notable constituents of Fr. 11. However, they are unlikely to be of significance in the flavor of Fr. 11 as they have weak flavors and relatively high flavor thresholds. As is known by a large number of reports, alcohol can arise from the fat by mechanisms involving oxidation (Keeney, 1962). It is recognized that the fat used in this investigation was subjected to oxidation in the heating condition of shallow frying.

It is well known that methional possesses a broth-like odor (Patton, 1956; Patton and Barnes, 1958), and is formed from methionine by the Strecker degradation (Ballance, 1961). Although this compound has been reported to be of primary importance to irradiation off-odor in beef (Wick *et al.*, 1967), it has not been detected in heated meat flavor components because of its trace amounts and its decomposition to methyl mercaptan and acrolein (Ballance, 1961). Odor assessment of the observed peaks in gas chromatogram was of great help in identifying methional, and its typical odor was impressive when it eluted from the column. Methional has been found in roasted cocoa bean (van Praag *et al.*, 1968).

As shown by many model experiments, furan derivatives can be regarded as the typical products in the thermal degradation of carbohydrates (Fagerson, 1969) and the Maillard browning reaction (Hodge, 1967). 2-Acetylfuran and 2-furfuryl methyl ketone were identified as furan derivatives in the present investigation. The former have been found in heated glucose (Walter and Fagerson, 1968), lactose-casein model system (Ferretti *et al.*, 1970), sugar-amino acid system (Langner and Tobias, 1967), cocoa (Flament *et al.*, 1967), popcorn (Walradt *et al.*, 1970), and coffee (Stoll *et al.*, 1967), and the latter in deep fat-fried soybean (Wilkens and Lin, 1970b), lactose-casein model system (Ferretti *et al.*, 1970) and coffee (Stoll *et al.*, 1967). These compounds might also contribute significantly to the flavor of Fr. 11.

1-Methyl-2-acetylpyrrole is probably a Maillard browning reaction product. This compound has also been found in the degradation products from glucose in the presence of acetic acid and methylamine (Jurch, 1970), lactose-casein browning system (Ferretti and Flanagan, 1971), and coffee (Gianturco *et al.*, 1966).

The source of benzothiazole is unknown, although it has been found as a flavor component of heated milk (Scanlan *et al.*, 1968), stored casein (Ramshaw and Dunstone, 1969), soybean milk (Wilkens and Lin, 1970a), popcorn (Walradt *et al.*, 1970), and cocoa (Flament *et al.*, 1967). This compound has a characteristic rubber odor at a high concentration, and seems to be significant in the flavor of Fr. 11.

Odor appraisal of the effluent showed that no single peak was associated with the typical heated beef flavor in Fr. 11, although some compounds possessed characteristic odors. So, the typical part of heated beef flavor in Fr. 11 might be a complex sensation resulting from a mixture of several differ-

ent compounds: methional, 2-acetylfuran, 2-furfuryl methyl ketone, 1-methyl-2-acetylpyrrole, and benzothiazole, etc.

Acetol acetate and *O*-hydroxyacetophenone, which were the main constituents in Fr. 16', could be detected. The former has been found in lactose-casein browning system (Ferretti and Flanagan, 1971) and pineapple (Creveling *et al.*, 1968), and the latter in coffee (Stoll *et al.*, 1967) and cocoa (Flament *et al.*, 1967). These compounds might be related to the sour cream-like flavor.

#### ACKNOWLEDGMENT

The authors thank Kajuo Hayashi and Tetsuya Yanai, Hasegawa Co., Ltd., for their support.

#### LITERATURE CITED

- Ballance, P. E., *J. Sci. Food Agr.* **12**, 532 (1961).  
 Creveling, R. K., Silverstein, R. M., Jennings, W. G., *J. Food Sci.* **33**, 284 (1968).  
 Fagerson, I. S., *J. Agr. Food Chem.* **17**, 747 (1969).  
 Ferretti, A., Flanagan, V. P., *J. Agr. Food Chem.* **19**, 245 (1971).  
 Ferretti, A., Flanagan, V. P., Ruth, J. M., *J. Agr. Food Chem.* **18**, 13 (1970).  
 Flament, I., Willhalm, B., Stoll, B., *Helv. Chim. Acta* **50**, 2233 (1967).  
 Friedel, R. A., Shultz, J. L., Sharkey, A. G., Jr., *Anal. Chem.* **28**, 926 (1956).  
 Gianturco, M. A., Giammarino, A. S., Friedel, P., *Nature (London)* **210**, 1358 (1966).  
 Herz, W., *J. Org. Chem.* **22**, 1260 (1957).  
 Hodge, J. E., "Symposium on Foods. Chemistry and Physiology of Flavors," Schultz, H. W., Day, E. A., Libbey, L. M., Eds., AVI, Westport, Conn., 1967, p 465.  
 Jurch, G. R., Jr., *Carbohydr. Res.* **15**, 233 (1970).  
 Keeney, M., "Symposium of Foods. Lipids and Their Oxidation," Schultz, H. W., Day, E. A., Sinnhuber, R. O., Eds., AVI, Westport, Conn., 1962, p 79.  
 Langner, E. H., Tobias, J., *J. Food Sci.* **32**, 495 (1967).  
 Newman, M. S., Magerlein, B. J., "Organic Reactions," Adams, R., Bachmann, W. E., Blatt, A. H., Fieser, L. F., Johnson, J. R., Snyder, H. R., Eds., Wiley, New York, N.Y., 1957, p 413.  
 Patton, S., *Food Technol.* **10**, 60 (1956).  
 Patton, S., Barnes, I. J., *Food Res.* **23**, 221 (1958).  
 Pierson, E., Giella, M., Tishler, M., *J. Amer. Chem. Soc.* **70**, 1450 (1948).  
 Ramshaw, E. H., Dunstone, E. A., *J. Dairy Res.* **36**, 215 (1969).  
 Scanlan, R. A., Lindsay, R. C., Libbey, L. M., Day, E. A., *J. Dairy Sci.* **51**, 1001 (1968).  
 Stoll, M., Winter, M., Gautschi, F., Flament, I., Willhalm, B., *Helv. Chim. Acta* **50**, 628 (1967).  
 van Praag, M., Stein, H. S., Tibbetts, M. S., *J. Agr. Food Chem.* **16**, 1005 (1968).  
 Walradt, J. P., Lindsay, R. C., Libbey, L. M., *J. Agr. Food Chem.* **18**, 926 (1970).  
 Walter, R. H., Fagerson, I. S., *J. Food Sci.* **33**, 294 (1968).  
 Watanabe, K., Sato, Y., *Agr. Biol. Chem.* **32**, 191 (1968a).  
 Watanabe, K., Sato, Y., *Agr. Biol. Chem.* **32**, 1318 (1968b).  
 Watanabe, K., Sato, Y., *Agr. Biol. Chem.* **35**, 756 (1971a).  
 Watanabe, K., Sato, Y., *J. Agr. Food Chem.* **19**, 1017 (1971b).  
 Wick, E. L., Murray, E., Mizutani, J., Koshika, M., *Adv. in Chem. Ser. No. 65*, p 12 (1967).  
 Wilkens, W. F., Lin, F. M., *J. Agr. Food Chem.* **18**, 333 (1970a).  
 Wilkens, W. F., Lin, F. M., *J. Agr. Food Chem.* **18**, 337 (1970b).  
 Yamanishi, T., Nose, M., Nakatani, Y., *Agr. Biol. Chem.* **34**, 599 (1970).

Received for review September 2, 1971. Accepted November 8, 1971.