

## Isolation and Identification of Some Sulfur Chemicals Present in Pressure-Cooked Beef

Richard A. Wilson, Cynthia J. Mussinan, Ira Katz,\* and Anne Sanderson

Lean ground round beef was slurried with water and cooked in a sealed stainless steel vessel at 163 and 182°. Atmospheric distillation, followed by, in the case of the lower temperature product, continuous countercurrent ether extraction or, for the 182° material, salt saturation and continuous

liquid-liquid ether extraction, yielded extracts which, after concentration, were analyzed by gas-liquid chromatography (glc) and glc coupled with mass spectrometry (glc-ms). Forty-six sulfur-containing chemicals were identified.

Recent interest in improved meat flavors and the desirability of employing flavors comprised of naturally occurring components have led to the current investigations of the flavor volatiles in beef. Beef flavor, as well as that of other meats, has been shown to be the product of the heating of a complex system of flavor precursors. In this respect, the chemistry of meat flavors is different from that of fruit, dairy, or fermented flavors. In fact, it is this very difference which has determined to a great extent the approach to their study by many investigators.

A glycoprotein isolated by Batzer *et al.* (1960, 1962) produced an odor reminiscent of broiled steak when heated in fat with the coisolated nucleotide, inosinic acid. Subsequent investigations of aqueous extracts of raw and/or cooked beef by numerous groups have led many (Flament and Ohloff, 1971; Hornstein and Crowe, 1960; Mabrouk *et al.*, 1969; Macy *et al.*, 1964a,b; Schutte and Koenders, 1972; Tonsbeek *et al.*, 1969; Wasserman, 1972; Wasserman and Spinelli, 1972) to conclude that the development of meat flavor and aroma is, at least in part, associated with a Maillard-type reaction. As a result, there has been extensive study of model browning systems in attempts to duplicate or simulate meat flavors (Arroyo and Lillard, 1970; Heydenreich, 1971; Kitada *et al.*, 1970; Kyowa Fermentation Industry, 1968; May, 1960; May and Morton, 1961; Morton *et al.*, 1960; Rothe and Voight, 1963; Scheide, 1969; Wasserman and Spinelli, 1970). Raw beef has been thoroughly analyzed for its amino acid-sugar content (Bender *et al.*, 1958; Coassini-Lokar and Baradel, 1967; Mabrouk *et al.*, 1969; Macy *et al.*, 1964a, 1970; Wasserman and Gray, 1965; Wasserman and Spinelli, 1970, 1972; Wood, 1956, 1961; Wood and Bender, 1957) and many combinations have been formulated and subjected to various cooking conditions. The results, although indicative of the important contribution these reactions must play in the formation of meat flavors, have clearly demonstrated that the chemistry is more complex than originally thought. As a result, there has been increasing emphasis on the characterization of the flavor volatiles from cooked beef (Brennan and Bernhard, 1964; Halvarson, 1972; Watanabe and Sato, 1971b, 1972; Yueh and Strong, 1960). Hornstein and Crowe (1960) suggested that "... the characteristic flavor differences in pork and beef reside in the fat." Sanderson *et al.* (1966) amplified this to explain the flavor differences between roasted and boiled beef. This concept was expanded to include the organoleptic differentiation of beef, pork, lamb, and veal based on the fat-soluble flavor components (Wasserman and Talley, 1968). The volatile flavor components from heated meat fats have been intensively investigated (Nakanishi and Wa-

tanabe, 1966; Watanabe and Sato, 1968a,b, 1969a,b, 1970a,b, 1971a; Yamato *et al.*, 1970) and Pepper and Pearson (1969, 1971) and Wasserman and Spinelli (1972) have speculated concerning the possible role as flavor precursors of the water-soluble components of meat adipose tissues.

In 1968, Chang *et al.* reported the isolation from boiled beef of two heterocyclic compounds, 2,4,5-trimethyl- $\Delta^3$ -oxazoline and 3,5-dimethyl-1,2,4-trithiolane, which, although not novel to the synthetic organic chemist, were new in the field of flavor chemistry. These disclosures were followed closely by a report of the isolation of 4-hydroxy-5-methyl-3(2H)furanone and its 2,5-dimethyl homolog from beef broth (Tonsbeek *et al.*, 1968). Of particular interest in the recent literature have been the reports of sulfur compounds such as dimethyl sulfone (Liebich *et al.*, 1972), 2-acetyl-2-thiazoline (Tonsbeek *et al.*, 1971), and 1-methylthio-ethanethiol and thialdine (Brinkman *et al.*, 1972).

It now appears that the chemical pathways involved in the formation of meat flavor compounds must include not only Maillard browning reactions and fatty acid oxidation but also inter- and intramolecular cyclizations as well as numerous mechanisms which are made possible by the reactivity of such substances as ammonia, hydrogen sulfide, and mercaptans, and other undefined intermediates, especially at elevated temperatures.

It was the purpose of this research program to investigate cooked beef flavor with the goal of identifying new compounds which were formed as a result of these pathways. A review of the literature revealed that much of the previous work had been done on beef cooked under atmospheric conditions, *i.e.*, either in a boiled or pan-fried system. Initial efforts by our group confirmed much of the results of these investigations. Therefore, it was decided to cook at elevated temperatures in a sealed system; 182° for 15 min was selected. Also prepared and analyzed was

**Table I. Mercaptans Isolated by Alkaline Extraction Followed by AgNO<sub>3</sub>-Celite Chromatography**

Compound	Beef preparation in which found		<i>I<sub>E</sub></i> <sup>a</sup> on unknown	<i>I<sub>E</sub></i> <sup>a</sup> on known
	163°	182°		
3-Methyl-2-butanethiol	X	X	2.91 <sup>b</sup>	2.90 <sup>c</sup>
2-Methyl-1-butanethiol	X <sup>d</sup>	X	3.20 <sup>b</sup>	3.52 <sup>c</sup>

<sup>a</sup> The *I<sub>E</sub>* is the glc retention index relative to a series of ethyl esters of normal alkanolic acids. <sup>b</sup> *I<sub>E</sub>* on 10 ft × 1/8 in. stainless steel column packed with 5% Carbowax 20M, terminated with terephthalic acid, on 60-80 mesh, AW-DMCS Chromosorb G. <sup>c</sup> *I<sub>E</sub>* on 20 ft × 1/8 in. stainless steel column packed with 5% Carbowax 20M terminated with terephthalic acid, on 80-100 mesh, AW-DMCS Chromosorb G. <sup>d</sup> The identification was confirmed in the nonalkali-treated extract of the 163° sample.

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Table II. Sulfur Compounds Identified in the Beef Volatiles Extract

Compound	Beef preparation in which found		$I_E^a$ on unknown	$I_E^a$ on reference	Source of reference chemical
	163°	182°			
Mercaptans					
Methyl mercaptan	×		0.93 <sup>b</sup>	<1.00 <sup>b</sup>	Commercial
Isobutyl mercaptan	×		2.69 <sup>b</sup>	2.65 <sup>b</sup>	Commercial
Naphthalene thiol <sup>c</sup>	×	×	10.32 <sup>b</sup>	10.93 <sup>d</sup>	Commercial
Sulfides and disulfides					
Methyl sulfide	×		<1.00	0.65	Commercial
Methyl disulfide	×	×	4.55	4.48	Commercial
Ethyl methyl disulfide	×	×	4.58 <sup>b</sup>	4.43 <sup>b</sup>	Mukaiyama and Takahashi (1968) <sup>e</sup>
Methyl vinyl disulfide <sup>f</sup>	×		6.4		No reference compound available
Ethyl disulfide		×	5.94	6.00	Commercial
Thiophenes					
Thiophene		×	4.02	4.00	Commercial
2-Methyl thiophene	×	×	4.62	4.61	Commercial
2-Ethyl thiophene	×	×	4.72 <sup>b</sup>	4.75 <sup>b</sup>	Commercial
2- <i>n</i> -Butyl thiophene	×		7.32	7.34	Grey <i>et al.</i> (1960)
2- <i>n</i> -Amyl thiophene	×		8.25	8.27	Grey <i>et al.</i> (1960)
<i>n</i> -Octyl thiophene <sup>g</sup>		×	10.50		No reference compound available
<i>n</i> -Tetradecyl thiophene <sup>g</sup>	×	×	18.00		No reference compound available
2-Thiophene carboxaldehyde	×	×	10.45	10.54	Commercial
3-Thiophene carboxaldehyde	×	×	6.27 <sup>b</sup>	6.27 <sup>b</sup>	Commercial
5-Methyl-2-thiophene carboxaldehyde	×	×	11.43	11.43	Commercial
2,5-Dimethyl-3-thiophene carboxaldehyde	×	×	8.10 <sup>b</sup>	8.23 <sup>b</sup>	Weston and Michaels (1950)
2-Thiophene acrolein		×	9.56 <sup>b</sup>	9.58 <sup>b</sup>	Hari (1958) <sup>e</sup>
2-Acetylthiophene	×	×	11.31	11.44	Commercial
3-Acetylthiophene <sup>f</sup>	×	×	11.50		No reference compound available
5-Methyl-2-acetylthiophene	×	×	12.29	12.38	Farrar and Levine (1950)
1-(2-Thienyl)-1-propanone <sup>i</sup>	×		11.61	12.06	Heid and Levine (1948) <sup>e</sup>
1-(2-Methyl-5-thienyl)-1-propanone <sup>i</sup>	×		9.15 <sup>b</sup>	9.43 <sup>b</sup>	Heid and Levine (1948) <sup>e</sup>
2-Thiophene methanol	×	×	12.86	12.67	Commercial
Tetrahydrothiophen-3-one	×	×	9.31	9.29	Commercial
2-Methyl tetrahydrothiophen-3-one	×	×	9.00	9.09	Woodward and Eastman (1946)
Thiazoles					
Thiazole		×	6.36	6.21	Commercial
2-Methylthiazole	×	×	6.15	6.08	Hantzsch (1888)
4-Methylthiazole	×	×	6.55	6.55	Clarke and Gurin (1935)
2,4-Dimethylthiazole	×	×	6.56	6.49	Commercial
5-Ethyl-4-methylthiazole <sup>i</sup>	×		8.10	8.45	Buchman (1936) <sup>e</sup>
4-Ethyl-2-methylthiazole	×	×	7.23	7.23	Hantzsch (1888) <sup>e</sup>
2,4,5-Trimethylthiazole	×	×	6.10 <sup>b</sup>	6.12 <sup>b</sup>	Hantzsch (1890)
2,4-Dimethyl-5-vinylthiazole <sup>f</sup>	×		7.20 <sup>b</sup>		No reference compound available
2-Acetylthiazole <sup>f</sup>	×		10.15	10.18	Pittet (1972)
Benzothiazole <sup>i</sup>	×		13.36	12.91	Commercial
Polysulfur heterocycles					
3,5-Dimethyl-1,2,4-trithiolane (2-isomers)	×	×	9.78 <sup>i</sup>	9.69 <sup>i</sup>	Assinger <i>et al.</i> (1959)
5,6-Dihydro-2,4,6-trimethyl-1,3,5-dithiazine (thialdine)	×	×	11.38	11.44	Marckwald (1886)
2,4,6-Trimethyl- <i>s</i> -trithiane (trithioacetaldehyde)		×	9.62 <sup>b</sup>	9.61 <sup>b</sup>	Baumann and Fromm (1889)
2,2,4,4,6,6-Hexamethyl- <i>s</i> -trithiane (trithioacetone)		×	9.72 <sup>b</sup>	9.77 <sup>b</sup>	Baumann and Fromm (1889)
Miscellaneous					
Methylthioacetate	×		3.00 <sup>b</sup>	3.00 <sup>b</sup>	Commercial
1,1-Bis-methylthioethane	×		6.73	6.66	Yamanishi <i>et al.</i> (1952)

<sup>a</sup>  $I_E$  values are for a 500 ft × 0.03 in. Carbowax 20M stainless steel open-tubular column unless noted otherwise. <sup>b</sup>  $I_E$  on a 500 ft × 0.03 in. SF-96 stainless steel open-tubular column. <sup>c</sup> Isomer uncertain. Tentative identification. <sup>d</sup>  $I_E$  on 2-naphthalenethiol on a 500 ft × 0.03 in. SF-96 stainless steel open-tubular column. <sup>e</sup> An extension of the published procedure was employed. <sup>f</sup> No reference compound available. Tentative identification. <sup>g</sup> The position of the alkyl moiety is uncertain. <sup>h</sup> Weak mass spectrum. Tentative identification. <sup>i</sup> Poor correlation of retention indices. Tentative identification. <sup>j</sup> The  $I_E$  is for the first isomer.

the 163° sample described by Mussinan *et al.* (1973). The choice of these parameters was arbitrary; however, it was felt that they represented temperatures normally encountered during the roasting of beef. The one condition of the heating process which might be considered nonrepresenta-

tive was the sealed vessel which was employed to attain the elevated temperatures in an aqueous system.

#### EXPERIMENTAL SECTION

With the exception of the temperature, the cooking pro-

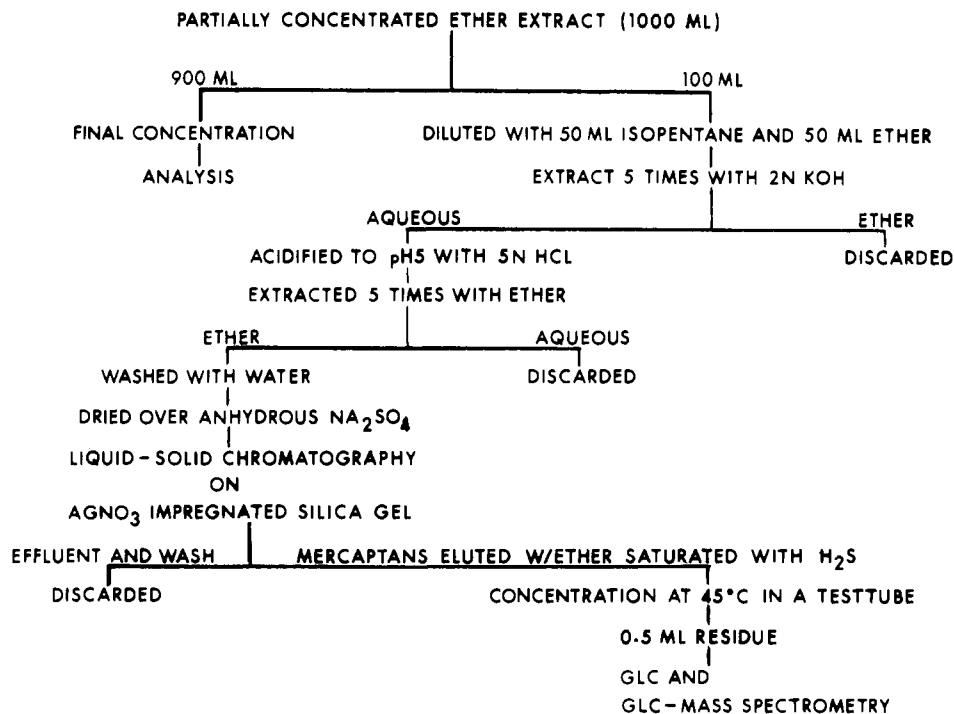


Figure 1. Scheme for the isolation of mercaptans.

cedure was identical for both preparations. The color of each was dark brown, resembling the interior of well-done hamburger loaf, and the odors were similar, reminiscent of well-done lean roast beef. The flavor extract of the higher temperature sample was prepared by exhaustive atmospheric distillation of the aqueous beef slurry, saturation of the distillate with sodium chloride, and continuous liquid-liquid extraction with distilled diethyl ether. Concentration of the extract and analysis of the residue were essentially as previously described (Mussinan *et al.*, 1973), with the following exception.

One-tenth of the partially concentrated ether extract was subjected to selective analysis for mercaptans, as shown in Figure 1. The residue was analyzed by glc-ms using a Hewlett-Packard 5750B gas chromatograph coupled to a Hitachi RMU-6E mass spectrometer. A 10 ft  $\times$   $\frac{1}{8}$  in o.d. stainless steel column packed with 5% Carbowax 20M, terminated with terephthalic acid, on 60-80 mesh AW-DMCS Chromosorb G was used.

As an aid in the detection of sulfur compounds, the mercaptan concentrate and the residue from the untreated extract were also analyzed by glc using a Tracor MT-220 gas chromatograph equipped with a flame photometric detector operated in the sulfur sensitive mode. This greatly facilitated the interpretation of mass spectra, especially where mixed spectra were involved.

## RESULTS

Two sulfur-containing peaks were detected in the mercaptan isolate. Both had molecular weights of 104. The isotope ratios ( $M^+ + 2 = 4.4\%$ /S atom) indicated each contained one sulfur atom, and the fragmentation patterns suggested branched-chain mercaptans which were subsequently identified by comparison with commercially available samples, as shown in Table I.

The sulfur compounds which were identified in the nonalkali-treated extract are shown in Table II. These include three mercaptans not detected by the method just described.

In summary, flavor concentrates from beef cooked su-

peratmospherially were prepared. Subsequent analysis by glc and glc-ms yielded 46 sulfur-containing compounds which included mercaptans, mono- and disulfides, thiophenes, thiazoles, polysulfur heterocycles, a thioester, and a thioacetal.

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## Potential Precursors of *N*-Nitrosopyrrolidine in Bacon and Other Fried Foods

Donald D. Bills,\* Kjell I. Hildrum, Richard A. Scanlan, and Leonard M. Libbey

The possibility of producing *N*-nitrosopyrrolidine from several compounds in a system designed to simulate the frying of fatty foods containing small amounts of water was investigated. With the exception of tests on *N*-nitrosoproline, equimolar amounts of the compound to be tested and sodium nitrite were added to the system prior to

heating. With a heat-up time of 10 min plus 10 min at 170°, *N*-nitrosopyrrolidine was produced from *N*-nitrosoproline, pyrrolidine, spermidine, proline, and putrescine in yields of 2.6, 1.0, 1.0, 0.4, and 0.04% theoretical, respectively. *N*-Nitrosopyrrolidine was not produced from glutamine, glutamic acid, and hydroxyproline.

*N*-Nitrosopyrrolidine (NPY), a carcinogen, has been isolated from fried bacon as reported in FDA Papers (1972), by Crosby *et al.* (1972), and by Sen *et al.* (1973). The first reference indicates that preliminary studies on four different brands of bacon showed that NPY was formed when the bacon was cooked in a conventional manner but was not present in raw bacon. Levels ranged from 30 to 160 ppb in the cooked product. Crosby *et al.* found NPY in 13 of 24 samples of fried bacon of various origins. In the positive samples, concentrations ranged from a trace (<1 ppb) to 40 ppb. In addition to NPY, Crosby *et al.* also reported finding the *N*-nitroso derivatives of dimethylamine, diethylamine, and piperidine in some samples of fried bacon. In 8 of 16 samples of fried and raw bacon, Sen *et al.* found NPY in concentrations of 4 to 25 ppb and dimethylnitrosamine in six samples in concentrations of 2 to 30 ppb. NPY was not found in any uncooked samples but dimethylnitrosamine was found in one.

Lijinsky and Epstein (1970) speculated that the cooking of foods might result in the formation of certain secondary amines. Pyrolysis of protein could yield proline. Putrescine, to the extent that it might be present in a whole-

some food product, could be converted to pyrrolidine. In the presence of nitrite, such secondary amines may be nitrosated under certain conditions to form the corresponding *N*-nitroso derivative.

The purpose of this investigation was to evaluate some possible precursors for the production of NPY under simulated conditions of pan-frying meat products such as bacon.

### EXPERIMENTAL PROCEDURES

**Heating System.** To simulate the physical conditions present in a system such as bacon being pan-fried at a pan temperature of 170°, a 200-ml balloon flask was fitted with a reflux condenser. Into the flask was introduced 100 ml of steam-stripped Wesson oil (processed soybean and cottonseed oil), 1 ml of water, a boiling chip, an internal standard (40 mg of methyl myristate), and 0.005 mol of the compound to be tested. With the exception of tests for the production of *N*-nitrosopyrrolidine from *N*-nitrosoproline, 0.005 mol of sodium nitrite was also added to the system. The balloon flask was immersed in a bath of silicone oil heated on a hot plate. The temperature of the contents of the flask was brought to 170° in about 10 min and held at 170° for an additional 10 min. During the heating period the water in the system was under constant

\* Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331.