were produced. Chromatogram C shows that a portion of the added 2-pentanone was converted to 2-pentanol, and in chromatogram D it can be seen that 2-pentanol was oxidized to 2-pentanone.

Table V summarizes the ketone-alcohol conversions observed and the compounds identified in the culture headspaces. The *Mycoderma*, *Geotrichum*, *Torulopsis*, and *Penicillium* cultures actively interconverted 2-pentanone and 2-pentanol, and acetone and 2-propanol. Headspace of the *Mycoderma* and *Torulopsis* cultures contained large amounts of ethyl acetate. *S. lactis* had no effect on any substrate, and *B. linens* could only convert 2-pentanol to 2-pentanone. The *B. linens* cultures had a very strong putrid aroma; however, no peaks other than the added substrates were present in the chromatograms. *P. roqueforti* mycelia were much more active than the spores in reducing the ketone to the alcohol. It appears that yeasts associated with Blue cheese ripening could influence the formation of secondary alcohols as well as produce certain esters and alcohols.

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BEEF COMPONENTS

Effect of Cooking Procedure on Flavor Components of Beef. Carbonyl Compounds

ANNE SANDERSON, A. M. PEARSON, and B. S. SCHWEIGERT

Department of Food Science, Michigan State University, East Lansing, Mich.

Gas-liquid chromatography was employed to determine the carbonyl compounds resulting from beef cooked by two procedures. Aldehydes and ketones were collected as their 2,4-dinitrophenylhydrazine derivatives and regenerated as carbonyls with levulinic acid for injection into the chromatograph. Tentative identifications were made by comparing the retention times with known carbonyl compounds using two different column packings. Beef cooked with water gave the same number of aldehydes and ketones as beef cooked in fat, although in varying amounts. Results suggest that the characteristic differences in flavor and aroma of roasted and boiled beef may arise from the volatile carbonyl compounds.

THE question of origin of volatile flavor components has been a topic of wide speculation. Many workers agree that the flavor precursors can be extracted from raw meat with water (*1, 3, 5, 8*), and that the characteristic meat flavors can be produced by heating the isolated precursors with fat (*1, 3, 4*). Hornstein and Crowe (*3, 6*) suggested that the isolated precursors from most meats produce a similar aroma regardless of the species, while the characteristic odor of a particular meat is due to the fat. Researchers have isolated and identified volatile carbonyl compounds during cooking of beef (*6, 9*), chicken (*10, 12, 13*), pork (*3*), cured meats (*2, 11*), and lamb (*4*). Hornstein and his associates (*3, 4, 6*) determined the volatile carbonyl compounds evolved on heating the water-soluble precursors and then the previously separated fat.

The present investigation was undertaken to compare the amount and nature of the carbonyl compounds produced upon cooking beef in water and fat.

Apparatus and Materials

The meat used in this study was removed from the lumbar region of the longissimus dorsi muscle of 20-month-old cattle (U. S. Choice and Prime grade). The meat was finely ground and stored at −20° C. prior to cooking.

Figure 1 shows the cooking apparatus used for water-cooked beef. The apparatus for fat-cooked beef was identical, except that no condenser was necessary. After cooking, the regenerated carbonyl compounds were chromatographed on a Barber-Colman Model 20 gas chromatograph equipped with a radium ionization detector. A 6-foot, $^1/_4$-inch o.d. copper column packed with 10% diisodecyl phthalate on acid-washed Diaport W (60/80-mesh) separated mixtures of known aldehydes and ketones satisfactorily. Ockerman, Blumer, and Craig (*11*) reported good results using similar procedures for separating regenerated carbonyl compounds from cured ham. The column was operated at 100° C., with the flash heater and detector cell temperatures at 150° C. Argon was utilized as the carrier gas at 8 p.s.i., giving a calculated flow rate of 43.5 ml. per minute through the column. A voltage setting of 1250 volts was used.

A second column of 6-foot, $^1/_4$-inch o.d. copper tubing packed with 10% Carbowax 20M on acid-washed Diaport W (60/80-mesh) was prepared for confirmatory identification of the volatile aldehydes and ketones from cooked beef. It was operated at 75° C. with the detector cell and flash heater at 155° C. As before, the cell voltage was 1250 volts and the argon carrier gas was 8 p.s.i., giving a flow rate of 43.5 ml. per minute through the column.

Experimental Procedure

A total of 500 grams of ground beef was cooked with 1000 ml. of distilled water in the cooking apparatus (Figure 1). All volatile constituents were removed by constantly sweeping the surface of the cooking slurry with nitrogen gas and then trapping the volatiles in a series of reagent traps. The volatiles were bubbled through a capillary tube into two traps containing a saturated solution of

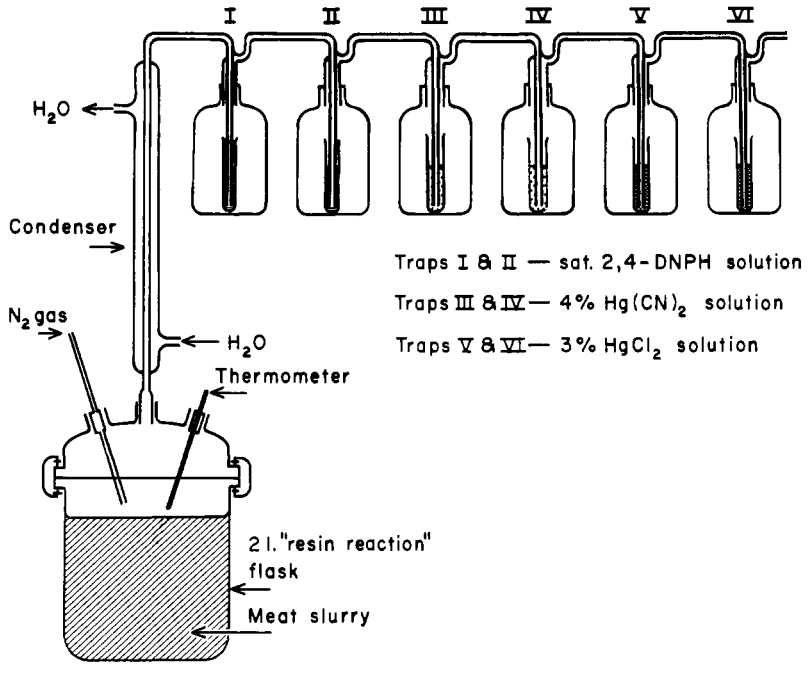

Figure 1. Schematic diagram of cooking apparatus and trapping system used for collection of volatile components from beef cooked in water

2,4-dinitrophenylhydrazine (2,4-DNPH) made up according to Pippen *et al.* (*13*), then through two traps containing 4% mercuric cyanide and through two traps of 3% mercuric chloride. The sulfur compounds precipitated in the last four traps were retained for further investigation. The slurry was slowly brought to the boiling point and then cooked for 8 hours. At the end of this time the off-odors emanating from the meat were considered unlike cooking beef and cooking was discontinued.

The volatile components from fat-cooked beef were collected by the procedure used when cooking the beef in water. A total of 500 grams of fat was melted and placed in the cooking apparatus to prevent sticking or burning prior to addition of 500 grams of ground beef. The temperature of the volatiles never exceeded 100° C. To rule out the possibility that the fat was breaking down to contribute significant amounts of carbonyl compounds on heating, an identical sample of ground beef fat was heated without the addition of ground beef. The quantity of carbonyls collected was too small to permit identification of the regenerated carbonyls.

After the 8-hour cooking period, the traps were disconnected and the orange precipitate of mixed 2,4-dinitrophenylhydrazones (2,4-DNPS) was centrifuged, washed with 2*N* HCl and distilled water, and then dried in a desiccator. The 2,4-DNPS yield from six water-cooked and six fat-cooked samples was recorded. The carbonyl compounds were regenerated from their 2,4-DNPS with 5 ml. of levulinic acid mixture (*7*) and 1 ml. of distilled water in a distillation flask. As the temperature of the flask was raised very slowly over a period of $\frac{1}{2}$ hour to 100° C. in an oil bath, the levulinic acid exchanged with the 2,4-DNPS, liberating the free carbonyls. If regeneration was too fast, levulinic acid breakdown occurred. The aldehydes and ketones, which were freed by this procedure, were distilled over, along with a small amount of water, into a small tube chilled in ice water. Since the gas chromatograph was not suited for samples containing water, the carbonyls were then extracted with an organic solvent. Most of the more commonly used solvents, such as ethyl ether, were unsatisfactory, as they masked the earlier emerging carbonyl peaks on the chromatograph. Methyl phenyl ether was finally chosen as a suitable solvent, as it emerged from the column at least 25 minutes after the last of the carbonyl peaks from the regenerated sample. This procedure was chosen, as the extremely small amounts of 2,4-DNPS obtained could be successfully regenerated and separated by gas chromatography.

Retention times of known carbonyls were employed to characterize the unknown carbonyls using both columns.

Table I. Yield of 2,4-DNPS by Two Methods of Cooking

	Grams of 2,4-DNPS from 500 Grams of Beef	
Replication	Cooked in water	Cooked in fat
1	0.126	0.161
2	0.049	0.190
3	0.033	0.040
4	0.009	0.107
5	0.054	0.256
6	0.048	0.287
Mean	0.05	0.17

Results and Discussion

The yield of 2,4-DNPS from the beef cooked in fat was three times as large as the yield on cooking in water, with means of 0.17 and 0.05 gram, respectively. The data for each replication of both cooking treatments are presented in Table I. It is possible that the condensor used when cooking in water may have caused small amounts of carbonyl compounds to be retained with the refluxing water and not carried over with the other volatile components. However, the fat itself may have contributed carbonyl compounds, since other workers (*3*, *4*) have reported the origin of carbonyls from fat.

Immediately after regeneration of the 2,4-DNPS with levulinic acid, the carbonyl compounds released from the

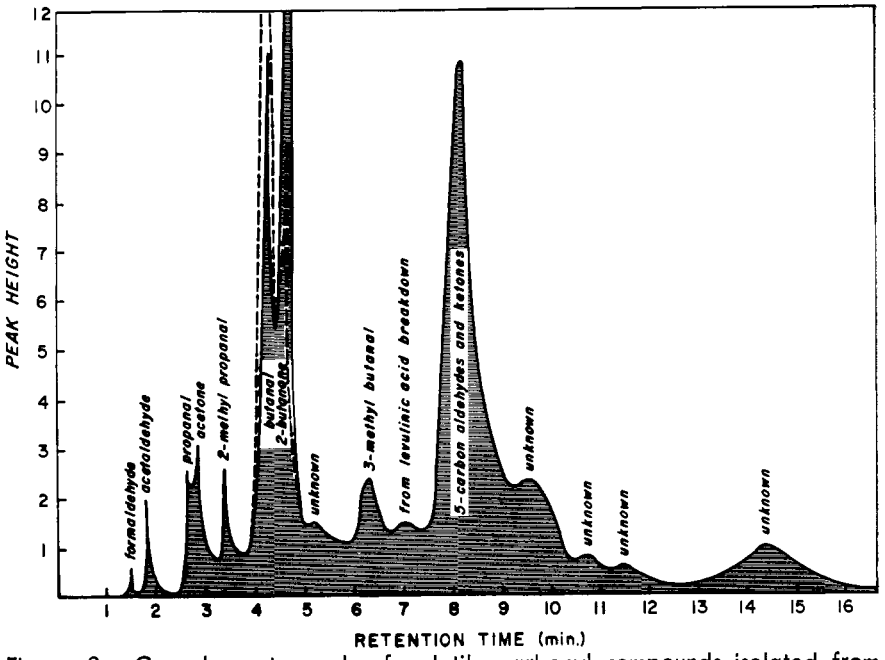

Figure 2. Gas chromatograph of volatile carbonyl compounds isolated from beef cooked in fat

Broken lines indicate where volatile carbonyls from beef cooked in water differed from that of beef cooked in fat. Made with diisodecyl phthalate column at 100°C.

Table II. Retention Times of Carbonyl Compounds Regenerated from 2,4-DNPS by Exchange with Levulinic Acid

Carbonyl Compound	Retention Time on Column, Min.			
	Diisodecyl Phthalate Column at 100° C.		Carbowax 20M Column at 75° C.	
	Unknown	Known	Unknown	Known
Formaldehyde	1.5	1.5	2.0	2.1
Acetaldehyde	1.8	2.0	3.4	3.4
Propanal	2.6	2.7	5.2	5.1
Acetone	2.8	2.8	5.9	5.9
2-Methylpropanal	3.4	3.4	6.8	6.6
Butanal	4.2	4.3	8.4	8.3
2-Butanone	4.6	4.5	9.7	9.7
3-Methylbutanal	6.3	6.2	11.0	10.8
5-Carbon carbonyls	8.1	8.2	16.0	15.7

beef cooked in water and fat had a sweet, faint caramel odor. Formaldehyde, acetaldehyde, propanal, acetone, 2-methylpropanal, butanal, 2-butanone, and 3-methyl butanal were identified from both fat-cooked and water-cooked beef. Another peak corresponding to the 5-carbon aldehydes and ketones, which could contain pentanal, 2-pentanone, 3-pentanone, or any combination of the three compounds, was observed. As 2,3-butanedione and other known di- and polycarbonyl compounds gave either broad or reverse peaks, they could not be used for identification. Five small peaks were also obtained but, because of the small sample size, tentative identification was not possible. The water-cooked beef yielded more butanal than 2-butanone, whereas the fat-cooked sample usually had more 2-butanone than butanal and was less consistent in the amounts of carbonyls volatilized. Hornstein and Crowe (*4*) noted similar variation in volatile carbonyl compounds released from heated lamb fat. A typical chromatograph of the carbonyl compounds from beef cooked in water and fat is shown in Figure 2.

Although beef cooked in fat has a greater yield of volatile carbonyls than water-cooked beef, both cooking procedures produced the same number of aldehydes and ketones in varying proportions. Butanal, 2-butanone, and the 5-carbon carbonyls were found to be the major carbonyl compounds, with smaller amounts of 3-methylbutanal, 2-methylpropanal, acetaldehyde, formaldehyde, acetone, and propanal. The low concentrations of formaldehyde and acetaldehyde, especially the former, were undoubtedly due to losses by volatilization. Sweeping the surface of the cooking meat slurry with air or oxygen instead of nitrogen might have produced a greater yield of carbonyl compounds. However, neither air nor oxygen entrainment, nor even vacuum distillation of flavor volatiles, paralleled so-called "normal" cooking any more closely than nitrogen entrainment. Perhaps the differences in the flavor volatiles produced by beef cooked in water and fat may arise from the quantity and composition of the aldehydes and ketones released on heating. However, no attempts were made in the present study to relate the differences in the flavor volatiles associated with the two methods of cookery to the different flavors developed.

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FLAVOR COMPOUND

Isolation of *S*-Methyl Methionine Sulfonium Salt from Fresh Tomatoes

FRANCIS F. WONG and J. F. CARSON

Western Regional Research Laboratory, Western Utilization Research and Development Division, U. S. Department of Agriculture, Albany, Calif.

A sulfonium compound has been isolated from fresh tomato fruit. Infrared spectra, melting points, and paper chromatograms establish that it is a salt of S-methyl methionine sulfonium. The compound decomposes to yield homoserine and dimethyl sulfide. The behavior of the isolated sulfonium salt and its concentration in the tomato suggest it is an important precursor in tomato aroma.

INCREASING RESEARCH in flavors is uncovering organic sulfur compounds that are important volatile flavor components of foods (*2, 6, 10*). Although these compounds are by nature malodorous in large quantities, trace quantities are often pleasant. The principal flavor component of garlic (*Allium sativum*), for example, has been reported to be allylthio allylsulfinate (*4*); those of onions (*Allium cepa*) are methyl and *n*-propyl thiosulfinates and corresponding disulfides (*2*). Vegetables of the *Brassica* species (cabbage, etc.) give off varying amounts of methyl sulfide as well as isothiocyanates, which are found also in radishes (*8, 23*). Small amounts of methyl sulfide are given off by raw tomato fruit and appreciable amounts are produced during cooking or prolonged heating (*12*). Indeed, there is strong evidence that methyl sulfide is the principal volatile flavor component of processed tomato products (*12*).

Sulfur-bearing amino acids and their derivatives have drawn renewed interest, especially as precursors of volatile sulfur components of flavor (*19*). Allyl cysteine sulfoxide has been shown to be the flavor precursor in garlic (*16*).