EFFECT OF COOKING PROCEDURE ON THE FLAVOR COMPONENTS OF BEEF

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ABSTRACT

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by Anne Sanderson

The chemical components volatilized when beef was cooked in water were compared and contrasted with those obtained on cooking beef in fat. Equal quantities of fat-free beef were cooked by the two procedures for eight hours in an inert atmosphere. The flavor volatiles produced were swept through a series of reagent traps with nitrogen gas. Special emphasis was placed on the carbonyl and sulfur compounds in the flavor volatiles produced by both methods of cooking.

The carbonyl compounds in the flavor volatiles were precipitated as their 2,4-dinitrophenylhydrazones (2,4-DNPS). The yield of 2,4-DNPS from beef cooked in fat was three times as large as that from beef cooked in water, with mean values of 0.17 g. and 0.05 g., respectively. An equivalent amount of beef fat cooked alone yielded less than 0.01 g.

The aldehydes and ketones were released from their 2,4-DNPS by exchange with levulinic acid. The carbonyl compounds from beef cooked in both fat and water had a sweet and faintly caramel aroma, which was indicative of their role in producing a cooked beef aroma. When chromatographed, the carbonyl compounds from beef cooked in water and fat separated into 14 peaks. Butanal, 2-butanone and the 5-carbon carbonyls were identified as the major components of the carbonyl compounds volatilized by both cooking procedures. Smaller amounts of formaldehyde, acetaldehyde, propanal, acetone, 2-methyl propanal and 3-methyl butanal were shown to be present. Five smaller peaks, too small to identify,

were also obtained. In addition there was one small peak which was found to be a breakdown product of levulinic acid.

Chemical tests for polycarbonyl compounds, such as 2,3-butanedione, with 2% alcoholic potassium hydroxide showed that polycarbonyls were not present in the 2,4-DNPS derivatives from the flavor volatiles produced by both methods of cooking. Other chemical tests for confirmatory identification of the aldehydes and ketones proved unsatisfactory.

Although beef cooked in fat yielded the same number and type of aldehydes and ketones as beef cooked in water, the quantities of carbonyl compounds volatilized varied with cooking procedure. Beef cooked in water consistently yielded less 2-butanone than butanal, whereas, beef cooked in fat produced more 2-butanone than butanal. Since 2-butanone was the carbonyl compound found in greatest proportion on heating fat alone, it appears that the increase in 2-butanone in the flavor volatiles of beef cooked in fat was mainly due to the fat. However, smaller quantities of 2-butanone were found in the flavor volatiles obtained on cooking beef in water, together with larger amounts of butanal, so it appears that these two carbonyls play an important role in producing typical beef aroma and flavor.

Hydrogen sulfide and the mercaptans in the flavor volatiles were precipitated in a reagent trap containing 4% mercuric cyanide, while any volatile sulfides passed through this trap and were precipitated in 3% mercuric chloride. The mercaptans were released from their corresponding mercuric mercaptides with acid and then were oxidized to disulfides with iodine in ether for easier separation and identification. Only one mercaptan was identified in the flavor volatiles of beef cooked in both fat and water; namely methyl mercaptan. The black hydrogen sulfide precipitate remained in the reaction flask as it was not attacked by acid.

Identification of the precipitated sulfides by most normal techniques was not possible due to their extremely small amounts. On regeneration of the sulfides with concentrated sodium hydroxide, the sulfide in greatest proportion was determined by its characteristic odor. Dimethyl sulfide was tentatively identified in this manner.

Although no differences were found in the number and type of sulfur compounds in the flavor volatiles of beef cooked in both water and fat, there is no doubt that slight variations in the concentration of methyl mercaptan, hydrogen sulfide and/or dimethyl sulfide could account for some of the flavor differences. This is especially true since the aroma and flavor of any sulfur compound is very dependent on concentration. When instrumentation and techniques have been developed to measure the minute amounts of methyl mercaptan and dimethyl sulfide and their interaction with the carbonyl compounds, it should be possible to identify the exact compound or compounds responsible for the difference in flavor between beef cooked in fat and water.

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INTRODUCTION

With the expanding population of the modern world, problems of an insufficient food supply have already arisen. It is becoming increasingly apparent that the use of animals as a major source of food is inefficient and costly, and that in future man may have to rely more heavily on plant material. However, it is likely that man will be reluctant to relinquish his taste for meat, and for beef in particular. Consequently, it is necessary to elucidate the exact nature of the flavor constituents that make meat palatable. By the turn of the century, much of our "meat" will probably consist of protein derived from plant sources with chemicals being added to produce the desired flavors. There have been several attempts to produce "synthetic meat". Although the texture is similar, the flavor leaves much to be desired.

The characteristic aroma and flavor of meat have long interested the researcher. Since the field of flavor chemistry began over a century ago, the question of origin of the volatile flavor components has been a topic of wide speculation. Raw meat has little flavor with the characteristic "meaty" aroma developing on heating. Flavor precursors can be extracted from raw meat with water and the characteristic odor of meat can be produced by heating the isolated precursors in fat, (Batzer *et al.*, 1962). Hornstein and Crowe (1964) suggested that the aroma derived on heating the water-soluble precursors from meat is the same regardless of the type of meat, while the characteristic species flavor differences are due to the contribution of the volatiles derived from the fatty tissues.

The composition and quantity of flavor volatiles varies with the method of cooking. Both temperature and time of cooking influence the yield of the volatile components. The quantity and composition of the flavor volatiles is also affected by the collection conditions. This was demonstrated by Lineweaver and Pippen (1961) who showed that the amount and type of volatile carbonyls could be varied by cooking in an inert atmosphere of nitrogen, an oxidative atmosphere of oxygen or by distillation. The influence of fat on the yield of flavor volatiles is of special interest since there are large amounts of fat present in meat, which readily undergo oxidation on heating.

The present study was undertaken to compare and contrast the flavor volatiles derived from beef cooked in water and in fat. Particular emphasis was placed on the identification of the carbonyl and the sulfur compounds.

LITERATURE REVIEW

Meat Flavor

The definition of flavor is as complex as the sensation itself. Of the many definitions, that of Kazeniac (1961) appears to be the most appropriate. He described flavor as a combination of taste, aroma, body or texture and mouth satisfaction.

In 1847 Justus von Leibig published the first analysis on meat extract. He found no difference between the flavor of meat from ox, pork, chicken, doe or fox after cooking and concentrating the extracts. He concluded that the odor and taste of cooked muscle extract was similar to that from roast meat. More recently, Wood and Bender (1957), Bender *et al.* (1958) and Bender and Ballance (1961) have studied meat extracts. Wood and Bender (1957) reported the isolation of more than 30 volatile and non-volatile compounds from commercial ox-muscle extract.

Crocker (1948) studied the flavor volatiles of beef, pork and chicken. He found ammonia, hydrogen sulfide and acetaldehyde present in the flavor volatiles derived from all three sources of meat. He came to the conclusion that the flavor components originated from the fibers of the meat instead of from the expressible fluid. Further studies by other researchers (Kramlich and Pearson, 1958), showed that the expressible fluid developed more flavor on cooking than the fibers, although the flavor components were more strongly bound to the cooked than to the raw fibers. This was further substantiated by Hornstein *et al.* (1960), who found that water-extracted hamburger was essentially tasteless and odorless.

However, the water extract developed a definite "beefy" aroma on heating. Kramlich and Pearson (1960) identified acetaldehyde, acetone, carbon dioxide, methyl mercaptan and tentatively methyl sulfide from a cooked beef slurry.

In a series of articles, Hornstein and Crowe (1960, 1963, 1964) and Hornstein _et al_. (1960, 1963) studied the flavor of beef, pork, whale and lamb. They found that lean meat regardless of source, gave a basic "meaty" aroma on cooking. They postulated that the species differences in flavor are due to the heated fat. The characteristic odor of lamb was found to be due to the volatiles from the fat, a major portion of which was contributed by the carbonyl compounds. They reported finding ammonia, acetaldehyde, acetone, formaldehyde, hydrogen sulfide and carbon dioxide in the volatiles obtained on cooking both beef and pork. In addition they isolated formic, acetic, propionic, butyric and isobutyric acid from beef broth.

Pippen _et al_. (1954) found that more flavor was produced on cooking a cold water extract of chicken meat than the fibers. They further indicated that the fat contributed to the overall chicken flavor. More recently, Pippen _et al_. (1958), Pippen and Nonaka (1960, 1963) and Lineweaver and Pippen (1961) stressed the importance of the volatile carbonyl compounds in chicken flavor. They isolated and identified many carbonyls present in the volatile fraction. They observed an increase in the yield of carbonyls on cooking chicken in an oxidative atmosphere as compared to a non-oxidative atmosphere. Bouthilet (1950, 1951a), working with chicken, reported that fat played an important role in producing chicken flavor. He also found that hydrogen sulfide was a major component in the volatiles from cooked chicken. Fractionation of chicken broth gave a sulfur-containing fraction with a definite "meaty" odor, and a fraction having a typical "chickeny" flavor. He concluded that the "meaty" aroma of chicken was due to a compound associated with the meat fibers, which could be extracted with water, and stressed the importance of the sulfur compounds in producing the "meaty" odor on heating.

Pippen and Eyring (1957) identified the sulfur-containing volatiles from cooked chicken as consisting mainly of hydrogen sulfide. They suggested that the hydrogen sulfide released on cooking played an important role in chicken flavor as well as in the flavor from other meats. Mecchi _et al_. (1964) quantitatively analyzed the hydrogen sulfide produced by heating chicken muscle. They indicated glutathione as the precursor of hydrogen sulfide. Since the sulfur in glutathione is present as cystine and/or cysteine, they suggested that the rate of hydrogen sulfide evolution from cooked chicken could be approximately predicted from the cystine content.

Pippen and Eyring (1957) failed to detect mercaptans in the flavor volatiles of cooked chicken, but recent work on the flavor of chicken by Minor _et al_. (1965a, b, c) has indicated the presence of not only mercaptans but also of disulfides. In a comparative study of the flavor volatiles from old and young hens, Minor _et al_. (1965b) found few, if any, qualitative differences. Minor _et al_. (1965c) identified carbonyl compounds, hydrogen sulfide and ammonia in the volatile fraction. Pippen and Eyring (1957) identified the volatile nitrogen as consisting almost completely of ammonia, and showed by organoleptic tests that ammonia was not important to chicken flavor. In fact, the volatile ammonia in chicken flavor may exert a negative influence according to a report by Lineweaver and Pippen (1961).

Pippen and Nonaka (1963) reported a comparison of the volatile flavor components of chicken and turkey, although they did not identify any of the compounds isolated. They found that carbonyl compounds played an important role in the production of rancidity in chicken. The importance of carbonyl compounds was stressed by Jacobson and Koehler (1963), who suggested that the differences in flavor between breeds of sheep could be due to differences in the quality and composition of the carbonyls.

Until recently, very little information was available on the flavor components of cured meats. Ockerman et al. (1964) presented a gas chromatographic analysis of the volatiles derived from dry-cured hams. They found that the spectrum of compounds was very similar to that reported by other investigators for the volatiles of uncured meat. Cross and Ziegler (1965) found that the main differences between cured and uncured ham were due to the different proportions of the carbonyl compounds. Uncured ham yielded appreciable quantities of n-valeraldehyde and hexanal on cooking, but these compounds were barely detectable in the volatiles from cured ham. Acetone was found to be a major constituent in the volatiles from both cured and uncured ham. Although the authors found methyl mercaptan and hydrogen sulfide present in the volatiles from both cured and uncured ham, they did not attempt to compare the relative amounts of the sulfur compounds from the two types of meat.

Apart from the identification of hydrogen sulfide in the flavor volatiles of beef, less emphasis has been placed on the sulfur compounds. However, many workers agree that sulfur plays an extremely important role in production of the typical "meaty" aroma. Yueh and Strong (1960), in addition to hydrogen sulfide, reported finding small quantities of dimethyl sulfide. Increased amounts of hydrogen sulfide were evolved on prolonged heating. On quantitative analysis, Hornstein et al. (1960) determined that 0.1 mg. of hydrogen sulfide was recovered per gram of dried beef powder after vacuum distillation at 100°C. Neither groups of researchers found any trace of mercaptans or disulfides, even though Yueh and Strong (1960) suggested the possibility of oxidation of the mercaptans to disulfides during cooking. After cooking beef in an inert atmosphere by bubbling nitrogen gas through the cooking slurry, Kramlich and Pearson (1960) identified methyl mercaptan in the volatile flavor constituents. They also tentatively identified dimethyl sulfide, which is in agreement with Yueh and Strong (1960).

Sulfur compounds in high concentrations are also known to be responsible for off-odors. Batzer and Doty (1955) found methyl mercaptan and hydrogen sulfide present in the off-odors from gamma irradiated beef. Although low concentrations of sulfur compounds may greatly enhance the flavor of a food, they are probably responsible for many unpleasant odors.

Extremely low concentrations of sulfur compounds can be detected by the human nose, i.e., one part ethyl mercaptan per fifty billion parts of air can be detected by the olefactory nerve cells of the human nose (Noller, 1965). The majority of the flavor volatiles from onions (Carson and Wong, 1961) and garlic (Oaks et al., 1964) are sulfur compounds, such as mercaptans, sulfides and disulfides, which are normally considered to have a displeasing aroma.

Flavor Precursors

In the water extract of meat, Justus von Liebig (1847) isolated inosine, creatine, creatinine, lactic acid and amino acids. Over a century later, inosinic acid, in combination with a ribose-5-phosphate complex, was shown to be important in the development of browning and "meaty" flavor by Wood (1961). Bouthilet (1951b) indicated glutathione was a major precursor for the "meaty" flavor of chicken, and stressed the importance of the sulfur-containing fraction to meat flavor. Mecchi et al. (1964) found that glutathione was the precursor of hydrogen sulfide in cooked chicken.

After an elegant separation procedure, Batzer et al. (1960, 1962) isolated a low molecular weight glycoprotein, which upon heating with glucose, inosine and inorganic phosphate in fat, produced an odor similar to that of broiled steak. In a review of meat flavor and flavor precursors, Doty et al. (1961) indicated that the glycoprotein fraction could be isolated from beef, pork or chicken and resulted in the same basic "meaty" aroma regardless of the source. Further studies using the fractionation procedure of Batzer et al. (1960) have been carried out by Wasserman and Gray (1965), who have questioned the role of sugars as one of the "meaty" flavor precursors. In addition to the low molecular weight fraction, which gave a broiled steak odor, they isolated a high molecular weight fraction having a brothy odor on heating. The fraction responsible for the steak aroma was separated on ion-exchange resins and a complete amino acid analysis was carried out on all fractions. Hornstein and Crowe (1960, 1963) found a similar water-soluble, low molecular weight compound in the lean meat portions of pork, beef and lamb, which they indicated to be a precursor of the typical "meaty" odor.

Method of Cooking

In one of the earliest reports on meat flavor, Crocker (1948) boiled various types of meat in water in an open beaker. Since then numerous refinements have been made to simulate "actual cooking conditions" and to trap the volatile flavor components, either by fractionation in a series of cold traps or by trapping in various reagents. In their studies on beef, pork, lamb and whale, Hornstein _et al._ (1960, 1963) and Hornstein and Crowe (1960, 1963) removed the volatiles from meat by vacuum distillation and then captured them in a series of traps held at the temperature of either liquid nitrogen or of a dry-ice isopropyl alcohol mixture. They chose 100°C as the most satisfactory operating temperature for vacuum distillation. A similar temperature fractionation procedure was used by Kramlich and Pearson (1960) to separate the flavor volatiles produced by cooking beef in a non-oxidative system. A second method for identification was also employed by bubbling the volatiles through a solution of 2,4-dinitrophenylhydrazine (2,4-DNPH) and a solution of lead acetate in order to identify the carbonyl and sulfur compounds, respectively. Bouthilet (1949, 1950, 1951a,b) found that the flavor components could be released from the fibrous material of chicken muscle by simmering in tap water but were further released from the resulting chicken broth using either steam or vacuum distillation. He stressed the importance of the interrelationship between the fat and lean meat and the production of flavor, and suggested that the flavor components may be soluble in the fat. This was later explained by Kazeniac (1961) in a review on chicken flavor. He suggested that the fat might serve as a trapping agent for some of the volatiles. Steam distillation of chicken broth with some fat gave a more desirable flavor than broth from which the fat was removed. In earlier work, Pippen _et al_. (1954) also found that fat contributed to the aroma of chicken broth. They found that cooking method and isolation procedure strongly influenced the yield of carbonyl compounds. When chicken was cooked under non-oxidative conditions by passing a stream of nitrogen through the system, the lowest yield of carbonyls was obtained. Under oxidative conditions, utilizing air to entrain and sweep the volatile components into reagent traps containing 2,4-DNPH, they obtained the greatest yield of carbonyl compounds. Under "normal cooking conditions" or distillation, they found an intermediate yield of 2,4-dinitrophenylhydrazones (2,4-DNPS). In a later paper, Pippen _et al_. (1958) identified the 2,4-DNPS released by air-entrainment of cooking chicken. The volatile carbonyls produced under these conditions included those which arose through oxidative processes not present under normal cooking conditions, and may contribute to both typical and off-flavor.

Steam distillation produced aldehydes with longer carbon chains than air-entrainment (Pippen and Nonaka, 1960). Chicken boiled in the presence of air yielded not only a larger volatile fraction, but a more complex fraction (Pippen and Nonaka, 1963). Temperature was found to be important in the production of flavor and aroma, as shown by the fact that raw chicken had little flavor as compared to chicken cooked at 100°C. They found that n-2,4-decadienal was produced on cooking fresh chicken in the presence of oxygen, and also showed that this compound was a major volatile component of cooked rancid chicken. This suggests that n-2,4-decadienal may be an immediate precursor of stale or rancid chicken. Nonaka and Pippen (1966) found that steam distillation gave a greater yield of volatiles than vacuum distillation and suggested that the off-flavor of fried chicken might be due to volatile products produced by the oxidation of lipid material. They also concluded that the relative amounts of n-2,4-decadienal could be used as an indication of freshness.

Utilizing the "oxidation-inhibiting conditions" of Pippen _et al._ (1958), Minor _et al._ (1965a, b, c) separated and identified a variety of flavor volatiles using both a temperature fractionation procedure and a reagent trapping system. They indicated that the sulfur compounds were responsible for the "meaty" odor of chicken, while the typical "chickeny" flavor was due to the carbonyl compounds.

The difficulty of transferring the isolated volatiles from any system for concentration, identification and analysis has always been a problem to the flavor chemist, because of the minute quantities of compounds volatilized, and their often unstable nature. Precipitation in reagent traps is only suitable for certain classes of volatile compounds, and temperature fractionation can lead to large losses of volatiles by evaporation. Libbey et al. (1962) developed a refrigerated trap for the collection of food volatiles. In this system the volatiles could be directly transferred to a gas chromatograph by gently heating. Hornstein and Crowe (1962) used a similarly designed helical trap, but added a chromatographic column packing of 25% Castorwax on 30/60 mesh Chromosorb W. This gave the added advantage of a preliminary separation before transferring the sample to the gas chromatograph. A greatly improved yield of flavor volatiles was obtained for analysis, although the authors did not mention the problem of samples containing considerable quantities of water, which is usually the case with most of the volatiles produced on cooking meat.

EXPERIMENTAL PROCEDURE

Sample Preparation

Most of the meat used in this study was removed from the lumbar region of the *longissimus dorsi* muscle of U. S. Choice or Prime grade cattle of approximately 20 months of age. After removal of all the subcutaneous and intermuscular fat, the meat was finely ground and stored at -20°C prior to cooking. The fat used for cooking was removed from the same carcasses as the meat samples. The fat was chilled, finely ground and stored at -20°C until used. Before cooking, the samples were allowed to thaw slightly at room temperature.

Cooking Procedure

Method of Cooking in Water. A total of 500 g. of ground beef was cooked with 1000 ml. of distilled water in the cooking apparatus shown in Fig. I. A "resin reaction" flask was used as a cooking vessel as it offered special advantages for cleaning after cooking. The cooking flask was heated with a heating mantle, which could be held at a constant temperature using a rheostat. The meat slurry was simmered gently for eight hours after it reached the boiling point. All volatile constituents were removed from the cooking vessel by constantly sweeping the surface of the cooking slurry with nitrogen gas, and then trapping the volatiles in a series of reagent traps. At the end of eight hours of cooking, off-odors began emanating from the meat and were considered to be atypical of cooked beef, so cooking was discontinued.

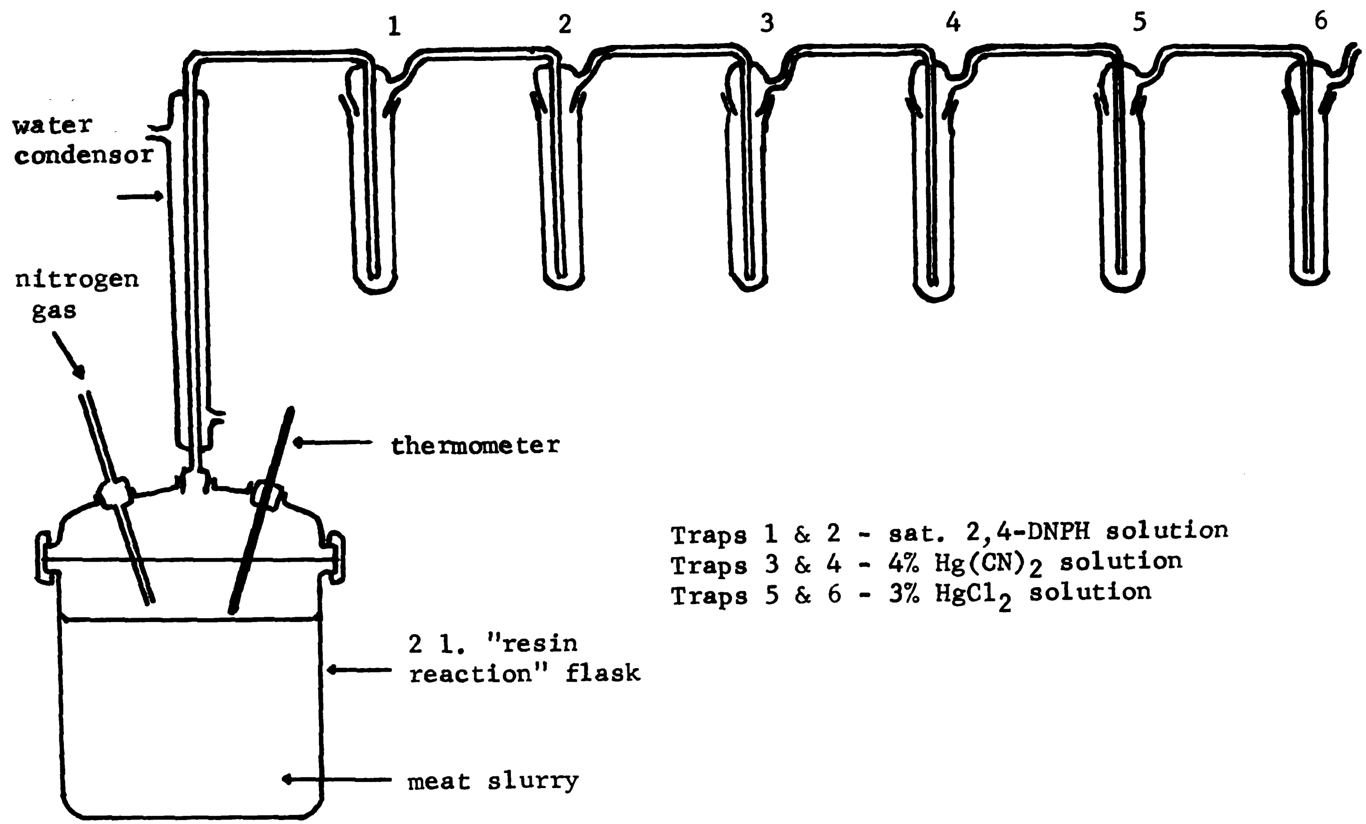

Figure I. Schematic diagram of the cooking apparatus and trapping system used for the collection of the volatile components from beef cooked in water.

Method of Cooking in Fat. The apparatus used for cooking beef in fat was similar to that used for cooking beef in water except that no condensor was utilized. Instead, any water that was produced was collected in a 500 ml. trap immersed in crushed ice before the remaining volatiles were swept through the reagent traps. A total of 500 g. of ground beef was cooked with 500 g. of fat. The fat was melted in the cooking flask prior to the addition of the meat in order to prevent sticking or burning. The meat was cooked in fat for eight hours so that results would be comparable to those for the sample cooked in water. The temperature at which the volatiles emerged was recorded.

Separation of Carbonyl Compounds

The volatiles from the cooking meat were bubbled through a capillary tube into a saturated solution of 2,4-DNPH in 2N HCl. A second tube of 2,4-DNPH was used to prevent any loss of carbonyls, although it was not necessary to change either the first or the second 2,4-DNPH trap in this study. The water collected on cooking the meat in fat was added to a solution of saturated 2,4-DNPH and the precipitated 2,4-DNPS were added to those in the first 2,4-DNPH trap. After the eight hour cooking period, the traps were disconnected and the yellow-orange precipitate of 2,4-DNPS was centrifuged, washed with 2N HCl and distilled water and then dried in a desiccator. The yield of 2,4-DNPS from six samples cooked in fat and six cooked in water was recorded.

<u>Separation of Sulfur Compounds</u>

The volatile sulfur compounds were passed through a series of traps containing 4% mercuric cyanide and then 3% mercuric chloride according to the method of Challenger (1959). This method is based on the fact that hydrogen sulfide and mercaptans form an insoluble precipitate with mercuric cyanide, whereas, alkyl sulfides do not form a precipitate. Alkyl sulfides form an insoluble white precipitate with mercuric chloride. Thus, any alkyl sulfides produced on cooking would pass through the mercuric cyanide traps and be precipitated in the mercuric chloride traps. This method was useful in that it separated the mercaptans from the sulfides and disulfides.

After the eight hour cooking period, the traps were disconnected. The small yield of sulfur compounds from cooking in either fat or water made it necessary to combine the precipitates from two runs, giving a total of three identifications from beef cooked in fat and three from beef cooked in water. A greater yield of sulfur compounds resulted when the mercuric cyanide and mercuric chloride traps were placed before the 2,4-DNPH traps. After the first trial, this procedure was considered to be necessary. Mercuric cyanide was added to the water collected from the beef cooked in fat to give a 4% solution, and the resultant precipitate was added to that in the other mercuric cyanide trap. The black precipitate from the mercuric cyanide traps was centrifuged and washed with distilled water prior to regeneration.

Precipitation of the sulfur compounds as their mercuric salts was chosen to preference to their lead salts because of the ease of regeneration. In comparison to the mercuric salts, the lead salts of sulfur compounds tend to be amorphous and are, therefore, very difficult to crystallize or obtain pure (Challenger, 1959).

Regeneration of Carbonyls from 2,4-DNPS

A stock mixture of one volume of water to nine volumes of levulinic acid was made up according to Keeney (1957). For the regeneration of the 2,4-DNPS, 5 ml. of the stock solution and 1 ml. of distilled water were added to the dried hydrazones in a small distillation flask. The temperature of the flask was raised very slowly to 100°C in an oil bath over a period of 30 minutes. The levulinic acid exchanged with the 2,4-DNPS liberating the free carbonyl compounds, which were distilled together with a little water into a small tube chilled in ice-water. If regeneration were carried out too rapidly, levulinic acid breakdown occurred. The oil bath was held at 100°C for a further period of five minutes, in order to distill over the majority of the carbonyl compounds.

Ockerman et al. (1964) used α-ketoglutaric acid to free the carbonyl compounds from their 2,4-DNPS. With this method it is difficult to attain reproducible results, as all the 2,4-DNPS do not always exchange in the dry state. Cross and Ziegler (1965) have reported good results using 20% H_2SO_4 to regenerate carbonyls from their 2,4-DNPS, however, levulinic acid was preferred in this study, because of the strong oxidizing properties of sulfuric acid.

Since the gas chromatograph used was not suitable for samples containing water, the carbonyl were extracted with an organic solvent. Most of the more commonly used solvents, such as diethyl ether, were not considered to be satisfactory, as they masked the earlier emerging carbonyl peaks on the gas chromatograph. Methyl phenyl ether was finally chosen as a suitable solvent, as it emerged on the chromatograph at least 25 minutes after the last of the carbonyl peaks from the regenerated sample. After extraction, approximately 0.1 g. of powdered anhydrous sodium sulfate was added to remove any traces of water.

The procedure using levulinic acid described above was chosen since the extremely small amounts of 2,4-DNPS released on cooking could be successfully regenerated and separated by gas chromatography. Retention times of known reagent-grade aldehydes and ketones were employed to characterize the carbonyls from beef cooked in both water and fat on two separate columns under differing conditions.

<u>Regeneration of Mercaptans from Mercuric Salts Precipitated by Mercuric Cyanide</u>

Challenger (1959) reported the occasional formation of co-ordination compounds with mercuric cyanide and mercaptides of the type $(RS)_2Hg.Hg(CN)_2$ as well as mercaptides such as $(RS)_2Hg$. The co-ordination compounds can be removed by boiling or shaking the solution of the mercaptides, but this was found to be unnecessary in the present study. The mercuric mercaptides were regenerated with acid, whereas the mercuric sulfide formed from hydrogen sulfide was not reduced (Challenger, 1959). This chemical test was utilized to distinguish the difference between mercaptides ($(RS)_2Hg$) and mercuric sulfide (HgSHg). The test was found to be extremely useful, since the large quantities of black mercuric sulfide normally would mask the small quantities of white mercuric mercaptides. The free mercaptans were liberated from the mercaptides with 1N H_2SO_4, which did not appear to cause much oxidization of the mercaptans to their corresponding acids.

Trapping the liberated mercaptans presented a particularly difficult problem due to their extremely volatile nature, their unpleasant odor and the small amounts present. Collecting the mercaptans in a gas-tight bottle and subsequent injection into the gas chromatograph with a gas-tight syringe also resulted in problems. At the high sensitivities required, contamination of the large surface area of the syringe and the septum in the injection port of the gas chromatograph could lead to extraneous peaks on subsequent analyses. Trapping the regenerated mercaptans in a coil held at liquid nitrogen temperatures for direct release into the chromatograph could be used to eliminate losses in the gas transfer system. However, the small amounts of water produced during the regeneration procedure made this method unsatisfactory, since the gas chromatograph was not suitable for samples containing water.

Adams et al. (1960) reported a technique for trapping malodorous, sulfur-containing gases from waste processes of the paper industry. By passing the regenerated mercaptans through a tube of Drierite at 55°C, it was possible to trap all traces of water. The mercaptans, still in the vapor state, passed over to a "U" tube of silica gel at -78°C. The mercaptans were absorbed on the silica gel and could easily be transferred to the gas chromatograph for analysis by gentle heating with a heating tape. Although this method is ideal for large amounts of known samples, it was not practical for the extremely minute amounts of regenerated mercaptans obtained from the mercuric salts of the volatile mercaptans in the present investigation.

Because of the difficulties encountered in transferring small amounts of liberated mercaptans into the gas chromatograph, the possibility of converting them into a more stable form was investigated. Sporek and Danyi (1963) reported good yields on converting mercaptans to a series of disulfides by a simple and fast oxidation with iodine in ether. The disulfides were easily soluble in ether, and owing to their relatively high boiling points and chemical stability were more convenient to handle than the original mercaptans. As mixed disulfides are formed from two or more mercaptans, and give rise to a series of peaks on the gas chromatograph, this offers an additional check on the existence of a particular thiol.

The black precipitate was transferred to an amber flask fitted with a water condensor. A total of 10 ml. of diethyl ether and 25 ml. of 1N H_2SO_4 was added, and the mixture stirred vigorously with a magnetic stirrer for ten minutes at room temperature. After the flask and contents were cooled in ice-water for five minutes, 10 ml. of diethyl ether containing an excess of iodine (approximately 0.5 g.) were added to the flask through the condensor. The mixture was stirred for a further five minutes and then decanted into a separatory funnel. The resultant liquid contained considerable unchanged black mercuric sulfide and was brown in color due to excess iodine. The aqueous phase was discarded and the organic layer washed with 25 ml. of 0.1M sodium thiosulfate to remove the excess iodine. Again the aqueous layer was discarded and the organic layer washed with a solution of saturated sodium chloride. After washing three times with distilled water followed by saturated sodium chloride, the organic layer was concentrated under vacuum. The concentrated disulfides were dried with anhydrous sodium sulfate powder and injected into the gas chromatograph.

Mercuric salts of known mercaptans were prepared by adding the thiol to a 4% mercuric cyanide solution. The white mercaptides precipitated and were recrystallized from ethyl alcohol. The melting points of these mercaptides are recorded in Table 1 and are compared with those given by Wild (1960).

Table 1. Values of prepared mercaptan derivatives.

		Mercury salt	
Mercaptan	Boiling point (°C)[a]	Melting point of prepared mercaptide (°C)	Melting point reported by Wild (°C)[a]
Methyl	6	168	175
Ethyl	36	74	76
n-propyl	68	71	72
Iso-propyl	59	63	63
n-butyl	98	85	86
Iso-butyl	88	--	95
n-amyl	127	66	75

[a]Values reported by F. Wild (1960) in "Characterisation of Organic Compounds", Cambridge University Press, Cambridge, England p. 104.

Retention times of the disulfides regenerated from the unknown mercuric mercaptides were compared with the retention times of the disulfides synthesized from the known, crystalline mercuric mercaptides.

Regeneration of Sulfur Compounds from Mercuric Salts Precipitated by Mercuric Chloride

Even after several runs, the amount of white precipitate in the first mercuric chloride trap was almost negligible, making identification extremely difficult. On regeneration of the sulfides with cold, concentrated sodium hydroxide according to Challenger (1959), the amount of material available was so small that identifications were not possible except by comparing the odor with that of known sulfides. By comparison with known dialkyl sulfides, the sulfide in greatest proportion could be detected by its characteristic odor.

Gas Chromatography of Carbonyl Compounds

The instrument used was a Barber-Colman, Model 20 gas chromatograph equipped with a radium ionization detector. A six foot, 1/4 inch O.D. copper column packed with 10% diisodecylphthalate on acid washed Diaport W (60/80 mesh) was shown to satisfactorily separate mixtures of known aldehydes and ketones. Ockerman et al. (1964) reported good results using a similar column packing to separate regenerated carbonyl compounds from the volatiles of cured ham. The column was operated at 100°C, with the flash heater and detector cell at a temperature of 155°C. Argon was utilized as the carrier gas at 8 p.s.i., giving a calculated flow rate of 43.5 ml./minute through the column. The current across the detector cell was set at 1250 volts.

A second six foot, 1/4 inch O.D. copper tubing column, packed with 10% Carbowax 20M on acid washed Diaport W (60/80 mesh) was prepared for confirmatory identification of the volatile aldehydes and ketones of cooked beef. It was operated at 75°C with the detector cell and flash heater settings at 155°C. The cell voltage setting was 1250, and argon was used as the carrier gas at 8 p.s.i., giving a flow rate of 43.5 ml./ minute through the column.

Retention times of known carbonyl compounds were employed to characterize the unknown aldehydes and ketones regenerated from their 2,4-DNPS using both columns.

Gas Chromatography of Sulfur Compounds

The disulfides regenerated from the mercuric mercaptides were analyzed with an Aerograph Model 204 gas chromatograph equipped with both an electron capture and a hydrogen flame detector. The combination detector was especially suitable since the electron capture detector is extremely sensitive to sulfur-containing compounds. Oaks *et al.* (1964) used a similar dual channel system in the analysis of sulfur compounds in garlic with excellent results. The flame detector responds almost equally to all organic compounds, while the electron capture detector is sensitive only to certain organic compounds such as halogenated compounds, conjugated carbonyls, nitro compounds and sulfur-containing compounds. The sensitivity between the two detectors varies with the type of compound and for this reason the ratio of the electron capture response to the flame response becomes an important means of component identification. Oaks et al. (1964) designated this value as ∅. The value ∅ is an empirical function of the two detectors and is based on the same attentuation for both detector electrometers and assumes a 1:1 split in the column effluent going into each detector. Thus, ∅ coupled with retention time gives a definite and almost complete proof of identification when compared to known standards.

A six foot, 1/4 inch O.D., copper column was packed with 30% (w/w) Triton X-305 on acid washed Chromosorb W (30/60 mesh). This column was found to satisfactorily separate known mixtures of sulfur-containing compounds without the extensive bleeding obtained with some other columns. Adams and Koppe (1959) investigated several Triton series from Rohm and Haas as stationary phase solvents for the separation of mercaptans, sulfides and disulfides. They obtained the best separation with low retention times using the Triton X-305 solvent on Chromosorb W.

The column was operated at 155°C with the flash heater and both detectors set at 175°C. The flame detector was operated with a hydrogen generator set at a hydrogen flow rate of 25 ml./minute. Both detectors were operated at a fixed potential of 90 volts. Nitrogen was used as a carrier gas with a flow rate of 50 ml./minute, giving a flow of 25 ml./minute through each detector, with a 1:1 split after the column. The responses from both detectors were recorded on a two-pen Westronics recorder, Model D11A, which allowed identical retention times for each channel with a single compound. Both channels were operated on a range of 1, usually with an attenuation of 16 times.

Retention times of the disulfides regenerated from the unknown mercuric mercaptides were compared with the retention times and the $\emptyset$ values of the disulfides synthesized from the prepared mercuric mercaptides.

RESULTS AND DISCUSSION

Carbonyl Compounds

The yield of 2,4-DNPS from beef cooked in fat was three times as large as that obtained from beef cooked in water. The mean values were 0.17 and 0.05 g., respectively. The data from each cooking replication are presented in Table II. Although there was considerable variation between yields of 2,4-DNPS in each replication by both methods of cooking, the yields were consistently higher on cooking beef in fat. The greater yield on cooking in fat could have been due to the fact that the condensor used for cooking beef in water may have caused the retention of carbonyl compounds with the refluxing water, and may have thereby reduced the yield on cooking in water. Other aldehydes and ketones may have resulted from the reaction of the meat with the heated fat on cooking beef in fat. It is also quite possible that the fat itself contributed volatile aldehydes and ketones, especially since Hornstein and Crowe (1960, 1963), Cross and Ziegler (1965), and other workers have reported carbonyl compounds to originate from fat. Nevertheless, cooking the meat in fat appears to have been responsible for the greater yield of 2,4-DNPS. The fact that cooking 500 g. of fat alone yielded only 0.0098 g. of 2,4-DNPS after six hours of cooking suggested that the greater yield on cooking the meat in fat, as compared to water, was actually responsible for the difference in yields between the two methods of cooking.

Table II. Yield of 2,4-DNPS by two different methods of cooking.

	Grams of 2,4-DNPS from 500 g. beef	
Replication	Cooked in water	Cooked in fat
1	0.1255	0.1610
2	0.0492	0.1895
3	0.0330	0.1070
4	0.0092	0.0403
5	0.0540	0.2564
6	0.0481	0.2874
Mean:	0.05	0.17

The 2,4-DNPS obtained on cooking fat alone were difficult to regenerate with levulinic acid, and on chromatographic analysis gave one peak which corresponded to 2-butanone. Other very small peaks obtained were too minute to identify. The low yield of 2,4-DNPS on cooking in fat alone would suggest that the differences in yield between the two methods of cooking meat were largely the result of variation in the cooking procedures.

Immediately after regeneration of the 2,4-DNPS with levulinic acid, the carbonyl compounds released from both the beef cooked in water and that cooked in fat had a sweet, faint caramel aroma. This characteristic aroma of the aldehydes and ketones is indicative of their role and relative importance in the flavor volatiles from cooking meat. A typical gas chromatogram of the carbonyl compounds from beef cooked in water is shown in Fig. II. The aldehydes and ketones from beef cooked in water included formaldehyde, acetaldehyde, propanal, acetone, 2-methyl propanal, butanal, 2-butanone and 3-methyl butanal. Another peak, corresponding to the 5-carbon aldehydes and ketones, which may have contained either pentanal, 2-pentanone or 3-pentanone, or any combination of these three compounds was observed. However, it was not possible to separate and identify known reagent-grade standards of pentanal, 2-pentanone and 3-pentanone by altering the conditions of the gas chromatograph, since the range in boiling point of the three carbonyls is within 1°C.

Five small peaks were also obtained from the regenerated carbonyl compounds volatilized by cooking beef in water, but due to the small sample size identification was not possible. None of the peaks corresponded to known samples of carbonyls such as hexanal, 2- or 3-hexanone, 2-methyl butanal, 3-methyl 2-butanone, or similar short chain aldehydes and ketones. Another small peak was identified as a breakdown product of levulinic acid. Butanal, 2-butanone and the 5-carbon carbonyls were found to be the major carbonyl compounds produced by cooking beef in water, with smaller amounts of 3-methyl butanal, 2-methyl propanal, acetaldehyde, formaldehyde, acetone and propanal. The low concentrations of formaldehyde and acetaldehyde, and especially of the former, were undoubtedly due to losses by volatilization during the regeneration procedure.

For identification of the regenerated carbonyls from the flavor volatiles obtained on cooking beef, the retention times of these aldehydes

Column 10% diisodecylphthalate on

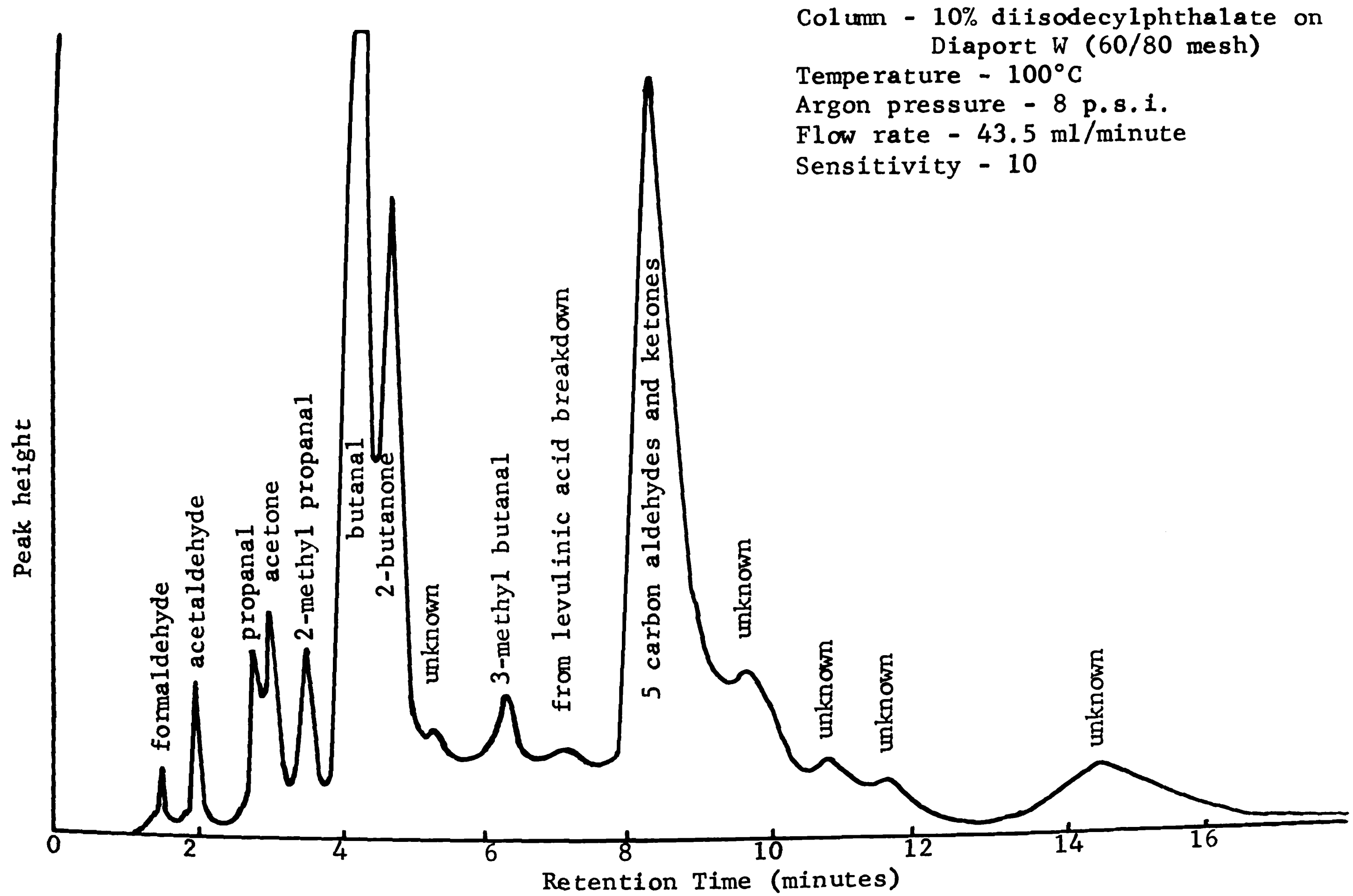

Figure II. Gas chromatogram of the volatile carbonyl compounds isolated from beef cooked in water.

and ketones were compared with those of known reagent-grade standard carbonyl compounds. The retention times are shown in Table III. The agreement between the retention times of known standards and unknown carbonyls on both chromatographic columns was excellent.

Fig. III shows a typical gas chromatogram of the regenerated carbonyl compounds obtained on cooking beef in fat. Although the beef cooked in fat gave a greater yield of volatile carbonyl compounds than beef cooked in water, both cooking procedures produced the same number and type of aldehydes and ketones. However, the relative proportion of the various carbonyl compounds differed according to the method of cooking. A comparison of the gas chromatograms shown in Fig. II and III will show that beef cooked in water yielded more butanal than 2-butanone, whereas, the beef cooked in fat usually had more 2-butanone than butanal. The relative amounts of carbonyls volatilized from beef cooked in fat were less consistent than those from beef cooked in water. Hornstein and Crowe (1960) have also noted similar variations in volatile aldehydes and ketones released from heated lamb fat.

As the major carbonyl compound produced by heating beef fat alone was 2-butanone, it appears highly probable that the increase in this compound on cooking beef in fat was due to the contribution of the fat itself. At first thought this may suggest that 2-butanone does not contribute to the aroma of beef itself. However, this is not necessarily true since the fat present in the meat itself may result in production of 2-butanone, and thus, it could still be responsible for the characteristic difference in aroma between beef cooked in fat and that cooked in water.

...ated from 2,4-DNPS by

Table III. Retention times of carbonyl compounds regenerated from 2,4-DNPS by exchange with levulinic acid.

Carbonyl compound	Boiling point (°C)	Retention time on column in minutes			
		Diisodecylphthalate column at 100°C		Carbowax 20M column at 75°C	
		Unknown	Known	Unknown	Known
formaldehyde	-21	1.5	1.5	2.0	2.1
acetaldehyde	21	1.8	2.0	3.4	3.4
propanal	49	2.6	2.7	5.2	5.1
acetone	56	2.8	2.8	5.9	5.9
2-methyl propanal	64	3.4	3.4	6.8	6.6
butanal	74	4.2	4.3	8.4	8.3
2-butanone	80	4.6	4.5	9.7	9.7
3-methyl butanal	93	6.3	6.2	11.0	10.8
5-carbon carbonyls	102-103	8.1	8.2	16.0	15.7

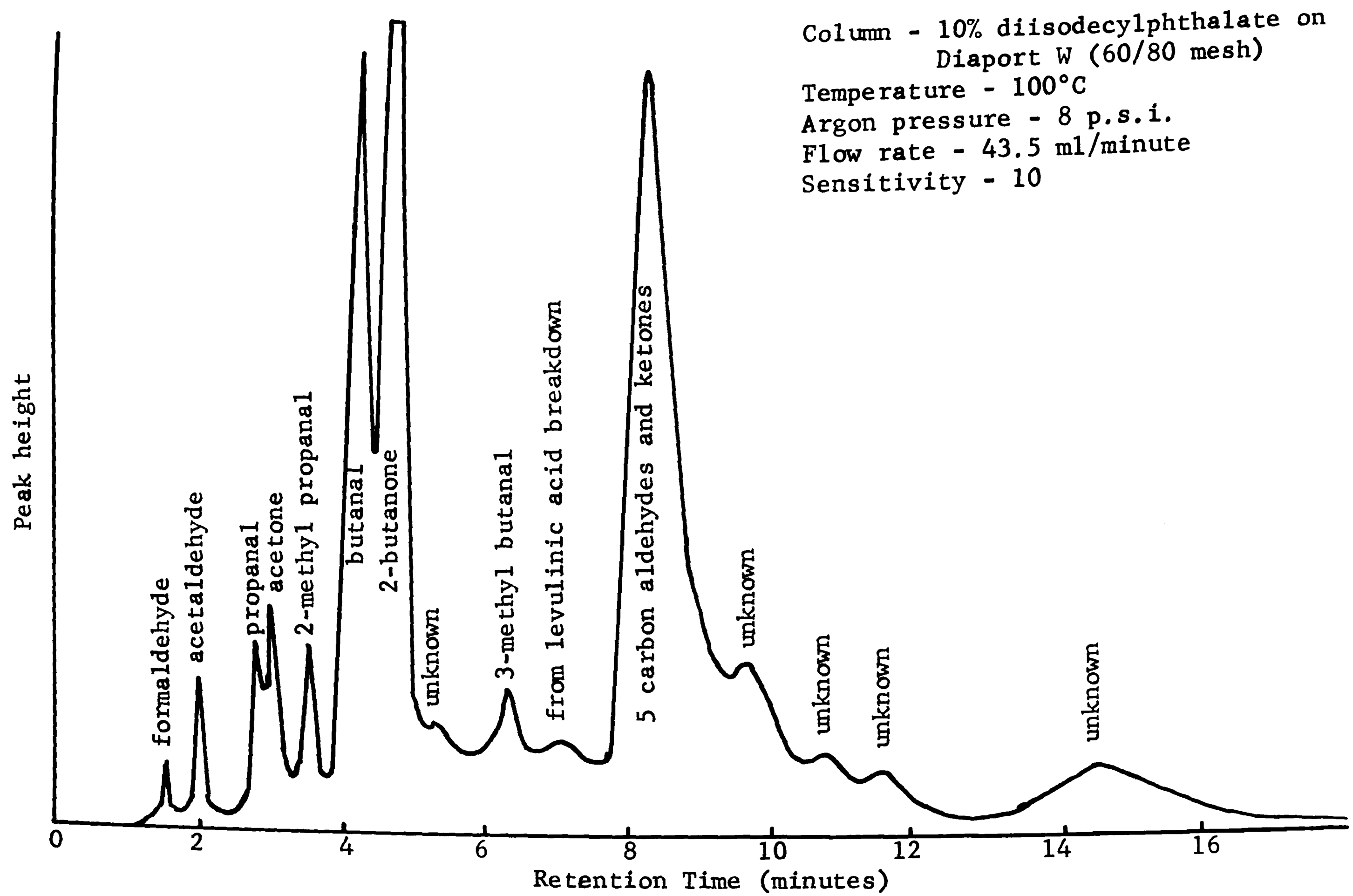

Figure III. Gas chromatogram of the volatile carbonyl compounds isolated from beef cooked in fat.

Butanal, 2-butanone and the 5-carbon carbonyls appeared to be the carbonyl compounds in greatest proportion in the flavor volatiles obtained from beef cooked in fat. Smaller amounts of formaldehyde, acetaldehyde, acetone, propanal, 2-methyl propanal and 3-methyl butanal were also identified. In addition, there were five small peaks which were too small to be identified, and one small peak which emerged before the 5-carbon carbonyl compounds. The latter peak was due to the breakdown of levulinic acid.

Known samples of poly- and dicarbonyls, such as 2,3-butanedione (diacetyl), either gave very broad or reverse peaks and could not be used for identification. As the peaks from the regenerated carbonyl compounds from beef cooked in water and fat were sharp, it appears that di- and polycarbonyls were not present in appreciable quantities. As another test for polycarbonyls, a 2% solution of alcoholic potassium hydroxide was added to the 2,4-DNPS dissolved in ethyl acetate. If any polycarbonyl compounds were present, the 2,4-DNPS solution would have turned brownish-purple. No color change was observed in the solution of 2,4-DNPS from beef cooked in either fat or water, indicating that polycarbonyls were absent in the flavor volatiles produced by both methods of cooking.

Any α-hydroxy aldehydes and ketones present in the flavor volatiles would have formed osazones with 2,4-DNPH. For example, 2,3-butanedione would have been formed on regeneration of the osazone of 3-hydroxy butanone (acetoin). The absence of polycarbonyls in the regenerated carbonyl sample from the flavor volatiles eliminated the possibility of the existence of any α-hydroxy aldehydes and ketones as well as of polycarbonyls.

A soluble ring compound is formed when 2,4-DNPH in 2N HCl is added to 2,4-pentanedione, and the hydrazone does not precipitate. If 2,4-pentanedione were present in the flavor volatiles, it is unlikely that it would precipitate as a 2,4-dinitrophenylhydrazone. It is possible that 2,4-pentanedione could be present in either or both beef cooked in fat and water, but, if present, it would not be identifiable as a hydrazone. Although 2,4-pentanedione has been identified as a constituent of the flavor volatiles from the breast and leg muscle of chicken by Minor et al. (1965a), there have been no reports of the isolation of 2,4-pentanedione from the volatiles obtained on cooking beef.

The possibility of oxidation of the regenerated aldehydes to their corresponding acids with Tollen's reagent was investigated for confirmatory identification. If ammoniacal silver nitrate is added to a basic solution of aldehydes, it is reduced to a silver mirror, while the aldehydes are oxidized to their corresponding acids (Noller, 1965). Tollen's reagent will oxidize a variety of organic compounds, including aldehydes, although it will not affect ketones. Although some of the aldehyde peaks from the regenerated sample were decreased on the gas chromatograph, consistent results were unattainable, so the method could not be used for confirmatory identification of the aldehydes.

According to Wild (1960), aldehydes form crystalline derivatives with an excess of dimedone (5,5-dimethyl cyclohexa-1,3-dione). As the reaction is specific for aldehydes, and no precipitate is formed with ketones, the possibility of precipitating the aldehydes of the flavor volatiles was investigated for confirmatory identification. Unfortunately, the dimedone derivatives are formed rather slowly, sometimes taking days to crystallize, thereby making the method unsatisfactory for precipitation of the aldehydes of the volatiles obtained by cooking beef.

In a review on meat flavor, Hornstein and Crowe (1964) listed the aldehydes and ketones found in the volatiles obtained on cooking beef, which has been reported by other investigators. These included formaldehyde, acetaldehyde, propanal, 2-methyl propanal, 3-methyl butanal, 2-butanone and acetone. These are in agreement with the findings in the present study. In addition, butanal and the 5-carbon carbonyls were shown to be present in the flavor volatiles obtained in the present investigation.

Yueh and Strong (1960) isolated 2,3-butanedione from cooked beef broth. When reagent-grade 2,3-butanedione was chromatographed, either a very broad or a reverse peak was obtained. If present, 2,3-butanedione could have been masked by the large peaks of butanal and 2-butanone on the gas chromatogram. However, it is unlikely 2,3-butanedione was present in the flavor volatiles since chemical tests with 2% alcoholic potassium hydroxide on the 2,4-DNPS were negative.

Hornstein and Crowe (1960) isolated hexanal and small quantities of nonanal from steam distilled beef fat. They also reported finding unsaturated aldehydes and dialdehydes. If they heated beef fat in air without steam distillation, dialdehydes and unsaturated aldehydes were not produced. The three carbonyl compounds they obtained by heating beef fat in air at 100°C were acetaldehyde, propanal and acetone. In the present investigation, the beef was cooked in an inert atmosphere, in the absence of air or oxygen, by both methods. The temperature of the emerging volatiles from both beef cooked in fat and water did not rise above 100°C, although when beef was cooked in fat the temperature of the fat itself was considerably higher than that of the emerging volatiles. It is probable that sweeping the surface of the cooking meat with air or oxygen would have produced a greater yield of carbonyl compounds, especially from the beef cooked in fat.

It seems likely that the difference between the flavor volatiles produced by beef cooked in water and fat may arise in part from the quantity and composition of the aldehydes and ketones released on heating. However, it is not possible at present to identify the exact carbonyl or carbonyls responsible for the difference. The greater concentration of 2-butanone in the flavor volatiles obtained on cooking beef in fat, as compared to that cooked in water, was undoubtedly due to the contribution of the fat itself. This was evident when 2-butanone was shown to be the carbonyl obtained in the greatest proportion when fat was cooked alone. It is possible that the relative proportions of 2-butanone and butanal may account for part of the difference in aroma and/or flavor obtained on cooking in fat and water, but as yet this has not been verified. When it is possible to accomplish quantitative analysis of the carbonyls in the volatiles rather than just determining the relative proportions of the aldehydes and ketones, it will be possible to elucidate the exact differences between the carbonyl compounds obtained on cooking beef in fat and water.

Sulfur Compounds

Hydrogen Sulfide and Mercaptans It was not possible to obtain the yield of mercuric mercaptides because of the large quantities of mercuric sulfide present in the same mercuric cyanide trap. The white mercaptides were not visible in the first mercuric cyanide trap because the black mercuric sulfide precipitate masked their appearance. As hydrogen sulfide is the only sulfur compound which forms a black precipitate with mercuric cyanide, this is a positive identification for the presence of hydrogen sulfide in the flavor volatiles of both beef cooked in both fat and water. Mercurous oxide is another black mercury compound, but it would not be formed by bubbling the flavor volatiles of cooking meat through a 4% solution of mercuric cyanide.

Although white mercuric mercaptides were not visible in the mercuric cyanide trap, the mercaptans were released on regeneration with acid. Black mercuric sulfide was not decomposed with the 1N H_2SO_4. This showed that mercaptans as well as hydrogen sulfide were present in the flavor volatiles of beef cooked in both water and fat. After regeneration with 1N H_2SO_4, the mercaptans were converted to disulfides with excess iodine in ether according to the procedure described by Sporek and Danyi (1963). The resulting diethyl ether solution was pale yellow in color due to the disulfides and impurities. On submitting the mixture to gas chromatography, the impurities completely masked all peaks produced by the electron capture detector. Thus, it was necessary to wash the mercuric mercaptide precipitate with diethyl ether prior to regeneration in order to remove the impurities. Washing the mercaptides prior to regeneration and oxidation removed the majority of the masking impurities, which were produced on injection of the disulfide sample.

In order to identify the mercaptans, the retention time and the value of Ø (the ratio of the response of the electron capture detector to the response of the flame detector) for disulfides regenerated from known mercuric mercaptides were recorded. These values are reported in Table IV. Oaks et al. (1964) reported Ø values for mercaptans from 0.1 to 0.5, for monosulfides from 0.01 to 0.09 and for disulfides from 0.5 to 10.0. The highest Ø values were obtained for trisulfides, with an increase of 200- to 300-fold over the Ø value of the disulfides. High Ø values were observed in the present investigation, if oxidation of the mercaptans to disulfides were carried out too vigorously, presumably because of the formation of trisulfides.

Oaks et al. (1964) noted that the values for Ø may vary as much as 10% from day to day and that the reproducibility for a single compound within a given set of chromatographic conditions was within a deviation of 4%. Variation of the Ø value could be partially compensated for by normalizing with known standards. In the present study, the value of Ø varied considerably according to the amount of standard disulfide injected into the gas chromatograph. Injection of a consistent volume of known and unknown disulfides produced a more consistent Ø value. All the values of Ø obtained for disulfides were within the range of 0.5 to 10.0 as found by Oaks et al. (1964).

Table IV. Organic disulfide standards

Disulfide	Separated on Triton X-305 volumn at 105°C Retention time in minutes	Ø value
Dimethyl	2.4	1
Methyl ethyl	3.2	2
Methyl iso propyl	3.7	3
Diethyl	4.2	2
Methyl propyl	4.5	3
Diisopropyl	5.2	4
Ethyl propyl	5.9	3
Methyl butyl	6.5	1
Dipropyl	8.3	3
Dibutyl	12.2	3

The inconsistency of Ø is mainly due to the response of the electron capture detector. This response is dependent on the standing current, which is partially determined by the column bleed and the contamination of the tritium foil. In order to attain consistent results, the tritium foil was cleaned at least every two months according to the Atomic Energy Commission regulations. Oaks et al. (1964) reported Ø values for standard sulfur compounds to two significant places and one significant place for the unknown sulfur compounds extracted from garlic. Variations in Ø up to 1.6 between the standard and unknown disulfides made this accuracy appear unnecessary. In the present investigation, the value of Ø was reported to the nearest whole number.

The only disulfide produced from the unknown mercuric mercaptides from beef cooked in both water and fat was identified as dimethyl disulfide. A typical chromatograph of dimethyl disulfide from either beef cooked in water or fat is shown in Fig. IV. The first peak is diethyl ether, which was used as the solvent. The second peak is dimethyl disulfide obtained upon oxidation of the mercaptan in the flavor volatiles. It has a retention time of 2.4 minutes and a ∅ value of 1, which is in agreement with the values obtained for the standard sample of dimethyl disulfide and the values reported by Oaks et al. (1964). This indicates the presence of methyl mercaptan in the volatiles from beef cooked in both water and fat.

If more than one mercaptan had been present in the flavor volatiles of cooking meat, this would have given rise to a series of peaks on oxidation to disulfides. For example, if ethyl mercaptan had been present as well as methyl mercaptan, diethyl and dimethyl and methyl ethyl disulfide would all have been produced on oxidation. Oxidation of three mercaptans would have produced six disulfides, four mercaptans would have produced ten disulfides and five mercaptans would have given 15 permutations. Thus, if more than one mercaptan had been present in the flavor volatiles of beef cooked in either fat or water, a series of disulfides would have resulted on oxidation to disulfides. Within the sensitivity of the gas chromatograph, there is a definite indication that cooking beef by either method produced only one mercaptan; namely methyl mercaptan.

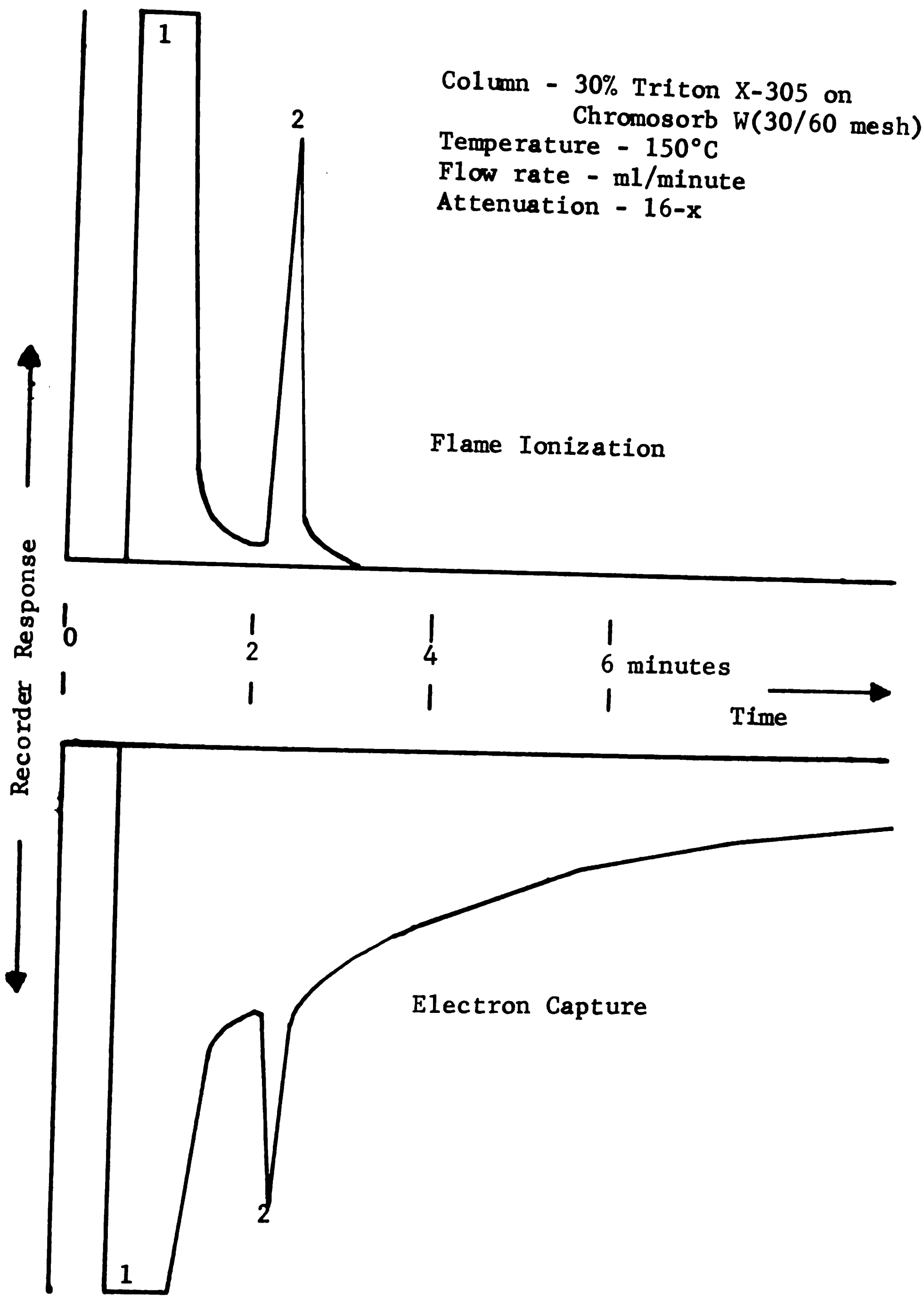

Figure IV. Gas chromatogram of the disulfide obtained on oxidation of the mercaptan volatilized by beef cooked in either water or fat.

Kramlich and Pearson (1960) found methyl mercaptan and hydrogen sulfide were released on heating a slurry of beef. Besides hydrogen sulfide and methyl mercaptan, Hornstein and Crowe (1964) noted that ethyl mercaptan had been found in the flavor volatiles of cooking beef. Ethyl mercaptan was not found in the flavor volatiles from beef cooked in either fat or water in the present investigation.

Sulfides The volatile sulfides obtained from cooking meat were passed through the mercuric cyanide trap and precipitated in the mercuric chloride trap. According to Challenger (1959), organic sulfides form co-ordination compounds with mercuric chloride of the type $R_2S.xHgCl_2$. The co-ordination complex from dimethyl sulfide with mercuric chloride has the composition $2(CH_3)_2S.3HgCl_2$, and that from diethyl sulfide with mercuric chloride has the formula $(C_2H_5)_2S.2HgCl_2$. There is no bond between the mercury and carbon as the sulfur donates electrons for the formation of the co-ordination compound with mercuric chloride. Because of this fact, the co-ordination compounds are readily decomposed by heat or hot water. When decomposed with cold sodium hydroxide, the characteristic odor of the sulfide is apparent.

The combined precipitate from several cookings was filtered and washed with distilled water. Cold concentrated sodium hydroxide was added to the precipitate and the odor produced was compared with dimethyl, diethyl, methyl ethyl and dipropyl sulfide. A brilliant yellow precipitate of mercuric oxide was produced as the dialkyl sulfide or sulfides was/were released. According to Challenger (1959) the mercuric oxide produced does not oxidize the dialkyl sulfides either in hot or cold suspension.

A strong odor, characteristic of dimethyl sulfide, was produced when sodium hydroxide was added to the white precipitate from the mercuric chloride trap of beef cooked in both fat and water. This indicates that dimethyl sulfide is the sulfide in greatest proportion in the flavor volatiles of beef cooked in both water and fat. This is in agreement with Kramlich and Pearson (1960) who tentatively identified dimethyl sulfide in the flavor volatiles of a cooking beef slurry. Yueh and Strong (1960) have also identified dimethyl sulfide in the flavor volatiles of cooking beef.

Only hydrogen sulfide and methyl mercaptan were positively identified from the volatile sulfur derivatives obtained on cooking beef. However, dimethyl sulfide was tentatively identified by its characteristic odor. These same three compounds appeared to be present in both beef cooked in fat and that cooked in water. It is possible that the amounts of these compounds may have varied quantitatively, but the methods used were only qualitative, and the differences could not be determined.

The fact, that no differences were found in the type of sulfur compounds produced by beef cooked in either fat or water, does not rule out the possibility of their role in producing flavor differences by the two methods of cooking. The odor of hydrogen sulfide, mercaptans and sulfides varies considerably with concentration. Hornstein et al. (1960) have already determined the quantitative amounts of hydrogen sulfide produced when beef is cooked in water and work on the hydrogen sulfide produced from heated beef fat is currently under investigation. Although quantitative analysis of the minute amounts of methyl mercaptan and dimethyl sulfide presents a more formidable task, there is no doubt that the improved instrumentation and techniques of the future will determine the exact role and importance of each flavor constituent.

SUMMARY AND CONCLUSIONS

Chemical components volatilized on cooking beef in water were compared with those obtained on cooking an equal amount of beef in fat. The flavor volatiles were separated in reagent traps, regenerated and identified by gas chromatography.

The yield of carbonyl compounds precipitated as their 2,4-DNPS from beef cooked in fat was three times that from beef cooked in water, with mean values of 0.17 and 0.05 g., respectively. Although the fat itself contributed some carbonyl compounds, these carbonyls did not completely account for the increase in 2,4-DNPS on cooking beef in fat. The greater proportion of 2-butanone volatilized by beef cooked in fat was probably due to the contribution of the fat, although it could still be a major contributor to the aroma and/or flavor of beef cooked in fat.

On regeneration with levulinic acid, the carbonyl compounds from the volatiles obtained on cooking beef in both fat and water had a sweet, faint caramel aroma. A total of 14 peaks was obtained from the aldehydes and ketones regenerated from their 2,4-DNPS from beef cooked in both fat and water. Butanal, 2-butanone and the 5-carbon carbonyl compounds constituted the major aldehydes and ketones produced by both methods of cooking. Smaller amounts of formaldehyde, acetaldehyde, acetone, propanal, 2-methyl propanal and 3-methyl butanal were also identified. Five other peaks were obtained but identification was not possible due to the small sample size. A small peak caused by the breakdown of levulinic acid was observed on the gas chromatograms of the carbonyl compounds from beef cooked in both water and fat.

Polycarbonyls, such as 2,3-butanedione, were shown to be absent in the regenerated carbonyls from the flavor volatiles obtained on cooking beef in fat and water. Various chemical tests for the confirmatory identification of the aldehydes and ketones were found to be unsatisfactory.

The carbonyl compounds in the flavor volatiles obtained on cooking beef in water had a greater concentration of butanal than 2-butanone, whereas, beef cooked in fat yielded more 2-butanone than butanal. Since 2-butanone was found to be the carbonyl compound released in greatest proportion from heated beef fat, it is possible that the larger proportion of 2-butanone found in the volatiles obtained on cooking beef in fat may have been partially due to the fat itself.

Free mercaptans were released on regeneration of the precipitated mercuric mercaptides with acid. The freed mercaptans were oxidized to their corresponding disulfides for easier identification and analyzed by gas chromatography. Methyl mercaptan was the only mercaptan identified in the flavor volatiles obtained on cooking beef in either fat or water.

Large quantities of hydrogen sulfide were found to be present in the flavor volatiles obtained from beef cooked in both water and fat. In addition, dimethyl sulfide was tentatively identified as the sulfide in greatest proportion in the volatiles of beef cooked in water and fat.

The importance of the sulfur compounds in flavor is well known. Although no differences were found in the type of sulfur compounds produced by the two methods of cooking, it is evident that further investigations are necessary to elucidate the exact role and importance of each component. Studies to increase the knowledge of interactions between the carbonyl and sulfur compounds in the flavor volatiles of meat is also necessary.

BIBLIOGRAPHY

Adams, D. F., and R. K, Koppe. 1959. Gas chromatographic analysis of hydrogen sulfide, sulfur dioxide, mercaptans, and alkyl sulfides and disulfides. TAPPI 42:601.

Adams, D. F., R. K. Koppe and D. M. Jungroth. 1960. Adsorption sampling and gas chromatographic analysis of sulfur compounds in waste process gases. TAPPI 43:602.

Batzer, O. F., and D. M. Doty. 1955. Nature of undesirable odors formed by gamma irradiation of beef. J. Ag. Food Chem. 3:64.

Batzer, O. F., A. T. Santoro, W. A. Landmann and B. S. Schweigert. 1960. Precursors of beef flavor. J. Ag. Food Chem. 8:498.

Batzer, O. F., A. T. Santoro and W. A. Landmann. 1962. Identification of beef flavor precursors. J. Ag. Food Chem. 10:104.

Bender, A. E., and P. E. Ballance. 1961. A preliminary examination of the flavor of meat extract. J. Sci. Food Ag. 12:683.

Bender, A. E., T. Wood and J. A. Palgrave. 1958. Analysis of tissue constituents. Extract of fresh ox muscle. J. Sci. Food Ag. 9:812.

Bouthilet, R. J. 1949. A note on the nature of a flavor constituent from poultry meat. Food Technol. 3:118.

Bouthilet, R. J. 1950. Chicken flavor: The source of the meat flavor component. Food Res. 15:322.

Bouthilet, R. J. 1951a. The fractionation of volatile constituents. Food Res. 16:118.

Bouthilet, R. J. 1951b. Chicken flavor: Separation and concentration of its volatile components from broth. Food Res. 16:201.

Challenger, F. 1959. Aspects of the Organic Chemistry of Sulfur. Butterworth's. London, England.

Crocker, B. C. 1948. Flavor of meat. Food Res. 13:179.

Cross, C. K., and P. Ziegler. 1965. A comparison of the volatile fractions from cured and uncured meat. J. Food Sci. 30:610.

Carson, J. F., and F. F. Wong. 1961. The volatile flavor components of onions. J. Ag. Food Chem. 9:140.

Doty, D. M., O. F. Batzer, W. A. Landmann and A. T. Santoro. 1961. Meat flavor. Proceedings Flavor Chemistry Symposium. Campbell Soup Co., Camden, N. J. p. 7.

Hornstein, I., P. F. Crowe and W. L. Sulzbacher. 1960. Constituents of meat flavor: Beef. J. Ag. Food Chem. 8:65.

Hornstein, I., and P. F. Crowe. 1960. Meat flavor chemistry: Studies on beef and pork. J. Ag. Food Chem. 8:494.

Hornstein, I., and P. F. Crowe. 1962. Gas chromatography of food volatiles - An improved collection system. Anal. Chem. 34:1354.

Hornstein, I., and P. F. Crowe. 1963. Meat flavor: Lamb. J. Ag. Food Chem. 11:147.

Hornstein, I., P. F. Crowe and W. L. Sulzbacher. 1963. Flavor of beef and whale meat. Nature 199:1252.

Hornstein, I., and P. F. Crowe. 1964. Meat flavor - A review. J. Gas Chromatography 2:128.

Kazeniac, S. J. 1961. Chicken flavor. Proceedings Flavor Chemistry Symposium. Campbell Soup Co., Camden, N. J. p. 37.

Keeney, M. 1957. Regeneration of carbonyls from 2,4-dinitrophenylhydrazones with levulinic acid. Anal. Chem. 29:1489.

Kramlich, W. E., and A. M. Pearson. 1958. Some preliminary studies on meat flavor. Food Res. 23:567.

Kramlich, W. E., and A. M. Pearson. 1960. Separation and identification of cooked beef flavor components. Food Res. 25:712.

Libbey, L. M., D. D. Bills and E. A. Day. 1963. A technique for the study of lipid-soluble food flavor volatiles. J. Food Sci. 28:329.

Liebig, Justus von. 1847. Über die Bestandtheile der Flüssigkeiten des Fleisches. Annalen der Chemie und Pharmacie 62:257.

Lineweaver, H., and E. L. Pippen. 1961. Chicken flavor. Proceedings Flavor Chemistry Symposium. Campbell Soup Co., Camden, N. J. p. 21.

Mecchi, E. P., E. L. Pippen and H. Lineweaver. 1964. Origin of hydrogen sulfide in heated chicken muscle. J. Food Sci. 29:393.

Minor, L. J., A. M. Pearson, L. E. Dawson and B. S. Schweigert. 1965a. Chicken flavor: The identification of some chemical components and the importance of sulfur compounds in the cooked volatile fraction. J. Food Sci. 30:686.

Minor, L. J., A. M. Pearson, L. E. Dawson and B. S. Schweigert. 1965b. Gas chromatographic analysis of volatile constituents from cooked carcasses of old and young chickens. Poultry Sci. 44:535.

Minor, L. J., A. M. Pearson, L. E. Dawson and B. S. Schweigert. 1965c. Separation and identification of carbonyl and sulfur compounds in the volatile fraction of cooked chicken. J. Ag. Food Chem. 13:298.

Noller, C. R. 1965. <u>Chemistry of Organic Compounds</u>. W. B. Saunders Co., Philadelphia.

Nonaka, M., and E. L. Pippen. 1966. Volatiles and oxidative flavor deterioration in fried chicken. J. Ag. Food Chem. 14:2.

Oaks, D. M., H. Hartman and K. P. Dimick. 1964. Analysis of sulfur compounds with electron capture/hydrogen flame dual channel gas chromatography. Anal. Chem. 36:1560.

Ockerman, H. W., T. N. Blumer and H. B. Craig. 1964. Volatile chemical compounds in dry-cured hams. J. Food Sci. 29:123.

Pippen, E. L., A. A. Campbell and J. V. Streeter. 1954. Flavor studies. Origin of chicken flavor. Poultry Sci. 34:1139.

Pippen, E. L., and E. J. Eyring. 1957. Characterization of volatile nitrogen and sulfur fractions of cooked chicken and their relation to flavor. Food Technol. 11:53.

Pippen, E. L., A. A. Campbell, F. T. Jones and F. Stitt. 1958. Volatile carbonyl compounds of cooked chicken. I. Compounds obtained by air entrainment. Food Res. 23:103.

Pippen, E. L., and M. Nonaka. 1960. Volatile carbonyl compounds of chicken. II. Compounds volatilized with steam during cooking. Food Res. 25:764.

Pippen, E. L., and M. Nonaka. 1963. Gas chromatography of chicken and turkey volatiles; the effect of temperature, oxygen, and type of tissues on composition of the volatile fraction. J. Food Sci. 28:334.

Sporek, K. F., and M. D. Danyi. 1963. Detection and identification of mercaptans by gas liquid chromatography. Anal. Chem. 35:956.

Wasserman, A. E., and N. Gray. 1965. Meat flavor. I. Fractionation of water soluble flavor precursors of beef. J. Food Sci. 30:801.

Wild, F. 1960. Characterisation of Organic Compounds. Cambridge University Press, Cambridge, England.

Wood, T. 1961. Browning of ox muscle extracts. J. Sci. Food Ag. 12:61.

Wood, T., and A. E. Bender. 1957. Analysis of tissue constituents. Commercial ox muscle extract. Biochem. J. 67:366.

Yueh, M. H., and F. M. Strong. 1960. Some volatile constituents of cooked beef. J. Ag. Food Chem. 8:491.