SEPARATION AND IDENTIFICATION OF BEEF FLAVOR COMPONENTS

By

William Edward Kramlich

AN ABSTRACT

Submitted to the College of Agriculture
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ABSTRACT

Flavor is one of the most important attributes of all food products, yet little is known concerning its chemical nature or the contributing components. In spite of the importance of flavor to acceptability and saleability of meat items, little research effort has been directed toward the area of meat flavor. The first objective of this study was to characterize and to ascertain if the components responsible for meat flavor were water extractible and whether they were located in the extractible juices, or the fibers from both cooked and raw meat. The second objective was to fractionate, collect and identify the volatile components contributing to cooked meat flavor by using a combination of gas chromatography and qualitative tests.

Flavor was characterized by having a group of judges suggest descriptive terms until the group was satisfied that the taste sensations were adequately described. Raw meat, cooked meat, press fluid and press cake were characterized. The threshold at which flavor could be detected was determined by submitting a series of water dilutions to a panel of judges and having them record the number of the beaker in which they could first obtain a definite flavor.

The volatile compounds from cooked beef were collected in a series of cold traps--wet-ice, dry-ice and ethanol and liquid air. The volatiles were then further fractionated and tentatively identified in a vapor fractometer. Qualitative organic tests, in addition to comparing the retention volumes of known and unknown compounds in the vapor fracto-

meter, were used to identify the fractionated compounds. Carbonyl compounds were collected as their 2, 4-dinitrophenylhydrazones and chromatographed on paper.

The character of the flavor varied greatly between the raw and the cooked fractions, yet upon heating the raw fractions, the flavor appeared to differ only in intensity. It was evident that press fluid had a highly concentrated flavor. Results showed the flavor constituents were largely water soluble in both the cooked and the raw fractions. However, cooking prior to extraction increased the flavor threshold, indicating that the full flavor development may be due to heating the juice and fibers together. Leaching of the meat with water resulted in a complete loss of flavor with both raw and cooked meat, but the cooked meat maintained its flavor over a longer period of time.

Of the volatile carbonyl compounds collected and chromatographed on paper as their 2, 4-dinitrophenylhydrazone derivatives, only acetaldehyde was identified; however, the presence of at least two, and possibly more carbonyls was indicated by the presence of two large, poorly resolved spots. Of the three columns (didecylphthalate, "carbowax 400" and dinonylphthalate) used in the vapor fractometer to study cooked beef volatiles, dinonylphthalate found was to be the most suitable.

The "carbowax 400" column resolved the volatile mixture into 3 peaks, identified as carbon dioxide, methyl mercaptan and acetaldehyde. The didecylphthalate column resolved the mixture into 6 peaks. Four of the compounds were tentatively identified as carbon dioxide, methyl

mercaptan, acetaldehyde and acetone, but identification was not accomplished for the other two peaks. Six peaks were resolved on the dinonylphthalate column and were identified as carbon dioxide, methyl mercaptan, acetaldehyde, methyl sulfide, acetone and water.

An infrared spectrum of the entire volatile mixture showed but three relatively small absorption maxima. No conclusions as to the nature of the compounds in the mixture could be drawn from the graphs, because the quantity of volatiles was too small for identification by this method.

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INTRODUCTION

Flavor is one of the most important attributes of all food products, yet little is known concerning its chemical nature or the contributing components. In spite of the importance of flavor to acceptability and saleability of meat items, little research effort has been directed toward the area of meat flavor.

The study and eventual solution of many flavor problems confronting the meat industry would be greatly aided by basic knowledge of the chemical and physical components contributing to meat flavor. Solutions to such far-reaching and diverse flavor problems as those involving irradiated meats, boar flavor and also mutton flavor would be logical outgrowths of more extensive knowledge of the normal flavor of meat.

Crocker (8) found the flavor of raw meat to be present in the juice but not in the fiber. However, he attributed the flavor of cooked meat to chemical changes taking place in the fiber rather than in the juice. Unfortunately, the methods of analysis available to the researcher until a few years ago made it a tedious and difficult task to determine the chemical components responsible for meat flavor. Much depended on the keenness and accuracy of the sense of smell of the individual investigator in interpreting organic chemical odors, a situation open to criticism and possible error. However, with the application of gas chromatography and mass spectrometry to problems involving food flavor, new methods and approaches can be employed in an attempt to unlock the secret of meat flavor.

EXPERIMENTAL OBJECTIVES

The objectives of this study were as follows:

1. To determine whether the flavor of beef, both raw and cooked, was present only in the fibers, in the extractable juice or in both the fiber and juice.

2. To ascertain whether the flavor constituents were water soluble.

3. To detect, collect and identify the volatile constituents of cooked beef.

REVIEW OF LITERATURE

General

The field of food flavor research in general, and meat flavor research in particular, has been relatively unexplored; consequently, little actual research data have been published.

Hammond (16) stated that in general, the strength of meat flavor is closely linked with meat color and that color could be used for judging flavor, there being an optimum color and flavor for each class of meat.

Solamon (31) attributed the flavor of cooked meat to disintegration of the proteins into cleavage products-through the proteoses, peptones, peptides and to the amino acids. He named glutamic acid as being chiefly responsible for meat flavor. Howe and Barbella (18) reported the constituents of meat flavor to be a composite of salts, acids and a group of products resulting from heating extractives, possibly disintegration products of proteins and lipids.

Wood (36) examined the chemical composition of cooked meat extract by freeing the extract from its protein and submitting the extract to paper chromatography. By the use of a wide range of color reagents the following compounds were identified: glutamic acid, serine, alanine, glycine, proline, methyl histidine, carnosine, anserine, guanidine, methyl guanidine, creatine, creatinine, choline, carnitine, hypoxanthine, inosine, urea and citrulline. No sugars or their derivatives were detected in the extract. However, no attempt was made to relate the components of the

extract to flavor, and it is likely that many of these components contribute little or nothing to flavor. Furthermore, the method of preparing and analyzing the extract unquestionably resulted in breakdown products not normally present after the usual methods of cookery.

Alexander and Elvehjem (1) using ion exchange and paper chromatographic procedures separated and identified the nitrogenous compounds of beef. Approximately one hundred percent of the nitrogen present in meat was accounted for as amino acid nitrogen, ammonia and other nitrogenous constituents, including vitamins, purines, creatine, creatinine, carnitine and methyl guanidine. Since the objective of this study was determination of the nitrogenous components, no attempt was made to associate the compounds present with flavor.

Crocker (8) reported the flavor of cooked beef to be quite complicated chemically and to consist more of odor than taste. He indicated that hydrogen sulfide, amines of several kinds, including a low simple form, one of the piperdine type, and possibly indole were present.

Bouthilet (4) reported the flavor of chicken to consist of volatile compounds which were sufficiently stable to be stripped from broth and concentrated in a fractionating column. At a pH of 5.8, it was found by Bouthilet (3) that cooking chicken elicited a continuous savory odor. It was assumed that the flavor was most volatile at this pH. Bouthilet (5) further demonstrated the presence of volatile nitrogen and volatile sulfur fractions in chicken broth. Pippen et al. (27) characterized the volatile nitrogen fraction of chicken broth as being entirely ammonia and the sulfide fraction to be hydrogen sulfide. Pippen et al. (27)

concluded that additional factors and substances are involved in the production of chicken flavor.

By the use of a glass train designed to trap the volatile sulfur components of cooked cabbage, Dateo (9) has identified dimethyl sulfide and hydrogen sulfide. Analysis indicated that methyl sulfide was the main constituent and was largely responsible for the odor of cooked cabbage.

Methional has been suggested by Patton (23) as a compound of general importance in the flavor of foods because of its broth-like qualities. He claimed that it is odorless when freshly vacuum distilled and that within minutes after exposure to air it takes on an extremely potent meat-like character. It has been further proposed that this odor results from methional sulfoxide. However, Witting and Batzer (35) have questioned whether methional has any odor.

Physical Components Associated with Flavor

One of the first attempts to determine which meat components were actually responsible for flavor was made by Crocker (8). In this study the juice and fibers were separated by pressing and subsequent leaching with water. The raw meat fiber so treated had no apparent flavor, but developed a distinct meaty flavor after being cooked. However, the press juice upon being boiled, produced very little odor and did not appreciably increase in taste intensity. Thus, it was concluded that cooking developed the meaty flavor, apparently owing to chemical changes taking place in the fiber rather than in the juice. He demonstrated that bones contributed little to beef flavor while marrow and tissue fats may have

supplied aroma but contributed nothing to the development of a meaty flavor.

According to Solamon (31) raw meat has no particular flavor and it is only upon cooking that meat develops a characteristic flavor, partly derived from heated fat.

According to Bouthilet (6) the flavor of chicken meat is derived from a substance which is not associated with fat, but is attached to the fibers and cannot be removed by pressing. He found it to be a soluble substance, which is extractable from meat with dilute tri-chloro-acetic acid or 60% methyl alcohol.

Peterson (25) reported fat and skin to contribute little to the flavor of chicken broth; while Pippen et al. (26) stated that chicken meat was a much better source of flavor than either the bones, fat or skin or a composite of all three components.

Cold Water Extraction

Work by Bouthilet (6), Pippen et al. (28) and Peterson (25) indicated that the "meaty-flavor" can be extracted from chicken meat by steeping in cold water. Pippen et al. (26) lyophilized the extract and were able to restore the flavor to chicken broth by adding back the neutralized ash of the extract.

Volatile Carbonyl Components

One of the chief obstacles to research in the field of food flavors has been the lack of methods and techniques that could be used to study various volatile food flavors. In recent years, the volatile carbonyl compounds believed to be associated with food flavors have been studied

by converting the carbonyls to their 2, 4-dinitrophenylhydrazone derivatives. Identification of the carbonyls has been accomplished using paper chromatography and by melting point determinations on the isolated compounds. This technique has been employed by several investigators to study the flavor of milk (11), cheese (24), fruits (14)(17), vegetables (10) and recently, chicken (29).

Day et al. (11) reported the presence of five carbonyl compounds in irradiated skim milk and identified them as acetaldehyde, acetone, butanone, formaldehyde and n-hexane, but he was able to find only acetaldehyde and acetone in unirradiated skim milk. The carbonyls were studied as their 2, 4-dinitrophenylhydrazone derivatives. Identification of the compounds was made by determining melting points and by chromatographing the compounds on paper. Similar studies by Patton et al. (24) on cheddar cheese indicated the presence of six carbonyls, heptone-2, butanone-2, acetone, acetaldehyde, formaldehyde and 3-OH butanone. Morgan et al. (22) found the principal volatile carbonyl compounds produced in skim milk to be furfural and acetaldehyde.

Spencer and Stanley (32) reported vacuum distillates of tomatoes contained esters at a level of 2 p.p.m. and volatile acids at 1 p.p.m. in both fresh and cooked juice. However, they stated that the main components of the juice were volatile carbonyl compounds. Acetaldehyde and isovaleraldehyde were the principal compounds identified, but eighteen other carbonyls were reported to be present in such small amounts as to make identification impractical. Acetaldehyde constituted seventy percent

of the total carbonyl weight from the raw tomato and eighty percent of the total carbonyl weight from the cooked tomato.

David and Josyln (10) reported the major volatile present in green peas to be acetaldehyde, but at least four other carbonyls were observed in the peas in such minute quantities as to make identification impossible. Henze et al. (17) were able to separate chromatographically eighteen volatile carbonyls from stored apples, but only acetaldehyde, acetone and propionaldehyde were identified. The 2, 4-dinitrophenylhydrazone derivatives of acetone, acetaldehyde, 2-hexanal and biacetyl were isolated from strawberry puree by Dimick and Makover (14). Identification was accomplished by means of melting point determinations, ultraviolet absorption spectra and x-ray diffraction patterns. The carbonyl content, however, was not thought to be related to the flavor intensity of the strawberries.

Pippen et al. (29) separated and identified eighteen separate carbonyl compounds present in chicken. Conclusively identified were derivatives of diacetyl, acetone, normal aliphatic saturated aldehydes containing 2, 3, 4, 5, 6, 8, and 9 carbon atoms, normal aliphatic 2-en-1-als containing 5, 6, 7, 10 and 11 carbon atoms and n-hepta, 2, 4-dien-1-al. Evidence was also obtained indicating the presence of 2, 4-dinitrophenylhydrazones of methyl-ethyl-ketone and of two unidentified 2, 4-dien-1-als. However, to increase the yield of minor components, the chicken slurry was simmered for twenty hours with air being constantly bubbled through it. It was recognized by the authors that this method of isolation involved a prolonged cooking time under conditions which

favored oxidation. Therefore, they stated it is possible that the volatile carbonyls found under these conditions could have arisen from oxidative processes or could be present in greater amounts than would be found in chicken cooked under normal conditions.

Batzer et al. (2) reported the presence of at least four 2, 4-dinitrophenylhydrazones in both the fat and the lean of beef subjected to sterilizing doses of gamma radiation. No attempt was made to identify these carbonyl compounds.

Burnett et al. (7), working with the volatile components of vacuum-packed dehydrated pork, isolated and identified acetaldehyde as being the only volatile carbonyl present in pork samples stored at both -20°F. and 94°F. Ammonia was detected in samples stored at 100°F. and 160°F.

Gas Chromatography and Mass Spectrometry

Until the development of gas-liquid partition chromatography in 1952, analysis of flavor fractions was carried out by chemists using distillation techniques on a micro scale followed by chemical analysis. Pain staking efforts and elaborate equipment often resulted in only group separation, and the high boiling materials were often badly decomposed and poorly resolved, according to Dimick and Corse (13). The development and application of gas chromatography to the analysis of food flavors has provided analytical tools for solving problems which were heretofore considered unsolvable. According to Jennings (19) a limited sample size makes it difficult to recover appreciable quantities of any given component, yet the components must be reasonably concentrated. The sample components must also be volatile and stable under the conditions

employed. Sullivan (34) observed that greatly improved separation could be achieved by gradually increasing the temperature of the gas chromatographic column during the time of elution. Nevertheless, it has been recognized by Day et al. (12), Stahl (33), and Drew et al. (15) that gas chromatography is not always the entire answer to the identification of various volatile compounds. The use of mass spectrometry along with gas chromatography has proven a valuable tool in making conclusive identification. According to Drew et al. (15), the mass spectrometer is best used after the volatile components of a mixture have been separated by gas chromatography. Thus, usage of both gas chromatography and mass spectrometry allows for more complete analysis of flavor components.

Rhoades (30) using gas chromatography to analyze the volatiles from ground roasted coffee, obtained sixteen peaks. Using infra red spectrometry and comparative retention times of knowns, acetaldehyde, acetone and methyl alcohol were identified. Tentative identification was made of dimethyl sulfide, propionaldehyde, methyl-ethyl ketone and diacetyl.

Mattick et al. (20) isolated and identified the main steam distillable acid of apple juice as n-caproic acid. Identification of the volatile acid was accomplished by means of vapor phase, paper and silicic acid chromatography in addition to the neutralization equivalent and the melting points of the analide and p-bromo-phenacyl ester derivatives.

Day et al. (12) used a low temperature-reduced pressure distillation apparatus to remove the volatiles from skim milk. The vapors were fractionated by a series of cold traps and introduced into a vapor fractometer

for separation and tentative identification. Conclusive identification was made by mass spectrometry. Only acetone was revealed in control samples of skim milk and sodium caseinate, while methyl sulfide, acetaldehyde, acetone, butanone, methyl and ethyl alcohol were identified in irradiated skim milk. With the exception of methyl alcohol, the same compounds were present in the caseinate. In addition, methyl mercaptan was found to be present. Using the same equipment and procedures as were used by Day et al. (12), Patton et al. (24) identified dimethyl sulfide, ethanol, acetone and diacetyl as being volatile components of cheddar cheese.

Wynn (37) used a low temperature-reduced pressure distillation apparatus similar to that used by Day et al. (12) to remove the volatiles from fresh milk. Separation and identification of the volatiles was accomplished by means of vapor phase chromatography. Acetone, acetaldehyde, methyl sulfide and an unidentified carbonyl were found to be present in the milk.

Stahl (33), using distillation and trapping methods similar to those described by Day et al. (12) was able to separate by means of gas chromotography and identify with the aid of the mass spectrometer, at least thirty eight different volatile compounds from beef given a beta radiation dosage of four megareps. He separately identified twenty five volatiles as being present in beef given two megareps of beta radiation. However, only five compounds were found to be present in the meat vapor released from the control sample which had not been irradiated. These compounds were carbon dioxide, carbon monoxide, hydrogen sulfide, methyl and ethyl mercaptans.

Merritt *et al.* (21) used the combination of gas chromatography and mass spectrometry to analyze the volatiles obtained from fresh and irradiated beef. Acetaldehyde, acetone, and methyl ethyl ketone were found to be present in both the fresh and irradiated meat. In addition, trace amounts of methyl mercaptan, dimethyl sulfide, ethyl mercaptan, methanol and ethanol were identified in fresh beef. These same compounds were observed to be present in irradiated meat, but in addition dimethyl sulfide and isobutyl mercaptan were found.

Experimental Procedure

This investigation was conducted in two segments, the first being an attempt to characterize and ascertain the intensity of flavor in various beef and beef juice fractions, while the second dealt with the fractionation and identification of the volatile flavor components.

Beef Flavor Characterization and Intensity

Source of meat.

The meat used in all phases of this investigation consisted of the *Longissimus dorsi* muscle stripped from a U. S. Good grade beef rib. Roasts were frozen and removed as needed for flavor studies.

Flavor threshold determinations.

To determine the threshold at which the flavor could be detected, a series of water dilutions were made and submitted to a panel of 3 to 5 judges. Each judge was asked to record the flask number in which he could first obtain a definite flavor. De-ionized distilled water was used to make all dilutions. Samples were tested at room temperature. Whenever a filtrate was used, the solution was filtered through acid-washed Whatman 41-H filter paper, previously washed with de-ionized distilled water to remove the acid taste.

Values reported as the flavor threshold in this study are those reported by the majority of the judges. However, there was generally excellent agreement between different judges, and real discrepancies were apparent only on a few occasions.

Characterization of Flavor.

Flavor was characterized by having the entire group of judges suggest descriptive terms until the group was satisfied that the taste sensations were adequately described. Raw meat, cooked meat, press fluid and press cake (both raw and cooked) were characterized.

Raw meat.

The raw meat was made into a slurry of 1 part meat to 2 parts of water in a Waring blender and used for flavor threshold determinations. After the initial flavor threshold was ascertained, the slurry was filtered and the filtrate collected.

Raw meat filtrate.

After the maximum dilution at which flavor could be detected was determined, the filtrate was heated for one hour over a steam bath at 93°C. The original filtrate was cooled and the flavor threshold was again determined to see if heating had any effect on intensity of flavor. The previously heated filtrate was again filtered to remove the slight precipitate that formed on heating, and to determine whether the flavor was concentrated in the precipitate or the aqueous portion.

Raw meat residue.

A portion of the residue remaining on the filter paper after the initial filtration was tasted and the flavor characterized. The remaining residue was resuspended in water, heated over a steam bath at 93°C. for one half hour, cooled, and the flavor characterized again to ascertain the influence of heating upon the raw meat fibers.

Cooked meat.

The meat was roasted to an internal temperature of 74°C. in an electric oven held at 149°C. A meat-water slurry was prepared in the same manner as with the raw meat; the same procedure was followed as that used for studying the slurry, filtrate, and residue of the raw meat.

Press fluid from raw beef.

The meat juice was extracted from the fibers with a Carver Laboratory Press. Three grams of shredded filter paper were mixed with every 100 g. of coarsely ground meat before pressing. Each 100 g. sample of meat was pressed for 10 minutes at a pressure of 6,000 lbs. per square inch, after the pressure was attained gradually during a 5-minute period, and the volume of press fluid was recorded. The flavor was characterized by the panel and the flavor threshold was determined by dilution. The remainder of the press fluid was heated for one hour over a water bath at 93°C., cooled, and the flavor threshold was again determined to ascertain the effect of heating on the flavor of press fluid from raw beef. The material which had coagulated upon heating was removed by filtering, and a flavor threshold determination was made upon the filtrate.

Press cake from raw beef.

The press cake from raw beef was tasted by the panel and its flavor characterized. Then a 1-3 meat-water slurry was prepared and simmered at 93°C. for one hour. The slurry was cooled to room temperature, tasted by panel members and the flavor was characterized. The slurry was then filtered to remove the fibers and fat particles and the filtrate tasted.

Press fluid from cooked beef.

The meat was prepared by roasting in the manner previously described. Press fluid was collected by the procedure used for the raw beef, and flavor threshold determinations were made both before and after filtering.

Press cake from cooked beef.

Two parts of water by weight were added to one part of press cake. The suspension was heated the same way as the raw press cake, tasted, filtered and the filtrate tasted.

Leaching studies on meat flavor.

Raw beef.

Seventy grams of raw beef were cut into 1-inch cubes, covered with 300 ml. of de-ionized distilled water and placed in a refrigerator held at approximately 4°C. At 24-hour intervals the beaker was removed and the meat squeezed by hand. Leachings were collected and used for further studies. The meat was again covered with 300 ml. of previously cooled water and returned to the refrigerator. The flavor threshold was determined for the leachings and the remainder of the leachings was heated over a 93°C. steam bath for one hour. The heated leachings were then cooled and threshold determinations made.

Cooked beef.

A beef roast was roasted as described previously. The meat was cut into cubes and treated the same as the raw meat except that the leachings were not simmered.

Chemical Analysis.

Dry matter and ether extract were determined for the raw beef roast, raw press fluid, cooked beef roast, cooked press fluid, raw press cake and cooked press cake. In the case of the raw beef roast, the sample was ground through a 5/64 inch plate, sealed in a glass jar and frozen at -20°F. for subsequent analysis. Samples of the cooked beef roast, raw and cooked press fluid and raw and cooked press cake were frozen intact. Moisture determinations were made by placing 3-5 g. of the thawed sample in a tared aluminum dish and placing in a 100°C. oven for 24 hours. At the end of the heating period, the dishes were removed from the oven and cooled in a desiccator for 20 minutes. The dishes were then weighed and the loss in weight calculated as per cent moisture. The per cent moisture subtracted from 100 gave the per cent dry matter.

Ether extract was determined on the moisture-free sample, which had been dried in a disposable aluminum weighing dish. Each dried sample was folded in the weighing dish and inserted into an alundum cup, which was placed in a metal sample container and extracted with anhydrous ethyl ether for four hours with a Goldfisch Fat Extractor. The excess ether was evaporated, whereupon the beaker and other soluble residue was dried to a constant weight in a 100°C. oven.

A Beckman model G pH meter was utilized to take pH readings at each step throughout the investigation.

Fractionation and Identification of Volatile Beef Flavor Components

Source of meat.

The beef used in this portion of the investigation consisted of the round muscles from ungraded cow rounds. The round was separated in 3

muscle groups, and roasts of approximately 2 pounds cut from each group. The first group consisted of the semimembranosus and adductor; the second, biceps femoris and semitendinosus while the third consisted of the sartorius, vastus lateralis, vastus intermedius, vastus medialis and rectus femoris. All roasts were trimmed closely of all external fat, wrapped in laminated freezer paper, frozen and subsequently stored at -20°F. Twelve hours prior to use, a roast was removed from the freezer and thawed in the paper at 37°F.

Fractionation and Identification of Volatile Sulfide and Carbonyl Compounds

Fractionation.

The beef was ground once through a quarter inch plate and then weighed. Sufficient de-ionized distilled water was added to make a 1-3 meat-water dilution by weight. The water and meat were mixed by means of an electric mixer and then poured into a 12 liter, triple necked, round bottom flask. Eight hundred grams of meat were cooked vigorously for 24-hours after which the flask was emptied and then recharged with an additional 700 g. of meat. Cooking was continued for another 96 hours. Nitrogen gas was used as a carrier to convey the meat volatiles through the system of traps. At the end of the cooking period, the hydrazones in traps 2 and 3 were filtered off, washed with acidified water and dried under vacuum.

The apparatus used for isolation of volatile sulfides and carbonyls consisted of a series of traps and is shown in figure 1. The first trap contained lead actate solution while the second and third traps each contained 300 ml. of a saturated 2,4-dinitrophenylhydrazine solution (2 g. per liter in 2 N HCl).

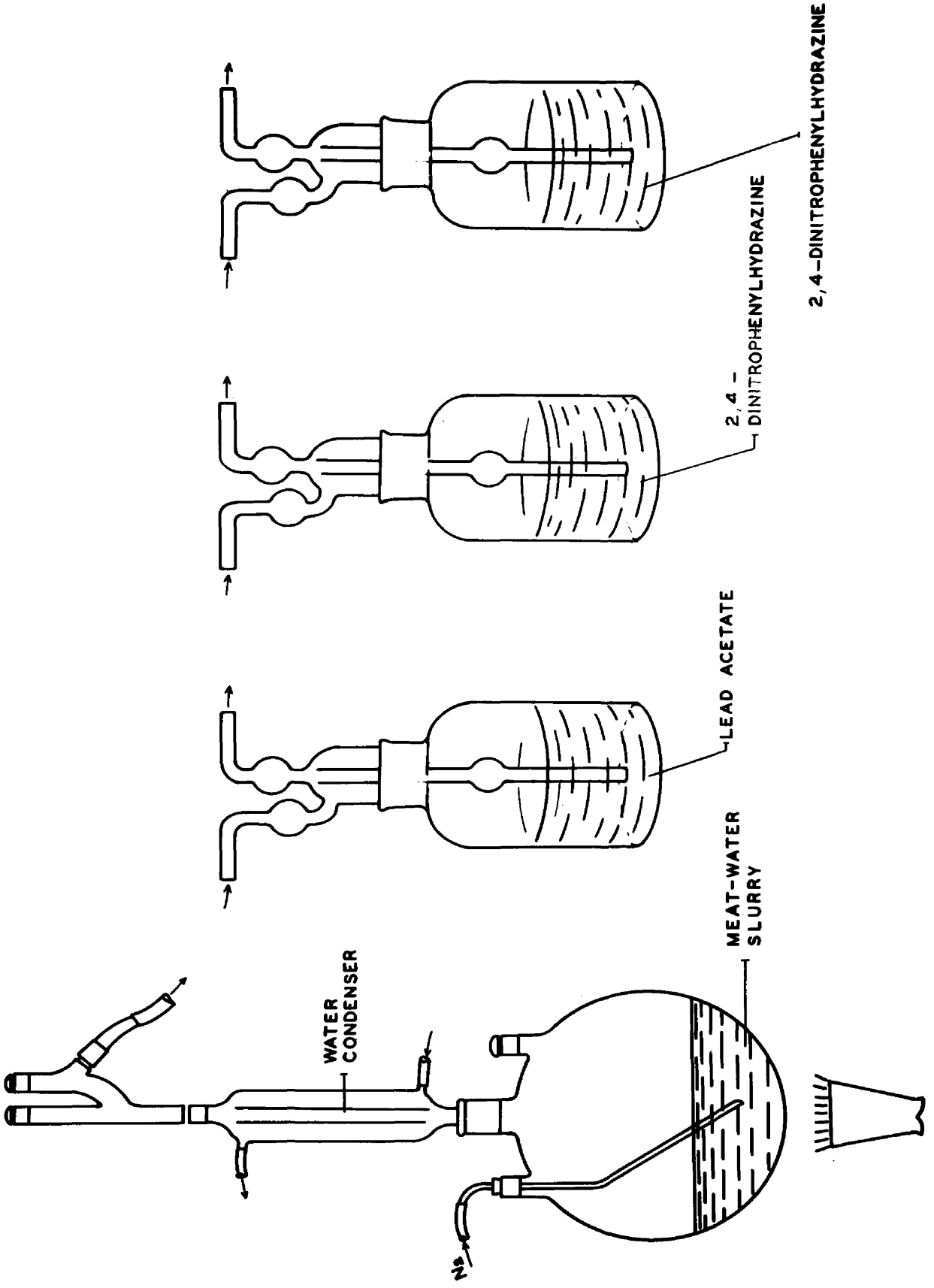

Figure 1. Diagram of collection apparatus for volatile sulfide and carbonyl compounds from cooked beef.

Identification of hydrazones was attempted employing paper chromatography. Ascending chromatographic methods were used with the procedure being carried out in a large bell jar. The solvent used was a mixture of 95% ethyl ether and 5% petroleum ether. Identification was accomplished by spotting known compounds adjacent to the unknown and comparing the relative movement of the known with the unknown on the strip of chromatographic paper.

Fractionation and Identification of Total Cooked Beef Volatiles.

Fractionation.

A 1-3 meat-water slurry was prepared as described previously for the detection of volatile carbonyls and poured into a 12 liter round bottom flask. The apparatus used for the fractionation of total cooked beef volatiles is pictured in figure 2. The first trap was immersed in a beaker of wet ice, the second in a beaker containing ethanol and dry ice. The third and fourth traps were immersed in liquid-air, a block of Styrofoam being utilized to contain the liquid-air. Nitrogen gas was bubbled through the meat slurry for the purpose of transporting the volatiles through the trapping system. Heat was applied to the flask by means of a Fisher burner and the slurry cooked for 5-7 hours. After the cooking was completed the traps were disconnected, stoppered and placed aside for subsequent fractionation of the volatile components in a Perkin-Elmer, Model 154-B, vapor fractometer. The fractometer together with a Leeds and Northrup recorder is pictured in figure 3. Figure 4 illustrates a schematic flow diagram of the vapor fractometer. The sample inlet system was modified slightly so that a trap containing volatiles for fractionation could be

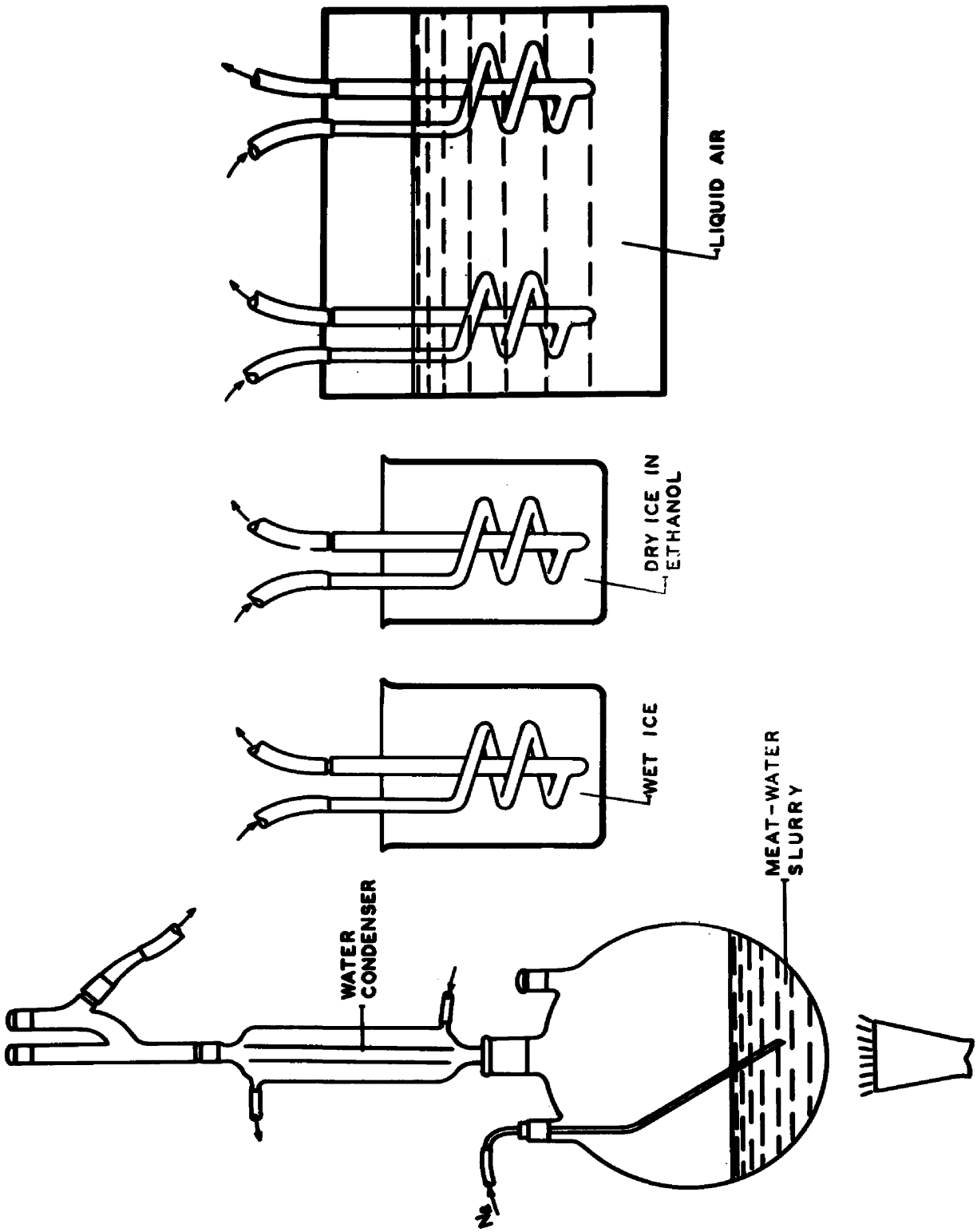

Figure 2. Diagram of collection apparatus for total cooked beef volatiles.

Figure 3. Perkin-Elmer Model 154-B vapor fractometer with its matching Leeds and Northrup recorder.

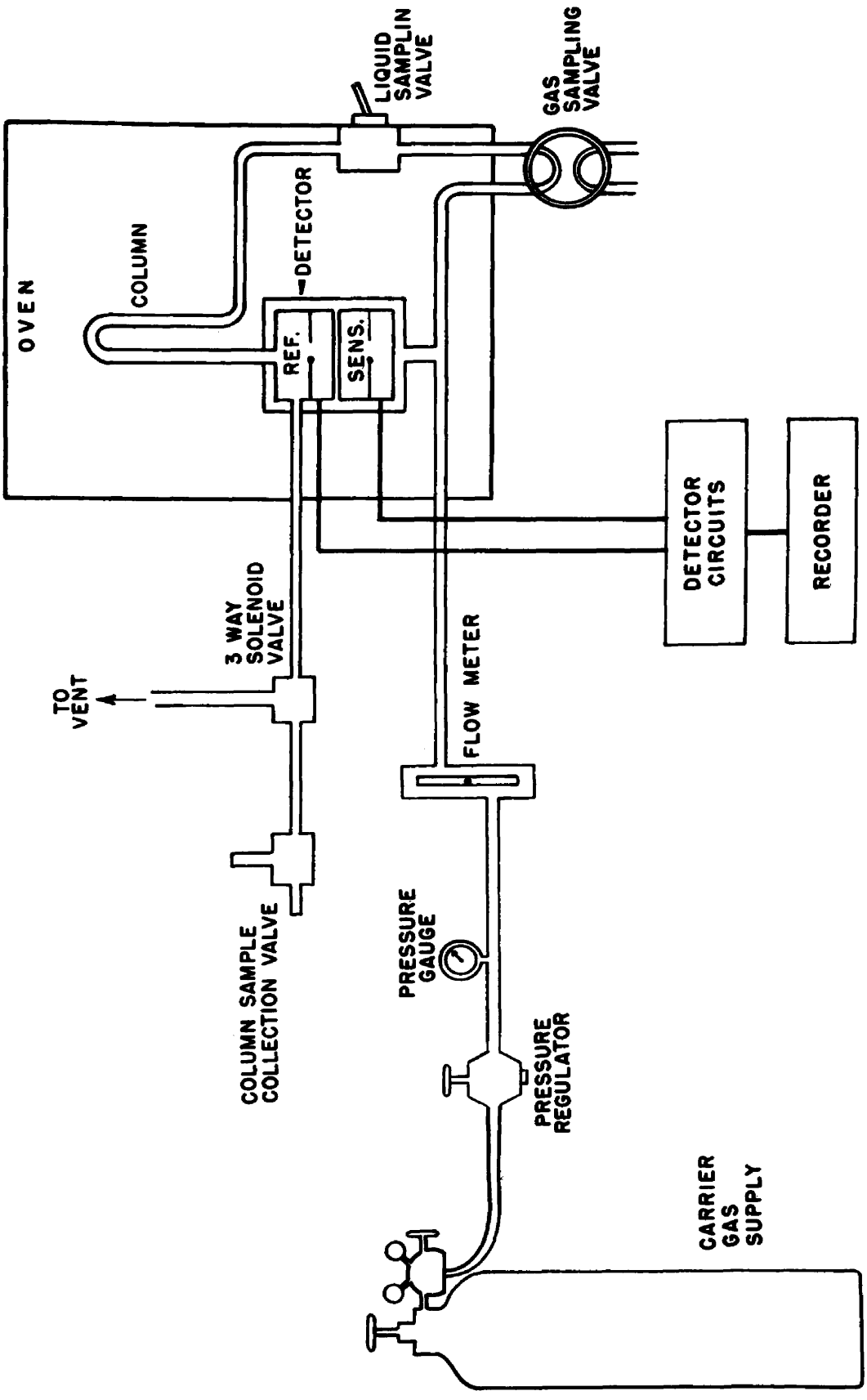

Figure 4. Schematic flow diagram of the vapor fractometer.

connected and the material conveniently introduced into the fractometer as shown in figure 5.

For the purposes of this study, several columns, each employing a different type stationary phase, were used. After initial experimentation, the column containing dinonylphthalate as the stationary phase was determined to be the best all around column to use and was subsequently employed for the major share of the investigation. The stationary phase was carried on 30 to 60 mesh acid washed celite.

The columns were prepared from packing material purchased from the Burrel corporation. Six foot sections of one-quarter inch copper tubing were packed with approximately 20 g. of packing material while the tube was being constantly vibrated by an electric vibrating needle. The ends were plugged with glass wool and the column bent into a V shape by means of a pipe bending tool. To remove any possible contamination, all columns were flushed with helium for several hours at 100°C. before initial use.

Different operating temperatures were used initially for the oven of the fractometer but a temperature of 78-80°C. was finally selected as being the most ideal for the fractionation of meat volatiles and subsequently used for the major share of this investigation. The pressure and carrier gas flow used for each column was determined by running known mixtures through the column under different operating conditions. The trap containing the volatiles was removed from the liquid air or dry ice and ethanol and immersed in hot Dow-Corning 550 oil or heated directly with the flame of a Bunsen burner to bring about faster volatilization. In

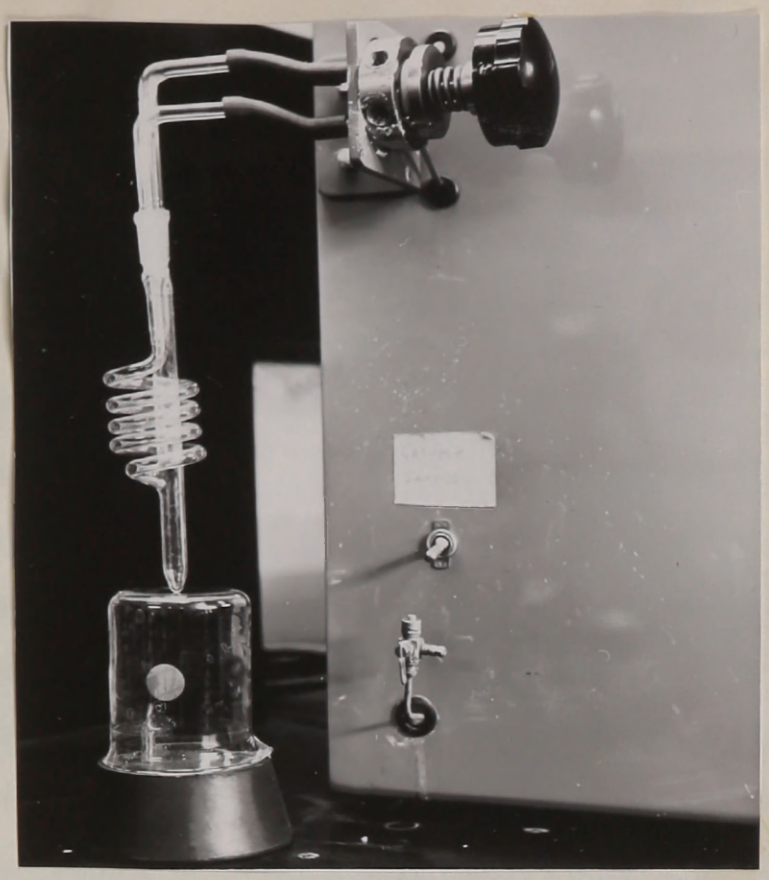

Figure 5. Gas sample inlet valve of the vapor fractometer with a liquid-air trap attached.

Figure 6. Gas sample outlet valve of the vapor fractometer with hypodermic needle and glass tube attached.

either case the trap was kept stoppered until attached to the vapor fractometer as shown in figure 5. Helium was diverted into the trap for fifteen seconds to flush the volatiles out of the trap and into the column. These various volatile components passed through the column according to their individual chemical and physical properties. Detection was accomplished by means of a thermal conductivity cell. The components were observed on a recording chart as they emerged from the column.

Identification.

Identification of the fractionated components was attempted by comparing retention volume in the column for the unknown volatile components with retention volume of known compounds. The same column and operating conditions were used for detecting known compounds as for the unknowns.

A second method of identification employed the use of specific qualitative tests for determination of various fractions. Each of these tests involved the bubbling of the gas responsible for a particular peak through the test solution as it emerged from the column. Figure 6 shows the device used for this procedure. The compounds tested for and the tests employed were as follows:

Methyl mercaptan.

Isatin test - A 1% solution of isotin in concentrated H_2SO_4 was prepared. As the unknown gas was bubbled through the isotin solution, five drops of 95% ethanol were added. The appearance of a green color indicated a positive test.

Nitroprusside - A 1% solution of sodium nitroprusside was prepared. Three drops of 10% NaOH were added to the nitroprusside solution where-

upon the unknown gas was bubbled through the solution. A deep wine color is indicative of a positive test for mercaptans as well as hydrogen sulfide.

Lead Acetate - A saturated solution of lead acetate in ethanol was prepared and the gas bubbled through it. A yellow precipitate indicated a positive test.

Hydrogen sulfide.

Lead acetate - A saturated lead acetate solution was prepared and the unknown gas bubbled through the solution. A black precipitate was indicative of a sulfide.

Carbon dioxide.

Calcium oxide - The unknown gas was bubbled through a saturated calcium oxide solution. A white precipitate indicated a positive test.

Carbonyl Compounds.

2, 4-dinitrophenylhydrazine - The gas was bubbled through a 2, 4-dinitrophenylhydrazine solution. A positive test was indicated by the precipitation of yellow hydrazones.

Infrared spectrophotometry.

A Perkin-Elmer Model 21 double-beam infrared spectrophotometer equipped with a salt prism was used for analysis of the total volatile mixture. Collection was accomplished as previously described for gas chromatographic analysis. The gas cell was evacuated, the liquid air trap containing the volatile compounds was attached and the cell was filled by opening the valves. The cell was closed and placed along side the reference cell in the spectrophotometer for analysis. The sample beam window was set to

approximately 100 per cent transmission and a scanning range of 2-14.5 microns was used.

EXPERIMENTAL RESULTS AND DISCUSSION

Characterization and Flavor Threshold Determination

Studies on raw beef.

Characterization of flavor.

Raw beef was characterized as blood-like, slightly salty, and somewhat pleasant. When the taster held his nostrils, a slightly astringent effect was noted but no other flavor was perceptible. On releasing the nostrils, a flood of flavor filled the mouth.

Flavor threshold.

Figure 7 shows the flavor threshold determinations of the various raw beef fractions studied. The maximum dilution at which the raw beef could be detected was 1-50, and after filtering it could still be detected at 1-50. Thus, it is apparent that the raw beef flavor was primarily located in the juice. After simmering the raw beef filtrate, the flavor threshold was found to be 1-90. Consequently, the flavor was intensified on cooking the juice as shown by the change in flavor threshold. In addition, it was shown that the raw juice developed a cooked meat flavor on heating, which shows that at least part of the cooked meat flavor is present in the juices.

The raw residue remaining after filtration had a chalky, bitter, and unpleasant flavor. Heating the water-residue mixture resulted in development of a pleasant, somewhat bland, slightly meaty flavored solution. Filtering the mixture did not change the flavor characteristics of the filtrate. Thus, it is evident that the meaty flavor is water soluble and was largely removed in the aqueous portion of the original filtrate from the meat-water slurry.

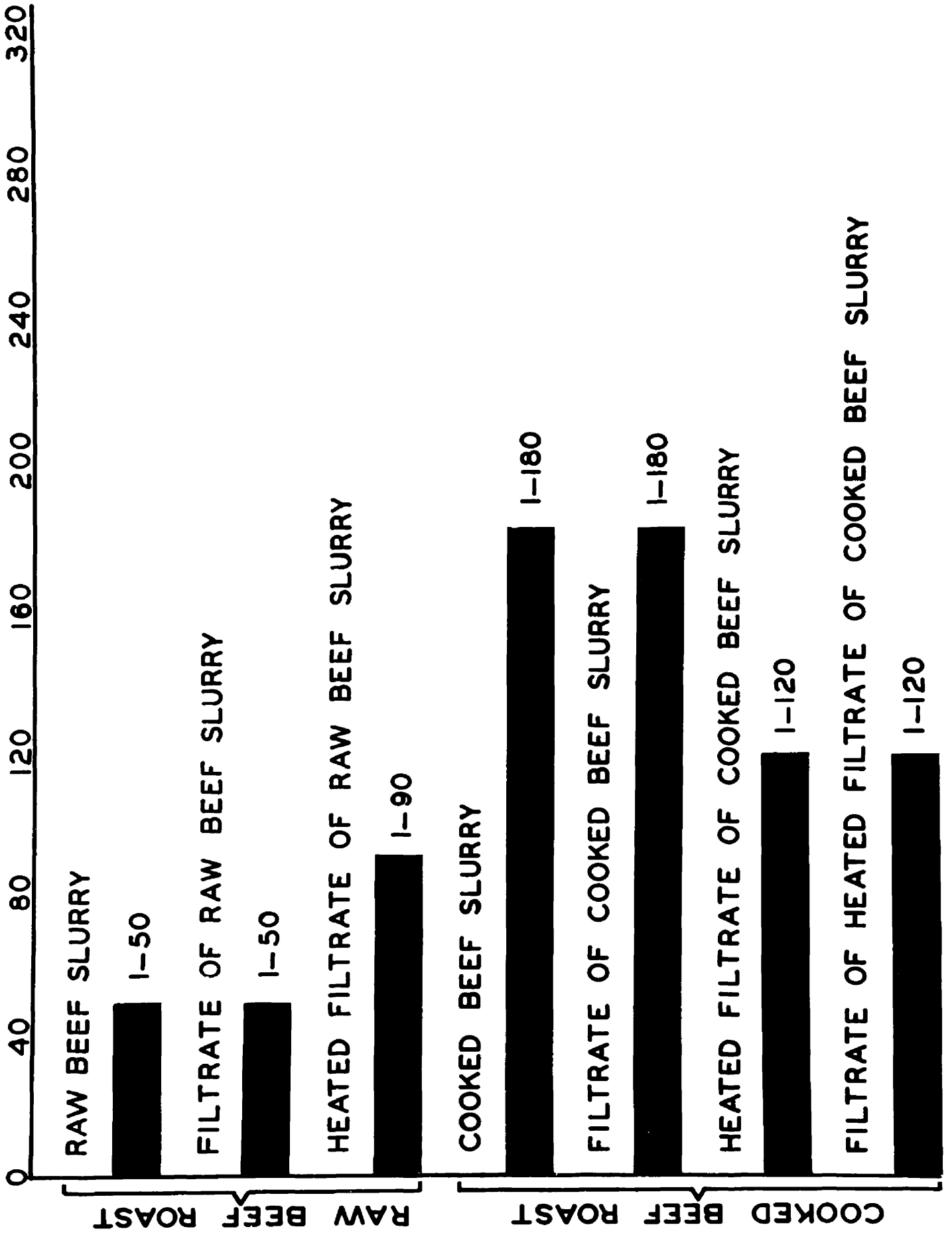

Figure 7. Flavor threshold determinations for raw and cooked beef.

Studies on cooked beef.

Characterization of flavor.

Cooked beef was characterized as being pleasant and meaty. When the taster held his nostrils, no flavor was detectable, but on releasing the nostrils, a flood of pleasant meaty flavor filled the mouth. Thus, the flavor of cooked beef appeared to be closely allied with the sense of smell.

Flavor threshold.

Flavor threshold values for different roast beef fractions are presented in figure 7. The original roast beef could be detected at 1-180, and filtering did not change the threshold. It is evident that the flavor components are water soluble and are not bound to the fibers. When the water filtrate was heated over a steam bath, the flavor threshold was lowered to 1-120, which did not change on filtering off the coagulable portion. The drop in the flavor threshold is probably due to a loss of the volatile flavor constituents during heating. Since the amount of fat-free dry matter is greater in the heated filtrate than in the original filtrate (.22 compared to .15), it is obvious that the flavor changes observed are not a function of dry matter.

The difference in flavor thresholds between raw and cooked meat indicates that cooking develops or intensifies the meat flavor. Examination of the differences in fat-free dry matter (Table 1) between raw and cooked beef at the flavor threshold reveals that the cooked beef can be detected at a greater dilution of fat-free dry matter. Since the flavor was not nearly as apparent on heating the raw fibers in water, it is postulated

	Flavor threshold	pH	Dry matter	Ether extract	Fat free dry matter
			%	%	%
Raw beef roast	---	--	29.87	8.63	21.24
Raw beef slurry	1-50	5.50	.60	.17	.42
Filtrate of raw beef slurry	1-50	5.50	.60	.17	.42
Heated filtrate of raw beef slurry	1-90	5.72	.33	.10	.24
Cooked beef roast	---	--	41.50	15.19	26.31
Cooked beef slurry	1-180	6.10	.23	.08	.15
Filtrate of cooked beef slurry	1-180	6.10	.23	.08	.15
Heated filtrate of cooked beef slurry	1-120	6.10	.35	.13	.22
Filtrate of heated filtrate of cooked beef slurry	1-120	6.10	.35	.13	.22
Raw press fluid	---	--	14.02	.21	13.81
Raw press fluid	1-70	5.34	.20	.003	.20
Heated raw press fluid	1-260	5.70	.05	.0008	.05
Filtrate of heated raw press fluid	1-260	5.70	.05	.0008	.05
Raw pressed fiber	---	5.44	59.02	6.81	52.21
Heated raw pressed fiber	---	5.70	---	---	---
Cooked beef press fluid	---	--	11.62	0.31	11.31
Cooked beef press fluid	1-300	6.00	.03	.001	.038
Filtrate of cooked beef press fluid	1-300	6.00	.03	.001	.038
Cooked pressed fiber		5.92	67.35	7.45	59.90

Table 1. Flavor thresholds and chemical determinations on various meat fractions (Fat, dry matter and fat-free dry matter percentages are based on the original composition of each roast.)

that heating the juices and fibers together may intensify or enhance the development of flavor.

The cooked meat residue remaining after the first filtration had a mild, pleasant roast beef flavor. On heating a water suspension of the residue, it developed a somewhat stronger roast beef flavor. Thus, indications are that the flavor is retained more tenaciously by the fibers after heating. On filtering the water-residue suspension, the filtrate was strong and slightly bitter, whereas the remaining residue was bland, flat and slightly beefy.

Press Fluid Studies.

Characterization of flavor.

Undiluted raw press fluid was characterized as being strong, unpleasant and viseral-like. The flavor of the press fluid from cooked beef was less objectionable than that from raw beef, but it was rather strong and somewhat viseral or animal-like in taste. The flavor of both the raw and cooked press fluid appeared to be predominately odor.

Flavor threshold.

The raw press fluid had a flavor threshold of 1-70, which increased to 1-260 upon heating (figure 8). Removal of the fat and coagulated material did not alter the level of detection. Furthermore, the flavor of the filtrate was very strong but meaty. Thus, the flavor components in beef could be pressed from the fiber. In addition, cooking develops or enhances the flavor of the press fluid. It was noted that the fat-free dry matter at the detection threshold of heated press fluid was 1.05% or about one-fourth as high as that of the raw press fluid, which contained .20 % fat-

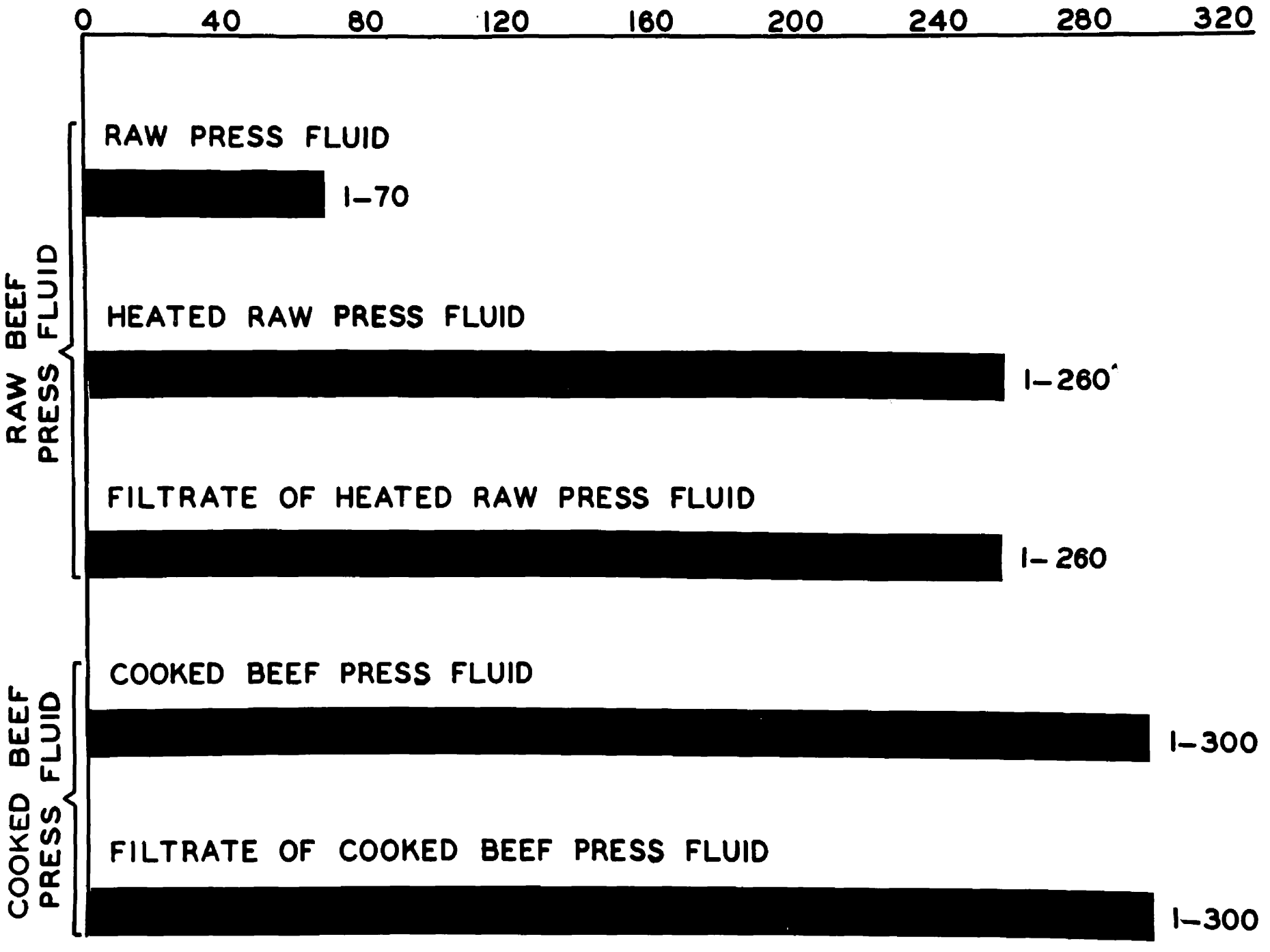

Figure 8. Flavor thresholds for raw and cooked beef press fluid.

free dry matter at the flavor threshold. It is therefore evident again that flavor is not a function of dry matter.

The flavor threshold for press fluid from roast beef was found to be 1-300 and was not altered by filtration. Cooking prior to pressing appeared to intensify the flavor of the press fluid, which again indicates cooking of the fibers and juice together resulted in greater flavor development.

On heating the raw press cake in water, a pleasant beefy flavor was developed. Although a fatty film was present on the surface after heating, the flavor was still present in the filtrate to about the same extent. Hence, flavor did not appear to be associated with the fatty portion. A good example of this is found in the case of the cooked press fluid (Table 1) which has the highest flavor threshold (1-300) but a very low percent of ether extract (.001). Examination of the other data in Table 1 on percentage fat at different flavor thresholds also verifies the fact that intensity of flavor and fat content are not related.

Chemical components.

Cooking increased the ether extract of the roasts from 8.63 to 15.19%, or an increase of 6.56% (Table 1). Along with the increase in ether extract an 11.63% increase in dry matter content was observed or a change from 29.87 to 41.50%. However, upon cooking raw press fluid, the percent ether extract increased from 0.21 to 0.31 while the dry matter content of the press fluid decreased 2.40%, or changed from 14.02 to 11.62%. There was a tendency for flavor threshold determinations and dry matter

content of the raw press fluid to be inversely related or, as dry matter decreased, the flavor threshold increased. The raw meat slurry had a pH of 5.50 while it was 6.10 for the cooked slurry. The raw press fluid had a pH of 5.34 while the pH of the cooked beef press fluid was 6.00. In all instances, an increase in pH was noted upon cooking the various meat and meat juice fractions.

Leaching studies.

The flavor of raw meat leachings was characterized as being slightly blood-like and bland; the flavor of the leachings from cooked meat was meaty and slightly astringent. The raw meat was devoid of flavor after leaching for 3 days, while 5 days were required to leach all the flavor from cooked meat.

Flavor thresholds of the raw leachings were 1-50, 1-20 and 1-5 at the end of the first, second and third 24-hour periods of leaching, respectively (figure 9). The detection threshold was found to increase upon heating the leachings to 1-100 for first period, 1-60 for the second and 1-30 for the third 24-hour period. With the cooked meat leachings, the threshold was 1-40 after the first period, 1-15 for the second, 1-10 for the third and 1-5 after both the fourth and fifth 24-hour leaching periods (figure 9). Leaching could not be carried out longer with either the cooked or raw series because spoilage ensued.

Results of the leaching studies show the flavor of beef can be leached out of both the cooked and raw beef, although the cooked beef fibers appear to bind the flavor components more tenaciously. The flavor binding effect of the fibers is evident by the lower initial flavor threshold of

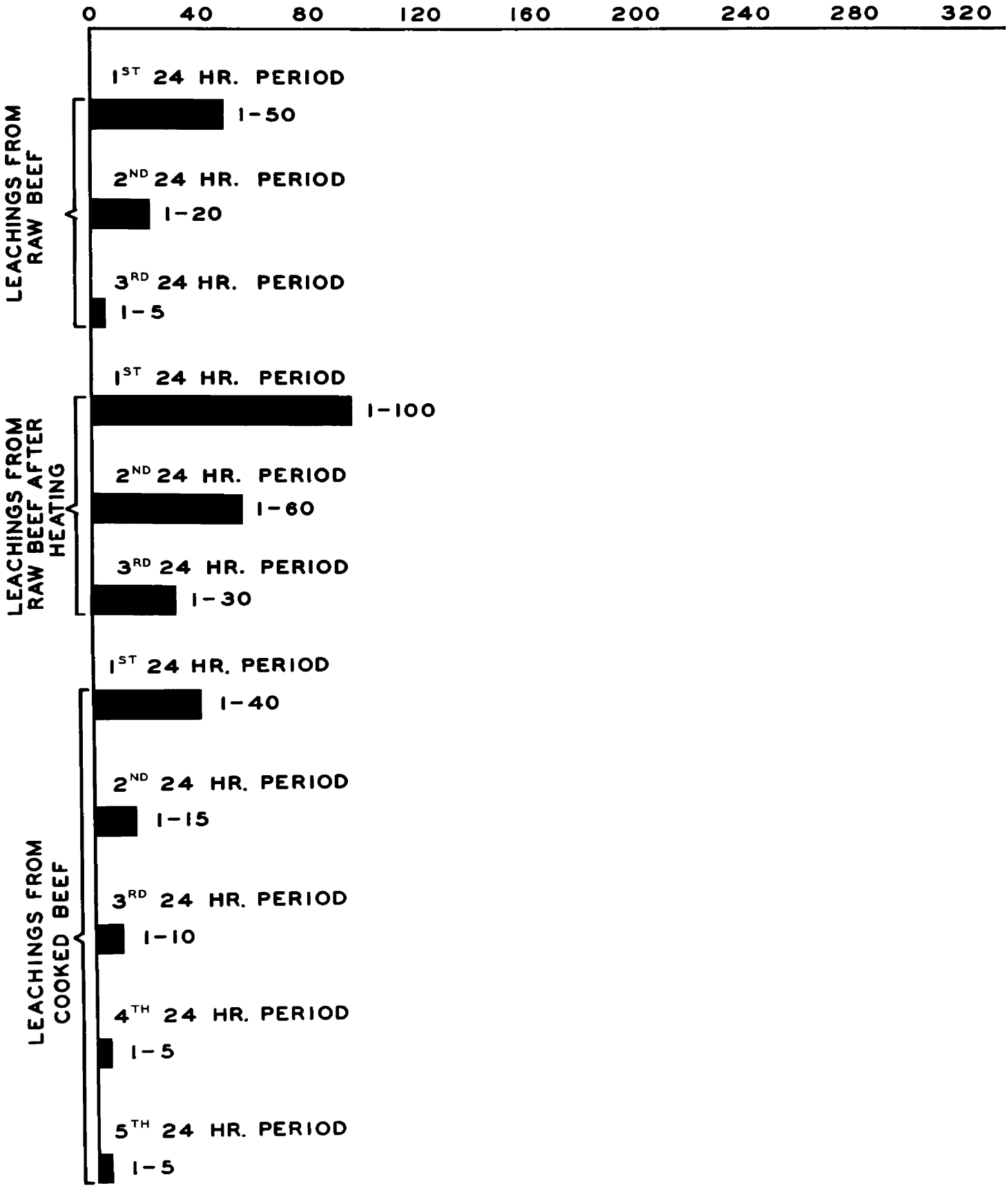

Figure 9. Flavor thresholds for raw and cooked beef leachings.

the cooked leachings and by the longer period of time over which flavor could be leached from the cooked meat.

Identification of Volatile Beef Flavor Compounds

Identification of Volatile Sulfide and Carbonyl Compounds.

The presence of a black precipitate in the lead acetate trap was observed, this being indicative of the presence of volatile sulfide compounds. However, no attempt was made to separate and identify the sulfur containing compounds in the precipitate.

Figure 10 shows a paper chromatogram of the carbonyl compounds chromatographed as their hydrazone derivatives. Acetaldehyde was identified as being one of the spots but the other two spots showed evidence of excessive trailing, making identification impractical. A solvent system composed of 1 part anhydrous methanol to 1 part heptane was also tried, but the resulting chromatogram showed poor separation and a great deal of trailing.

Identification of Total Cooked Beef Volatiles by Gas Chromatography and Qualitative Tests.

The major share of the identification of the volatile compounds fractionated in the various cold traps, and then further fractionated in a vapor fractometer, was accomplished by comparing retention volumes from gas chromatograms. Retention volumes were calculated from a standard calibration curve for both known compounds and the fractionated volatiles. Due to the nature of the curve, all retention volumes based on the curve are considered to be approximations and not exact values. All retention

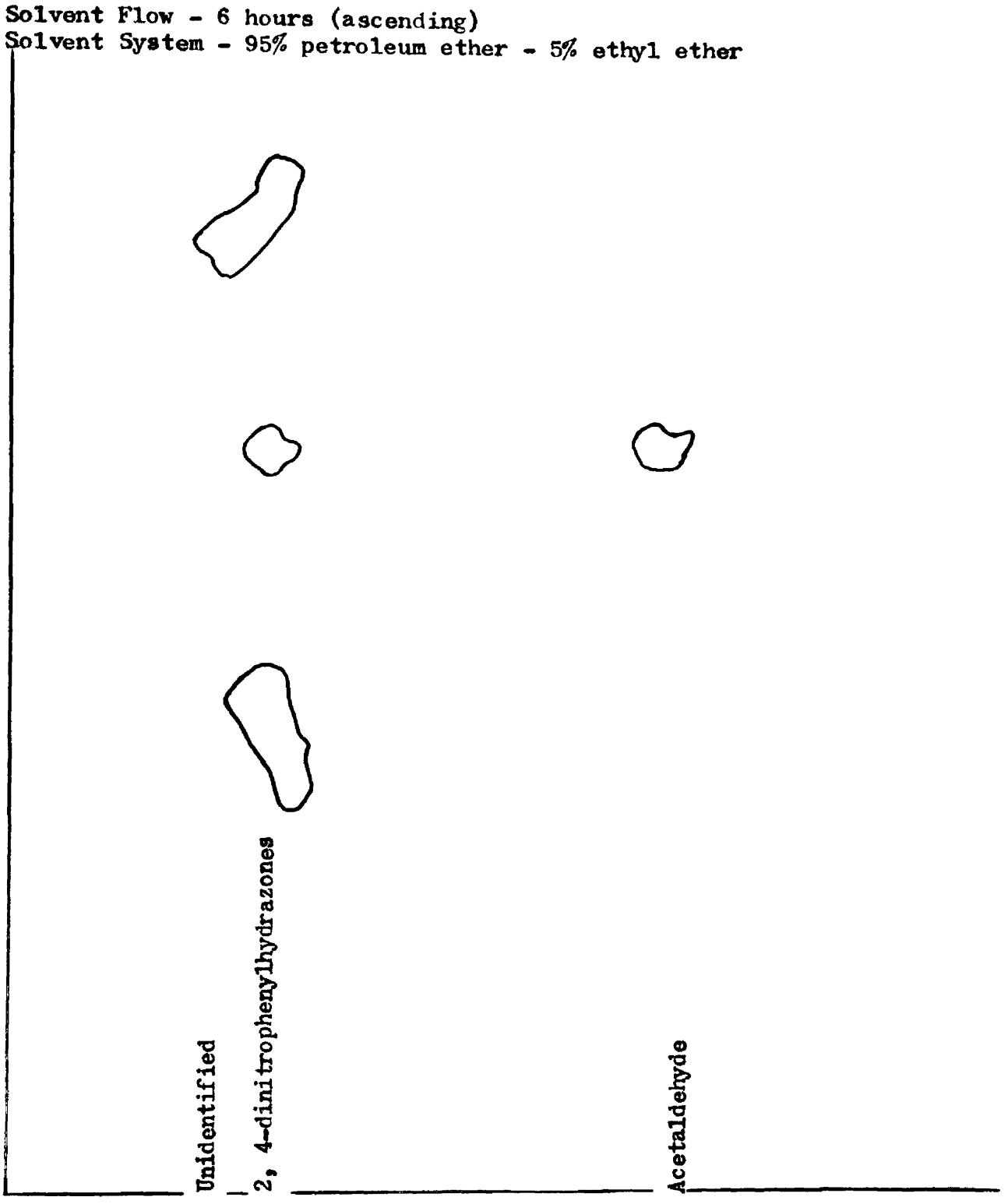

Figure 10. Paper chromatogram of the volatile carbonyl compounds of cooked beef chromatographed as their hydrozone derivatives.

values were measured from the injection point to the center of the peak in question. Three different columns were used, didecylphthalate, dinonylphthalate and "carbowax 400". The dinonylphthalate and didecylphthalate resolved the volatiles collected in the liquid air trap into 6 peaks while the "carbowax 400" column would only resolve the mixture into 3 peaks. Retention volumes differed greatly between the three columns. All samples were allowed a 15 minute injection period.

Didecylphthalate Column.

Figures 11, 12 and 13 depict gas chromatograms of methyl mercaptan, acetaldehyde and acetone, respectively. Retention volumes are as follow: methyl mercaptan 112, acetaldehyde 152 and acetone 227.

Figure 14 shows a gas chromatogram of cooked beef volatiles collected in a liquid air trap. The trap was removed from the liquid air and immersed in warm oil (approximately 45°C.) for 2 minutes before injection into the fractometer. Figure 15 pictures the same mixture after being immersed in the oil for 10 minutes. All didecylphthalate chromatograms are actual size with the exception of the air peak, which is two thirds of its actual height. Six distinct peaks were resolved.

Peak one was identified as carbon dioxide by bubbling the gas as it emerged from the vapor fractometer through a saturated calcium oxide solution and observing the white precipitate formed. Attempts were made to obtain a retention volume for known carbon dioxide by placing dry ice in a gas wash bottle and allowing it to volatilize. However, unsatisfactory results were obtained when the gas was injected into the vapor fractometer. Greatly rounded and skewed peaks were observed.

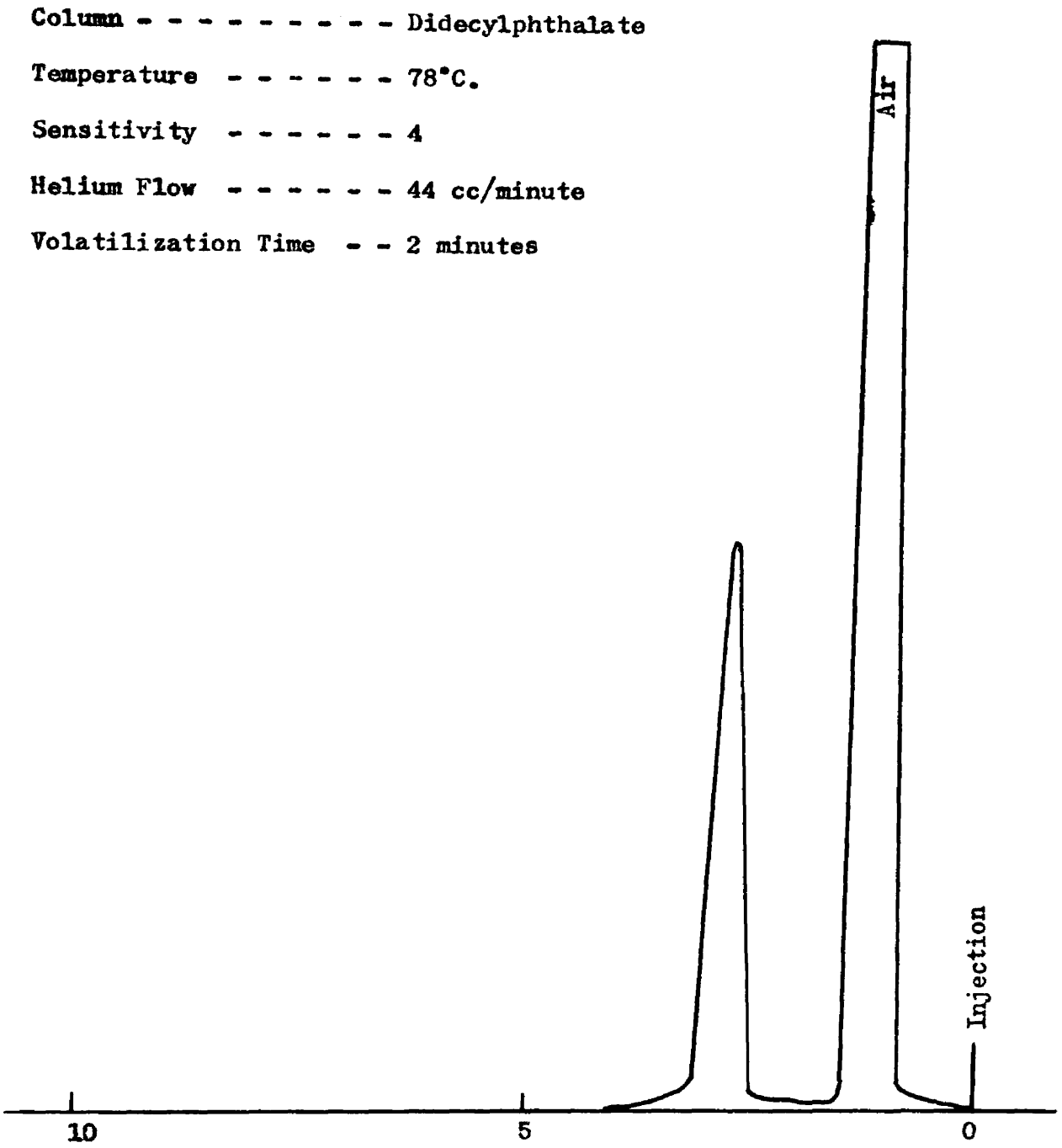

Figure 11. Gas chromatogram of methyl mercaptan resolved on a didecylphthalate column.

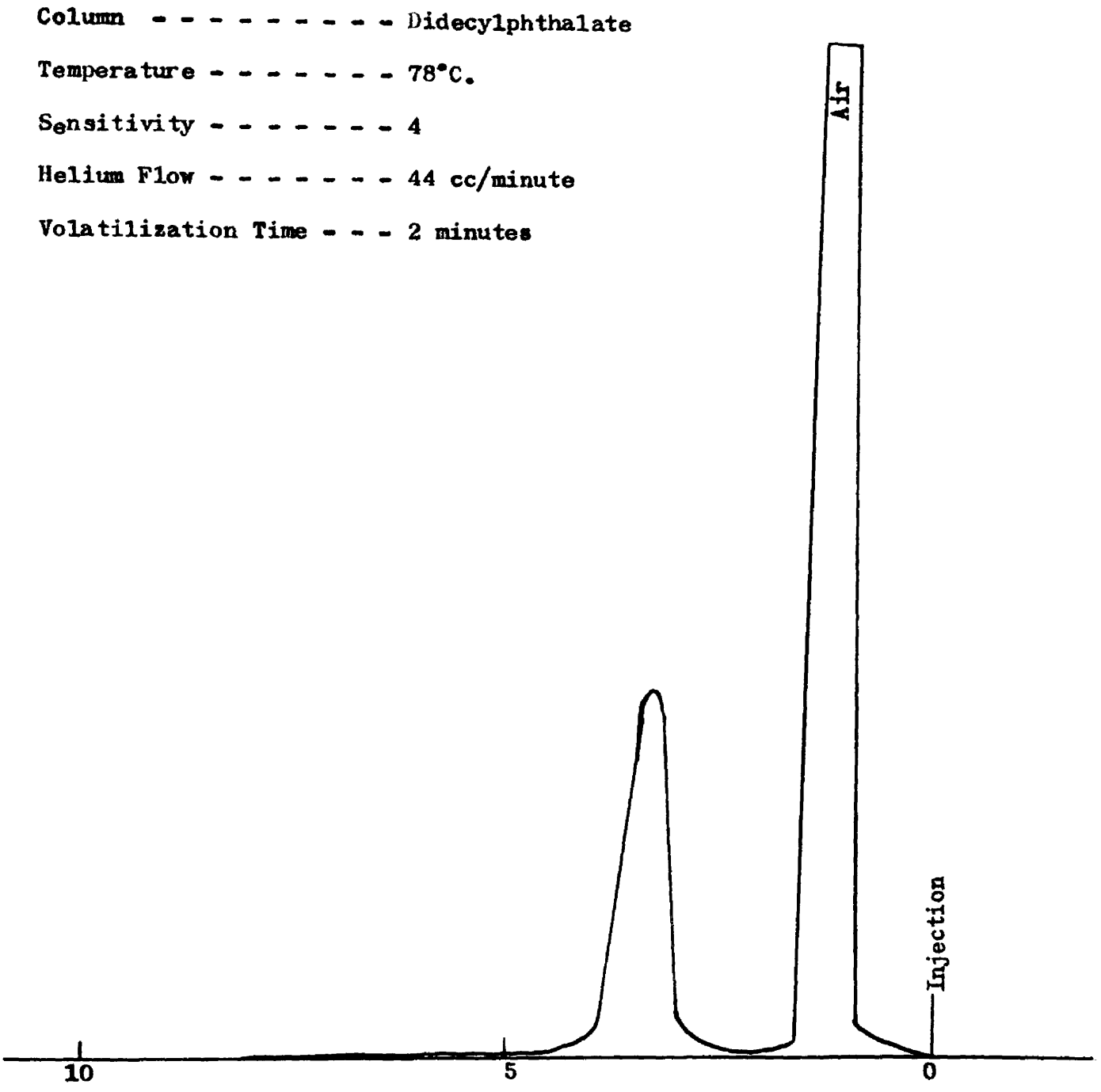

Figure 12. Gas chromatogram of acetaldehyde resolved on a didecylphthalate column.

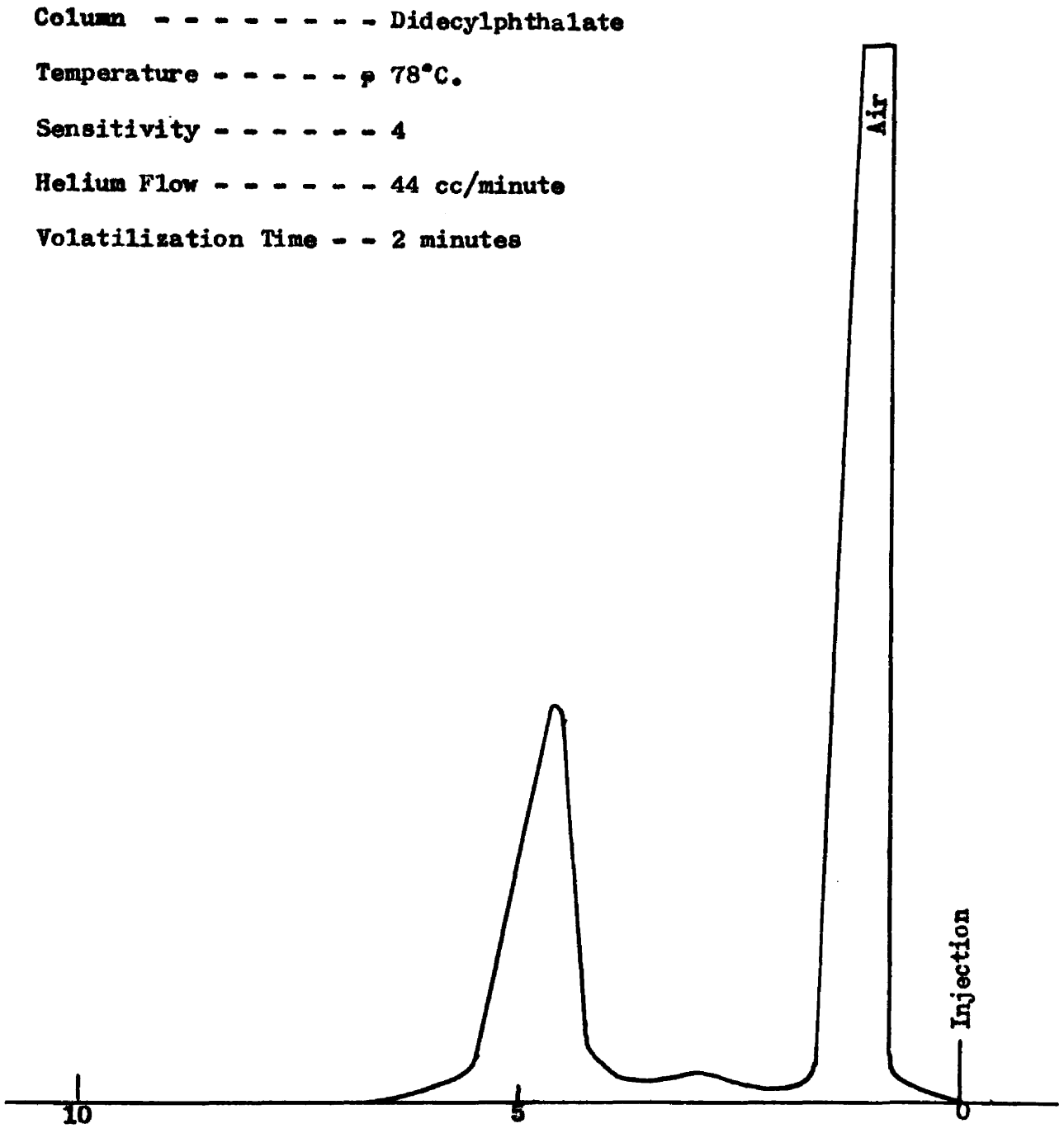

Figure 13. Gas chromatogram of acetone resolved on a didecylphthalate column.

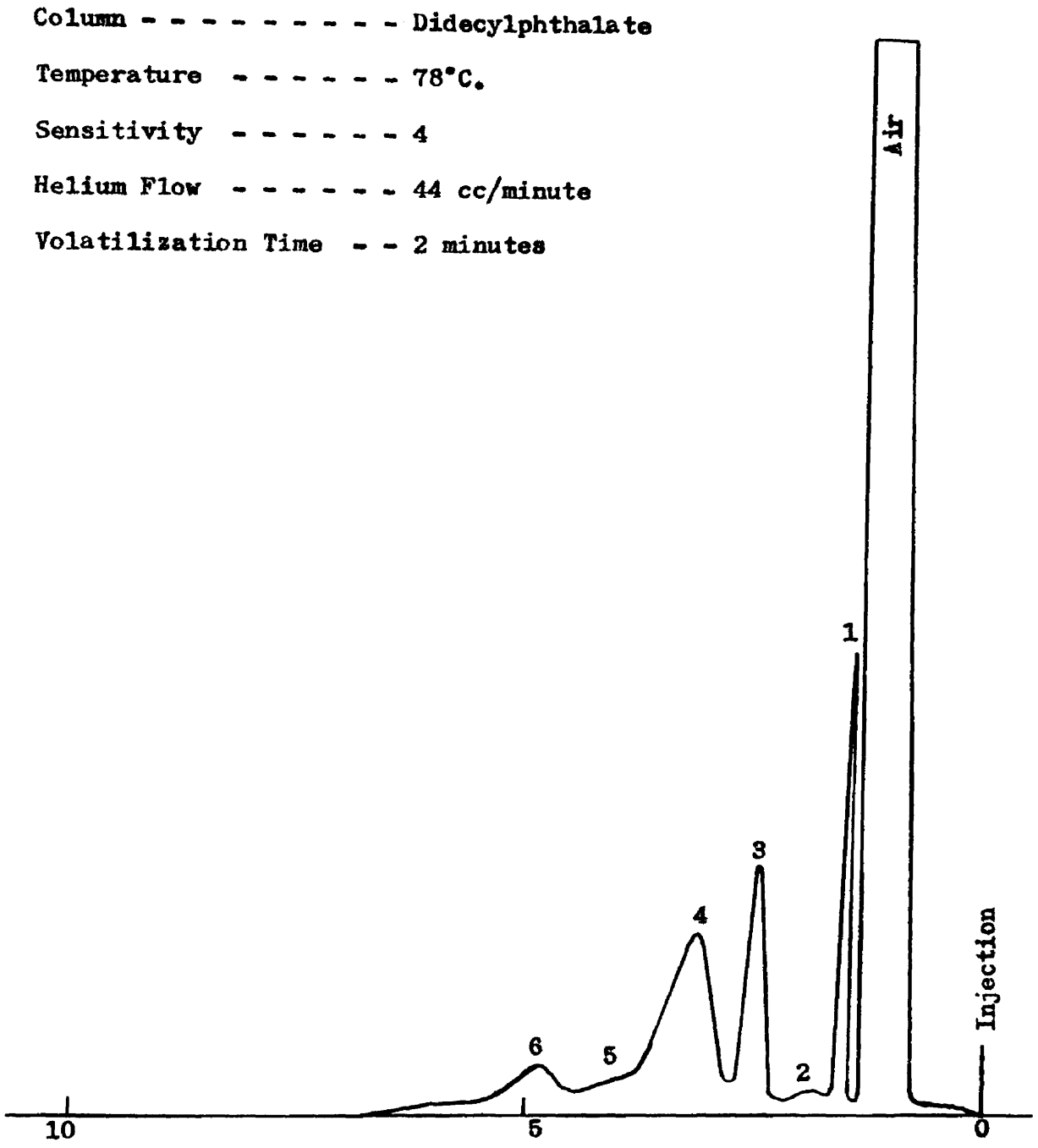

Figure 14. Gas chromatogram of cooked beef volatiles collected in a liquid air trap and resolved on a didecylphthalate column.

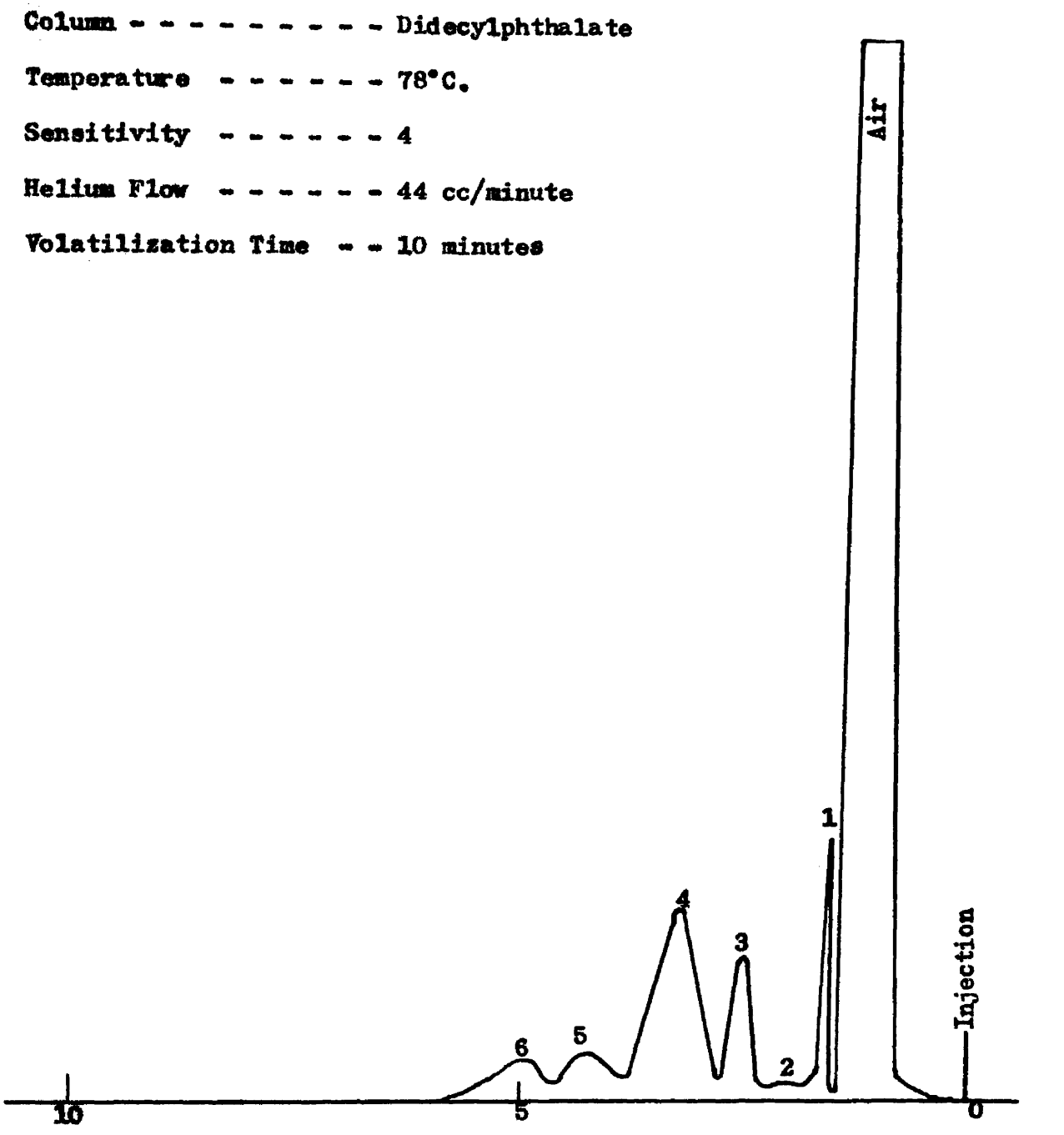

Figure 15. Gas chromatogram of cooked beef volatiles collected in a liquid air trap and resolved on a didecylphthalate column.

Peak two was not identified. Its lack of size and rounded contour did not lend itself to identification by comparison with known compounds, nor did the small quantity of gas present allow for identification by means of various qualitative tests.

The third peak had a retention volume of 120 which is similar to that for methyl mercaptan. A yellow precipitate was observed when the gas was bubbled through a saturated solution of lead acetate in ethanol. Thus, the peak was identified as methyl mercaptan by both the gas chromatogram and the positive qualitative test.

The retention volume of peak four was 167, which is approximately that of acetaldehyde. Upon bubbling the gas through a solution of 2, 4-dinitrophenylhydrazine, hydrazones were precipitated, which indicated the peak to be a carbonyl compound. Due to lack of good separation and resolution, peaks five and six could not be identified.

Figure 16 shows a mixture of cooked beef volatiles resolved on a didecylphthalate column. The trap was heated for five minutes prior to injection. Only three peaks were obtained when the contents of the trap were injected into the vapor fractometer. Peaks 1 and 2 as seen in figures 14 and 15 were no longer resolved while peaks 5 and 6 (figures 14 and 15) were resolved as one large peak in place of the two small ones seen in figures 14 and 15. Apparently prolonged heating increased the size of peaks 3 and 4 in figures 14 and 15, which are shown as peaks 1 and 2 in figure 16. Peak three (figure 16), which corresponds to peaks 5 and 6 in figures 14 and 15, was found to have a retention volume of 214, similar to that of acetone. Hydrazone crystals were observed to precipitate when

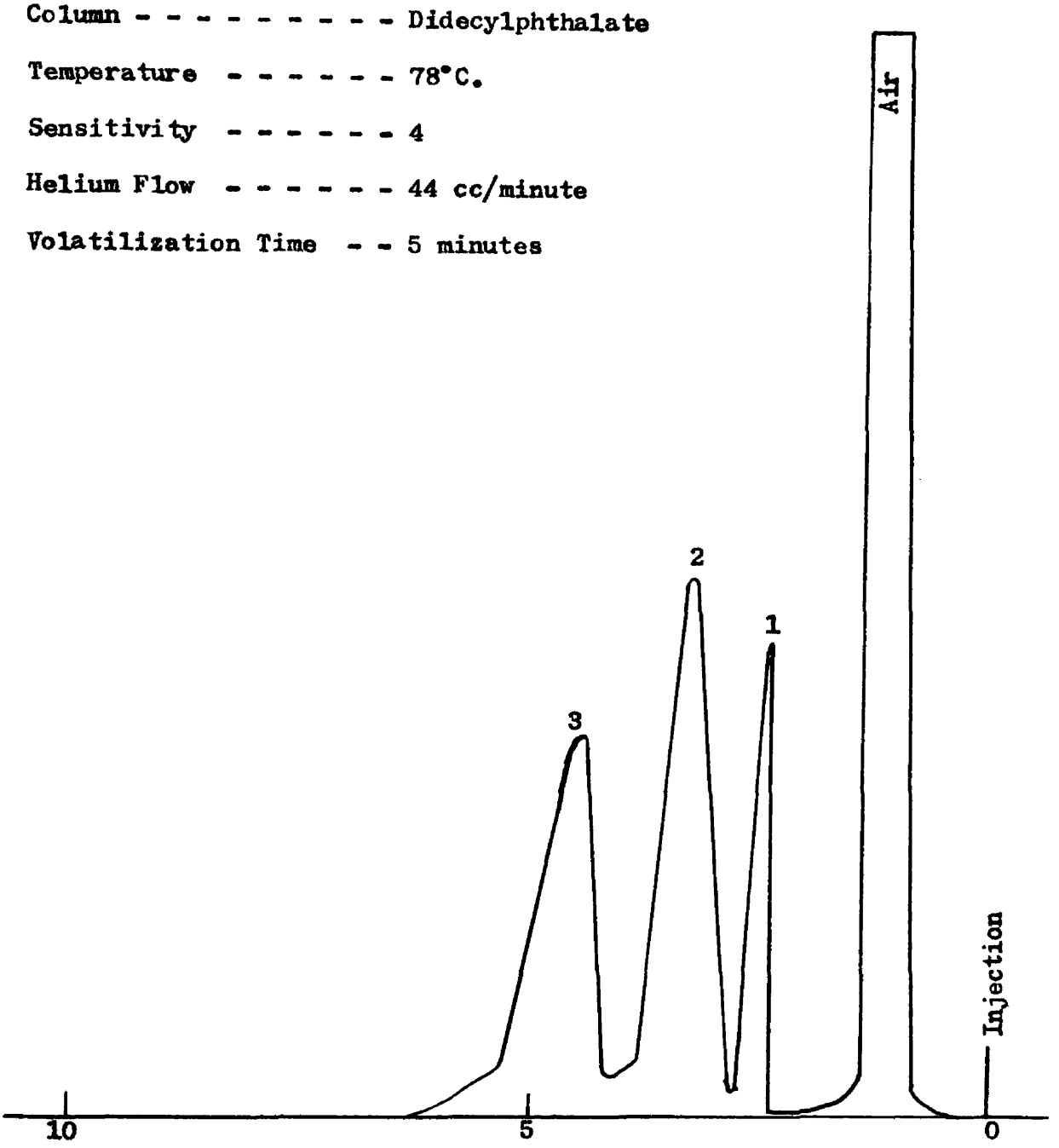

Figure 16. Gas chromatogram of cooked beef volatiles collected in a liquid air trap. The trap was heated for five minutes with a Bunsen burner flame.

the gas was bubbled through a 2, 4-dinitrophenylhydrazine solution. Thus, peak 3 (figure 16) appears to be a carbonyl compound, probably acetone.

Retention volumes for all known and unknown compounds resolved on the didecylphthalate column are shown in Table 2.

"Carbowax 400" Column.

All "carbowax 400" chromatograms are actual size, however, the air peak was reduced to two thirds its original size. Figures 17 and 18 show known methyl mercaptan and acetaldehyde as they appeared when resolved on a "carbowax 400" column. Methyl mercaptan had a retention volume of 387 while 605 was the retention volume observed for acetaldehyde.

Figures 19 and 20 picture successive chromatograms of the same mixture, the difference being only in volatilization times. Figure 19 represents a volatilization time of 2 minutes, while figure 20 represents a volatilization period of 10 minutes. The "carbowax 400" column resolved only 3 peaks compared to the 6 peaks resolved by the phthalate columns. Peak one was identified as carbon dioxide by observing a white precipitate when the gas was bubbled through a calcium oxide solution. Peak two showed a retention time of 367, which is similar to that of methyl mercaptan. The peak did not give a reaction to the isatin and nitroprusside tests for methyl mercaptan, but did give a slight yellow precipitate when bubbled through an ethanol solution of lead acetate. The retention volume of peak three was 611. This compared favorably with acetaldehyde which had a retention volume of 625. Hydrazones were formed when the gas was bubbled through 2, 4-dinitrophenylhydrazine, indicating peak three to be a carbonyl compound. Thus carbon dioxide, methyl mercaptan and acetaldehyde were

Known Compounds

Compound	Retention Volume (c.c. He.)
Methyl Mercaptan	112
Acetaldehyde	152
Acetone	227

Unknown Compounds

Figure No.	Peak No.	Retention Volume (c.c. He.)
14 & 15	1	70
	2	99
	3	120
	4	167
	5	187
	6	220
16	1	122
	2	165
	3	214

Table 2. Retention volumes of known and unknown volatile compounds resolved on a didecylphthalate column.

Column - - - - - - - - - "Carbowax 400"

Temperature - - - - - - - 78°C.

Sensitivity - - - - - - - 4

Helium Flow - - - - - - - 121 cc/minute

Volatilization Time - - 2 minutes

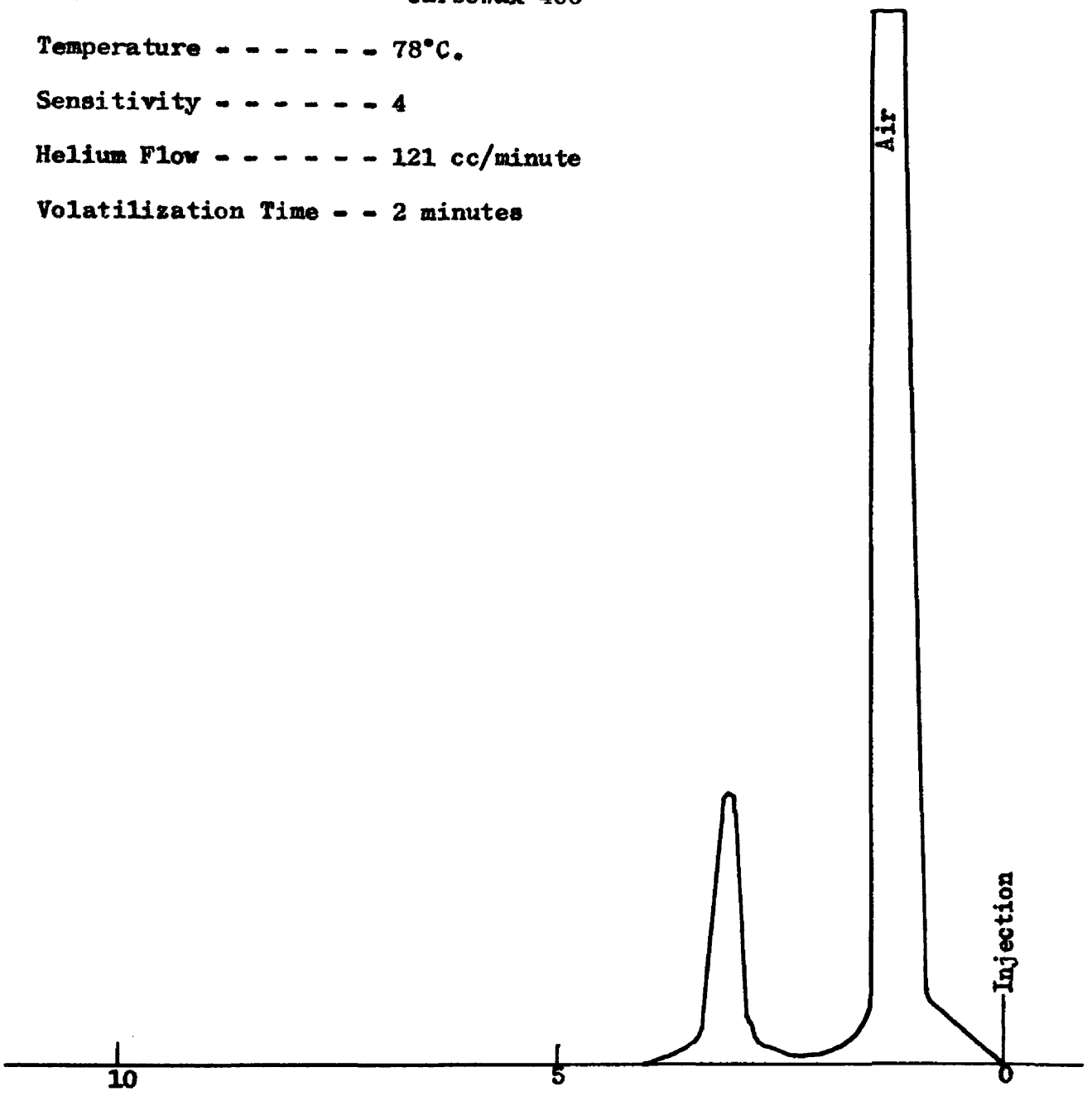

Figure 17. Gas chromatogram of methyl mercaptan resolved on a "Carbowax 400" column.

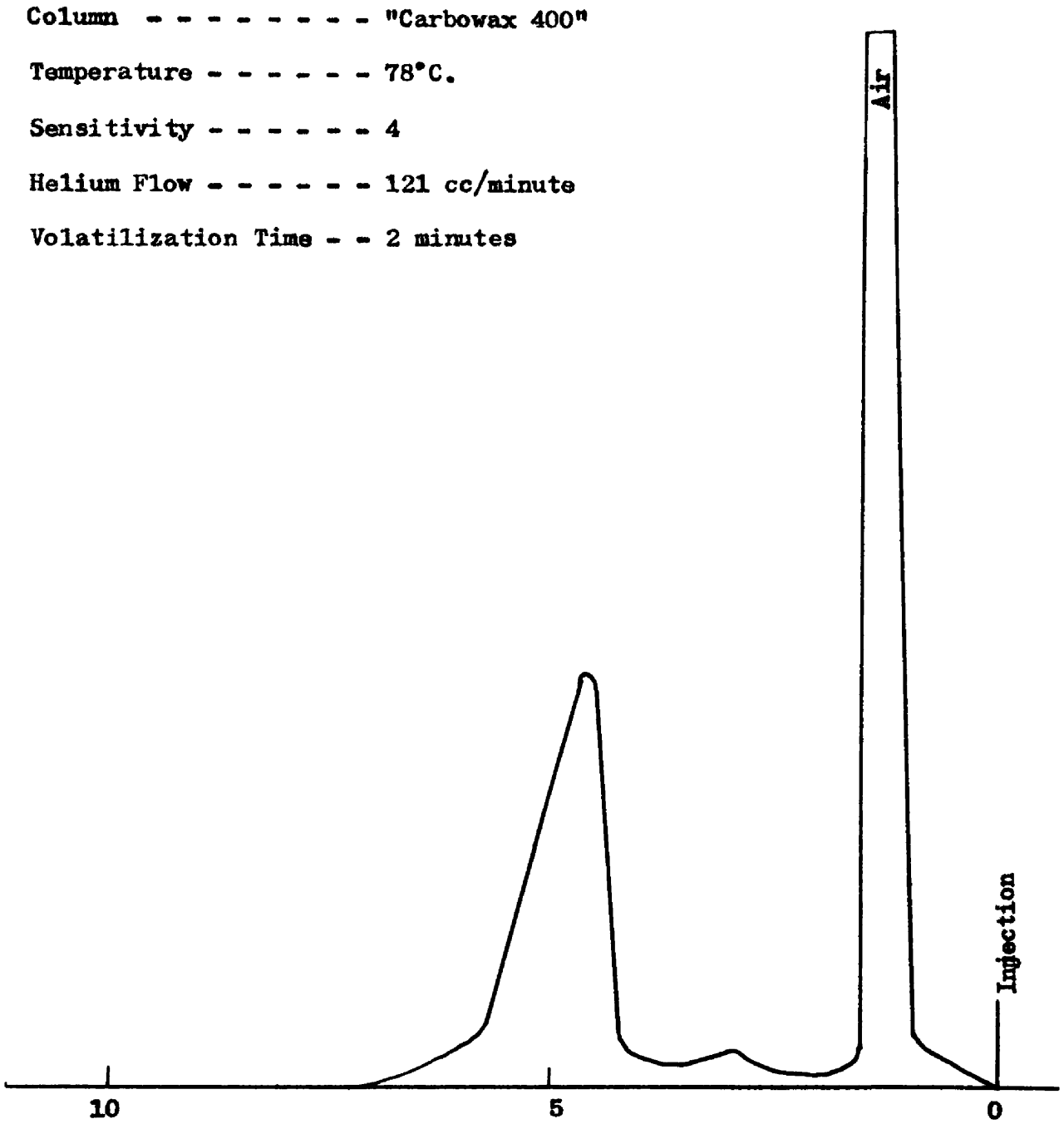

Figure 18. Gas chromatogram of acetaldehyde resolved on a "Carbowax 400" column.

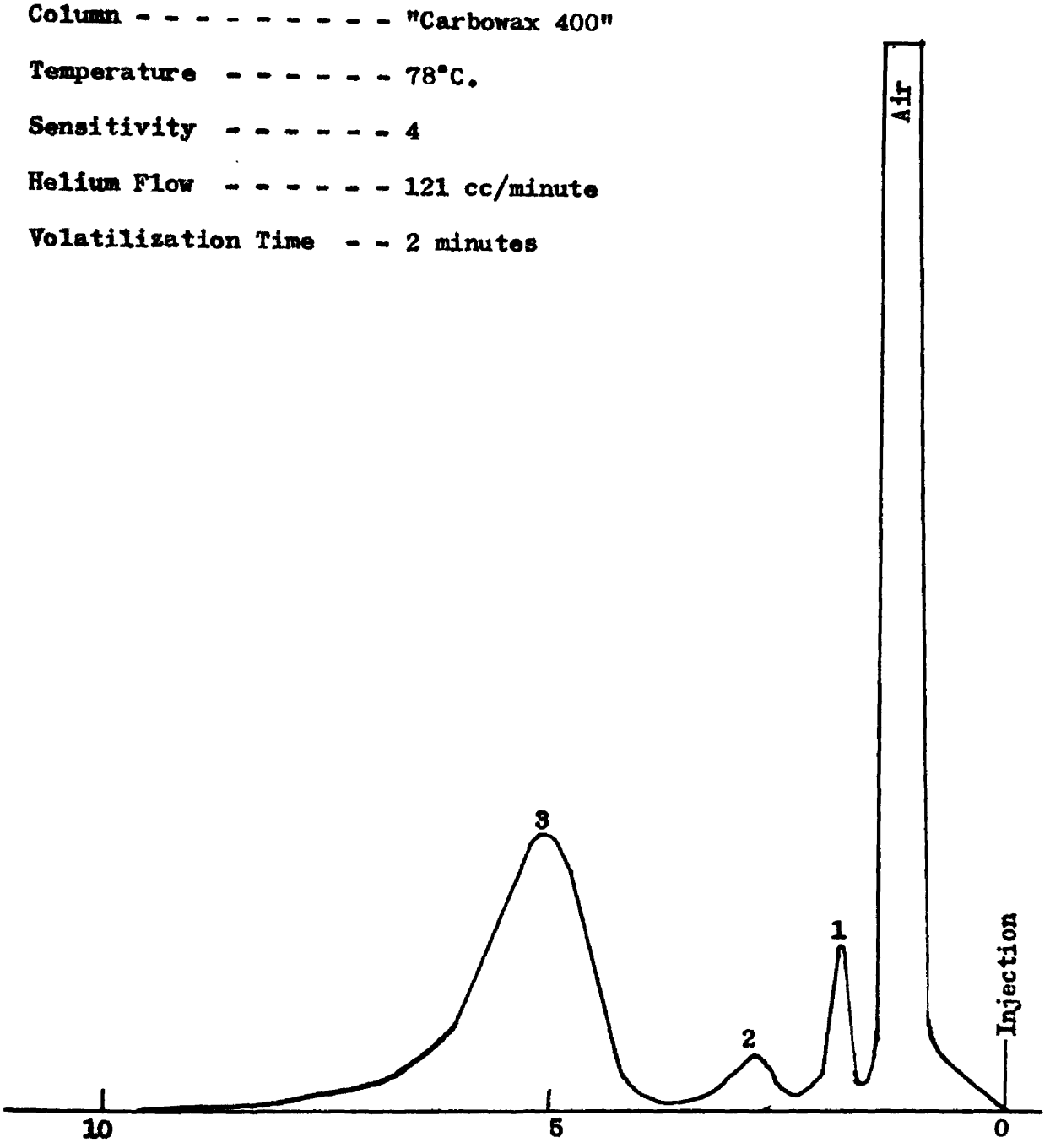

Figure 19. Gas chromatogram of cooked beef volatiles collected in a liquid air trap and resolved on a "Carbowax 400" column.

Column - - - - - - - - -"Carbowax 400"

Temperature - - - - - - - 78°C.

Sensitivity - - - - - - - 4

Helium Flow - - - - - - - 121 cc/minute

Volatilization Time - - 10 minutes

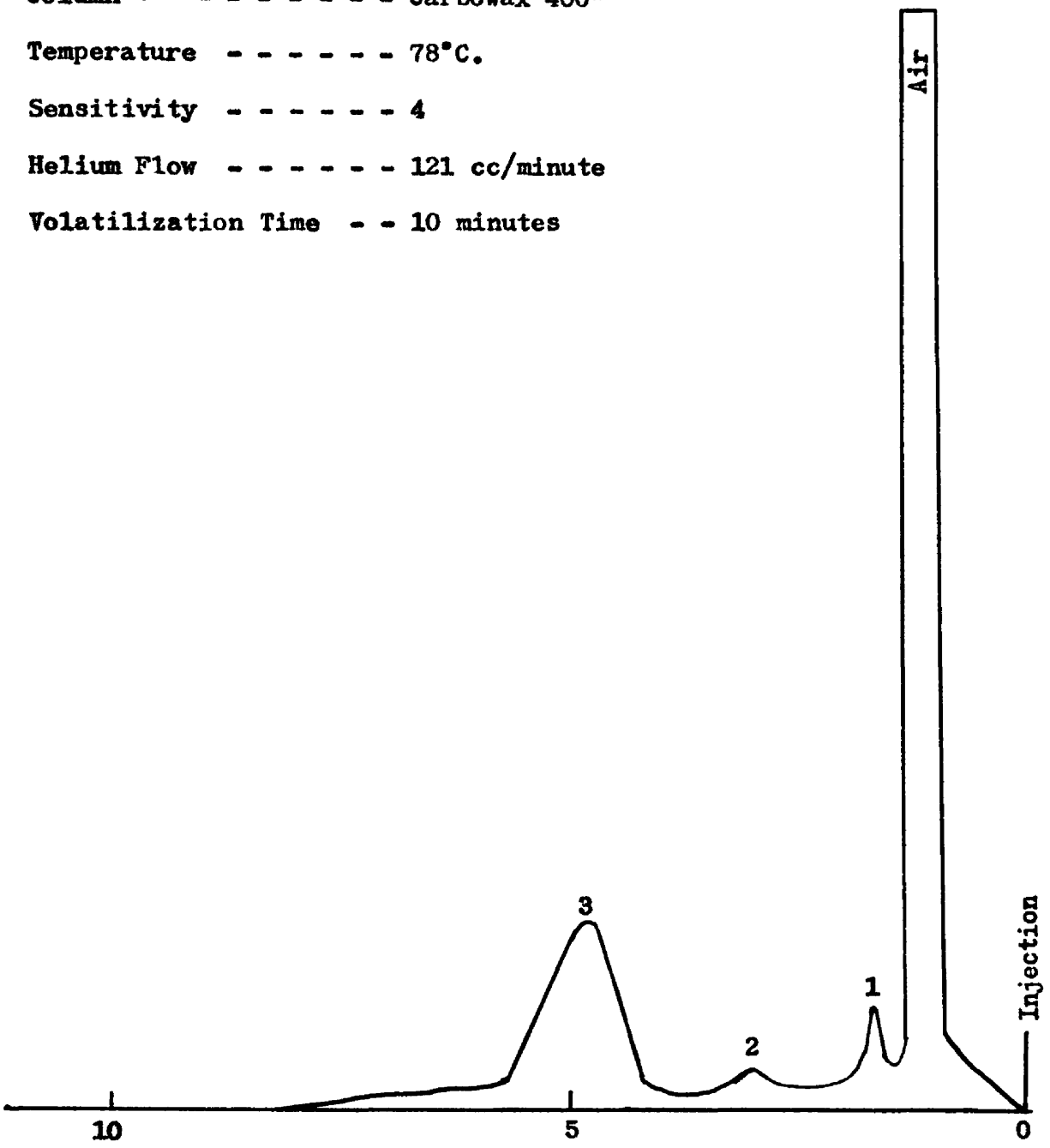

Figure 20. Gas chromatogram of cooked beef volatiles collected in a liquid air trap and resolved on a "Carbowax 400" column.

identified using the "carbowax 400" column. As was shown previously, these same compounds were also resolved and identified using a didecylphthalate column.

A slight, but nevertheless distinct odor emanated from the dry ice-ethanol trap after each collection was completed. This indicated that one or more volatile compounds were being collected in this trap and not allowed to pass over to the traps immersed in liquid air. Figure 21 represents a gas chromatogram of cooked beef volatiles collected in a dry ice-ethanol trap. Two peaks were obtained, the first being poorly resolved and could not be identified, while the second was tentatively identified as water.

Table 3 shows retention volumes for all known and unknown compounds resolved on the "carbowax 400" column.

Dinonylphthalate column.

All dinonylphthalate chromatograms are reduced to two thirds their original size. Figures 22, 23, 24, 25 and 26 represent gas chromatograms of methyl mercaptan, acetaldehyde, methyl sulfide, acetone and water, respectively. Retention volumes for these compounds are shown in Table 4.

This column resolved the volatile mixture of the compounds obtained upon heating beef into six peaks as seen in figure 27. Figure 28 represents a sample chromatographed successively from the same mixture as figure 27. Peak one was identified as carbon dioxide by passing the gas through calcium oxide and observing the precipitate.

Peak two showed a retention volume of 93, approximating that of methyl mercaptan. A positive test for mercaptan was noted when the gas was bubbled through a solution of lead acetate in 95% ethanol. A retention volume

Known Compounds

Compound	Retention Volume (c.c. He.)
Methyl Mercaptan	367
Acetaldehyde	625

Unknown Compounds

Figure No.	Peak No.	Retention Volume (c.c. He.)
19 & 20	1	198
	2	381
	3	611
21	1	636
	2	1102

Table 3. Retention volumes of known and unknown volatile compounds resolved on a "carbowax 400" column.

Known Compounds

Compound	Retention Volume (c.c. He.)
Methyl Mercaptan	105
Acetaldehyde	180
Methyl Sulfide	229
Acetone	279
Water	323

Unknown Compounds

Figure No.	Peak No.	Retention Volume (c.c. He.)
27 & 28	2	93
	3	172
	4	225
	5	298
	6	304
29	1	89
	2	157

Table 4. Retention volumes of known and unknown volatile compounds resolved on a dinonylphthalate column.

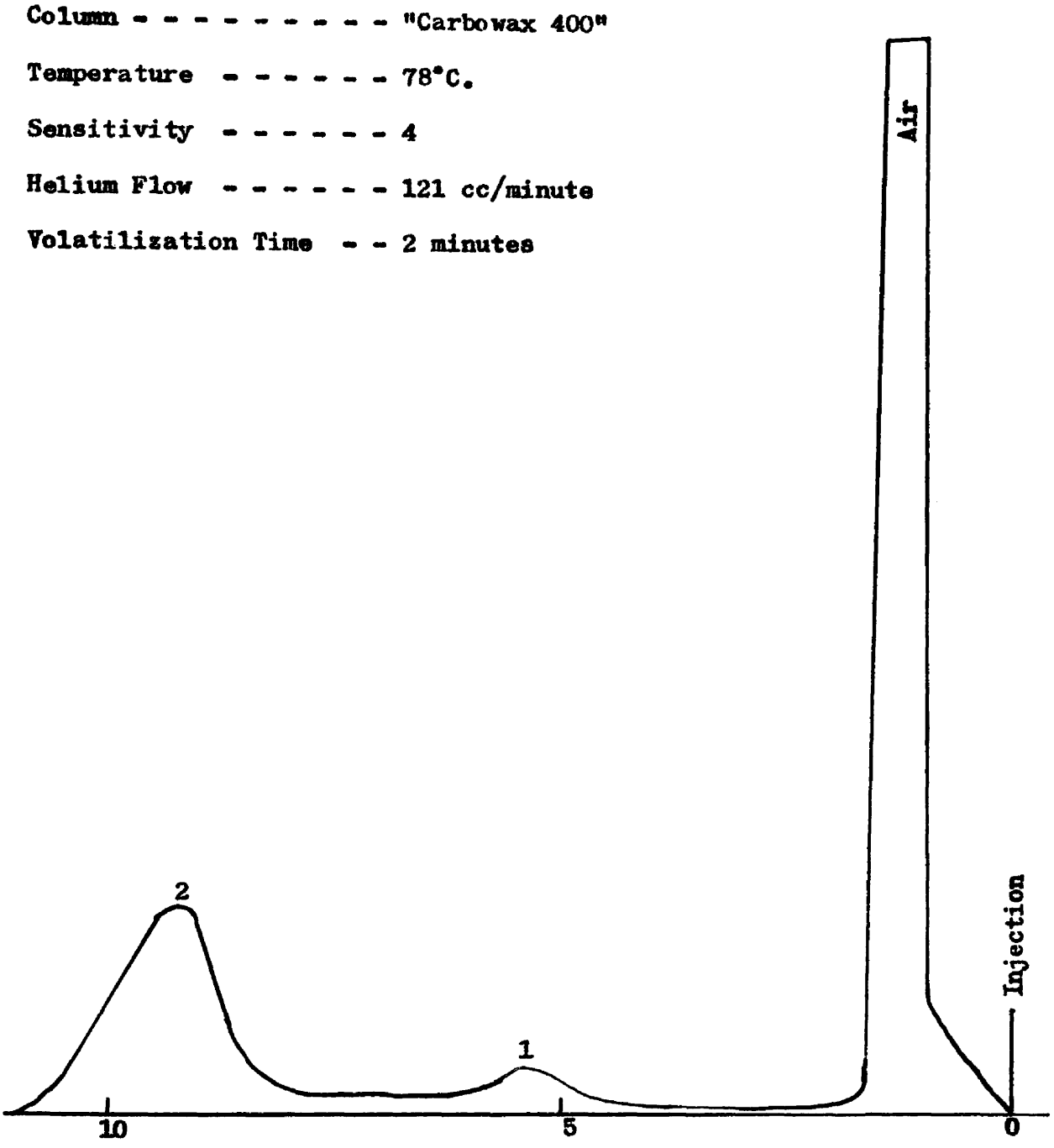

Figure 21. Gas chromatogram of cooked beef volatiles collected in a dry ice-ethanol trap and resolved on a "Carbowax 400" column.

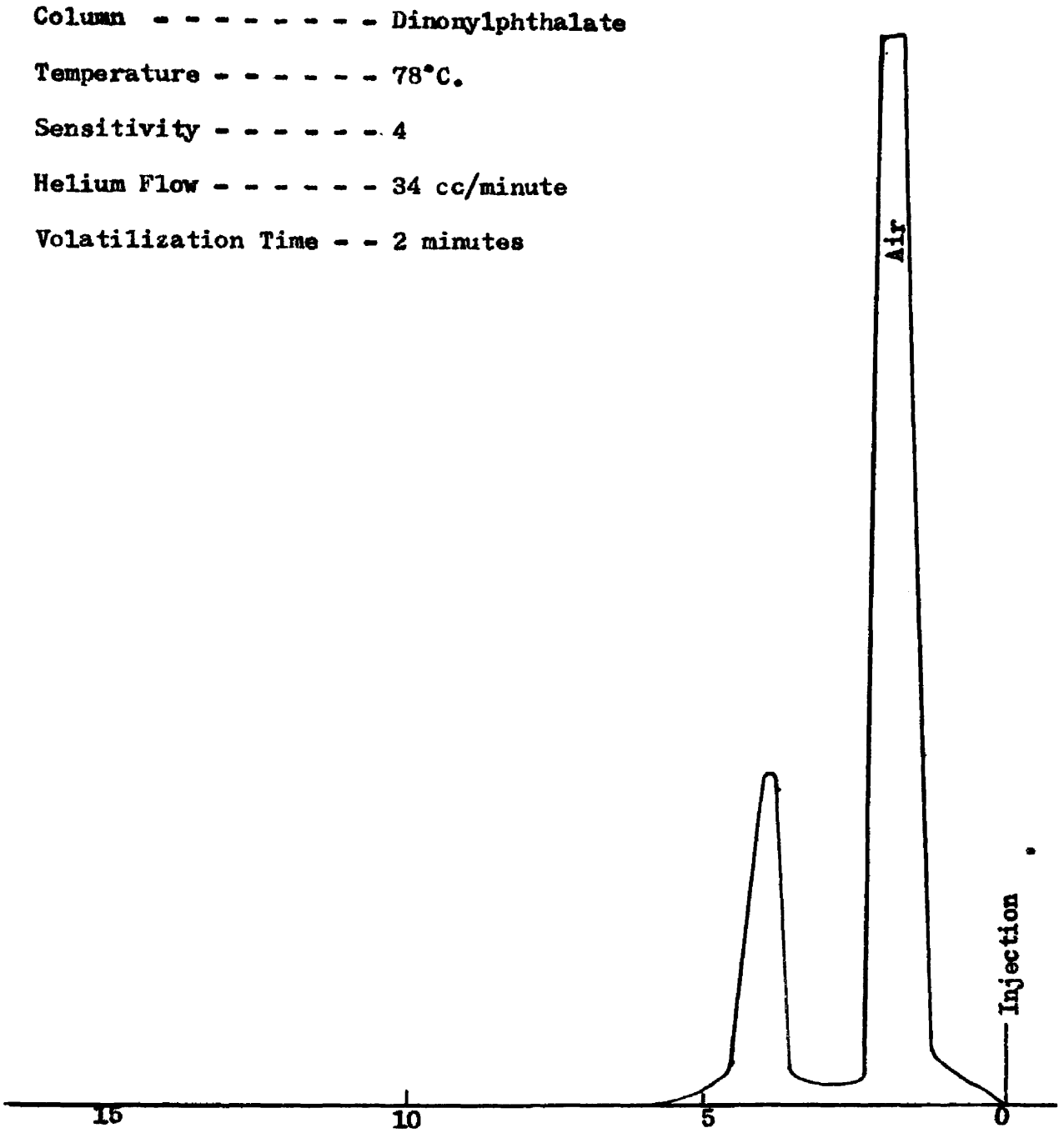

Figure 22. Gas chromatogram of methyl mercaptan resolved on a dinonylphthalate column.

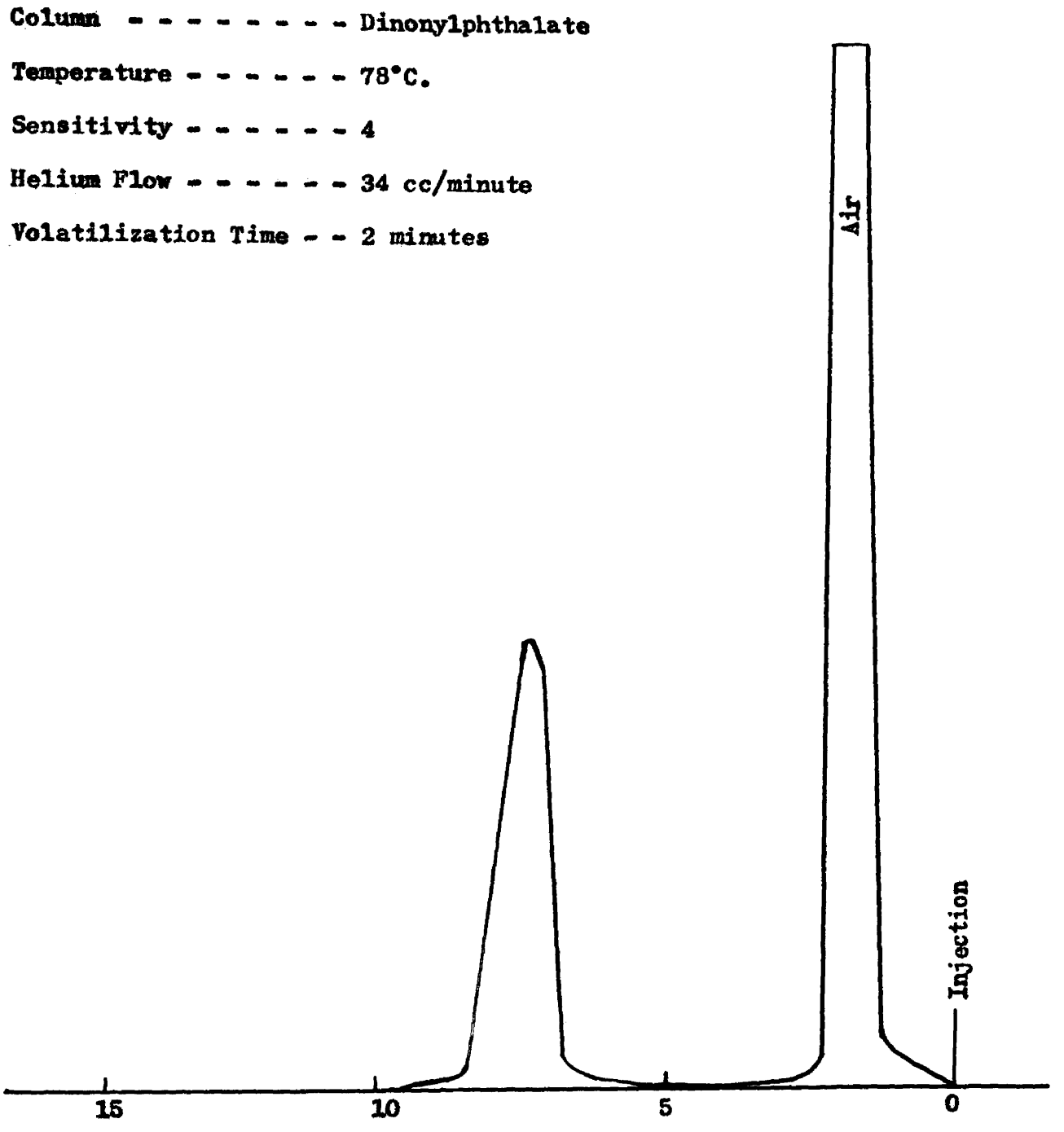

Figure 23. Gas chromatogram of acetaldehyde resolved on a dinonylphthalate column.

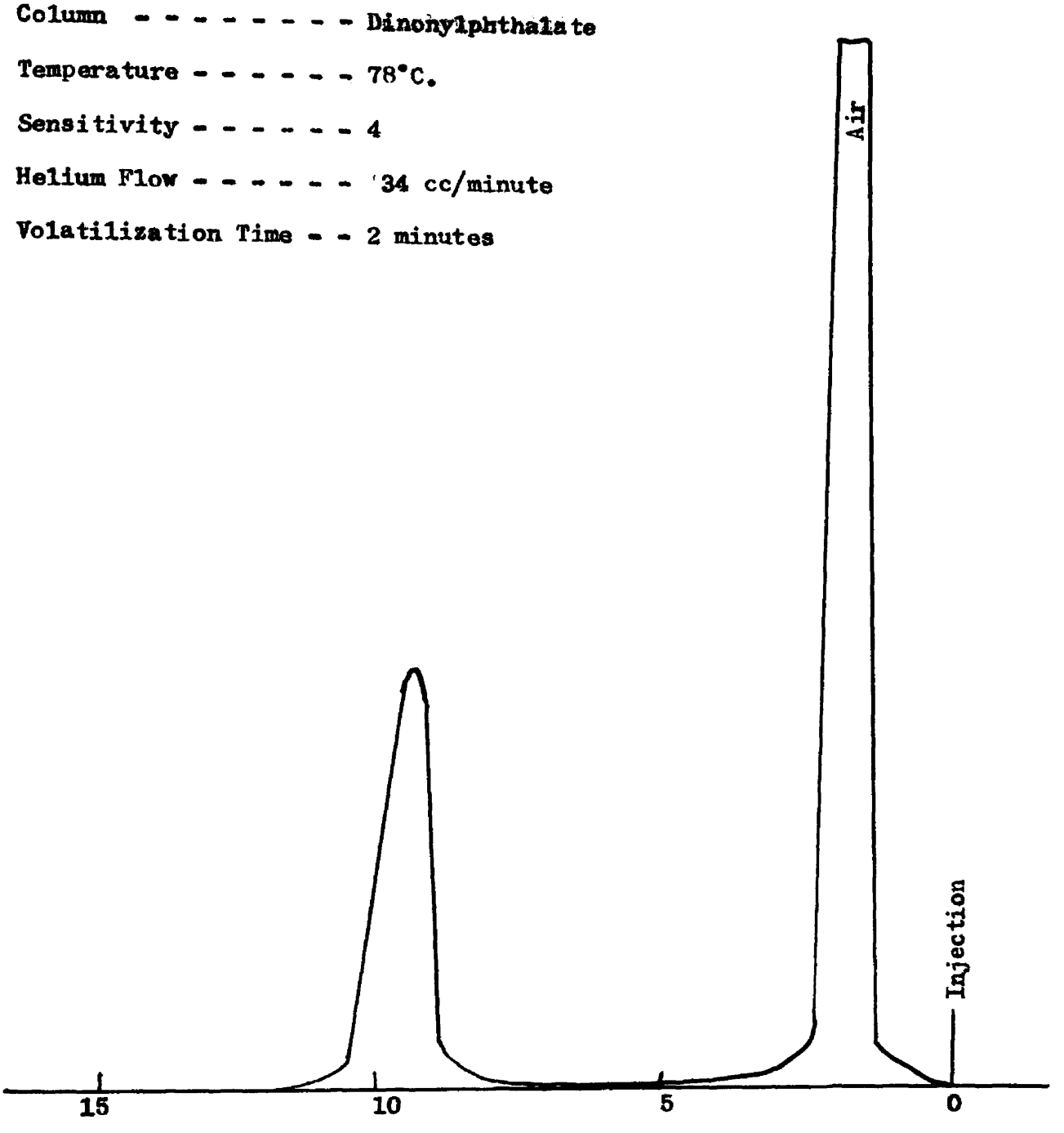

Figure 24. Gas chromatogram of methyl sulfide resolved on a dinonylphthalate column.

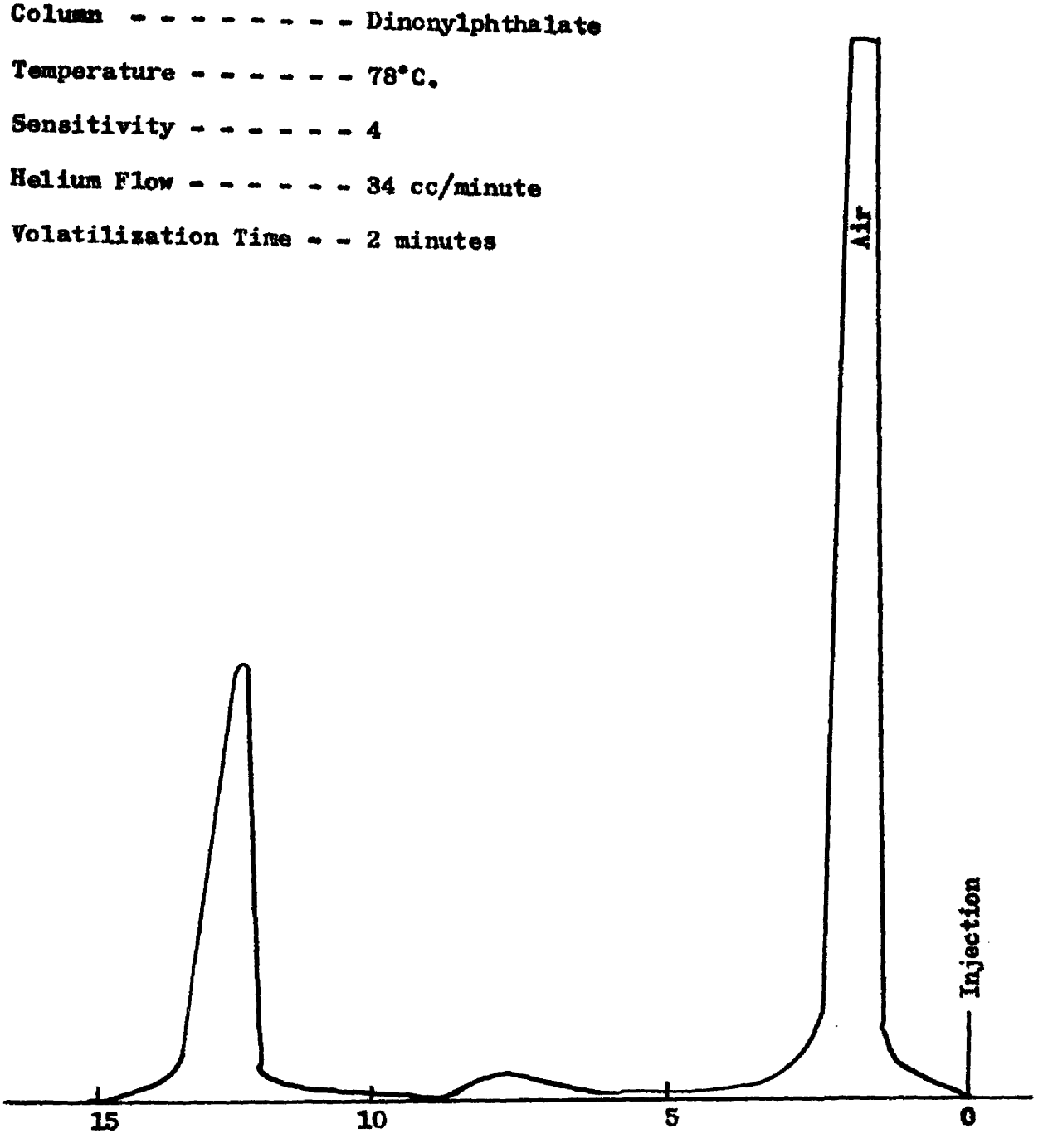

Figure 25. Gas chromatogram of acetone resolved on a dinonylphthalate column.

Column - - - - - - - - Dinonylphthalate

Temperature - - - - - - 78°C.

Sensitivity - - - - - - 4

Helium Flow - - - - - - 34 cc/minute

Volatilization Time - - 10 minutes

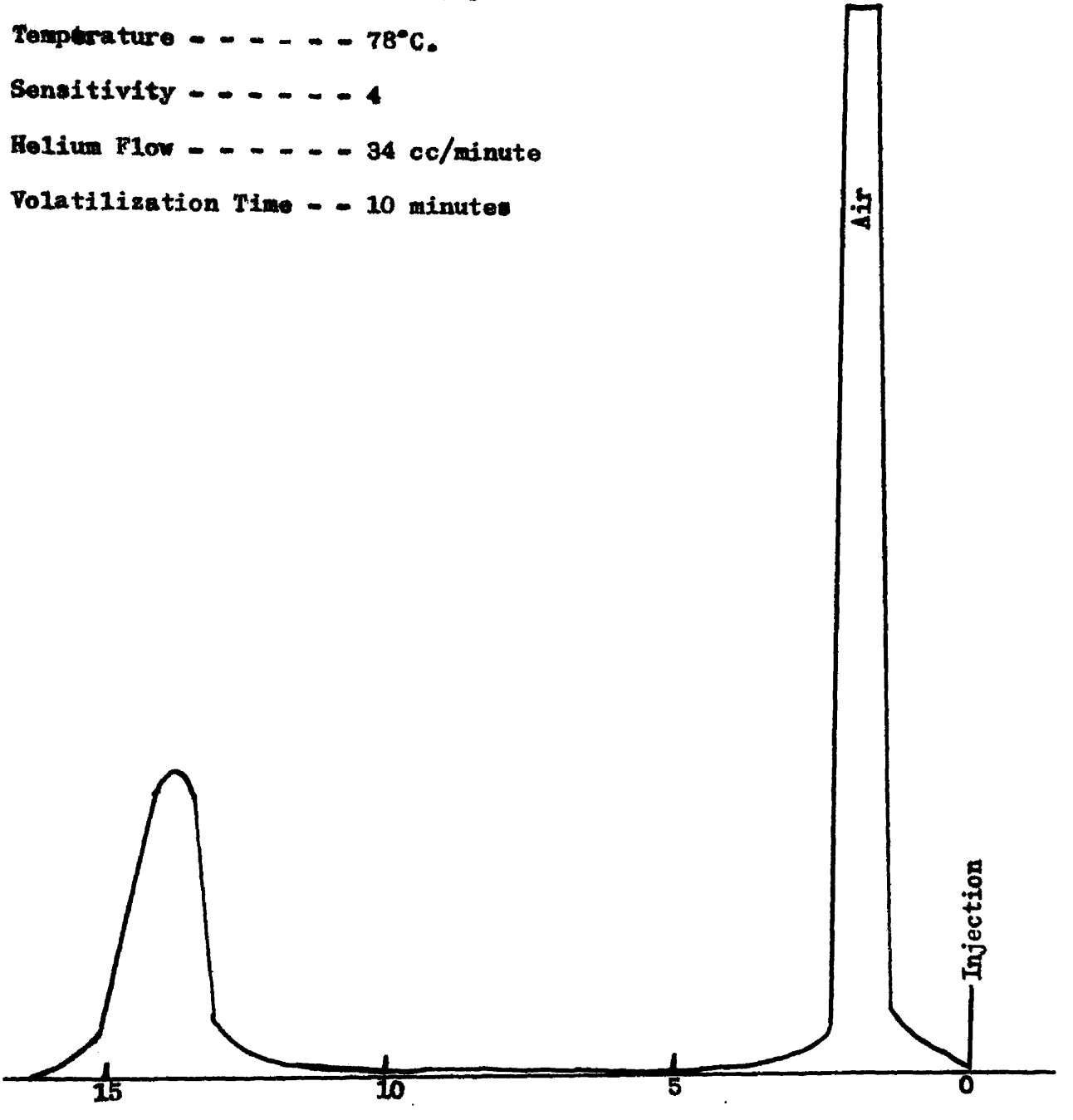

Figure 26. Gas chromatogram of water resolved on a dinonylphthalate column.

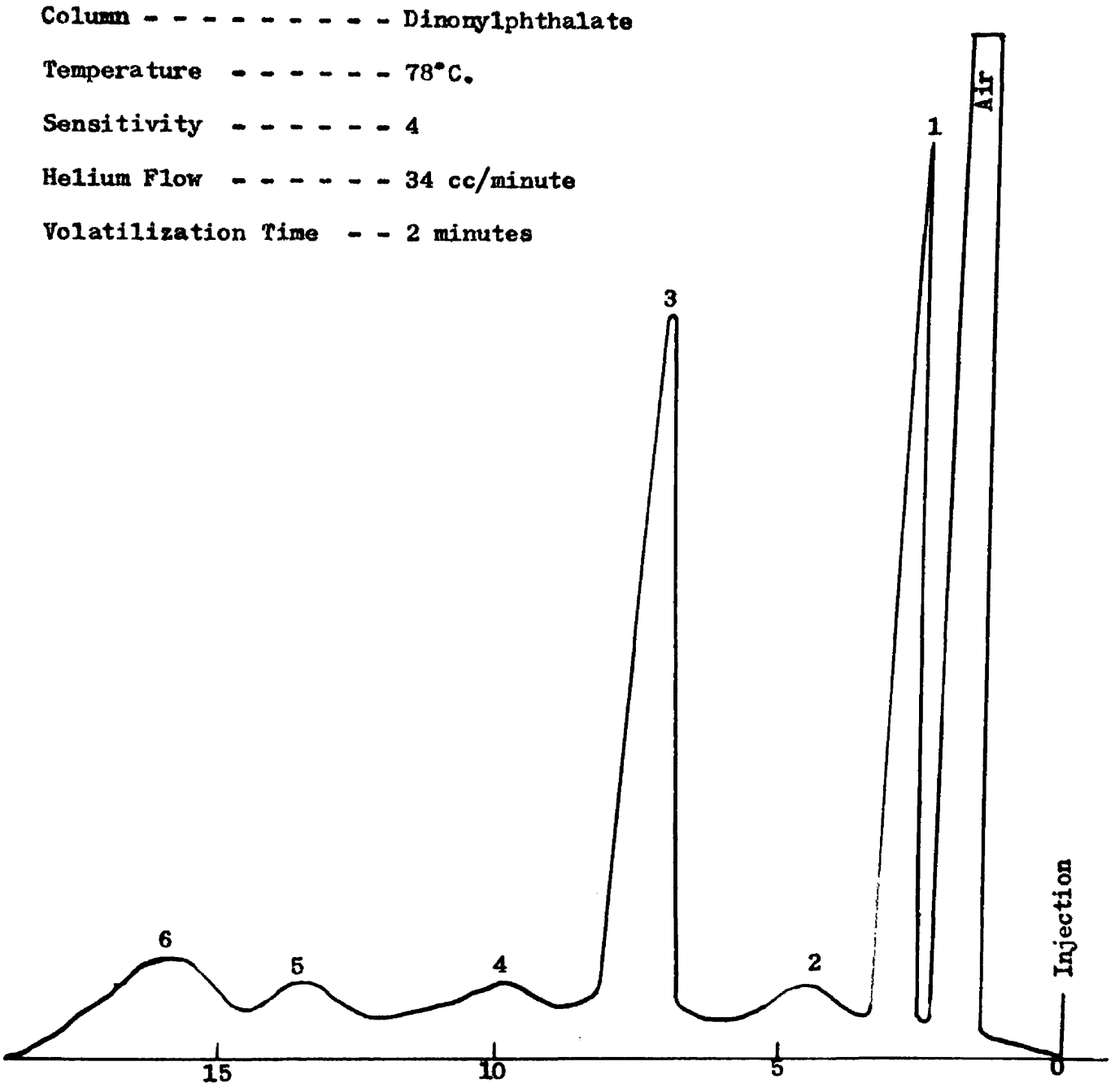

Figure 27. Gas chromatogram of cooked beef volatiles collected in a liquid air trap resolved on a dinonylphthalate column. - 2 minute volatilization period.

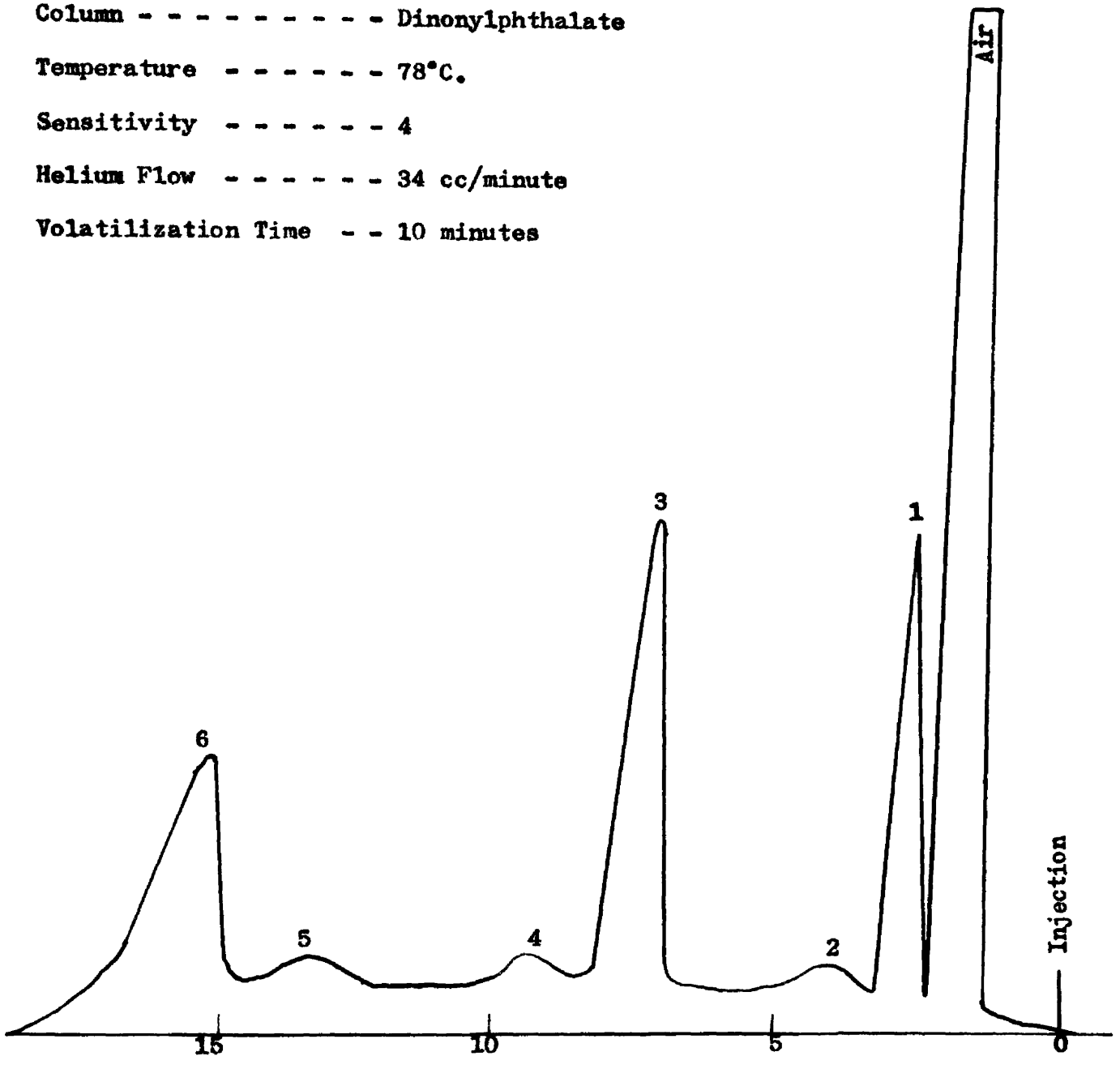

Figure 28. Gas chromatogram of cooked beef volatiles collected in a liquid air trap resolved on a dinonylphthalate column. - 10 minute volatilization period.

of 172 was observed for the third peak, which closely parallels that of acetaldehyde. Bubbling the gas through 2, 4-dinitrophenylhydrazine precipitated hydrazones, indicative of a carbonyl compound.

Peak four was found to have a retention volume of 225, close to that of methyl sulfide. However, bubbling the gas through a lead acetate solution failed to give a positive test for sulfides.

Peak five produced a few hydrazone crystals when bubbled through 2, 4-dinitrophenylhydrazine, indicating a probable carbonyl compound. This peak had a retention volume of 298 which more closely approximated the retention volume of acetone than any other compound tested.

Peak six showed poor resolution at the end of two minutes but was much more satisfactorily resolved after a volatilization period of 10 minutes. A known water standard was obtained by applying gentle heat with a Bunsen burner to a gas wash bottle containing water. This gave a retention volume of 323 for water. The peak was resolved more clearly as the volatilization time was increased from 2 to 10 minutes (figure 26). The retention volume of 304 was comparable to that obtained for water. Thus, carbon dioxide, methyl mercaptan and acetaldehyde were identified as being present in the volatile mixture, which agrees with the results obtained using the didecylphthalate and "carbowax 400" columns. In addition, acetone was resolved, as it was on the didecylphthalate column.

It had been suggested that the use of a carrier gas was unnecessary to carry the flavor volatiles from the heated flask through the system of acid traps. Figure 29 illustrates a typical chromatogram observed when no carrier gas was bubbled through the meat slurry. Two small poorly re-

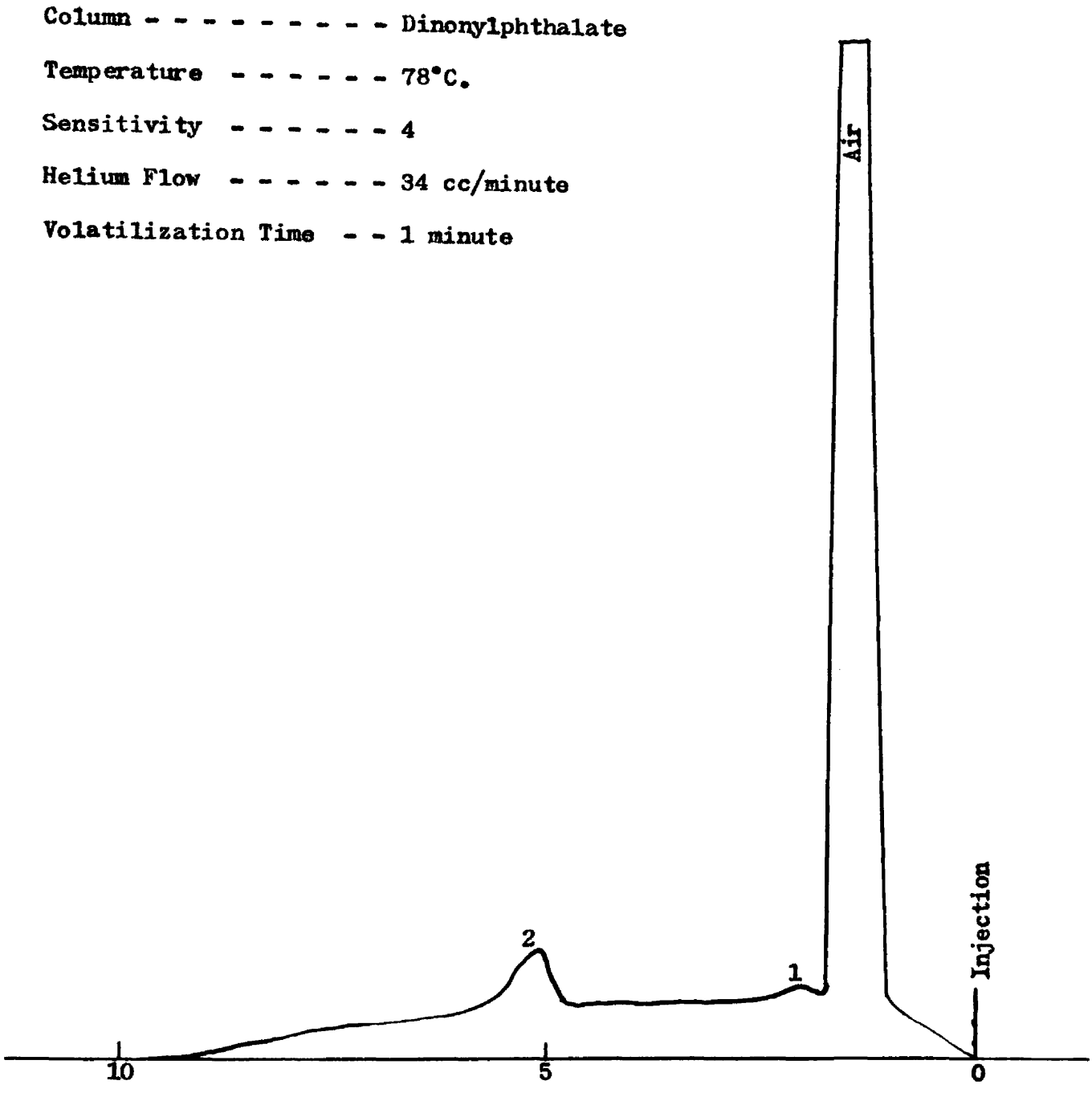

Figure 29. Gas chromatogram of cooked beef volatiles collected in a liquid air trap without the use of a carrier gas resolved on a dinonylphthalate column.

solved peaks were recorded but not identified. Thus, the use of a carrier gas appeared to be necessary.

Infrared spectrophotometry.

Figure 30 illustrates the infrared spectrum of a mixture of cooked beef volatiles collected in a trap immersed in liquid air. Infrared absorption maxima were noted at 4.3, 6.6 and 12.2 microns. However, no conclusions as to the identification of various components could be drawn from this graph. Apparently, the volume of the various components was too small to be accurately identified by this method.

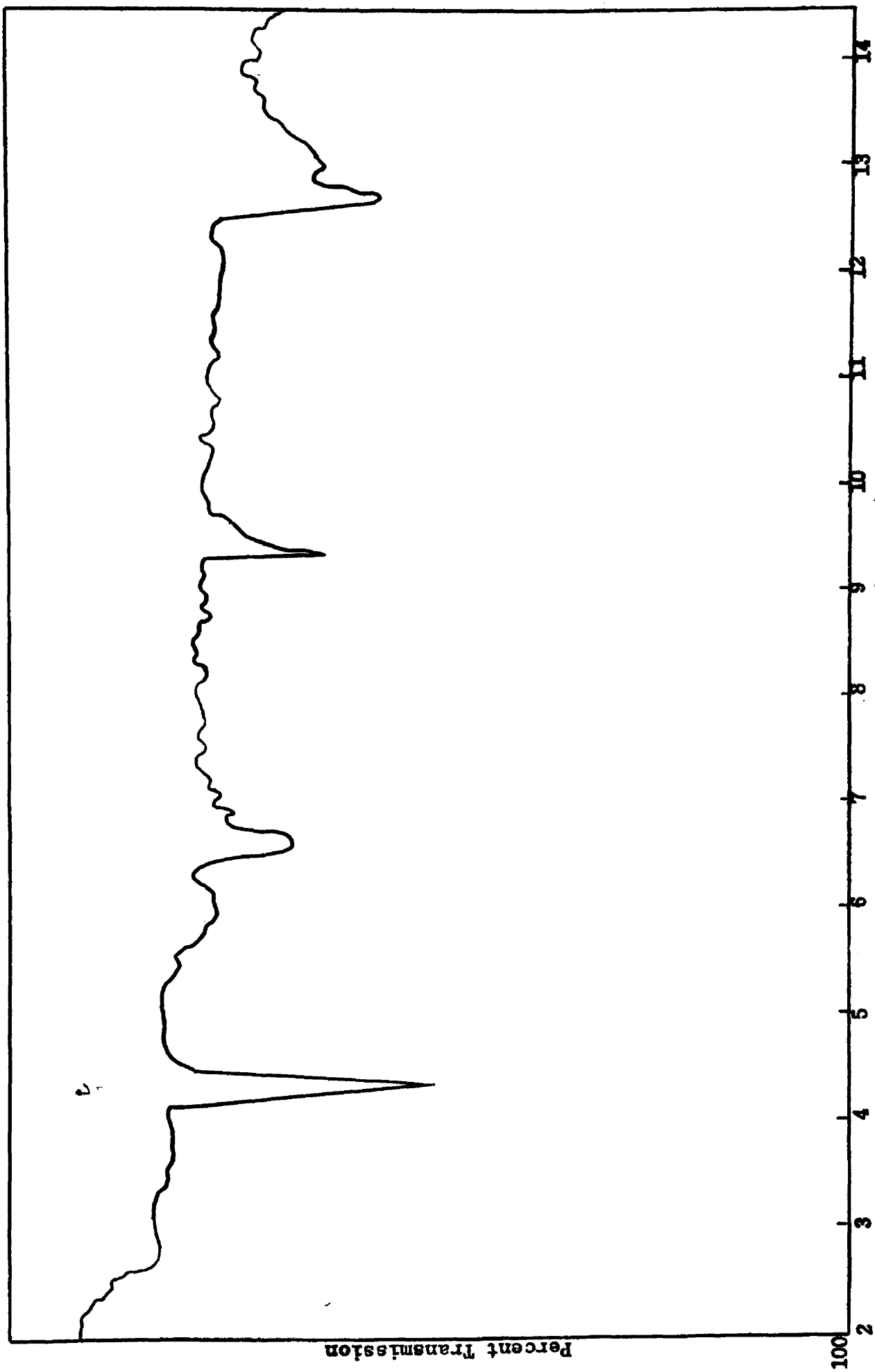

Figure 30. Infrared spectrum of total cooked beef volatiles.

SUMMARY AND CONCLUSIONS

The first part of this investigation was undertaken to characterize and ascertain the intensity of flavor in various meat and meat juice fractions. A dilution technique of ascertaining the detection threshold with a panel was used for the experiment. Raw and cooked beef water slurries, raw and cooked press fluid and filter cake, and raw and cooked meat water leachings with the various filtrates, both before and after heating, were used to study flavor changes.

The character of the flavor varied greatly between the raw and cooked fractions, yet upon heating the raw fractions the flavor appeared to differ only in intensity. It was evident that press fluid had a highly concentrated flavor. Results showed the flavor constituents were largely water soluble in both the cooked and raw fractions. However, cooking prior to extraction increased the flavor threshold, indicating that full flavor development may be due to heating of the juice and fibers together. Leaching of the meat with water resulted in a complete loss of flavor with both cooked and raw meat, but the cooked meat maintained its flavor over a longer period of time.

Comparisons of the flavor threshold with gross chemical analysis showed that neither fat content nor fat-free dry matter was responsible for differences in flavor. Although increases in pH occurred on heating the various meat and meat extracts, such changes did not appear to be responsible for flavor differences.

The second portion of this investigation was concerned with fractionation and identification of the volatile components of cooked beef.

Of the volatile carbonyl compounds collected and chromatographed on paper as 2, 4-dinitrophenylhydraxone derivatives, only acetaldehyde was identified; however, the presence of at least two, and possibly more carbonyls was indicated by the presence of two large, poorly resolved spots.

The presence of sulfur compounds in the mixture was indicated by passing the volatiles through a lead acetate solution and observing the black precipitate formed.

Of the three columns (didecylphthalate, "carbowax 400" and dinonylphthalate) used in the vapor fractometer to study cooked beef volatiles, dinonylphthalate was found to be the most suitable.

The three peaks resolved by the "carbowax 400" column were identified as were all six peaks resolved by the dinonylphthalate column. However, only three of the six peaks resolved by the didecylphthalate column were identified. Figure 31 shows a diagramatic sketch of the 6 compounds tentatively identified.

Carbon dioxide, methyl mercaptan and acetaldehyde were identified by qualitative tests or retention volumes, or both, with all three columns. In addition, acetone was identified with both the phthalate columns and methyl sulfide and water were tentatively identified with the dinonyphthalate column.

An infrared spectrum of the entire volatile mixture showed but three relatively small absorption maxima. No conclusions as to the identification of the various components could be drawn from the graphs.

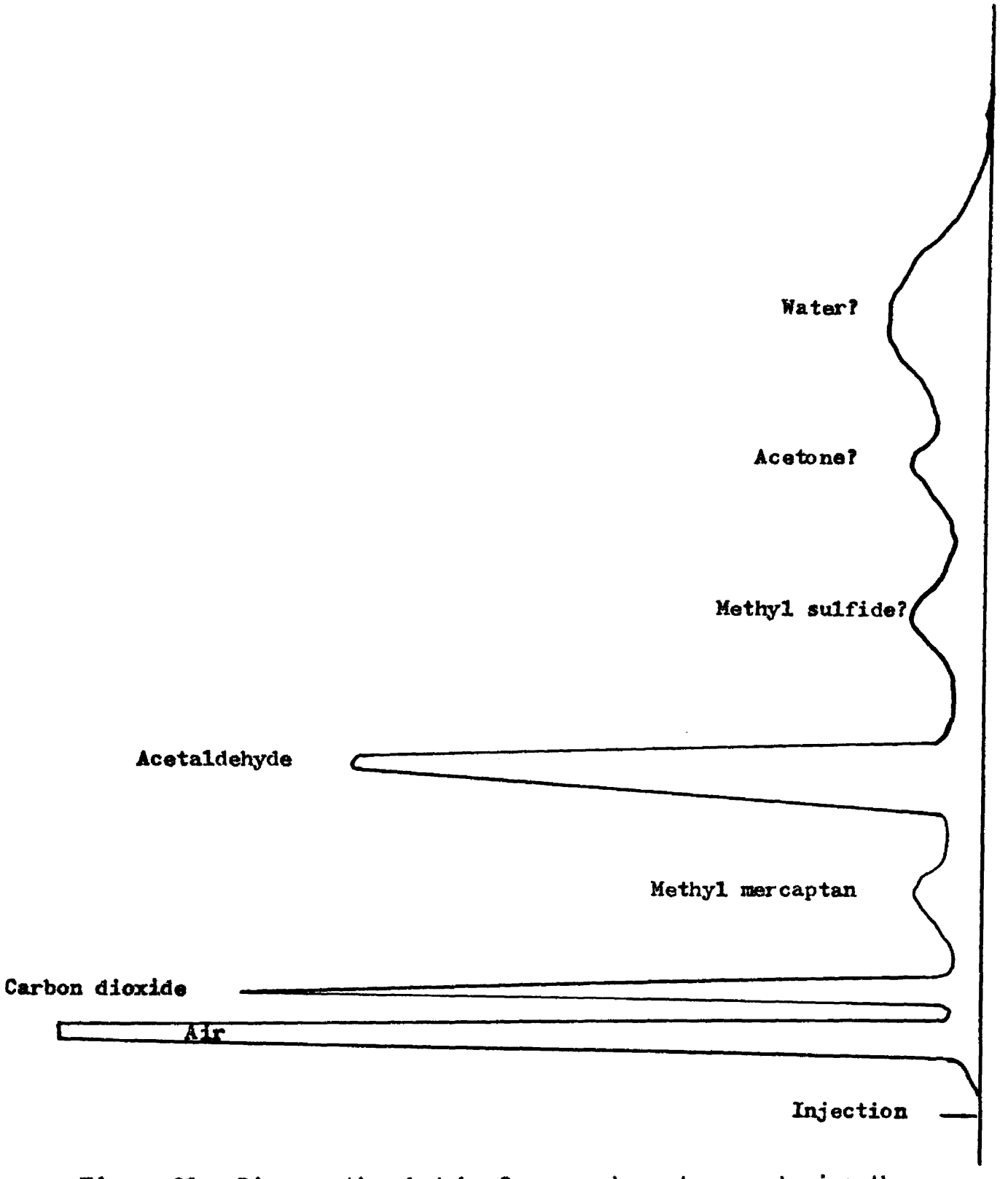

Figure 31. Diagramatic sketch of a gas chromatogram showing the compounds fractionated and tentatively identified as contributing to cooked beef flavor.

The compounds fractionated and identified in this study are believed to contribute to the flavor of cooked beef. The results obtained with paper chromatography, plus the fact that on occasions more than six peaks were obtained on the phthalate columns in the vapor fractometer would seem to indicate that more compounds are involved in the flavor of cooked meat than were detected during this investigation. Such additional compounds, even though present in small quantities, could conceivably contribute greatly to meat flavor. With the application of new techniques and the development of more sensitive instruments, it seems reasonable to believe that all the components responsible for meat flavor will some day be isolated and identified.

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