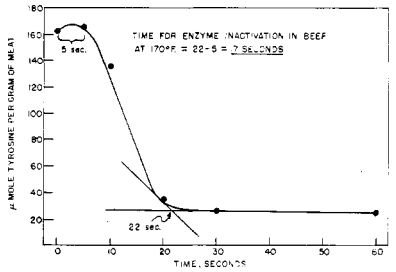

Figure 1. Enzyme inactivation at 170° F. *vs.* time in beef

Measured by the amino-N content of sample irradiated with a 5-megarad dose and stored at 100° F. for 6 weeks

tion. Plotting time on a log scale *vs.* temperature gave straight lines. This is in agreement with the generally accepted theory that the heat inactivation of enzyme systems follows a first order reaction curve.

The regression lines for the inactivation of beef and of pork are shown in Figure 2. The regression functions (y = log seconds) are as follows:

$y_{beef} = 11.893 - 0.0623\ x$ (std. error of estimate = 0.0165 and correlation coefficient = 0.9971)

$y_{pork} = 11.664 - 0.0600\ x$ (std. error of estimate = 0.0224 and correlation coefficient = 0.9953)

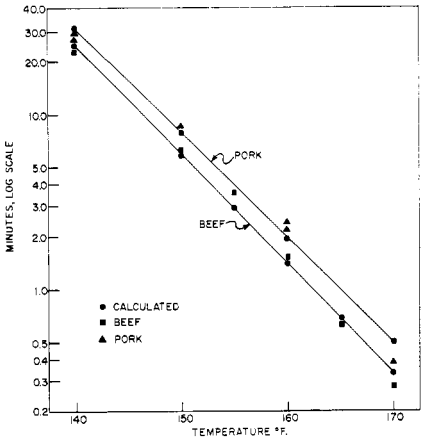

Figure 2. Time *vs.* temperature plot of regression lines for enzyme inactivation in irradiated beef and pork

This figure, then, shows the relationship of time and temperature which would be required to inactivate enzymes in radiation-sterilized beef and pork products. The difficulty of controlling any heat process, in order to achieve an internal temperature for the specified length of time should compensate for any errors which have been made because of assumptions concerning the point of inactivation at each temperature.

Whether this same time-to-temperature relationship is true of tissue proteolytic enzymes of organ meats, and of other species of animals, and what time-to-temperature ranges for complete inactivation of enzymes will produce the most acceptable product in terms of flavor, odor, color, texture, and storage stability is still to be determined.

Acknowledgment

The assistance of Robert Baldwin in performing analyses of the pork samples is gratefully acknowledged.

Literature Cited

(1) Doty, D. M., Wachter, J. P., J. AGR. FOOD CHEM. **3,** 61 (1955).
(2) Drake, M. P., Giffee, J. W., Jr., Ryer, R., III, Harriman, H., *Science* **125,** 23 (1957).
(3) Kempe, L. L., *Appl. Microbiol.* **3,** 346 (1955).
(4) Pratt, G. B., Ecklund, O. F., *Food Technol.* **10,** 496 (1956).
(5) Rosen, H., *Arch. Biochem. Biophys.* **67,** 10 (1957).

Received for review April 2, 1959. Accepted August 5, 1959. Division of Agricultural and Food Chemistry, 134th Meeting, ACS, Chicago, Ill. Paper No. 954 in series approved for publication. The views or conclusions are those of the authors. Mention of company products does not constitute endorsement of the department over others not named.

FOODSTUFF VOLATILES

Determination of Volatile Components of Foodstuffs. Techniques and Their Application to Studies of Irradiated Beef

CHARLES MERRITT, Jr., S. R. BRESNICK, M. L. BAZINET, J. T. WALSH, and PIO ANGELINI[1]

Pioneering Research Division, U. S. Army, Quartermaster Research and Engineering Center, Natick, Mass.

Techniques have been developed for the isolation, separation, and identification of volatile components of various foodstuffs such as meat, fish, vegetables, and coffee. Isolation and separation are accomplished by low-temperature, high-vacuum distillation techniques and by gas chromatography. Three main fractions are usually obtained by the low-temperature, high-vacuum technique: a carbon dioxide fraction, a center cut, and a water fraction. Further separation is required before final identification of the components by mass spectrometry can be made. The efficiency and advantages of the different separation techniques are discussed. Some results of studies of the volatile components isolated from samples of irradiated beef are given.

THE GREAT POTENTIAL of the mass spectrometer, as an analytical device, for the determination of volatile components of foodstuffs has been described (*2*). In order to utilize fully the capabilities of the mass spectrometer, however, reliable methods must be available for the collection and separation of the samples of volatile components to be analyzed.

In these studies, volatile components are considered to be those compounds which may be distilled under a high vacuum from the food material at room temperature. This method is more rapid than flushing the sample with an inert gas. The vacuum distillation method is also believed to be superior to steam distillation or solvent extraction methods. When steam distillation is used, many compounds may be collected which are not appreciably volatile under ordinary conditions of temperature and pressure, whereas the use of solvent extraction methods introduces the added complication of solvent removal in subsequent separation steps.

Collection and Separation

The basic high-vacuum, low-temperature distillation apparatus employed for

[1] Present address, Department of Agricultural Engineering, Michigan State University, East Lansing, Mich.

collecting the samples and for subsequent separations consists of two gas bottles fitted with stopcocks attached to a vacuum manifold. The sample is placed in one of the gas bottles and cooled to −196° C. with liquid nitrogen while air is pumped from the system. A final pressure of about 1 micron can usually be obtained. The vacuum pump is then closed off, and the sample is allowed to warm up to room temperature. The volatile compounds are now condensed in the receiver flask by cooling it with liquid nitrogen.

A photograph of a typical setup is shown in Figure 1. The sample is contained in the large flask shown at the left. This flask will hold a sample of ground meat, fish, or vegetable weighing up to 500 grams. If larger flasks are employed, stopcocks should have a correspondingly larger bore to permit the system to come to equilibrium within a reasonable time. Volatiles were collected with this apparatus from 200-gram samples of irradiated meat.

The sample of volatile components of the foodstuff collected in the receiver is called the total condensate and contains practically all of the odorous compounds present in the original material. The residue remaining after the volatile components have been removed appears to retain very little of its characteristic odor. Furthermore, the odor of the total condensate is strong and highly characteristic of the original foodstuff. The sample of total condensate, however, consists mainly of water and carbon dioxide. The odorous compounds constitute less than 1% of the volume of the total condensate. It is, therefore, necessary to separate efficiently the trace amounts of the odorous materials from the bulk of the carbon dioxide and water. This can be done by further high-vacuum low-temperature distillation.

The apparatus used for separations is the same as that used for the initial collection of total condensate, except that the gas bottles are smaller. At the right of Figure 1 are shown two gas bottles used for the separation of a low-temperature fraction. The total condensate might be cooled to −140° C. while the receiver is again at liquid nitrogen temperature. The apparatus shown with a stopcock in the center of the manifold for isolating each half of the vacuum system permits two pairs of flasks to be used in this type of bulb-to-bulb distillation on the same manifold.

The low-temperature, high-vacuum fractionation of the total condensate is based on differences in the vapor pressure–temperature relationships of the compounds present. A Clausius-Clapeyron plot of pressure–temperature data for some of the compounds which may be present in a sample of total condensate is shown in Figure 2. The graph shows that at −140° C. carbon dioxide has a vapor pressure of about 400 microns, whereas all the other components except hydrogen sulfide, carbonyl sulfide, etc., have a vapor pressure of less than a micron. By cooling the sample of total condensate to −140° C. and condensing the carbon dioxide in a receiver at −196° C., the carbon dioxide is efficiently separated from the rest of the sample. Any hydrogen sulfide or carbonyl sulfide present will be collected with the carbon dioxide.

Many components of the mixture may be separated from water in a similar manner at −80° C. Higher boiling components of the mixture remain unseparated in the water fraction.

A flow sheet for the separation of a sample of total condensate is shown in Figure 3. In order to accomplish the separations more rapidly, the carbon dioxide and the other more volatile components of the sample which are called the center cut are first distilled from the

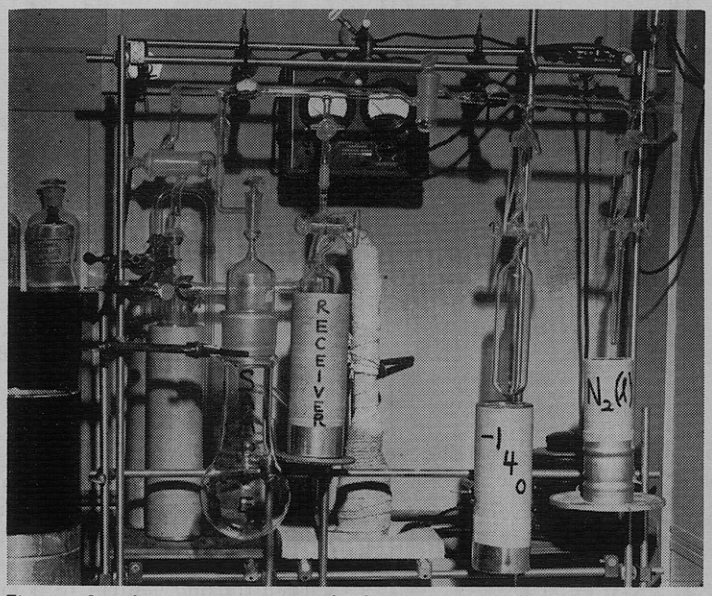

Figure 1. Low-temperature, high-vacuum distillation apparatus

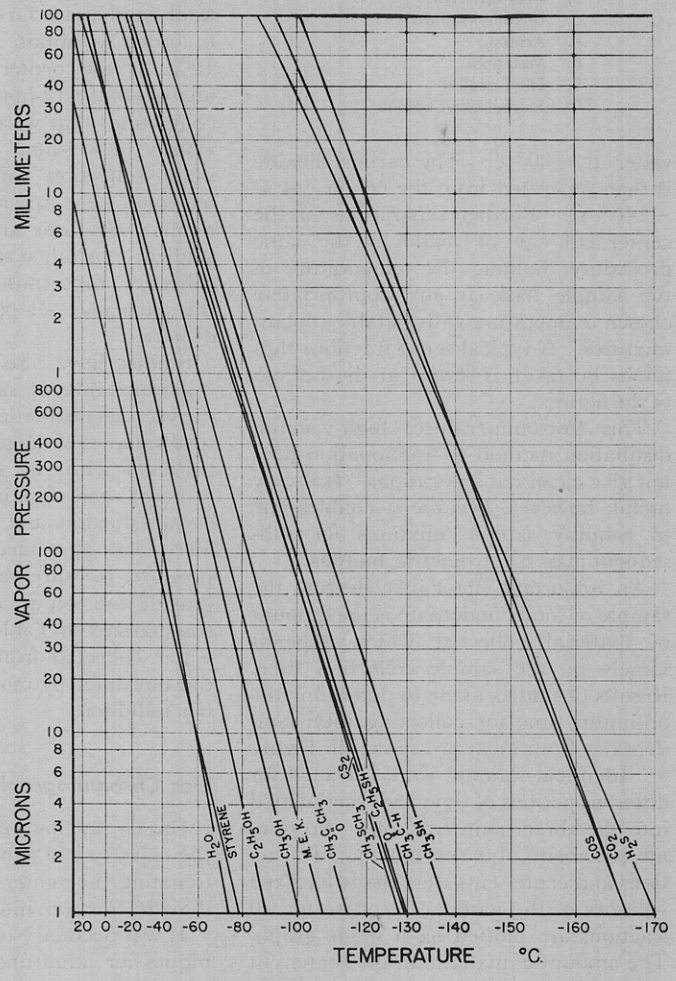

Figure 2. Vapor pressure–temperature relationships

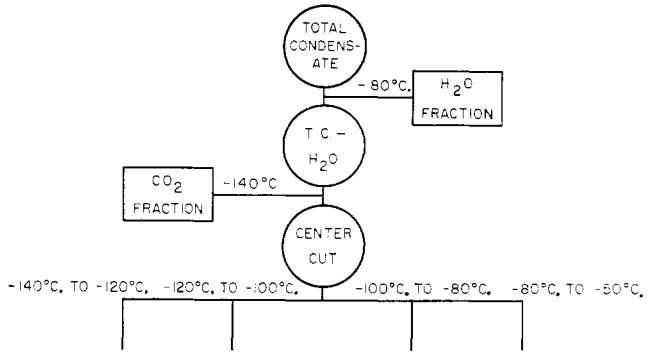

Figure 3. Flow sheet for fractionation of total condensate

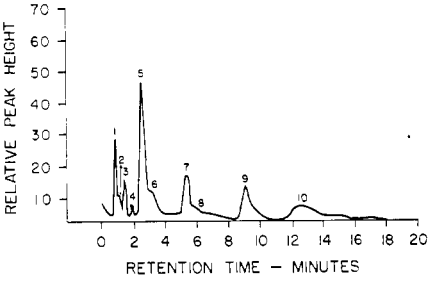

Figure 5. Gas chromatogram of a meat odor center cut

Compounds identified

1. Air
2. Carbon dioxide
3. Methyl mercaptan
4. Methyl formate (tentative)
5. Acetaldehyde
6. Dimethyl sulfide
7. Acetone
8. Methanol
9. Ethyl acohol
10. Methyl ethyl ketone

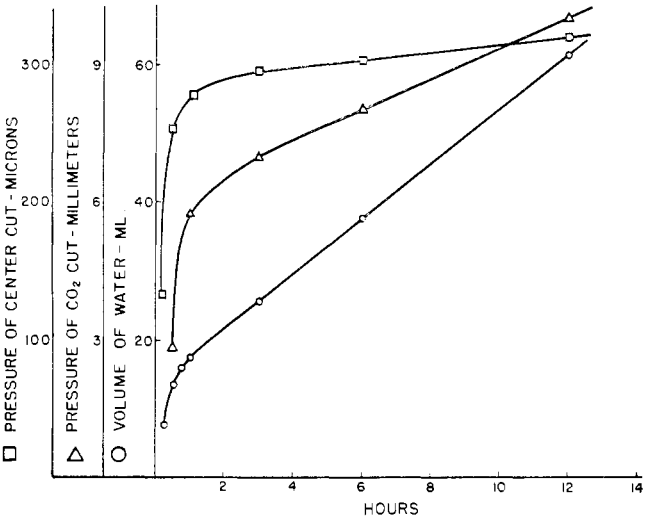

Figure 4. Graph showing effect of collection time on total condensate

water at −80° C. The carbon dioxide is then separated from the center cut at −140° C. Further separations of the center cut can be made by the same procedure, holding the temperature of the sample flask at any appropriately chosen temperature with suitable coolant mixtures. A typical set of fractions that might be taken is shown at the bottom of the figure.

This low-temperature, high-vacuum distillation method of fractionation does not give clean-cut separations. It is very useful, however, because it enables one to simplify sample mixtures for subsequent mass spectrometric analysis.

An important factor in collecting the sample of total condensate is the amount of material collected which depends largely on the sample collection time. Results of studies made to determine the optimum time for collecting total condensates from meat are shown in Figure 4. Identical samples were collected for different periods of time and fractionated into the three main fractions. Pressure measurements of the carbon dioxide fractions and center cuts were made for fixed volumes of the gases. The data for all fractions are plotted on a single graph. The amount of material in the center cut tends to level off after about 3 hours, whereas the amount of carbon dioxide and water continues to increase. As subsequent separations are accomplished more easily by keeping the water and carbon dioxide content to a minimum, it is obviously undesirable to collect the total condensate for more than 3 hours in order to obtain an additional 5 to 10% of the center cut. Subsequent analysis of the 12-hour sample showed no compounds that were not identified previously in a 3-hour sample. The optimum collection time of 3 hours determined here applies only to the particular apparatus and samples of meat studied. In other studies the optimum collection time must be determined for the particular set of experimental conditions.

Studies have also been made of the reproducibility of sample collection by the vacuum distillation procedure. Identical samples of volatiles from meat were collected with the same apparatus for 3 hours and fractionated into water, carbon dioxide, and center cut fractions. Very close agreement was obtained for pressure measurements on the carbon dioxide and center cuts. The results are summarized in Table I. The volume of water collected from each sample also did not differ by more than a few tenths of a milliliter.

Gas Chromatographic Separations

Gas chromatographic separations offer the most satisfactory means of fractionating the center cut and the carbon dioxide fraction into their components.

It was necessary to devise special techniques for admitting the sample to the chromatography apparatus and for collecting the separated components. The usual method employed is to transfer an aliquot of the center cut or carbon dioxide fraction into a special type of sampling tube (*1*). The transfer is accomplished on a vacuum manifold by evacuating the sampling tube, immersing it in liquid nitrogen, and then opening the tube to the original center cut or carbon dioxide fraction. After transfer, the sampling tube is attached to the chromatography apparatus and, after warming slightly, the sample is swept onto the chromatographic column with helium as the carrier gas. As the eluted components emerge from the column they are collected successively in separate cold traps at liquid nitrogen temperature.

The results of a typical gas chromatographic separation of a meat odor center cut are shown in Figure 5. The column used consisted of 30% carbowax on 30- to 60-mesh firebrick and was operated at 60° C. with a helium flow rate of 70 ml. per minute. The separated components were collected and analyzed by mass spectrometry. The identity of the various peaks established by mass spectrometric analysis is also indicated in Figure 5. The compounds identified in this way agree with the results obtained when separation was accomplished by low temperature vacuum distillation. The chromatographic separation may be accomplished in about 0.5 hour, whereas the separation by vacuum distillation requires a day or more.

Studies of Irradiated Meat

The techniques described in the preceding sections have been used to identify some of the volatile components

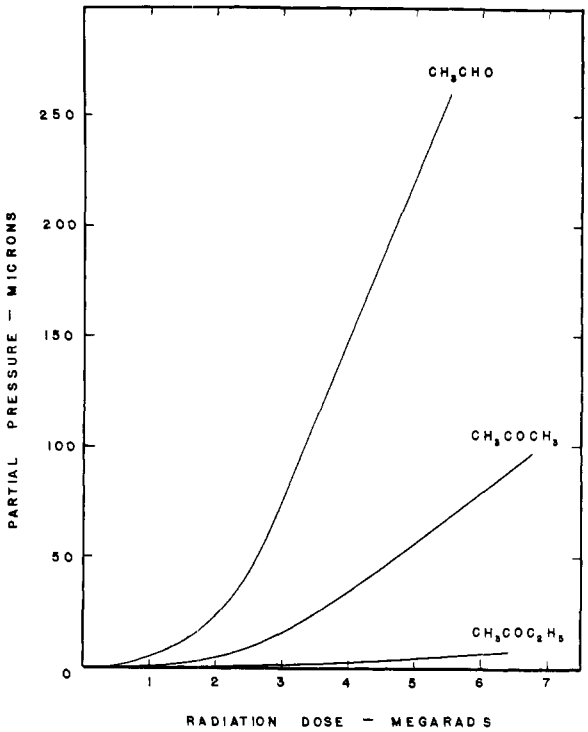

Figure 6. Relative amounts of carbonyl compounds as a function of radiation dose

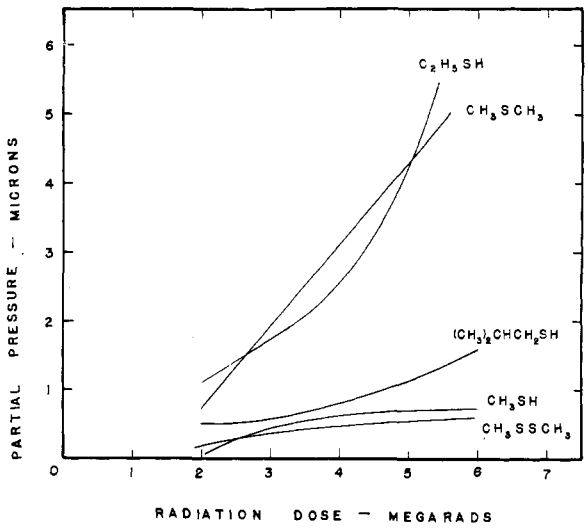

Figure 7. Relative amounts of sulfur compounds as a function of radiation dose

Table I. Precision of Collection of Sample

Sample	Pressure of CO_2 Cut, Mm.	Pressure of Center Cut, μ
A	6.81	66
B	6.79	60
	δ = 0.3	δ = 1.0

isolated from irradiated ground beef.

A sample of ground beef, usually about 200 grams, is packaged in the form of a pattie in a 3-mil Mylar bag. The sample is flushed with argon before sealing the package. The sample is then irradiated with the appropriate dose of electron radiation in a 1.0-m.e.v. Van de Graaff electron accelerator.

After irradiation, the packages are immersed in liquid nitrogen until the meat is thoroughly frozen. The packages are then opened and the frozen pieces of meat are transferred to the glass sample container which in turn is fitted to the high-vacuum manifold. The irradiated sample of meat now contained in the flask attached to the vacuum system is held at liquid nitrogen temperature (−196° C.) while air and argon are pumped off. The sample is then allowed to thaw, and when it has come to equilibrium at room temperature (25° C.) the total condensate is collected and subsequently separated and analyzed.

Analyses were made of duplicate samples of a control and of ground beef irradiated at 2, 4, and 6 megarad. The following compounds were found in the center cuts of the irradiated samples:

Acetaldehyde	Methyl mercaptan
Acetone	Dimethyl sulfide
Methyl ethyl ketone	Dimethyl disulfide
Methanol	Ethyl mercaptan
Ethyl alcohol	Isobutyl mercaptan

All of these compounds except dimethyl disulfide and isobutyl mercaptan were also found in the controls. It should not be inferred, however, on the basis of the present data that dimethyl disulfide and isobutyl mercaptan are compounds which may be significantly associated with irradiated meat, as the possibility exists that they may also be present in the nonirradiated meat in amounts too small to be detected by the techniques used in this investigation. On the other hand, it should not be concluded that the compounds shown represent a complete list of those present in the center cut.

In general, the amounts of the compounds increased in the irradiated samples. Furthermore, the inerease was proportional to the radiation dose. The relative increase in the amount of compound with increasing dose is indicated by the graphs shown in Figures 6 and 7 for carbonyl and sulfur compounds, respectively. The curves in Figure 7 originate at the lowest dose level, as there is no apparent continuity in the concentration of sulfur compounds as a function of radiation dose between zero and some finite radiation level. The trace amounts of sulfur compounds found in the controls do not show on the graph. The amount of methyl and ethyl alcohols increased with increasing radiation dose.

The relation of the compounds found in the center cut to the odor of the irradiated beef is unknown at present, except that they probably contribute in part. It is known, however, that other compounds which are odorous remain unseparated in the water fraction. Studies are in progress to isolate and identify these compounds.

Acknowledgment

This work would not have been possible without the assistance of W. T. Hall, who constructed most of the glassware. The authors are also indebted to J. R. Strom, J. H. Sullivan, D. H. Robertson, W. J. Balke, and Roslyn E. Kramer for their contributions to the experimental work.

Literature Cited

(1) Bazinet, M. L., Walsh, J. T., Research Rept., Anal. Chem. Series **10,** Quartermaster Research and Engineering Command, Natick, Mass.
(2) Herk, L. F., Jr., Levy, E. J., O'Brien, D. C., Stahl, W. H., Division of Agricultural and Food Chemistry, 130th Meeting, ACS, Atlantic City, N. J., September 1956.

Received for review April 20, 1959. Accepted July 10, 1959. Division of Agricultural and Food Chemistry, 134th Meeting, ACS, Chicago, Ill., September 1958.