Dr. Pearson, members of the Conference,--

Before beginning my presentation, I think I should comment that, in spite of the subject of this panel, I can tell you very little about the components responsible for meat flavor. Nevertheless, the mass spectrometer has been found to be a very useful device for the analysis of volatile compounds which may be isolated from meat, and some of these very likely contribute to its flavor. In order to effectively utilize the mass spectrometer it is necessary to have reliable techniques for collection and separation of the volatile compounds. I shall confine my remarks, therefore, to a discussion of these techniques and to a brief description of the mass spectrometric method of analysis.

Slide 1

**ANALYSIS SCHEME**

Odorous Sample
Collection of volatiles

Total Condensate
Fractionation
Gas chrom. or vac dist.

Fractions

Mass Spectrometer Analysis

Here we see an outline of our general analysis scheme. In most of our work, since we have been concerned mainly with odor compounds, the method of collecting samples has been limited to vacuum distillation. Actually, this method of isolation can be generally useful, because it affords a non-destructive technique for isolating compounds from natural products, whatever the interest may be. The material isolated from the initial distillation, the total condensate, as we call it, is usually a complex mixture containing large amounts of water and carbon dioxide as well as the volatile flavor and odor compounds. This mixture must be separated
before mass spectrometric analysis can be performed. The separation may be accomplished by further vacuum distillation or by gas chromatography. The use of gas chromatography for this purpose is a long story in itself, so I shall have to limit my discussion today to the use of vacuum distillation.
This slide shows the basic high vacuum-distillation apparatus employed for collecting samples, and for subsequent separations.

The apparatus 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 one micron can usually be attained. 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.

The volatile components collected in the receiver we call the "total condensate." It consists mainly of carbon dioxide and water, and contains less than 0.01 or perhaps even 0.001% of the odor or flavor compounds. It is necessary, therefore, to separate efficiently the trace amounts of the flavor and odor compounds from the bulk of the water and carbon dioxide. This can be done by further high vacuum distillation at low temperature.

Efficient separation depends on the selection of an appropriate temperature.

Slide 3 is a Clausius-Clapeyron plot of the pressure-temperature relationships for some of the compounds which may be present in a meat total condensate.

Looking at the graph, it is seen that at -140°C, carbon dioxide has a vapor pressure of about 400 μ, whereas all the other components except hydrogen sulfide, carbonylsulfide, or possibly a few other low molecular weight gases, have a vapor pressure of less than a micron. By cooling the total condensate to -140°C and condensing the carbon dioxide in a receiver at -196°C, the carbon dioxide is quite efficiently separated from the rest of the sample. Any H2S present will of course be collected with the CO2. Similarly, at -80°C most of the components shown here have an appreciable vapor pressure, whereas the vapor pressure of the water is less than a micron. In this case, the other components of the sample are distilled into the receiver at liquid nitrogen temperature.
Here is shown a flow sheet for the separation of the total condensate. In order to accomplish the separations more rapidly, the carbon dioxide and other more volatile components of the sample, which we call the center cut, are first distilled away from the water at $-80^\circ C$. The carbon dioxide is then separated from the center cut at $-140^\circ C$. 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 are shown at the bottom of the chart.

Slide 5 is a picture of a typical vacuum distillation apparatus employed in our laboratory. The type of bottle used to hold the meat sample is shown on the extreme left and the volatiles are collected in the receiver bottle shown immersed in the dewar. An example of a setup for the carbon dioxide separation is shown on the right half of the vacuum manifold.

I should like to digress for a moment to consider an important factor in collecting the total condensate. The amount of material collect-
ed, especially the water and carbon dioxide, depends largely on the sample collection time. Results of studies made to determine the optimum time for collecting the total condensate from meat are shown in the next slide.

Slide 6

- Pressure of center cut (microns)
- Pressure of CO₂ (millimeters)
- Volume of water (ml)

Graph showing the changes over time.
The study was made by collecting identical samples, for different periods of time, and fractionating into the three main fractions. The pressure measurements of the CO₂ and center cuts were made for fixed volumes of the gases. The data for each fraction are plotted here on a single graph.

It can be seen that the amount of material in the center cut tends to level off after about three hours, whereas the carbon dioxide and water content continue to increase. Since subsequent separations are effected 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 three hours in order to obtain an additional 5-10% of center cut. Subsequent analysis of the twelve-hour sample showed no additional compounds to be identified that were not found in the three-hour sample.

### Slide 7

<table>
<thead>
<tr>
<th>PRECISION OF COLLECTION OF SAMPLE</th>
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<tbody>
<tr>
<td>SAMPLE</td>
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<tr>
<td>--------</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>S = 0.3</td>
</tr>
</tbody>
</table>

Studies have also been made of the reproducibility of collecting the samples of volatiles by the vacuum distillation procedure. Identical samples of volatiles from meat, A and B, were collected with the same apparatus for three hours and fractionated into a water, a carbon dioxide and a center cut. As shown on the slide, very close agreement was obtained for the pressure measurements on the carbon dioxide and center cuts. The volume of water collected from each sample also did not differ by more than a few tenths of a milliliter.

Since the final analysis is accomplished by mass spectrometry, I should like to describe the mass spectrometer briefly and to mention some of the problems encountered in the multi-component analysis of trace amounts of compounds.

Slide 8 is a schematic diagram of the magnetic deflection type mass spectrometer. The sample must be admitted to the inlet system as a gas under reduced pressure. The gas is allowed to diffuse through a small leak (actually a pin hole in a piece of gold foil) into an ionization chamber where the molecules are bombarded by a stream of electrons and a number of ion fragments are produced. The ions are accelerated by means of
a high potential electric field and emerge into a perpendicular magnetic field where they are deflected in a circular path. Ultimately, they reach the collector plate, give rise to an ion current which is amplified and recorded. The ions produced by any given compound or mixture can be resolved by varying the accelerating electric field so that ions having different mass-to-charge ratios can be successively focused on the collector plate. The relative abundance of the ions can be determined from the amount of ion current recorded for each ion. A mass spectrum is, therefore, a plot of ion current, or as we say more simply, peak height, against mass-to-charge ratio of the ions produced in the ionization chamber.

Slide 9 is a photograph of our mass spectrometer. I will not take time here to describe the various components of this instrument.

Slide 10 shows the mass spectrum of acetaldehyde. The major peaks are 44, the molecular ion peak, resulting from the loss of an electron by the molecule; and 15 and 29 resulting from a cleavage of the molecule between the methyl and the aldehyde groups. The other, smaller peaks result from secondary fragmentation of the major ions.
4 HEXENAL - 1

M.W. = 98

CH₃ - CH=CH - CH₂ - CH₂ - C=O

15 83 41 57 55 43 69 29

MASS / CHARGE
This is the spectrum of 4-hexenal. As you might expect, as the molecule becomes larger, the spectrum is more complex. We have a peak at 98, due to the molecular ion and several other large peaks due to cleavages indicated at the various bonds in the structural formula of the compound.

Here we see compared the mass spectra of three carbonyl compounds we might expect to find in a meat center cut. Although the spectrum of each compound is different, they all have some peaks in common, such as the 15, 29 and 43 peaks. In order to identify these compounds in a mixture, it is necessary to be able to see enough of the spectrum to pick out the more characteristic, but less intense molecular ion peaks. However, if the concentration of one component in a mixture is much larger than any of the others, the smaller but more characteristic ion peaks of the compounds in lesser concentration may not appear at all in the spectrum of the mixture.
The graph in the top portion of the slide represents the major peaks one obtains on a typical mass spectrum of the vapor in equilibrium with a sample of the total condensate. The two main peaks are 18 and 44 due to water and carbon dioxide respectively. One also finds small 29 and 43 peaks. However, these do not show the presence of any particular compound, e.g., CH₃CHO, since several other compounds have the same peaks in common. Any contribution to the 44 peak by acetaldehyde is masked by CO₂. The graph in the lower portion shows a typical mass spectrum of one of the fractions separated by low temperature-high vacuum distillation from the center cut. The 29 and 43 peaks result from contributions from acetaldehyde, acetone and methyl ethyl ketone. However, the acetaldehyde can be identified from the 44 peak, since carbon dioxide is removed, and the acetone and methyl ethyl ketone from the 53 and 72 peaks respectively. One can check the validity of this identification since the total peak heights of 29 and 43 must be the sum of the contributions from each of these three compounds—calculated from the known ratio of the peak heights of 29 and 43 to the molecular ion peaks for each of the compounds.

It can also be seen that the peaks at 47 and 48 due to methyl mercaptan and at 61 and 62 due to dimethyl sulfide appear in the mass spectrum of the -140 to -120°C fraction. This is because these compounds have been concentrated in this fraction and the dilution effect of water and carbon dioxide does not obscure them as it did in the total condensate spectrum.

The low-temperature, high-vacuum distillation method of separation, using, as we have, a bulb-to-bulb technique without a fractionating column, does not give clean-cut separations. It is very useful, however, because it enables one to simplify the mixtures which must be analyzed by mass spectrometry.

Recently we have developed a method of obtaining more clean-cut fractions by carrying out the low-temperature distillation directly on the inlet system of the mass spectrometer. I shall refrain from discussing this in detail in favor of some still newer developments which are probably more significant.

Although we have analyzed a fairly large number of meat center cuts, and have obtained some rewarding results, we have always been disturbed by the fact that odorous compounds remained unseparated and unidentified in both the water and the carbon dioxide fractions. We have finally, I think, discovered a way to obtain spectra from these fractions which will lead to the identification of the compounds present.

Slide 14 is a photograph of a vacuum distillation setup, similar to our more conventional type, except that the receiver flask contains a quantity of No. 5A molecular sieves which have been heated and evacuated. A portion of the equilibrium vapor over a water fraction is allowed to equilibrate with the sieves and the flask with the sieves is then transferred to the spectrometer inlet system. Spectra may now be obtained of the vapor in equilibrium with the sieves and with the vapors that may be distilled from the sieves by heating.
COMPARATIVE HI-MASS SPECTRA
OF A WATER FRACTION

DISTILLED FROM SIEVES

SAMPED DIRECTLY

MASS/CHARGE RATIO
Slide 15 shows a comparison of the spectra obtained in the high molecular weight region of the same water fraction sampled directly and distilled from the molecular sieves. The gain in sensitivity is apparent. The low molecular weight region spectra show the effect even more strikingly, but unfortunately I have no slide available to show. However, the water peak at 18 is found to be off scale more than 10,000 divisions for the directly sampled water fraction whereas the 18 peak shows less than 50 divisions for the sample over molecular sieves.

We have been able to achieve similar results with carbon dioxide fractions using ascarite as an absorbing medium and sampling in the same way.
MASS SPECTRA OF THE
CARBON DIOXIDE
FRACTION

as collected

over Ascarite
In the upper spectrum shown here, we see peaks that can be attributed only to carbon dioxide. In the lower spectrum carbon dioxide is virtually absent as indicated by the absence of the 22 peak from the spectrum. From the peaks remaining several compounds can be identified. Actually shown here is only a part of the whole spectrum and in this particular sample, peaks were obtained as high as mass 70.

I should like to conclude by briefly describing the low voltage ionization technique.

Slide 17
Here we see several spectra of acetaldehyde obtained at various energies of the ionizing beam of electrons. In the lowest spectrum, at 10 V, no peaks are obtained because the energy of the electrons is below the ionization potential of the compound. At 11.0 V, a peak due only to the molecular ion is present. This is because the loss of one electron is the lowest energy ionization process which can occur in a molecule. More drastic ruptures occur at higher energies. At 11.5 V, a hydrogen atom is cleaved producing a mass 43 ion and at 14.0 V, the methyl is cleaved from the aldehyde giving a 29 and a 15, not shown. Finally at 70 V, the voltage normally used, the familiar spectrum is obtained. It should be apparent that low voltage spectra should greatly simplify the interpretation of spectra of complex mixtures.
SLIDE 18
MASS SPECTRA OF A COFFEE
AROMA FRACTION AT HIGH
AND LOW VOLTAGE

MASS/CHARGE RATIO

32 44 58 60 62 68 72 76 82

CH₃CHO
CH₃OH
C₂H₅CHO
HCOOCH₃
CH₃CO₂H₅
CS₂
CH₃SCH₃
Unfortunately, I have no slide showing this behavior for a meat sample, but this slide of the comparative high and low voltage spectra obtained from a coffee aroma center cut fraction illustrates the point quite graphically. The upper spectrum is the complex spectrum obtained from a nine-component mixture at 70 V, whereas the lower spectrum of the same mixture at 12.0 V shows only the molecular ion peaks of the compounds.

In conclusion, I should perhaps apologize for not presenting some results of the applications we have made of these techniques, but the truth of the situation is that we have spent so much time developing these methods that there is now a lot of work we have to do to find out which compounds we identify are responsible for meat flavor. I hope that on another occasion I can give you this side of the story.

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CHAIRMAN PEARSON: I want to thank you, Dr. Merritt, for coming here and being with us this afternoon and presenting some of your work on methodology. We certainly appreciate this.

The next part of our program deals with a discussion of some flavor problems and analytical procedures. This is a little bit in opposition to the program as scheduled. The head of our Food Science Department of the State University will lead the discussion for the remainder of the program.

MR. SCHWEIGERT: Thank you, Al. Al is really a good man. He is going to be a full Professor on July 1st.

If you think that controlled discussion next door was good, wait until you hear what we have to offer. We instead of having blanket participation are going to lead off with some more heavy water on a very excellent program that Dr. Pearson has planned. I will reserve my comments until these controlled speeches are presented.

First, I would like to call on Dr. Ramsey of the University of Kentucky, who will give a few thoughts with reference to mutton flavor. Dr. Ramsey.

DR. RAMSEY: Mr. Chairman, fellow members of the Conference and friends:

I am about to start off by saying I am no longer with the University of Kentucky. Dr. Kemp turned me loose and I am now with the University of Tennessee. I took the position vacated by Dr. Lawrence.

As you have heard, my talk concerns mutton flavor and I have had quite a bit of kidding today because I was giving this talk. I haven't figured out yet why I got it. I think Dr. Pearson couldn't find anybody else to take it, is the reason I got it. And one of my friends went so far as to change my first name from Boyd to "Matton."

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