Future progress in the field of meat technology will become increasingly dependent on physical or physiochemical technics and this is particularly true for the investigations of flavors and odors or similar problems. The purpose of this paper is to give a broad view of the potential utilization of electrophoresis and infrared spectroscopy to the studies of flavor and odor molecules from meat and meat products.

Three important steps are involved in the study and identification of flavor and odor compounds. These are: 1) concentration, 2) separation or fractionation and 3) identification. Electrophoresis as a tool in the study of flavor molecules is largely a method of separating flavor compounds or their precursors. Infrared spectroscopy, on the other hand, is primarily a physical procedure which can be used to determine molecular structure and for chemical group analysis and best results are usually obtained with purified compounds.

Electrophoresis, or as it was once called, cataphoresis, involves movement of a solid phase in respect to a liquid under the influence of an external electrical field. It is simply the migration of charged particles in a fluid between two electrical poles. Many different technics have been used to separate particles from mixtures according to their electrical properties, but the ones concerning us here are moving boundary electrophoresis and zone electrophoresis.

In the moving boundary method (slide 1) the experimenter measures the movement of the boundary of a mass of particles. The material to be studied is poured into the bottom of a U-tube; on top of this is placed an electrolyte (buffer) solution and this is laid on carefully so that there is a sharp boundary between the two solutions. An electric current is then passed through the solution by means of a positive electrode inserted in the top of one arm of the tube and a negative electrode in the other. The object is to study the movement of boundaries under the urge of electricity. Most developments in this field were primarily due to the efforts of Arne Wilhelm Kaurin Tiselius, whose technics employed in measuring the moving boundary are so refined and important that most modern types of moving boundary equipment are commonly called the Tiselius apparatus. A photograph of the Tiselius-Longsworth electrophoresis cell is shown in slide 2.

Both solid and liquid anticovectants are used in zone electrophoresis to stabilize solutes. This expedient makes it possible to separate the individual components of a colloid mixture completely without incurring
the risk of convection and hence of boundary instability. By far the most frequently used solid carrier is filter paper, although other solids as starch, silica gel, sand, powdered glass, etc. have also been used. Numerous technics and arrangements have been used in paper electrophoresis and many instruments are available. One type which may be of extreme importance to the separation and study of meat components is the continuous paper electrophoresis of Durrum (slide 3).

This is one of the most promising two-dimensional methods developed during the last few years. The solution of the materials to be separated is applied to a suitable location on a vertical paper sheet in the form of a slow trickle. A uniform stream of buffer solution is slowly allowed to flow down the sheet or curtain at the same time. An electric field is applied to the system at right angles to the direction of buffer flow. As the solute particles enter the electrical field they are deflected from their original vertical direction depending on their own charge and other environment conditions. For preparation purposes, individual fractions are collected for further analysis.

Still another approach to the separation of relatively large quantities of material is the use of packed columns from which zones are eluted after separation under a potential gradient. Such a column is depicted in the next slide (slide 4). The cellulose column is contained in a water-jacketed pyrex tube resting on a perforated teflon disk. The lower end of the column is immersed in an electrode vessel filled with electrolyte solution. Contact with the other electrode vessel is obtained as shown in the figures.

Electrophoresis has been widely used in the separation of components which might be of importance to the study of flavors and odors in meats. These include many naturally occurring constituents as well as their degradation and recombination products. Examples are proteins, peptides, amino acids, nucleic acids, choline derivatives, tristearin and similar esters, fatty acids, amino compounds, certain carbohydrates and sterols.

Certain of the free amino acids have characteristic flavors which are important as well as certain of their degradation products as amines, aldehydes, amides and fatty acids. Some of these can be separated and studied by the various types of electrophoresis. Many other components of meat such as various sugars carry no charge on their surface and remain stationary in an electric field and it is therefore useless to attempt to separate them unless charged complexes can be formed.

Probably the most important use of electrophoresis in the study of meat flavors and odors will be as an aid in the elucidation of the biological mechanisms by which flavors are generated. Undoubtedly, some of the flavor compounds in meats are produced from flavor-precursors by the catalytic effect of enzymes. Numerous autolytic enzymes are present in meat which are constantly catalyzing chemical changes which may be important to the production of flavor and odor compounds. Bacterial proteases, peptidases, lipases, decarboxylases and deaminases may also produce characteristic flavors during the aging and storage of meat products.
Electrophoresis can serve to help purify these proteinaceous catalysts and determine their purity so their importance in the formation of flavor from various precursors can be determined. It may be possible to concentrate and purify these enzymes so that they might be directly added to meat to enhance production of desirable flavors.

An early use of electrophoresis to purify a flavor compound was that of Ikeda and S. Suzuki, who obtained a patent for "a method of making a nutritional and flavoring substance". These investigators hydrolyzed vegetable protein with acid and after removing the excess acid electrolyzed the amino acid mixture in a three compartment cell. The material collecting at the anode compartment proved to have a very desirable flavor when added to meats and other foods; it was sodium glutamate.

The use of electrophoresis as a fractionation procedure along with other methods of fractionation such as chromatography, counter-current distribution, preferential solvent extraction, sublimation, fractional crystallization, molecular distillation, dialysis, centrifugation and freeze drying have permitted the examination of the infrared absorption characteristics of almost every essential group of compounds found in meat products. These include nucleic acids, purines, and pyrimidines, proteins, polypeptides, amino acids, lipides, fatty acids, carbohydrates, porphyrins and vitamins.

The infrared region as usually defined, lies between the visible and the radio portion of the spectrum, i.e., wavelengths between 0.001 mm to 1 mm. The range most useful in the studies of biological materials is between 0.0025 mm and 0.015 mm. The conventional frequency unit in infrared spectroscopy is the wave number (cm\(^{-1}\)), which expresses the number of waves in 1 cm. For example 0.01 mm (wave length) is equivalent to 1000 cm\(^{-1}\) (waves per centimeter, wave number).

When infrared radiation is passed through a compound, the compound characterizes itself by the wavelength it absorbs and those it transmits. The bonds within the compound have a natural frequency of vibration determined by the masses of the two atoms held by the bond and the restoring forces of the bonds. A light wave with this frequency of oscillation will have most effect on the bond, its energy will greatly increase the natural vibration of the atoms. The molecule will absorb part of the energy of the light at this resonant frequency and an absorption detector will show an absorption peak for that wavelength. The infrared spectrum is simply a display of the resonant frequencies of the sample, but not all resonant frequencies give rise to absorption.

In general there are enough active resonant frequencies in a molecule so that the infrared spectrum is characteristic of the atom, the bonds and the geometrical arrangement of the molecule--and here we see the reason for the great value of infrared, its specificity. The infrared spectrum is the most nearly unique property of a substance. Even geometrical isomers, which have the same bonds but differ in arrangement can be distinguished by infrared.
An example of the type of information given by an infrared spectrum is shown on the next two slides (slide 5 and 6), which is the spectrum for acrylonitrile. The curve represents the amount of energy transmitted by a sample of the material as the wavelength increases (from left to right). Dips indicate frequency at which energy is strongly absorbed, showing that a natural frequency of molecular vibration has been reached. Vibration causes minima as indicated.

In the study of flavor compounds from meat we are interested in using infrared spectroscopy primarily as a means to establish the identity of organic substances. The only serious disadvantage of infrared analysis is that the use of water as a solvent is generally not desirable because of its intense absorption over much of the working range. One great advantage is that liquids, solids or gases can be examined equally well by these techniques and usually on a few milligrams of sample, or with care with a few micrograms. This method of measurement alters the sample being measured very slightly and temporarily; samples may be recovered unchanged.

The general line of attack is to compare the spectrum of the compound whose structure is unknown with the spectra of compounds of known structure containing groups of atoms identical with and in a similar environment to those suspected of occurring in the unknown. Extensive collections of infrared spectra have now been compiled by various laboratories and organizations, and the comparison can be made relatively rapidly by various mechanized sorting devices. This, plus the fact that three of four spectra an hour from 5000 to 625 cm\(^{-1}\) may be obtained with present commercial instruments makes infrared a rapid and convenient way to scan fractions of unknowns from meat extracts.

One good illustration of the use of infrared as a means of identifying flavor compounds was the work of Jackson and Morgan who identified the substance causing a malty aroma in milk--cultures of *Streptococcus Lactis Var. Maltigenes*. After isolating neutral carbonyls as their phenylhydrazones these investigators separated them by chromatography and analyzed the individual derivatives by infrared. Slide 7 is a comparison of one of the unknown phenylhydrazones with 2-methylbutanal and 3-methylbutanal. It was concluded that the unknown odoriferous compound was 3-methylbutanal.

Further studies by these workers on this problem showed that the organism involved or enzymes produced by this organism could convert leucine and isoleucine to their corresponding pentanals.

Another example of a degradation product of amino acids which has been identified as a flavor compound producing a broth-like flavor is methional (3-methylthiopropanal). This compound has been identified in milk by infrared as its phenylhydrazone after purification by gas and paper chromatography. It is formed by oxidative deamination through a Strecker degradation from methionine. The widespread occurrence of methionine and the fact that light, heat and bacteria are all capable of inducing Strecker-like degradations of amino acids to flavorful aldehydes indicates the importance of this general pathway to flavors in meat and meat products.
A further example of the use of infrared in the study of flavor extracts, this time from a product close to the hearts of all present, is that of Hornstein (private communication), who compared the infrared spectra of volatile fractions of aqueous extracts from beef and pork. Both fractions had a "meaty"-type odor and produced practically the same infrared spectrum (slide 8). There apparently was no qualitative difference in the fractions involved.

Further fractionation of these volatiles by chromatography yielded two very similar, but different compounds as determined by their infrared spectra (slide 9).

Numerous other workers, particularly in the dairy and horticulture fields have used infrared spectroscopy as an aid in identifying flavor components. Many of these compounds were produced from molecules common to meat and certainly techniques utilized by these investigators will require special scrutiny on the part of workers interested in flavors from meat.

In conclusion I might say that the use of the various forms of electrophoresis may be somewhat limited to most approaches to flavor and odor studies of meat but should be extremely useful as a means of fractionating many flavor precursors in meats; infrared, supplemented by other forms of spectroscopy and the classical methods of organic analysis will be an important phase in the final meat flavor structural analysis by those fortunate enough to have it available. The infrared spectrum is certainly not a magic crystal ball in which one reads the structural formula of an unknown compound but through its use and those techniques offered by electrophoresis we can shorten tremendously the time required to complete certain jobs on problems concerning meat flavors.

SELECTED BIBLIOGRAPHY


---

CHAIRMAN PEARSON: Thank you, Milton.

Our next topic this afternoon deals with Paper Chromatography. This paper will be handled by Dr. Wendell A. Landmann of the American Meat Institute Foundation.

# # # # # # # # #