ISOLATION OR IDENTIFICATION OF COMPONENTS RESPONSIBLE FOR MEAT FLAVOR: Paper Chromatography

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Because of time limitations and the familiarity of this audience with the subject of paper chromatography, it is hardly necessary to go into any detailed discussion of this technique. However, I should like to give a general background of the methods commonly used, and illustrate their application to meat flavor research.

Paper chromatography consists of the fractionation or separation of a mixture of solutes on filter paper through a process of adsorption and desorption mediated by a solvent which is passed through the paper. Fractionation occurs as a result of counter-current distribution between the solvent and the stationary water phase held by the cellulose fibers, or as a result of the attractive forces between cellulose and the solutes, or most likely, as a combination of both. The solvent and paper on which a spot of the sample has been placed are equilibrated in a tightly sealed chamber--in other words, allowed to stand until the solvent vapors, water vapor, and paper have reached equilibrium. A supply of solvent is then introduced and allowed to flow across the paper in ascending or descending fashion by capillary attraction. Two dimensional chromatography can be carried out after the first run by turning the paper ninety degrees and allowing a second solvent to migrate in this new direction.

Preparative chromatography is usually carried out on thick filter paper, such as Whatman 3 MM. A two to ten-fold increase in sample size per spot can be achieved in this way without a loss in resolving power. Since single dimension chromatography is best for this purpose, it is possible to streak a sample or place numerous spots across the line of origin over the full width of the paper. After development of the chromatogram, narrow strips are removed from both edges of the sheet and treated with the detection re-agent to locate the spots. The remainder of the paper can be cut into strips at these positions, and the material eluted. These purified compounds can then be subjected to other chemical tests or re-chromatographed in another system for identification.

Quantitative paper chromatography is often carried out by preparing such eluates, which are subjected to colorimetric analysis. It is also possible to compare the color density of the spots on the paper with a series of spots of standard or known concentration by means of a photoelectric densitometer.

A wide choice of solvent systems is available for all classes of compounds. Means of detection of the spots range from fluorescence under ultraviolet light, to radiation counters or films, to chemicals, including

concentrated sulfuric acid. Almost any colorimetric reaction can be adapted as a spray or dipping solution for paper chromatograms.

The paper itself is available in different densities, which offers a range of speeds, capacities, and resolving ability. The paper may further be modified by impregnating it with silicone, silicic acid, organic solvents or buffers. Glass fiber "paper" is also available, and has found application in the chromatography of unreactive organic compounds which can be detected only by charring with concentrated sulfuric acid.

Paper chromatography also has ramifications in paper electrophoresis and electro-chromatography. These techniques are frequently used to supplement the regular technique. Thus paper chromatography can be seen as an extremely versatile tool for identification or isolation work.

Limitations, however, do exist. Volatile substances cannot be determined except as non-volatile derivatives. Many systems will not give satisfactory results unless preliminary purification or desalting procedures are employed. This, however, can usually be accomplished by the use of chromatographic columns or ion-exchange resins. The most serious limitation, perhaps, is that sample sizes are necessarily small, thus limiting the amount of material which can be isolated. The small sample size, on the other hand, is a distinct advantage for identification work where often only a small quantity of material is available.

All these techniques are brought to bear in meat flavor research. Early work in this field was concerned primarily with volatile components of flavor, or off-flavor, particularly in irradiated or cooked meat (1,2,3). The substances involved were found to consist of amines, hydrogen sulfide, mercaptans, carbon dioxide, and carbonyl compounds. Paper chromatographic identification was for a time limited to the carbonyls, which could be readily converted to the 2,4-dinitrophenylhydrazones and chromatographed as such (4). The amines have only recently been chromatographed as their hydrochloride derivatives (5,6,7). Acetaldehyde and acetone were found as the main constituents of volatile carbonyls, while methyl amine, ethyl amine, and ammonia comprised the greater part of the volatile bases. As far as methods for the identification of volatile constituents of flavor are concerned, paper chromatography has been superseded by the more sensitive direct determinations with gas chromatography.

Paper chromatography is of greatest value in studies of the water soluble non-volatile constituents of meat extract, particularly when used in combination with other isolation procedures as dialysis, electrodialysis, and ion-exchange or column chromatography. The most complete work of this type has been carried out by Wood, Bender and Palgrave (8,9), in their work with commercial meat and fresh muscle extracts. The qualitative and quantitative composition of these extracts was determined almost entirely by the use of paper chromatography after prior separations into more or less homogeneous groups of compounds. These fractions were obtained by the methods mentioned previously, and consisted of the following classes of compounds: inorganic ions, acidic, neutral, and basic organic compounds, and proteins. By extensive chromatography in several systems, and comparison with chromatograms of known compounds, Bender, et al, were able to identify some thirty or more compounds in each of the extracts.

More recent studies of irradiation flavor and meat flavor have been concerned with the identification of the precursors of the odor or flavor constituents. In principle the methods consist of the isolation of the odor - or flavor-bearing fractions, followed by paper chromatographic separation and identification of components. For example, one of the precursors of an irradiation off-flavor has been found in a phospholipid fraction of irradiated meat. (10). This fraction was obtained by solvent extraction of the meat, and was subjected to a preliminary purification by chromatography on a silicic acid column. The odor-containing fraction from this column was then examined by the paper chromatography on paper impregnated with silicic acid. Several known phospholipids and associated degradation products were identified, although several unidentified compounds were present as well. By elution of these materials from the paper, it was possible to collect small quantities for further studies. In cases where solvents which leave no residual odor in the paper could be used, it was possible to test the odor of the individual spots directly on the paper.

Very recent developments in the field of paper chromatography will undoubtedly give an added impetus to its use in flavor research. Papers loaded with ion-exchange resins have been developed and have just begun to be studied.(11) These would combine the effects of ion-exchange columns with paper chromatography. One of the more obvious techniques for their use at present is to employ, in the first direction, an aqueous buffer system to take advantage of the ion-exchange or desalting capacity of the resin paper. This could be followed in the second direction with an organic system designed to suppress the ion-exchange effects and allow the partitioning effects to occur.

Modified cellulose has in recent years found extensive use in the column chromatography of proteins. Phenomenal separations have been achieved, even with large proteins. Modified cellulose papers are now also available, (12, 13) which should make possible the paper chromatography of proteins without the difficulties encountered heretofore.

Considering the wide range of problems which arise in meat flavor research, a promising new tool as this should indeed be investigated thoroughly, and should be added to the list of those already commonplace in flavor laboratories.

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Dr. LANDMANN: Thank you, Dr. Pearson.

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CHAIRMAN PEARSON: We want to thank Dr. Landmann for his excellent discussion on Paper Chromatography.

We will now continue the program by discussing Gas Chromatography. And this will be handled by Irwin Hornstein of the U. S. Department of Agriculture. Dr. Hornstein.

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