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FLAVOR CHEMISTRY

Constituents of Meat Flavor: Beef

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The flavor precursors of cooked beef are water-soluble. Lyophilization of a water extract yields a powder concentrate that on heating develops a flavor similar to that of cooked beef. A standard technique for the heat treatment and fractionation of the flavor constituents in this powder is described. Small amounts of carbonyls, ammonia, and hydrogen sulfide have been found in the most volatile fraction. An oily, viscous, liquid mixture of very low vapor pressure, but with a strong aroma, has also been isolated.

ALTHOUGH AN UNDERSTANDING of the chemical composition of meat flavor is basic to many aspects of meat technology, an actual knowledge of meat flavor constituents is nearly nonexistent. Bouthilet (*1*, *2*) and Pippen and coworkers (*10*, *11*) studied the flavors of chicken broth and separated and identified several of the volatile constituents of chicken broth distillate. Classical studies of beef flavor chemistry (*9*) dealt largely with the analysis of beef extract, a product which is formed by the subsequent concentration of beef tissue hydrolyzates and is thus different from beef per se.

The present investigation was undertaken as part of a program on fundamental studies of meat composition. The flavor aspects of this program are expected to result eventually in identifying the naturally occurring substances which are responsible for flavor in beef, pork, and lamb, and in various products made from these meats. This paper deals with the methods used to fractionate fresh beef into potentially flavorful and nonflavored portions and with the preliminary chemical investigation of some of the isolated products.

A beef powder extract separated from raw beef has been heated under vacuum and the total volatiles have been condensed at low temperature. This condensate, in turn, has been analyzed for carbonyls, and the most volatile fraction has been examined for acidic and basic components.

Experimental

Freeze-Dried Beef Powder Extract. Fresh meat was aged for 10 days at 36° to 38° F. Several of the muscles were then dissected and stored at 0° F. As needed, 1.5 kg. of meat was thawed, fat and connective tissue were removed, and the trimmed beef was ground in an electric grinder maintained at 32° F. One part by weight of the ground beef was blended for 1 minute with 1.5 parts by volume of ice-cold, distilled water. The slurry was allowed to stand overnight at 32° F., blended again for 1 minute, and centrifuged at 4000 r.p.m. for 20 minutes in a refrigerated centrifuge kept at 28° F. The supernatant liquid was decanted, mixed with 1% w./v. of Filter-aid, and filtered under vacuum through a Büchner funnel. The filtrate was shell-frozen in a dry ice–isopropyl alcohol bath and lyophilized. The yield of dry powder was approximately 3.5% of the weight of the trimmed meat.

Distillation and Fractionation. A vacuum of better than 10^{-5} mm. of mercury was maintained by means of a two-stage oil diffusion pump backed by a mechanical vacuum pump. The fractionation train consisted of three fraction collectors. The vacuum system is shown schematically in Figure 1. Thirty to 35 grams of dried powder were placed in flask *a*. Vapor from a boiling liquid in flask *b* rose through the jacket, *c*, surrounding *a* and returned to *b* by the condenser. An auxiliary heating tape was wrapped around the jacket to ensure that vapor condensation did not take place around *a*. By the appropriate choice of liquid any desired temperature could be maintained.

In a given experiment, traces of moisture were first removed from the dried powder by evacuating the system ($<10^{-5}$ mm. of mercury) at room temperature for 4 hours. A Dewar flask containing liquid nitrogen was then placed around trap *d*. Maintaining the same vacuum, the solvent in *b* was brought to a boil and heating was continued for 6 hours. At the end of the heating period the vacuum stopcocks at *h* and *i* were turned off, the liquid nitrogen trap was removed from *d* and placed around trap *g*, and a dry ice–isopropyl alcohol freezing mixture placed around trap *e*.

Spontaneous distillation took place as the contents of trap *d* came to room

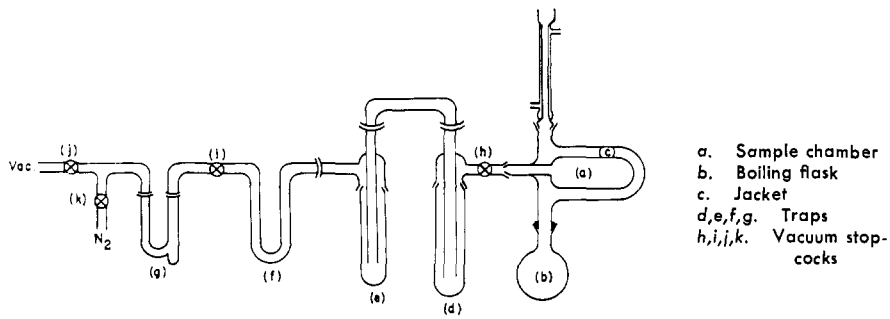

Figure 1. Vacuum system for fractionation of volatile flavor components

temperature. After 16 hours, dry nitrogen was admitted into the system.

A series of tests was first conducted to select an optimum operating temperature. At room temperature no odor products were trapped out in liquid nitrogen at 10^{-5} mm. of mercury. At 78° C. (ethyl alcohol) the over-all odor profile was essentially pleasant and fruitlike. At 100° C. (water) a similar, but more accentuated odor was noted. At 157° C. (hexyl alcohol) the predominant odor pattern was ammoniacal and unpleasant. At 188° C. (propylene glycol) the over-all odor impression was strongly ammoniacal and decidedly unpleasant; in addition, yellow crystals, not further investigated, sublimed to the top of the beef powder. At higher temperatures (200° C.) massive decomposition took place and odors characteristic of burned meat were obtained. As a result of these observations an operating temperature of 100° C. was selected and adhered to in all further experiments.

Chemical Identifications. Carbonyls in *d*, *e*, and *g* were converted to their respective 2,4-dinitrophenylhydrazones by the addition of a 2*N* hydrochloric acid solution saturated with 2,4-dinitrophenylhydrazine. In a separate experiment the acidic compounds in the most volatile fraction—trap *g*—were converted to their ammonium salts by the addition of 5 ml. of 5% ammonium hydroxide, and, in another run, basic compounds were converted to their hydrochlorides by the addition of 5 ml. of 2*N* hydrochloric acid. The residue in trap *d* consisted of a very small amount of a colorless viscous liquid, which was water-soluble and of a pleasant fruity odor. On standing exposed to air, this liquid slowly darkened and assumed a desirable meaty aroma. This material has been separated into at least two constituents by ascending paper chromatography using butyl alcohol saturated with water as the solvent. Two spots were detected by spraying the dried paper with aqueous potassium permanganate. Identification of the compounds in this mixture is under way at present.

Determination of Carbonyls. Carbonyls were identified by the paper chromatographic technique of Gaddis and Ellis (*5*, *6*). The combined phenylhydrazones were extracted from the aqueous phase with carbon tetrachloride. The solution was evaporated almost to dryness and the residue washed with carbon tetrachloride onto a column of chromatographic alumina. The chromatogram was developed with benzene and a monocarbonyl fraction eluted. The benzene was removed by distillation and the residue taken up in a minimum of carbon tetrachloride spotted on No. 3 Whatman paper. The chromatogram was developed with petroleum ether (boiling point 37° C.). Two spots appeared; these were rechromatographed on paper treated with propylene glycol using Skellysolve C (essentially heptane) as the mobile phase. The R_F values for these spots corresponded to those for authentic samples of acetone, acetaldehyde, and formaldehyde. Less than 0.05 mg. of each of these carbonyls was recovered for each gram of dried beef powder heated.

Analysis of Acid Volatiles. A portion of the fraction trapped in ammonium hydroxide on acidification evolved a gas, the major constituent of which was carbon dioxide. The remainder of the fraction was analyzed for free sulfide ion. The analytical procedure was essentially the standard reaction between hydrogen sulfide and *N*,*N*-dimethyl-*p*-phenylenediamine to form methylene blue. Recent modifications of this procedure by Marbach and Doty (*8*) and by Sastry (*12*) have been used to estimate sulfides released after zinc reduction from gamma irradiated beef and from plant materials. In the present procedure, zinc reduction was omitted, and free sulfide (H_2S) was determined on a 1-ml. aliquot of the total acid fraction, trapped in ammonium hydroxide.

Absorption was measured at 667 mμ, using a Beckman DU spectrophotometer. The actual concentration of hydrogen sulfide could be read from a standard curve prepared from known concentrations of sulfide ion obtained from sodium sulfide. Approximately 0.1 mg. of hydrogen sulfide was recovered per gram of dried beef powder.

Analysis of Basic Volatiles. The entire volatile fraction trapped in 2*N* hydrochloric acid was lyophilized to yield approximately 150 mg. of a white crystalline salt. The addition of one drop of sodium hydroxide to a few crystals of this material gave the characteristic odor of ammonia. To determine whether or not any other hydrochlorides, presumably of low boiling aliphatic primary amines, were present, 1 mg. of the salt was spotted on No. 1 Whatman paper and the chromatogram was developed with methanol. Spots were detected by spraying with an indicator similar to the one used by Davies, Wolfe, and Perry (*4*) to detect primary amines. The indicator contained 0.5% ninhydrin in 70% ethyl alcohol to which 20% pyridine v./v. had been added. Ammonium chloride did not give a spot with this indicator. However, known mixtures of ammonium chloride containing 1% of the primary methyl- and ethylamine hydrochlorides gave characteristic purple spots.

A very faint purple spot of the R_F for methylamine hydrochloride was obtained for the unknown. This spot was far less intense than the corresponding spot for the control containing 1% of the primary amines. When similar chromatograms of the unknown were sprayed with methyl orange as an indicator, an acid spot with the R_F value corresponding to ammonium chloride was obtained.

Assuming that the salt was essentially ammonium chloride, approximately 1.7 mg. of ammonia were obtained from each gram of dried beef powder heated.

Reproducibility of Results. Most of this work was done using the *longissimus dorsi* muscle. To check the reproducibility of the results from animal to animal and also from muscle to muscle, the infrared spectra of the least volatile, and, by coincidence, the most appetizing, odor constituent were obtained for several of the batches run. These spectra were similar in nature, and the general odor characteristics were identical, an indication that good reproducibility could be achieved in experimental work by the use of the isolation techniques described.

Results and Discussion

The distribution of flavors and their precursors between beef solids and juices has been studied by Crocker (*3*), and more recently by Kramlich and Pearson (*7*); their results indicated that flavor contributors were water-soluble. Preliminary experiments confirmed these findings. For example, hamburgers prepared from water-extracted ground beef were essentially tasteless and odorless; the water extract, on the other hand,

developed a "beef" aroma when heated. Freeze-drying of the unheated extract yielded a water-soluble, friable powder. This dried powder, when heated, developed an odor reminiscent of roast beef; a water solution of the powder, upon heating, evolved a boiled beef aroma. As the extraction and lyophilization procedure concentrated the flavor precursors and simultaneously simplified the system under study by the removal of fat, water, and water-insoluble matter, this concentrate has been the starting point for this work, with the realization, however, that under usual cooking conditions where fat and moisture are present, the isolated products may differ in some respects from those obtained in the present study.

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MEAT AGING AND FREEZING

Post-Mortem Changes in the Water-Soluble Proteins of Bovine Skeletal Muscle during Aging and Freezing

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Alteration of the water soluble proteins of bovine skeletal muscle is of particular interest, because these proteins have enzymatic character and are involved in biochemical processes occurring in meat. The effect of aging meat for 7 days and of freezing at −20° C. for 5 weeks was studied by electrophoresis and ultracentrifugation, as well as by several chemical methods. Systematic differences in protein content and in the content of the enzyme aldolase were noted from muscle to muscle. Both aging and freezing of meat were shown to result in a decreased extractability of water soluble proteins, as well as in a loss of specific electrophoretic and ultracentrifugal components.

POST-MORTEM CHANGES occurring in meat are known to be rather profound. Without doubt, a number of biochemical changes identified with alterations in tissue properties are associated with the salt extractable, structural proteins such as actin and myosin. On the other hand, many processes are controlled by enzymes found in the water-soluble myogen fraction—e.g., lactic acid production. While such protein enzymes may comprise only a small weight percentage of meat, the significance of the reactions that they catalyze may render their alteration during aging or freezing of tissue of the greatest importance.

The effect of aging and freezing on the extractability of the enzyme aldolase and the other water-soluble proteins has been studied. These data, as well as more specific information as to the alteration of the water soluble proteins by ultracentrifugal and electrophoretic analysis of muscle extracts and subfractions derived from these extracts by fractional salt precipitation, are presented.

Experimental

Materials and Methods. Details of analytical and preparative methods have been described previously (*8*).

Four animals were used in this study. Animal 6 was a 5-year-old cow, while animals 7, 8, and 9 were 18-month-old steers of choice grade. Muscle dissected from the left side of the animal approximately 20 minutes post mortem—fresh muscle—was compared with muscle taken from the right side of the same animal held at 3° C. for 7 days. For the sake of brevity the latter muscle has been referred to as aged. Samples of the fresh muscle, which had been chilled in ice for transportation to the laboratory, were frozen in a deep freeze unit about 3 hours post mortem. Frozen muscle was stored at −20° C. for 5 weeks after which it was thawed for about 24 hours at about 5° C. prior to extraction. Aqueous extracts were prepared in the manner described previously (*8*).

In the case of animal 6, an average muscle sample was taken over much of its length. In the case of animals 7, 8, and 9, sections were made according to published diagrams (*4*) and individual muscles were then removed from the proper section (Table I). Shown in the remaining columns of Table I are the locations at which sections were made, the illustration plate number of the reference cited, and the commercial cut of meat corresponding to this section.

Moving Boundary Electrophoresis and Ultracentrifuge Measurements. The methods and equipment employed have been described previously (*8*). Both types of measurements were carried out on protein solutions in pH 8.17, 0.05 ionic strength tris(*N*,*N*,*N*-hydroxymethyl) methylamine (Tris) buffer. Prior to measurement, solutions were centrifuged in the Spinco Model L at 60,000 × G in order to remove trace amounts of insoluble protein.

Because it was virtually impossible to carry out electrophoresis or ultracentrifuge measurements at identical concentrations, it was necessary to normalize the curves for purposes of calculation. The areas of projected traced