# IDENTIFICATION OF SOME CHEMICAL COMPONENTS IN CHICKEN FLAVOR

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ABSTRACT

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IN CHICKEN FLAVOR

by Lewis J. Minor

Some of the chemical components from cooked light and dark chicken tissue have been identified using gas, column, thin-layer and paper chromatography, UV and I-R absorption spectra, a trap reaction technique utilizing functional group analysis, and a solubility classification method.

Precursors and non-volatiles were studied by chemical methods. The protein, fat, moisture and ash contents were determined for the raw muscle and cooked-freeze-dried slurry from light and dark meat for three classes of birds. Concentrations of inosinic acid, creatine/creatinine, diacetyl/acetoin, sulfhydryl compounds, inorganic sulfides, cystine and methionine, and pH values were also determined for these samples. Yield studies gave average values of 52.7% for raw meat and 36.3% for cooked meat from roasters; 48.8 and 33.5%, from heavy hens and 47.7 and 35.5% from light hens, respectively. Organoleptic studies showed that after 50 hrs. cooking-distillation at 180°F, broth from heavy hens had better flavor than that from roasters or light hens. Broth from light muscle had typical chicken flavor, while broth from dark muscle tended to resemble beef broth in flavor. After 50 hrs. cooking-distillation, the pH of the heavy hen breast meat slurry increased from 5.8 to 6.2 and that from the heavy hen leg meat slurry increased from 6.1 to 6.3 indicating that acidic and neutral constituents were distilled off. Some basic constituents (possibly sulfides and methylamine) were also distilled over

as evidenced by a change in the pH of the water trap (8.1-9.6) through which the volatiles were passed. The higher molecular weight acidic, neutral and basic compounds remained in the aqueous phase. Volatiles that were trapped in a liquid nitrogen medium (-196°C) had pH values of 8.0 or higher.

Gas chromatographic studies showed that 20 month old hens contained the same volatile components as 12 week old birds of identical origin that were fed the same ration, but some of the cooked volatile components from the older birds were present in higher concentrations. Chemical tests indicated that the intestinal contents from the heavy hens contained carbonyls, sulfides, disulfides and mercaptans, but in lesser amounts than for the meat from the same birds.

Gas chromatography of the volatile fraction released upon cooking fryer breasts resulted in identification of ethane, propane and carbon dioxide. Hydrogen sulfide and carbon dioxide were identified in the fraction distilling off at -140°F. Ammonia was identified chemically in the cooked volatile fraction. Odor tests indicated the presence of carbonyl sulfide, although further identification was not made.

A steam distillate from heavy hen breast muscle contained two phosphatidyl lipid components, which were tentatively identified as cardiolipin and either phosphatidic acid or phosphatidyl inositol. Identifications were made using thin-layer chromatography.

Volatiles derived by cooking-distillation of young birds in an oxygen containing atmosphere were trapped in 2,4-dinitrophenylhydrazine (2,4-DNP) and lead acetate solution, respectively. A total of nine carbonyls

were identified. Eight were monocarbonyls; namely, acetaldehyde, propanal, n-butanal, n-pentanal, n-hexanal, n-octanal, ethyl-methyl ketone and acetone. One was a polycarbonyl; namely, diacetyl. Results were in agreement with those of earlier workers. Sulfides, disulfides and mercaptans were identified by preparing their derivatives from the cooked volatiles and identifying the derivatives by acid decomposition followed by gas chromatography and chemical methods.

Model systems demonstrated the importance of glutathione as a precursor for the sulfur containing amino acids (specifically, cystine and cysteine) and of 2,3-butanedione as an active carbonyl in Strecker degradation. Copious amounts of hydrogen sulfide were evolved by this degradation which occurred in a warm aqueous solution. The product had a taste resembling chicken broth.

Some of the chemical components of heavy hen leg muscle volatiles that were identified by the functional group trapping technique included a total of 29 compounds from 30 peaks separated from the total cooked volatile fraction. These compounds were methyl mercaptan acetone, methanol, dimethyl sulfide, methyl-ethyl sulfide, methylamine, diethyl sulfide, ethanol, acetaldehyde, methyl-iso-propyl sulfide, 2,3-butanedione, methyl disulfide, acetoin, ethyl-n-propyl sulfide, ethyl disulfide, ethyl mercaptan, dipropyl sulfide, n-propyl mercaptan, n-hexanal, 2,4-pentanedione, iso-amyl alcohol, n-amyl alcohol, n-heptanal, ethanol-amine, n-hexanol, 2-heptanone, n-heptanol, ethane and propane.

By the same method using an Apiezon-L column, a flame-ionization detector and temperature programming from 100-250°C with an F and M model

500 gas chromatograph, a total of twenty-five peaks were obtained from heavy hen breast muscle. Compounds that were identified by means of the functional group analysis trapping technique were ethane, propane, acetone, methanol, dimethyl sulfide, methylamine, methyl formate, diethyl sulfide, ethanol, acetaldehyde, 2,3-butanedione, methyl disulfide, acetoin, n-pentanal, ethyl-n-propyl sulfide, iso-butanol, ethyl disulfide, n-butanol, dipropyl sulfide, n-propyl mercaptan, n-hexanal, 2,4-pentanedione, n-heptanal, 2-heptanone and n-heptanol.

The concentrations of acetoin and diacetyl in light and dark muscle samples were determined by steam distillation followed by a colorimetric procedure. Results confirmed reports of earlier workers. Inosinic acid determinations were made using a convenient UV absorption method at a wavelength of 250 mu. Results were obtained for the raw light and dark tissue samples from roasters, heavy- and light-weight hens.

Creatine/creatinine, cystine, methionine, sulfhydryl and inorganic sulfide determinations were made by colorimetric procedures on raw light and dark muscle samples from heavy hens, roasters and light hens, and also on the cooked-freeze-dried muscle slurries from these birds. In addition, cystine and methionine were determined by a microbiological method.

Several important flavor characteristics of light and dark chicken muscle and of young and old chickens were revealed by these studies. Young and old chickens had the same components in their respective volatile fractions, but certain constituents were present in higher concentrations from older birds. Using a model system, glutathione was decomposed in

the presence of moist heat and 2,3-butanedione. Glycine, glutamic acid and cystine were formed and copious amounts of hydrogen sulfide, ammonia and carbon dioxide were liberated. A chicken-like flavor was obtained by this decomposition. Both glutathione and 2,3-butanedione are contained in chicken muscle and were shown to be precursors of hydrogen sulfide, organic sulfides, disulfides and mercaptans. Sulfur esters may be important flavor constituents also, but their existence in chicken muscle samples was not verified. Light meat contained more inosinic acid, creatine/creatinine, and acetoin/diacetyl than dark meat. Inosinic acid enhanced chicken flavor. Acetoin/diacetyl imparted a cooked, buttery and oily note to flavor, which confirmed earlier reports. Creatine/creatinine were bitter but may have imparted desirable additive and/or synergistic flavor effects. Some steam-distillable phosphatidyl components, when freshly prepared, contained "chicken essence", but the desirable aroma disappeared in a few hours. Results indicated that the phosphatides may have acted as electrovalent bonding agents for the flavor volatiles. Sulfur compounds possessed a characteristic "meaty" aroma of greater importance to flavor than carbonyls. However, carbonyls may function at sub-threshold concentrations by exerting synergistic and additive flavor effects. Differences between flavor from light muscle versus dark muscle was not traceable to differences in the concentration of precursors. There may be a relationship between heme compounds and the characteristic flavors of the various red meats. Electropositive metallic bonds present in the iron of the heme moiety of myoglobin and hemoglobin could serve as binding media for electronegatively charged

flavor volatiles. It is certain that the removal of sulfur compounds by mercuric chloride and mercuric cyanide solutions resulted in an almost complete loss of "meaty" odor from the cooked volatiles of light or dark chicken meat.

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By

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# INTRODUCTION

For nearly two centuries scientists have studied the chemical composition of meat extracts searching for the key to meat flavor. Hydrolyzed vegetable proteins, casein digests and yeast autolysate extracts are preparations having characteristic flavors of their own, yet are compatible with certain meats or meat and vegetable mixtures. Monosodium glutamate, disodium guanylate and disodium inosinate have also proven useful in improving the flavor scores of some meat and vegetable mixtures (Caul and Raymond, 1963; Kurtzman and Sjöstrom, 1963; Toi et al., 1963), but none of these products or any known combination of them can replace meat flavor. Their primary functions are to provide additive or synergistic flavor improvement and to extend meat flavor. Of all the condiments, meat extract provides the truest resemblance to the mouth satisfaction indigenous to freshly prepared meat stock.

Relatively little is known of the many chemical reactions that occur on heating meat. The complexity of meat and its associated flavors are responsible for the difficulties encountered in elucidating the chemical basis of meat flavor. Despite the efforts that many workers have expended for more than one hundred and fifty years, the available information concerning meat flavor is at best fragmentary.

In addition to the continuing research on meat extract, elucidation of the volatile and non-volatile flavor components associated with beef, pork, mutton, chicken and fish has been undertaken. Scientists in many countries have contributed to the present status of our knowledge of meat

flavor. The natural resources of individual nations have played an important role in determining whether support and research emphasis should be given to investigations on fish, fowl, animal flavor or to the flavor of meat extract.

Fat flavor has been of special interest due to the large amounts of fat present in meat and the tendency of fat to undergo chemical changes, even at sub-freezing temperatures. Lipid oxidation in lean tissues has been reviewed by Watts (1961). Some components present in pork fat and associated with sex odor have been reported by Craig and Pearson (1959). Hofstrand and Jacobson (1960) stated that the presence or absence of fat did not affect the taste of mutton broth, but that water soluble compounds in depot fats volatilize and influence the aroma.

Off-flavors in meat caused by irradiation have been the subject for numerous investigations, as any value that this method for preserving meat may have is limited by palatability scores.

Development of new techniques and scientific instruments has made flavor research more objective. Recent developments in instrumentation provide both accuracy and sensitivity that supplements chemical and organoleptic testing procedures, which have been established and standardized. Physical methods have now become indispensable to the chemist or food scientist. Every modern laboratory has its spectrographic and chromatographic equipment. To these methods gas chromatography has been added, which is in a state of flux due to constant improvement in the sensitivity of instruments and new identification techniques. Enthusiasm is generated from the fact that gas chromatographic analysis, as it is

practiced today, not only achieves separation, but also identification and quantitative determination. By means of gas chromatography joined with other modern processes an automatic analysis is almost within reach. It may now be possible to achieve an automatic analysis of volatile constituents from cooked meat by passing the volatiles through a gas chromatograph to separate them, an infrared and/or ultraviolet spectrograph to identify molecular configuration, a time-in-flight mass spectrometer to determine molecular weight and nuclear magnetic resonance equipment to confirm the molecular structure of each compound.

Molecular separations of non-volatiles can now be made by dialysis through membranes of controlled porosity size, by elution through columns known as molecular sieves, and ion-exchange or neutral columns. Ion exchange paper is also available for rapid separation of nucleotides as reported by Smillie (1959). Thin layer chromatographic media and techniques are likewise undergoing rapid development. X-ray diffraction can be used to identify different compounds present in a single impure crystal. Complete protein analysis can be made in less than 24 hours. Histochemical techniques and enzyme research developments will undoubtedly assume important roles in developing the future knowledge concerning meat flavor and its precursors.

## EXPERIMENTAL OBJECTIVES

The overall experimental objective of this study was to elucidate the components responsible for chicken flavor; more specifically, the objectives were as follows:

1. To develop methods for separating and collecting the volatile compounds from cooked chicken in order to identify them by chemical and gas chromatographic analysis.

2. To determine some chemical compounds in raw-frozen and cooked-freeze-dried light and dark muscle from three classes of chicken that would indicate changes in precursors and differences in flavor volatiles.

3. To place special emphasis on the identification of sulfur compounds in cooked chicken volatiles and to determine their precursors.

4. An added objective was to determine meat yields from three classes of birds, which were used as a major source of material for the flavor studies.

## REVIEW OF LITERATURE

Numerous definitions of flavor can be given but the one chosen is that of Kazeniac (1961), in which he used four designations as proposed by Sjöstrom (1955). The first was taste, consisting of saltiness, sweetness, sourness and bitterness. The second was aroma, which was used to describe perceptible odor sensations. The third was body, which referred to the texture or sensation caused by chewing, but having nothing to do with taste or aroma. The fourth was mouth satisfaction, which implied increased salivary stimulation, blending and pleasantness, but had little to do with taste or aroma. Thus described, flavor is an interaction of these four basic sensations.

### Meat Flavor

A classical report on the constitution of meat extract and its mode of preparation from lean muscle was published in 1847 by the immortal chemist, Justus von Liebig (1803-73). Liebig related how Berzelius, Gmelin, Braconnot and Chevreul some 40 years earlier had tried without success to find a meat substitute. Liebig extracted not only ox, but chicken, fish, pork, deer and fox muscle as well. His procedure was to mix 10 lbs. of muscle with 5 lbs. of water by hand. The solution was pressed out and collected. He repeated this procedure three times. The combined extracts were then filtered through linen cloth and placed in a large glass flask which was immersed and heated in a water bath. Fat was removed constantly as it rose to the top of the solution. When all of the fat was separated, Liebig filtered the solution and simmered it

in a water bath. After prolonged heating the solution darkened, and was concentrated under a vacuum to complete the process.

Liebig (1847) determined the creatine and creatinine contents of the muscle extracts, and concluded that creatinine is an important constituent of muscle. He extracted the remaining concentrate with alcohol, after first removing the creatine and creatinine and isolated a new acid, which he called inosinic acid after the Greek word meaning muscle. When inosinic acid had been extracted, lactic acid remained behind. He then determined the amount of lactic acid and inorganic constituents in the extract. Liebig also isolated sarcosine (methyl aminoacetic acid), which he named after the Greek word meaning meat, and tyrosine, which he named after the Greek word meaning cheese. After examining these extracts, he concluded that the odor and taste coming from the lean muscle resembled roast meat. After they were cooked and concentrated, there was no fundamental difference between ox, doe, fox, pork or chicken muscle. The amount of albumin released by heating depended upon the age of the animal, with lesser amounts coming from older animals. Liebig's extract is recognized in commerce as a derivative of ox or beef muscle. Many scientific studies have been devoted to the elucidation of its chemical nature and value. Papers on meat extract have been published by Micko (1908, 1909, 1914a,b), Wolff (1913), Krimberg and Israilskii (1914), Salkowski (1914), Smith (1916), Waser (1917), Geret (1918), Kluender (1920), Korchow (1927), Kappeler-Adler and Stern (1931), Hordh (1936), Boon (1937), Kokovikhina (1942), Tempus (1956), Wood (1956, 1957, 1961), Lissi et al. (1961), Bender and Ballance (1961), Pocchiari

et al. (1962) and Wood et al. (1962).

In a review on meat flavor, Doty et al. (1961) indicated that modern objective techniques have replaced the purely subjective approaches that were used in earlier flavor research. He stated that two approaches have been used for meat flavor research. The first involves the isolation and identification of both volatile and non-volatile flavor components from cooked meat. A second method depends upon the isolation and characterization of flavor precursors from raw meat. He cited some obvious advantages and disadvantages inherent in each of these approaches. He pointed out that one may isolate many compounds from cooked meat, yet have no means of determining the relative importance of each of the isolated constituents to the overall meat flavor. This would entail quantitative determinations of the volatile and non-volatile constituents, and then recombining them at the correct concentration to again achieve the original cooked meat flavor. Doty pointed out that if one isolates and identifies the precursors of meat flavor from raw meat, it is still necessary to elaborate the chemical changes which are responsible for the development of typical meat flavor.

## Beef Flavor

Waser (1920) extracted dried beef broth with alcohol and isolated an aromatic fraction from the alcohol insoluble portion, which he then fractionally dialyzed through parchment paper. Twelve percent of the dialysate was assumed to contain inosine, inosinic acid, carnine and similar substances. Analysis of the remaining 88% of the dialysate

indicated that it was composed of 47% inorganic and 53% organic constituents. The organic fraction consisted of the following compounds in percentage: taurine or cystine 1.6, ammonia 4.4, creatine 5.4, creatinine 2.7, hypoxanthine 1.4, carnosine 16.6, methylguanidine 1.3, glutamic acid 7.0, formic acid 1.4, acetic acid 23.9, lactic acid 12.9 and organic phosphorous 2.4. Of the total ash, 10% was KCl and the balance was composed of various phosphates.

Feulgen and Voit (1925) histologically demonstrated the existence of plasmal, which was derived from the lipid, plasmologen. They noted a relationship between plasmal and the odor of cooked beef.

In early work, Crocker (1948) stated that typical meaty flavors were contained in the fibers of cooked meat rather than in the expressible fluid. Barylko-Pikielna (1957) reported taste panel evaluation indicated that the typical flavor of roast beef was present in the water-insoluble residue, whereas, the water-soluble fraction had a very intense but atypical beef flavor. More recently, Kramlich and Pearson (1958) found that fluids expressed from raw meat developed a concentrated flavor upon cooking, and that the cooked beef fibers bound the flavor components more tenaciously than the raw fibers. In further studies on beef flavor, Kramlich and Pearson (1960) and Hornstein et al. (1960) removed the flavor precursors from raw beef with cold water, and identified some of the volatile compounds emanating from cooked beef.

The classical report on ox muscle extract by Wood and Bender (1957) exemplifies the painstaking investigations on meat flavor. These workers identified more than 30 volatile and non-volatile compounds from ox muscle extract.

Melanoidins, which form as a result of interactions between free amino acids and reducing sugars, were noted in meat broth by Lobanov and Wolfson (1958). By means of two-dimensional paper chromatography, they separated and identified glucose and glucose-6-phosphate, cystine, histidine, aspartic acid, glutamic acid, serine, glycine, lysine, arginine, threonine, alanine, proline, tyrosine, methionine, valine, leucine, phenylalanine, carnosine, and anserine. In a model system, they produced a broth-like odor by refluxing a solution containing 1% of a mixture of certain amino acids and 0.2% of a mixture of glucose and glucose-6-phosphate. The glucose and glucose-6-phosphate had disappeared upon evaporation to dryness, thus showing evidence of the browning reaction.

Browning of the acetone-soluble constituents of ox muscle extract was studied by Wood (1961). Nucleic acid decomposition was an important factor in browning and meaty flavor. He isolated inosinic acid and ribose-5-phosphate as the active ingredients. He described the taste of inosinic acid as meaty. By using model systems, the relative efficacies of ribose, ribose-5-phosphate and glucose with amino acids were studied. Ribose-5-phosphate was more active in browning than the other reducing sugar moieties.

Major emphasis at the American Meat Institute Foundation has been placed on the study of flavor precursors from raw beef. This work was summarized in a review by Doty et al. (1961). This approach was selected due to the fortuitous observation that the fat fraction from the third acetone extract of raw ground beef gave a typical broiled steak odor

when heated. Subsequent research on this fraction revealed that the flavor was not due to the fatty material per se, but was caused by substances that diffused through a semi-permeable membrane upon dialysis with water. On separation of this fraction, these workers found a white granular material. This fraction was unstable and became a brown, tarry, mass when stored under vacuum. Ammonia and/or amines were released as browning progressed, and the deteriorated material assumed a characteristic stale meat odor. Concurrently, Wood (1961) also reported the same or a similar acetone-soluble material from ox muscle extract that decomposed in an identical manner.

Batzer et al. (1960) undertook the formidable task of trying to characterize and identify the components in the water-soluble fraction that yielded the typical meat odor on heating. Upon further fractionation of this diffusate by dialysis through a sausage casing and separation of the dialysate on a Sephadex G-25 column, two fractions were obtained. One was a protein fraction, and the other was characterized as the basic meat flavor precursor in beef. By extracting chicken or pork loin tissue and treating the extracts in the same manner as that used for ground beef, they were successful in isolating similar protein fractions. These fractions had almost the same basic odor as that isolated from beef muscle. In a classical series of experiments, the same investigators showed that the basic meat flavor precursor was a glycoprotein, which gave a strongly positive carbohydrate reaction prior to hydrolysis with perchloric acid and a strongly positive phosphate test after hydrolysis. Ultra-centrifugation at 60,000 rpm resulted in

no distinct peak. Thus, the compound was established as being of relatively low molecular weight. Ninhydrin tests were positive after 8 spots had been separated by paper chromatography of the acid hydrolysate. Two of the ninhydrin positive spots could not be positively identified, but the others revealed the presence of leucine, proline, isoleucine, alpha alanine, valine, serine and beta alanine, with trace amounts of glycine and glutamic acid. When the diffusate fraction from the secondary dialysate was separated on Dowex-50 ion-exchange resin and eluted with acid, a spectra of nucleotide peaks matching published results for hypoxanthine, inosinic acid and inosine were obtained. Some further studies on the identification of beef flavor precursors by Batzer et al. (1962) resulted in the identification of inosinic acid, inosine and a glycoprotein with a glucose moiety. It was concluded that these are simple, water-soluble components of beef muscle tissue.

Batzer and Landmann (1963) stated that the consistent isolation of the glycoprotein from various runs was a function of the efficacy of the sausage casing which was used for dialysis. Old casings were ineffective and fractions obtained failed to exhibit the characteristic broiled steak odor when the standard test was applied. Furthermore, they were unsuccessful in identifying the eighth amino acid of the peptide group in the glycoprotein moiety. When a mixture of the glycoprotein, inosine or inosinic acid, inorganic phosphate and glucose was heated in fat, an odor similar to the original broiled steak odor was obtained. It is interesting that the phosphorous-containing nucleic acid fraction isolated in these studies was previously reported as present in ox muscle extract

by Wood (1956). The glycoprotein fraction was not reported present in beef extract and Doty et al. (1961) concluded that this was understandable, since the glycoprotein undoubtedly decomposed upon heating.

With reference to precursors, first Crocker (1948) and then Kramlich and Pearson (1958) and Hornstein et al. (1960) demonstrated that flavor precursors can be leached from raw beef with cold water, although none of these workers attempted to establish their identity. In a recent report on the flavor of beef and whale meat, Hornstein et al. (1963) made a chemical comparison between freeze-dried extracts of lean whale and beef tissue and showed the volatile fractions were identical, thereby confirming their earlier conclusions. In working with beef and pork (1960a, b) and lamb (1963), the same authors found an identical meaty aroma associated with the lean portion of all red meats, whereas, flavor differences resided in the fat.

A paper by Harries et al. (1963) described the evolution of their methods for assessing beef quality which have become standard since 1955. Organoleptic techniques were perfected by rigidly standardizing cooking and judging procedures. Judges were chosen for their flavor evaluating acuity. All samples were coded at random, and no more than four samples were judged at any one session. Only one session was held per day. Bread and water were used as palate cleansers and tasters were encouraged to take their time and retaste as often as necessary. Hot and cold tasting results were compared.

Pork and Mutton Flavor

Only a few studies have been reported on pork and mutton flavor volatiles. Hornstein and Crowe compared the volatiles from lean pork, beef and lamb in three separate studies (1960a, b, 1963). Common components of the volatile fraction from these three different meats were carbon dioxide, formaldehyde, acetaldehyde, acetone, ammonia and hydrogen sulfide. The same investigators also theorized that fundamental flavor differences between meat reside in lipid-soluble foreign compounds, and the capability of a specific fat to produce different flavor components in different ratios. This was in contrast to the report of Crocker (1948), who suggested that bones contribute little to meat flavor while marrow and tissue fats supply aroma, but add nothing to flavor.

Observations on the objectionable reaction of some individuals to mutton flavor were made by Ziegler (1958) and Kean (1959), and gave impetus to research on the chemical constituents in mutton flavor. Mutton flavor studies by McInnes et al. (1956) indicated that a group of steam volatile fatty acids occurred in mutton fat, which may be important in mutton flavor. They found a series of normal acids from formic to capric ($C_1$ to $C_{10}$) together with isobutyric, isovaleric, and alpha methyl butyric acid. Hofstrand et al. (1960) proved that lamb broths lost their identifying aromas and the fat odor was volatilized by the application of heat.

Jacobson et al. (1962) characterized mutton flavor as "fragrant," "oily," "sweet" and somewhat "musty." By comparing the volatiles from cooked lamb with chemical odors, they found similarities to ethyl oleate,

diacetyl and a number of sulfur compounds. An extensive study on lamb flavor was reported by Jacobson et al. (1963). They found glucose, fructose, inositol and 19 amino constituents in water extracts from raw and cooked lamb. Aroma was strongly affected by the numerous carbonyls present, including several alkanals, alkanones and possibly 2-methyl-cyclopentanone.

Williams (1962) obtained a patent on a process for improving the tenderness and flavor of mutton. Freshly slaughtered old crop (yearling) lamb and mutton were pumped by the stitch method with a solution containing 0.4-0.6 oz. monosodium glutamate (MSG/lb) at a dosage level of 3% by weight of the carcass. The carcass temperatures were raised to 108-115°F by microwave heating or in a steam room. He claimed that after chilling the carcass, the meat had the flavor and tenderness of spring lamb.

Fish Flavor

A voluminous literature on the chemistry of fish has been compiled by Borgstrom (1961). However, no attempt was made to correlate the chemical data with flavor. Jacquot's (1961) contribution on the organic constituents of fish and other aquatic animal foods provides an insight into some similarities and differences between fish and mammalian muscle. He reported that seafoods contain a series of specific basic proteins. These protamines are indigenous to a certain species and differ from one another due to the nature, content and position of the amino acids. Salmine from salmon, iridine from rainbow trout, fontinine from brook

trout and clupeine from carp all have polypeptide chains ending in proline. Sturine from sturgeon has alanine and glutamic acid at the terminal end of the protamine molecule.

Fat composition varies between ocean and fresh water fish according to Lovern (1950). In fresh water fish, 20% of the unsaturated acids were $C_{10}$, 40% $C_{18}$, 13% $C_{20}$ and 2.5% $C_{22}$, whereas, in ocean fishes 10% of the unsaturated acids are $C_{10}$, 25% $C_{18}$, 25% $C_{20}$ and 15% $C_{22}$. Wide variations were found in the composition of lipids due to species, diet, temperature and salinity of the environment, selective mobilization and selective distribution. Lovern stated that fish lipids contained little or no linoleic acid. Palmitic acid was the predominant saturated fatty acid in fish oil and constituted 10-18%. Myristic and stearic acids occurred in lesser quantities, with the latter rarely exceeding 2%. Lovern also concluded that the three alcohol sites on the glyceride molecule could be esterified, resulting in the same or different fatty acids.

Lovern (1950) also reported that the most common unsaturated fatty acids encountered in fish oils could be classified as monoenoic acids and polyenoic acids. The principal monoenoic acids reported were palmitoleic with $C_{16}$, gadoleic with $C_{20}$, cetoleic with $C_{22}$ and selacoleic acid with $C_{24}$. The latter acid is abundant in shark oil but has not been encountered so far among the telosteans. The principal polyenoic acids found were clupanodonic with $C_{22}$, which is the most abundant and contains five double bonds, arachidonic with $C_{20}$ and four double bonds, hiragonic with $C_{16}$ and three double bonds, and minisic and thynnic acids with $C_{24}$, whose compositions are so far not well understood despite numerous studies.

According to Tarr (1950) and Jones (1958) glucose and ribose are the principal free sugars in fish. Original evidence for sugar phosphates was obtained by Tarr (1950), who used a non-specific barium fractionation procedure. Recently, Jones and Burt (1960) used an ion-exchange column and separated fructose-1-phosphate, fructose-6-phosphate, ribose-1-phosphate and ribose-5-phosphate. These workers characterized the flavor as being "sweetish-salty" at pH 6.8.

Amino acids and peptides differed between fatty and non-fatty fish according to Lukton and Olcott (1958). Fatty species contained considerable histidine in the flesh, whereas, non-fatty species contained anserine (beta alanyl-1-methylhistidine). Jones (1961) stated that anserine and taurine have pronounced pleasant effects on the tongue, which were described as "mouthfulness" by the panel. Aspartic and glutamic acids were detected at low concentrations and described as "acid." The other amino acids were characterized as "sweetish," with proline being rated as the sweetest. He also found that a combined amino acid solution simulating non-fatty fish muscle had a "pleasant, sweet-sour, meaty, yeasty flavor."

Tarr (1958) reviewed the biochemistry of fishes. This article included sections on proteins, non-protein nitrogen, nucleic acids and related compounds, phospholipids, enzymes, sterols and the nutritional value of marine invertebrates. A total of 265 references are included in this comprehensive review.

Extensive investigations have been made on nucleotides and nucelotide changes in fish muscle by Tarr and his associates (1957, 1958, 1959,

1960 1962), Fujimaki and Kojo (1953), Shewan and Jones (1957), Jones and Murray (1957) Saito and Arai (1958), Tomlinson and Creelman (1960), Tomlinson et al. (1961) and Olley (1961). Jones (1961) reported a high inosine-5'-monophosphate content in fresh fish and showed that it was one of the major flavoring components. It was described as possessing a strong salty-acid flavor, with overtones described as "meat extract," "yeasty," "almonds."

An electrodialysis method was devised by Yoshida and Kogeyama (1956) for separating inosinic acid from dried fish muscle. A contemporary patent granted to Motozaki et al. (1963) described the preparation of inosine from mutants of Bacillus subtilis, which require adenine and an amino acid for growth in an aerobic media containing carbon and nitrogen sources. Such nutrients can be yeasts, or yeast derivatives, meat extract or corn steep liquor. The pH must be maintained between 4 and 9 preferably between 5.0 and 6.7 during fermentation. It is interesting to note that the first commercial production of inosinic acid was from fish muscle by the Japanese.

Saito and Arai (1957) reported that noteworthy changes occurred in carp muscle when it was held at low temperatures. At 16°C, ATP and ADP broke down to give inosine monophosphate (IMP) as the major product, while in liquid air no change took place. According to Jones (1961), inosinic acid broke down to form hypoxanthine after 1--12 days storage of fresh fish on ice. Hypoxanthine was produced and gave bitter flavor, which was indicative of breakdown.

Several important chemical factors are responsible for undesirable flavors in fish. Tanikawa(1959) traced sourness in stored fish to an increase in free fatty acids. The sour taste was noticed after 90 hours at 10-13°C in dry storage or after 21 days on ice. Spoiled flavors in fish (Hughes 1960) resulted when a reducing atmosphere prevailed, thus causing formaldehyde, dimethylamine and trimethylamine to be formed and volatilized. Hughes (1961) also reported that the combined creatine-creatinine fraction plus histidine were reduced during heating, and then large amounts of ammonia were liberated. Tanikawa (1958) measured the amount of increase in some objectionable amines in fatty fish. He found that as cold storage time increased so did the content of histamine, cadaverine, putrescine, agmatine and iso-amylamine. Reay and Shewan (1949) reported that hydrogen sulfide increased in direct proportion to the time the fish were chill-stored. Methyl mercaptan was found in aged samples.

In working with fish oils, Lundberg (1957) attributed the oxidized flavor and odor accompanying deterioration, to an increase in the unsaturated carbonyl and dicarbonyl compounds. Privett et al. (1958) suggested that malonaldehyde is the active carbonyl compound in the thiobarbituric acid test (TBA test) for oxidative rancidity. Mangan (1959a,b) demonstrated the presence of acetaldehyde, methanol, ethanol and probable dicarbonyl and alpha hydroxy-compounds in frozen haddock, and suggested that they may be important flavor and odor components. The production of carbonyls in heat-processed herring has been reported by Hughes (1961, 1963). He correlated the degree of freshness

of iced herring prior to canning with total carbonyl production and the classes of carbonyls that were produced. He isolated five major carbonyls, namely; acetaldehyde, propionaldehyde acetone, iso-butyraldehyde and 2-methylbutyraldehyde from the cooked flesh of herring. These were the main components of the neutral volatile fraction with smaller quantities of longer-chain carbonyl compounds.

Jones (1961) characterized the most acceptable raw and cooked fish odor as "fresh seaweedy." The most undesirable odors were described as "sulfide," "ammoniacal" (due to amines) and "indole." Sweet flavors were found in fresh fish and flavors ranging from "bitter," "rubber-like" and "sulfide-like" to "nauseating and putrid" occurred as spoilage ensued. He described some intermediate odors as "sour" and "condensed milk" and some intermediate flavors as "chewing cotton wool" or "sourness with no bitterness."

Undesirable Meat Flavor

The influence of browning in foods may be desirable if controlled, but is undesirable if excessive. Maillard (1912) first explained the fundamental browning mechanism as an interaction between the carbonyl group of reducing sugars and the amino group of amino acids, peptides, polypeptides or proteins. This is known as nonenzymatic browning. Lea and Hannan (1950a,b) reported on the reaction between proteins and reducing sugars in the dry state, and on the biochemical and nutritional significance of browning. In 1952, Lea and Schwartz reported further studies on the mechanism of browning in the dry state. Speck (1952)

showed that primary and secondary amines catalyze dealdolization and conversion of six carbon sugars to pyruvaldehyde and diacetyl. Lewin (1957) investigated the effect of initial pH on the behavior of glucose and amino acid-glucose interactions. Subsequently, he studied the reactions of amino and imino compounds with reducing sugars, including the reaction of histidine with glucose. Later Lewin and McCall (1957) reported on the interaction of glycylglycine with glucose.

A classic study on the Strecker degradation of amino acids was made by Schönberg et al. (1952). Previously these workers (1948) showed that degradation of alpha amino acids to aldehydes and ketones resulted from interactions between these amino acids and certain carbonyl compounds. Alpha amino acids are degraded to the corresponding aldehydes or ketones containing one less carbon atom by the action of specific carbonyls. Degradations were carried out in water or water-glycerol media by holding the mixture at the boiling point for 15 minutes in a $CO_2$ atmosphere. Active carbonyls were the CO:CO type such as glyoxal, methyl glyoxal, pyruvic acid, diacetyl (acetoin), ketoglutardialdehyde, alloxan, phenyl pyruvic acid and others. Various mechanisms were postulated, but experimental evidence favored Strecker degradation with the accompanying loss of $CO_2$ and $NH_3$ in an oxygen atmosphere. Schiff base intermediates were formed between the amino acid and the active carbonyl. Degradation products of the amino acids were identified by their 2,4-dinitrophenylhydrazones (2,4-DNPHS).

Akazawa and Conn (1958) obtained little experimental evidence supporting the pyridoxal cleavage theory of amino acid degradation.

This mechanism requires the oxidation of reduced pyridine nucleotide by peroxidase in the presence of catalytic amounts of $Mn^{++}$ and certain phenols. The active phenols were found to be monohydric phenols or resorcinol. The reaction was inhibited by catalase, certain phenols, cysteine, cupric ions and inhibitors of heavy metal enzymes.

It was shown by Mazelis (1962) in his studies on the potential precursors of volatile sulfur compounds that highly purified horseradish peroxidase will not oxidatively decarboxylate methionine unless both pyridoxal phosphate and $Mn^{++}$ are present. Phenols were found to be catalysts of this reaction. In a separate study (Mazelis et al. 1962) methylthiopropionamide was identified as a product of decarboxylation of methionine.

Glucosone is regarded as an important intermediate of browning. Kato (1960, 1962, 1963) has presented data on its formation from N-glucoside and proposed a mechanism for the reaction. D-glucosone was isolated from the browning degradation mixture of N-D-glucosyl-n-butylamine in methanol solution neutralized with acetic acid. It was identified by its 2,4-dinitrophenylhydrazone derivative. The author also found 3-deoxy-D-glucosone and showed it to be an intermediate substance in the browning reaction. The yield of N-D-glucosone was far less than that of 3-deoxy-D-glucosone, but increased considerable when air was bubbled through the reaction mixture. This indicated that its formation is dependent on the amount of dissolved oxygen.

Burton et al. (1963) showed that non-enzymatic browning of phenolic compounds in the presence of nitrogen occurs more rapidly than non-

nitrogenous browning, regardless of whether in the solid state or in weakly acid solution. Sulfite was effective in retarding the onset and development of such browning. They stated that this is clearly an example of the browning associated with carbonyl and amino groupings, which sulfites will prevent in some foodstuffs. Both carbonyl groups and reducing agents also effectively retarded this particular browning reaction.

Lobanov and Wolfson (1958) have studied browning of meat broth while Wood (1961) studied browning of meat extract. According to Wood, reducing substances in fresh muscle include the reduced form of diphosphopyridine nucleotide (DPNH), sugar phosphates and free reducing sugars. He stated that the Maillard reaction involves splitting of ortho-phosphate in aged muscle, by which free sugars, principally ribose, are released to react and impart the brown color and meat flavor characteristic of ox muscle extract. Wood used model systems, in which various amino acids were reacted with glucose and glucose-6-phosphate, or ribose and ribose-5-phosphate to produce effects similar to those observed in foods.

Recently, Ballance (1961) showed that Strecker degradation of methionine by ninhydrin resulted in formation of methional, methyl mercaptan, dimethyl sulfide, isobutyraldehyde and acrolein. Schönberg *et al.* (1948, 1952) demonstrated that many compounds apart from ninhydrin will decompose amino acids on heating. This included several that are indigenous to living tissues.

Off-flavors in meat caused by irradiation have been studied by numerous workers. Papers on irradiated meat flavor have been published

by Proctor et al. (1955), Batzer and Doty (1955), Groninger et al. (1956), Marbach and Doty (1956), Witting and Batzer (1957), Burke et al. (1957), Cavallini et al. (1959), Pearson et al. (1959), Merritt et al. (1959), and Hedin et al. (1960). According to Pearson et al. (1959), irradiation flavor was associated with high levels of hydrogen sulfide, methyl mercaptan and carbonyls. Their studies were made on irradiated pork, beef and veal by both chemical and organoleptic evaluation procedures.

Oxidative rancidity of adipose and lean tissues of meat was reviewed by Watts (1961). She reported that lean muscle tissues of land animals or fin fish tended to oxidize very rapidly following heat denaturation of heme proteins. The hematin compound was found to be the active catalyst in this oxidation. In heat-processed meats, the oxidation occurred only to a limited extent due to the development of antioxidants during the severe heat treatment. Watts stated that a promising approach to the elimination of lipid oxidation is through the use of water soluble antioxidants which can be incorporated in raw meat or applied to the surfaces of the cooked meat.

Chicken Flavor

According to Lineweaver and Pippen (1961), the ten-fold increase in per capita consumption of broilers since 1947 constituted strong evidence that broilers as presently produced, processed and marketed have highly acceptable flavor. They also cited the fact that the low cost of poultry compared with other meat has been an important factor in acceptance.

However, there is still a lack of scientific knowledge on the effects of modern production upon chicken flavor. Lineweaver mentioned the laxity of the poultry industry in failing to rate the comparative efficacy of modern broilers and old-fashioned roasting chicken. He stated that on the basis of current experimental evidence it is difficult to determine any flavor difference among various types of birds, and then concluded that differences are ordinarily negligible.

Studies on the chemical nature of chicken flavor began when Liebig extracted various meat, including hen meat in 1847. He quantitatively determined creatine, creatinine, lactic acid, inorganic salts and other constituents of lean chicken muscle. He then isolated and identified inosinic acid in extracts from chicken.

Osborne and Heyl (1908) hydrolyzed the edible parts of mature hens, which had been bled and freed from fat and connective tissue. After hydrolysis the amino acids were determined to be present in the following percentages: glycine 0.68, alanine 2.28, leucine 11.29, proline 4.74, phenylalanine 3.63, aspartic acid 3.21, glutamic acid 16.48, tyrosine 2.16, arginine 6.50, histidine 2.47, lysine 7.24 and ammonia 1.67, thus comprising a total of 62.75%. Tryptophane was present. Cystine and hydroxyproline were not determined. Valine and serine were not identified in the hydrolysate. It was concluded that except for the higher lysine content, the protein of hen muscle is similar to the protein of leguminous seeds.

Houghton (1911) studied the effects of low temperature storage on ground meat. After five months at 35°F, ammonia increased slightly in

light meat. There was a large increase in the water-soluble nitrogen content of light meat and a slight increase in dark meat. There was an increase in free amino acids and proteoses, and a decrease in peptones. Insoluble phosphorous increased during the first 90 days storage, especially in dark meat. There was also an increase in the iodine number of the fat. Five different lipolytic enzymes were detected in the stored fat and muscle fractions.

Sadikov et al. (1934) ascribed an important role to glutathione as a flavor precursor in chicken. These workers found that hydrogen sulfide was formed on cooking chicken muscle. The hydrogen sulfide appeared to be due to the total decomposition of glutathione and the partial decomposition of cystine and methionine. They concluded that part of the hydrogen sulfide was reabsorbed by the protein, but that larger amounts were lost by formation of free sulfur.

Modern studies on the chemical nature of chicken flavor in the United States probably began with a classic distillation of tissues from chicken, pork and beef by Crocker (1948). Some of the same simple compounds, namely; hydrogen sulfide, ammonia and acetaldehyde were found in each of the three distillates. Crocker concluded that all meats may possess identical fundamental flavor factors, and that individual species differences may be due to low concentrations of specific compounds characteristic of the particular species. Bouthilet (1951) replaced Crocker's simple distillation procedure by a high vacuum distillation technique, and used it in fractionating chicken broth. Hydrogen sulfide and ammonia were found in the distillates. Upon completion of numerous tests using

chicken broth. Bouthilet (1949, 1950, 1951a,b) postulated that glutathione is the major muscle precursor of chicken flavor. He further concluded that fat affects the aroma of the broth.

Distillation studies were made on the aqueous phase of chicken broth by Pippen and Eyring (1957). These workers also found hydrogen sulfide and ammonia in the distillate and demonstrated that removal of the ammonia from the distillate resulted in enhancement of chicken flavor. Thus Bouthilet's (1949) conclusion that a progressive lowering of pH raised the chicken flavor level in the broth distillate was confirmed. Pippen and Eyring also confimed Bouthilet's work showing that upon standing, desulfuration of the broth continued as long as true chicken flavor existed. Results led both groups of workers to conclude that sulfides are important in chicken flavor. From these experiments, Pippen and Eyring also concluded that chicken flavor is associated with the neutral or acidic constituents.

A concentrated chicken flavor precursor was prepared by Peterson (1957) using a water extract of lyophilized lipid-free muscle. He concluded that fat does not contribute to the taste of chicken broth but contributes to its aroma. A flavorful broth was made by heating a water solution of the chicken flavor precursor. These results confirmed the earlier findings of Pippen et al. (1954).

A classical study on the carbonyl compounds in the volatile fraction of cooked chicken was published in two parts by Pippen et al. (1958, 1960). Many carbonyl compounds were isolated, separated and identified as their 2,4-dinitrophenylhydrazone derivatives (2,4-DNPHS). Elegant

separations were made by column and paper chromatography, and identifications of the carbonyls were substantiated by melting point comparisons with known compounds, as well as by infrared spectroscopy. When sufficiently large fractions of derivative were available to permit carbon, hydrogen and nitrogen analyses, empirical formulas for the 2,4-DNPHS were determined. These same investigators showed that the cooking procedure and isolation method had a pronounced influence on the yield of carbonyls. Normal cooking consisted of heating an equal weight of water and raw cut-up chicken at 100°C for 4 hours in a vessel arranged for distillation. Cooking in an oxidative atmosphere consisted of refluxing the chicken-water mixture for 16 hours. The carbonyls were carried past the low-efficiency condenser with an air stream passing through the trap containing 2,4-dinitrophenylhydrazine solution (2,4-DNP) at a rate of two bubbles per second. Carbonyl compounds were condensed along with the water from the cooking mixture. Cooking in an inert atmosphere consisted of passing nitrogen through the system instead of air.

Pippen et al. (1958, 1960) found that the yield of volatile carbonyls was influenced markedly by the cooking method employed. Large yields resulted from cooking the chicken in an oxidative atmosphere. Carbonyl production was 30 times greater when an oxidative atmosphere was used than when a nitrogen atmosphere was utilized. Normal cooking gave an intermediate yield of carbonyl derivatives. By using the oxidative atmosphere for cooking, the yields of 2,4-DNPHS was so large that the recognition of minor carbonyl compounds was facilitated. Diacetyl, acetaldehyde, n-hexanal, n-hept-2-enal and n-deca-2,4-dienal were present

in the largest amounts. Many other carbonyls were identified including n-butanal, n-pentanal, n-heptanal, n-octanal, n-nonanal, acetone, propanal, methyl-ethyl-ketone, 2-pentanone, 2-heptanone, n-pent-2-enal, n-butenal, n-hepta-2,4-dienal, n-hex-2-enal, n-dec-2-enal, n-oct-2-enal, and n-non-2-enal. A total of 7.8 g. of 2,4-DNPH precipitate was obtained from 31.4 kg. of chicken. Long chain aldehydes from $C_{16}$ to $C_{18}$ that were found in the normal cooking did not appear in the volatile fraction after air entrainment. N-hept-2-enal, which was one of the most abundant carbonyl compounds present in the air entrainment volatile fraction, was found only as a minor component after the normal cooking procedure.

Lineweaver and Pippen (1961) stated that specific chemical knowledge concerning the precursors of carbonyls is incomplete or non-existent. Pippen et al. (1960) determined that the concentration of acetoin exceeded that of diacetyl by a factor of 18 in cooked aqueous extracts of chicken meat. It was concluded that acetoin is the immediate precursor of some if not all of the diacetyl. By studying the effects of heating time and temperature on the concentration of acetoin in aqueous extracts of raw chicken meat, they found that heat labile precursors of acetoin were present. These authors concluded that the remaining carbonyls may originate from either the triglyceride or phospholipid fractions. Rapport (1959) has demonstrated that the plasmologens or acetal-phospholipids can yield long chain aldehydes. Lineweaver and Pippen (1961) concluded that this phospholipid fraction may be the precursor of $C_{16}$ to $C_{18}$ aldehydes in chicken meat, but is not the precursor of decadienal.

Experimental evidence for the importance of carbonyls to chicken flavor is not yet conclusive. According to Lineweaver and Pippen (1961), carbonyls represent some of the end products of chemical reactions that occur during cooking, and probably have a definite role in the flavor of cooked chicken. Pippen and Nonaka (1960) estimated that the average carbonyl concentration in chicken broth samples was approximately 14 x $10^{-5}$ moles/liter. Lea and Swoboda (1958) determined that n-decanal could be tasted at a level of 5 x $10^{-8}$ moles/liter. According to Patton et al. (1959) decadienal was detected at concentrations as low as 0.5 part per billion in water. Lineweaver and Pippen (1961) found that decadienal had a deep-fat-fried odor, that may contribute to the flavor of foods where fat mingles with moisture at elevated temperatures. Thus appears to be a staling or rancidity factor.

Limited tests were made by Pippen et al. (1960) to ascertain whether diacetyl and acetoin contribute to the flavor of chicken broth. They concluded that normal concentrations of acetoin and diacetyl in chicken broth cannot be detected. However, if substantial amounts of acetoin was oxidized to diacetyl, its presence could easily be detected. They postulated that diacetyl contributes a transient buttery-oily type aroma in freshly cooked chicken.

The conclusions reached by Lineweaver and Pippen (1961) after reviewing the available knowledge on chicken flavor indicated that flavor is fairly independent of the type of bird. Meat was the most important flavor component in the carcass. The chemical composition of the volatile fraction from cooked poultry included sulfide and carbonyl compounds,

which are probably of importance to flavor. Although diacetyl/acetoin may contribute to flavor, further work is needed to confirm the importance of carbonyls to chicken flavor.

Kazeniac (1961) reported on a project aimed at isolating and identifying the components and precursors of chicken flavor, establishing the optimum conditions necessary for flavor development, developing processing procedures that will maintain high levels of flavor, discovering suitable ways for preventing flavor deterioration during storage and raising chickens high in flavorful components and precursors. He postulated that fat may be a trapping agent for flavor volatiles, since broth with some fat gave a more desirable flavor than broth with the fat removed. Diacetyl values were found to be higher for cooked skins than for raw skins or dark meat, and for light meat than dark meat. Light meat broth had stronger taste and mouth satisfaction, which was attributed to a higher inosinic acid content, while dark meat had more body and a stronger aroma. Taste in chicken broth was attributed to the various classes of compounds, including a mixture of amino acids, peptides, carbohydrates, inorganic salts, sulfides, carbonyls and non-amino nitrogen compounds, such as ammonium sulfide, creatine/creatinine, carnitine, hypoxanthine, inosine and inosinic acid. Addition of either glutathione or homocysteinethiolactone improved broth flavor. Other sulfur compounds such as cysteine, cystine, and homocysteine increased the broth sulfide content after heat-processing, but also gave objectionable off-flavors. Kazeniac concluded that sulfide released must be considered as a direct contributor, as well as a possible indirect indicator of flavor. He found that

the sharp aldehydic flavor of light meat distillates became similar to the flavor of dark meat distillates by the addition of ammonia, and stated that the sulfide/ammonia relationship has apparently been overlooked in flavor problems. Ammonium sulfide contributed a sweet taste to broth and improved the aroma.

Kazeniac (1961) stated that acetoin gave a desirable buttery, oily-type flavor with improved body, but when levels were too high, the characteristic sour notes of diacetyl made the flavor undesirable. Acetaldehyde gave a scorched flavor note, which is common to chicken broth flavor profiles. Carbonyls derived from chicken skins contributed a very bitter off-flavor, whereas, those higher aldehydes derived from muscle added to the aroma and body, as characterized by oily-fatty notes in the broth. Creatine/creatinine content was higher in light than in dark meat, and was related by Kazeniac to the bitter after-taste more often found in light meat than in dark meat broths. Carnitine enriched broths developed strong fishy aromas and showed intense browning. Hypoxanthine and inosine imparted a bitter taste, whereas, inosinic acid made a major contribution to mouth satisfaction and intensified the effects of other compounds. Collagen and lipids gave more body to the flavor of chicken broth. Kazeniac showed that when certain amino acids including lysine, arginine, alpha alanine, glutamic or aspartic acid were added to chicken broth the overall flavor was improved. Glutamic acid gave greatest mouth satisfaction at levels of 0.02-0.04%. Lysine with glutamic acid gave the highest intensity of mouth satisfaction when levels of glutamic acid were 0.02-0.05% and lysine between 0.05 and 0.08%.

Alpha alanine imparted a sweet taste to broth together with some mouth satisfaction. Lactic acid contributed to the sour, astringent taste in broths and improved mouth satisfaction slightly at levels of 0.02-0.04% lactic acid and 0.06-0.08% lysine or arginine.

Kazeniac (1961) further reported that glucose, fructose and ribose were the principal sugars present in chicken broths, and that inositol was suspected. Dialysates of the raw meat extracts showed appreciable amounts of ribose, but upon boiling the extracts turned brown with noticeable losses of ribose. Results were similar to the effect found by Wood (1961) in ox muscle extract and Doty et al. (1961) using an acetone extract from raw beef. In Kazeniac's work he found that sulfhydryl compounds such as glutathione decreased browning and improved broth flavor. He reported that the salty taste in broth was derived from inorganic salts and salts of the amino acids. He also found that the alkaline ash of chicken meat improved broth flavor slightly, whereas, Pippen and Klose (1955) found that the neutral ash of water extracts of chicken produced a similar effect. Kazeniac (1961) attributed the improvement in flavor to the added inorganic phosphates and to the alkalinity of the ash, which raised the pH and released ammonium sulfide. Ammonia in the presence of hydrogen sulfide gave the broth a sweet aroma and taste, provided the concentration of ammonia was relatively high. Kazeniac concluded that chicken flavor is a complex blend of different compounds, and that precursors hold more promise for improvement in chicken flavor than the volatile flavor fraction. He planned in future research to use radioactive sulfur compounds to study the reactions of sulfur containing compounds.

Glucose and fructose were shown to be the principal free sugars in chicken muscle by Lilyblade and Peterson (1962). Red muscle contained more than twice as much free inositol as white. Inositol, fructose and ribose increased during storage in both kinds of muscle in both older and younger birds.

Carnosine levels were found by Davey (1957) to be 1.04 g/100 g. in the breast and 0.18 g./100 g. in the leg muscle of the hen, whereas, anserine levels were 0.58 g./100 g. and 0.28 g./100 g., respectively. The free amino acid level in the cock was reported to be approximately 0.22 g./100 g. by Florkin (1957). All of these values were obtained on lean somatic muscle.

Phosphatide components in fowl were reported by Kates and James (1961). These workers found phosphatidyl ethanolamine in the sphingomyelin fraction together with lysophosphatidyl ethanolamine and small amounts of phosphatidyl inositol, phosphatidyl serine, lysolecithin and phosphatidic acid. Each had a different fatty acid composition. The phosphatidyl ethanolamine fraction had a higher proportion of polyunsaturated fatty acids, and stearic acid had a lower proportion of palmitic acid than lecithin. Both phosphatides were also present in plasmologen as the palmitaldehyde and stearaldehyde moieties.

Effects of Feed on Flavor

Early ration studies by Maw (1935) suggested that fat replacement of moisture in the bird is due to ration, and that fat carries the flavor. Thus, a higher fat content in tissues would mean more flavor.

Wheat-fed birds had dry tissue, whereas, the tissue of corn-fed birds was moist, soft in texture, best in flavor and had the highest nutritional value. In single grain rations after corn, barley ranked second, oats third and wheat last. Broilers fed single grain rations, such as wheat and oats or wheat and barley, were found by Odland et al. (1955) to be equal or superior to those raised on more complex rations of barley, wheat, bran shorts, wheat bran and/or corn. Fish solubles produced no off-flavors, but did not improve palatability scores.

When levels of 8% animal fat were fed to broilers for 10 weeks by Essary (1961), the tissues contained more fat than birds raised on a standard commercial diet. In separate studies reported by Marble et al. (1938), Carrick and Hauge (1926) and Asmundson et al. (1938) it was found that fish meals or fish oils imparted a fishy flavor to chicken meat. A study on the effect of milk products on broilers by Weisberg (1956) indicated that addition of 1.5-5.0% of dry milk solids to the rations improved the flavor of the meat.

Brant et al. (1958) found the ration to have no effect when comparing the flavor of modern-type broilers with that of those raised on older type rations. In a separate study, Hanson et al. (1959) compared the 1930 (old-type) diet with the 1956 (new type). No significant flavor difference was found to be associated with diet with either equal age or equal weight birds. By feeding a low-fat purified diet and a standard diet as a control from the chick stage to an age of 8 to 10 weeks, Lewis et al. (1956) demonstrated that birds raised on a standard diet had more intense flavor in the broth and in both light and dark meat.

An attempt was made by Newman et al. (1958) to season poultry meat by feeding flavor ingredients to eight week old White Rock broilers for two weeks prior to slaughter. Garlic, celery seed, allspice, cloves or monosodium glutamate were added at a level of 3 to 5 ozs./100 lb. feed. Taste panels rated the garlic-flavored birds the least acceptable, while the monosodium glutamate fed birds were slightly more acceptable than the controls. Spice-fed birds were rated about the same as the controls.

No flavor differences occurred when birds were fed either semi-synthetic or standard commercial rations according to Kahlenberg et al. (1960).

Oxidized oils, such as those reclaimed after potato-chip manufacture, caused an edemous condition in chickens when included in standard rations according to Brew et al. (1950). A high chlorine content occurred in these birds, especially in the pectoral muscle, but was liberated during cooking. Both the broth and meat from the birds fed oxidized oil had an off-flavor. The effect was attributed to a compound contained in the unsaponifiable fat fraction of the toxic fat. A concentrate was obtained by column chromatography that was 3200 times as toxic as that contained in the original fat. The molecular weight was determined by a mass spectrograph, and the UV spectrum confirmed the compound as a benzene substituted material or a product of cholesterol.

Patrik (1962) reported better growth resulted when 2% of phosphatides from vegetable oils were added to a chick ration. The meat from phosphatide-fed birds graded higher, and a higher yield of edible meat with lower muscle water content was obtained.

How Cooking Method Affects Flavor

Simmering or pressure-cooking was recommended by Hanson et al. (1950) to increase tenderness in cooked poultry. They reported that roasting had no advantage over simmering or pressure-cooking in producing typical "roast turkey flavor." Furthermore, roasting had the disadvantage of accelerating rancidity development. A study of the relationship of cooking method, grades and frozen storage to quality of cooked mature Leghorn hens was reported by Swickard et al. (1954). They found that the meat of steamed hens rated higher than that obtained by pressure-cooking.

Conventionally-cooked 10-week old fryers were compared with the similar electronically-cooked birds by Schano and Davidson (1958). Electronically-cooked chicken was inferior to roasted or rotisserie-cooked birds, although the yields by the former method were higher. Old fowl was cooked by boiling, simmering and pressure-cooking in experiments by Kahlenberg and Funk (1960). Simmering gave higher yields, and pressure-cooking more tender meat, but with a lowered fat content. A comparison of the effects of various cooking methods on old fowl with and without the incorporation of salt in water was made by these workers. Cooking losses, degree of tenderness in the breast meat and amount of fat in the thigh meat was determined. Cooking in salt solutions had no advantage over cooking in water. Significantly lower non-fat cooking losses were obtained by simmering than by boiling. Pressure-cooking resulted in no significant change in non-fat cooking losses as compared to simmering. Pressure-cooking increased tenderness of breast meat, but the fat content

of the thigh meat was reduced by pressure-cooking as compared to boiling or simmering. A statistical treatment of palatability scores showed that cooked dark meat was significantly more flavorful, juicier and more tender than light meat from the same bird.

Effects of Processing and Storage

In a study made on cold storage of chickens (1907), Pennington reported that after 10 months storage the birds degenerated as evidenced by microscopic appearance and taste. Subsequently Pennington and Hepburn (1913) reported the presence of catalases, oxidases and lipases in chicken fat, and showed that lipolysis occurred even in fat that was frozen solid. Storage time ranged from 3 days at 40°F to 151 days at -9.4°F. Stewart et al. (1945) showed that quick-frozen broilers lost flavor during 51 days storage at -10°F. In a recent review, Brant (1963) expressed concern over the possibility that the broiler and fryer industries may decide to market their products frozen rather than fresh. He pointed out that 85% of all turkeys sold in the United States are marketed frozen, whereas, an almost equal percentage of broilers and fryers are sold fresh. Consumers prefer cooked meat of fresh fryers over the frozen according to Mountney et al. (1960). Cellophane-wrapped fryers were evaluated by a panel of 1500 visitors at the 1957 Texas State Fair. Each was given 3 cooked samples to taste; namely, (a) fresh, (b) frozen-stored 3 months, and (c) frozen-stored 9 months. Half of the panel preferred the fresh fryers over those frozen and stored 3 months. About 60% preferred the fresh over frozen-stored 9 months. They concluded

that there is enough flavor difference to create a slight product resistance toward frozen chicken stored for 3 to 9 months.

Biochemical changes occurring in chickens stored at above freezing temperature were reported by Van den Berg et al. (1963). Poultry meat was stored at 0°C. under an inert atmosphere of nitrogen. Changes in odor, flavor, tenderness and juiciness of the meat were observed after cooking. Odor and flavor scores differed markedly from that of control samples held at -40°C. After 5 weeks storage at 0°C. the breast meat was less tender. Tenderness and juiciness increased in leg meat during the first week of storage, indicating tenderization during that period. The amount of extractable protein in leg meat also increased appreciably during this period, whereas, no change was noted for breast meat during storage. Proteolysis was appreciable in both breast and leg meat, resulting in free amino acids and other breakdown products. Ion-binding properties as measured by loss of weight and minerals during cooking changed markedly during storage. The water-binding capacity of breast meat decreased considerably during the first week of storage, whereas, no appreciable change occurred in leg meat. In a sequel to this study, Khan and Van den Berg (1963) reported on the quantitative changes in myofibrillar, sarcoplasmic, stroma and non-protein-nitrogen fractions of breast and leg muscles from chickens 10 weeks, 4 months and 8 months of age stored under aseptic conditions at 0, 2 and 5°C for 7 weeks. Changes were small in the stroma-protein fraction, actomyosin fraction, and the myosin-adenosine triphosphatase activity of the actomyosin fraction. The myosin fraction increased during storage except in breast

muscle of 10 week old birds. The sarcoplasmic-protein fraction decreased in the leg muscle of 10 week old birds and the breast of 4 and 8 month old abirds but not in the breast of 10 week old birds. Non-protein-nitrogen and protein breakdown products increased in both muscles regardless of bird age. Proteolysis increased with storage time and temperature. Results obtained in this study were compared with those for storage at below-freezing temperatures.

Zabik and Dawson (1963) compared poultry coated with acetylated monoglyceride with controls wrapped in polyvinylidene film. When stored in the absence of other foods, flavor scores for the coated breast meat and the control were about equal. In the presence of other foods, the coated meat scored significantly lower than the control.

Other Factors

Chilling: Broth from half carcasses immersed in ice water for as little as 5 hours was found by Pippen et al. (1954) to have less flavor than broth from halves cooled in air. A subsequent study of the effect of chilling by immersion in ice water on chicken flavor was made by Pippen and Klose (1955). They demonstrated that loss of flavor resulted from ice water chilling, and that part of the decrease in flavor was traceable to the loss of neutralized ash by leaching. A comparison of broilers chilled for 21 hours by immersion or with "spin-chill," first in water then in water plus ice by Kahlenberg et al. (1960) resulted in a 22.7% gain by "spin" and a 13% gain by immersion. No significant

difference was noted in the flavor of thigh meat. Greater losses of flavor occurred in liquid-chilled poultry as compared to air-chilled according to Hurley et al. (1958). After water leaching by par-boiling, no detectable differences in roasted meat flavor were noted between either chilling procedure.

Sex and Age: A notable difference in flavor between sexes was demonstrated by Gilpin et al. (1960). Males yielded more meat and were tastier, but females yielded a higher percentage of breast meat and fat. The age factor was found to have an important effect on flavor in poultry by Peterson et al. (1959). These workers found the dark muscles were more flavorful from old hens than that from 3 month old pullets. Also with older birds, the dark muscles were more flavorful than the breast muscle. A comparison of freeze-dehydrated breast muscle from 28 month-old, 19 week-old and 9 week-old White Leghorn females by Wells et al. (1962) indicated that although color was best in the 19 week-old birds, tenderness and flavor were best in the 28 month-old birds.

Aging and Handling: Scalding caused flavor loss and toughening in poultry according to Wise and Stadelman (1961), but could be reduced by aging the birds for 24 hours. Greater cooking losses resulted in lowered flavor scores, and were shown by Ziolecki (1963) to increase from 17.8 to 22.8%, when chickens were aged 24 hours as compared to 7 days at 2-4°C.

De Fremery and Pool (1960) demonstrated that mechanical handling resulted in a rapid onset of rigor and excessive losses of ATP. They also showed that muscles became tough and glycogen was lost more quickly by any treatment which caused rapid lowering of pH. Working with red meats, Briskey and Wismer-Pedersen (1961) reported that rapid chilling slowed the rate of glycolysis and retarded pH change.

## MATERIALS AND METHODS

### Chickens

For the first phase of this study, old and young laying hens of known origin, which had been raised on identical rations (Appendix Table 1) were obtained. The birds were slaughtered, dry-picked and eviscerated. The entire carcass was ground in a meat grinder. Four of the laying hens were used; whereas, seven of the young hens were required to give approximately an equal weight of meat after grinding (8.25 lbs.). In order to preclude bacterial spoilage, the meat was frozen immediately after grinding.

The young hens, which were used in the preliminary chemical identification of cooked volatiles, were prepared as described above. After 20 old laying hens had been killed, the intestinal contents were collected (1.5 lbs.) and saved for subsequent analysis. The old hens were then processed in the same manner as described earlier. The intestinal contents were used for subsequent studies, in which attempts were made to relate the volatiles from the cooked intestinal contents with those obtained from eviscerated ground whole carcasses.

Fresh fryer breasts of unknown origin were purchased from a retail store, skinned and boned. They were subjected to gas chromatography and used for the determination of some of the gaseous components, including oxygen, nitrogen and carbon dioxide together with some lower alkane hydrocarbons.

Sixty heavy weight hens (Cornish cross hens), 65 light weight hens (White Leghorn hens), and 65 roasters (Cornish cross males) were selected

from known sources. All of the birds were raised and maintained on standard commercial rations to an age of 16 months for the hens and 16 weeks for the roasters. Evisceration was carried out in the usual manner, after which the birds were divided into two separate lots. One of these lots was used for yield studies and the other for identification of chemical components.

Thirty-five heavy weight hens, 35 light weight hens and 35 roasters were selected for the yield studies. The birds were packed in ice and held 14 hrs. at 30°F for further processing.

The remainder of this group of birds consisting of 25 heavy hens, 30 light hens, and 30 roasters were used for the identification of chemical components. The birds were packed in ice and held at 30°F overnight before preparing them for frozen storage. The birds were then de-iced, packaged as individual whole carcasses, and sealed individually under vacuum in Cryovac bags. Each bird was tagged for identification purposes. The birds were then separated according to class and placed on the freezer racks to be individually quick-frozen at -30°F. The birds were next crated according to class in marked crates and stored at -10°F for further processing.

Prior to analysis, the frozen birds were unpacked, sawed into halves at 60°F., and then the frozen halves were sawed in a manner that gave a crude separation of light from dark meat. Skin was removed after partial thawing. Kidney fat was removed. Then the bones, tendons and veins were cut away to give a careful separation of light from dark muscle. After grinding, the two kinds of muscle from each of the three classes

of birds were weighed as individual 100 g. portions and packaged in 4 x 6 inch Cryovac bags. The bags were sealed to preclude air, tagged individually for identification, quick-frozen at -30°F, and stored at -10°F for subsequent analysis.

This group of birds was used for the following purposes: (1) to compare gas chromatograms of the cooked volatiles from light and dark muscle; (2) to identify sulfur compounds and functional groups present in the cooked volatile fractions of light and dark muscle; (3) to tentatively identify a phosphatidyl component in a "chicken essence" distillate; (4) to determine the diacetyl/acetoin and inosinic acid contents of raw light and dark muscle from the three classes of birds; and (5) to also determine pH, and possible precursors or precursor indicators including creatine, creatinine, cystine, methionine, sulfhydryl (glutathione equivalent) and inorganic sulfide in both light and dark raw muscle and the cooked-freeze-dried meat-broth slurry for all three classes of birds.

After cooking and distilling 2 kg. of muscle and 3 l. of deionized distilled $H_2O$ slurry in a 12 l. flask for 50 hrs. at 180°F (Variac at 60), the flask contents were emptied into an 18 qt. stainless steel pail and mixed by hand. When the mixture containing broth, collagenous and fibrous residues became homogeneous, it was poured into two 12 x 12 x 2 inch stainless steel trays to a depth of 1 inch. The trays were covered with aluminum foil, cooled to room temperature and placed in a freezer overnight at -10°F. Then the trays were put on separate shelves in a Stokes Model 2003 F-2 freeze-dryer and dried 36 hrs. at a tray temperature of 42°C. Vacuum in the freeze-dryer compartments was maintained

between 75-100 u Hg. In this manner the cooked-freeze-dried meat-broth slurry aliquots of light and dark muscle from roasters, heavy- and light-weight hens were obtained for the study of changes in precursors in raw meat which occurred after prolonged cooking. The freeze-dried muscle-broth aliquots were friable and broke up when packaged in Cryovac bags and were held for analysis at -10°F.

Proximate Analysis

Moisture: Moisture was determined by placing 10 g. tissue in a loosely covered aluminum dish (75 mm. dia. x 15 mm.), and drying to a constant weight for 24 hrs. at 95°C. Moisture was calculated using the following formula:

$$\% H_2O = \frac{\text{original sample wt. - sample wt. after drying}}{\text{original sample wt.}} \times 100$$

Ether Extract: The dried sample obtained after moisture analysis was weighed into a Soxhlet extraction thimble of a porosity that permitted rapid extraction of the sample with anhydrous ether. Complete extraction of the fat from the tissue required 16 hours in a Soxhlet Extractor at a solvent condensation rate of 4 drops per second. The extracted tissue was dried 30 min. at 100°C, cooled in a desiccator and weighed (A.O.A.C. 1960). Ether extract was calculated using the following formula:

$$\% \text{ ether extract} = \frac{\text{sample wt. after drying - sample wt. after ext'n.}}{\text{original sample wt.}} \times 100$$

Protein: The Kjeldahl method followed the procedure outlined by Benne et al. (1956). A sample of tissue weighing 1.5-2.0 g. was weighed on 2 x 2 inch vegetable parchment paper. The paper was then rolled

around the meat and dropped into a Kjeldahl flask. For digestion of the meat, the HgO or Hg used in the A.O.A.C. (1960) method was replaced with 1 g. $CuSO_4$. Five grams of anhydrous $Na_2SO_4$ and 40 ml. of $H_2SO_4$ (N-free -93-98%) were added. The flask was heated gently until frothing ceased, and was boiled briskly until the solution cleared and then for an additional hour. Before distilling, 200 ml. of $H_2O$ were added to the digestion mixture, and the mixture was cooled by placing the flask in the cooler for 2 hrs. Then 4 drops of mineral oil and a few granules of mossy Zn were added. The flask was tilted and 100 ml. of a pre-cooled 50% NaOH solution was added to form a layer without inter-mixing. The flask was immediately connected to the distilling bulb on the condenser. The tip of the condenser was immersed beneath the surface of the standard acid in the 250 ml. Erlenmeyer receiver, which contained 50 ml. of 0.2N $H_2SO_4$ and 2 drops of methyl red indicator. The excess standard acid was back titrated with standard 0.2N NaOH. Protein was determined by multiplying the number of g. of nitrogen by 6.25 and expressing as percentage protein in the meat.

Ash: Two grams of tissue were weighed into a porcelain crucible and placed in a muffle furnace that had been preheated to 600°C. It was held at this temperature 2 hr. The crucible was transferred directly to a desiccator, cooled and weighed. Ash was calculated as follows:

$$\% \text{ Ash} = \frac{\text{sample wt. after ashing}}{\text{original sample wt.}} \times 100$$

Creatine and Creatinine: Several colorimetric methods for determining creatine and creatinine were compiled by Snell and Snell (1954). The

method chosen for this study was that of Peters (1942). However, the tissue samples were prepared for colorimetric analysis by the water extraction method described by A.O.A.C. (1960).

A 10 g. sample of tissue was weighed into a 150 ml. beaker, 10 ml. of cold (15°C) deionized-distilled $H_2O$ were added and stirred to a homogeneous paste. Then 50 ml. cold $H_2O$ were added, and stirred at 3 min. intervals for 15 min. After standing 2-3 min., the liquid was decanted through a quantitative filter, and the filtrate was collected in a 500 ml. volumetric flask. The beaker was drained by pressing the liquid out of the meat fibers with a glass rod. Fifty ml. of cold $H_2O$ were added to the fiber residue. It was stirred for 5 min., allowed to stand for 3 min., and decanted as before. The fibers were removed from the filter and returned to the beaker with a glass rod. Extractions of the fiber residue were repeated using two 50 ml. and four 25 ml. portions of cold $H_2O$. After the final extraction, the entire fiber residue was transferred to the filter and washed with three 10 ml. portions of $H_2O$, allowing time to drain between washings. The filtrate was diluted to the 500 ml. mark and mixed thoroughly.

A 150 ml. aliquot of the extract was measured into a 250 ml. beaker and evaporated to 40 ml. on a steam bath with occasional stirring. The solution was neutralized using phenolphthalein to spot test small aliquots. Chromogens were removed by adding 1 ml. of 0.1N acetic acid and boiling gently for 5 min. The coagulum was filtered on quantitative filter paper, and the beaker was washed 4 times with hot $H_2O$. The coagulum on the

filter was washed 3 times and the residue was discarded.

For duplicate measurements of both creatine and creatinine, 5 ml. of the diluted extract were placed in a flask, and 40 ml. N/12 $H_2SO_4$ and 5 ml. sodium tungstate solution were added. The mixture was shaken thoroughly, and the protein was removed by filtering. Eight ml. of the protein-free tungstic acid filtrate were measured into each of 4 colorimetric tubes, while 8 ml. of $H_2O$ were measured into a fifth tube as a blank. The mouths of two of the tubes were covered with aluminum foil, and the tubes were autoclaved for 45 min. at 15 lbs. pressure. The tubes were removed from the autoclave and cooled to room temperature. A fresh solution of alkaline picrate was prepared (important to prepare just 5 min. prior to use) by adding 1 volume of 10% NaOH to 5 volumes of the picric acid solution.

Four ml. of the alkaline picrate solution were added to each of the 5 tubes (the 2 which had been autoclaved, the 2 containing unautoclaved filtrate and the water blank). The tubes were set aside for 30 min. to permit complete development of color before colorimetric readings were taken. After color development, the readings were taken in a Model 6A Coleman spectrophotometer at 490 mu. The blank tube was read first with the galvanometer set to give a transmittance reading of 100. The colors of the other tubes were then read in the usual manner.

The galvanometer readings were converted to creatinine by interpolation on a curve (Fig. 1) constructed by plotting readings for standard solutions of creatinine zinc chloride (Hawk and Bergeim 1937). The standard semi-logarithmic curve was obtained by plotting percent trans-

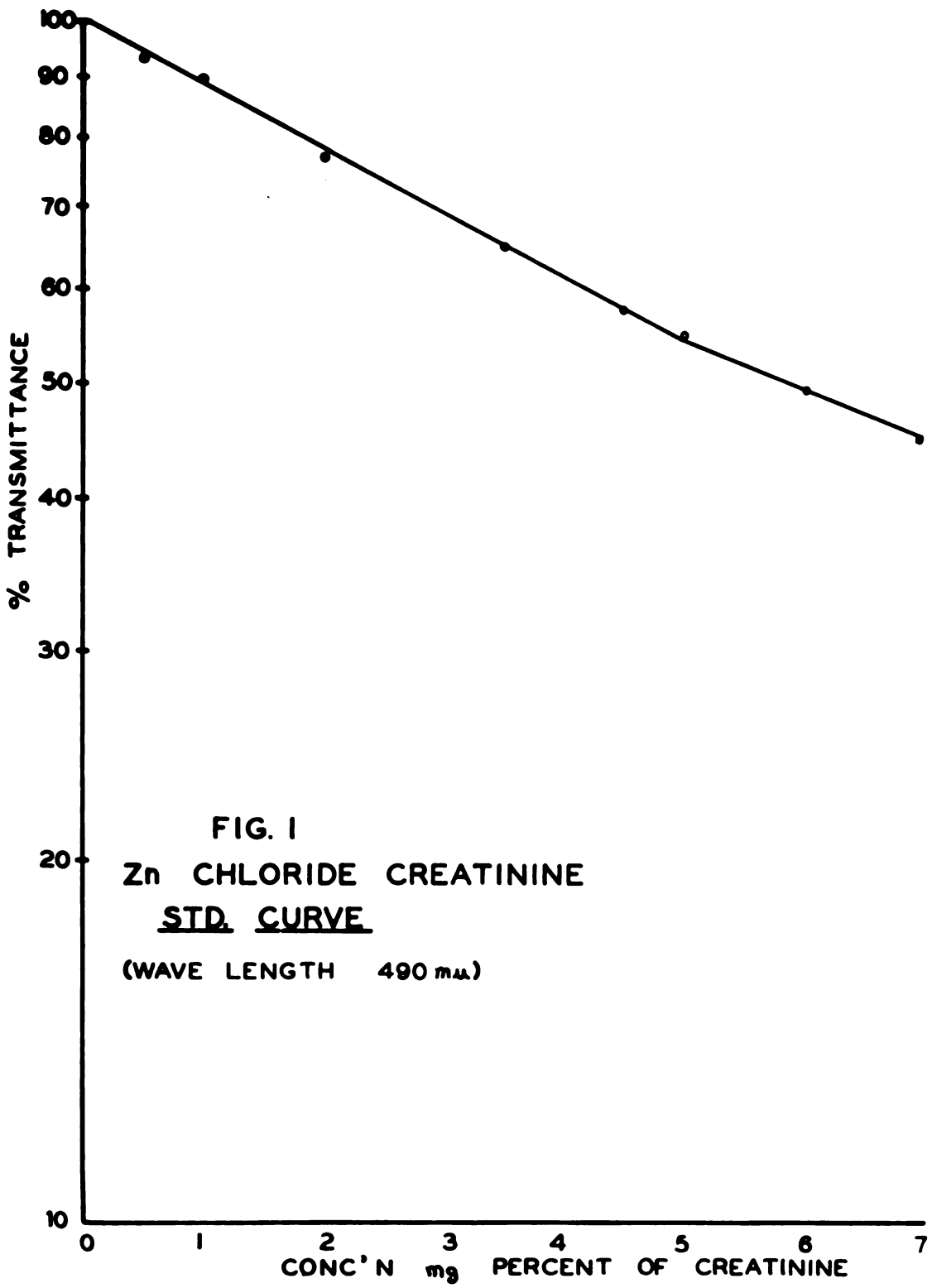



FIG. 1

Zn CHLORIDE CREATININE

STD. CURVE

(WAVE LENGTH 490 mμ)

mittance against concentration of creatinine zinc chloride in mg. percent of creatinine. The difference between readings of autoclaved and unautoclaved samples represented creatinine in terms of creatine. This was converted to creatine by multiplying by the factor 1.16. Dilutions of the tissue extract were made so that readings could be taken from the curve in the concentration 1 to 5 mg. percent. In this range, the accuracy was ± 4%. At concentrations higher than 5 mg. percent, the curve gave larger deviations.

Diacetyl and Acetoin: Spectrophotometric determinations of the diacetyl and acetoin contents of raw-frozen light and dark muscle samples were made by the method of Prill and Hammer (1938) as modified by Stotz and Raborg (1943) and Pippen et al. (1960).

For diacetyl, a 50 g. sample of tissue was weighed into a 250 ml. beaker, and 50 ml. of deionized-distilled $H_2O$ were added while stirring with a glass rod to make a uniform slurry. The slurry was transferred to a 500 ml. distillation flask and the beaker was rinsed with 10 ml. $H_2O$, which was likewise added back to the slurry. Several granite chips were added to prevent bumping. The flask was connected to the distillation apparatus and an inert atmosphere of carbon dioxide was maintained in the flask during a 3 hr. refluxing period. Steam was introduced slowly below the surface of the sample, and 5.0 to 5.2 ml. of distillate were collected during 25-30 min. Collection of the distillate in 1 ml. of hydroxylamine acetate solution facilitated the partial conversion of diacetyl to dimethylglyoxime. The stoppers of the tubes of distillate were loosened and the tubes were heated in a water bath at 85°C for 1 hr. to complete the for-

mation of the dioxime. The tubes were then removed from the water bath and while still warm, 1 ml. of acetone-disodium phosphate was added to each and allowed to react for 30 min. This removed any excess of hydroxylamine. Then 0.3 ml. of ammonium hydroxide and 2.2 ml. of saturated tartrate solution were added to each of the tubes, which were then stoppered and inverted repeatedly to facilitate mixing. Then 0.2 ml. of ferrous sulfate solution was added to each tube and mixed by inverting the tubes. Thirty minutes were required to develop the color. A reagent blank was prepared by adding 5 ml. deionized-distilled $H_2O$ to an equivalent amount of reagents. The blank was treated in the same manner as the diacetyl tubes. After color development, readings were taken in a Model 6A Coleman spectrophotometer at 530 mu (Krishnaswamy and Babel 1951). The blank tube was used to adjust the galvanometer to give a transmittance of 100. The colors of the other tubes were then read against the blank. The concentration of diacetyl was measured as ammono-ferous dimethylglyoxime by referring to a standard semi-logarithmic curve prepared for the spectrophotometer (Fig. 2).

Acetoin plus diacetyl was determined using a 50 g. sample of tissue, which was weighed into a 250 ml. beaker. Then 60 ml. of a 40% ferric chloride solution was added gradually with stirring to facilitate thorough mixing. The mixture was carefully transferred to a 500 ml. distilling flask and refluxed for 3 hrs. to oxidize acetoin to diacetyl. Due to the high concentration of diacetyl, 10.0-10.5 ml. of distillate were collected in 2 ml. of hydroxylamine acetate solution by careful steam distillation for 25-30 min. The development and measurement of the color were the same

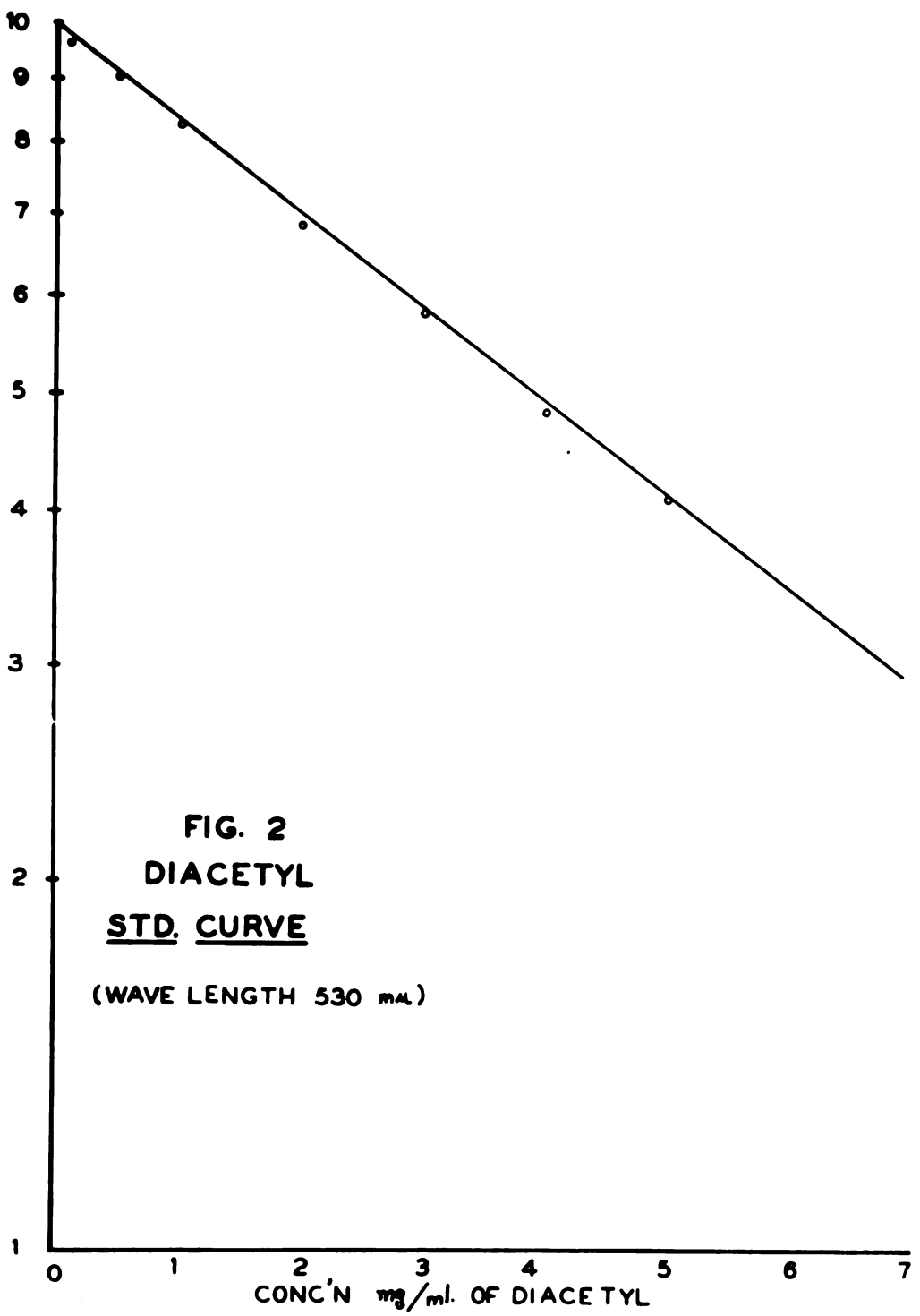



FIG. 2

DIACETYL

STD. CURVE

(WAVE LENGTH 530 mμ)

as in the diacetyl measurements, except that the amount of reagents were doubled throughout.

Inosinic Acid: Inosinic acid was determined for the raw, light and dark muscle samples using a semi-quantitative spectrophotometric method suggested by Kazeniac (1963). After cooking and distillation of the raw muscle slurry, the collagenous material coagulated and floated to the top of the solution. The fibrous material collected at the bottom of the flask and a comparatively clear broth formed an intermediate layer. A 50 ml. aliquot of the clear broth was pipetted off, and the slight haze which remained therein was removed by adding chloroform (v/v) and centrifuging. After one-half hour of centrifugation, the haze concentrated at the interface between the aqueous and chloroform layers. The clear supernatant was then pipetted into a cuvette and ultraviolet absorption was measured at 250 mu with a Beckman DU Model 2400 spectrophotometer using a hydrogen lamp. According to Kazeniac (1963), 90% of the absorbancy in chicken broth samples at 250 mu was due to inosinic acid. Absorbancies were compared with those of a standard curve for inosinic acid (Fig. 3). Values reported were based on the assumption that 90% of the nucleotide absorption at 250 mu was due to inosinic acid, and were corrected by calculation to a 100% inosinic acid equivalent basis.

Hydrogen Ion Concentration (pH): The pH measurements in this study were made with a Beckman Model G battery operated pH meter equipped with small glass and calomel electrodes, or with a Beckman Zeromatic line-operated pH meter using a standard calomel half-cell and glass electrode. The instruments were standardized with reference buffer solutions to read

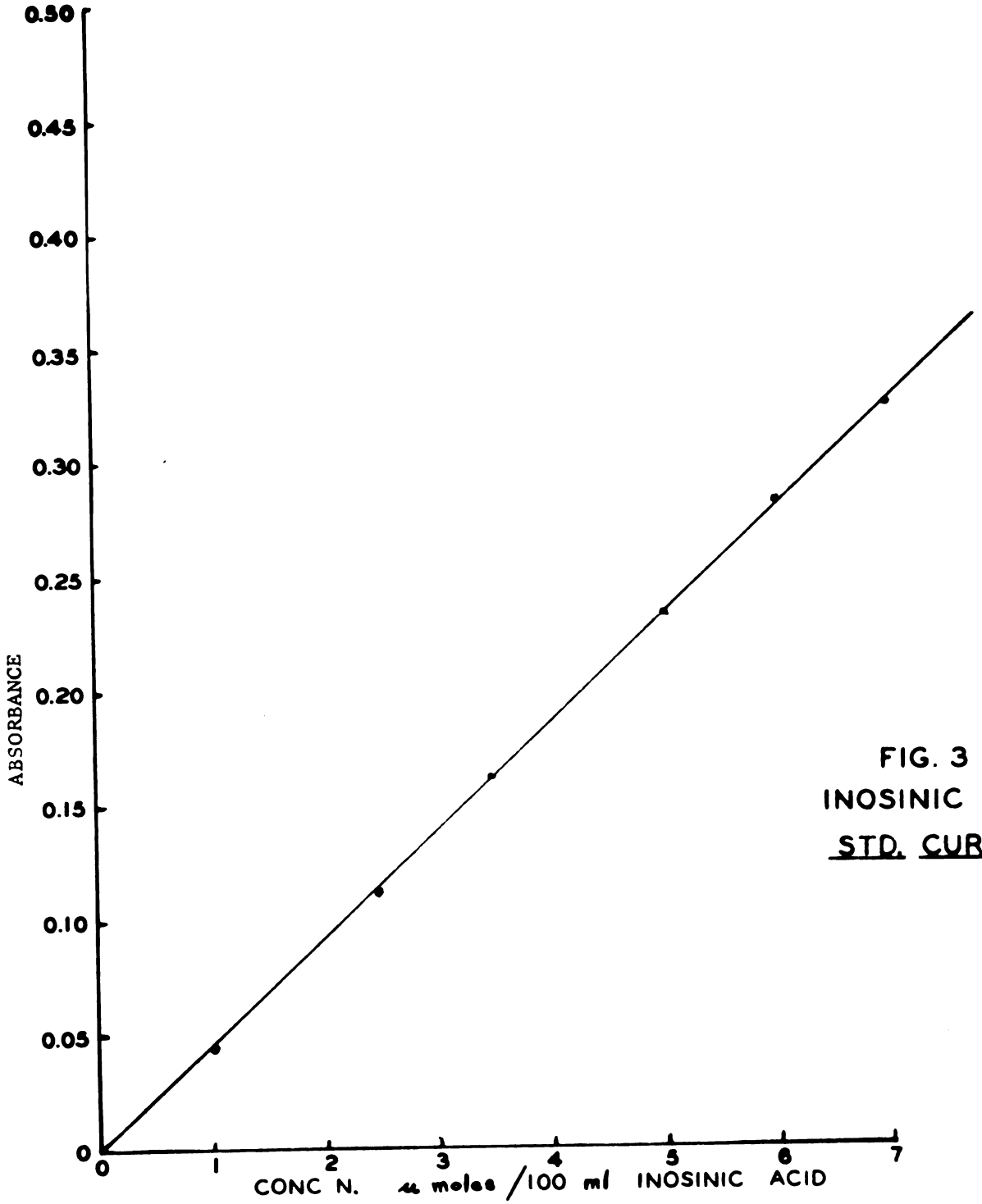



FIG. 3
INOSINIC ACID
STD. CURVE

accurately in the range of pH 6.8-7.0 with the temperature adjustment knob set at that of the test solution. The results were expressed to the nearest one-tenth pH unit.

Inorganic Sulfide Determination: Sulfide determinations were made on the raw-frozen and cooked-freeze-dried light and dark muscle samples, by the spectrophotometric method of Sands et al. (1949). A 10 g. sample of tissue was extracted with water by the procedure used in the creatine/creatinine determinations. A 25 ml. aliquot of the water extract was added to 25 ml. of zinc acetate solution. Then 5 ml. of the diamine reagent were added and stirred. One ml. of ferric chloride solution was stirred in next. The resulting blue color was allowed to develop for 15 min. before reading the absorbance at 745 mu. A standard curve was developed using standard sulfide solutions (Fig. 4). Readings were made on a Model 6A Coleman spectrophotometer at 745 mu by comparing the transmittance readings with a reagent blank set at 100% transmittance.

Cystine and Methionine: Cystine/methionine contents of light and dark muscle from raw, frozen roasters, heavy- and light-weight fowl and from the cooked-freeze-dried slurries of these same birds were determined by the microbiological assay method of Henderson and Snell (1948). The method utilizes specific strains of lactic acid producing bacteria with a single medium, which is made deficient in any single amino acid. The method can be used to determine any of 14 different amino acids titrimetrically. The colorimetric method of Friedmann and Graeser (1933) as modified by Barker and Summerson (1941) was also used for determining cystine and methionine values.

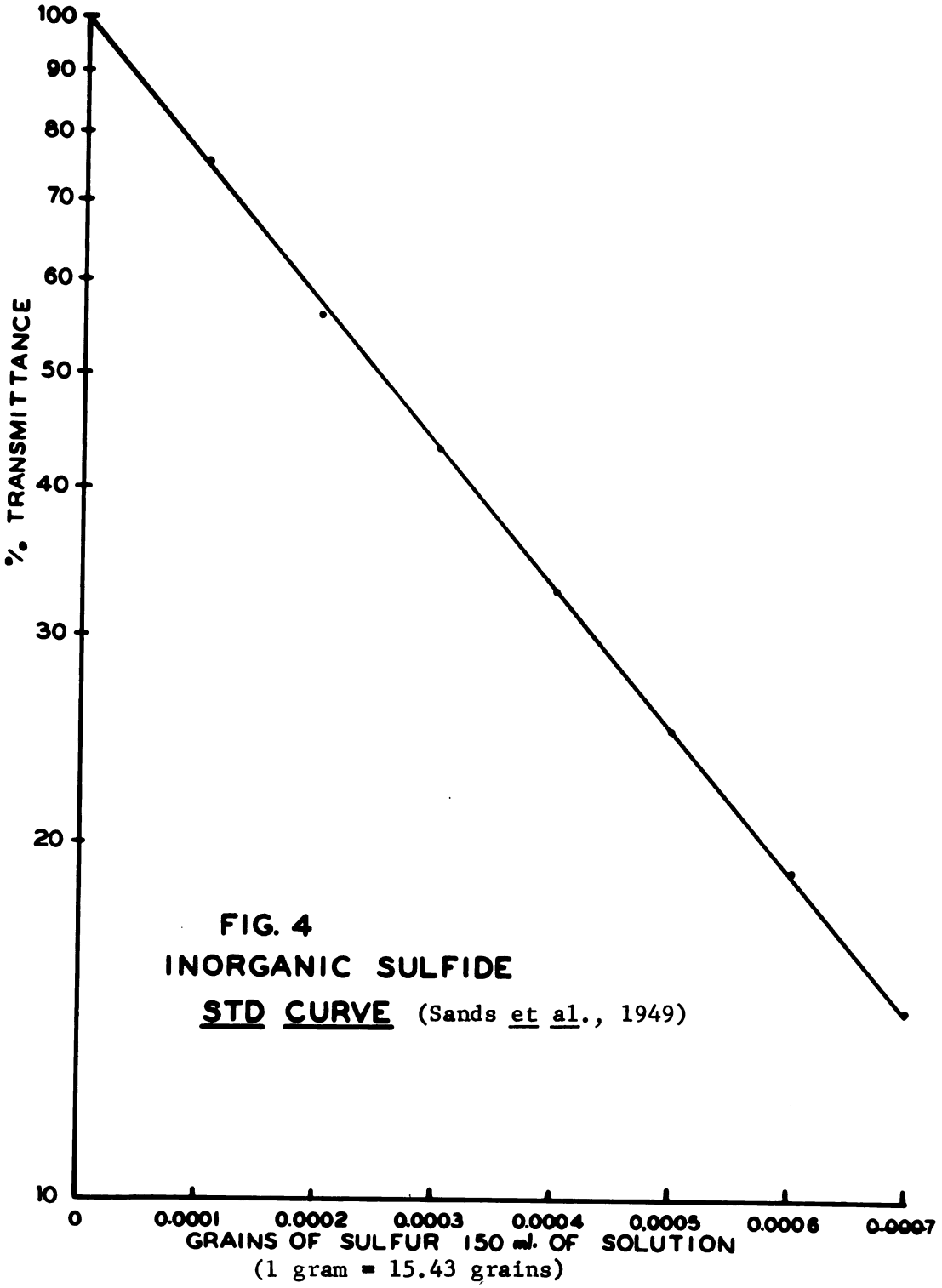



FIG. 4
INORGANIC SULFIDE
STD CURVE (Sands et al., 1949)

Assay media were obtained from Digestive Ferments Co., Detroit, Michigan. The media were specific, with one being deficient in cystine and the other in methionine. A stock culture of Leuconostoc mesenteroides P-60, ATCC 8042 was obtained from the American Type Culture Collection, 2029 M. St. N.W. Washington, D. C. Stab cultures and inocula were maintained and grown by standard procedures (A.O.A.C. 1960). Duplicate tubes containing 0, 0.2, 0.4, 0.6 and 1.0 ml. of standard solutions of L-cystine and L-methionine were used to construct titrimetric and colorimetric standard curves. One or 10 g. raw, frozen or cooked-freeze-dried meat slurry were hydrolyzed by refluxing with 2 N HCl overnight for cystine determinations or with 3 N HCl for 24 hrs. for methionine determinations. The hydrolysates were neutralized carefully to pH 6.8 and diluted as necessary to conform with the makeup of the standard curves. Then from 0.2 to 2.0 ml. were added to 5 or 10 incubation tubes, respectively. Titrimetric determinations were made using a Beckman Automatic Titrator and checking the end point at pH 6.8 with bromothymol blue indicator. Milligrams of cystine or methionine per gram of original samples were calculated from ml. 0.0987 N KOH used by referring to the standard curves for cystine and methionine (Figs. 5 and 6). Colorimetric determinations were made using a Spectronic 20 colorimeter set at a wavelength of 560 mu. Percentage transmission values for the various samples were read off from the standard curves for cystine and methionine (Figs. 7 and 8). The milligrams of cystine or methionine per gram of original sample were then calculated.

Sulfhydryl Content: The sulfhydryl content of the raw-frozen light and dark muscle of roasters, heavy- and light-weight hens and the cooked-

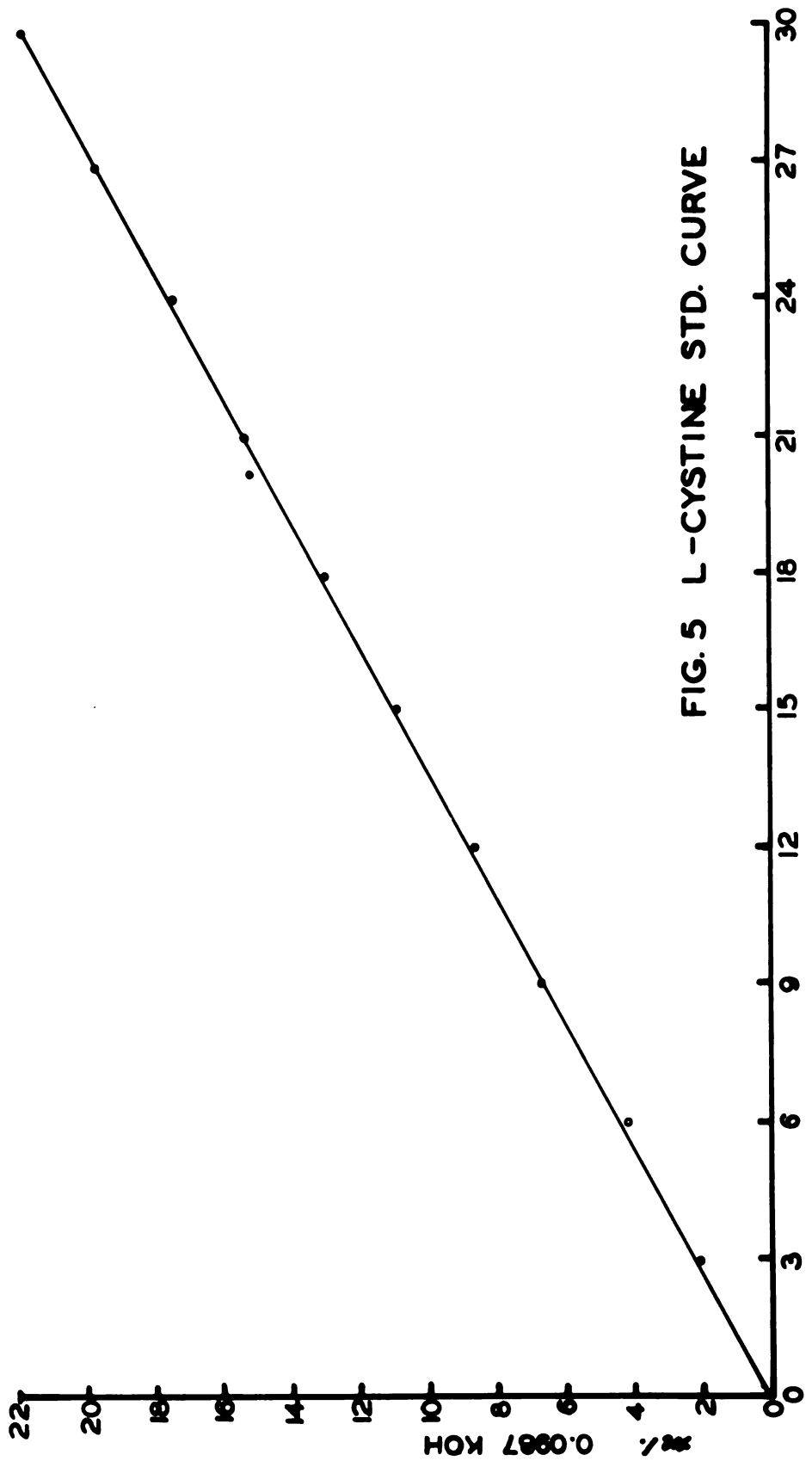



FIG. 5 L-CYSTINE STD. CURVE

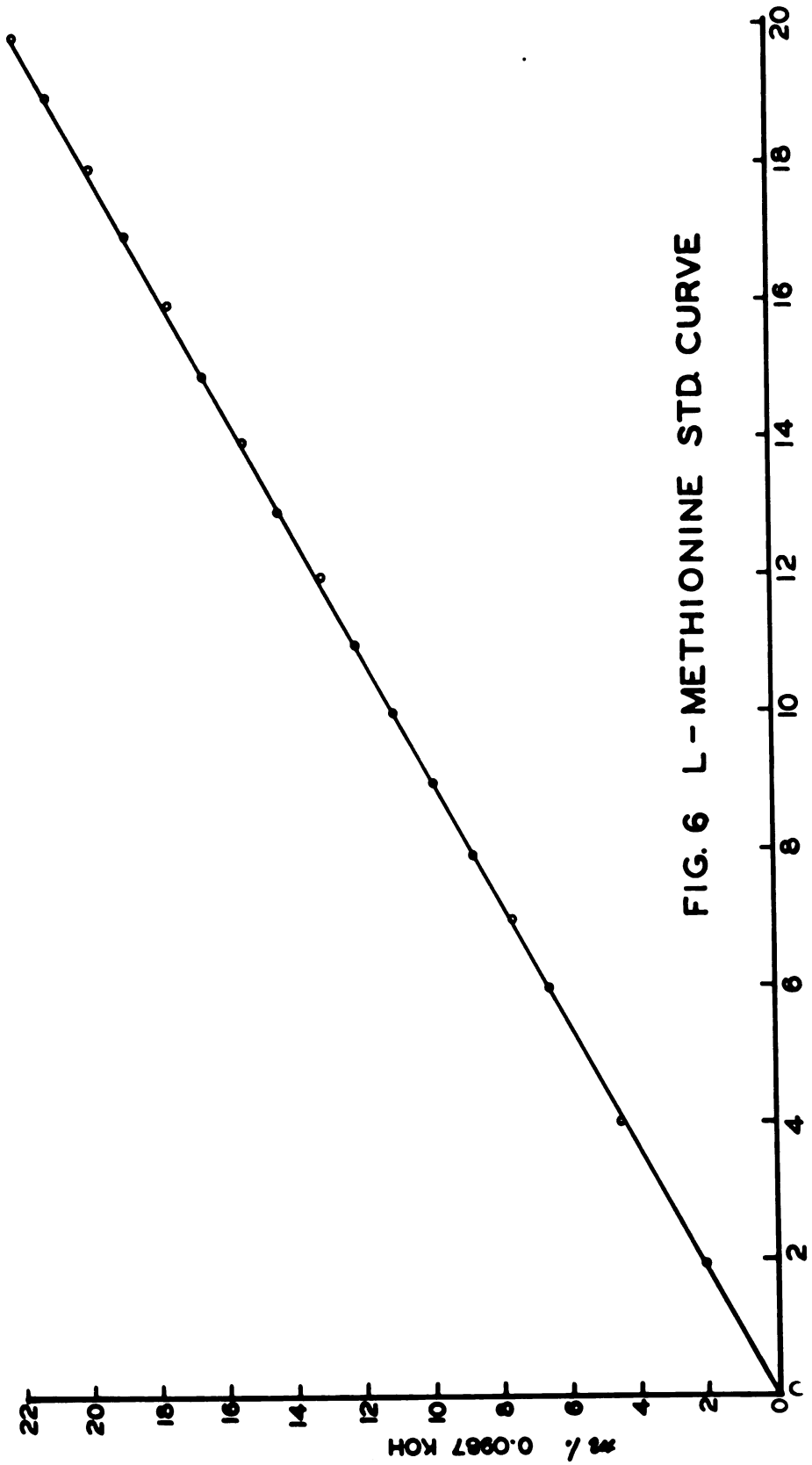



FIG. 6 L-METHIONINE STD CURVE

FIG. 7

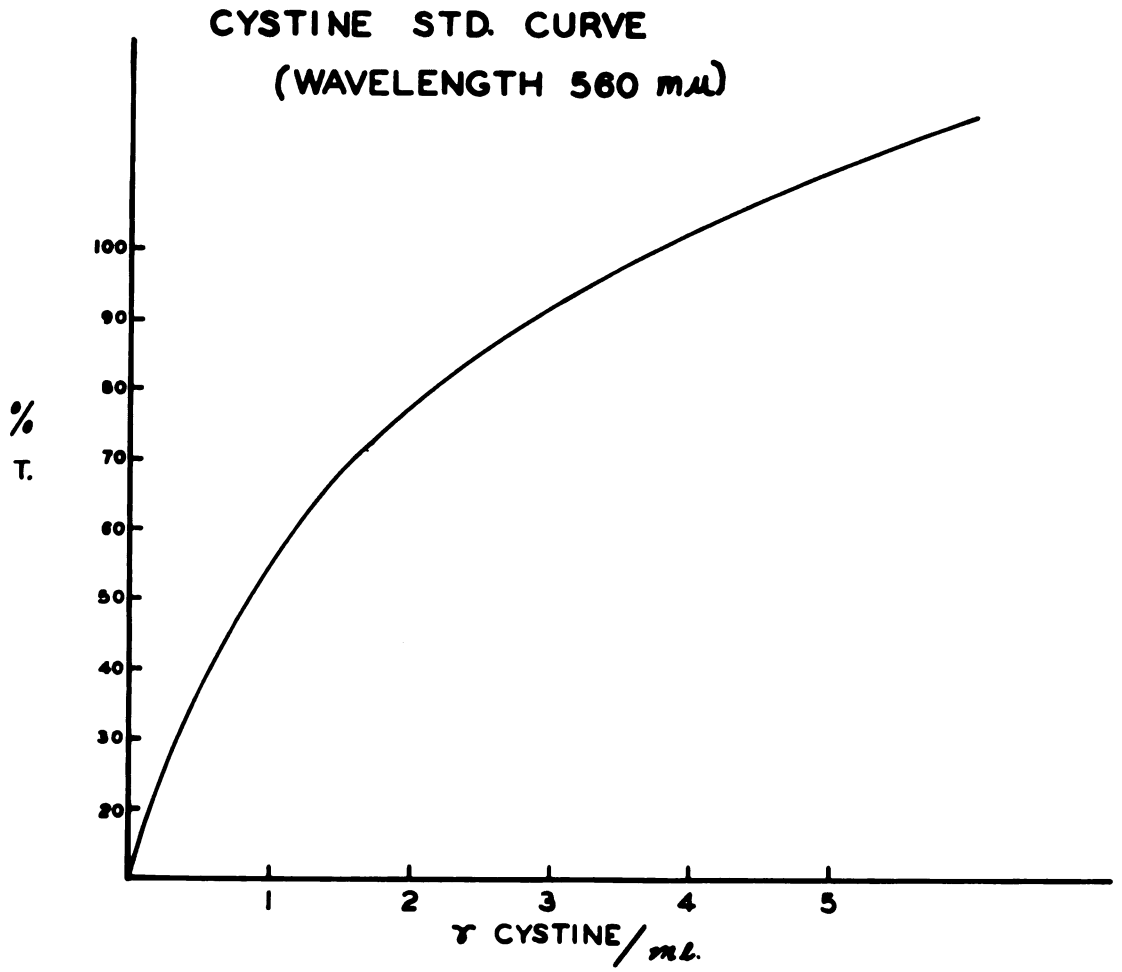
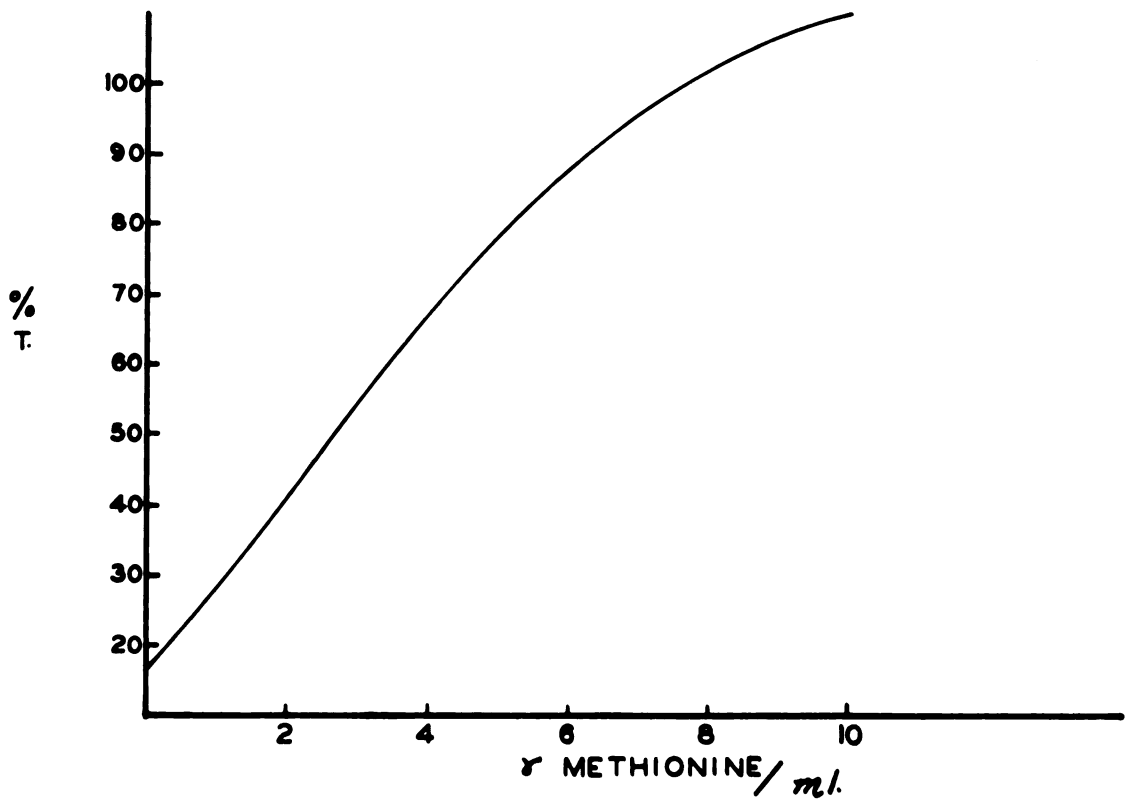

CYSTINE STD. CURVE
(WAVELENGTH 560 mμ)
%
T.
100
90
80
70
60
50
40
30
20
1
2
3
4
5
γ CYSTINE/ml.

FIG. 8

METHIONINE STANDARD CURVE

(WAVELENGTH 560mμ)



freeze-dried meat-broth slurries from these same birds was determined spectrophotometrically by the method of Grunert and Phillips (1951) as modified by Batzer and Doty (1955). A standard curve was constructed in the range of 10 to 200 ug of glutathione per 10 ml. of solution. The color intensity was directly proportional to the concentration of glutathione. Absorbance was plotted against glutathione concentration.

In order to decrease the possibility of errors due to non-homogeneity of the sample, a relatively large sample was used. Either 10 g. of meat or of dried slurry was mixed in a Waring Blendor with 60 ml. cold 3% metaphosphoric acid and 20 ml. cold distilled $H_2O$ for 1 min. About 30 g. NaCl was added for saturation and the mixture was mixed for an additional 1 min. The solution was centrifuged for 10 min. and the supernatant was filtered into a 100 ml. volumetric flask and made to volume with a saturated NaCl solution. Eight ml. of this solution were added to each cuvette, and allowed to stand for 10 min. to equilibrate. One ml. of 2% nitroprusside solution and 1 ml. of sodium carbonate-0.067M sodium cyanide solution were added. Each tube was read immediately in a Coleman Model 6A spectrophotometer at 520 mu. Two percent metaphosphoric acid solution saturated with NaCl was used as a blank. The standard curve (Fig. 9) was prepared from the appropriate amounts of reduced glutathione in a 2% metaphosphoric acid-saturated sodium chloride solution. Absorbance values were proportional to the amount of glutathione in the range of 0 to 200 gammas of glutathione in 10 ml. of final solution. Determinations were made in duplicate.

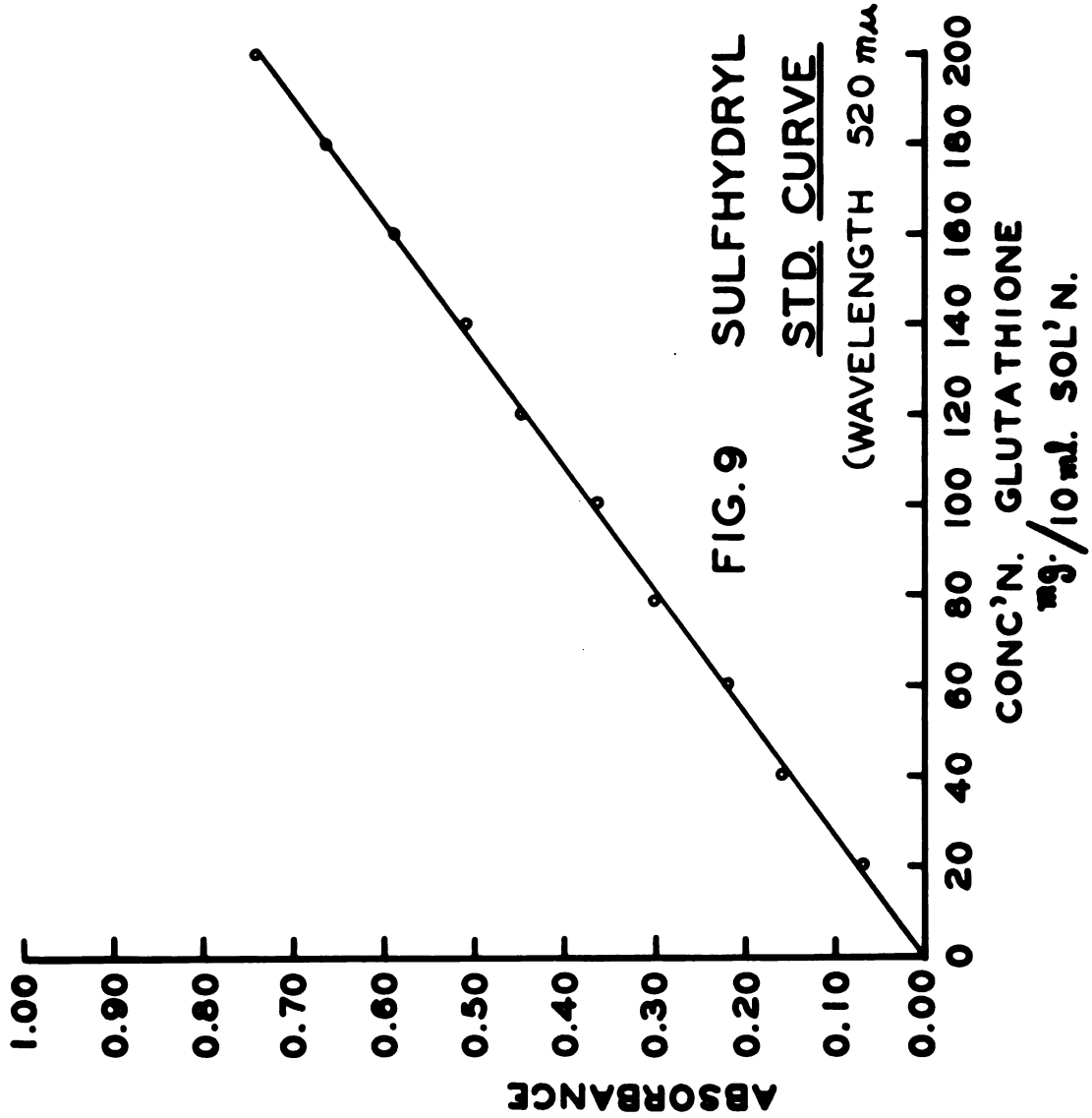


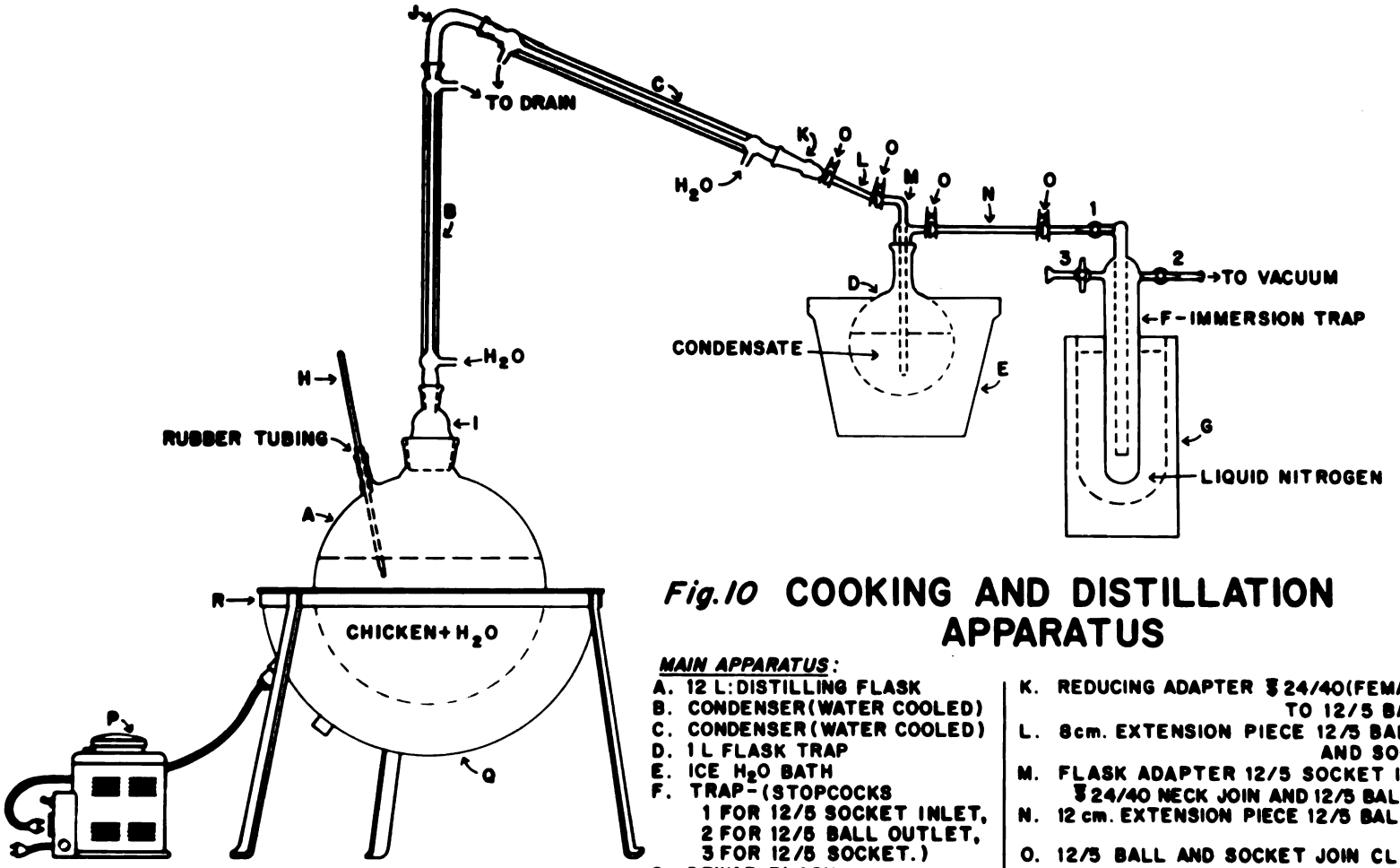

FIG. 9 SULFHYDRYL STD. CURVE (WAVELENGTH 520 mμ)

Gas Chromatography of Chickens Differing in Age

Within 1-2 days after freezing, 8.25 lbs. of the ground chicken mixture was placed in a stainless steel bucket and immersed in a hot-water bath. After adding 4 l. deionized-distilled $H_2O$, a smooth slurry was prepared by kneading the cold meat and warm water together by hand. This slurry was filtered into a 12 l. flask, and the flask was attached to the apparatus shown in Fig. 10. The slurry was cooked under influx at 102°C for 4 hrs. with the normal cooking conditions described by Pippen et al. (1958).

After cooking 4 hrs., the cooling water in Condenser B was shut off and distillation began. The distillate was collected in a 1 l. flask (Fig. 10 - Trap D) and cooled in ice-water. The volatiles, which passed through the ice-water trap were collected in a liquid nitrogen trap (Fig. 10 - Trap F). When 2/3 l. of watery distillate had accumulated, the ice-water cooling trap (Fig. 10 - Trap F) was disconnected at K.

A UV absorption test for carbonyls was made on the watery distillate by placing an aliquot of distillate in a cuvette and reading the O.D. maxima at 228 mu for monoenals and 282 mu for dienals (Kazeniac, 1961). Part of the absorption in the 228 mu range was likely due to sulfide, which according to Kazeniac (1961) also gives a maximum absorption in this region. No attempt was made to correct for sulfide as the main object was to verify that volatiles had been entrained by the watery distillate. The watery distillate was then subjected to vacuum and heated to 45-50°C in order to expel the volatiles into Trap F. After 25-30 min. under vacuum, distillation was stopped, and an aliquot was pipetted from the 1 l. flask into a cuvette for another UV absorption reading. This time the test was




## *Fig.10* COOKING AND DISTILLATION APPARATUS

***MAIN APPARATUS:***

A. 12 L: DISTILLING FLASK
B. CONDENSER (WATER COOLED)
C. CONDENSER (WATER COOLED)
D. 1 L FLASK TRAP
E. ICE $H_2O$ BATH
F. TRAP - (STOPCOCKS
1 FOR 12/5 SOCKET INLET,
2 FOR 12/5 BALL OUTLET,
3 FOR 12/5 SOCKET.)
G. DEWAR FLASK

***ACCESSORIES:***

H. THERMOMETER
I. REDUCING ADAPTER - ⅄ 55/50 OUTER TO ⅄ 24/40 INNER.
J. ELBOW ADAPTER ⅄ 24/40 (2-MALE)
K. REDUCING ADAPTER ⅄ 24/40 (FEMALE) TO 12/5 BALL.
L. 8cm. EXTENSION PIECE 12/5 BALL AND SOKET.
M. FLASK ADAPTER 12/5 SOCKET INLET, ⅄ 24/40 NECK JOIN AND 12/5 BALL OUTLET.
N. 12 cm. EXTENSION PIECE 12/5 BALL AND SOCKET.
O. 12/5 BALL AND SOCKET JOIN CLAMPS
P. VARIAC CONTROLLER.
Q. HEATING MANTLE.
R. HEATING MANTLE SUPPORT.

negative indicating that the volatiles had been transferred into Trap F (Fig. 11). The liquid nitrogen trap was then sealed by closing and tightening all three Teflon stopcocks.

Separation and Identification of $CO_2$, $H_2S$ and Carbonyl Sulfide: The vapor pressure differential between $CO_2$, $H_2S$ and carbonyl sulfide and the other volatile components was utilized in making the separation. A Clausius-Clapeyron plot of pressure temperature relationships of a typical condensate of a volatile meat fraction condensed at liquid nitrogen temperature was made by Merritt et al. (1959). This showed that hydrogen sulfide and carbon dioxide each have a vapor pressure of about 400 u and carbonyl sulfide 300 u at -140°C. At this same temperature the other components have vapor pressures of less than 1 u. The sealed trap (Trap 1) containing the total condensate was transferred from the liquid nitrogen bath into a Dewar flask containing a mixture of 2-2-4 trimethylpentane, dry ice and liquid nitrogen, which maintained a temperature of -140°C. An identical trap, which will be referred to as Trap 2, was first evacuated to a final pressure of less than 10 u and immersed in a liquid nitrogen bath (-196°C). To effect the transfer of low boiling components from Trap 1 at -140°C to Trap 2 at -196°C the following steps were taken: (1) The socket joint of Trap 2 which was controlled by stopcock 3, was connected to the ball joint of Trap 1 (stopcock 2) by means of a 12/5 ball and socket clamp. When the traps had equilibrated to -140 and -196°C, respectively (15 min.), stopcocks 2 and 3 were opened. This allowed the low boiling fraction consisting of $CO_2$, $H_2S$, carbonyl sulfide and possibly some low boiling hydrocarbons to be drawn into the liquid nitrogen trap

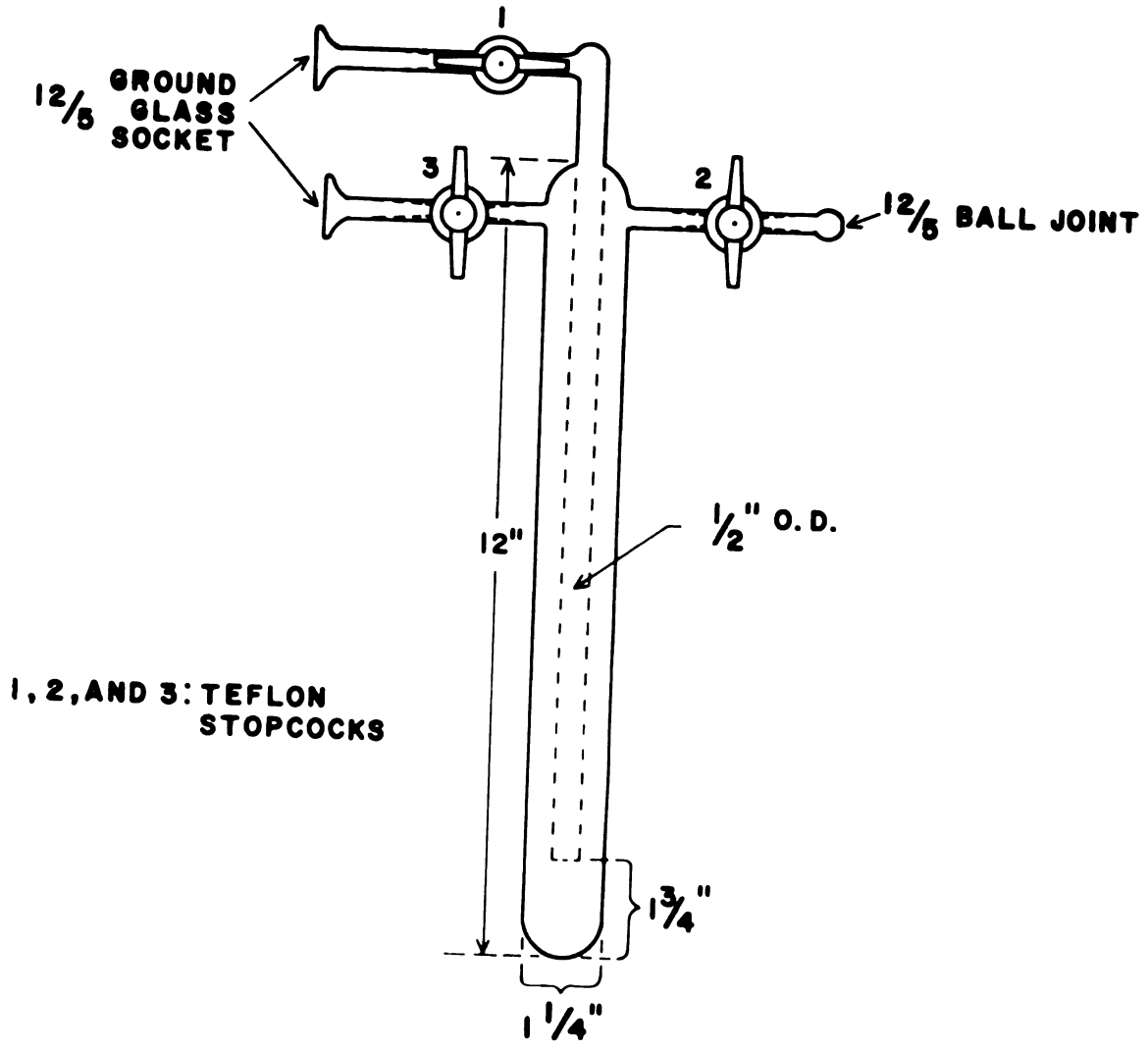



*Fig. 11* LIQUID-$N_2$-DIPPED TRAP

and condensed at -196°C. The traps were then resealed and disconnected as the separation had been effected.

Three cold-finger type traps having internal dimensions of 1 cm. X 6 cm. and made up with 12/5 ground glass ball and socket joints and containing the appropriate reagents were joined together in series by 12/5 ball and socket clamps. The first trap contained 3 ml. of 2% cadmium acetate solution (pH 6.5); the second contained 3 ml. of 0.01M $NaHCO_3$ solution; and the third contained 3 ml. of saturated CaO solution. This series of small traps was equilibrated at 0°C in an ice bath for 15 min. and joined to the large trap, which contained the low boiling fraction (-196°C) at the ball joint controlled by stopcock 2 using a 12/5 ball and socket clamp. Nitrogen gas was used to purge the gases from the large trap and provide ebullition through the three small absorption traps. A ball joint was connected to the nitrogen controller by gum-rubber tubing and to the large trap by a 12/5 ball and socket clamp at the socket joint controlled by stopcock 3. When stopcocks 2 and 3 were opened the gas flow rate was adjusted to 5 ml./min., and after 4 hrs. of purging the removal of the gases from the large trap was completed.

Gas Chromatographic Analysis: The volatiles remaining in Trap 1 were subjected to gas chromatographic analysis. The ball joint controlled by stopcock 2 was covered with a rubber surgical seal which was secured with a 12/5 clamp. Samples were taken by inserting the needle of a 10 ml. Hamilton gas-tight syringe through the surgical seal and momentarily opening stopcock 2. A uniform sample was obtained by filling and emptying the syringe several times to thoroughly mix the gases inside the trap

before removing the 10 ml. sample. After the sample was removed from the trap, it was injected immediately through the silicon rubber seal which covered the injection port of the Beckman GC 2 Gas Chromatograph which was attached to a Bristol recorder. Volatiles from old laying hens and younger chickens were separated on a 1/4 inch x 13 ft. copper column packed with 5% Di-(2 ethyl-hexyl) Sebacate (liquid phase) on Haloport F (solid phase) using helium gas as a carrier at a flow rate 36 and 50 ml./min. A thermal conductivity cell detector was used. Chromatograms were obtained for samples taken at room temperature, and after warming the trap to 75°C.

Gas Chromatographic Comparison of Total Cooked Volatiles from the Carcass and Intestinal Content of Old Hens

Cooking and distillation of the carcass was in the manner described in the previous experiment. The 1.5 lbs. of intestinal contents obtained was mixed with 500 ml. of distilled water and cooked under reflux in a 3 l. flask. Otherwise the cooking and distillation apparatus was identical to that shown in Fig. 10 for trapping volatiles in a liquid nitrogen bath (trap F). The set-up for chemical analysis will be described later in the appropriate section.

Sampling was done in the same manner used in the study of birds differing in age only. Some qualitative tests for sulfide, $CO_2$ and carbonyl sulfide were made on the low boiling fraction that was released at -140°C. The remaining volatiles in the trap were then analyzed by gas chromatography in the manner already described except that a hydrogen

flame detector replaced the thermistor type previously used and the sample size was reduced from 10 to 5 ml. Chromatograms were obtained for the whole carcass and intestinal content volatiles by warming Trap F to 70°C, sampling and chromatographing on the same column as before using a flow rate of 36 ml./min.

Gas Chromatographic Analysis of the Low Boiling Fraction in Fryers of Unknown Origin

A slurry consisting of 500 g. breast muscle and 500 ml. deionized-distilled $H_2O$ was pre-cooked and put in a pre-warmed 2 l. flask. Then the flask was connected to a high vacuum Buchler lyophilization apparatus. The flask was kept warm by heating water in the warming tray of the apparatus. The pressure in the system was reduced to 75 mm. Hg by a vacuum pump. The apparatus had a slight leak which caused an accumulation of liquid air in the liquid nitrogen trap. The trapping system consisted of 4 large cold-finger type traps having internal dimensions of 4 x 16 cm. These traps were joined in series between the lyophilization flasks and the vacuum source. The first two traps were immersed in an ethanol-dry ice bath maintained at -80°C. The second two traps were immersed in liquid nitrogen at -196°C. After 2 hrs. the vacuum was released, and the apparatus returned to atmospheric pressure. The liquid nitrogen traps contained considerable amounts of liquid air. The first trap was filled to a depth of 6 cm. Samples were removed directly from a liquid air layer by means of a 5 ml. gas-tight Hamilton syringe fitted with a special needle 12 cm. long. Two ml. samples were injected in a Perkin-Elmer

Vapor Fractometer, Model 154 B using an absorption column and a partition column in parallel. The first column was a Linde molecular sieve 5A 30-60 mesh packed into a 1/4 in. dia. copper column 1 m. long. The partition column was 1/4 in. dia. x 2 m. in length packed with 12 ml. hexamethylphosphoramide on 60 g. 30-40 mesh Chromasorb W. The columns were maintained isothermally at room temperature. The helium flow rate was 40 ml./min. Samples of 2 ml. of the volatiles (-196°C) were removed and injected immediately into the vapor fractometer.

Cooked Meat Yields from Roasters, Heavy- and Light-weight Hens

The birds were de-iced and weighed before cutting. The parts were removed from each bird and weighed. They were packed into 0.5 lb. tinplate C-enameled cans (404 x 700). The cans were sealed on a double-seamer, cooked 4 hrs. in a retort at 220°F, and then water-cooled. Each can was numbered to retain each bird's identity. Two cans were required for each roaster and heavy hen, and one can sufficed for each light hen.

After cooking, each can was opened, and the liquid (fat and broth) was drained onto a U.S. Std. stainless-steel sieve. Then the fat and broth were separated and weighed. Each of the cut portions was weighed individually after removal of skin and bone, which were also weighed. The skin from the breast and legs of the uncooked birds was weighed and discarded; whereas, skin reported for the cooked birds was obtained from the back, neck, and wings as this skin could not readily be removed before cooking.

Meat, bone and skin from the cooked birds was separated on a stainless-steel table in an air-conditioned room maintained at approximately

65°F and 45% relative humidity. Each separate component was weighed and the data recorded for the individual birds.

The data were submitted to analysis of variance. Calculations were made on the basis of both percentage and weight for the uncooked and cooked birds. Standard deviations were also calculated for each class of bird.

Method of Distillation and Thin Layer Chromatography of a Phospholipid Fraction Containing "Chicken Essence."

An experiment aimed at using smaller amounts of muscle for gas chromatographic and chemical analysis of cooked chicken volatiles failed. However, as a result of changing the distillation procedure an interesting fraction, later identified as a phospholipid, was recovered.

Cooking: A thick slurry was prepared from 300 g. heavy hen breast muscle and 200 ml. deionized-distilled $H_2O$. The slurry was prepared in the manner already described, and placed in a 1 liter 2-neck round bottom flask with ℧ 24/40 openings. The flask was set in a heating mantle controlled by a Variac resistance controller set at 60. A reflux condenser with ground glass ℧ 24/40 joints on each end was connected to the flask and the cold water put through the jacket. An elbow with ℧ 24/40 tapered glass joints on either end and bent at an angle of 105° was connected to the top of the reflux condenser. An adapter with a female ℧ 24/40 joint on one end and a 12/5 ball joint on the other was connected to a series of traps all fitted with 12/5 ball and socket joints. The first was a cold-finger type trap having internal dimensions of 1 x 6 cm. which con-

tained 1 ml. of deionized-distilled $H_2O$. The second, third, and fourth traps each had internal dimensions of 24 mm. x 16 cm., and these were bubbler-type absorption traps. The bubbler tubes were 5 mm. in diameter, extended to within 5 mm. of the bottom of the trap, and the end was tapered to provide a capillary opening. The second trap contained 25 ml. of a saturated solution of 2,4-dinitrophenylhydrazine (2,4-DNP) in 2N HCl (Pippen et al. 1958). The third trap contained 3.6 g. 2,4-dinitrochlorobenzene in 25 ml. methanol. The fourth trap contained 25 ml. of bismuth nitrate solution made up according to Koren and Gierlinger, (1953). The fourth trap's 12/5 ball joint was connected to an empty 2 l. suction flask which in turn was connected to another 2 l. suction flask which had Drierite absorbent in the bottom to a depth of 3 cm. This second suction flask was joined to the vacuum line by suction tubing. The vacuum valve was partially opened. Then a T 24/40 male joint made up with a stop-cock controlled capillary tube which extended to within 2 cm. of the bottom, when fitted snugly, was inserted in the second neck of the 1 l. flask. The stopcock was left open to preclude plugging the capillary. After insertion the stopcock was adjusted to allow air intake through the system at the rate of 50 ml./min. The condenser water was turned on and cooking under "oxidation-favoring conditions" was carried out as described by Pippen et al. (1958). A gentle reflux was maintained throughout the run which lasted for 6 hours. At the end of the run by a fortuitous circumstance some colored material had collected in the water trap. The water solution contained some yellow, green, brown and amber colored material, which apparently had been steam-distilled over into

the first trap and amounted to about 4 ml. of solution. The pH was checked with a Beckman Model G pH meter. The solubility of the solution in various solvents was tested, and the odor was noted.

Thin Layer Chromatographic Procedure: The adsorbent film used on the chromatoplates in this study was Silica Gel G prepared according to Stahl (1958). This material was obtained from Terra Chemicals Co., 500 Fifth Avenue, New York 36, N.Y. The adsorbent contained a small amount of calcium sulfate which served as a binder for the silica gel. Five (20 x 20 cm.) clean chromatoplates were centered on a (22 x 113 cm.) plastic applicator board. Two 5 x 20 cm. end plates were put in place, one at each end of the 5 chromatoplates. A drop of water beneath each plate prevented the plates from sliding on the board. A Desaga fixed-thickness chromatofilm spreader (Research Specialties Co., 200 South Garrard Blvd., Richmond, California) was used to provide a uniform film thickness of 250 u.

Exactly 25 g. Silica Gel G was weighed into a clean 250 ml. beaker, and 50 ml. deionized-distilled $H_2O$ was measured into a clean graduate cylinder. The water was added in 3 equal parts while stirring each portion with the silica gel. The slurry had to be smooth and bubble-free. It was then poured into the Desaga spreader and the plates were coated in one uniform sweep of the spreader. The entire operation required about 3 min. The coated plates were placed on a storage rack so that the easily scratched surfaces were not disturbed. The coatings were dried at room temperature for 20-25 min., and the prepared plates were stored in a desiccator cabinet. Just prior to application of the samples,

the films were activated by placing the chromatoplates in an oven at 80°C. for 45-60 min. A marking template was used, which facilitated application of the samples at equal intervals along a straight line. Holes 100 mm. apart permitted marking the ends at the start and finish lines. The plates were inspected and any irregularities in film thickness were scraped off or the plate in question was discarded. The corners of the plates were scraped as the film was thicker at these points. These precautions were taken as variation in film thickness caused the front to change and gave an uneven line.

A sample of pork lecithin provided by Mr. Chung-yen Peng of the Food Science Department at Michigan State University was used as a control. The yellow distillate solution from the water trap and the pork lecithin control in carbon disulfide solvent were measured with a 10 ul syringe. A 200 ul aliquot of unknown solution was placed in each of 3 micro test tubes (internal dimensions 5 x 25 mm.), and diluted with an equal amount of appropriate solvent. Chloroform, carbon disulfide and ethanol were the solvents used. Spot size was limited to a maximum diameter of 5 mm. by drying the solution rapidly in a stream of nitrogen. One hundred ul of the original distillate solution and 100 ul of the control solution were applied. Three spots were applied per plate, consisting of the pork lecithin control and the unknown in two separate spots on each side of the known. One plate was used for each sample dilution, making a total of three chromatoplates per run.

Ascending chromatography was used to develop the plates. The plates were placed on chromatofilm racks and developed in Model 200-D Research

Specialties development chambers. The solvent system was chloroform-methanol-$H_2O$ mixture in a proportion of 195:75:12. The developing chambers were equilibrated overnight at room temperature to assure a uniform vapor phase. The plates were developed in 75 min., and then were evaluated by Skidmore's (1962) spray technique.

A second run was made using Skipski et al's. (1962) method for separating phosphatidyl ethanolamine, phosphatidyl serine, and other phospholipids by thin layer chromatography. Plates were prepared as before. However, in addition to the "neutral" plates, "basic" plates were prepared by substituting a 0.01M $Na_2CO_3$ solution for the water in the silica gel slurry. The chromatoplates were spotted with the known standard and the unknown distillate as before. A different solvent system was used which gave a different $R_f$ value. For the "neutral" and "basic" plates the best separation was achieved with solvent mixtures of chloroform-methanol-glacial acetic acid-$H_2O$ mixtures of 65:25:8:4 and 50:25:8:4, respectively. Spots were detected using the Dragendorf reagent (Skidmore, 1962) spray and the molybdic acid spray (3 g. ammonium molybdate dissolved in 50 ml. distilled $H_2O$, 5 ml. 6 N HCl and 13 ml. 70% perchloric acid). Dragendorf's reagent was used to test for free choline (purple spot if present), and for choline compounds (orange spot if present). The molybdic acid spray was used to test for phosphatides (blue spot with white background if present).

Separation and Identification of Carbonyls and Sulfur Compounds in the Volatile Fraction of Cooked Chicken

Cooking and Distillation: A total of 3 kg. of freshly ground skin, bones and muscle from 10 eight-week-old female chickens, and 4 l. of deionized-distilled $H_2O$ was made into a slurry and placed in a 12 l. flask. The cooking and distillation assembly was similar to that shown in Fig. 10. However, instead of attaching cold traps to the distillate condenser (Fig. 10-C) a series of three 2 l. Erlenmeyer flasks were used as liquid absorption traps. Each flask was made up with a ℧ 29/42 female neck joint. The bubbler tube was made from a piece of 5 mm. tubing. The outer arm was 6 cm. long overall, and it terminated in a 12/5 socket joint at the inlet end. The tubing was turned at a 90° angle, and the second arm extended to within 1 cm. of the bottom of the Erlenmeyer. The bottom of the tube was constricted to provide an opening about 1.5 mm. diameter. The outlet tube was also made of 5 mm. tubing turned at 90°. The inner arm extended to a position opposite the inside center of the ℧ 29/42 male joint. The outer arm was 6 cm. in length overall and terminated in a 12/5 ball joint. Both the bubbler inlet tube and the 5 mm. outlet tube were sealed in at the top of a small manifold (2 cm. in dia. x 2.5 cm.) which in turn was sealed to the top periphery of the ℧ 29/42 male joint.

Essentially, the cooking-distillation apparatus consisted of a 12 l. flask heated by a Variac-controlled heating mantle that was mounted on a steel tripod. A ℧ 55/50 bottom-inner and 24/40 top-outer adapter was fitted into the center neck of the flask. A highly efficient 60 cm.

Graham coil-type condenser (water-cooled) with T 24/40 tapered joints was connected to the flask via the adapter. Another adapter, a side-arm type, was connected at the top end of the condenser. This adapter had 2-T 24/40 male joints at the bottom and side openings and a T 10/30 top-joint opening for a thermometer. The side-arm of the adapter was sealed in at an angle of 120° so that the thermometer bulb was directly opposite the side-arm discharge opening, which led into a smaller (30 cm. in length) Graham coil condenser. The T 24/40 male joint at the discharge end of the condenser was connected to another adapter made up with a T 24/40 female joint at one end and a 12/5 ball joint at the other end (6 cm. in length overall). The 12/5 ball joint of the adapter was connected to the 12/5 socket joint on the 5 mm. bubbler tube of the first Erlenmeyer absorption reagent flask by means of a 12/5 ball and socket clamp. The other two Erlenemeyers were attached to the first in like manner. The vapor discharge outlet of the third Erlenmeyer remained open to the atmosphere. The first Erlenmeyer trap contained 1 l. of saturated lead acetate solution. The second contained 1 l. of DNP solution containing 2 g. of DNP/l. of 2N HCl. The third trap contained 1 l. of saturated lead acetate solution to minimize the escape of sulfur containing compounds.

The chicken slurry was cooked under influx for 3 hrs. using "oxidative-cooking conditions" (Pippen et al., 1958). The isolation of volatile compounds under "oxidation-inhibiting conditions" (Pippen et al., 1958) was effected by bubbling nitrogen gas at 400 ml./min. through the cooked slurry for 10 hrs. By passing cold water through the reflux

condenser the escape of steam was prevented. The volatile components were purged from the slurry, which was maintained at a temperature of 102°C. Upon completion of the run, the 2,4-DNPHS were filtered, washed with water, dried at 60°C. under 5 in. vacuum, and stored over Drierite in a desiccator until further analysis.

Fractionation and Identification of 2,4-DNPHS: The 2,4-DNPHS were first fractionated into mono- and polycarbonyl components (Pippen et al., 1958). The extreme insolubility of hydrazones of polycarbonyl compounds was reported by Malmberg (1954). The monocarbonyl derivatives were separated by the nature of their ready solubility in hot alcohol or chloroform; whereas, the polycarbonyl derivatives were only slightly soluble. Thus, the monocarbonyl 2,4-DNPHS were separated by suspending the total hydrazone precipitate (1.82 g) in 20 ml. of a (v./v.) mixture of hot alcohol (ethanol) and chloroform in a 50 ml. beaker, and filtering through a quantitative filter paper. The beaker and residue were rinsed with 10-10 ml. portions of the hot solvent mixture. The filtrate was collected in a 250 ml. beaker, and the monocarbonyl fraction was evaporated to dryness in a stream of nitrogen on the steam bath. The polycarbonyl derivatives remaining on the filter paper were washed off with near-boiling nitrobenzene, and allowed to crystallize. They were refiltered, washed with ethanol and oven-dried under 5 inches of vacuum at 100°C. for 24 hrs. The dried sample was stored in a desiccator over Drierite until analyzed.

Column Chromatography: Monocarbonyl 2,4-DNPHS in the 250 ml. beaker were dissolved in hot chloroform and chromatographed on columns as equal

aliquots by the method of Gordon et al. (1951) as modified by Pippen et al. (1958 ) and Jacobson et al. (1963). The petroleum ether that was used boiled at 40-50°C. After purifying it by treating it successively with concentrated sulfuric acid, deionized-distilled $H_2O$, 3N KOH, and deionized-distilled $H_2O$, it was dried over anhydrous sodium sulfate, and distilled to obtain the desired fraction (boiling range 40-50°C). The ether and chloroform were both redistilled.

Preparing the Columns: An 800 g. lot of silicic acid (Mallinckrodt-chromatography grade-100 mesh) was dry-blended with 400 g. of Celite (60 mesh Johns-Mansville). The adsorbent was washed successively with 8N HCl, deionized-distilled $H_2O$, absolute methanol and anhydrous ethyl ether (Corcoran, 1956), and dried under 5 inches of vacuum at 80°C for 24 hrs. The dry adsorbent was suspended as 100 g. aliquots in 1 l. of the solvent system (2% diethyl ether in petroleum ether). The chromatographic columns were 35 mm. in diameter and constricted to an 8 mm. opening at the bottom, where a piece of 10 mm. tubing 8 cm. long was sealed to the constriction. A sintered glass disc was secured in the bottom and a wad of glass wool was packed on top. The previously washed solvent slurry adsorbent was poured into the column in about 6 equal portions and packed with 5 lbs. nitrogen pressure to a height of 75 cm. Care was taken so that the column never became dry. Three circles of No. 1 filter paper were placed on the top of the adsorbent.

When the 11 columns had been prepared, the monocarbonyl fraction of 2,4-DNPHS was dissolved in chloroform (1.52 g. 2,4-DNPHS in 220 ml. chloroform). Equal 20 ml. aliquots were pipetted on each column, and 5

lbs. nitrogen pressure was applied to fix the 2,4-DNPHS solutions in the top adsorbent layer. Gradient elution was carried out using diethyl ether in petroleum ether and 5 lbs. nitrogen pressure. Elution began by adding 200 ml. 2% diethyl ether. The amount of diethyl ether was increased 2% after each 200 ml. addition to give a maximum of 30%. Thus, each of the columns was eluted with a total of 3 l. of gradient solvent. The solvent flow rate was 15 ml./hr./column.

Nine preliminary bands were eluted from each column and tentatively identified by the technique of Gaddis and Ellis (1959). The absorption in the 325 to 400 mu region in a chloroform solvent was read with a Beckman DU spectrophotometer. Two slow bands were cut from each of the columns. The hydrazones were recovered from the adsorbent by extraction with chloroform, and removal of the solvent by a stream of nitrogen on the steam bath. The fractions were recrystallized from hot ethanol. Comparable bands from the 11 columns were combined according to their order of elution, UV absorption spectra and melting points. These combined fractions were recrystallized from hot ethanol for infra-red analysis, paper chromatography, and for mixed melting point determinations using authentic compounds. At this stage, 7 of the 9 residual hydrazone fractions gave sharp melting points. Four of the fractions had distinctly different melting points, while the other 2 pairs of fractions were so nearly identical that this criterion alone did not provide a conclusive means of identification.

The alcoholic KOH test of Newberg and Strauss (1945) was used to

differentiate monocarbonyl 2,4-DNPHS (red color) from those having two or more adjacent carbonyl groups (violet color).

Melting Points: Melting points were determined using authentic 2,4-DNPHS prepared according to Pippen et al. (1958), and comparing with the various unknowns. Another set of 20 authentic 2,4-DNPHS prepared according to the method of Shriner et al. (1954) was provided by Dr. Roy Porter, formerly of the Food Science Department, Michigan State University.

A melting point tube containing the sample was attached to a calibrated mercury thermometer with a rubber band so that the crystalline material in the tube was adjacent to the middle of the mercury bulb of the thermometer. A Thiele melting point apparatus as modified by Robertson and Jacobs (1962), and containing a silicone-oil bath (Merriam, 1948) was used to determine the melting points of the authentic and unknown hydrazones together with the mixed melting points. The sample tube and thermometer were immersed in the silicone-oil bath and the temperature was raised slowly (less than 2°C./min.) by means of a micro-burner. Results were checked by determining the melting point for the same sample between cover glasses on an electrically heated stage, which had been pre-calibrated with a series of known compounds.

Paper Chromatography: Paper chromatography was carried out by the method of Lynn et al. (1956). However, instead of using 18 1/4 x 22 1/2 inch sheets of Whatman No. 1 paper, the sheets were cut into 4 x 45 cm. strips. A pencil line was drawn across each strip 5 cm. from one end. A piece of aluminum rod (8 mm. in dia. x 4 cm. long) was fastened to the

other end by doubling the paper over at a distance of 5 cm. from the end, inserting the rod, and stapling. The strips were impregnated by dipping them in 500 ml. of 10% 2-phenoxyethanol in acetone solution in a 24 x 40 x 4 cm. glass tray. The strips were air-dried for 10-20 min. before the 2,4-DNPHS were spotted. Standard solutions of several 2,4-DNPHS (conc'n. 5 mg./ml. chloroform), including the derivatives of formaldehyde, acetaldehyde, n-propionaldehyde, n-butanal, isobutyraldehyde, iso-valeraldehyde, n-valeraldehyde, n-hexanal, n-heptanal, n-octanal, n-nonanal, acetone, ethyl-methyl ketone and 2-butanone, were spotted on separate strips with a 10 ul. syringe. Five mg. samples (5 mg./ml. chloroform) of unknown from each of the 9 different 2,4-DNPH fractions were spotted on separate strips. On each strip, n-heptanal 2,4-DNPH was applied as a tag compound (Nonaka et al. 1959). Spots were limited to a maximum diameter of 5 mm. by rapidly drying them in a stream of nitrogen between applications. The spotted strips were put into the chromatographic cabinet (48 x 48 x 36 cm.) and suspended from troughs by inserting the end of the strip under a 1 x 5 cm. stainless steel bar. When all of the strips were in place, the troughs were filled with the n-heptane saturated 2-phenoxyacetone moving phase. The chromatographic chamber, which had been previously equilibrated with n-heptane vapor, was then sealed. The strips were developed 15 1/2 hrs. The strips were read in a dark room under UV light and a pencil outline was drawn around each spot. The separated spots averaged 2-3 cm. in diameter. The heptanal tag compound was used as a reference to check the mobility ($R_h$).

$$R_h = \frac{R_f \text{ DNPH - X (unknown)}}{R_f \text{ DNPH - n - heptanal}}$$

The 2,4-DNPH spots on the developed chromatogram were cut out and placed over the sample aperture of a Cary Model 14 spectrophotometer. The absorption spectrum was determined in the wavelength range of 300-450 mu. The wavelength at maximum absorption for each spot was determined. Infrared spectra in the range of 2 to 15 u were obtained for samples made up in the form of potassium bromide disks with a Model IR-3 Beckman spectrophotometer using sodium chloride prisms. The 2,4-DNPHS were dissolved in a few drops of chloroform and thus eluted from the paper spot. A 10-30 ul. aliquot of the eluate was mixed with a small amount of potassium bromide powder. The mixture was ground using a 2 cm. onyx mortar and pestle. The mixture was evacuated in a vacuum desiccator and pressed.

Preparation of Authentic 2,4-DNPHS: The known carbonyl compound was added to a solution of 2,4-DNP (2 g./1. on 2N HCl). A 10% excess of 2,4-DNPH reagent was used. If the carbonyl compound was immiscible in the 2,4-DNP solution, the mixture was agitated and warmed on a steam bath. The hydrazone was filtered off and washed with water. For moncarbonyl derivatives, this preparation was followed by recrystallization from ethanol, chromatography on silicic acid-Celite columns to remove impurities, and crystallization of the purified product from ethanol. In a few instances the melting points for the derivatives were higher than values reported in the literature (Pippen et al., 1958).

Separation of Sulfur Containing Volatiles: Since the black precipitates formed in the lead acetate traps in copious quantities, the traps were emptied as needed and another liter of saturated lead acetate solution was added to each trap. The precipitates were washed and dried.

Test for Thiols: A 0.2 g. aliquot of dried lead acetate precipitate was placed in a cold finger-type trap (internal dimensions 1 x 6 cm.) with a shortened inlet tube, which extended to 3 cm. above the trap bottom to permit the nitrogen gas to flow above the reaction mixture in the tube (Folkard and Joyce, 1963). The reaction trap was connected to a series of 6 identical traps. The first 4 traps contained distilled $H_2O$, bismuth nitrate solution (Koren and Gierlinger, 1953), mercuric acetate solution 4% w/v. (Folkard and Joyce, 1963) and mercuric chloride solution 3% w/v. Traps 5 and 6 each contained 3.0 ml. ethanol plus 0.02 g. solid NaOH. Each absorption trap contained 3 ml. of solution. Two ml. of 8N HCl were added to the lead acetate precipitate, the tube was sealed at once, with Dow-Corning silicone grease on the joints, and gently heated until the compound dissolved. It was then allowed to cool and the gas flow was started. The reaction trap was connected to a nitrogen source through a length of 1/4 in. gum-rubber tubing and a pressure-regulator. After passing nitrogen gas through the system at 10 ml./min. for 15 min., the gas flow was stopped. Inorganic sulfide was absorbed by the bismuth nitrate solution. Organic disulfides were removed by the mercuric acetate and mercuric chloride solutions and the thiol vapors were absorbed by the alkaline-ethanol solution. These solutions were

added slowly to separate volumes (1.25 ml.) of alcoholic 1-chloro-2,4 dinitrobenzene (saturated at 25°C.) and the solutions were cooled in ice. The crystals were removed by filtering, and a further small volume of reagent was added to the filtrate. The filtrate was then cooled, and if no crystals separated it was discarded (Folkard and Joyce, 1963).

Test for Sulfides: Emil Fischer's methylene blue method as modified by Marbach and Doty (1956) was used to detect sulfides. The solution containing the black precipitate, which resulted from passing cooked chicken volatiles through a saturated lead acetate solution was tested then tested. A suspension was made by stirring the solution containing the lead derivatives. A 5 ml. aliquot of this suspension was pipetted into a test tube and 1.5 ml. N,N-dimethyl-p-penylenediamine reagent solution and 0.5 ml. Reissner solution were added. A deep blue color indicated the presence of sulfides.

Test for Organic Disulfides: A 2 ml. aliquot of the unknown lead derivative suspension was placed in a small absorption trap (1 x 6 cm.). The trap was connected to a purified nitrogen source and to a series of 2 identical traps containing ethanolic sodium hydroxide in the same manner as for the thiol test. Stahl and Siggia (1957) demonstrated that the reduction of organic disulfides was the best means for their identification since alkyl sulfides and mercaptans do not interfere. Thus, 1 g. of sodium borohydride and 1 mg. of aluminum chloride were added to the unknown in the reaction trap. The gas flow was started and after 1/2 hr. at room temperature the gas flow was stopped. The two traps of ethanolic

sodium hydroxide were now added slowly to separate volumes (1.25 ml.) of alcoholic 1-chloro-2,4-dinitrobenzene (Rittner et al., 1962). The precipitate was formed in the same manner as in the procedure for mercaptans.

Separation and Identification of Carbonyls and Sulfur Compounds in the Volatile Fraction of Cooked Intestinal Contents of Chicken

Cooking and Distillation: The intestinal contents of 20 old laying hens (1.5 lbs.) were mixed with 500 ml. of deionized-distilled $H_2O$. They were cooked under reflux using "normal cooking conditions" for a period of 3 hrs. at 102°C. (Pippen et al., 1958) in a 3 l. (2-neck) round bottom distilling flask made up with ℧ 24/40 neck joints. The flask was heated by an electric mantle controlled by a Variac controller set at 60. At the end of 3 hrs. cooking, distillation began and continued for 17 hrs. under "oxidation-inhibiting conditions" (Pippen et al., 1958) which consisted of purging the volatiles from the cooked slurry by bubbling nitrogen gas through the slurry at a rate of 100 ml./min. As the volatiles left the second condenser (Fig. 10-C) they passed through a ℧ 24/40 female-12/5 ball joint adapter, and then into a Y tube (stream-splitter) made up from 5 mm. glass tubing with a 12/5 socket joint at the straight end and 2-12/5 ball joints at the branch ends. Each branch was controlled by a 2-way Teflon stopcock.

One branch was used for the collection of volatiles for gas chromatography while the other was for chemical identification. The branch for chemical identification was connected to a series of two 2 l. Erlenmeyer flasks made up as absorption traps. The first trap contained 1 l. of

2,4-DNP solution (2 g./l. in 2N HCl). The second contained 1 l. of saturated lead acetate solution. All of the traps were connected by 12/5 ball and socket clamps to 12/5 ball and socket joints.

Gas chromatographic analysis of the contents from the liquid nitrogen trap was made as described previously. Then the branch used for gas chromatography was shut off by means of that Y branch stopcock, and the entire stream of volatiles was passed through the 2,4-DNP and lead acetate solutions for 17 hrs. After the total cooking and distilling time of 20 hrs. had elapsed, the ebullition of nitrogen gas through the cooked slurry and absorption traps was terminated and the run was stopped.

Fractionation and Identification of 2,4-DNPHS: The 2,4-DNPHS were filtered and the solid DNPHS were washed and treated with solvent as before in order to effect a separation of mono- from polycarbonyls. Column chromatography of the monocarbonyls was carried out in the manner already described.

Melting points of the 2,4-DNPHS were determined by the electrically-heated stage method previously described since the amounts available were smaller than in the previous experiment. Authentic 2,4-DNPHS were used as standards and for the mixed melting point determinations.

Paper Chromatography: Due to the limited amount of 2,4-DNPHS available, the capillary-ascent test tube method (Rice et al., 1951), with a few of the modifications of Ellis et al. (1958), was used for separating ug. quantities of the 2,4-DNPHS. These techniques were adapted from methods developed earlier, for the separation of ug. quantities of amino acids, by Rockland and Dunn (1949) and Rockland et al. (1951). Strips

10 1/2 x 1 3/8 x 1 in. were cut from sheets (45.7 x 56.3 cm.) of Whatman No. 1 filter paper. The strips were pierced in the center about 4 mm. from the broad end to facilitate suspending them with a bent paper clip during spraying. The 2,4-DNPHS were dissolved in chloroform. No drying was necessary before or after development of the chromatograph. Pencil dots were placed 5 mm. from the bottom and top ends of each strip in order to fix the solvent ascent boundary. A light source was necessary behind the tubes to improve the observation of the solvent ascent boundary.

The 2,4-DNPH samples were applied with a Gilmont-ultramicroburet 0.01 ml. capacity. A single spot ($10^{-4}$ ml.) was dried for 1 min. in a nitrogen stream. Spots were applied 10 times without increasing the spot size. Approximately 5 ml. of 5% diethyl ether in petroleum ether (boiling point 65-110°C.) was pipetted into the bottom of a 11 13/16 x 1 1/2 in. test tube. The strip was carefully inserted into the solution without the sides of the strip touching the wall except at the upper end and the tube was stoppered. Immediately after the solvent boundary reached the second pencil mark, the paper strip was removed from the tube, suspended by the bent paper clip on a wire holder against a white poster board and sprayed with a 10% solution of KOH in $H_2O$. While still wet, the strips were placed on a white background and the vividly colored areas, which showed up as red for monocarbonyls and violet for polycarbonyls, were outlined with a pencil. A conventional atomizer could not be used for spraying the strips. For carefully defined spots an artist's airbrush was used.

A rectangular museum jar 12 x 12 x 6 in. with 3 1/4 in. wooden dowels inserted in the grooves provided at the top of the jar was advantageous for determining the $R_f$ values of a number of 2,4-DNPHS simultaneously while using a single solvent mixture. Single dimension ascending chromatography was used in this study. A difference of 0.05 cm. in $R_f$ value was necessary before separation could be observed.

Pippen et al's. (1958) methods for the preparation of authentic 2,4-DNPHS, absorption spectra and polar microscopy were used to complete the identification.

The tests for thiols, sulfides and organic disulfides were carried out as previously described.

An Apparatus for Splitting and Trapping the Volatile Fraction of Cooked Chicken

A radial-manifold volatile fraction splitter was designed and made up for simultaneously splitting the volatile stream into 8 or less fractions. The 5 mm. 90° elbow inlet tube was made up with a 12/5 socket joint at the inlet end and was sealed to the hemispherical shaped manifold at the other end. The 8 outlet tubes were also 5 mm. but made up with 12/5 ball joints, and these were sealed symmetrically around the periphery at the bottom of the manifold. The overall radius of the splitter was 8 cm. and the overall height was 5 1/2 cm. The manifold bulb was 3 cm. in diameter at the bottom and 2 1/2 cm. in diameter on the hemispherical portion, which was sealed to the inlet tube. The 8 radial branches were made up from 5 mm. tubing and were 2 cm. in overall

length, including the ball joints. Each arm of the inlet elbow was 3 cm. in overall length (Fig. 12).

Cold-finger type absorption reagent traps were made up in assorted sizes. The 5 mm. inlet tubes were connected to 12/5 socket joints turned at a 90° angle and sealed into an inlet-outlet manifold made up as a 8 male tapered ground glass joint. After the turn, the 5 mm. tube was extended to within 1 cm. of the bottom of the trap and constricted to a bubbler-type opening of approximately 1 mm. in diameter. The outlet tubes were made up of 5 mm. tubing turned at 90° to extend to the center of the ground glass joint in the large traps (internal dimension 2.3 x 13.5 cm.) and having 12/5 ball joints at the outlet ends. The small traps (internal dimension 1 x 6 cm.) had the outlet tube sealed at the periphery near the top of the inlet-outlet manifold. The opening at the discharge end of the small trap was 2.5 mm. in diameter.

Before a run, the bubbler traps were filled with the appropriate reagents, tagged for identification, clustered around the radial manifold, and connected to the manifold by 12/5 ball and socket clamps. A photograph of two types of traps that were commonly used is shown in Fig. 12. A 6 inch plastic rule illustrates the relative trap sizes and the manifold size in the photograph. The small trap was assembled for photography as if a sample for gas chromatographic analysis had just been removed. If this had been the case, the volatiles would have been trapped at -196°C in a liquid nitrogen bath. However, prior to sealing, pressure would have been released by transferring the open trap to another bath containing 2-2-4 trimethlpentane-dry ice-liquid nitrogen maintained at -140°C.

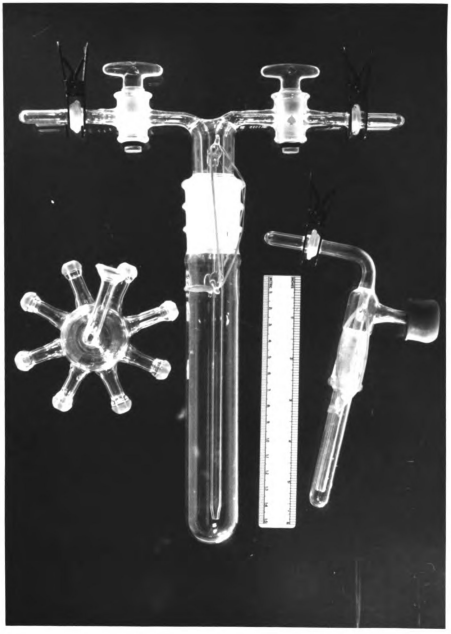


Fig. 12 (l. to r.) Radial-manifold stream-splitter. Large absorption trap with ball and socket plugs clamped on .A 12 cm. rule. Small absorption trap with surgical seal and plug in place to retain volatiles for gas chromatographic analysis.

This operation would eliminate the low boiling fraction. Then a rubber seal would have been forced over the 12/5 ball-joint to facilitate removal of the sample by a Hamilton gas-tight syringe. A 12/5 ball-joint plug (2.5 cm. in length overall) would have sealed the 12/5 socket-joint inlet of the trap by connecting it with a 12/5 ball and socket clamp. Dog-ear projections were provided 5 mm. above and 5 mm. below the 14/35 ground glass taper joints of the trap and these were pointed in opposite directions. This made it possible to maintain a slight pressure by means of a taut rubber band which held the trap together. Silicone vacuum-type stopcock grease (Dow Chemical Co., Midland, Michigan) was used to seal all ground glass tapered joints. Ball- and socket-joints (12/5) of ground glass were sealed adequately by glass to glass contact with pressure applied by 12/5 ball and socket clamps. The larger absorption trap having 2.3 x 13.5 cm. internal dimensions was made up for photography to illustrate that the 12/5 ball outlets could be sealed with 12/5 socket joints joined to 5 mm. tubing and having an overall length of 2.5 cm. in lieu of a rubber surgical seal. The 12/5 socket inlet was sealable by means of a 12/5 ball-joint plug joined to 5 mm. tubing and having an overall length of 2.5 cm. The seals were made secure by means of 12/5 ball and socket clamps. This trap was made up with ₹ 29/42 ground glass joints. A pair of 2-way stopcocks were used to regulate ingress and egress from the bubbler-type absorption trap. Dog ear projections were provided 1 cm. above and 1 cm. below the ground glass joints of the trap so that a slight pressure could be maintained on the joint by using a taut rubber band (Fig. 12).

A photograph of the entire apparatus was taken as it appeared during a run (Fig. 13). In this run, a classification of the volatile fraction from cooked chicken was being made in 5 of the absorption-reagent traps while simultaneously derivatives of carbonyls and sulfur compounds were being prepared in the other 3 traps. A slurry consisting of 2 kg. of ground chicken muscle and 3 l. of deionized-distilled $H_2O$ was being cooked and distilled in a 12 l. 3-neck flask by means of an electric heating mantle, mounted on a steel tripod ring support, and connected to the line current through a Variac-controller. An inlet Y with stopcocks on both branches was made up from 5 mm. tubing put through and sealed to a manifold-top made up from a ♀ 24/40 ground glass joint. The 5 mm. tubing extended to within 2 cm. of the flask bottom and was drawn to a bubbler-tip of 1 mm. diameter at that point. A piece of 1/4 in. diameter gum-rubber tubing was connected at one end to a purified nitrogen source through a pressure-regulator. The other end of the tubing was connected to the Y inlet which extended into the cooked chicken slurry. Nitrogen gas was passed through at a rate of 40 ml./min. The other 24/40 neck opening of the flask was sealed with a ♀ 24/40 plug secured with a clamp except when intermittent temperature readings were taken with a mercury thermometer. The temperature was maintained at 185°F to provide a gentle reflux throughout these runs which lasted for 50 hrs. A ♀ 55/50 to 24/40 inner adapter was used to connect the ♀ 24/40 reflux condenser which was cooled with cold tap water (50°F). A ♀ 24/40 male-jointed elbow was securely clamped to provide a tight connection to the reflux condenser.

Fig. 13. Perspective view of cooking-distillation apparatus showing nitrogen gas tube - chicken slurry in flask - water trap - and cluster of reagent traps surrounding the radial-manifold stream-splitter.

The other end of the elbow was connected to an adapter made up with a ℱ 24/40 tapered ground glass female joint on one end and a 12/5 ball-joint on the other end. This bubbler was connected to a 12/5 socket-joint bubbler tube leading to the bottom of a 1 l. round bottom distilling flask made up with ℱ 24/40 joints and a 12/5 socket-joint on the inlet tube side and a 12/5 ball-joint on the outlet tube side. About 200 ml. of deionized-distilled $H_2O$ was put into the flask before the start of a run. An extension tube made up as 5 mm. tubing with 12/5 ball- and socket-joints was used to connect the water trap to the radial-manifold volatile fraction splitter. Then a cluster of absorption reagent traps each containing 25-30 ml. of the appropriate reagent were connected to the 8-12/5 ball-joint branches of the splitter. Auxiliary traps containing secondary reagents were connected to absorption reagent traps for sulfur compounds. These small traps contained 3-4 ml. of the secondary reagent, and were attached either singly or in tandem pairs.

A closeup photograph of the trapping assembly was taken (Fig. 14). This shows clearly how the water trap was connected to the adapter-elbow-condenser assembly on one side, and the radial-manifold on the other. However, the radial-manifold itself was obscured by the cluster of traps which surrounded it. The small auxiliary traps were partially obscured. Light and dark precipitates in the absorption reagent traps were visible.

A third photograph was taken during an actual run when gas chromatographic analysis was in progress (Fig. 15). The volatile fraction from cooked chicken was being carried through the radial manifold by a stream

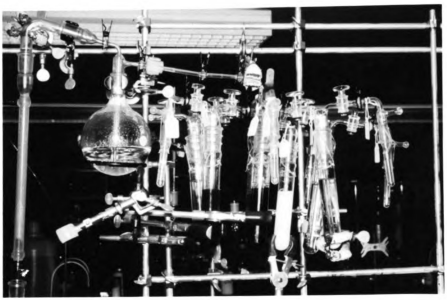


Fig. 14. Closeup view of water-trap and a cluster of 8 reagent traps surrounding the radial-manifold stream-splitter. Small auxiliary reagent traps are also shown by this view.

**Fig. 15.** Closeup view of cooking-distillation set-up for trapping cooked volatiles with dry ice-ethanol and liquid nitrogen traps connected in series to a small trap containing a functional group reagent which in turn is connected to the radial manifold stream-splitter.

of nitrogen. The 2-way stopcocks, which controlled the inlet tubes to 7 of the absorption reagents were turned to the perpendicular position to shut off the stream of nitrogen and cooked volatiles. The flow rate was reduced from 200 to 10 ml./min. The eighth trap which contained a fresh solution of mercuric chloride (3% w/v), and an auxiliary trap containing fresh mercuric cyanide (4% w/v) was connected to the branch outlet that was most nearly parallel to the inlet elbow of the radial-manifold. The auxiliary trap in turn was connected to a trap immersed in an ethanol-dry ice mixture at -80°C and a second trap immersed in liquid nitrogen at -196°C. A platform was improvised from 2 x 8 x 14 in. concrete blocks, 2 x 4 x 8 in. bricks and some books in order to support the cold baths which were contained in Dewar vacuum bottles.

Functional Group Analysis of Components from the Volatile Fraction of Cooked Chicken by Qualitative Chemical Tests

The auxiliary chemical identification of functional groups contained in the volatile fractions from various samples of light and dark muscle was carried out by means of the stream-splitting and trapping system already described (Figs. 12, 13, 14 and 15). The reagents used were chosen from among those employed by Walsh and Merritt (1960), Cheronis and Entrikin (1961) and Shriner et al. (1956).

An auxiliary or secondary stream-splitter was made up according to the design of Walsh and Merritt (1960). Two modifications were made. Glass tubing 5 mm. in diameter was used instead of 1/4 in. O.D. stainless steel tubing. A 12/5 socket joint was sealed to the inlet and two 12/5

ball joints to the outlet branches of the stream-splitting device. The vials used were the same as those described by Walsh and Merritt (1960). They were made up in clusters of five vials, with each positioned in a separate hole contained in a large-cork holder just as the authors had illustrated. The reagent preparation and the procedure were essentially the same as those described by Walsh and Merritt (1960).

The 12/5 socket inlet joint of the auxiliary stream-splitting device was coupled to one of the 12/5 ball joint outlets leading from the radial-manifold splitter. The cooked chicken volatiles from light or dark muscle were passed simultaneously through all reagents for 6 to 7 hrs. at room temperature (approximately 23°C). Thus, color tests were made during the early part of a run by coupling two of the auxiliary splitters to two of the radial-manifold outlets. Of the six remaining outlets, two were used for the preparation of sulfide, disulfide and mercaptan derivatives, and four were used for the solubility classification of the volatile fraction.

Color Tests: Color tests for various classes of compounds were carried out as follows:

Alcohols: The presence of primary and secondary alcohols was tested by passing the volatiles through a vial containing nitrochromic acid solution for 6 to 7 hours (Cheronis and Entrikin, 1961). A yellow to blue-gray color appeared if primary and/or secondary alcohols were present. Ceric nitrate reagent prepared according to Walsh and Merritt (1960) was used to test for tertiary alcohols. An amber to red precipitate appeared if a tertiary alcohol was present.

Carbonyls: The 2,4-DNP reagent was used to test for the presence of aldehydes and ketones. A yellow precipitate indicated if carbonyls were present (Cheronis and Entrikin, 1961; Shriner et al., 1956).

Schiff's reagent: Schiff's reagent was used to differentiate between aldehydes and ketones (Cheronis and Entrikin, 1961; Shriner et al., 1956). A pink color indicated the presence of aldehydes. With ketone derivatives, the yellow 2,4-DNPH did not change.

Esters: The presence of esters was tested with hydroxylamine absorption reagent according to Cheronis and Entrikin (1961). Then after the sample had passed through the solution for 5 or 6 hrs, a few drops of 2N HCl and 10% ferric chloride were added to complete the test. If esters were present, a pink color developed.

Alkyl Nitriles: The presence of nitriles was tested with hydroxylamine reagent (Cheronis and Entrikin, 1961) made up in propylene glycol solution. After the volatiles were passed through the reagent for 5 to 6 hrs., the tube was heated to boiling and cooled. Then 1 or 2 drops of 10% $FeCl_3$ were added to the clear and colorless solution. If present nitriles gave a red-wine color.

Mercaptans: Alcoholic silver nitrate reagent was used to test for mercaptans (Shriner et al., 1956). This reagent gives a white precipitate with mercaptan(s), and a black precipitate with hydrogen sulfide.

Mercaptans: Lead acetate was used to test for mercaptans (Cheronis and Entrikin, 1961). Lead acetate gives a yellow color with mercaptan(s), and a black color with hydrogen sulfide.

Mercaptans: Isatin reagent was also used to detect mercaptans (Cheronis and Entrikin, 1961). Isatin reagent reacts with mercaptan(s) to give a green color.

Mercaptans: Sodium nitroprusside reagent was used as a test for mercaptans (Grote, 1931). Sodium nitroprusside reagent reacts with mercaptans, sulfides and disulfides to give a red color.

Alkyl Sulfides: The sodium nitroprusside test (Walsh and Merritt, 1960) was used for alkyl sulfides as described for mercaptans earlier.

Alkyl Sulfides: Sulfides were oxidized to sulfones by permanganate reagent (Cheronis and Entrikin, 1961). A change in the odor of the effluent stream from the vial occurred if sulfides were present.

Alkyl Disulfides: The isatin test was used for alkyl disulfides as for the mercaptans (Cheronis and Entrikin, 1961). A green color developed if disulfides were present.

Alkyl Disulfides: Sodium borohydride was used to test for alkyl disulfides (Stahl and Siggia, 1957). When the sample stream passed through a vial containing the sodium borohydride solution, any disulfides in the cooked chicken volatile fraction were reduced. A mercaptan odor developed that was detectable in the effluent stream coming from the vial.

Amines (primary, secondary and tertiary): Primary and secondary amines were tested with sodium nitroprusside reagent (Cheronis and Entrikin, 1961). When present, primary amines give a red color; whereas, secondary amines give a blue color. Tertiary amines do not interfere.

Tertiary Amines: Tertiary amines were tested with a few drops of pyridine followed by 10% NaOH and Hinsberg's benzenesulfonylchloride

reagent (Cheronis and Entrikin, 1961). If present, primary amines give a pale to bright yellow color, secondary amines tend to give tan to dark brown colors, and tertiary amines give a rose to deep purple color.

Aromatic Nucleus and Aliphatic Unsaturation: The presence of aromatic nuclei and aliphatic unsaturation was tested with LeRosen's formaldehyde-sulfuric acid reagent (Cheronis and Entrikin, 1961). Either group gives a range of color if present. However, the colors for most compounds that give positive tests change to various shades of brown and black upon standing.

Nitroparaffins: The ferric chloride test was used for nitroparaffins (Cheronis and Entrikin). A red color develops in the test solution if primary or secondary nitroparaffins are present. Tertiary nitroparaffins do not react.

Phenols and Enols: The presence of enolic structures was tested with ferric chloride reagent (Cheronis and Entrikin, 1961). The colors produced by a large number of phenols have been published by Wesp and Brode (1934) and Soloway and Wilen (1952).

Ammonia: The presence of ammonia was tested with Folin's method (Hawk and Bergeim, 1937). The volatiles were passed through a vial containing a few drops of Congo Red in a 0.1N sulfuric acid solution. A blue tint appeared in the solution when ammonia was present.

Preparation of Sulfide, Disulfide, Mercaptan and Carbonyl Derivatives

The reagent used for trapping carbonyls was 25 ml. of the 2,4-DNP solution of Pippen et al. (1958). Sulfides and disulfides were separated by

the methods of Challenger and Rawlings (1937), Hasselstrom (1957), Salvador et al. (1962) and Folkard and Joyce (1963).

For preparation of carbonyl derivatives, 25 ml. of 2,4-DNP reagent (Pippen et al., 1958) was placed in a large trap (Fig. 12). The volatiles were passed through by connecting the trap to a 12/5 ball joint outlet leading from the radial-manifold.

Three series of three traps each were used for trapping the sulfur compounds in the volatile fraction. In each series, one large and two small traps were used (Fig. 12). The large (primary) trap in the first series contained 25 ml. of mercuric cyanide (4% w/v), and the small (secondary) traps each contained 3 ml. mercuric chloride (3% w/v). In the second series, the large trap contained 25 ml. of mercuric chloride (3% w/v), and each of the two small traps contained 3 ml. of mercuric cyanide (4% w/v). In the third series, the large trap contained 25 ml. of bismuth nitrate reagent (Koren and Gierlinger, 1953), and each of the two small traps contained 3 ml. of mercuric acetate (4% w/v). Each series of traps was coupled to a 12/5 ball outlet and the radial-manifold stream-splitter.

Solubility Classification of Compounds: The solubility classification system of Cheronis and Entrikin (1961) was used in all runs for both light and dark muscle. The ether solvent trap (Division S) was not used. A water trap containing 300 to 500 ml. of deionized-distilled $H_2O$ was connected between the reflux condenser and the radial-manifold splitting device as shown in Fig. 14. A large trap (Fig. 12) containing 25 ml. 1.2N HCl was coupled to a 12/5 ball joint outlet and the radial-

manifold in order to absorb most of the amines (Division B). The amines if present are insoluble in water but soluble in dilute acid. A second large primary trap containing 25 ml. of 2.5N $NaHCO_3$ and two small secondary traps, each containing 3 ml. of 2.5N NaOH solution, were coupled in series to a 12/5 outlet from the radial manifold. This system was used to absorb any "Division $A_2$" compounds (Cheronis and Entrikin, 1961) present. The compounds trapped in this system are insoluble in water and 2.5 N $NaHCO_3$ solution, but are soluble in 2.5N NaOH solution. A large primary trap containing 25 ml. of 1.5N $NaHCO_3$ solution and two secondary traps each containing 3 ml. of 2.5N NaOH solution, were coupled to a 12/5 outlet and the radial-manifold. This system absorbed any "Division $A_1$" compounds. These compounds are insoluble in water and 1.5N $NaHCO_3$ solution but are soluble in 2.5N NaOH. A fourth large trap containing 25 ml. of concentrated sulfuric acid was coupled to one of the 12/5 outlets and the radial-manifold (Fig. 14). This trap absorbed "Division N" compounds if present. These compounds are insoluble in water but soluble in concentrated sulfuric acid.

Not all of the trapping systems described were used for all runs of light and dark muscle. To do this, 10 outlets would need to be provided from the radial-manifold (Fig. 12). However, the solubility classification reagents were used in all of the runs as were the trapping systems for sulfur compounds.

Functional Group Analysis by Gas Chromatography with the Chemical Trap Reaction Technique

In studying the cooked chicken volatiles, the "syringe reaction technique" as described by Hoff and Feit (1963) and the "head space gas" analysis method of Bassette et al. (1962) were modified as a trap reaction technique.

After cooking the chicken slurry under "oxidation-inhibiting conditions" for 36 hrs. (Pippen et al., 1958), the 2-way stopcocks leading to the solubility classification and derivative preparation traps were closed. As ebullition of the gas through the traps ceased, the gas flow rate through the slurry was proportionally decreased by partially closing the stopcock, which controlled the nitrogen flow rate through the chicken slurry. To prevent plugging of the inlet tube, the gas flow was maintained at 5 ml./min. during the manipulations which followed.

Gas Chromatography: An F and M Model 500 Gas Chromatograph (F and M Scientific Corporation, Avondale, Pennsylvania) was used for the analysis of the cooked chicken volatiles from light and dark chicken muscle samples. The chromatograph had both thermal conductivity and flame-ionization (Model 1609) detectors. The flame-ionization side of the instrument was used in this study.

A 1/8 in. O.D. x 8 ft. coiled copper column was used. The column was packed with 15% by weight of Apiezon L (liquid phase) on Chromasorb W (60-80 mesh) solid support. The column was pre-conditioned at 250°C for 72 hrs. before being used for analyses. The column was also conditioned for 16 hrs. prior to each run or until the signal response from the column produced a steady base line.

The block heater was set at about 200°C and the injection port at 100°C. Air pressure to the hydrogen flame detector was maintained at 7 lbs. and hydrogen at 2 lbs. Helium pressure was held at 50 lbs. to maintain a constant gas flow rate of 55 ml./min. A sight gauge indicator attached to the instrument showed any fluctuations which occurred in the flow of helium, hydrogen and air. Limits of flow rate were established on all 3 scales and verified by runs with known compounds, such as redistilled acetone and methanol. Vapor samples of 0.5 ml. were taken above the liquid level of these solvents.

The chromatograph was operated in its most sensitive range. The attenuator was set on 8 at the start of each run. The chart speed was 80 seconds per inch, and the temperature programmer was set to provide a 15°C/min. increase in column temperature. The starting temperature was 100°C and a final temperature of 250°C was used.

Sampling for Gas Chromatography: The following procedures were used in sampling the cooked chicken volatiles before and after passing through the traps which contained the functional group reagents.

Control Samples: An open position was made by removing a trap from one branch outlet of the radial-manifold (Fig. 12). The position chosen was that located most nearly opposite and parallel to the manifold inlet tube. In place of the trap, a 12/5 ball and socket joint extension piece (Fig. 14) was coupled on at this position. A pair of small, empty absorption traps (Fig. 12) were coupled in series and immersed in cold baths. The first trap coupled to the extension piece was immersed in an ethanol-dry ice bath (-80°C), and the second was immersed in a liquid nitrogen

bath (-196°C). Volatiles were passed through the traps for one half hour using a nitrogen flow rate of 5 ml./min. Both traps were then disconnected and prepared for gas chromatographic analysis as previously described. When the trap had reached room temperature (23°C), a 2 ml. sample was removed with a gas-tight syringe and injected into the chromatograph.

The 1 l. water trap (Fig. 14) was disconnected, and an identical flask containing 100 g. of anhydrous sodium sulfate was connected in its place. Care was taken to see that the bubbler tube remained slightly above the reagent so that the outlet would not get plugged. When the 1 l. flask containing the dehydrating agent had been coupled into position, the nitrogen flow rate was increased to 100 ml./min. With the radial-manifold outlet open, the system was purged for 1/4 hr. The gas flow rate was reduced again to 5 ml./min. A series of small traps (Fig. 12) were connected to the radial-manifold outlet and immersed in cold traps as in the previous run. The second 2 ml. control sample was then taken from the nitrogen trap after warming to room temperature and injected as before. However, before taking the trap out of the cold bath, the low boiling fraction was released by warming the trap slightly in a 2-2-4 trimethylpentane-dry ice bath (-140°C). This was a standard procedure for all samples.

Functional Group Analysis Samples: The functional group analysis trapping reagents that were commonly used for the light and dark muscle volatiles included acidic and basic hydroxylamine (Bassette et al., 1962), mercuric chloride and mercuric cyanide (Folkard and Joyce, 1963), saturated potassium permanganate solution and sodium borohydride solution (Hoff and

Feit, 1963). Acetic anhydride solution was used with additional treatment of the volatiles in a secondary trap containing sodium bicarbonate solution (Hoff and Feit, 1963). Concentrated sulfuric acid and 1.2N HCl reagents were also used (Hoff and Feit, 1963). In addition to the regular treatment, other functional group reagents that were used on occasion included mercuric perchlorate, bismuth nitrate followed by mercuric acetate (Salvador et al., 1962), 1-Cl-2,4-DNB (Rittner et al., 1962), 2,4-DNP (Pippen et al., 1958) and ferric chloride (Cheronis and Entrikin, 1961).

The functional group reagent trap(s), containing 3 ml. of reagent, was/were coupled to the extension piece as previously described for the control samples. Then a series of cold traps was coupled on as previously described for the control samples, except that now they were coupled to the functional group reagent trap via an additional 12/5 ball and socket joint extension piece as shown in Fig. 15. The cooked volatiles were allowed to pass through the reagent, ethanol-dry ice and liquid nitrogen traps in that order at a flow rate of 5 ml./min. About 1 ml. of liquid nitrogen accumulated in the liquid nitrogen trap together with the volatile fraction. This was eliminated with the low boiling fraction by prewarming the trap slightly in the 2-2-4 trimethylpentane-ethanol-dry ice bath (-140°C). The release of gases relieved the pressure so that the trap could be warmed to room temperature without disturbing either the trap joints or seals.

Samples were taken as already described for the controls. Approximately 1 hr. was required to obtain a sample and chromatogram from a

single functional group reagent test on the cooked volatiles. For an average total functional group analysis, 14-18 hrs. were required to complete the manipulations and tests for 2 control samples and 10-16 functional group reagent-treated-samples, and also to dismantle the glass equipment for cleaning.

Preparing the Glassware: In early attempts to clean the glassware that had been exposed to the cooked chicken volatiles, a variety of cleaning solutions and solvents were tried. None of these, or no combination of them, effectively removed the odoriferous compounds, especially those containing sulfur. The glassware procedure adopted was as follows: (1) overnight immersion in double detergent (Alconox) solution followed by rinsing in hot water and cold distilled water; (2) immersion overnight in a (v/v) concentrated sulfuric acid and concentrated nitric acid mixture followed by a hot water and distilled water rinse and drying, and (3) heating in a muffle furnace at 400-500°C for 6 hrs. The silicone stopcock grease that was used to seal the T joints, and the mercuric precipitates were especially difficult to remove from the surface and pores of the glass traps. However, the procedure described proved adequate.

Another problem was the gas-tight syringe. Cleaning agents and excessive heat tended to loosen the Teflon plunger-tip. Accordingly the syringe was placed in a vacuum oven at 60°C under a 5 in. vacuum between runs. The plunger was inserted to the zero position of the syringe before removing the syringe from the oven. Care was taken at all times not to pump any of the laboratory atmosphere into the syringe when analyses were in progress. Upon the removal of samples from the trap, the syringe was flushed with the sample 8-10 times prior to taking the sample for injection.

Organoleptic Testing

Emphasis was not placed on organoleptic testing. However, an evaluation of effluent odors was made after the cooked volatiles had passed through derivative-preparation or solubility-classification traps. The odor and taste of the cooked chicken slurries were checked at the conclusion of the light and dark muscle runs. The taste of the freeze-dried muscle samples was evaluated on a comparative basis. The odors from the liquid nitrogen and ethanol-dry ice traps were noted immediately after the gas chromatographic analysis. The odors emanating from vials containing functional group reagents were checked. After gas chromatographic analysis of samples the odors that remained in the functional group reagent traps were noted.

Hydrogen Ion Concentration Tests

Tests of pH were made on both light and dark muscle before and after cooking using either a Beckman Model G or Beckman Zeromatic pH meter or both. The pH of the water trap was checked at the beginning and end of each run. The pH of volatiles trapped in the cold baths was checked following gas chromatographic analysis of the sample by adding 1 or 2 ml. of deionized-distilled $H_2O$ and taking the pH reading with a Beckman Model G pH meter.

Method of Preparation, Sampling and Gas Chromatographic Analysis of Sulfur Derivatives

The mercuric chloride and mercuric cyanide derivatives of cooked chicken volatiles from light and dark muscle samples were filtered on an

M-porosity sintered glass filter under vacuum. The precipitate was recrystallized from hot water and hot ethanol and dried overnight at 80°C under 5 inches of vacuum. The precipitates were tested separately by the methods of Self et al. (1963) and Folkard and Joyce (1963). About 50 mg. of derivative was placed on a stainless steel spatula and measured directly into the 1 x 6 cm. reaction trap, that was previously described. A small trap (Fig. 12), containing 3 ml. of water, and two cold traps of the same size were immersed in ethanol-dry ice at -80°C and in liquid nitrogen at -196°C. These traps were connected in series in the order named. Nitrogen gas was passed through the trapping system at a flow rate of 5 ml./min. The derivative was decomposed by adding 1 ml. 8N HCl or 10% NaOH solution. The volatiles were purged from the reaction trap with nitrogen and passed through the water trap and into the cold traps. After 1/2 hr. of ebullition, a sample from the liquid nitrogen trap was injected into the gas chromatograph under the conditions already described.

Model Experiments

Two model experiments were conducted. The purpose of the first was to determine whether volatiles obtained by heating an aqueous solution consisting of sodium sulfide, lactic acid, carbamyl phosphate and ammonium hydroxide had any characteristics resembling those found in the cooked volatile fraction of light or dark chicken muscle. The purpose of the second experiment was to determine whether glutathione would decompose when heated in an aqueous media containing acetoin, lactic acid, ammonium hydroxide, phosphoric acid and glutathione to yield the volatile sulfur

compounds found in the cooked volatiles from light and dark chicken muscle. Glutathione and acetoin are both indigenous to light and dark chicken muscle. Glutathione resides in the protein portion of chicken muscle and acetoin is found in the lipid fraction.

Model Experiment with Sulfide and Lactate Formation: This experiment was performed in a set of small traps (Fig. 12). A pure nitrogen source was used to purge the volatiles from the reaction trap and to transfer them into a liquid nitrogen immersion trap for subsequent analysis by gas chromatography.

A mixture consisting of 3 ml. of deionized-distilled $H_2O$, 1 mg. of sodium sulfide and 1 mg. of carbamyl phosphate was made into a clear solution by warming on a steam bath in a 10 ml. beaker. The solution was cooled and ammonium hydroxide was added dropwise until the pH was adjusted to a constant reading of 7.9 using a Beckman Model G pH meter. The solution was transferred into a small trap (Fig. 12) which was connected in series to two identical traps on the outlet side and to a source of pure nitrogen on the inlet side as previously described. The first trap after the reaction chamber contained 3 ml. deionized-distilled $H_2O$ and the second trap was connected via a 12/5 ball and socket connector 8 cm. long as previously described. The latter trap was immersed in a liquid nitrogen bath at -196°C. The reaction trap was heated to 180°F by immersing it in a water bath. The second trap, containing pure $H_2O$, remained at room temperature. Nitrogen was passed through the system at a rate of 5 ml./min. for 1/2 hr. The trapped volatiles were tested for hydrogen sulfide as previously described. A 5 ml. sample of the volatiles

was removed from the liquid nitrogen trap with a gas-tight syringe after the trap had been warmed to room temperature. The sample was injected into an F and M 500 gas chromatograph equipped with a hydrogen flame detector as previously described. The reagent and water trap contents were tested organoleptically for odor and taste.

Model Experiment with Sulfide and Lactate Formation and Glutathione Decomposition: The glutathione model system was identical to that used in the sulfide and lactate formation experiment excepting that 1 mg. of reduced glutathione and 0.1 ml. of 2,3 butanedione were added to the reagents previously used. The pH was adjusted to 7.9 with ammonia as before. The trapping system was the same as that used in the previous experiment, and the glutathione reaction trap was heated to 180°F. in a water bath prior to purging the volatiles with nitrogen as previously described. The trapped volatiles were tested for hydrogen sulfide and a 5 ml. sample was subjected to gas chromatography as previously outlined. The contents of the glutathione trap were tested for pH, odor, and taste. Spot tests were made on the contents of the reaction and water traps for sulfides, mercaptans and disulfides (Cheronis and Entrikin, 1961).

## EXPERIMENTAL RESULTS AND DISCUSSION

### Comparing Total Cooked Chicken Volatiles from the Whole Carcasses of Young and Old Female Chickens

The purpose of this study was to determine whether any differences could be demonstrated between volatiles from old and young chickens. This was accomplished using gas chromatography and chemical identification. The cooking-distillation apparatus shown in Fig. 10 and the cold trap detailed in Fig. 11 were used in this study. After trapping the volatiles in a liquid nitrogen immersion trap at -196°C., the low boiling fraction (minor volatile fraction) was distilled off at -140°C. The distillate was trapped in a series of three traps containing 2% cadmium acetate solution (pH 6.5), 0.01M $NaHCO_3$ solution and saturated CaO solution in that order as previously described.

Hydrogen Sulfide: Hydrogen sulfide was positively identified in the cadmium acetate trap by Emil Fischer's methylene blue method as modified by Marbach and Doty (1956). After adding 0.3 ml. of amine solution and 0.1 ml. of Reissner solution the absorption tube was stoppered, shaken well and let stand at room temperature for 30 minutes. The dark blue color which developed indicated that sulfide was present in the cooked volatiles from both young and old chickens. Dilution tests of 1/10, 1/100 and 1/1000 gave visual evidence that the methylene blue concentration was higher in the solution containing trapped volatiles from older chickens than in that containing volatiles from young birds.

Carbon Dioxide: Carbon dioxide was positively identified in the second and third traps by two different methods. Identification was made in the second trap by Marriott's method (1916) using 0.01M $NaHCO_3$ reagent and Cresol Red indicator. The colorimetric result was checked against the pH values using a Beckman Model G pH meter. The pH decreased from 8.2 to 6.7 when the volatiles from older chickens passed through the reagent; whereas, with the volatiles from younger birds, the pH dropped from 8.2 to 6.9. No colorimetric differences between samples could be demonstrated due to the lack of color intensity in the original solutions. However, the results of both tests demonstrated that carbon dioxide was present in the chicken volatiles. The pH results showed that the sample obtained from older birds contained more carbon dioxide than the sample from younger birds.

The third trap which contained saturated CaO solution gave a white precipitate of $CaCO_3$. Gravimetric results for $CO_2$ as $CaCO_3$ were not determined for the $CO_2$ contents of the cooked volatiles from old and young birds that were trapped in saturated CaO solutions.

Carbon dioxide has no flavor value per se. However, carbon dioxide may combine with hydrogen sulfide, ammonia, water or other volatiles from cooked chicken, and in this way may have some role in chicken flavor. The amount of carbon dioxide present in the volatile fraction might possibly serve as an indication of the extent of precursor degradation that occurs with different samples of chicken muscle or with the same chicken muscle under varying conditions of cooking and distillation. One of the most probable pathways to carbon dioxide formation and evolution in cooked

chicken volatiles is through the degradation of muscle glutathione (Mason, 1931). Cleavage of glutathione may result in the formation of cysteine, glycine and alpha amino glutamic acid. Then the formation of a Schiff's base could follow by an interaction between a carbonyl compound and one of the free alpha amino acids. In the presence of acetoin after heating to boiling for 15 minutes, the alpha amino acids are degraded to the corresponding aldelydes or ketones containing one less carbon atom (Schönberg et al., 1952), while ammonia and carbon dioxide are then liberated.

Carbonyl Sulfide: Carbonyl sulfide was not chemically identified. However, a strong similarity was noted between the odor of the total trapped volatile fraction and that of a pure sample of carbonyl sulfide. Carbonyl sulfide has been found as a trace impurity in carbon dioxide, which is used for carbonating beverages (Hall, 1962). Hall also reported that when carbonyl sulfide comes in contact with water it hydrolyzes slowly to hydrogen sulfide and carbonic acid.

The presence of hydrogen sulfide, ammonia and carbonyls has been demonstrated in cooked chicken volatiles by Crocker (1948), Bouthilet (1949, 1950, 1951a,b), Pippen and Eyring (1957), and Pippen et al. (1958). Copious amounts of carbon dioxide were also found in the present study, which increases the likelihood of carbonyl sulfide formation. There is a good chance of its presence in cooked chicken volatiles, although probably in a relatively low concentration.

Gas Chromatographic Analysis: Table 1 shows the results for gas chromatographic analysis of the major volatile fractions from older laying hens and younger female chickens. Samples were taken after warming the

liquid nitrogen trap (Trap F - Fig. 10) to 23°C. and 75°C. A total of seven peaks were obtained from the cooked volatiles from young or old female birds. The peaks had essentially identical retention times. Fig. 16 and Table 1 show that the retention times were slightly longer for samples taken at 23°C than for those taken at 75°C (Fig. 17). However, the increase in helium flow rate in the second comparative analysis (Fig. 17 and Table 1) was probably the principal cause of this difference. Nevertheless, the retention times for comparative sample peaks remained identical for each particular analysis at each temperature, irregardless of which helium flow rate was used.

Table 1 and Figs. 16 and 17 also show the total retention volumes that were obtained by adding together the respective retention volumes taken from each of the seven peaks at both sampling temperatures. The retention volumes were higher for older hens than for the younger birds. The comparative retention volumes were calculated by multiplying the retention time for each peak by the peak height for that particular peak. A difference between the total retention volumes of less than 5 (Table 1) when pertinent to the same peak for younger chickens and for old hens at a given temperature was not regarded as a significant difference. Then the designation (N.S.D.) meaning no significant difference between the peaks was used. For example, the values for total retention volume, Peak 1--Table 1--23°C. were 4 for old hens and 6 for young chickens. This difference in total retention volume being less than 5 was not significant. On this basis, the volatiles taken from the liquid nitrogen trap (Trap F--Fig. 10) at 23°C. gave four peaks (Peaks 2, 3, 5 and 7)

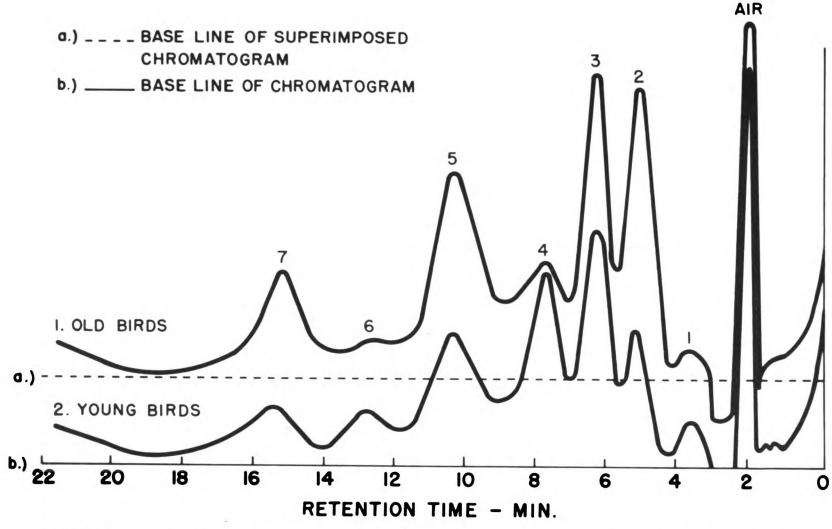



FIGURE 16. EXPERIMENT 1-A GAS CHROMATOGRAMS OF 10 ML. VOLATILES FROM COOKED WHOLE CARCASS OLD HENS AND YOUNG BIRDS TRAP WARMED TO ROOM TEMPERATURE (23°C) 1/4" X 13' (DEHS) COLUMN - 70°C AND 21 P.S.I. HELIUM.

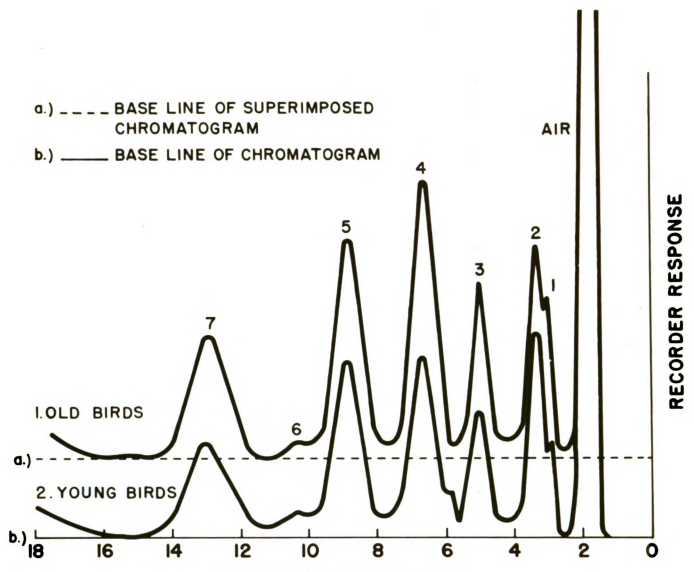



FIGURE 17. EXPERIMENT I-B GAS CHROMATOGRAMS OF 10 ML. VOLATILES FROM COOKED WHOLE CARCASS HENS AND YOUNG BIRDS TRAP WARMED TO 75°C 1/4" X 13' (DEHS) COLUMN - 70°C AND 30 P.S.I. HELIUM.

Table 1. Volatile components separated from old hens and younger female chickens.

| Volatiles taken from trap F[a] at 23°C. | | | | Volatiles taken from trap F[a] at 75°C | | | |
|---|---|---|---|---|---|---|---|
| | | Comparative retention volumes (retention time x peak height) | | | | Comparative retention volumes (retention time x peak height) | |
| Peak No. | Time (min.) | Old hens | Young chickens | Peak No. | Time (min.) | Old hens | Young chickens |
| air | 2 1/4-3 | - | - | air | 1 1/2-2 1/2 | - | - |
| 1 | 4 | 4 | 6 (N.S.D.)[b] | 1 | 3 1/4 | 5 | 7 (N.S.D.) |
| 2 | 5 1/2 | 46(+)[c] | 22 | 2 | 3 1/2 | 21 | 21 |
| 3 | 6 1/2 | 57(+) | 43 | 3 | 5 1/4 | 26(+) | 18 |
| 4 | 8 | 26 | 43(+) | 4 | 6 3/4 | 54(+) | 35 |
| 5 | 10 1/2 | 62(+) | 39 | 5 | 9 | 57(+) | 46 |
| 6 | 13 | 15 | 19 (N.S.D.) | 6 | 10 1/3 | 12 | 14 (N.S.D.) |
| 7 | 15 1/2 | 46(+) | 23 | 7 | 13 1/3 | 47(+) | 36 |

[a]Trap F - liquid nitrogen immersion trap.
[b]N.S.D. - no significant difference.
[c]The sign (+) indicates that the peak for this kind of chicken is higher than the comparative peak for the other kind of chicken.

in Table 1 which were appreciably higher for older hens than for younger birds. The volatiles obtained from younger birds gave only one peak out of seven at 23°C, Peak 4, that was higher than the corresponding peak for the old hens. Two of the seven peaks, Peaks 1 and 6, obtained from each type of bird were small peaks and these showed no significant difference in retention volumes. Sampling at 75°C. gave four peaks out of seven (Peaks 3, 4, 5 and 7) in Table 1 which were appreciably higher for older hens than for young birds. The three remaining peaks at 75°C showed no significant differences in height for either type of chicken.

The results of gas chromatographic analysis indicate that the volatiles from young and old chickens were identical in chemical composition. This was demonstrated by the good agreement between retention times. The retention volumes for the older birds were consistently higher.

This experiment indicated that the cooked volatiles from whole carcasses of young and old chickens were essentially alike, but that larger quantities of certain volatile components were evolved from older birds upon prolonged heating. The origin and chemical character of these volatiles were not elucidated. Studies on cooked chicken volatiles by Bouthilet (1949, 1950, 1951a,b), Pippen and Eyring (1957), Pippen *et al.* (1958), Pippen *et al.* (1960), Kazeniac (1961) and Pippen and Nonaka (1963) show the importance that these workers attached to the relation of the composition of the volatile fraction to chicken flavor. The results obtained in the present study substantiate Lineweaver and Pippen's (1961) observation that the flavor of chicken *per se* is fairly independent of type of bird. Although the flavor components of old hens and young female

chickens were found to be the same, the older birds appeared to have more flavor per unit of cooked volatiles than the younger birds. Whether the particular portions of the volatile fraction of old hens that were present in higher concentrations indicated a better flavor rather than simply more flavor cannot be decided from the results alone. In fact, our present knowledge of chicken flavor includes scarcely more than speculative information as to the origin of the so-called flavor volatiles.

It is entirely possible that cooking serves to eliminate or reduce the amounts of certain undesirable flavor components in addition to producing the desirable "chickeny" flavor that develops only after heating the meat. For example, Pippen and Eyring (1957) showed that the flavor of chicken broth was improved as a result of removal of ammonia. Kazeniac (1961) stated broth with a high hydrogen sulfide content had an undesirable "eggy" taste; whereas, the addition of a high concentration of ammonia to the solution reduced the egginess and gave the solution a sweet flavor and an improved aroma. Kazeniac (1963) suggested that ammonium sulfide and ammonium polysulfide may make a positive contribution to chicken broth flavor. Kazeniac (1961) also showed the need for ammonia flavor by ammonia and sulfide balance experiments. Carbonyls contribute to overall chicken flavor, both positively and negatively, according to Pippen (1958) and Kazeniac (1961). Accordingly, further evidence would be needed in order to conclude that old hens have a more desirable flavor than young birds simply because a higher concentration of certain volatile components was obtained by cooking and distilling meat from old hens as compared to younger females.

This study of heavy hen and younger female chicken volatiles showed that the volatile components from the two kinds of birds were essentially alike. It was also shown that the concentration of volatile constituents from old hens was higher than from young female chickens. This concentration difference was verified by gas chromatography and chemical tests. Hydrogen sulfide and carbon dioxide were identified in the low boiling portion of the volatile fraction which was distilled from the total cooked volatile fraction at -140°C. Sulfide and carbon dioxide concentrations were markedly higher in the low boiling fraction from old hens than in that from young female chickens.

Vapor Fractometer Tests on Cooked Breast Muscle from Fryers

The first peak from cooked chicken volatiles as determined with a hydrogen flame detector and a Model 500 F and M instrument would not react with any of the functional group reagents, including concentrated sulfuric acid (Hoff and Feit, 1963). Thus, the presence of low molecular weight paraffin(s) was suspected. To determine whether this was true, vapor fractometer tests were made using pre-packaged fryer breasts that were obtained from a local market. The manner of preparation of the muscle has been previously described.

The presence of oxygen, nitrogen and carbon dioxide in the volatile fraction was verified using the method of Brenner *et al.* (1959). The sample passed through the Linde 5A molecular sieve within 5 min. and 3 peaks were obtained. These peaks were tentatively identified as oxygen, nitrogen and carbon dioxide, in order of emergence from the column by using a

standard chromatogram for comparison (Table 2). The presence of nitrogen and oxygen may have been due to a leak in the Buchler apparatus, which would allow liquid air condensed from the laboratory atmosphere to accumulate in the liquid nitrogen trap. The presence of carbon dioxide in the concentration indicated by the retention volume of that peak could not be attributed to the small amount of carbon dioxide that was present in the laboratory atmosphere. A test sample from the laboratory atmosphere verified the low concentration of carbon dioxide and indicated that the carbon dioxide must have originated from the cooked chicken.

Table 2. Vapor fractometer separations of the low boiling fraction in fryer breast muscle distillate

| Peak No. | Retention time (min.) columns[a] | | | | Tentative identity column | |
|---|---|---|---|---|---|---|
| | Linde 5A molecular sieve | | HMPA on chromasorb | | Linde 5A molecular sieve | HMPA on chromasorb |
| | Unknown | Known | Unknown | Known | | |
| 1 | 2 | 2 | 1/2 | 1/2 | oxygen | nitrogen |
| 2 | 4 | 4 | 1 | 1 | nitrogen | ethane |
| 3 | 6 | 6 | 2 | 2 | | carbon dioxide |
| 4 | | | 2 1/2 | 2 1/2 | | propane |
| 5 | | | 5 3/4 | none available | | unknown |

[a]Columns were connected in parallel.

By using the tentative method for natural gas analysis of the National Gasoline Association of America (421 Kennedy Building, Tulsa 3, Oklahoma), which utilizes gas chromatography, the presence of ethane and propane was tentatively verified. Upon passing through the HMPA column

a total of 5 peaks were recorded on the chromatogram. Four of these peaks were identified as nitrogen, ethane, carbon dioxide and propane. The fifth peak was not identified. All of the peaks were eluted within 6 minutes. When authentic samples of ethane and propane were injected into the fractometer, the retention times matched those obtained on the chromatogram of the cooked breast meat condensate taken at -196°C. as shown in Table 2.

A Phospholipid Fraction Containing "Chicken Essence"

When an experiment aimed at using smaller amounts of muscle for gas chromatographic and chemical analysis of cooked chicken volatiles failed, a modified distillation procedure resulted in the isolation of a phospholipid fraction. By cooking under "oxidation-favoring conditions" (Pippen et al., 1958) and refluxing under a partial vacuum some yellow, green, amber and brown colored material collected in the small water trap (Fig. 12). This material had been steam-distilled over into the first trap and amounted to about 4 ml. of solution. Members of the laboratory staff agreed that the freshly prepared distillate had a several-fold concentration of true chicken odor.

After standing overnight the color changed from dark amber to clear yellow. The pH was determined on a Model G pH Meter and was 8.6. After 48 hrs. the "chickeny" aroma was nearly gone, which was probably due to the escape of sulfur compounds (Bouthilet, 1951; Pippen and Eyring, 1957). The fugitive nature of sulfur components contained in onion has been reported by Challenger and Greenwood (1949).

The solubility of the fraction was tested in water, ethanol, ammonia water, chloroform, and carbon disulfide. The material was readily soluble in carbon disulfide, water and ammonia water; moderately soluble in ethanol, and slightly soluble in chloroform.

The fraction was subjected to thin layer chromatography. Two unknown spots moved faster than the lecithin control spot. The two unknown spots were close together. By changing the phase from "neutral" to "alkaline" the spots moved more slowly. However, they still moved faster than the lecithin standard during the 75 min. development time at room temperature (Table 3). The identity of the spots was tested using ninhydrin spray for amines, fuchsin-sulfurone reagent for aldehydes, hydroxylamine-ferric chloride reagent for esterified fatty acids, ammoniacal silver nitrate for glycerol and inositol, ferric chloride-sulfosalicylic acid reagent for phosphate groups, molybdic acid for phosphatides and Dragendorf's reagent for choline compounds (Skidmore, 1962). The lecithin spot developed with the Dragendorf reagent spray. The slower moving unknown spot reacted positively to the ammoniacal silver nitrate. Both of the unknown spots reacted to give a positive test with the molybdic acid spray.

Table 3. Thin layer chromatographic results for "chicken essence" from heavy hen breast muscle

| Sample | $R_f$ values in cm. | | |
|---|---|---|---|
| | 1[a] | 2[b] | 3[b] |
| Lecithin | 11 | 10 | 8 |
| Unknown-1 | 19 1/2 | 14 | 12 |
| Unknown-2 | 20 | 16 | 14 |

[a]Neutral" chromatoplates. Chloroform-methanol-$H_2O$ (195:75:12)
[b]"Basic" chromatoplates. Chloroform-methanol-glacial acetic acid-$H_2O$ (62:25:8:4)
[c]"Basic" chromatoplates. Chloroform-methanol-glacial acetic acid-$H_2O$ (50:25:8:4)

Authentic compounds were not available to test the $R_f$ values of the two unknown compounds. From the literature values of Skipski et al. (1962), it appeared that these spots were due to phosphatidyl-ethanolamine and lyso-phosphatidyl-ethanolamine which Kates and James (1961) had previously isolated from old hens. However, the fact that a negative ninhydrin test was obtained ruled these compounds out. It seems likely that these compounds may be cardiolipin and phosphatidyl inositol which Gray and Macfarlane (1961) have isolated from pigeon breast muscle.

Bouthilet (1951) extracted an aqueous solution of redistilled and strongly flavored chicken broth distillate with isopropane. He obtained three fractions, and found that desulfuration continued upon standing so long as true flavor was evidenced by hydrogen sulfide formation. He concluded that chicken flavor consists of at least two portions, a sulfur containing compound and a fatty acid-like material. Bouthilet (1951) postulated that the sulfur compound that is the precursor for the transitory hydrogen sulfide contributing to chicken flavor is glutathione. Peterson (1957) also found that the principal aroma component in chicken is sulfide, which he stated possibly arises from the heat treatment of the protein. Kazeniac (1961) found that steam distillates obtained from the fat fraction of chicken broth showed faint, chicken-like aroma, which indicated that fat may serve as a trapping agent for some of the volatiles.

The volatile sulfur component(s) present in the phospholipid fraction isolated in the present study were not due to the presence of glutathione as the ninhydrin spray test (Skidmore, 1962) was negative. This indicates that the phosphatide itself may serve as a trapping agent for small amounts

of sulfur compounds, since only small amounts are needed to produce organoleptic response (Guadagni et al., 1963). Furthermore, Schöberl (1933, 1936) observed that butylmercaptan decomposes in the presence of oxygen in a mild alkaline media to liberate dipropyl disulfide, hydrogen sulfide and propionaldehyde. Thus, results suggest that the phosphatide may serve as an electrostatic binding media for both the acidic mercaptans and the sulfides which have two unshared pairs of electrons on the sulfur atom or the disulfides which have one unshared pair of electrons on each sulfur atom. (Challenger, 1959). Due to the pH of the fraction (8.6), which was probably associated with the hydrogen ion binding properties of the aqueous sulfide, it appears that the only sulfur compounds present would either be sulfides or disulfides or both. Mercaptans would be converted to disulfides and/or aldehydes (Schöberl, 1933, 1936) under the basic condition of the media. Aldehydes and alcohols, due to their acidic natures (Fieser and Fieser, 1956), would also be bound by the media. In conclusion, evidence indicates that a phosphatidyl lipid fraction or fractions in chicken may be responsible for the observation of Kazeniac (1961) that chicken fat may serve as a trapping agent for some of the volatiles. This lipid fraction could also be the fatty acid-like material that Bouthilet (1951) found was responsible for cooked chicken flavor, together with a highly labile sulfur compound contained in the chicken flesh, probably glutathione.

Comparison of Total Cooked Chicken Volatiles from the Whole Carcasses of Old Hens with the Volatiles from the Cooked Intestinal Contents of the Same Birds

The purpose of this study was to determine whether any chemical similarities could be demonstrated between the intestinal contents of older hens and the cooked carcasses from the same birds. This was an exploratory type of experiment in which comparative parameters were not closely controlled.

The intestinal contents collected from 20 old laying hens (1.5 lbs.) were mixed with 500 ml. deionized-distilled $H_2O$ and cooked by refluxing under "normal cooking conditions" for three hours and then by cooking and distilling under "oxidation-inhibiting conditions" for 17 hours.

Gas Chromatography: The results of gas chromatographic analysis are shown in Fig. 18. None of the peaks was identified. A hydrogen flame detector was used in this study so that a positive correlation of retention volumes with those obtained for the whole carcasses of old birds as shown in Figs. 16 and 17 could not be made as the parameters were different. However, Fig. 18 represents a chromatogram for the total cooked volatiles from the intestinal contents of old hens.

Chemical Identifications: Prior to gas chromatography, the low boiling fraction was condensed at -196°C. and boiled off at -140°C. The results obtained matched those for the same fraction of volatiles obtained from cooking the whole carcasses of heavy hens. Carbon dioxide and hydrogen sulfide were positively identified and carbonyl sulfide was tentatively identified.

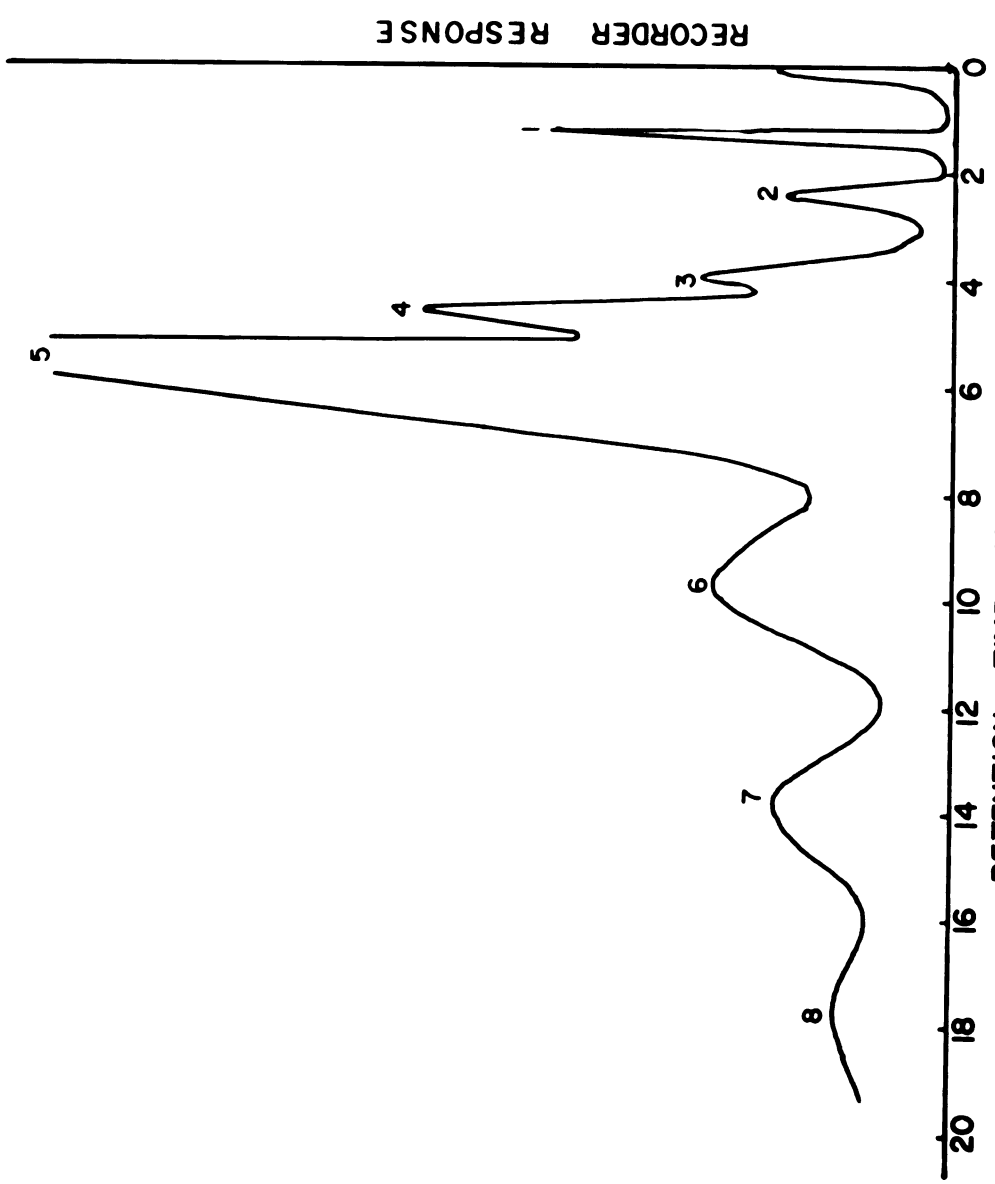



**Figure 18** Experiment 3, Gas Chromatogram Of 5 Ml. Volatiles From Cooked Intestinal Contents Of Old Hens. Trap Warmed To 23°C. 1/4" X 13' (DEHS) Column-70°C. And 20 P.S.I Helium.

Results of Carbonyl Tests: A small quantity of DNPH was isolated (200 mg.). By means of column chromatography, UV and I-R spectra and paper chromatography, acetaldehyde, acetone and n-hexanal were tentatively identified from the monocarbonyl fraction. Polar microscopy and I-R spectra were used to identify diacetyl, which was separated from the polycarbonyl fraction by fractional crystallization. (Rice et al., 1951; Ellis et al., 1958; and Pippen et al., 1958). Results for the carbonyl identification are shown in Table 4.

Table 4. Physical properties of 2,4-DNPHS of carbonyl compounds from the cooked intestinal contents of old hens

| Column band No. | 2,4-DNPH derivative | $R_f$ unknown cm. | $R_f$ known cm. | UV absorption maxima Unknown mu | UV absorption maxima Known mu | Additional methods of characterization |
|---|---|---|---|---|---|---|
| forerun | unknown | - | - | - | - | - |
| 1 | n-hexanal | 0.82 | 0.80 | 360 | 361 | 1 |
| 2 | acetone | 0.88 | 0.86 | 363 | 363 | 1 |
| 3 | acetaldehyde | 0.81 | 0.79 | 354 | 355 | 1 |
| 4 | unknown | - | - | - | - | - |
| - | diacetyl | 0.00 | 0.00 | - | - | 1, 2 |

1. The infrared spectrum was identical to that of the authentic sample.
2. Microscopic examination showed that the crystals had identical refractive indices and phase transformations. A polar microscope was used.

Three monocarbonyls, namely, acetone-, acetaldehyde- and n-hexanal - 2,4-DNPHS, and one polycarbonyl--diacetyl--were tentatively identified in the cooked volatile fraction from the intestinal contents of old hens. The small amount of derivative recovered from the solvent cuts taken from

the center of each band (center cuts), which had sharp absorption maxima, was too small to make melting point determinations practical. Tests on the forerun solvent fraction (which contained no clearly defined bands) and on the chloroform extract of bands remaining on the column adsorbent, showed that in addition to those compounds which were tentatively identified, other monocarbonyls and polycarbonyls were present. A violet color with alcoholic KOH gave evidence of the presence of at least one or more additional polycarbonyls, other than diacetyl. By reading the UV absorption maxima in chloroform solvent, evidence was found that 2-en-1-al with a UV absorption maximum of 373 mu and 2,4-dien-1-al with a UV maxima of 339 mu may be present.

Results of Sulfide, Disulfide and Mercaptan Tests: The quantity of lead acetate derivative obtained from the cooked volatiles from the intestinal contents was extremely small as compared to the amounts obtained in the whole carcass experiments. Sulfides, disulfides and mercaptans were present but in low concentrations.

The exploratory experiment for evaluating the characteristics of cooked intestinal contents indicates that the same classes of compounds probably exist in this media as in the whole carcass of chicken but in varying proportions and in lower concentrations.

## The Chemical Identification of Carbonyls and Sulfur Containing Compounds Present in the Volatile Fraction of Cooked Chicken

The purpose of this study was to attempt to confirm the classical work of Pippen et al. (1958) on carbonyls, and also if possible, to determine some of the classes of sulfur containing compounds that were present

in the cooked volatile fraction of chicken. Although the cooking was done under the atypical "oxidation-favoring conditions" used by Pippen et al. (1958) for carbonyls, the distillation was carried out under "oxidation-inhibiting" conditions by using nitrogen gas to purge the whole carcass chicken slurry and to provide a carrier gas for ebullition of the cooked volatiles through the 2,4-DNP and saturated lead acetate reagent absorption traps. The apparatus used is shown in Fig. 10, but was modified by adding an absorption train as previously described.

Carbonyls: The fractionation of the hydrazone precipitate was carried out according to the scheme described by Pippen et al. (1958). A total of 1.8 g. of crude precipitate of 2,4-DNPHS was obtained from 3 kg. of chicken, which was a considerably higher yield than that reported by Pippen et al. (1958). It may have been caused by a more efficient air entrainment method associated with modifications in the cooking and distilling apparatus of Pippen et al. (1958).

The separation and isolation of diacetyl-2,4-DNPH was made by a direct crystallization from a nitrobenzene solution of the polycarbonyl derivatives in the manner described by Pippen et al. (1958). A yield of 0.15 g. of polycarbonyl-derivatives was obtained, which was taken up in hot nitro-benzene and filtered. After several days, the crystals that separated were washed with ethanol and dried. Comparison of the melting point and I-R spectra of this unknown material with those of authentic diacetyl-2,4-DNPHS showed that they were identical. During chromatography of monocarbonyl 2,4-DNPHS, other polycarbonyl 2,4-DNPHS were found as small bands. These were eluted from the column following acetaldehyde-

2,4-DNPH. Treatment with alcoholic KOH gave a blue-violet color which is characteristic for 2,4-DNPHS having carbonyl groups on adjacent carbon atoms (Newberg and Strauss, 1945). The amounts of 2,4-DNPH derivatives present in the bands were small, and this fact as well as the lack of authentic derivatives precluded any further study.

The unknown bands were easily collected, except for the butanal and ethyl-methyl ketone bands, which ran together (Pippen et al., 1958). To separate these 2,4-DNPHS, the eluted fraction was dried, taken up with chloroform and separated by chromatography according to the method of Lynn et al. (1956). An average distance of 5 cm. separated the centers of the two spots, which were outlined on the paper by pencil as the spots were examined in a dark room under UV light. Pure ethyl-methyl ketone-- and butanal--2,4-DNPHS were recovered by elution from the paper with chloroform, drying and recrystallizing from hot ethanol.

A single passage of equal amounts of the monocarbonyl derivatives through 11-75 cm. beds of adsorbent gave 9 preliminary bands by gradient elution. Two slower bands were cut out of the column adsorbent media and these were eluted from the adsorbent with hot chloroform. Solvent cuts, that were taken from the centers of the 9 preliminary bands, were tested for UV absorption maxima in chloroform solvent in the range of 300-425 mu using a Beckman DU Spectrophotometer. Additional UV maxima readings were taken on the dried spots following paper chromatography according to the method of Nonaka et al. (1959). The results are shown in Table 5.

Melting and mixed melting point results (Table 5) indicate that the center cuts taken from bands 9, 8, 7, 4, 3 and 1 gave sharp melting

Table 5. Properties of 2,4-DNPHS of carbonyl compounds isolated from cooked chicken volatiles

| Column band No. | Recrystallized fraction M.P., °C | Carbonyl compound identified | Authentic sample M.P., °C | Mixed with authentic sample M.P., °C | Additional methods of characterization |
|---|---|---|---|---|---|
| forerun | 87-89 | unknown | - | - | - |
| 1 | 104-107 | n-octanal | 107-108 | 104-107 | a, e |
| 2 | 130-137 | 2,4-dien-1-al | unavailable | - | c |
| 3 | 105-108 | n-hexanal | 108-110 | 105-109 | a, d, e |
| 4 | 108-110 | n-pentanal | 108-110 | 108-110 | a, e |
| 5 | 122-123 | n-butanal | 123-124 | 122-124 | a, e |
| 6 | 111-115 | methyl-ethyl ketone | 114-115 | 111-114 | b, d, e |
| 7 | 154-158 | n-propanal | 155-157 | 154-158 | a, e |
| 8 | 124-126 | acetone | 125-127 | 124-127 | b, d, e |
| 9 | 164-168 | acetaldehyde | 168-169 | 164-168 | a, e |
| 10 | 94-95 | unknown | - | - | - |
| poly- | - | unknown | - | - | - |
| carbonyl | d. above 300 | diacetyl | d. above 300 | d. above 300 | e, f |

[a]Shows ultraviolet absorption characteristics of an n-alkanal derivative.
[b]Shows ultraviolet absorption characteristics of 2 2-alkanone derivative.
[c]Shows ultraviolet absorption characteristic of 2,4-dien-1-al derivative.
[d]Migration on paper chromatograms was checked with authentic samples.
[e]The infra-red spectrum was identical to that of the authentic sample.
[f]Microscopic examination of crystals with a polarizing microscope showed that the crystals were alike with respect to refractive indices and phase transformations.

points. The center of band 10 was cut out of the adsorbent, eluted with hot chloroform (UV reading taken), dried and recrystallized from hot ethanol. Band 11 was small and poorly defined so that no tests were made on it. The center cuts from bands 3 and 4 and 5 and 9 gave melting points that were so nearly alike that paper chromatography together with UV and I-R spectra readings were required to establish their respective identities. The UV readings are shown in Table 6 together with the $R_f$ and $R_h$ values for 8 of 11 bands. Results of I-R spectra studies on acetone, diacetyl, ethyl-methyl ketone and n-hexanal are shown in Figs. 19, 20, 21 and 22. No authentic samples were available for confirming the identities of fractions 2, 10 or 11.

On the basis of melting point and mixed melting point results (Table 5) together with paper chromatography and UV absorption results (Table 6) fraction 1 was identified as n-octanal-2,4-DNPH. Using melting points (Table 5) and UV absorption data (Table 6), band 2 was tentatively identified as 2,4-dien-1-al-2,4-DNPH (Pippen et al., 1958).

A total of 8 monocarbonyls were identified by 3 or more criteria including melting point, chromatographic behavior, crystallographic properties, ultraviolet and infrared absorption spectra. Two ketone derivatives, namely; methyl ethyl ketone and acetone were isolated and identified together with 6 aldehydes; namely, the n-alkanals containing 2, 3, 4, 5, 6 and 8 carbon atoms. One polycarbonyl, namely, diacetyl was separated by the solubility and crystallization method of Pippen et al. (1958). Identity of diacetyl was also confirmed by melting point, I-R spectra and microscopic examination of its crystallographic properties.

Table 6. Paper chromatography and UV absorption maxima of spots of 2,4-DNPH derivatives

| Column band No. | 2,4-DNPH derivative | $R_f$ unknown cm. | $R_f$ known cm. | $R_h$ unknown cm. | $R_h$ known cm. | UV absorption maximum mu |
|---|---|---|---|---|---|---|
| forerun | unidentified | - | - | - | - | - |
| 1 | n-octanal | 35 | 33 | 1.20 | 1.14 | 370 |
| 2 | unidentified | - | - | - | - | 389 |
| 3 | n-hexanal | 25.1 | 26.0 | 0.87 | 0.90 | 370 |
| 4 | n-pentanal | 21.0 | 22.0 | 0.73 | 0.76 | 370 |
| 5 | n-butanal | 16.9 | 15.5 | 0.58 | 0.54 | 370 |
| 6 | methyl-ethyl ketone | 27.3 | 27.5 | 0.94 | 0.95 | 374 |
| 7 | n-propanal | 13.1 | 12.0 | 0.45 | 0.41 | 370 |
| 8 | acetone | 13.2 | 13.5 | 0.46 | 0.47 | 373 |
| 9 | acetaldehyde | 10.3 | 9.0 | 0.35 | 0.31 | 368 |
| 10 | unidentified | - | - | - | - | - |
| 11 | unidentified | - | - | - | - | - |

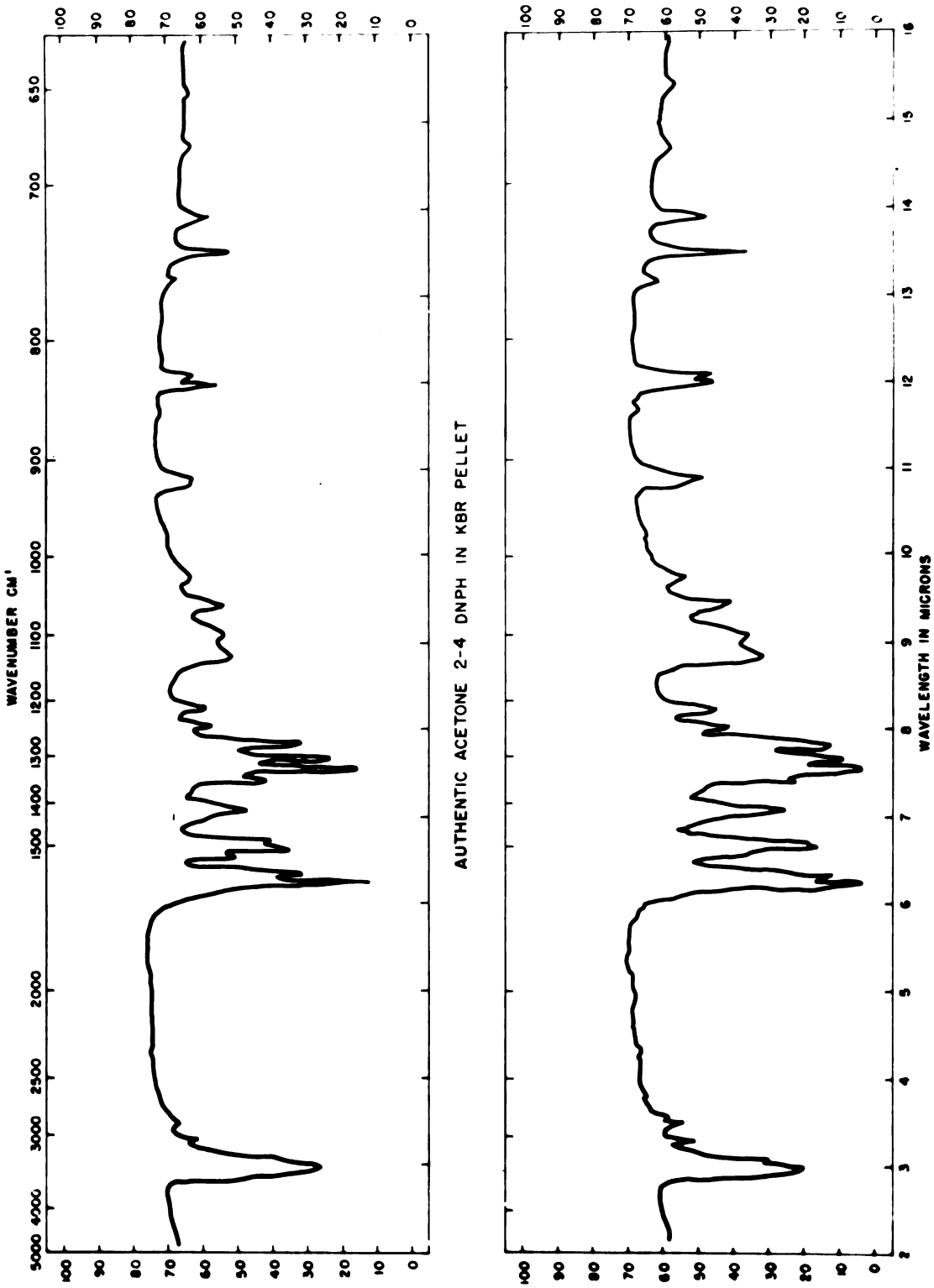



Fig. 19. Infra-red spectra for the authentic and unknown samples of acetone-2,4-DNPH.

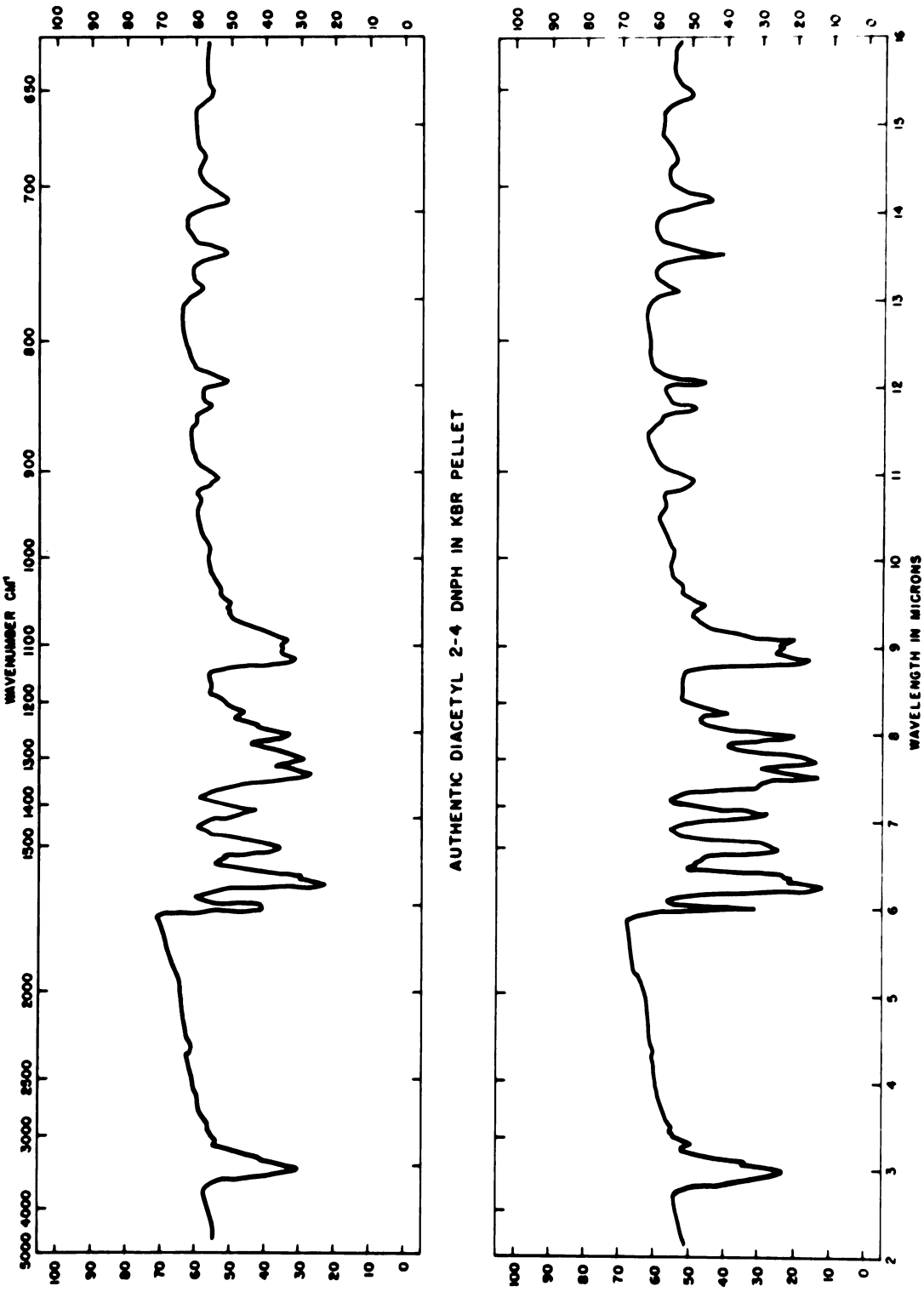



Fig. 20. Infra-red spectra for the authentic and unknown samples of diacetyl-2,4-DNPH.

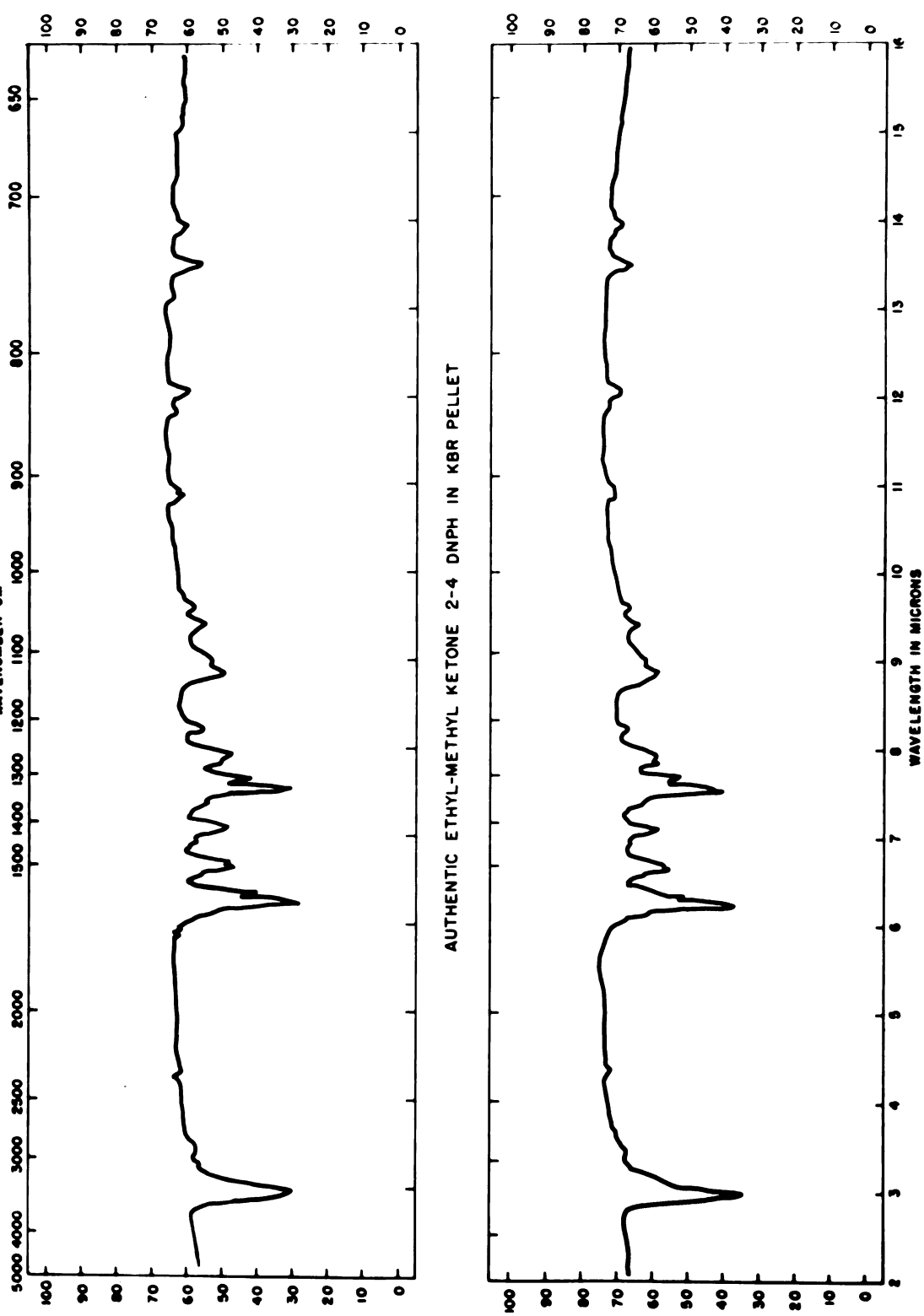



Fig. 21. Infra-red spectra for the authentic and unknown samples of ethyl-methyl-ketone-2,4-DNPH.

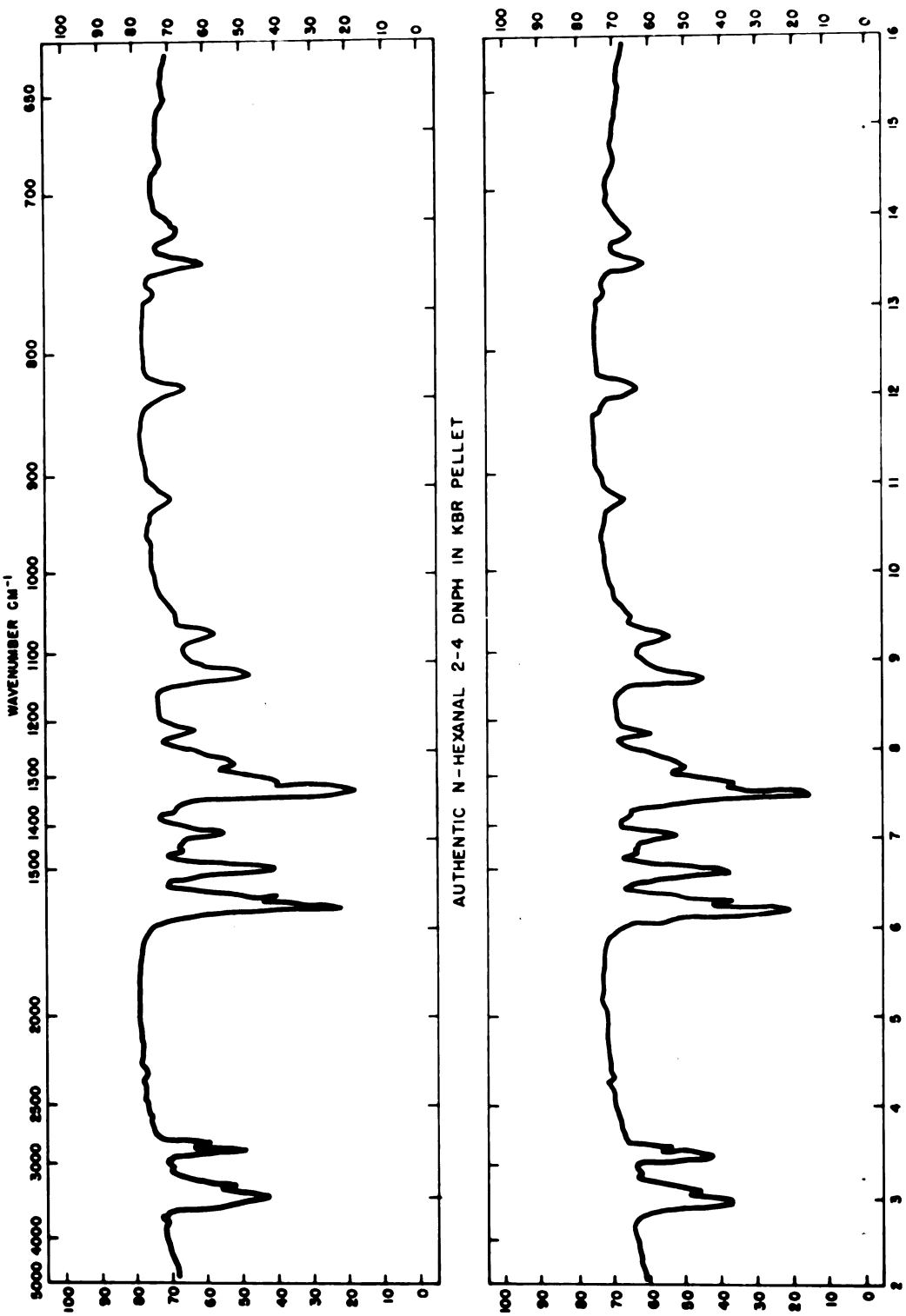



Fig. 22, Infra-red spectra for authentic and unknown samples of n-hexanal-2,4-DNPH.

The elegant investigation of carbonyls in the cooked volatile fraction of chicken by Pippen et al. (1958) was confirmed in a limited manner by the results obtained in the present study for 8 monocarbonyls and 1 polycarbonyl. Pippen et al. (1958) used larger chickens that probably had a higher fat content than those used in the present study. This may have been the reason that a larger number of carbonyl fractions were obtained than in the present study. Nevertheless, a total of 9 carbonyl derivatives were obtained, which had the same physical properties as 9 of the 18 carbonyls that were identified by earlier workers. This showed that the cooked volatiles from both kinds of birds were similar, at least in respect to the 9 carbonyl components.

Lineweaver and Pippen (1961) have pointed out that the importance of carbonyls to chicken flavor is not well understood. In a recent study using gas chromatography, Pippen and Nonaka (1963) found that n-hexanal and n-2,4-decadienal were two of the principal components in the cooked volatiles of rancid chicken, whereas, n-hexanal was prominent in the cooked volatiles from either fresh chicken or turkey, as well as in rancid chicken volatiles. Larger amounts of these two carbonyls were found in chicken skin and the fat attached to the skin than in the lean leg or breast muscle of chicken. The present study seems to confirm Pippen and Nonaka's (1963) findings concerning carbonyls in fresh chicken, although the identity of the n-2,4-decadienal derivative was merely tentative.

Sulfur Compounds in the Cooked Volatile Fraction: Sulfur derivatives formed in the reagent traps in prolific amounts. Their formation started

within one-half hour after the cooking-distillation temperature of 180°F was reached and continued without abatement until the end of the cooking-distillation period. This same phenomena was previously reported by Yueh and Strong (1960) in their studies on cooked beef volatiles.

Mercaptan Test: A positive test for mercaptans was obtained in the form of a light yellow precipitate of 2,4-dinitrophenyl thioether(s). This test was made using the method of Folkard and Joyce (1963) in the manner previously described.

Sulfide Test: A positive test for sulfide(s) was obtained as evidenced by the formation of methylene blue using the method of Marbach and Doty (1956).

Test for Disulfides: A positive test for disulfides was obtained using the methods of Stahl and Siggia (1957) and Rittner et al. (1962).

Bouthilet (1951), Pippen and Eyring (1957) and Kazeniac (1961, 1963) have recognized and stressed the importance of sulfides and other sulfur compounds in chicken flavor. In the present study, sulfur derivatives were visible in the traps within one-half hour after heating of the chicken-water slurry and ebullition of the volatiles through the reagent traps began. In contrast, the formation of carbonyl derivatives took place slowly, and a visible precipitate of 2,4-DNPH was not visible until about two hours after heating began. Thus, it appears that organic sulfur compounds are probably the first odorous compounds obtained in quantity in the volatile fraction, apart from hydrogen sulfide and ammonia both of which have low boiling points. This suggests that at least part of the typical cooked chicken flavor may be related to sulfur compounds; princi-

pally, hydrogen sulfide, organic sulfide(s), organic disulfide(s) and to a lesser extent to mercaptan(s).

Speculatively, carbonyls may have a split role. The lower carbonyls, principally acetaldehyde may play a major part in the "browning reaction". Acetaldehyde has also been described as adding a scorched flavor note in flavor profiles of chicken broth (Kazeniac, 1961). Low flavor threshold values have been found by Lea and Swoboda (1958) for many carbonyls; for example, n-decanal was detectable in water at a concentration of $5 \times 10^{-8}$ moles per liter. Patton et al. (1959) reported that n-2,4-decadienal, which Pippen and Nonaka (1963) had shown to be a major component in cooked chicken volatiles, has a "deep-fat fried aroma." However, Pippen and Nonaka (1963) have noted that it can also have a desirable cooked fat or fried chicken odor. On the other hand, these workers, found that on exposure to air at room temperature, n-2,4-decadienal first developed stale and then rancid odors. Furthermore, n-hexanal was found to be the most prominent carbonyl compound in rancid chicken by Pippen and Nonaka (1963). The concentration of n-hexanal in rancid chicken was found to be much higher than in fresh chicken (Pippen and Nonaka, 1963). Accordingly, it can be concluded that the higher carbonyls, such as n-hexanal and n-2,4-decadienal, can contribute to desirable aroma but may also be immediate precursors of stale or rancid odors. This leads to the speculation that carbonyls may be responsible for over-cooked and off-flavors as well as to some desirable flavors in cooked chicken volatiles. Perhaps, some pleasing flavors in cooked chicken may be due to the presence of low or even sub-threshold concentrations of specific carbonyls. The

importance of acetoin and n-2,4-decadienal, which may impart transient buttery-oily or fried chicken odors, respectively, has been shown by Pippen and Nonaka (1960, 1963).

Cooked Meat Yields from Roasters, Heavy- and Light-Weight Hens

The purpose of this study was to determine the cooked meat yield from three classes of chickens. This was accomplished by dividing the carcasses into wings, necks, backs, legs, breasts, giblets, and skin, which constitute the so-called solid portion of the chicken, and into the broth and fat, which constitute the liquid portion. The waste due to cutting and handling was also determined for each class of bird.

Table 7 shows that the uncooked White Rock roasters were two times and Cornish Cross hens two and one-half times as heavy as the White Leghorn hens. On a percentage basis the breast meat content of the light hens was higher than that of either the roasters or heavy hens, but on a weight basis was considerably less. Breasts from the heavy hens weighed considerably more than those from roasters. On a percentage basis, the difference was significant at the 5% level. Legs from roasters and heavy hens were about equal on a weight basis, but on a percentage basis the roaster legs showed a greater yield with the light hens second and heavy hens last. Heavy hens yielded the heaviest backs.

From Table 8, it is evident on a percentage basis that cooked roasters yielded more leg meat than light hens, which in turn yielded more than heavy hens. On a weight basis, the light hens yielded less than one-half as much leg meat as heavy hens or roasters. The breast meat yield from

Table 7. Summary of the average uncooked yields obtained from 35 chickens of each class by weight and percentage

| Component | Light hens | | Heavy hens | | Roasters | |
|---|---|---|---|---|---|---|
| | Grams | % of total | Grams | % of total | Grams | % of total |
| **Wings** | 126** ± 12 | 11.6" ± 0.7 | 277 ± 35 | 10.4" ± 1.3 | 268 ± 39 | 12.8" ± 1.5 |
| Neck | 40** ± 11 | 3.7" ± 1.1 | 77** ± 20 | 2.9" ± 0.7 | 94** ± 21 | 4.5" ± 0.9 |
| Legs | 267** ± 27 | 24.6" ± 1.5 | 595 ± 59 | 22.4" ± 1.5 | 586 ± 59 | 28.0" ± 1.5 |
| Back | 228** ± 38 | 21.0 ± 2.0 | 665** ± 109 | 25.1" ± 2.9 | 443** ± 59 | 21.2 ± 2.0 |
| Breast meat | 261** ± 28 | 24.0" ± 1.9 | 596** ± 82 | 22.4 ± 1.5 | 457** ± 61 | 21.9 ± 1.6 |
| Skin[a] | 72** ± 18 | 6.7 ± 1.4 | 217** ± 64 | 8.3' ± 2.1 | 152** ± 40 | 7.3 ± 1.7 |
| Internal fat | Insig. | - - - | 103 ± 57 | 3.9 ± 2.0 | Insig. | - - - |
| Giblets | 82 ± 28 | 7.5" ± 2.4 | 105** ± 35 | 3.9 ± 1.2 | 70 ± 22 | 3.4 ± 1.1 |
| Losses | 11 ± 4 | 0.9 ± 0.3 | 23 ± 8 | 0.8 ± 0.3 | 20 ± 7 | 0.9 ± 0.3 |
| Total | 1087** ± 106 | 100.0 ± 0.4 | 2658** ± 305 | 100.0 ± 0.6 | 2090** ± 197 | 100.0 ± 0.4 |

[a]Skin represents the combined total of skin from legs and breast which was removed prior to cooking.
[b]Back skin, wing skin, and neck skin were removed and weighed after cooking, breast and leg skin before cooking.
[c]Skin represents skin from backs, necks, and wings removed after cooking. [d]Standard deviations.
*Significant at 5% level. **Significant at 1% level. 'Significant at 5% level. "Significant at 1% level.

Table 8. Summary of the average cooked yields obtained from 35 chickens of each class by weight and percentage

| Component | Light hens | | Heavy hens | | Roasters | |
|---|---|---|---|---|---|---|
| | Grams | % of total | Grams | % of total | Grams | % of total |
| Wings | | | | | | |
| Meat | 42** ± 9 | 3.9 ± 1.0 | 86 ± 19 | 3.2" ± 0.7 | 81 ± 17 | 3.9 ± 0.6 |
| Bone | 38** ± 7 | 3.5" ± 0.6 | 55 ± 7 | 2.1 ± 0.3 | 49 ± 11 | 2.3 ± 0.5 |
| Skin | 21** ± 6 | 1.9 ± 0.6 | 52** ± 21 | 2.0" ± 0.9 | 70** ± 21 | 3.3 ± 0.9 |
| Neck | | | | | | |
| Meat | 20** ± 4 | 1.8 ± 0.4 | 35 ± 11 | 1.4" ± 0.4 | 37 ± 8 | 1.8 ± 0.4 |
| Bone | 15** ± 3 | 1.8" ± 0.4 | 22* ± 6 | 0.8" ± 0.3 | 25** ± 5 | 1.2" ± 0.2 |
| Skin[b] | 14** ± 4 | 1.7" ± 0.4 | 25** ± 11 | 1.0 ± 0.4 | 23* ± 5 | 1.1 ± 0.2 |
| Legs | | | | | | |
| Meat | 125** ± 17 | 11.5' ± 2.1 | 284 ± 36 | 10.7" ± 0.9 | 274 ± 33 | 13.1' ± 1.1 |
| Bone | 51** ± 7 | 4.7" ± 0.9 | 92** ± 9 | 3.5 ± 0.3 | 75** ± 15 | 3.6 ± 0.8 |
| Back | | | | | | |
| Meat | 50** ± 11 | 4.6' ± 0.9 | 137* ± 21 | 5.1' ± 0.7 | 120* ± 24 | 5.7" ± 1.1 |
| Bone | 60** ± 13 | 5.5" ± 1.3 | 107** ± 21 | 4.0 ± 0.8 | 83** ± 13 | 4.0 ± 0.7 |
| Skin | 58** ± 11 | 5.4 ± 1.9 | 154* ± 55 | 5.8 ± 1.9 | 129* ± 33 | 6.2 ± 1.5 |
| Breast | | | | | | |
| Meat | 147** ± 16 | 13.5' ± 1.7 | 348** ± 53 | 13.1 ± 1.2 | 259** ± 36 | 12.4' ± 0.9 |
| Bone | 29** ± 7 | 2.7" ± 0.6 | 43 ± 6 | 1.6" ± 0.2 | 40 ± 10 | 2.2" ± 0.5 |
| Giblets | 48 ± 11 | 4.4" ± 1.7 | 52 ± 15 | 1.9 ± 0.6 | 38** ± 11 | 1.8 ± 0.7 |

See footnotes Table 7.

light hens (percentage basis) was greater than that from heavy hens, and from heavy hens was greater than from roasters. On a weight basis, the heavy hens yielded more than twice as much breast meat as the light hens and one and one-third times that from roasters. Back meat ranged from 50 grams, or 4.6 percent from light hens, to 120 grams or 5.7 percent from roasters, and 137 grams or 5.1 percent from heavy hens. Neck meat varied from 20 grams or 1.8 percent from light hens, to 35 grams and 1.8 percent from roasters and 35 grams or 1.4 percent in heavy hens. Wing meat amounted to 42 grams or 3.9 percent from light hens to 81 grams or 3.9 percent from roasters and 86 grams or 3.2 percent from heavy hens. Waste, in Table 9, refers to veins and cartilaginous material plus drip. Results are graphically summarized in Fig. A.

In Table 10, the average cooked lean meat content with reference to the uncooked eviscerated carcass weight is given. Light hens yielded 35.5 percent of lean meat, heavy hens 33.5 percent and roasters 36.3 percent.

Table 10 also shows the average yield of cooked total edible meat (lean, skin and fat) based on the uncooked eviscerated carcass weight. Light hens yielded 47.7 percent of total cooked edible meat whereas heavy hens yielded 48.8 percent and roasters yielded 52.7 percent. A graphical summary of these results is shown in Fig. B.

The significance of these data may be limited by several factors including boning method, cooking method, meat recovery method, eviscerated weight and eviscerated price per lb. as follows:

Table 9. Summary of the waste, solids, separation loss and liquids obtained from 35 chickens of each class by weight and percentage.

| Component | Light hens | | Heavy hens | | Roasters | |
|---|---|---|---|---|---|---|
| | Grams | % of total | Grams | % of total | Grams | % of total |
| Waste | 5** ± 1 | 0.5 ± 0.1 | 21 ± 8.5 | 0.8 ± 0.5 | 22 ± 11 | 1.1 ± 0.5 |
| Solids | | | | | | |
| Meat | 384** ± 34 | 35.5' ± 4.6 | 890** ± 106 | 33.5" ± 2.1 | 758** ± 114 | 36.3' ± 3.7 |
| Bone | 195** ± 21 | 18.0" ± 2.6 | 319** ± 25 | 12.0" ± 0.7 | 278** ± 34 | 13.3" ± 1.9 |
| Skin[c] | 96** ± 24 | 9.0 ± 2.3 | 233 ± 45 | 8.8" ± 1.5 | 221 ± 43 | 10.6 ± 2.0 |
| Separation loss | 56 | .51 | 71 | .27 | 74 | .35 |
| Solids total | 731** ± 47 | 67.3' ± 7.7 | 1513** ± 135 | 56.9" ± 3.1 | 1331** ± 122 | 63.7' ± 3.7 |
| Liquids | | | | | | |
| Broth | 320** ± 98 | 29.4 ± 7.3 | 973** ± 147 | 36.2" ± 2.7 | 647** ± 107 | 30.9 ± 3.3 |
| Fat | 36** ± 11 | 3.3" ± 0.9 | 172** ± 26 | 6.9" ± 0.6 | 112** ± 20 | 5.4" ± 0.6 |
| Total | 356** ± 109 | 32.7 ± 8.1 | 1145** ± 135 | 43.1" ± 3.1 | 759** ± 126 | 36.3 ± 3.8 |

See footnotes Table 7.

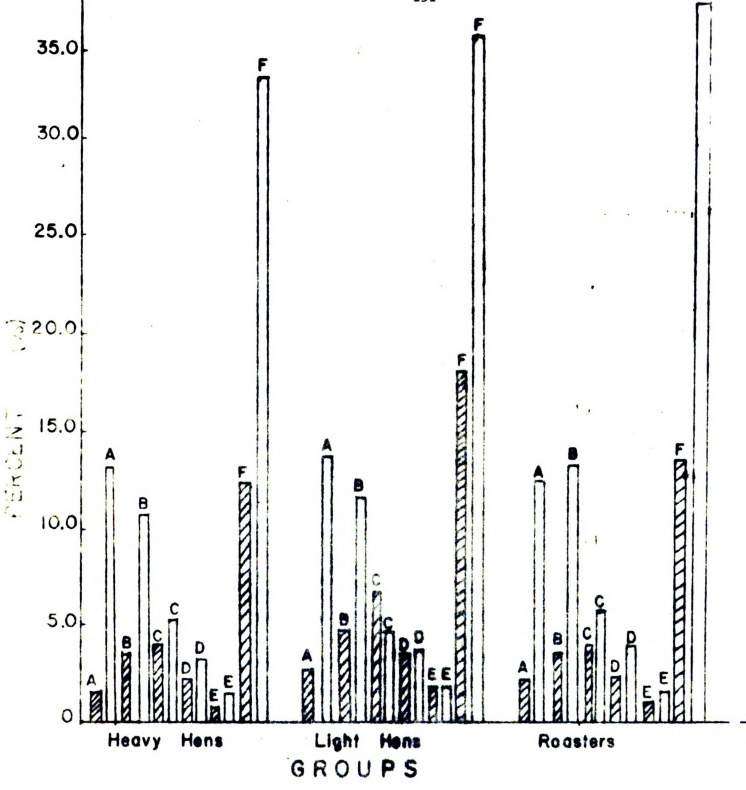



Fig. A. Percentage of cooked meat as: A-breast, B-leg, C-back D-wing, E-neck and F-total compared with the percentage of bone for these same components, in heavy hens, light hens and roasters.

Bone  Meat

Table 10. Average yields of cooked lean meat and total cooked edible meat based on uncooked eviscerated weight (EW)

| | Cooked lean meat | | | Total cooked edible meat | | |
|---|---|---|---|---|---|---|
| | Uncooked EW | Av. yield | | Uncooked EW | Av. yield | |
| Class of bird | Grams[a] | Grams | % of total | Grams | Grams | % of total |
| Light hens[b] | 1087 ± 106 | 384 ± 34 | 35.5 ± 4.6 | 1087 ± 106 | 516 | 47.7 |
| Heavy hens[c] | 2658 ± 305 | 890 ± 106 | 33.5 ± 2.1 | 2658 ± 305 | 1294 | 48.8 |
| Roasters[d] | 2090 ± 197 | 758 ± 114 | 36.3 ± 3.7 | 2090 ± 197 | 1091 | 52.7 |

[a]Standard deviation.

[b]White Leghorn hens (16 months old).

[c]White Cornish cross hens (16 months old).

[d]White Rock roasters (16 weeks old).

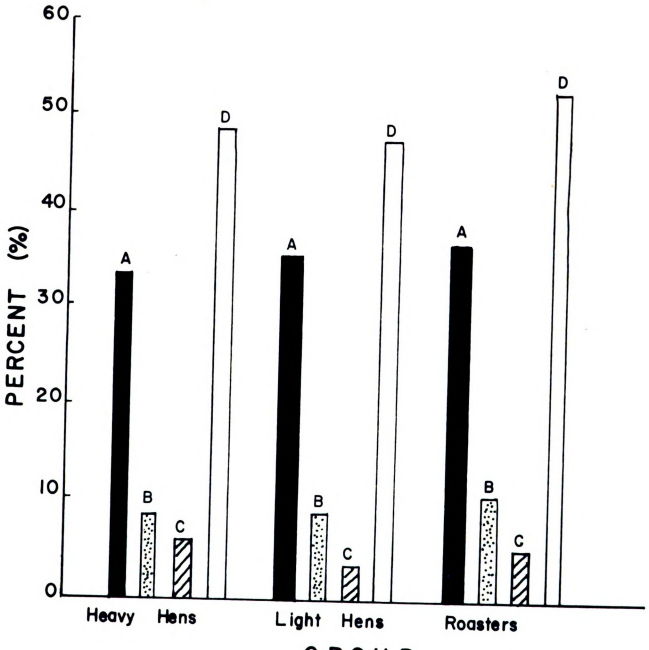



Fig. B. Percentage of A– meat, B– skin, C– fat, D–total cooked edible portion in heavy hens, light hens and roasters.

(1) A preliminary study revealed that when the carcass was boned prior to cooking, the cooked meat yield obtained was lower than when the carcass was cut up before cooking, but not boned until after cooking.

(2) Conventionally cooked chicken was compared with electronically cooked 10 week old fryers by Schano and Davidson (1958). They showed that flavor quality of electronically cooked chicken was lower but that the yield was higher than with roast or rotisserie cooked birds.

(3) Old fowl was cooked by boiling, simmering and pressure-cooking as reported by Kahlenberg and Funk (1961). Simmering gave higher yields and pressure-cooking more tender meat but with a lowered fat content.

(4) In the present study it was noted that the same number of man-hours were required to separate the lean meat from the birds whether roasters, heavy or light hens were used. Since the yield of lean meat from the 35 light hens by weight was about one-half of that obtained from the other classes, the cost of hand separation was doubled. However, cooked lean meat from light hens could be separated commercially by the Harris flotation method as described by Waskiewicz (1962), which would make the cooked lean meat from light hens less expensive than that from heavy hens or roasters.

(5) It was also noted that heavy hens which gave the highest yield of cooked lean meat on a weight basis required less man-hours of labor per unit weight of lean meat separated. In other words, heavier birds resulted in a reduction in the cost of hand separation.

Fig. C provides a chart for each of the three classes of chickens. A horizontal line from the price per lb. eviscerated weight of the class

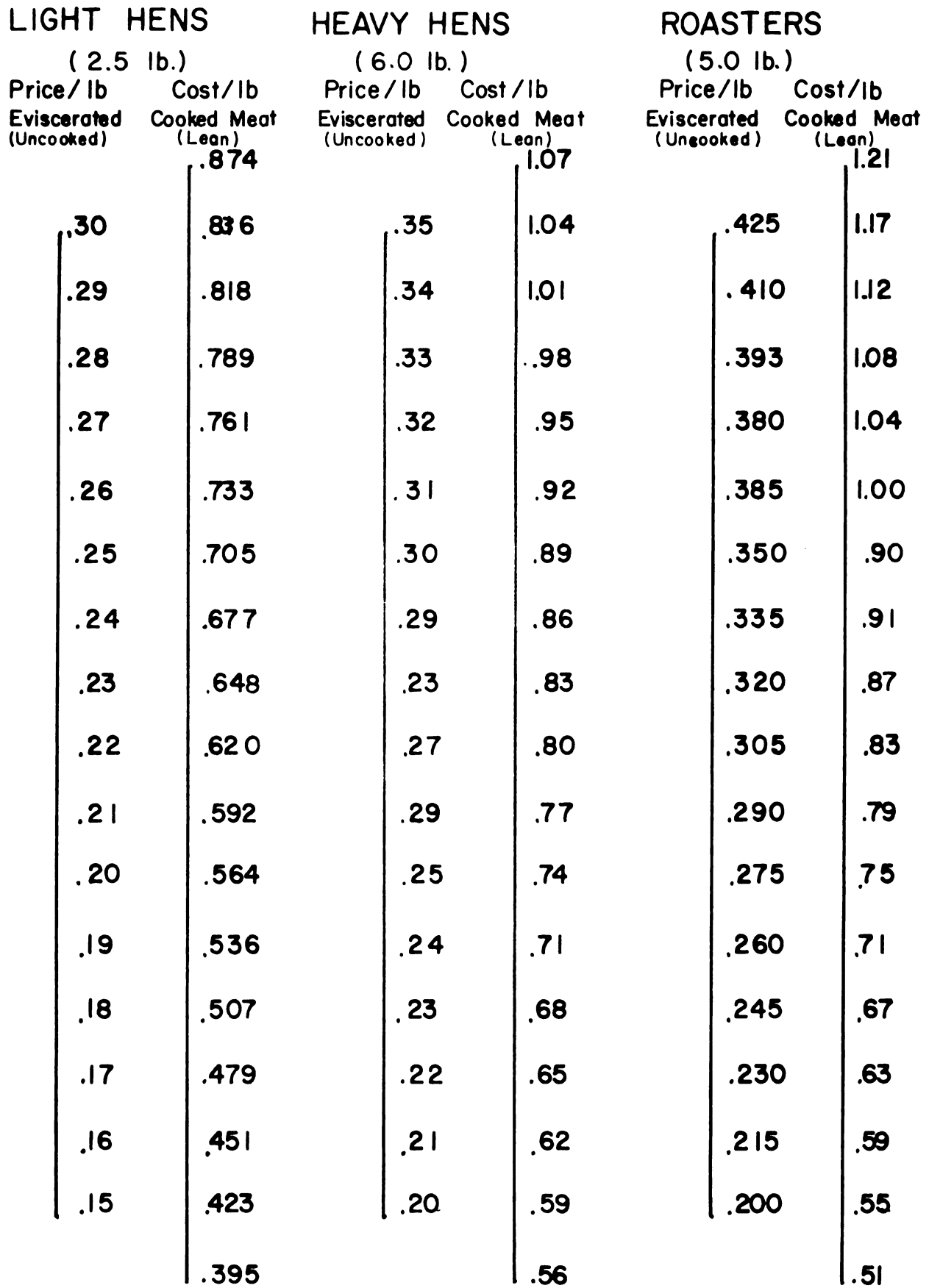



Fig. c. Chart for calculating the cost/lb of cooked lean meat for three classes of chickens.

of interest extended through the cost per lb. on the cooked lean meat axis will indicate the cost/lb. of cooked lean meat. This cost does not include labor or overhead and may vary up or down with lighter or heavier birds and in accord with the other parameters which have already been discussed.

Summary: A total of 105 birds (35 roasters, 35 heavy fowl, and 35 light-weight hens) were weighed and cut up, and individual parts were then weighed to ascertain the uncooked weights and yields of these various parts. The parts of each bird were then sealed in coded containers and pressure cooked. The meat was then removed from the bones so that the meat and bone contents of each part could be determined. Skin from legs and breasts was removed and weighed prior to cooking. Neck, back, and wing skin was separated and weighed after cooking. Broth and fat of each bird were separated and weighed. A composite value for the total cooked weight was ascertained for each bird. Analysis of variance was applied to the data to test for differences among classes, and standard deviations were calculated. Roasters yielded the highest percentage of cooked lean meat (36.6%), light hens next, and heavy hens the least. Yield of total edible product (lean, fat, and skin) was likewise highest for roasters (52.7%), followed by heavy hens and light hens. Light hens had the highest percentage of bone, whereas heavy hens yielded the highest percentage of total liquid (broth and fat). Light hens yielded the highest percentage of cooked breast meat (calculated from dressed weight); whereas, roasters had the highest percentage of cooked lean meat from the legs. The percentage of breast meat was higher from heavy hens than from roasters,

but lower than from light hens. From the data, a chart was developed for sight-calculating the cost/lb. of cooked lean meat for these three classes of chickens.

Chemical Analyses

The purpose of the chemical studies was to correlate the chemical composition of a given raw or cooked muscle sample with that of the cooked volatile fraction. Besides determining the proximate analysis for each raw muscle sample and each sample of cooked-freeze-dried slurry several other chemical analyses were made on these samples for: Creatine/creatinine, cystine, methionine, sulfhydryl compounds, inorganic sulfide and pH. Determinations were made on the raw muscle samples only for acetoin/diacetyl and inosinic acid concentrations. These results are shown in Table 11.

Representative paired samples taken from a 100 g. aliquot of either the raw muscle or the cooked-freeze-dried slurry of each kind of muscle from each class of bird was analyzed. Table 11 shows the protein, fat, ash, and moisture content of the various samples. Attempts to relate differences in composition to the organoleptic characteristics which were noted for the same samples gave negative results.

The cooked breast muscle slurry from heavy hens had better flavor than other samples.

Acetoin/diacetyl: Table 11 shows a higher acetoin and diacetyl content and a lower pH value for heavy hen breast muscle. These results agree with those obtained by Pippen et al. (1960) and Kazeniac (1961). Acetoin is 2,3 butanedione, which can be oxidized to diacetyl (acetylmethyl carbinol) by heating it in an oxidizing atmosphere. Acetoin in

Table 11. Components of raw-frozen and cooked-freeze-dried breast and leg muscle and cooked-freeze-dried meat-broth slurry from roasters, heavy- and light-weight hens

| Sample No. | Nitrogen % | Protein % | Fat % | Ash % | $H_2O$ % | Creatine % | Creatinine % | Cystine mg./g. |
|---|---|---|---|---|---|---|---|---|
| 1 r | 3.3 | 20.6 | 4.8 | 1.2 | 72.7 | 0.45 | 0.39 | 2.5 |
| 2 r | 2.8 | 17.5 | 5.7 | 1.9 | 75.0 | 0.36 | 0.35 | 2.3 |
| 3 r | 3.5 | 21.9 | 4.2 | 1.0 | 72.4 | 0.37 | 0.32 | 2.7 |
| 4 r | 3.2 | 20.0 | 5.4 | 1.5 | 73.6 | 0.35 | 0.31 | 2.4 |
| 5 r | 3.5 | 21.9 | 3.0 | 1.0 | 74.5 | 0.36 | 0.31 | 2.5 |
| 6 r | 3.2 | 20.0 | 3.2 | 1.0 | 75.1 | 0.31 | 0.26 | 2.3 |
| 1 c | 14.0 | 87.5 | 7.2 | 1.8 | 1.6 | 1.13 | 0.98 | 8.5 |
| 2 c | 13.8 | 86.3 | 8.6 | 2.4 | 1.1 | 0.86 | 0.74 | 8.0 |
| 3 c | 14.2 | 88.8 | 5.9 | 1.7 | 1.8 | 1.03 | 0.89 | 9.5 |
| 4 c | 13.7 | 85.6 | 8.1 | 2.3 | 2.0 | 0.82 | 0.71 | 8.5 |
| 5 c | 14.7 | 91.9 | 2.8 | 1.6 | 1.7 | 1.08 | 0.74 | 9.5 |
| 6 c | 14.5 | 90.6 | 4.9 | 2.0 | 1.3 | 0.67 | 0.58 | 9.0 |

1. Sample code: 1-heavy hen breast; 2-heavy hen leg; 3-roaster breast; 4-roaster leg; 5-light hen breast, and 6-light hen leg. (r: raw-frozen muscle) (c: cooked-freeze-dried meat-broth slurry).

Table 11. Components of raw-frozen and cooked-freeze-dried breast and leg muscle and cooked-freeze-dried meat-broth slurry from roasters, heavy- and light-weight hens (continued)

| Sample No. | Methionine mg./g. | Sulfhydryl ppm. | Sulfide[2] ppm. | Acetoin[3] ug./100 ml. | Diacetyl[3] ug./100 ml. | pH | Inosinic acid um/g. |
|---|---|---|---|---|---|---|---|
| 1 r | 6.5 | 37 | not present | 161.2 | 8.8 | 5.8 | 3.3 |
| 2 r | 5.7 | 73 | or less | 60.4 | 2.4 | 6.1 | 2.9 |
| 3 r | 6.9 | 58 | than 1 ppm. | 137.6 | 8.7 | 5.8 | 2.9 |
| 4 r | 5.1 | 48 | | 51.8 | 2.1 | 6.6 | 2.6 |
| 5 r | 6.6 | 98 | | 127.8 | 6.9 | 6.0 | 2.3 |
| 6 r | 7.0 | 27 | | 26.5 | 1.8 | 6.2 | 2.1 |
| 1 c | 27.8 | 233 | | | | 6.2 | |
| 2 c | 24.6 | 300 | | | | 6.3 | |
| 3 c | 24.4 | 383 | | | | 6.2 | |
| 4 c | 30.4 | 138 | | | | 6.8 | |
| 5 c | 27.2 | 368 | | | | 6.3 | |
| 6 c | 28.0 | 133 | | | | 6.4 | |

1. Sample code: 1-heavy hen breast; 2-heavy hen leg; 3-roaster breast; 4-roaster leg; 5-light hen breast, and 6-light hen leg. (r: raw-frozen muscle) (c: cooked-freeze-dried meat-broth slurry).

sufficient concentration imparts a transient buttery-oily aroma to cooked chicken or to chicken broth, whereas, high diacetyl levels impart sour notes (Kazeniac, 1961). Bouthilet (1951) showed that lower pH values enhance chicken flavor. Thus, these two effects, that is a higher acetoin content and lower pH value for heavy hen breast muscle than the other samples, may contribute to the superior flavor characteristics of breast muscle from the heavy hens.

Using model systems, Self et al. (1963) recovered hydrogen sulfide from the decomposition of cystine or cysteine in air or nitrogen atmospheres. Furthermore, methanethiol, dimethyl sulfide and dimethyl disulfide formed when methionine was decomposed in air. Only methanethiol formed in a nitrogen atmosphere.

Cystine and methionine as well as glutathione (glutamyl-cysteinyl-glycine) are indigenous to chicken muscle. Sulfhydryl concentration is used as an index to glutathione content. From the concentrations of methionine, cystine and sulfhydryl in Table 11, it is evident that these compounds were present in larger amounts in the samples from light weight hens than in the heavy hen or roaster muscle samples.

Studies by Kazeniac (1961) showed that sulfide and nitrogen balance was an important factor in good chicken broth flavor. The principal source of ammonia and carbon dioxide is from Strecker degradation of amino acids (Self et al., 1963). The importance of an active poly-carbonyl compound in such amino acid decompositions was demonstrated by Schönberg et al. (1948, 1952). Acetoin (2,3-butanedione) which is indigenous to chicken muscle is one of these active carbonyls. Therefore, it is conceivable that the amount of amino acid decomposition encountered in

heating muscle samples is a function of the active polycarbonyl concentration present in the sample. On this assumption, the flavor acceptability would improve if conditions prevailed favoring ammonia and sulfide balance. This could explain the richer and more "chickeny" flavor of the heavy hen breast and leg muscle samples. It would also clarify the inconsistency of organoleptic and gas chromatographic results when compared to the concentrations of sulfur compound precursors shown in Table 11.

Inosinic Acid: Inosinic acid is an important non-volatile flavor constituent. The concentrations of inosinic acid ranged from 2.3 to 3.3 um/g. in the raw breast muscle samples, and from 2.1 to 2.9 um/g. in the raw leg muscle samples. Heavy hen leg and breast muscle had the highest concentrations of inosinic acid with the roaster muscle samples next highest and light-weight hen muscle samples contained the lowest concentrations. The higher inosinic acid content in heavy hen breast and leg muscle samples than in the other samples probably contributed to their superior taste.

Creatine/creatinine: According to Kazeniac (1961), creatine/creatinine make up a major part of the non-amino nitrogen compounds in chicken muscle. The creatine/creatinine contents of raw and/or cooked muscle in the present study were higher in light than in dark meat (Table 11). Heavy hen muscle samples had a higher creatine/creatinine content than the other samples (Table 11). Both compounds have a bitter taste and in high concentrations impart a bitter after-taste to the muscle slurry. In his work, Kazeniac (1961) found that after 0.2 g. of creatine, 100 g. of ground light chicken meat and 100 ml. of water were made into a slurry,

canned and processed 30 min. at 120°C, the creatine additive had little effect on the volatiles. The role of creatine/creatinine in flavor is probably additive and synergistic. Due to the high content of creatine/creatinine in chicken, it is possible that they may serve a useful purpose as precursor tag compounds. It is interesting to note that the creatinine concentrations found in the raw muscle samples were almost equal to those of creatine. These results were in contrast to Kazeniac's (1961) observation that creatinine contents were very low if present at all in raw meat extracts. Creatine is converted to creatinine on heating. However, the creatine/creatinine concentrations in the cooked-freeze-dried muscle-slurry were about equal. Furthermore, results (Table 11) did not indicate that any appreciable conversion had occurred. However, some results (Table 11) indicated that losses of both creatine and creatinine occurred after 50 hrs. cooking-distillation.

Cystine and Methionine: Methionine concentrations were considerably higher for both raw and cooked samples than cystine concentrations. However, the ratio of concentrations between raw and cooked samples was about the same for methionine as for cystine. The pathway to cystine from glutathione via cleavage and oxidation is well known. That of methionine in cooked muscle has not been clearly defined. The role of glutathione and methionine in the production of hydrogen sulfide and methyl mercaptan during irradiation of meat has been reported by Martin et al. (1962). Recently Self et al. (1963) in their work on potato volatiles have defined the roles of cysteine, cystine and methionine in model systems. Again, the production of hydrogen sulfide from cystine and cysteine was

observed as a result of Strecker degradation of these alpha amino acids. Methionine degradation resulted in the formation of methyl mercaptan and dimethyl disulfide.

The cystine concentrations of all the samples were approximately equal, but were somewhat higher for the light than for the dark muscle samples (Table 11). Uncooked heavy hen and roaster breast muscle samples had considerably higher methionine concentrations than the corresponding leg muscle samples. The light-weight hen raw leg muscle sample had a markedly higher concentration of methionine than the breast muscle sample. There was no clearly defined trend in the cooked-freeze-dried slurry samples (Table 11).

Sulfhydryl Content: Both raw and cooked heavy hen breast muscle samples had a considerably lower sulfhydryl concentration than either the roaster or light-weight hen samples. Yet the sulfhydryl concentration was notably higher in raw heavy hen leg muscle than in either the roaster or light-weight hen samples. Sulfhydryl is used as an index of glutathione concentration. However, the cystine concentrations do not reflect the results one would expect by comparing sulfhydryl to cystine concentrations.

Ammoniacal Nitrogen: Ammoniacal nitrogen values were obtained for the raw muscle samples only and varied between 0.05 and 0.06%.

The significance of the chemical analyses (Table 11) probably extends beyond the limited data obtained. For example, the absence of sulfide indicates that a loss of ammonium sulfide and other sulfides must have occurred as a result of freeze-drying. It likewise confirms the fact that sulfide formation is the result of degradations and possibly con-

densations and rearrangements which occur when the raw muscle is cooked. Unfortunately, the sulfide determinations were not made on the cooked muscle slurry prior to freeze-drying. Such determinations would have given more significant results for sulfide.

Organoleptic Evaluations of the Cooked Broth Samples

The purpose of this study was to evaluate the flavor of the cooked broth samples from heavy hen, roaster and light-weight hen breast and leg muscle samples.

Table 12 shows that broth from heavy hen breast muscle had the most acceptable, strongest and truest chicken flavor of any of the samples following 50 hrs. of cooking-distillation at 180°F. Broth from heavy hen leg muscle had a good strong flavor, but it was not "chickeny". The odor of the hot broth resembled that of a kettle of hot pork fat while being rendered. With the atypical "oxidation-inhibiting conditions" (Pippen et al., 1958) used in these experiments, the broth from roaster breast muscle was not pleasant smelling or tasting and that from roaster leg muscle was less pleasing. Chemical volatiles that were tentatively identified as chlorine and ammonia and possibly nitrite were liberated from the reagent traps. This phenomena occurred only in the experiments on roaster muscle. The least acceptable of any of the broth samples were those from light-weight hen breast and leg muscle. Objectionable flavors in the breast muscle broth sample described as "oniony" was noted. This may have been due to the presence of higher concentrations of disulfide, sulfide and/or mercaptan than normally occur in chicken broth.

Table 12. Organoleptic evaluation of broth samples after 50 hrs. cooking at 180°F under "oxidation-inhibiting conditions", and pH values for original muscle/$H_2O$ slurry and cooked broth.

| Broth sample | pH | | Odor and flavor characteristics |
|---|---|---|---|
| | Slurry | Broth | |
| Heavy hen breast muscle | 5.8 | 6.2 | excellent, rich, "chickeny", pleasant after taste that lingered rich, "chickeny" odor taste, strong glutamate-inosinate effect |
| Heavy hen leg muscle | 6.1 | 6.3 | bland, sweet, odor like hot pork fat, not "chickeny". - taste, strong glutamate-inosinate effect. |
| Roaster breast[1] muscle | 5.8 | 6.2 | bland, no odor of chicken, strong taste but not "chickeny", metallic, soapy, astringent |
| Roaster leg[1] muscle | 6.6 | 6.8 | bone-stock overtone, astringent metallic after taste, taste like wet chicken feathers, nauseating unpleasant odor, wet chicken feather odor, grassy or seaweedy, soapy or tallowy, fecal taint. |
| Light-weight hen breast muscle | 6.0 | 6.3 | weak odor of chicken, strong sulfury odor, wet chicken feather odor and taste, disagreeable taste (oniony) |
| Light-weight hen leg muscle | 6.2 | 6.4 | sweet, rubbery and burnt odor - astringent, wet chicken feather taste, disagreeable, gags. fecal taint |

1. Copious amounts of chlorine and ammonia were liberated during these two runs. All of the laboratory glassware surrounding the apparatus and the apparatus itself was covered with a dense coating of ammonium chloride.

In making up the raw muscle slurries from the frozen dark muscle samples, a pronounced "beefy" odor was noted on warming the meat-water mixture in a hot-water bath. Similarities in flavor between the red meats, such as beef, pork, lamb and whale, have been reported by Hornstein and Crowe (1960a,b, 1963, 1964). Chicken leg muscle also belongs in this category according to its organoleptic characteristics and the presence of heme compounds. The presence of heme compounds was proven by extracting roaster leg muscle with water at 30°F overnight and then taking UV readings on a Beckman dU spectrophotometer on aliquots of the clear solution. Absorption maxima readings of 425 mu for heme compounds and 675 mu for metmyoglobin were obtained. Oxymyoglobin was lost as a result of prolonged frozen storage of the muscle samples prior to analysis. The role of heme compounds in meat flavor chemistry is not yet known.

Solubility Classification of the Volatile

Fraction of Cooked Chicken: The purpose of this study was to separate the volatile fraction according to solubility characteristics (Cheronis and Entrikin, 1961) by passing the volatile fraction through a cluster of large traps (Figs. 12, 14) containing the reagents previously described. As the volatiles were swept through the radial manifold stream-splitting system, the odor characteristics of the effluent streams emanating from each of these traps were evaluated. After a run was completed, the contents of each trap were subjected to specific qualitative tests. Separate cooking and distillation experiments, lasting for 50 hrs. at 180°F were made on light and dark muscle from heavy hens, roasters and light-weight hens. The organoleptic evaluations are summarized in Table 13.

**Table 13. Organoleptic evaluation of volatile fraction components by the solubility classification method (Cheronis and Entrikin, 1961)**

| Volatile sample | Water trap Div. $S_2$[a] | 1.2N HCl Div. B | 2.5N $NaHCO_3$::2.5N NaOH Div. $A_2$ | 1.5N $NaHCO_3$::2.5N NaOH Div. $A_1$ | Concentrated $H_2SO_4$ Div. N |
|---|---|---|---|---|---|
| Heavy hen breast muscle | roasted peanuts, sweet, buttered popcorn, tickles nostrils, sulfury, ammonia | chicken odor, sulfury | sweet, rubbery, fatty acid, strong chicken odor sl.black ppt. | chicken odor with roast beef background, oniony, (gray ppt.) | sweet, rubbery, rancid celery, fatty, alcohol, ester odor. |
| Heavy hen leg muscle | roasted peanuts, popcorn, ammonia, sulfury | sulfide beef odor. | pronounced sulfide roast beef odor | sulfide roast beef | sweet, alcohol, ester |
| Roaster breast muscle | burnt popcorn, ammonia, rotten egg | sulfide roast beef odor, sweet, pronounced odor of chlorine | pronounced sulfide roast beef odor, mild chlorine odor black ppt. | mild sulfide roast beef odor, strong chlorine odor | alcohol, ester, sweet rubbery |
| Roaster leg muscle | (sweet, vanillin, methanolic before $H_2O$ trap in connectors) sulfide rubbery, ammonia, rotten egg, burnt popcorn | sl.ammoniacal halogen odor, lead acetate paper test +, roast beef odor | roast beef, and sulfide halogen odor bone stock. Lead acetate paper test +, fishy odor (trimethylamine?) | rotten eggs, halogen odor, lead acetate paper test + ammonia, benzene | chlorine and acrid, sweet odor, alcohol ester. Lead acetate paper test +. |
| Lt.wt. hen breast muscle | popcorn, ammonia, rotten eggs, wet feathers | sl.ammoniacal, sweet, sl.chickeny | onion and fecal odor strong sulfide odor sl. beefy | onion, fecal. sulfide, | alcohol, ester, fatty acid, rubbery |
| Lt.wt. hen leg muscle | popcorn, ammonia, rotten eggs, wet feathers | sl.ammoniacal, sulfide, sl.roast beef odor, glue odor | oniony, fecal odor, sl.roast beef odor, rotten eggs | rotten eggs, fecal taint, sl.roast beef odor, bone stock | alcohol, ester, fatty acid, rubbery, acrolein |

[a] $S_2$ - B - $A_2$ - $A_1$ and N are solubility classification divisions (Cheronis and Entrikin, 1961)

Table 13 indicates that a more characteristic chicken odor was obtained from heavy hen breast muscle volatiles than from any of the samples tested. There was a prevalence of sulfide and roast beef odor in the effluent streams emanating from the traps which contained alkaline solutions as well as from the HCl trap. A stronger roast beef-like odor came from the more alkaline (Div. $A_2$) trap than from the 1.2N HCl (Div. B) trap. This suggests that certain components of the volatile fraction having an additive effect in producing a roast beef-like aroma were absorbed in the alkaline trap but were not absorbed in the HCl trap. Thus, alkaline conditions seemed to favor the maximum development of a roast beef-like odor. According to the solubility classification tables of Cheronis and Entrikin (1961) the alkaline trap (Div. $A_2$) removes sulfonic and sulfinic acids. The HCl trap (Div. B) removes amino thiophenols and amino sulfonamides. The alkaline trap (Div. $A_2$) also removes mercaptans and thiophenols, aminosulfonamides, amino sulfonic acids, amino thiophenols, sulfonamides and thioamides. Another possible explanation for the stronger beef-like odor from the alkaline trap is that it may remove compounds which mask or nullify the roast beef odor.

Panel judges of chicken broth as reported by Peterson (1957) classified the odor as meat broth, sulfide, bready, burnt and ammonia-like. This substantially agrees with the results found in the present study.

In addition to the reagents shown in Table 13, other reagents were used including nitrochromic acid, mercuric chloride, mercuric cyanide, bismuth nitrate 2,4-DNP, 1-Cl-2,4-DNB, and ceric ammonium nitrate (Table 14). One of the most surprising results was that the volatile effluent

Table 14. Organoleptic evaluation of volatile fraction effluent aroma from various reagent traps

| Reagent trap | Some meaty and non-meaty odor characteristics of effluent stream | pH of effluent trapped in $H_2O$ after 40 hrs[a] |
|---|---|---|
| nitrochromic acid | sweet, toasted marshmallow, beefy, water solution of effluent had a pronounced beef taste from breast muscle of heavy hens (heavy dark green ppt) | 7.0 |
| mercuric chloride | sweet, chemical odor from reagent only | 6.6 |
| mercuric cyanide | chemical odor from reagent, acidic odor | 6.9 (black ppt) |
| bismuth nitrate reagent | strong saurkraut odor, acidic, sulfide odor, sl. beefy (brown-black ppt.) | 4.3 |
| 2,4-DNP | strong sulfide and pronounced roast beef odor (orange, yellow-brown ppt.) | - |
| 1-cl-2,4-DNB | sulfide, beefy (yellow ppt.) | - |
| ceric ammonium nitrate | sweet sulfury chicken odor with roast beef background aroma, lead acetate paper gave + test. (yellow-amber ppt.) | - |
| Div. $A_1$ | sweet, fatty, oily (gray ppt.) | 6.9 |
| Div. $A_2$ | sweet, rubbery, strong chicken aroma from heavy hen breast muscle (black ppt.) | 8.9 |
| Div. B | glue odor | 7.0 |
| Concentrated $H_2SO_4$ | sweet, ester, alcohol, aldehydes, acrolein (black ppt.) | |
| water trap | roasted peanuts, burnt popcorn, pungent strong sulfide and rubber smell | |

[a]After 40 hrs. of cooking and distilling, the samples were taken.

from the concentrated sulfuric acid trap had a pH value of 7.0 after being trapped in water. In addition, carbonyl removal by 2,4-DNP did not detract from the roast beef aroma emanating from this trap, but rather seemed to enhance it. The sauerkraut odor from the bismuth nitrate reagent and the toasted marshmallow aroma from the nitrochromic acid trap were other surprising phenomena that were observed. The effluent stream from nitrochromic acid also contained a high concentration of roast beef-like aroma. The roast beef aroma was obtained from either chicken breast or leg muscle by passing the cooked volatile fraction through an acid oxidizing media, an alkaline media, an acidic solution of DNP, an acidic solution of 1-Cl-2,4-DNB or through 1.2N HCl. Results indicated that chicken flavor probably contains the same volatile components as beef plus some additional sulfur containing compounds, phospholipid fractions, enols and other reduced forms of compounds, which are responsible for the "chickeny flavor".

The importance of sulfur compounds in meat flavor was also made evident from the fact that mercuric chloride, mercuric cyanide, bismuth nitrate, and concentrated sulfuric acid removed the meaty aroma from the cooked volatile fraction. These findings confirmed the observation by Pippen et al. (1958) that chicken flavor is associated with the neutral or acidic constituents. Later, Kazeniac (1961) reported that "chickeny" sulfide flavor could be removed from broth by dialysis. Furthermore, separate observations by Bouthilet (1951) and Pippen and Eyring (1957) to the effect that sulfides are important in chicken flavor were also confirmed. The importance of carbonyls to normal meat flavor is questionable. Under the "oxidation-inhibiting conditions" of cooking-distillation, the carbonyl yield was negligible and the amounts of DNPH formed from the

volatiles of several runs were trivial, and amounted to much less than the DNPH obtained from a single run under "normal cooking conditions". Large amounts of sulfur derivatives were obtained under either type of cooking atmosphere, indicating the importance of sulfur compounds to flavor.

Qualitative Chemical Tests: In order to determine the efficacy of the solubility classification system of Cheronis and Entrikin (1961) as a means of separating various components which exist in the volatile fraction of cooked chicken muscle, a series of qualitative chemical tests were made on the contents of the reagent absorption traps according to the methods outlined by Cheronis and Entrikin (1961), Marbach and Doty (1956), Folkard and Joyce (1963), Siggia and Stahl (1957), and Rittner et al. (1962). The qualitative chemical tests are summarized in Table 15.

Table 15 shows the qualitative chemical results obtained by using the solubility classification system augmented by other absorption reagent traps. By this method, a total of 20 major classes of compounds and a few specific compounds were found to be present in cooked chicken volatiles. The compounds identified included nitro-sulfonic acid, ethanol, ethanal, methyl ketones, aldehydes, mono-, di- and polybasic acids, enols, phenols, sulfinic acid(s), ammonia, amines, mercaptans, alcohols, esters, hydrogen sulfide, organic sulfides and disulfides .

Chemical tests require concentrations of parts per million or more in order to get a reaction to occur. Furthermore, Guadagni et al. (1963) recently demonstrated that aromas may consist of several

Table 15. Results of qualitative chemical tests on the volatile fraction of cooked chicken based on the solubility classification method of Cheronis and Entrikin (1961) and for sulfur components by the method of Folkard and Joyce (1963).

| Division or compound tested | Reagent or method | Result | Major class (es) detected |
|---|---|---|---|
| $S_2$ | ferrous hydroxide test | + (red-brown ppt.) | nitro-sulfonic acid |
| | sodium hypoiodite | + (yellow ppt.) | ethanol, ethanal, methyl ketones |
| | cupric ions | + (yellow-red) | aldehydes |
| | iodate-iodide starch | + (blue) | acids (dibasic and polybasic) |
| | bromine in $H_2O$ etc. | + (tan to blue) | enols and phenols |
| | ferric chloride test | + (red) | sulfinic acid |
| | sodium hypochlorite phenol test | + (blue) | ammonia |
| B | ferricyanide | + (green-blue) | amines |
| $A_1$ | iodate-iodide starch | + (blue) | acids |
| | ferric chloride test | + (red) | sulferric acids |
| $A_2$ | iodate-iodide starch | + (blue) | acids |
| | 1-cl-2,4-DNB | + | mercaptans |
| N | iodic acid | + (brown) | alcohols |
| | nitrochromic acid | + (blue) | alcohols |
| | ferric chloride hydroxylanine | + (blue-red) | esters |
| Sulfur derivatives | Bismuth nitrate reagent, P-phenylene-diamine | + (blue) | hydrogen sulfide and sulfides (Marbach and Doty, 1956) |
| | Folkard and Joyce | + (ppt.) | mercaptans |
| | Stahl and Siggia (1957) and Rittner et al. (1962) | + (ppt.) | disulfides |
| | Folkard and Joyce (1963) | + (ppt.) | sulfides |

chemical entities, which exist in the fraction at subliminal concentrations but are organoleptically recognizable as a result of an additive effect. This means that odors may be perceptible in mixtures when each component is present in subthreshold concentrations.

Sulfur Compounds: Attempts to purify the mercuric cyanide and mercuric chloride derivatives obtained from cooked chicken volatiles by recrystallization from hot water, ethanol and ethyl acetate failed to provide material with a sharp melting point. This is supported by the earlier work of Challenger (1959), who found that mercury forms coordination complexes with sulfur compounds and makes the separation of mixtures of sulfur compounds as mercury derivatives extremely difficult.

Results of Sulfur Compound Identifications: The small trap method of testing for thiols, sulfides and disulfides was used as described on p. 85 in the experimental section. A 50 mg. aliquot of mercuric chloride or mercuric cyanide derivative was used for each of the tests. Thiol tests were made on the mercuric cyanide derivative; whereas, sulfide and disulfide tests were made on the mercuric chloride derivative as previously described.

Results for Sulfides: Hydrogen sulfide gave a black precipitate when moist lead acetate paper was exposed to the effluent stream emanating from the mercuric chloride trap when acid decomposition of the precipitate started.

Sulfide tests were also made on a spot test plate by the method of Marbach and Doty (1956). A few drops of solution contained in the water traps that were used for the disulfide and mercaptan test were pipetted

on a spot test plate. Upon the addition of a drop or two of N,N-dimethyl-p-phenylenediamine reagent and Reissner solution a deep blue color formed. This gave positive evidence that sulfides were present.

Disulfides and Mercaptans: Positive tests were obtained for both disulfides and mercaptans in the form of light yellow precipitates of their respective 2,4-dinitrophenyl thioethers as previously described.

Gas Chromatography: In addition to forming secondary derivatives of mercaptans sulfides and disulfides as their 2,4-dinitrophenylthioethers, which were identified qualitatively as colored precipitates, gas chromatographic evidence of their identities was also obtained. The amounts of 2,4-dinitrophenylthioethers recovered were not sufficient to permit recrystallizations for melting point determinations. Accordingly, to provide additional evidence of the presence of mercaptans, sulfides and disulfides, 25 mg. aliquots of mercuric chloride and mercuric cyanide derivatives were decomposed together in a small trap as previously described. This procedure was adopted instead of reacting 50 mg. aliquots separately. The washed volatiles from the mercury derivative decomposition trap were collected at -196°C and were then tested by gas chromatography. These results are shown in Fig. 23.

Fig. 23 shows that a total of nine peaks were obtained when the mercuric chloride and mercuric cyanide derivative mixture was decomposed with 8N HCl and the washed volatiles were subjected to gas chromatography. The retention times for these peaks were compared with those obtained using authentic known samples of the various sulfides, disulfides and mercaptans as shown in Table 16.

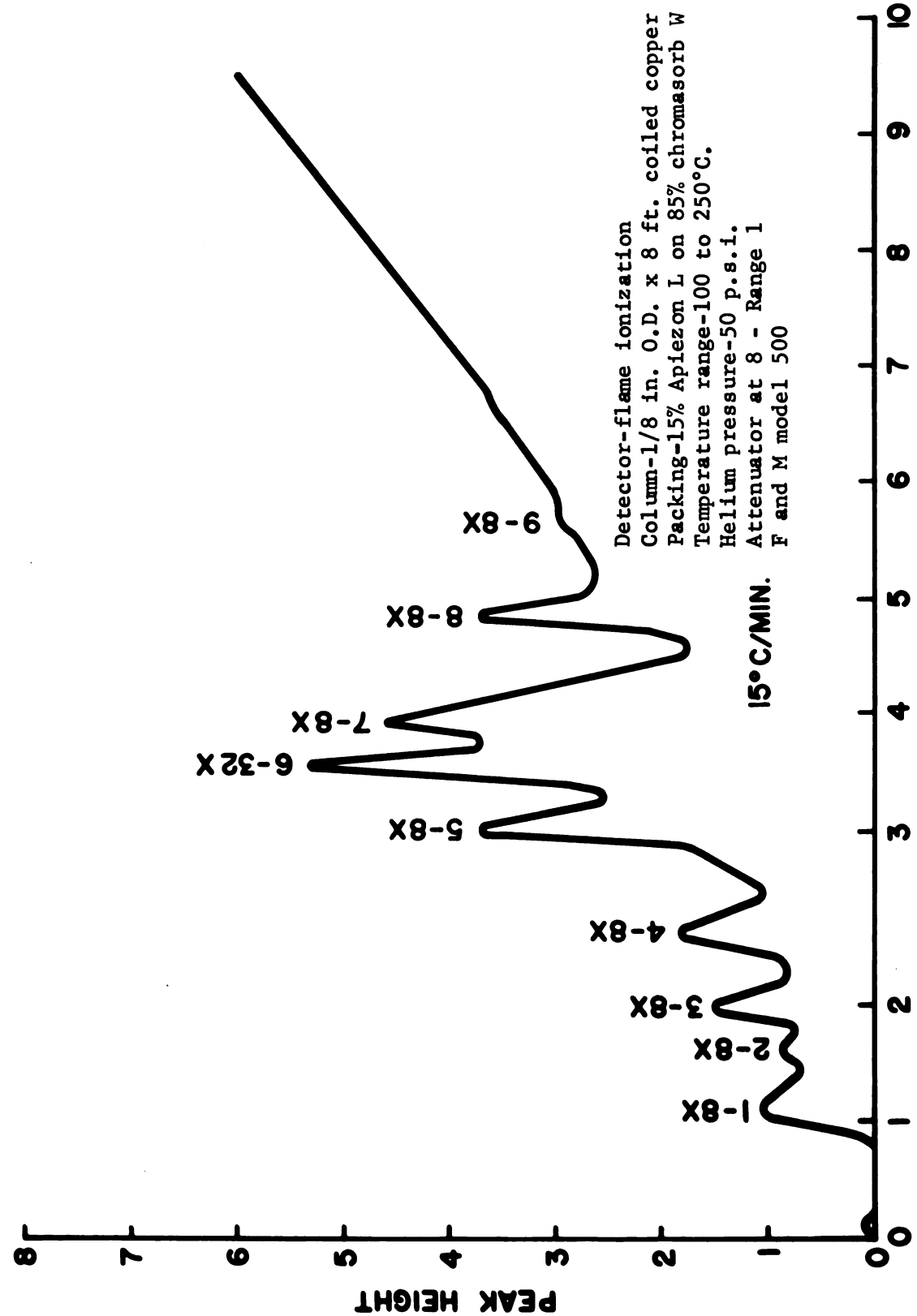



Fig. 23. Nonlinear temperature programmed separation of 5 ml. of volatiles from acid decomposition of mercuric cyanide and mercuric chloride derivatives of cooked chicken volatiles.

Table 16. Gas chromatographic analysis of volatiles obtained by acid decomposition of a mixture of the mercuric cyanide and mercuric chloride derivatives of cooked chicken volatiles.

| Peak No. | Retention times | | Tentative identity of peaks |
|---|---|---|---|
| | Unknown min. | Known min. | |
| 1 | 1.2 | 1.4 | dimethyl sulfide |
| 2 | 1.7 | 1.8 | ethyl mercaptan |
| 3 | 2.0 | 2.5 | methylethyl sulfide |
| 4 | 2.5 | 2.8 | n-Propyl mercaptan |
| 5 | 3.0 | 3.2 | diethyl sulfide |
| 6 | 3.7 | 3.8 | methyl disulfide |
| 7 | 3.9 | 4.1 | ethyl-n-propyl sulfide |
| 8 | 5.0 | 5.2 | Di-n-propyl sulfide |
| 9 | 5.8 | 6.0 | n-hexyl mercaptan |

Table 16 and Fig. 23 show that the principal compounds present in the mixture of sulfur derivatives were dimethyl sulfide, ethyl mercaptan, methyl-ethyl sulfide, n-propyl mercaptan, diethyl sulfide, methyl disulfide, ethyl-n-propyl sulfide, di-n-propyl sulfide and n-hexyl mercaptans. These sulfides, disulfides and mercaptans probably constitute the principal volatile flavor fraction in cooked chicken. Due to the low threshold levels, sulfides, disulfides and mercaptans are easily perceptible organoleptically but are not easily obtainable in sufficient amounts for chemical identification. Furthermore, there is prior evidence that sulfides are important constituents of chicken flavor (Pippen and Eyring, 1957; Bouthilet, 1951; Kazeniac, 1961). The absence of methyl mercaptan may be explained by the fact that its high vapor pressure might have

caused it to be lost instantaneously before the reaction trap could be closed following addition of the acid. Possibly a more precise technique would result in the recovery of methyl mercaptan. A small preliminary peak may have been due to methyl mercaptan since it has a retention time of about one-half minute at 100°C on the Apiezon L column.

Functional Group Analysis of Cooked Chicken Volatiles from Light and Dark Muscle After 50 hrs. Cooking and Distillation Under "Oxidation-inhibiting Conditions"

The purpose of this study was to tentatively identify the compounds present in the cooked volatile fractions from light and dark chicken muscle. As previously described, the volatiles were passed through specific reagents according to the methods of Walsh and Merritt (1960), Bassette et al. (1962) and Hoff and Feit (1963). A trap reaction technique was devised and used as previously described (Fig. 15).

A gas chromatogram of the total cooked chicken volatiles from heavy hen leg muscle is shown in Fig. 24. Chromatograms obtained after passing the total cooked chicken volatiles from heavy hen leg muscle through traps containing specific functional group reagents (Fig. 15) were compared with control chromatograms. Results of these comparisons are summarized in Fig. 25. The reagents used in obtaining the analytical results were as follows: (1) acidic hydroxyl-amine, (2) basic hydroxylamine, (3) a saturated solution of potassium permanganate, (4) mercuric chloride (3% w/v), (5) mercuric cyanide (4% w/v), (6) acetic anhydride, (7) sodium nitrite, (8) sodium borohydride, (9) 1.2N HCl, (10) concentrated $H_2SO_4$, and (11) deionized distilled $H_2O$ (control). Each of the

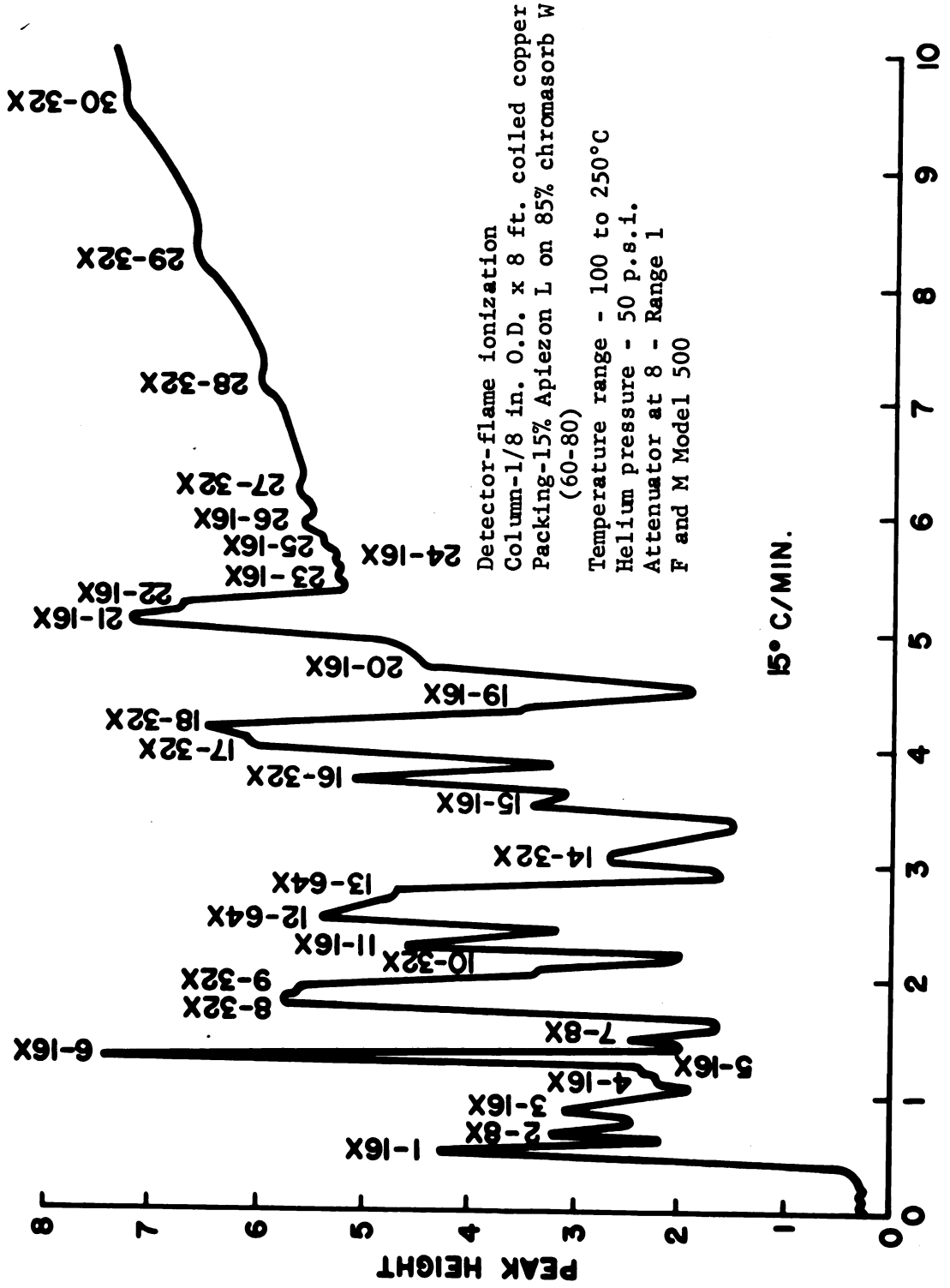



Fig. 24. Nonlinear temperature programmed separation of 2 ml. of total cooked volatiles from heavy hen leg muscle.

| PEAK NO | PEAK HEIGHTS HEAVY HEN LEG MUSCLE | FUNCTIONAL-GROUP REAGENT'S EFFECT | | | | | | | | | | FUNCTIONAL GROUP | TENTATIVE IDENTITY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ACIDIC $NH_4OH$ | BASIC $NH_4OH$ | $KMnO_4$ | $HgCl_2$ | $Hg(CN)_2$ | $NaNO_2$ | $NaBH_4$ | $AcCl_2O$ | $I_2$ in $KI$ | $H_2SO_4$ | | |
| 1 | 16× - 4.3 | | | | | | | | | | | R-H | ETHANE PROPANE |
| 2 | 8× - 3.2 | □ | □ | □ | | | □ | | □ | | | R-SH | METHYL MERCAPTAN |
| 3 | 16× - 3.1 | | | ■ | | | | □ | | | | RR'-CO | ACETONE |
| 4 | 16× - 2.2 | | | ◿ | | | □ | | □ | | | R-OH | METHANOL |
| 5 | 16× - 2.3 | | | □ | | | | | □ | | | R-S-R' | DIMETHYL SULFIDE |
| 6 | 16× - 7.4 | | | □ | | | | | □ | | | R-S-R' | METHYL ETHYL SULFIDE |
| 7 | 8× - 2.5 | | | | | | □ | | □ | | | $R-NH_2$ | METHYLAMINE |
| 8 | 32× - 5.7 | | | □ | | | | | □ | | | R-S-R' R-OH | DIETHYL SULFIDE ETHANOL |
| 9 | 32× - 5.6 | | | | | | | □ | ◿ | | | R-CHO | ACETALDEHYDE |
| 10 | 32× - 3.4 | | | □ | | | | | □ | | | R-S-R' | METHYL ISOPROPL SULFIDE |
| 11 | 16× - 4.6 | | | ◿ | | | □ | □ | □ | | | R-CO-CO-R | 2,3-BUTANEDIONE |
| 12 | 64× - 5.4 | ◿ | | | | | | □ | □ | | | R-S-S-R' | METHYL DISULFIDE |
| 13 | 64× - 4.7 | | | | | | | □ | | | | R-CO-CHOH-R | ACETOIN |
| 14 | 32× - 2.7 | | | □ | | | | | □ | | | R-S-R' | ETHYL-N-PROPYL SULFIDE |
| 15 | 16× - 3.4 | ◿ | | | | | | □ | □ | | | R-S-S-R' | ETHYL DISULFIDE |
| 16 | 32× - 5.1 | | | | | | | | | | | × | |
| 17 | 32× - 6.1 | □ | □ | □ | | | □ | | □ | | | R-SH | ETHYL MERCAPTAN SYM. TRITHIANE |
| 18 | 32× - 6.5 | | | □ | | | | | | | | R-S-R' | DI-PROPYL SULFIDE |
| 19 | 16× - 3.4 | □ | □ | □ | | | □ | | □ | | | R-SH | N-PROPL MERCAPTAN |
| 20 | 16× - 4.4 | | | | | | | □ | ◿ | | | R-CHO | N-HEXANAL |
| 21 | 16× - 7.2 | | | ■ | | | | □ | | | | RR'-CO | 2,4-PENTANE-DIONE |
| 22 | 16× - 6.7 | | | ◿ | | | □ | | □ | | | R-OH | ISO-AMYL ALCOHOL |
| 23 | 16× - 5.3 | | | ◿ | | | □ | | □ | | | R-OH | N-AMYL ALCOHOL |
| 24 | 16× - 5.3 | | | | | | | □ | ◿ | | | R-CHO | N-HEPTANAL |
| 25 | 16× - 5.4 | | | ◿ | | | □ | | □ | | | R-C-OH $NH_2$ | ETHANOLAMINE |
| 26 | 16× - 5.6 | | | ◿ | | | □ | | □ | | | R-OH | N-HEXANOL |
| 27 | 32× - 5.6 | | | ■ | | | | □ | | | | RR'-CO | 2-HEPTANONE |
| 28 | 32× - 6.0 | | | ◿ | | | □ | | □ | | | R-OH | N-HEPTANOL |
| 29 | 32× - 6.6 | | | | | | □ | | | | | × | × |
| 30 | 32× - 7.3 | | | | | | □ | | □ | | | × | × |

* ATTENUATION OF PEAK HEIGHT

| | ELIMINATION |
|---|---|
| □ | ELIMINATION NEW PRODUCTS |
| ◿ | SEVERE DECREASE |
| ◿ | SEVERE DECREASE NEW PRODUCTS |
| ■ | SLIGHT DECREASE |
| | NO EFFECT |

FIG. 25 COMPOSITE OF GAS CHROMATOGRAPHIC ANALYSIS OF COOKED VOLATILES FROM HEAVY HEN LEG MUSCLE BY A FUNCTIONAL GROUP METHOD

reagents traps removed one or more specific functional groups except for the water control trap.

Fig. 24 shows that a total of 30 peaks were obtained in the control chromatogram of the total cooked volatile fraction from heavy hen leg muscle. Fig. 25 shows the tentative identification made by functional group analyses followed by gas chromatography. A total of 6 sulfides, 3 mercaptans and 2 disulfides were tentatively identified, to give a total of 11 sulfur compounds. In addition, 3 aldehydes and 4 ketones were identified giving a total of 7 carbonyls compounds. There were 2 alkanes, 1 amine and 1 alcohol amine giving a total of 2 amines. There were 6 alcohols and acetylmethylcarbinol for a total of 7 alcohols, no esters, and 3 unidentified compounds. Thus, the principal components of the cooked volatile fraction from dark muscle were tentatively identified as being sulfur compounds and carbonyls. Two alkanes constituted one peak. Ethanal and dimethyl sulfide were found together in another peak. Thus a total of 32 compounds were present in 30 peaks.

Fig. 26 shows that a total of 25 peaks were obtained from gas chromatography of the total cooked volatile fraction from heavy hen breast muscle. Fig. 27 shows the compounds identified, which include: 4 sulfides, 2 disulfides and 1 mercaptan, giving a total of 7 sulfur compounds; 4 aldehydes, and 4 ketones, for a total of 8 carbonyls, 6 alcohols, 2 alkanes, 1 ester, 1 amine and 2 unidentified compounds. Two alkanes constituted one of the peaks. Ethanol and diethyl sulfide were contained in another. Thus, a total of 27 compounds were found in 25 peaks. The principal components of the total cooked volatile fraction from breast muscle were carbonyls.

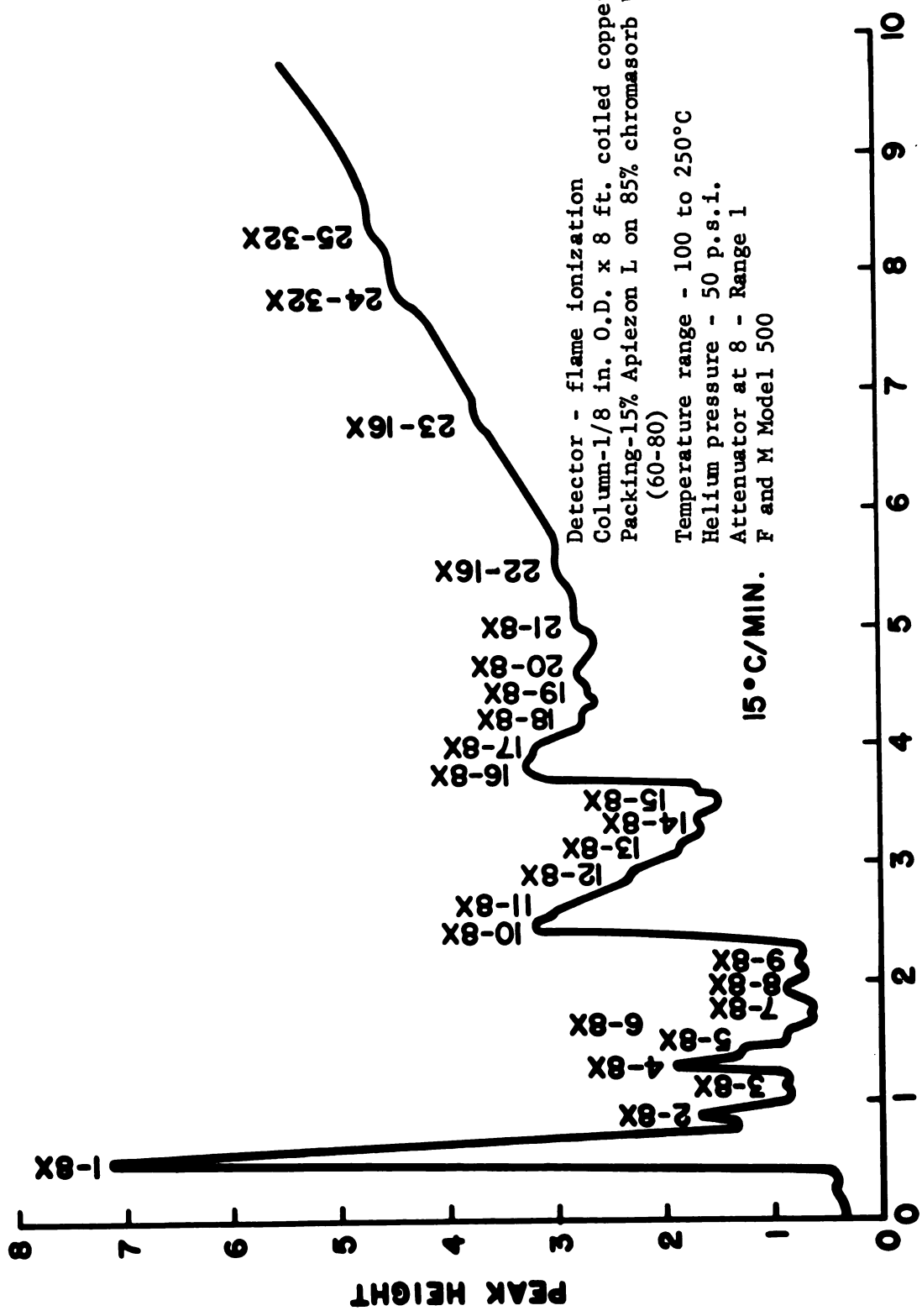



Fig. 26. Nonlinear temperature programmed separation of 2 ml. of total cooked volatiles from heavy hen breast muscle.

| PEAK NO. | PEAK HEIGHTS HEAVY HEN BREAST MUSCLE | FUNCTIONAL-GROUP REAGENT'S EFFECT | | | | | | | | | | FUNCTIONAL GROUP | TENTATIVE IDENTITY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ACIDIC $NH_4OH$ | BASIC $NH_4OH$ | $KMnO_4$ | $HgCl_2$ | $Hg(CN)_2$ | $NaNO_2$ | $NaBH_4$ | $(Ac)_2O$ | 1.2 N HCL | $H_2SO_3$ | | |
| 1 | 8x* - 7.0 | | | | | | | | | | | R-H | ETHANE PROPANE |
| 2 | 8x - 1.7 | | | ■ | | | | □ | ◿ | | | RR'-CO | ACETONE |
| 3 | 8x - 0.9 | ◿ | ◿ | ◿△ | | | □ | ◿ | □ | | | R-OH | METHANOL |
| 4 | 8x - 1.9 | ◿ | ◿ | □ | | | | | □ | | | R-S-R' | DIMETHYL SULFIDE |
| 5 | 8x - 1.3 | | | | | | □ | | □ | | | $R-NH_2$ | METHYLAMINE |
| 6 | 8x - 0.9 | | | | | | | | ■ | | | R-COO-R' | METHYL FORMATE |
| 7 | 8x - 0.7 | ◿ | ◿ | □ | | | | ◿ | □ | ◿ | | R-S-R', ROH | DIETHYL-SULFIDE ETHANOL |
| 8 | 8x - 0.9 | | | | | | | □ | ◿△ | | | R-CHO | ACETALDEHYDE |
| 9 | 8x - 0.8 | | ◿ | ◿△ | | | □ | □ | □ | | | R-CO-CO-R | 2,3-BUTANEDIONE |
| 10 | 8x - 3.2 | ◿△ | ◿ | | | | | □ | □ | ◿ | | R-S-S-R' | METHYL DISULFIDE |
| 11 | 8x - 3.0 | | | ◿ | | | | □ | ◿ | | | R-CO-CHOH-R' | ACETOIN |
| 12 | 8x - 2.3 | | | ◿ | | | | □ | ◿△ | | | R-CHO | N-PENTANAL |
| 13 | 8x - 1.8 | ◿ | ◿ | □ | | | | | □ | ◿ | | R-S-R' | ETHYL-N-PROPYL SULFIDE |
| 14 | 8x - 1.7 | | | | | | | | | | | R-OH | ISO-BUTANOL |
| 15 | 8x - 1.7 | ◿△ | ◿ | ◿ | | | | □ | □ | ◿ | | R-S-S-R' | ETHYL DISULFIDE |
| 16 | 8x - 3.3 | | | ■ | | | | □ | ◿ | | | R-CO-R' | × |
| 17 | 8x - 3.2 | ◿ | ◿ | ◿△ | | | □ | ◿ | □ | | | ROH | N-BUTANOL |
| 18 | 8x - 2.7 | ◿ | ◿ | □ | | | | | | ◿ | | R-S-R' | DIPROPYL SULFIDE |
| 19 | 8x - 2.7 | □ | □ | □ | | | □ | | □ | | | R-SH | N-PROPYL MERCAPTAN |
| 20 | 8x - 2.8 | | | | | | | □ | ◿△ | | | R-CHO | N-HEXANAL |
| 21 | 8x - 2.8 | | | ■ | | | | □ | ◿ | | | R-CO-R' | 2,5-PENTANEDIONE |
| 22 | 16x - 3.0 | | | | | | | □ | ◿△ | | | R-CHO | N-HEPTANAL |
| 23 | 16x - 3.7 | | | ■ | | | | □ | ◿ | | | R-COR' | 2-HEPTANONE |
| 24 | 32x - 4.4 | ◿ | ◿ | ◿△ | | | □ | ◿ | □ | | | R-OH | N-HEPTANOL |
| 25 | 32x - 4.7 | | | | | | | | | | | × | × |

| | |
|---|---|
| | ELIMINATION |
| □ | ELIMINATION NEW PRODUCTS |
| ◿ | SEVERE DECREASE |
| ◿△ | SEVERE DECREASE NEW PRODUCTS |
| ■ | SLIGHT DECREASE |
| | NO EFFECT |

* ATTENUATION OF PEAK HEIGHT

FIG.27 COMPOSITE OF GAS CHROMATOGRAPHIC ANALYSIS OF COOKED VOLATILES FROM HEAVY HEN BREAST MUSCLE BY A FUNCTIONAL GROUP METHOD

A comparison was made of retention volumes shown in Figs. 24 and 25 and Figs. 26 and 27 for the total cooked volatile fractions from leg and breast muscle, respectively. The cooked volatiles from heavy hen leg muscle contained a larger proportion of sulfur compounds than carbonyl compounds. Heavy hen breast muscle contained a larger proportion of carbonyls than sulfur compounds as judged by gas chromatography. However, this was not confirmed by total derivative yields. Furthermore, Guadagni et al. (1963) found that the subliminal concentrations of sulfur compounds are much lower than those of carbonyl compounds. Accordingly, the sulfur compounds would appear to dominate the overall flavor of the total cooked volatile fraction from both breast muscle and leg muscle, but to a somewhat lesser extent for the former. The presence of hydrogen sulfide and ammonium sulfide in typical volatiles from cooked chicken together with a higher carbonyl content resulting from oxidation under "normal cooking conditions" tended to increase the sulfide effect on overall flavor. No attempts were made to study the flavor effect of adding known compounds corresponding to those tentatively identified, in the proportions indicated by the comparative retention volumes. It is likely that the number of peaks found for total cooked volatiles of breast and leg muscle in the present study represent only a fraction of the true number of compounds present. Further studies using capillary columns and electron capture procedures are needed to probe deeper into the problem.

Neither were the acidic constituents detectable with the gas chromatographic procedures used in this study. Evidence for their presence was revealed, however, by the chemical identification system.

The complexity of the problem of chicken flavor has been well stated by Kazeniac (1961), and the present study confirms those observations. Quite recently Hornstein and Crowe (1964) stated in a review on meat flavor that the high boiling fraction contains the compound(s) responsible for the meaty aroma. This was also confirmed in the present study.

Organoleptic Evaluation of the Residual Effluents Contained in the Liquid Nitrogen Traps Following Gas Chromatography:

The purpose of this study was to determine the organoleptic characteristics and pH of the residual contents of the sample traps after removal of specific functional groups and gas chromatographic tests on the effluent.

The dry ice ethanol trap (-80°C) gave only three or four peaks for total volatiles in the low boiling range 100-175°C (0 to 5 min.) but peaks typical of those from the liquid nitrogen trap were eluted from the Apiezon L column in the 200-250°C range (7 to 10 min.). A pronounced sweet and roast beef odor was noted in the -80°C trap and a white precipitate was present on the trap inlet tube at that temperature. Upon warming the precipitate, it disappeared and may have been solid carbon dioxide. However, it was not identified. When the contents of the trap were treated with 2-3 ml. deionized-distilled $H_2O$, the pH was 8.0 or slightly over. The water solution of the volatiles residue tasted "meaty".

Table 17 summarizes the pH and odor characteristics of the liquid nitrogen traps (-196°C). Results for the effluent stream with no treatment (control) and treatment by several typical functional group reagents

Table 17. Organoleptic characteristics and pH values of liquid nitrogen trap, contents after passage of the total cooked volatile fractions of light or dark muscle through the reagent trap and chromatographic analysis

| Trap treatment | pH | Odor and taste characteristics |
|---|---|---|
| none | 9.0 | buttery, sulfury, petroleum odor, meaty taste |
| basic hydroxyl-amine | 10.0 | ammonia odor, bitter, disagreeable taste |
| acidic hydroxyl-amine | 8.4 | sweet-strong roast beef odor, beefy taste |
| acetic anhydride | 5.0 | sweet, beefy odor and taste |
| 1.2N HCl | | roast beef odor, sl. bitter, but meaty flavor |
| mercuric perchlorate | 8.0 | carbonyl, ester odor-not meaty, bitter taste |
| 2,4-DNP | - | strong-sweet roast beef odor, aromatic undertone, meaty taste |
| bismuth nitrate | - | sweet, sulfide odor, sauerkraut taste, acidic |
| 2.5N $NaHCO_3$::2.5N NaOH | - | sulfide, esters, aromatic odor, bitter, astringent, rubbery taste |
| 1.5N $NaHCO_3$::2.5N NaOH | - | sweet, fatty, ester odor, grassy, bitter astringent taste |
| permanganate | - | oxidized fat, acetone, sulfide odor, burnt bitter taste |
| sodium borohydride | - | strong mercaptan odor, disagreeable putrid flavor |
| mercuric cyanide | 9.0 | sweet and carbonyl odor, bitter, astringent, sweet, oily, bitter taste |
| mercuric chloride | 9.0 | sweet and carbonyl odor, bitter, astringent, bitter taste |
| sodium nitrite | | nitrite odor, bitter, astringent chemical flavor |

after the effluent passed from the reagent trap through the -80°C trap and into the -196°C trap are included. All tests were made on the -196°C trap only as random chromatographic tests on the contents of the -80°C trap showed only a few peaks as previously described.

Table 17 shows the effects of various functional group reagents on the odor and flavor of total cooked chicken volatiles. Carbonyl removal or reduction enhanced the meat flavor. An increase in mercaptans by the reduction of disulfides had an adverse effect on odor and taste. Oxidation of carbonyls and sulfides caused a burnt flavor and/or removed the meaty flavor component. Acidic reagents enhanced the meaty or roast beef-like flavor character. Basic reagents imparted a disagreeable bitter or rubbery flavor. Sulfide elimination caused a loss of the meaty taste and left a sweet, oily, bitter, burnt taste, which was probably due to esters, acids and carbonyls.

Perhaps some sulfur esters exist in the volatile fraction, which have not been identified. These would probably be found in the medium or higher boiling fractions. In a private communication, Kazeniac (1963) advised that Sasin (1962) prepared a series of sulfur esters. As a result of a private communication, Sasin (1964) advised that these thioesters had been sent out to another laboratory for spectrographic analysis and were not available. Although the mode of preparation of these thiol esters of fatty acids is quite simple, this line of investigation was not pursued further. Since the beef flavor of the volatile fraction was enhanced by the acetic anhydride treatment, this enhancement may have been due either to possible thiol ester formation, acetylation of amines or removal of basic sulfides. However, these effects were not studied.

Model Systems

The purpose of the model system studies was to attempt to relate chicken flavor to glutathione and to some simple compounds which are known to exist in chicken broth. These precursors and compounds included carbamyl phosphate, lactic acid, ammonium sulfide, glutathione, cystine and methionine. Organoleptic and gas chromatographic evaluations of three separate preparations were made.

In the first system, hydrogen sulfide was evolved by adding lactic acid to sodium sulfide. When carbamyl phosphate was added to this mixture and the mixture was warmed by a water bath to 180°F, an odor similar to chicken volatiles resulted.

In the second system sodium sulfide, lactic acid, carbamyl phosphate, and 2,3-butanedione were made up in solution and ammonium hydroxide was added to give a pH reading of 6.9. The mixture when warmed to 60°C gave only 3 small chromatographic peaks as compared to 7 small peaks that were obtained from the first system. Again a chicken-like flavor resulted. The importance of ammonium lactate as a component of meat flavor was recently reported by Hornstein (1964).

In the third system, which represented the concluding part of the model study, glutathione was added to a mixture of lactic acid, carbamyl phosphate and 2,3-butanedione. After adjusting the pH to 7.9, 3 ml. of the mixture were put into a small reagent trap (Fig. 12) and heated to 180°F in a water bath. The volatiles were trapped in a small liquid nitrogen trap (Fig. 12) and were subjected to gas chromatography. The chromatographic results are shown in Fig. 28 and Table 18.

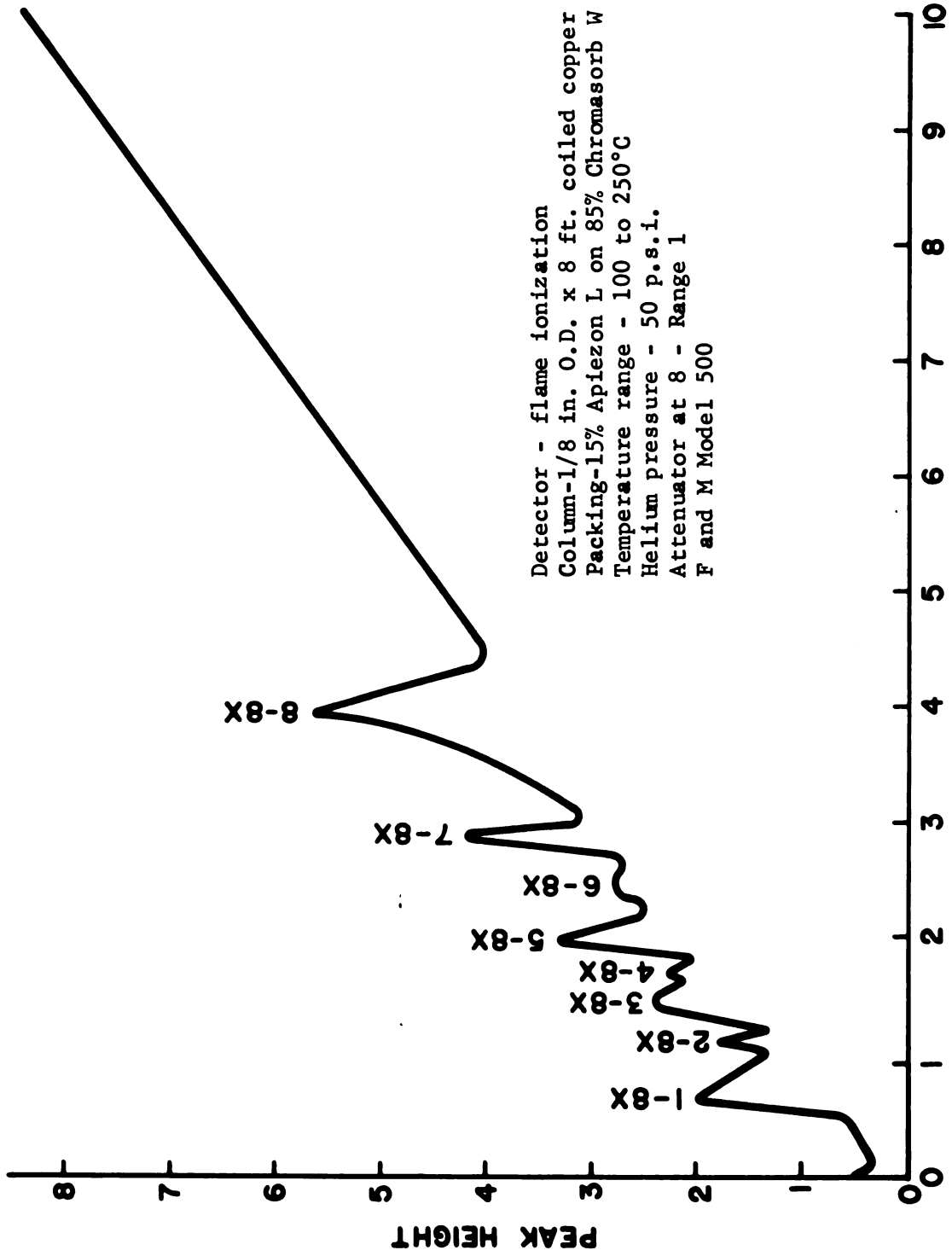



Fig. 28. Nonlinear temperature programmed separation of 5 ml. of volatiles from aqueous decomposition of reduced glutathione and other components of a model system.

Table 18. Gas chromatographic analysis of a model system in which glutathione was decomposed in an aqueous media containing other components of chicken muscle.

| Peak No. | Retention time Unknown min. | Retention time Known min. | Tentative identity of peaks |
|---|---|---|---|
| 1 | 0.7 | 0.7 | methyl mercaptan |
| 2 | 1.2 | 1.4 | dimethylsulfide |
| 3 | 1.6 | 1.6 | methylamine |
| 4 | 1.8 | 1.8 | acetaldehyde |
| 5 | 2.2 | 2.4 | methyl disulfide |
| 6 | 2.8 | 2.8 | 2,3-butanedione acetylmethyl carbinol |
| 7 | | | ethyl n-propyl sulfide |
| 8 | 4.0 | 3.8 | ethyl disulfide |

Fig. 28 shows that a total of 8 well-defined peaks are obtained. These were tentatively identified as 1 mercaptan, 2 disulfides and 3 sulfides plus 2,3-butanedione acetaldehyde and acetylmethyl-carbinol (Table 18). These results indicate that glutathione may be the source of methyl mercaptan, dimethyl sulfide, methylamine, acetaldehyde, methyl disulfide, ethyl-n-propyl sulfide and ethyl disulfide, which were previously shown to be indigenous to the total cooked volatile fraction of chicken leg muscle. With the exception of methyl mercaptan, all these compounds were likewise indigenous to the total cooked volatile fraction of breast muscle.

The final pH of the reaction products mixture was 4.7 as compared to the initial pH value of 7.9. The predominant taste was sulfide, and the flavor and odor resembled chicken broth. A mild mercaptan odor was

evident. However, the final product did not have a complete chicken broth flavor. A total of 50 ug of inosinic acid in a 1 ml. aliquot was added to 2 ml. of the reaction product solution. The pH was then adjusted to 5.6 with 0.01N NaOH and 1/4 mg. of creatine was added. These additives, in turn, improved the flavor but the product as before did not constitute a true chicken broth flavor.

Chemical Spot Tests: The sodium hypoiodite test (Cheronis and Entrikin, 1961) gave a positive reactinn for ethanal (acetaldehyde) in the form of a yellow precipitate. A few drops of the unknown solution on a spot plate reacted with ferricyanide reagent to give a green-blue color, which indicated that an amine was present. The addition of sodium borohydride to a few drops of the solution gave a mercaptan odor which indicated that disulfide(s) was/were present. A positive methylene blue test for sulfides was obtained with p-phenylenediomine reagent (Marbach and Doty, 1956). A positive test for mercaptans was obtained by adding 1 ml. of ethanolic NaOH and a few drops of 1-Cl-2,4-DNB to the solution which remained in the tube. A light yellow precipitate of the thioether derivative(s) separated upon cooling the tube in ice. Tests on the effluent stream from the liquid nitrogen trap gave a positive hydrogen sulfide test (black precipitate) with lead acetate paper.

The number and complexity of the reactions which would be required to produce these seven products from the original preparation envisages many reactions that are inexplicable by the Strecker degradation of amino acids alone. Studies on glutathione's decomposition in aqueous media have previously been reported by Kendall et al. (1930) and Mason

(1931). Cleavage of glutathione to cysteinyl-glycine and pyrollidone carboxylic acid occurred by incubating glutathione at 37-62°C. Judging from the reaction products obtained, further cleavage to cysteine, glycine and glutamic acid probably occurred at the higher temperature (180°F). The formation of acetylmethylcarbinol and methanethiol indicated that reduction reactions must have occurred in addition to Strecker degradation of the amino acids. There were also probably numerous rearrangements.

There is no evidence to support the finding of methanethiol as a product of cysteine and cystine. In their model systems, Self et al. (1963) obtained methanethiol from methionine, but not from either cystine or cysteine. Furthermore, their investigations showed that no sulfides or disulfides were formed from cystine or cysteine in either a nitrogen or oxygen atmosphere. They found that methionine yielded dimethyl sulfide in an oxygen medium, but not when nitrogen was used. Perhaps the presence of glycine glutamic acid, glutathione and the other compounds that were present with cystine were responsible for the mercaptans, sulfides and disulfides found in the present study. These compounds may also possibly result from interactions with hydrogen sulfide, cysteine and cystine in the vapor state. It is also conceivable that 2,3-butanedione may cause a different reaction pattern with cysteine and cystine than that observed by Self et al. (1963) with dehydroascorbic acid.

Further work is needed to verify the tentative identifications that were made in the present study of glutathione in a model system. Glutathione's role in hydrogen sulfide production upon heating chicken flesh as first reported by Sadikov et al. (1934) was substantiated by this study.

Glutathione also fits Bouthilet's (1951) description of a labile sulfur compound which together with a fatty acid-like material is responsible for cooked chicken flavor. A postulation was made by Bouthilet (1951) concerning a steam distillable, weakly acidic constituent of cooked chicken meat that had its origin in the glutathione of chicken muscle. He found that this constituent imparted a strong flavor to chicken broth in the pH range of 1 to 5. In the present study this characteristic was demonstrated by glutathione. Upon decomposition of glutathione, the pH of the original media dropped from 7.9 to 4.7. The association of desulfuration and sulfide losses with a reduction and final complete loss of "chickeny" flavor in broth as demonstrated by Pippen and Eyring (1957) was also observed in this study of glutathione. Addition of glutathione to a standard broth solution prior to heating resulted in a more "chickeny" flavor according to Kazeniac (1961) and his finding was further substantiated by the results obtained in the present study. Furthermore, the association of chicken flavor with neutral or acidic constituents by Pippen and Eyring (1957) was likewise confirmed.

## SUMMARY AND CONCLUSIONS

Chemical components from cooked light and dark chicken muscle were identified using gas, column, thin-layer and paper chromatography, UV and I-R absorption spectra, functional group analysis by a trap-reaction technique and a solubility classification method.

A total of 30 peaks were obtained by gas chromatographic separation of the total cooked volatile fraction from leg muscle and 25 peaks from breast muscle. Identifications were made by passing the volatiles through traps containing specific functional group reagents or by comparing chromatograms of the total cooked volatile fractions with those obtained using authentic compounds as reference standards.

Constituents identified in the total cooked volatile fraction from leg muscle were ethane, propane, methyl mercaptan, acetone, methanol, dimethyl sulfide, methyl-ethyl sulfide, methyl-amine, diethyl sulfide, ethanol, acetaldehyde, methyl-isopropyl sulfide, 2,3-butanedione, methyl disulfide, acetoin, ethyl-n-propyl sulfide, ethyldisulfide, ethyl mercaptan (symmetrical trithiane), dipropyl sulfide, n-propyl mercaptan, n-hexanal, 2,4-pentanedione, iso-amyl alcohol, n-amyl alcohol, n-heptanal, ethanol-amine, n-hexanal, 2-heptanone and n-heptanol for a total of 29 organic compounds.

Constituents isolated from the total cooked volatile fraction of breast muscle were ethane, propane, acetone, methanol, dimethyl sulfide, methylamine, methyl formate, dimethyl sulfide, ethanol, acetaldehyde, 2,3-butanedione, methyl disulfide, acetoin, n-pentanal, ethyl-n-propyl sulfide, iso-butanol, ethyl disulfide, n-butanol, dipropyl sulfide,

n-propyl mercaptan, n-hexanal, 2,4-pentanedione, n-heptanal, 2-heptanone and n-heptanol giving a total of 25 organic compounds.

Inorganic compounds identified in the cooked volatile fractions by cryogenic trapping of volatiles at -196°C followed by cryogenic distillation at -140°C and gas chromatography were hydrogen sulfide, and carbon dioxide. Ethane and propane were also identified. Ammonia and ammonium sulfide(s), hydrogen sulfide and carbon dioxide were identified using chemical and physical methods. The presence of carbonyl sulfide was indicated by odor tests, but further identification was not made.

Gas chromatographic studies showed that whole carcasses from 20 month old hens contained the same volatile components as 12 week old birds of identical origin and raised on the same ration. Intestinal contents from 20 month old hens had the same volatile organic constituents as meat from the same birds; namely, carbonyls, sulfides, disulfides and mercaptans, but in lesser amounts.

A steam distillable phosphatidyl lipid fraction was isolated from chicken breast meat. By subjecting this fraction to thin-layer chromatography, two compounds were identified; namely, cardiolipin and either phosphatidyl inositol or phosphatidic acid. The freshly prepared distillate had a several-fold concentration of true chicken odor and was called "chicken essence".

A total of 8 mono- and 1 poly-carbonyl were isolated from cooked chicken volatiles by preparing 2,4-DNPHS and identifying the derivatives by column and paper chromatography, UV and I-R absorption spectra, melting points, and mixed melting points using authentic samples, and microscopic examination. Acetaldehyde, acetone, n-propanal, methyl-ethyl ketone, n-butanal, n-pentanal, n-hexanal, n-octanal and diacetyl

were identified. A 2,4-dien-1-al was also present, but no authentic sample was available for comparison.

Gas chromatographic analysis of volatiles obtained by the acid decomposition of organomercuric derivatives of cooked chicken volatiles resulted in the identification of dimethyl sulfide, ethyl mercaptan, methylethyl sulfide, n-propyl mercaptan, diethyl sulfide, methyl disulfide, ethyl-n-propyl sulfide, di-n-propyl sulfide and n-hexyl mercaptan.

Chemical and physical methods were used to identify flavor precursors. Some quantitative results were obtained for protein, moisture, ash, ether extractibles, creatine-creatinine, diacetyl/acetoin, inosinic acid, sulfhydryl compounds, sulfides, cystine and methionine from light and dark meat from heavy- and light-weight hens and roasters. Hydrogen ion concentrations of raw slurries and cooked broths were also determined for these samples. Differences in flavor were not directly related to the concentrations of the precursors, although some interrelationships were indicated. Heme compounds may also have an associative effect with the characteristic red-meat flavor. Volatiles from dark chicken muscle resembled beef more than the "chickeny"flavor characteristic of light muscle. Desulfuration caused a loss of "chicken essence" contained in the phosphatidyl fraction. Removal of sulfur compounds by passing the volatiles through mercuric cyanide and mercuric chloride solutions removed nearly all of the "meaty" aroma. Carbonyls may function best at sub-threshold concentrations by exerting synergistic and additive flavor effects.

Using a model system, glutathione was decomposed in the presence of moist heat and 2,3-butanedione. In addition to carbon dioxide, ammonia

and copious amounts of hydrogen sulfide, eight organic compounds were evolved and identified; namely, methyl mercaptan, dimethyl sulfide, methylamine, acetaldehyde, methyl disulfide, 2,3-butanedione, ethyl-n-propyl sulfide and ethyl disulfide.

Emphasis was placed on determining the role of sulfur compounds in chicken flavor. In order to prevent oxidation of mercaptans, atypical "oxidation inhibiting conditions" were maintained during cooking-distillation in this phase of the work. Evidence was obtained which substantiated the importance of glutathione and 2,3-butanedione. These compounds are indigenous to chicken, and through Strecker degradation and/or other mechanisms may serve as precursors of at least part of the true chicken flavor.

Inosinic acid together with glutamic acid were demonstrated to be important non-volatile flavor constituents of chicken. Creatine and/or creatinine in pure solution was/were bitter, but may contribute to desirable flavor through additive and/or synergistic effects.

Sulfur components and their precursors were present in high concentrations in both the volatile fraction and the cooked-freeze-dried slurry from heavy hen breast meat. Even after 50 hrs. of cooking-distillation at 180°F there was no apparent abatement in their production. However, during this period the pH increased from 5.8 to 6.2, indicating that acidic and neutral constituents were also distilled over in the water-trap as manifested by an increase in pH from 7 to 9. These compounds were probably organic sulfides and methylamine.

## BIBLIOGRAPHY


Akazawa, T. and E. E. Conn. 1958. Oxidation of reduced pyridine nucleotides by peroxidase. J. Biol. Chem. 232:403.

Asmundson, V. S., F. H. Jukes, H. M. Fyler and M. L. Maxwell. 1938. The effect of certain fish meals and fish oils in the ration on the flavor of turkey. Poultry Sci. 17:147.

Association of Official Agricultural Chemists. 1960. Official Methods of Analysis of the Association of Official Agricultural Chemists. Washington, D. C. (9th edition).

Ballance, P. E. 1961. Production of volatile compounds related to the flavor of foods from the Strecker degradation of DL-methionine. J. Sci. Food Ag. . 12:532.

Barker, S. B. and W. H. Summerson. 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138:535.

Barylko-Pikielna, N. 1957. The components of meat flavor. Przemysl Spozywczy. 11:26. (CA 54, 18821 f).

Bassette, R., S. Ozeris, and C. H. Whitnah. 1962. Gas chromatographic analysis of head space of dilute aqueous solutions. Anal. Chem. 34:1540.

Batzer, O. F. and D. M. Doty. 1955. Nature of undesirable odors formed by gamma irradiation of beef. J. Ag. and Food Chem. 3:64.

Batzer, O. F., A. T. Santoro, W. A. Landmann and B. S. Schweigert. 1960. Precursors of beef flavor. J. Ag. and Food Chem. 8:498.

Batzer, O. F., A. T. Santoro and W. A. Landmann. 1962. Identification of beef flavor precursors. J. Ag. and Food Chem. 10:94.

Batzer, O. F. and W. A. Landmann. 1963. Personal communication.

Bender, A. E., T. Wood and J. A. Palgrave. 1958. Analysis of tissue constituents. Extract of fresh ox muscle. J. Sci. Food Ag. 9:812.

Bender, A. E. and P. E. Ballance. 1961. A preliminary examination of the flavour of meat extract. J. Sci. Food Ag. . 12:683.

Benne, E. J., N. H. VanHall and A. M. Pearson. 1956. Analysis of fresh meat. J.A.O.A.C. 39:937.

Birch, S. F. and D. T. McAllan. 1951. The separation of sulfur compounds by means of mercuric acetate. J. Inst. Petrol. 37:444.

Boon, W. R. 1937. The action of meat extracts and related substances as gastric stimulants in man. Brit. Med. J. II:412 (CA 31,79945)

Borgstrom, G. Editor. 1961. Fish as Food. Vol. 1. Production, Biochemistry and Microbiology. Academic Press. New York and London.

Bouthilet, R. J. 1949. A note on the nature of a flavor constituent from poultry meat. Food Technol. 3:118.

Bouthilet, R. J. 1950. Chicken flavor: Separation and concentration of its volatile components from broth. Food Res. 15:322.

Bouthilet, R. J. 1951. The fractionation of the volatile constituents. Food Res. 16:137.

Bouthilet, R. J. 1951. Chicken Flavor: The source of the meat flavor component. Food Res. 16:201.

Brant, A. W., H. Lineweaver and E. H. Dawson. 1958. The flavor of modern type broilers. 11th World's Poultry Congress Proc. Seccion V-3. Mexico City, Mexico, Sept. 1958.

Brant, A. W. 1963. Chilling poultry - a review. Poultry Processing and Marketing. 69:14.

Brenner, N. and E. Cieplinski. 1959. Gas chromatographic analysis of mixtures containing oxygen, nitrogen and carbon dioxide. N.Y. Acad. of Sciences. 72:705.

Brew, W. B., J. B. Dore, J. H. Benedict, G. C. Potter and E. Sipos. 1959. Characterization of a type of unidentified compound producing edema in chicks. J.A.O.A.C. 42:120.

Briskey, E. J. and J. Wismer-Pedersen. 1961. Biochemistry of pork muscle structure. I. Rate of anaerobic glycolysis and temperature change versus the apparent structure of muscle tissue. J. Food Sci. 26:297.

Burke, R. E. Jr., J. Esslinger and J. C. Lacey Jr. 1957. Flavor and chemical changes in foods during sterilization by irradiation. U. S. Dept. Commerce, Off. Tech. Serv. P.B. Rept. 143775.

Burton, H. S. and D. J. McWeeny. 1963. Nonenzymic browning reactions; consideration of sugar stability. Nature. 197:266.

Burton, H. S., D. J. McWeeny and P. N. Pandhi. 1963. Nonenzymic browning. Browning of phenols and its inhibition by sulfur dioxide. Nature. 199:659.

Carrick, C. W. and S. M. Hauge. 1926. The effect of cod liver oil upon flavor in poultry meat. Poultry Sci. 5:213.

Caul, J. F. and S. A. Raymond. 1964. Home-use test by consumers of the flavor effects of disodium inosinate in dried soup. Food Technol 18:95

Cavallini, D., B. Mondovi, B. Giovanella and C. DeMarco. 1959. Degradation of thiotaurine by ionizing radiations. Nature. 184: No. 4688:B.A. 61

Challenger, F. and A. A. Rawlings. 1937. Formation of organometalloidal and similar compounds by microorganisms. V. Methylated alkyl sulfides. The fission of the disulfide link. J. Chem. Soc. 868.

Challenger, F. 1959. Aspects of the organic chemistry of sulphur. Butterworth's. London. 253 pp.

Challenger, F. and D. Greenwood. 1949. Sulphur compounds of the genus allium. Detection of n-propylthiol in the onion. Fission and methylation of diallyl disulfide in cultures of Scopulariopsis brevicaulis. Biochem. J. 44:87.

Cheronis, N. D. and J. B. Entrikin. 1961. Semi-micro Qualitative Analysis. The Systematic Identification of Organic Compounds. Interscience Publishers, Inc., New York. 774 pp.

Corcoran, G. B. 1956. Chromatographic separation and determination of straight chain saturated monocarboxylic acids $C_1$ through $C_{10}$ and dicarboxylic acids $C_{11}$ through $C_{16}$. Anal. Chem. 28:168.

Craig, H. B. and A. M. Pearson. 1959. Some preliminary studies on sex odor in pork. J. Animal Sci. 18:1557 (abstract)

Craig, H. B., A. M. Pearson and N. B. Webb. 1962. Fractionation of the component(s) responsible for sex odor/flavor in pork. J. Food Sci. 27:29.

Crocker, E. C. 1948. Flavor of Meat. Food Res. 13:179.

Davey, C. L. 1957. Ion-exchange method of determining carnosine, anserine, and their precursors in animal tissue. Nature. 179:209.

Dawson, L. E., S. Walters, and J. A. Davidson. 1958. Cooked meat yields from four strains of chickens 6 and 16 weeks of age. Poultry Sci. 37:227.

De Fremery, D. and M. Z. Pool. 1960. Biochemistry of chicken muscle related to rigor mortis and tenderization. Food Res. 25:73.

Doty, D. M., O. F. Batzer, W. A. Landmann and A. T. Santoro. 1961. Meat Flavor. Proceedings Flavor Chemistry Symposium-1961. Campbell Soup Company, Camden, New Jersey, p 7.

Ellis, R., A. M. Gaddis and G. T. Currie. 1958. Paper chromatography of 2,4-DNPHS of saturated aliphatic aldehydes. Anal. Chem. 30:475.

Essary, E. O. 1961. The influence of added fat and protein in broiler rations on growth rate, dressing percentages and other carcass characteristics. Unpublished Ph.D. Thesis. Mich. State Univ., East Lansing, Mich.

Feulgen, R. and K. Voit. 1925. A widely distributed substance (plasmal, plasmologen) its histological demonstration and its relation to odor in cooked meat. Klin. Wochschr. 4:1330. (CA 19,2505).

Fieser, L. F. and M. Fieser. 1956. Organic Chemistry. Reinhold Publishing Co., New York. 1112 pp.

Florkin, M. 1954. Zoological aspects of aggregations of nonprotein amino acids. Bull. soc. zool. France. 79:369. (CA 52,10236 h)

Folkard, A. R. and A. E. Joyce. 1963. The collection and identification of thiols and disulphides. J. Sci. Fd. Agric. 14:510.

Friedmann, T. E. and J. B. Graeser. 1933. The determination of lactic acid. J. Biol. Chem. 100:291.

Fujimaki, M. and K. Kojo. 1953. Handling effect upon biochemical change in the fish muscle immediately after the catch--II. Changes of acid-soluble phosphorous compounds of frigate mackerel muscle. Bull. Japan. Soc. Sci. Fisheries. 19:499. (CA 49,7137 i)

Gaddis, A. and R. Ellis. 1959. Paper chromatography of 2-alkanone, n-alkanal, alk-2-enal, and alk.-2-4-dienal derivatives. Anal. Chem. 31:870.

Geret, L. 1918. New investigations of meat extract. Z. Nahr. Genussm. 35:412. (CA 14,201).

Gilpin, G. L., A. M. Harkin, R. A. Redstrom and E. H. Dawson. 1960. Quality and old-type chickens. Poultry Sci. 39:924.

Gordon, B. E., F. Wopat Jr., H. D. Burnham and L. C. Jones, Jr. 1951. Analysis of mixtures of aldehydes and ketones. Anal. Chem. 23:1754.

Groninger, H. S., A. L. Tapperl and F. W. Knapp. 1956. Some chemical and organoleptic changes in gamma irradiated meats. Food Res. 14:472.

Grote, I. W. 1931. A new color reaction for soluble organic sulfur compounds. J. Biol. Chem. 93:25.

Guadagni, D. G., R. G. Buttery, S. Okano and H. K. Burr. 1963. Additive effect of sub-threshold concentrations of some organic constituents of some organic compounds associated with food aromas. Nature. 200:1288.

Guadagni, D. G., R. G. Buttery and S. Okano. 1963. Odour thresholds of some organic compounds associated with food flavours. J. Sci. Food Ag. 14:761.

Gray, G. M. and M. Macfarlane. 1961. Composition of phospholipids of rabbit, pigeon and trout muscle, and various pig tissues. Biochem. J. 81:480.

Grunert, R. R. and P. H. Phillips. 1951. The inability of liver homogenates to convert uric acid to alloxan. J. Biol. Chem. 191:633.

Hall, H. L. 1962. Quantitative gas-solid chromatographic determination of carbonyl/sulfide as a trace impurity in carbon dioxide. Anal. Chem. 34:61.

Hanson, H. L., H. M. Winegarden, M. B. Horton and H. Lineweaver. 1950. Preparation and storage of frozen cooked poultry and vegetables. Food Tech. 4:430.

Hanson, H. L., A. A. Campbell, A. A. Kraft, G. L. Gilpin and A. M. Harkin. 1959. The flavor of modern and old-type chickens. Poultry Sci. 38:1071.

Harries, J. M., K. B. Jones, T. W. Houston and J. Robertson. 1963. Studies in beef quality. I. Development of a system for assessing palatability. J. Sci. Fd. Agric. 14:501.

Hasselstrom, T. 1957. Techniques employed in the study of the chemistry of cabbage flavor. Chemistry of Natural Food Flavors--A Symposium Quartermaster Food and Container Institute for the Armed Forces, Chicago, Ill. p. 76.

Hawk, P. B. and O. Bergeim. 1937. Practical Physiological Chemistry. The Blakiston Company, Philadelphia. 968 pp.

Hedin, P. A., G. W. Kurtz and R. B. Koch. 1960. Production and prevention of irradiated odor in beef. Food Res. 25:382.

Henderson, L. M. and E. E. Snell. 1948. A uniform medium for the determination of amino acids with various microorganisms. J. Biol. Chem. 172:15.

Hoff, J. D. and E. D. Feit. 1963. Functional group analysis in gas chromatography with the syringe reaction technique. Anal. Chem. 35:1298.

Hofstrand, J. and M. Jacobson. 1960. The role of fat in the flavor of lamb and mutton as tested with broths and depot fats. Food Res. 25:706.

Hordh, U. 1936. Extracts of beef, yeast and vegetables and their analytical differentiation. Industria y quin (Argentina). 1:99. (CA 30,4577[9]).

Hornstein, I., P. F. Crowe and W. L. Sulzbacher. 1960. Constituents of meat flavor: Beef. J. Ag. and Food Chem. 8:65.

Hornstein, I. and P. F. Crowe. 1960. Meat flavor chemistry: Studies on beef and pork. J. Ag. and Food Chem. 8:494.

Hornstein, I. and P. F. Crowe. 1963. Meat flavor: Lamb. J. Ag. and Food Chem. 11:147.

Hornstein, I., P. F. Crowe and W. Sulzbacher. 1963. Flavor of beef and whale meat. Nature. 199:1252.

Hornstein, I. and P. F. Crowe. 1964. Meat flavor--A review. J. Gas Chromatog. 2:128.

Houghton, H. W. 1911. The effects of low temperatures on ground chicken meat. J. Ind. Eng. Chem. 3:497.

Hughes, R. B. 1960. Chemical studies on the herring (Clupea harengus). IV.--Creatine in herring flesh and its behavior during processing. J. Sci. Food Ag. 11:700.

Hughes, R. B. 1961. Herring. (Clupea harengus). VI.--Carbonyl compounds formed during the heat-processing of herring. J. Sci. Food Ag. 12:822.

Hughes, R. B. 1963. Chemical Studies on the herring (Clupea harengus). VIII.*--Further observations on the production of carbonyls in heat-processed herring. J. Sci. Food Ag. 14:432.

Hurley, W. C., O. J. Kahlenberg, E. M. Funk, L. G. Maharg and W. L. Webb. 1958. Factors affecting poultry flavor. Poultry Sci. 37:1436.

Jacobson, M., M. Weller, M. W. Galgan and E. H. Rupnow. 1962. Factors in flavor and tenderness of lamb, beef and pork, and techniques of evaluation. Wash. Ag. Exp. Sta. Tech. Bul. 40.

Jacobson, M. and H. H. Koehler. 1963. Meat flavor. Components of the flavor of lamb. J. Ag. and Food Chem. 11:336.

Jacquot, R. Contributor. 1961. Fish as Food. Vol. 1. Borgstrom Editor. Academic Press. New York and London. 145.

Jones, N. R. and J. Murray. 1957. Nucelotides in the skeletal muscle of codling (Gadus callarias). Biochem. J. 66:5P.

Jones, N. R. 1958. Free sugars in chill-stored, trawled codling. (Gadus callarias). J. Sci. Food Ag. 9:672.

Jones, N. R. and J. R. Burt. 1960. The separation and determination of sugar phosphates with particular reference to extracts of fish tissue. Analyst. 85:810.

Jones, N. R. 1961. Fish Flavours. Proceedings Flavor Chemistry Symposium-1961. Campbell Soup Company, Camden, New Jersey. p. 61.

Kahlenberg, O. J., E. M. Funk, L. A. Voss, L. G. Maharg and N. L. Webb. 1960. Factors affecting poultry flavor. The effect of mechanical quick-chill cooling unit. Poultry Sci. 39:350.

Kahlenberg, O. J. and E. W. Funk. 1961. The cooking of fowl with various salts for pre-cooked poultry products. Poultry Sci. 40:668.

Kappeler-Adler, R. und E. Stern. 1931. Über die Fractionierungsversuche von Basengemischen inbesondere von Fleischextraktbasen mit Permutit. Biochem. Z. 235:390.

Kates, M. and A. T. James. 1961. Phosphatide components of fowl blood cells. Biochem. Biophys. Acta. 50:477.

Kato, H. 1960. Browning reactions between sugars and amino compounds. V. Isolation and characterization of new carbonyl compounds, 3-deoxysones formed from N-glycosides and their significance in the browning reaction. Bull. Ag . Chem. Soc. Japan. 24:1. (CA 54,10358 b)

Kato, H. 1962. Chemical studies on the amino-carbonyl reaction. I. Isolation of 3-deoxypentosulose and 3-deoxyhexosuloses formed by browning degradation of glycosylamines. Ag . Biol. Chem. (Toyko). 26:187. (CA 58,2495 g).

Kato, H. 1963. Chemical studies on amino-carbonyl reaction. II. Identification of D-glucosone formed by oxidative browning degradation of N-D-glucoside. Ag . Biol. Chem. (Tokyo) 27:461. (CA 59,9011 a)

Kazeniac, S. J. 1961. Chicken Flavor. Proceedings Flavor Chemistry Symposium-1961. Campbell Soup Company, Camden, New Jersey. p. 37.

Kazeniac, S. J. 1963. Personal communication.

Kean, G. R. 1959. Mutton-its value and uses. Proc. Recip. Meat Conf. 12:87.

Kendall, E. C., H. L. Mason and B. F. McKenzie. 1930. Glutathione. III. The structure of glutathione. J. Biol. Chem. 87:55.

Kahn, A. W., L. VanDenberg and C. P. Lentz. 1963. Effects of frozen storage on chicken muscle proteins. J. Food Sci. 28:425.

Kluender, U. 1920. The manufacture of Liebig's meat extract. Chem. Ztg. 44:837. (CA 15,712).

Kokovikhina, K. I. 1942. Determination of carnosine in meat extract. Biokhimiya. 7:238. (CA 38,1575$^{3}$).

Korchow, A. 1927. Über die Wirkung einiger nach dem Verfahren von Gulewitch und Krimberg gewonner Fraktionen des Liebigschen Fleischextrakts auf die Magensekretion. Biochem. Z. 190:188.

Koren, H. and W. Gierlinger. 1953. Beitrag zur kolorimetrischen Bestimmung von Sulfid-Ionen. Mikrochim. Acta. 1:220.

Kramlich, W. E. and A. M. Pearson. 1958. Some preliminary studies on meat flavor. Food Res. 23:567.

Kramlich, W. E. and A. M. Pearson. 1960. Separation and identification of cooked beef flavor components. Food Res. 25:712.

Krimberg, R. and L. Izrailskii. 1914. Zur kentniss der Extraktivestoffe der Muskeln. Über das Kreatosin, eine neue Base des Fleischextraktes. Z. physiol. Chem. 88:324.

Krishnaswamy, M. A. and F. J. Babel. 1951. Biacetyl production by cultures of lactic acid producing streptococci. J. Dairy Sci. 34:374.

Kurtzman, C. H. and L. B. Sjöström. 1963. The flavor modifying properties of disodium inosinate. Program 23rd Ann. Meeting Inst. of Food Technologists. Paper 110.

Lea, C. H. and R. S. Hannan. 1950. Reactions between proteins and reducing sugars in the dry state. Biochem. : Biophys. Acta. 3:313.

Lea, C. H. and R. S. Hannan. 1950. Biochemical and nutritional significance of the reaction between proteins and reducing sugars. Nature. 165:438.

Lea, C. H. and H. M. Schwartz. 1952. Reaction between proteins and reducing sugars in the "dry" state. Relative reactivity of the alpha and epsilon amino groups of insulin. Biochem. J. 50:713.

Lea, C. H. and P. A. T. Swoboda. 1958. The flavor of aliphatic aldehydes. Chem. and Ind. 1289.

Lewin, S. 1957. Some aspects of the effect of the initial pH on the behavior of glucose in the amino-acid-glucose interactions. Biochem. J. 65:30.

Lewin, S. and J. S. McCall. 1957. Some aspects of the interaction of glycylglycine with glucose. Biochem. J. 65:30.

Lewis, R. W., P. E. Sanford, A. F. Ericson, D. L. Harrison and R. E. Clegg. 1956. Flavor and aroma of birds fed purified and standard diets. Poultry Sci. 35:251.

Liebig, J. 1847. Ueber die Bestandtheile der Flüssigkeiten des Fleisches. Annalen der Chemie und Pharmacie. 62:257.

Lilyblade, A. L. and D. W. Peterson. 1962. Inositol and free sugars in chicken muscle post-mortem. Food Sci. 27:245.

Lineweaver, H. and E. L. Pippen. 1961. Chicken Flavor. Proceedings Flavor Chemistry Symposium-1961. Campbell Soup Company, Camden, New Jersey. p. 21.

Lissi, T. P. and M. T. Cuzzoni. 1961. Characterization of meat extracts. Farnaco Ed. Sci. 16:710. (CA 56,15892 b).

Lobanov, D. I. and G. Wolfson. 1958. The formation of melanoidins during preparation of meat and its effect on the gastric secretion of dogs. Nahrung. 2:660. (CA 53,12513 i)

Lovern, J. A. 1950. Some causes of variations in the composition of fish oils. J. Soc. Leather Trades' Chemists 34:7.

Lukton, A., and H. S. Olcott. 1958. Content of free imidazole compounds in the muscle tissue of aquatic animals. Food Res. 23:611.

Lundberg, W. O. 1957. Origin of oxidized flavor in fish oils. Comm. Fish.Rev. 19:5.

Lynn, W. S., Jr., L. A. Steele and E. Staple. 1956. Separation of 2,4-dinitrophenylhydrazones of aldehydes and ketones by paper chromatography. Anal. Chem. 28:132.

Maillard, L. C. 1912. Action of amino acids on sugars. Formation of melanoidins in a methodical way. Compt. rend. 154:66. (CA 6,11306).

Malmberg, E. W. 1954. A chromatographic study of carbonyl compounds present in a hydrocarbon flame. J. Am. Chem. Soc. 70:2061.

Mangan, G. F. 1959. Identification of some flavor compounds in haddock. Comm. Fish. Rev. 19:21.

Mangan, G. F. 1959. Dicarbonyl compounds as constituents of fish odor. Comm. Fish. Rev. 21(7):21.

Mangold, H. K. 1961. Thin-layer chromatography of lipids. J. Am. Oil Chemists' Soc. 38:708.

Mannan, A. and H. L. A. Tarr. 1961. Preparation of deoxynucleosides, purine and pyrimidine bases and deoxyribose 1-phosphate from deoxyribonucleic acid employing salmon enzyme systems. J. Fish. Res. Bd. Canada. 18:349.

Marbach, E. P. and D. M. Doty. 1956. Sulfides released from gamma irradiated meat as estimated by condensation with N,N-Dimethyl-p-phenylenediamene. J. Ag. and Food Chem. 4:881.

Marble, D. R., J. E. Hunter, H. C. Knandel and R. A. Dutcher. 1938. Fishy flavor and odor in turkey meat. Poultry Sci. 17:49.

Marriott, W. McK. 1916. Method for determining carbon dioxide in alveolar air. J. Am. Med. Ass'n. 66:1594.

Martin, G. B. and H. L. A. Tarr. 1961. Phosphoglucomutase, phosphoribomutase, and phosphoglucoisomerase of lingood muscle. Can. J. Biochem. Physiol. 39:298.

Martin, Salvador, O. F. Batzer, W. A. Landmann and B. S. Schweigert. 1962. The role of glutathione and methionine in the production of hydrogen sulfide and methyl mercaptan during irradiation of meat. J. Ag. and Food Chem. 10:91.

Mason, H. L. 1931. A study of glutathione. V. The spontaneous cleavage of glutathione in aqueous solution. J. Biol. Chem. 90:25.

Maw, W. A. 1935. How quality in poultry meat is affected by distribution of fat in the carcass. U.S. Egg and Poultry Mag. 41:32.

Mazelis, M. 1962. The pyridoxal phosphate-dependent oxidative decarboxylation of methionine by peroxidase. I. Characteristics and properties of the reaction. J. Biol. Chem. 237:104.

Mazelis, M. and L. L. Ingraham. 1962. The pyrodoxal phosphate-dependent decarboxylation of methionine by peroxidase. II. Identification of methyl-thiopropionamide as a product of the reaction. J. Biol. Chem. 237:109.

McInnes, A. G., R. P. Hanson and A. S. Jessop. 1956. The volatile acids of mutton fat. Biochem. J. 63:702.

Merriam, F. C. 1948. Modification of Hershberg melting-point apparatus for internal heating and silicone fluid. Anal. Chem. 20:1246.

Merritt, Jr., C., S. R. Bresnick, M. L. Bazinet, J. T. Walsh and P. Angelini. 1959. Determination of volatile components of foodstuffs. Techniques and their application to studies of irradiated beef. J. Ag. and Food Chem. 7:784.

Merritt, Jr., C. and J. T. Walsh. 1962. Qualitative gas chromatographic analysis by means of retention volume constants. Anal. Chem. 34:903.

Merritt, Jr., C. and P. Angelini. 1963. Personal communication.

Micko, K. 1908. Meat Extract: The portion not precipitated by salting out with zinc sulphate. Z. Nahr.-Genussm. 15:449. (CA 2,2109)

Micko, K. 1909. Über das Vorkommen von Monoaminosäuren im Fleischextrakt. Z. physiol. Chem. 56:180.

Micko, K. 1914. Food condiments and bouillon cubes. Z. Nahr.-Genussm. 26:321. (CA 8,537).

Micko, K. 1914. Über Bouillonwürfel, Speisewürzen und Fleischextrakt. Berlin: J. Springer.

Motozaki, S., T. Tsunoda, R. Aoki, S. Okomura, Y. Kondo, N. Muramatsu, H. Momose and Y. Tamagawa. 1963. Method for preparation of inosine. U.S. Patent 3,111,459. To Ajinomoto K.K. (Tokyo, Japan).

Mountney, G. R., R. E. Branson and W. C. Hurley. 1960. The effect on flavor of holding chicken for selected periods. Poultry Sci. 29: 287.

Newberg, C. and E. Strauss. 1945. Microdetermination of dicarbonyl compounds. On the coloring of nitro compounds in alkaline solutions. Arch. Biochem. 7:211.

Newman, S., P. J. Schaible and L. E. Dawson. 1958. Effect of spices in feed on poultry meat flavor. Quar. Bull. Mich. Ag. Expt. Sta., E. Lansing. 40 (4):747.

Nonaka, M., E. L. Pippen and G. F. Bailey. 1959. Separation and identification of n-alkanals, 2-alkanones, 2-alkenals and 2,4-alkadienals by paper chromatography and direct spectrophotometric examination. Anal. Chem. 31:875.

Odland, L. M., H. L. Mayfield and L. Page. 1955. The influence of palatability of certain grain combinations, fish solubles and vitamin B-12 aureomycin supplement in poultry rations. Poultry Sci. 34:822.

Olley, J. 1961. The phospholipases. Rept. Prog. Appl. Chem. 46:198.

Osborne, T. B. and F. W. Heyl. 1908. Hydrolysis of chicken meat. Am. J. Physiol. 22:433.

Patrik, I. A. 1962. Effect of phosphatides on chick growth and on the chemical content of their meat. World's Poultry Congr. Proc. 12th, Sydney, 205.

Patton, S., I. J. Barnes and L. E. Evans. 1959. n-Deca-2,4-dienal, its origin from linoleate and flavor significance in fats. J. Am. Oil Chemists' Soc. 36:280.

Pearson, A. M., R. N. Costilow, O. F. Batzer, R. A. Sliwinski and L. Chang. 1959. The relationship between panel scores and certain chemical components in recooked irradiated meats. Food Res. 24:228.

Peters, J. H. 1942. The determination of creatinine and creatine in blood and urine with the photoelectric colorimeter. J. Biol. Chem. 146:179.

Pennington, M. E. and S. J. Hepburn. 1913. Studies on chicken fat. U.S.D.A. Bur. of Chem., Circ. 103, 1.

Peterson, D. W. 1957. Surveys of subsistence problems. Quartermaster Food and Container Inst., Ser. I, No. 9:169.

Peterson, D. W. 1957. The source of chicken flavor. Proceedings of the Symposium on Chemistry of Natural Food Flavors. Quartermaster Research and Engineering Center Natick, Massachusetts. p. 167.

Peterson, D. W., M. Simons, A. L. Lilyblade and R. Martin. 1959. Some factors affecting intensity of flavor and toughness of chicken muscle. Food Technol. 13:204.

Phillips, J. H. 1962. A chart of UV absorption maxima of 2,4-dinitrophenylhydrazones. J. Org. Chem. 27:1443.

Pippen, E. L., A. A. Campbell and J. V. Streeter. 1954. Flavor Studies. Origin of chicken flavor. J. Ag. and Food Chem. 2:364.

Pippen, E. L. and A. A. Klose. 1955. Effects of ice water chilling on flavor of chicken. Poultry Sci. 34:1139.

Pippen, E. L. and E. J. Eyring. 1957. Characterization of volatile nitrogen and sulfur fractions of cooked chicken and their relation to flavor. Food Technol. 11:53.

Pippen, E. L., M. Nonaka, F. T. Jones, and F. Stitt. 1958. Volatile carbonyl compounds of cooked chicken. I. Compounds obtained by air entrainment. Food Res. 23:103.

Pippen, E. L. and M. Nonaka. 1960. Volatile carbonyl compounds of chicken. II. Compounds volatilized with steam during cooking. Food Res. 25: 764.

Pippen, E. L., E. J. Eyring and M. Nonaka. 1960. The occurrence and significance of acetoin in aqueous extracts of chicken. Poultry Sci. 39:922.

Pippen, E. L. and M. Nonaka. 1963. Gas chromatography of chicken and turkey volatiles: The effect of temperature, oxygen, and type of tissues on composition of the volatile fraction. J. Food Sci. 28:334.

Pocchiari, F., L. Gentori and G. Vivaldi. 1962. Presence of the dipeptide beta-alanyl-3-methylhistidine in whale meat extract. Sci. Repts. 1st. Super Sanita. 2:188. (CA 60,2256 a)

Porter, R. W., L. J. Bratzler and W. T. Magee. 1962. Yield of cooked edible meat from various retail beef cuts as influenced by weight and grade. Food Technol. 16:86.

Prill, E. A. and B. W. Hammer. 1938. A colorimetric method for the microdetermination of diacetyl. Iowa State Univ. Jour. Sci. 12:385.

Privett, O. S., J. R. Chipault, H. Schlenk, and W. O. Lundberg. 1958. The thiobarbituric acid test for oxidative rancidity. Comm. Fish. Rev. 20:18.

Proctor, B. E. and S. A. Goldblith. 1955. Cold sterilization of foods. Chem. Eng. Prog. 51:480.

Rapport, M. M. and B. Lerner. 1959. The structure of plasmologens. IV. Lipids in normal and neoplastic tissues of man and in normal tissues of rabbit and rat. Biochim. Biophys. Acta. 33:319.

Reay, G. A. and J. M. Shewan. 1949. The spoilage of fish and its preservation by chilling. Adv. in Food Res. 2:343.

Reay, G. A. and J. M. Shewan. 1949. The spoilage of fresh fish and its control. Chem. and Ind. 35.

Rice, R. G., G. C. Keller and J. G. Kirchner. 1951. Separation and identification of 2,4-dinitrophenylhydrazones of aldehydes and ketones and 3,5-dinitrobenzoates of alcohols by filter paper chromatography. Anal. Chem. 23:194.

Rittner, R., G. Tilley, A. Mayer, Jr., and S. Siggia. 1962. Identification of mercaptans and disulfides by X-ray diffraction of their respective 2,4-dinitrophenyl thioethers. Anal. Chem. 34:237.

Rittner, R. 1963. Personal communication.

Robertson, G. R. and T. L. Jacobs. 1962. Laboratory Practice of Organic Chemistry. 4th ed. MacMillan Co., New York.

Rockland, L. B. and M. S. Dunn. 1949. Capillary-ascent test-tube method for separating amino acids by filter-paper chromatography. Science. 109:539.

Rockland, L. B., J. L. Blatt and M. S. Dunn. Small-scale filter-paper chromatography. Anal. Chem. 23:1142.

Sadikov, V. S., A. F. Shoshin, K. M. Starukhina and M. I. Livshitz. 1934. Origin of hydrogen sulfide produced when chicken flesh is cooked. Compt. Rend. Acad. Sci. U.S.S.R. 3:39. (CA 28,6866[4]).

Saito, T. and K. Arai. 1957. Slow freezing of carp muscle and inosinic acid formation. Nature. 179:820.

Saito, T. and K. Arai. 1958. Organic phosphates in the muscle of aquatic animals. V. Changes in muscular nucleotides of carl during freezing and storage. Nippon Suisangaku Kaishi. 23:265. (CA 53,3528 i).

Salkowski, E. 1914. Über die Fallung der Purinebasen durch Zinksalze aus Fleichextrakt und Harn. Biochem. Z. 55:254.

Sands, A. E., M. A. Grafins, H. W. Wainwright and M. W. Wilson. 1949. Inorganic sulfide determination. Rept. of Investigations, R.I. 4547, U.S. Dept. of the Interior--Bureau of Mines, Sept. 1949.

Sasin, G. S. 1962. Sulfur derivatives of long chain acids. J. Am. Oil Chemists' Soc. 39:488.

Sasin, G. S. 1964. Personal communication.

Schano, E. A. and J. A. Davidson. 1958. A comparison of chicken cooked electronically with conventionally cooked chicken. Poultry Sci. 37:1239 (abstract)

Schöberl, A. 1933. Die Oxydation von Disulfiden zu Sulfonsäuren mit Wasserstoffsuperoxyd. Z. physiol. Chem. 216:193.

Schöberl, A. und H. Ech. 1936. Die hydrolytische Aufspaltung der Disulfidbindung. Ann. 522:97.

Schönberg, A., R. Moubacher, and A. Mostafa. 1948. Degradation of alpha amino acids to aldehydes and ketones by interaction with carbonyl compounds. J. Chem. Soc. 176.

Schönberg, A. and R. Moubacher. 1952. The Strecker degradation of amino acids. Chem. Rev. 50:261.

Schwartz, H. M. and C. H. Lea. 1952. Reaction between proteins and reducing sugars in the dry state. Biochem. J. 50:713.

Self, R., H. L. J. Rolley and A. E. Joyce. 1963. Some volatile compounds from cooked potatoes. J. Sci. Food Ag. 14:8.

Self, R., J. C. Casey, and T. Swain. 1963. The low-boiling volatiles of cooked foods. Chem. and Ind. 863.

Shewan, J. M. and N. R. Jones. 1957. Chemical changes occurring in cod muscle during chill storage and their possible use as objective indices of quality. J. Sci. Food Ag. 8:491.

Shriner, R. L., R. C. Fuson, and D. Y. Curtin. 1956. Identification of Organic Compounds. John Wiley and Sons, New York.

Siggia, S. 1963. Personal communication.

Sjöström, L. B. and S. E. Cairncross. 1955. Use of sugars and other carbohydrates in the food industry. Advances in Chemistry Series #12, American Chemical Society, Washington, D. C., 108.

Skidmore, W. D. 1962. Two-dimensional thin-layer chromatography of rat liver phosphatides. J. Lipid Res. 3:471.

Skipski, V. P., R. F. Peterson and M. Barclay. 1962. Separation of phosphatidyl ethanolamine, phosphatidyl serine and other phospholipids by thin-layer chromatography. J. Lipid Res. 3:467.

Sliwinski, R. A. and D. M. Doty. Determination of micro quantities of methyl mercaptan in gamma-irradiated meat. J. Ag. and Food Chem. 6:41.

Smillie, R. M. 1959. The separation of RNA nucleotides on ion exchange paper. Arch. Biochem. Biophys. 85:557.

Smith, W. B. 1916. Estimation of sugar in meat extracts. J. Ind. Eng. Chem. 8:1024.

Snell, F. D. and C. T. Snell. 1954. Colorimetric Methods of Analysis. D. Van Nostrand, New York.

Soloway, S. and S. H. Wilen. 1952. Improved ferric chloride test for phenols. Anal. Chem. 24:979.

Speck, J. C., Jr. 1952. Contributions of browning. Research to Ration Item Stability. Research and Development Associates, Food and Container Institute, U.S. Army Quartermaster Corps., Chicago, Ill.

Stahl, C. R. and S. Siggia. 1957. Determination of organic disulfides by reduction with sodium borohydride. Anal. Chem. 29:154.

Stahl, E. 1958. Thin-layer chromatography II. Standardization, detection, documentation and application. Chemiker Ztg. 82:323. (CA 53,27 c).

Stewart, G. F., H. L. Hanson, B. Lowe, and J. J. Austin. 1945. Effects of aging, freezing rate and storage period on palatability of broilers. Food Res. 10:16.

Stitt, F., R. B. Seligman, F. E. Resnik, Edith Gong, E. L. Pippen and David A. Forss. 1960. Spectrophotometric studies of the 2,4-dinitrophenylhydrazones of n-alka-2-enals and n-alka-2,4-dienals. Spectrochim. Acta. 17:51.

Stotz, E. and J. Raborg. 1943. A colorimetric determination of acetoin and diacetyl. J. Biol. Chem. 150:25.

Sullivan, J. H., J. T. Walsh, and C. Merritt Jr. 1959. Improved separation in gas chromatography by temperature programming. Application to mercaptans and sulfides. Anal. Chem. 31:1826.

Swickard, M. T., A. M. Harkin and P. J. Paul. 1954. Relationship of cooking methods, grades and frozen storage to quality of cooked mature Leghorn hens. U.S.D.A. Tech. Bull. 1077.

Tanikawa, E. 1958. Technical problems in the processing of canned salmon. Mem. Fac. Fisheries, Hokkaido Univ. 6:67. (CA 53,15411 e).

Tanikawa, E. 1959. Technical problems in the processing of canned salmon. Mem. Fac. Fisheries, Hokkaido Univ. 7:95. (CA 54,21530 g).

Tarr, H. L. A. 1950. The acid soluble phosphorous compounds of fish skeletal muscle. J. Fisheries Research Board Can. 7:608.

Tarr, H. L. A. 1957. Deoxyribose-1 : 5-diphosphate. Chem. and Ind. 562.

Tarr, H. L. A. 1958. Biochemistry of fishes. (Review). Ann. Rev. Biochem. 27:223.

Tarr, H. L. A. 1958. Lingood muscle purine nucleoside phosphorylase. Can. J. Biochem. and Physiol. 36:517.

Tarr, H. L. A. 1959. Lingood muscle phosphoribo-isomerase and ribulose 5'-Phosphate 3'-Opimerase. Can. J. Biochem. Physiol. 37:961.

Tarr, H. L. A. 1960. The enzyme 5-phosphorylribose pyrophophokinase in fish muscle. Can. J. Biochem. Physiol. 38:683.

Tarr, H. L. A. 1962. Acid-soluble phosphorous compounds and free sugars in fish muscle and their origin. Can. J. Biochem. and Physiol. 40:571.

Tarr, H. L. A. 1962. Personal communication.

Tempus, P. 1956. Purine bases in meat and yeast extracts in albumin hydrolysates. Mitt. Gebiete Lebensm. u. Hyg. 47:351. (CA 51,9028f).

Toi, B., S. Maeda, S. Ikeda and H. Furukawa. 1963. Flavoring and preparation thereof. U.S. Patent 3,109,741.

Tomlinson, N. and V. M. Creelman. 1960. On the source of free ribose formed post mortem in the muscle of lingood (Ophiodon elongatus). J. Fish. Res. Bd. Canada. 17:603.

Tomlinson, N., E. S. Arnold, E. Roberts and S. E. Geiger. 1961. J. Fish. Res. Bd. Canada. 18:321.

Toyama, Y. 1942. Occurrence of highly unsaturated acids in the oil of the leg of the chicken. J. Chem. Soc. (Japan). 63:1085.

Van den Berg, L., A. W. Khan and C. P. Lentz. 1963. Biochemical and quality changes in chicken meat during storage at above-freezing temperatures. Food Technol. 17(1):91.

Walsh, J. T. and C. Merritt, Jr. 1960. Qualitative functional group analysis of gas chromatographic effluents. Anal. Chem. 32:1378.

Waser, E. 1917. Nachweis und Bestimmung von Ameisensäure in Fleischextrakten. Z. physiol. Chem. 99:67. J. Chem. Soc. 112(2):343.

Waser, E. 1920. Investigations of meat broths. Z. Nahr-Genussm. 40: 280. (CA 15,1044).

Watts, B. M. 1961. The role of lipid oxidation in lean tissues in flavor deterioration of meat and fish. Proceedings Flavor Chemistry Symposium-1961. Campbell Soup Company, Camden, New Jersey, p. 83.

Weisberg, S. M. 1956. How milk products affect the eating quality of broilers. Broiler World. 4:16.

Wells, G. H., K. N. May and J. J. Powers. 1962. Taste panel and shear-press evaluation of tenderness of freeze-dried chicken as affected by age and pre-slaughter feeding of ions. Poultry Sci. 41:1693 (Abstract)

Wesp, E. F. and W. R. Brode. 1934. The absorption spectra of ferric compounds. I. The ferric chloride-phenol reaction. J. Am. Chem. Soc. 56:1037.

Williams, Beverly E. Jan. 9, 1962. Improving the tenderness and flavor of mutton. U. S. Patent 3,016,301.

Wise, R. G. and W. J. Stadelman. 1961. Tenderness of poultry meat: Effect of scalding temperatures. Poultry Sci. 40:1731.

Witting, L. A. and O. F. Batzer. 1957. Relation of methional to the odor of irradiated meat. Food Res. 22:237.

Wolff, H. 1913. The influence of meat extractives upon the thoroughness of digestion of vegetable food. Z. Klin. Med. 76:66. (CA 7,2056).

Wood, T. 1956. Some applications of paper chromatography to the examination of meat extract. J. Sci. Food Ag. 7:196.

Wood, T. and A. E. Bender. 1957. Analysis of tissue constituents. Commercial ox-muscle extract. Biochem. J. 67:366.

Wood, T. 1961. Browning of ox muscle extracts. J. Sci. Fd. Agric. 12:61.

Wood, T., E. P. Adams and A. E. Bender. 1962. Gastric-stimulating factor in meat extract. Nature. 195:1207.

Yoshida, T. and H. Kageyama. 1956. Inosinic acid salt as a condiment. Jap. patent. 732. (CA 51,P3870 b).

Yueh, M. H. and F. M. Strong. 1960. Some volatile constituents of cooked beef. J. Ag. and Food Chem. 8:491.

Zabik, M. E. and L. E. Dawson. 1963. The acceptability of cooked poultry protected by an edible acetylated monoglyceride coating during fresh and frozen storage. Food Technol. 17:87.

Ziegler, P. T. 1958. The Meat We Eat. The interstate Publishers and Printers. Danville, Illinois.

Ziolecki, J. 1963. The effect of aging period and pH value of chicken meat on cooking losses. 11th. Int'l Cong. of Refrign., Munich, Germany. (Abstract).

# APPENDIX

Table 1. Ration fed to White Leghorn laying hens (20 months old) and younger female chickens (12 weeks old) raised from the same flock.

| | | | |
|---|---|---|---|
| Ground corn | 54.8% | Soybean meal | 10.0% |
| Ground oats | 10.0% | Bone meal | 1.0% |
| Wheat mids | 10.0% | Iondine salt | 0.5% |
| Alfalfa meal | 5.0% | Fish meal | 2.0% |
| Dried milk | 2.0% | Oyster shell | 2.0% |
| Meat scrap | 2.0% | Fish oil | 0.25% |
| | | 1500 (I.U.) vitamin A | |
| | | 300 (I.U.) vi amin D | |

$MnSO_4$ 0.01%

Soluferm 500 0.38%

Vitamin B-12 supplement 0.03%

(9 mg./lb.)

Vitamin E supplement 0.03% (20,000 I.U./lb.)

Table 2. Determinations of L-cystine and L-methionine content of raw-frozen and cooked-freeze-dried meat-slurry from roasters, heavy- and light-weight hens by Henderson and Snell's (1948) microbiological method

| Sample No.[1] | L-cystine determination | | | | L-methionine determination | | | |
|---|---|---|---|---|---|---|---|---|
| | ml. .0987N KOH/ml. diluted hydrolysate | ug/ml. diluted hydrolysate | % in original sample | mg./g. in original sample | ml. .0987N KOH/ml. diluted hydrolysate | ug./ml. diluted hydrolysate | % in original sample | mg./g. in original sample |
| 1r | 18.0 | 25.3 | 0.25 | 2.5 | 14.4 | 13.0 | 0.65 | 6.5 |
| 2r | 17.8 | 24.2 | 0.24 | 2.4 | 11.2 | 10.2 | 0.51 | 5.1 |
| 3r | 18.6 | 26.4 | 0.26 | 2.6 | 12.6 | 11.4 | 0.51 | 5.7 |
| 4r | 17.0 | 23.3 | 0.23 | 2.3 | 14.6 | 13.2 | 0.66 | 6.6 |
| 5r | 19.6 | 27.1 | 0.27 | 2.7 | 15.4 | 14.0 | 0.70 | 7.0 |
| 6r | 17.0 | 23.3 | 0.23 | 2.3 | 15.8 | 14.0 | 0.70 | 7.0 |
| 1c | 15.2 | 21.3 | 0.85 | 8.5 | 15.6 | 13.9 | 2.78 | 27.8 |
| 2c | 14.7 | 20.1 | 0.80 | 8.0 | 13.6 | 13.3 | 2.46 | 24.6 |
| 3c | 16.8 | 23.8 | 0.95 | 9.5 | 13.5 | 12.2 | 2.44 | 24.4 |
| 4c | 17.0 | 21.3 | 0.85 | 8.5 | 16.7 | 15.2 | 3.04 | 30.4 |
| 5c | 16.8 | 23.8 | 0.95 | 9.5 | 15.2 | 13.6 | 2.72 | 27.2 |
| 6c | 16.4 | 22.5 | 0.90 | 9.0 | 15.4 | 14.0 | 2.80 | 28.0 |

1. Sample code: 1-heavy hen breast; 2-heavy hen leg; 3-roaster breast; 4-roaster leg; 5-light-hen breast; 6-light hen leg. (r: raw-frozen muscle) (c: cooked-freeze-dried meat-broth slurry)

Table 3. Composition of Henderson and Snell's (1948) single medium for determining 14 different amino acids with the appropriate amino acid omitted.

Composition of Medium

| Component | Amount/ 100 ml. | Component | Amount/ 100 ml. |
|---|---|---|---|
| Glucose | 2 g. | Ca Pantothenate | 100 ug. |
| Sodium Citrate (U.S.P. XIII) | 2 g. | Niacin | 100 ug. |
| Sodium acetate (anhydrous) | 0.1 g. | p-aminobenzoic acid | 20 ug. |
| Ammonium Chloride | 0.3 g. | Biotin | 1 ug. |
| $K_2HPO_4$ | 0.5 g. | Folic Acid | 1 ug. |
| Salts C | 2 ml. | D-L-Alanine | 100 mg. |
| Adenine-$SO_4$ | 1 mg. | D-L-Aspartic Acid | 100 mg. |
| Guanine-HCl | 1 mg. | L-Glutamic Acid | 100 mg. |
| Uracil | 1 mg. | L-Arginine-HCl | 20 mg. |
| Xanthine | 1 mg. | L-Lycine-HCl:$H_2O$ | 20 mg. |
| Thiamine | 100 ug. | Other Amino Acids L-forms | 10 mg. ea. |
| Riboflavin | 100 ug. | or DL-forms | 20 mg. ea. |
| Pyridoxal | 20 ug. | | |

Notes: 1) Magnesium and manganese ions from salts C overcome citrate toxicity.
2) Use a 2% sodium citrate buffer for Leuconostoc mesenteroides.
3) Hydrolyze the protein containing material for 5 hrs. at 15 lbs. pressure with 3N HCl.

Table 4. Colorimetric determination of percent transmission of cystine in diluted hydrolysate samples of raw-frozen muscle and cooked-freeze-dried meat slurry from roasters, heavy- and light-weight hens.

Standard Curve

A standard solution containing 5 ug of cystine was used.

| ml. standard solution | % Transmission of duplicate tubes | |
|---|---|---|
| 0.0 | 12 | 12 |
| 0.2 | 55 | 59 |
| 0.4 | 78 | 78 |
| 0.6 | 89 | 93 |
| 0.8 | 102 | 95 |
| 1.0 | 102 | 65 |

The % transmission values were plotted against ug of cystine on the standard curve (Fig. 5a).

Each hydrolysate sample was diluted 1-4000 and various amounts added to incubation tubes. The % transmission obtained were as follows:

| ml. of diluted sample | | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 |
|---|---|---|---|---|---|---|
| Sample No.[1] | 1 | 33 | 50 | 61 | 72 | 77 |
| | 2 | 34 | 52 | 62 | 71 | 78 |
| | 3 | 26 | 43 | 54 | 64 | 71 |
| | 4 | 27 | 44 | 55 | 63 | 68 |
| | 5 | 35 | 53 | 66 | 73 | 79 |
| | 6 | 34 | 55 | 61 | 71 | 81 |
| | 7 | 33 | 50 | 64 | 68 | 80 |
| | 8 | 31 | 49 | 63 | 69 | 78 |
| | 9 | 34 | 55 | 66 | 74 | 84 |
| | 10 | 38 | 55 | 68 | 72 | 76 |
| | 11 | 37 | 53 | 65 | 75 | 82 |
| | 12 | 38 | 54 | 66 | 74 | 82 |

Using the standard curve (Fig. 5a),

Milligrams of cystine per gram of sample were calculated from % transmission obtained.

1. Sample code: 1-heavy hen breast; 2-heavy hen leg; 3-roaster breast; 4-roaster leg; 5-light hen breast; 6-light hen leg. (r: raw-frozen muscle) (c: cooked-freeze-dried meat-broth slurry).

Table 5. Colorimetric determination of percent transmission of methionine in diluted hydrolysate samples from raw-frozen muscle and cooked-freeze-dried meat-slurry from roasters, heavy- and light-weight hens.

Standard Curve

A standard solution containing 10ug of methionine was used.

| ml. standard solution | % transmission of duplicate tubes | |
|---|---|---|
| 0.0 | 15 | 18 |
| 0.2 | 42 | 41 |
| 0.4 | 68 | 67 |
| 0.6 | 88 | 91 |
| 0.8 | 100 | 103 |
| 1.0 | 107 | 107 |

The % transmission values were plotted againstug of methionine on the standard curve (Fig. 6a).

Each hydrolysate sample was diluted 1-4500 and various amounts added to the incubation tubes. The % transmissions obtained were as follows:

| ml. of diluted sample | | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 |
|---|---|---|---|---|---|---|
| Sample No.[1] | 1r | 31 | 49 | 64 | 77 | 89 |
| | 2r | 31 | 50 | 62 | 78 | 86 |
| | 3r | 29 | 44 | 58 | 71 | 83 |
| | 4r | 30 | 43 | 61 | 70 | 80 |
| | 5r | 31 | 46 | 64 | 107 | 85 |
| | 6r | 30 | 48 | 63 | 91 | 89 |
| | 1c | 30 | 43 | 59 | 70 | 82 |
| | 2c | 29 | 43 | 58 | 72 | 81 |
| | 3c | 36 | 52 | 68 | 84 | 93 |
| | 4c | 35 | 53 | 67 | 82 | 92 |
| | 5c | 31 | 50 | 63 | 79 | 90 |
| | 6c | 32 | 50 | 64 | 78 | 86 |

Using the standard curve (Fig. 6a), milligrams of methionine per gram of sample were calculated from % transmission obtained.

1. Sample code: 1-heavy hen breast; 2-heavy hen leg; 3-roaster breast; 4-roaster leg; 5-light hen breast, 6-light hen leg. (r: raw-frozen muscle) (c: cooked-freeze-dried meat-broth slurry).

Campbell SOUP Company

CAMDEN 1, NEW JERSEY

November 1, 1963

Mr. L. J. Minor
Meat Laboratory
Food Science Department
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East Lansing, Michigan

Dear Mr. Minor:

It was a pleasure to talk to you and learn that you are doing research work on chicken flavor. I hope that the following comments may be helpful to you on this problem.

The major non-protein nitrogen compounds of chicken may be grouped as follows:

1. Creatine/creatinine
2. Amino acids/peptides
3. Purine derivatives
4. Carnitine
5. Ammonium salts (probably phosphates and lactate)
6. Possibly N-bases of phospholipids (e.g., choline, ethanolamine)

These are several procedures for the determination of creatine/creatinine, of which, the following would seem to be the best:

1. The alkaline picrate method (Methods of Analysis, Association of the Official Agricultural Chemists, p. 389, (1955)). Also see T. Wood and A. E. Bender, Biochem. J. 67, 366 (1957).

2. The alpha-naphthol/diacetyl color reaction (D. R. Anderson, C. M. Williams, G. M. Krise and R. M. Dowben, Biochem. J. 67, 258 (1957)).

3. The Sullivan-Irreverre test with greater specificity (M. X. Sullivan and F. Irreverre, J. Biol. Chem. 233, 530, (1958)).

Amino acids/peptides can be analyzed colorimetrically with ninhydrin by any of the various chromatographic methods or directly.

There are several good procedures for the determination of purine derivatives, using ion exchange resins. These compounds also can be estimated readily by thin-layer chromatography. The spots can be located with ultraviolet light, scraped from the glass plate, dissolved and measured by their ultraviolet absorption. Our early work indicated

that total purines with no differentiation as to type could be approximated by direct measurement of the absorption of the extract or the broth.

Dr. N. R. Jones at the Torry Research Station in Aberdeen, Scotland, has developed a method for determining purines enzymically with xanthine oxidase. The procedure is designed to measure the freshness of fish, since it is known that the nucleotides are at their highest level in fresh fish decreasing as the fish ages.

Carnitine can be determined either as the periodide (J. S. Wall, D. C. Christianson, R. J. Dimler and F. R. Senti, Anal. Chem. 32, 870, (1960)) or with bismuth triiodide (A. E. Bender, T. Wood and J. A. Palgrave, J. Sci. Food Agr. 9, 812, (1958)). Incidently, the latter paper contains many useful procedures for the analysis of meat extracts.

During our telephone conversation, you expressed interest in thiolesters. Dr. Sasin at the Drexel Institute in Philadelphia, Penna., has prepared thiolesters of long chain fatty acids and possibly might have samples available. Some of the references to his work are:

1. G. S. Sasin, J. Am. Oil Chemists' Soc. 39, 488 (1962).
2. G. S. Sasin, et al., J. Org. Chem. 24, 2022 (1959).
3. G. S. Sasin, et al., J. Org. Chem. 21, 852 (1956).

Details of the sulfide procedure we use are attached.

If you have any other questions, I shall be glad to try to answer them.

Good luck to you on chicken flavor.

Best regards to Dr. Pearson.

Sincerely yours,

S. J. Kazeniac

SJK/j

Inorganic Sulfide Determination
(A. E. Sands, M. A. Grafius, H. W. Wainwright and M. W. Wilson, Report of Investigations, R.I. 4547, United States Department of the Interior - Bureau of Mines, September 1949).

**Reagents:**

1. Zinc acetate solution - dissolve 20 g. zinc $(CH_3COO)_2$-$2H_2O$ in 500 ml. water. Add a few drops of glacial acetic acid to prevent precipitation of insoluble zinc salts. Precipitation may occur, if ammonia is present at high levels in solution being analyzed, but this does not seem to affect the results.

2. N,N-Dimethyl-p-phenylenediamine reagent - dissolve 150 mg. of N,N-dimethyl-p-phenylenediamine dihydrochloride in 150 ml. sulfuric acid (two volumes of concentrated sulfuric acid added to sone volume of water).

3. Ferric chloride reagent - dissolve 2.7 g. $FeCl_3$-$6H_2O$ in 100 ml. hydrochloric acid (one volume of concentrated hydrochloric acid in one volume of water).

**Procedure:**

Add 25 ml. of sample containing from 1-50 ppm sulfide to 25 ml. zinc acetate solution. Next add 5 ml. of the diamine reagent and stir. Then add 1 ml. of the ferric chloride solution, stir and allow blue color to develop for 15 minutes before reading absorbance at 745 mu. The curves attached are taken from the paper by Sands et al above.

We developed our own curve using sodium sulfide standard solutions. It checked closely with the reported values.

Method for preparation of derivatives: (2,4 DNPH)

I. Preparing 2,4 DNPH solution:

-to 0.4 g of 2,4-Dinitrophenylhydrazine in a 25 ml Erlenmeyer flask is added 2 ml. of concentrated $H_2SO$

-3 ml. of $H_2O$ is added dropwise with swirling or stirring until solution is complete

-10 ml of 95% ethanol is added to this solution

II. Preparing Carbonyl solution in ethanol:

dissolve 0.5 g of the compound in 20 ml of 95% ethanol

III. Making derivative:

the freshly prepared 2,4-DNPH in solution is added, and the resulting mixture is allowed to stand at room temperature, crystallization of the hydrozone usually occurs within 5-10 min. If no precipitate forms - mixture is allowed to stand overnight.