

Characteristics of Chicken Flavor-Containing Fraction Extracted from Raw Muscle

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Water extracts of ground, raw white and dark chicken muscle were dialyzed and taste tested. On heating, dialyzates produced significantly stronger chicken flavor than filtrates of non-dialyzable material. Dialyzates were fractionated using Sephadex G-25 and ultraviolet absorbance. The second of four fractions from both white- and dark-meat dialyzates produced, when heated, the chicken aroma and taste. The four fractions

were tested qualitatively for sugars, amino acids, amines, purine compounds, and sulfhydryls; pH values and infrared and ultraviolet spectra were determined. Flavor-forming fractions contained glucose, fructose, ribose, an unidentified sugar, lactic acid, amino acids (white 16; dark 11), amines, IMP, GMP, inosine, carbonyls, and sulfhydryls.

Recent experiments concerning the source of chicken flavor have investigated certain possible precursors and the chemical compounds derived from them under various physical treatments. Numerous tests have yielded divergent observations by which the characteristic flavor cannot as yet be fully explained.

Following the early discovery that chicken-flavor precursors are readily extracted from raw meat by cold water (Peterson, 1957; Pippen *et al.*, 1954; Pippen and Klose, 1955), several approaches have been taken toward establishing their identity. Analysis has been made of volatile carbonyl compounds formed during prolonged cooking of chicken meat and water with (Pippen *et al.*, 1958) and without (Pippen and Nonaka, 1960) air bubbling through the cooking mixture; numerous monocarbonyls and diacetyl-acetoin were identified. These workers postulated that diacetyl adds to the buttery note in the aroma of freshly cooked chicken (Pippen *et al.*, 1950). No specific role has been suggested for the monocarbonyls, although investigators (Kazeniak, 1961; Lineweaver and Pippen, 1961; Minor *et al.*, 1965; Pippen and Nonaka, 1963) emphasized their importance to chicken flavor.

Sugars (Lilyblade and Peterson, 1962), lactic acid, and amino acids (Miller and Dawson, 1965) have been determined in chicken meat and in broth (Kazeniak, 1961), but their specific relationship to flavor is uncertain. In experiments with beef, Hornstein and Crowe (1960) concluded that amino acids alone are not flavor precursors, but may interact with glucose and ribose in a Maillard reaction in the formation of meaty aroma. However, Wasserman and Gray (1965) report results suggesting that beef aroma could be produced without involving the Maillard reaction. Nucleotides, especially inosinic acid and inosine, have been judged to contribute "mouth satisfaction," to intensify flavors of other compounds (Kazeniak, 1961), and to impart a "meaty" flavor to beef (Wood, 1961) and to chicken (Chow, 1966).

Emphasis has been given to the contribution of sulfur compounds to chicken flavor. Volatile sulfide has been

determined (Pippen and Eyring, 1957) and its origin attributed to cystine-cysteine in protein and in glutathione (Mecchi *et al.*, 1964). Adding certain sulfhydryl compounds, particularly reduced glutathione, improved the flavor of chicken broths (Kazeniak, 1961). Comprehensive studies of sulfur compounds and other components of chicken flavor have recently been made by Minor *et al.* (1966), using model systems to combine glutathione, methionine, and 2,3-butanedione with sulfide, ammonia, carbonyl, lactate, and phosphate. Monosodium glutamate (MSG), disodium inosinate, and disodium guanylate were added as enhancers. A chicken-like flavor was observed after heating.

In the present study, water extracts, made separately from ground, raw dark muscles, were fractionated in two steps, by dialysis and gel filtration. Since, after exhaustive dialysis and concentration, chicken flavor was predominantly in the dialyzate, this fraction was further separated using Sephadex G-25. The resulting four fractions from each kind of muscle were tested for flavor and for presence of sugars, amino acids, amines, purine compounds, and sulfhydryls; spectra in the infrared and ultraviolet regions were determined. The over-all objective was to separate with minimum change and to characterize those fractions which were chickenlike in flavor.

Materials and Methods

Extraction and Fractionation. Chicken meat was obtained from standard flocks of the Washington State University Department of Animal Sciences. Breast, thigh, and leg meat from Leghorn hens was separated from skin, fat, bone, blood vessels, and tendons. White and dark muscles were ground separately. Portions of each were mixed with a volume of distilled water equal to three times the weight of the meat and stirred intermittently during 18 hours at 4° C. The pH of each meat-water mixture was measured. Each entire mixture was dialyzed against water at 4° C. in several portions with frequent agitation. Portions (200 ml.) were transferred to Visking Precision Cellulose C65 dialysis casing and suspended in 800 ml. of distilled water, which was replaced every 2 hours until five complete changes of water had been made. Approximately

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20 liters of dialyzate from white meat and the same from dark meat were obtained in each replication; these solutions were freeze-dried. Meat fiber was removed from the nondialyzable material by filtration in preparation for tests of pH and flavor. Freeze-dried dialyzates were reconstituted so that 1 ml. of dialyzate represented 4 grams of raw meat. The scheme for fractionation and analysis is shown in Figure 1.

Concentrated dialyzates were separated on a column of Sephadex G-25 (fine) using distilled water as eluant. Absorbance of the 110 3-ml. portions of eluate collected was determined at 280 and 260 $m\mu$ with a Beckman DU spectrophotometer. Four fractions, *A*, *B*, *C*, and *D*, were obtained; these were lyophilized to increase concentration.

Sensory Tests. Aroma and taste tests were carried out by experienced tasters at four stages of fractionation and analysis: extracted meat fiber *vs.* untreated ground meat; dialyzates *vs.* nondialyzable filtrates; individually on the fractions from gel filtration; and finally on recombinations of these fractions from both white and dark meats.

Extracted and untreated meats were baked in foil-covered beakers and submitted as paired comparisons to 10 tasters. Twenty comparisons were made of each pair.

Twelve tasters compared dialyzates with filtrates of nondialyzable material from white and dark meat extracts. Comparisons were made of aroma and taste before and after heating the dialyzates or filtrates just to boiling temperature.

A laboratory panel of two to 10 tasters was used to identify the presence or absence of characteristic chicken flavor in fractions obtained from gel filtration. The small quantity of certain fractions limited the size of these panels. Aroma and taste of the fractions were judged before and after heating to boiling. To determine possible effects of the gel filtration process on kind and proportion of components, equal aliquots of the unheated fractions from white meat and from dark meat were recombined, heated, and evaluated in the same way as the fractions.

Chemical Tests of Fractions. Sugars and lactic acid were identified using descending paper chromatography on Whatman No. 1 paper with 1-butanol-acetic acid-water (250:60:250) as solvent and ammoniacal silver nitrate as spray to detect spots (Block *et al.*, 1958). Comparisons were made with standard solutions of known sugars.

Amino acids were determined by two-dimensional paper chromatography (Block *et al.*, 1958) on Whatman No. 1 paper using butanol-acetic acid-water (250:60:250) followed by phenol-water-NH₄OH (100:20:0.1). Amino compounds were located by dipping in 0.25% ninhydrin in acetone and comparing with standard spots of 18 known amino acids. Further identification was made using Ehrlich's reagent (Smith, 1953) for tryptophan, 1-nitroso-2-naphthol test (Acher and Crocker, 1952) for tyrosine, and Sakaguchi reagent (Jepson and Smith, 1953) for arginine. Presence of amines was confirmed by the *p*-dimethylaminobenzaldehyde test (Feigl, 1956).

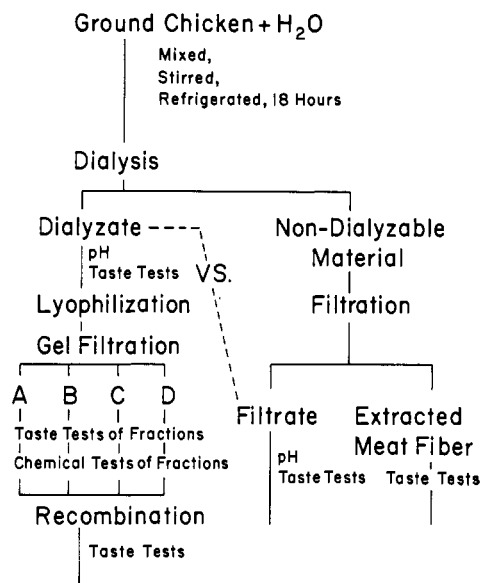


Figure 1. Scheme for fractionation and analysis

The biuret test (stable biuret reagent, Hycel No. 201) and the sodium nitroprusside test (Anson, 1941) were applied to the fractions from gel filtration.

Purine substances were separated by paper chromatography using butanol-acetic acid-water (65:15:25). Compounds were located by their absorption of ultraviolet light at 2537 Å. and were identified by their absorption spectra in the ultraviolet region (Beaven *et al.*, 1955) and by treating with Wood's silver nitrate-bromophenol blue reagent (Wood, 1955).

The pH of fractions was measured with a Beckman Model H-2 pH meter.

The *A* and *B* fractions were observed for electrophoretic movement using Sephaphore III in a Gelman electrophoresis chamber No. 51170.

Infrared and ultraviolet spectra of certain of the fractions were obtained and interpreted by the Chemistry Section, College of Engineering Research Division. Ultraviolet spectra were recorded with a Beckman DK-1 spectrophotometer. Infrared spectra were determined with a Perkin-Elmer Model 21 spectrophotometer after evaporating the fraction on a silver chloride window.

Results and Discussion

Location of Chicken Flavor. Taste panel comparisons of extracted meat fiber (Figure 1) *vs.* untreated chicken meat showed that both white and dark meats had lost their characteristic flavor through the process of water extraction. Extracted meat fiber was described as "flat," "tasteless," and "strawlike."

In taste comparisons of unheated dialyzate with filtrate from nondialyzable material (Figure 1), all samples were judged to have similar "raw meat" and "salty" tastes. But after white and dark meat dialyzates were heated, they had significantly ($P < 0.05$) more chicken flavor than filtrates from nondialyzable portions. Hence, dialyzates were fractionated and analyzed as

most promising sources of flavor components.

Flavor tests of fractions from gel filtration were made by the sniffing technique for aroma detection and by critical evaluation of taste responses. Each fraction was tested before and after heating to boiling. Results are summarized in Table I. The characteristic chicken flavor was found in white-meat *B* and in dark-meat *B*, although somewhat less pronounced in dark-meat *B*. No chicken flavor was detected in any of the other fractions.

The recombination of unheated white-meat fractions was judged to have a meaty odor slightly characteristic of chicken and to taste like chicken, but the unheated dark-meat recombination was described as having a "brothy," "meaty," and "sulfury" odor and taste not recognizable as "chicken." After heating to boiling, the white-meat recombination smelled and tasted strongly of chicken. There was no perceptible chicken flavor in the recombined dark-meat sample although it was described as being "brothy," "rich," "meaty," and "sulfury." The lack of detectable chicken flavor in the recombined sample of dark meat could have been due to dilution of flavor components or to change in their relative proportions during fractionation and reconstitution. Minor *et al.* (1966), however, point out that chicken leg muscle belongs organoleptically in the red-meat category, having a pronounced beef-like odor when mixed with water and warmed. Consequently, a predominance of "meaty, brothy" characteristics would be expected.

Chemical Analysis of Fractions. Results of tests of fractions separated from white- and dark-meat extracts by gel filtration are summarized in Table II. Amino acids identified are listed in Table III. These tables indicate differences in chemical composition which may aid in explaining the characteristic chicken flavor in the *B* fractions.

Both white and dark *B* fractions contained glucose, fructose, ribose, and lactic acid. White *B* had another (unidentified) sugar with an R_f value greater than that of ribose. Amino acids varied between white- and dark-meat fractions, there being 16 in white *B* and 11 in dark *B*. Both fractions contained alanine, aspartic acid, cysteine, glutamic acid, glycine, histidine, lysine, methionine, serine, taurine, tryptophan, and one unidentified ninhydrin-positive compound. White *B* had arginine, leucine-isoleucine, threonine, tyrosine, valine, and two unidentified amino substances not found in dark *B*. This variation in amino acid content may contribute to the difference in intensity of chicken flavor between white and dark *B* fractions. Kazeniak (1961) found that adding arginine, lysine, and glutamic acid to chicken broth improved the over-all flavor. He noted that arginine has a somewhat meaty taste and adds to mouth satisfaction. Flavor effects of the several amino acids identified in the present study are being explored at recognition threshold levels.

Although carbohydrates and amino acids undoubtedly contribute to the total taste of chicken meat, they have not been implicated as directly responsible for the specific chicken flavor. Wood (1961) and Hornstein and Crowe (1960) have suggested that glucose and ribose may interact with amino acids in a Maillard reaction to produce a meaty aroma. Since these compounds were present in the white and dark *B* fractions, such a reaction may have been responsible for at least the "meaty," "brothy" notes, if not for the characteristic chicken flavor. Lactic acid, noted by Kazeniak (1961) to be a major constituent of chicken broth, was found by him to improve the taste of chicken broth when added with arginine and lysine. Minor *et al.* (1966) used lactic acid in a model system which produced a chickenlike brothy taste. The lactic acid in the flavorful fractions in this study no doubt contributes

Table I. Flavor Descriptions of Fractions from Gel Filtration before and after Heating

Fraction	Odor		Taste	
	Before heating	After heating	Before heating	After heating
White meat				
<i>A</i>	Sl. buttery	Sweet, sl. meaty	Sl. buttery, astringent	Very bitter, haylike
<i>B</i>	Chickenlike	Chicken	Chicken, sulfury	Strong chicken, salty, sl. astringent
<i>C</i>	None	None	None	Sl. astringent
<i>D</i>	None	Sl. fragrant	Sl. "chemical"	Sl. astringent
Dark meat				
<i>A</i>	Sl. sweet	Meaty, sweet	Salty, metallic, very bitter	Salty, acid, very bitter
<i>B</i>	Brothy, meaty	Sweet, meaty	Bloody, chickenlike, sulfury	Chicken, full, meaty, rich, salty
<i>C</i>	Acid	Sl. sweet	Sl. sulfury	Sl. astringent, sl. sulfury
<i>D</i>	Sl. ammonia, "chemical"	Sl. sweet, rubbery	Sl. "chemical"	Sl. "chemical"

Table II. Analysis of Fractions Obtained from White and Dark Chicken-Meat Extract through Dialysis and Gel Filtration

	White Meat				Dark Meat			
	A	B	C	D	A	B	C	D
Chicken flavor	—	++	—	—	—	+	—	—
Carbohydrates ^a								
Glucose	+	+	—	—	+	+	—	—
Fructose	+	+	—	—	+	+	—	—
Ribose	—	+	—	—	—	+	—	—
Inositol	+	—	—	—	+	—	—	—
Unidentified sugar substance	+	+	—	—	+	—	—	—
Lactic acid	—	+	—	—	—	+	—	—
Amino acids ^a	9 ^f	16	6	0	14	11	2	0
Other ninhydrin-positive substances	3 ^f	4	0	0	2	3	0	0
Amines ^b	+	+	—	—	+	+	—	—
Purine substances ^c								
Hypoxanthine ^d	—	—	—	+	—	—	+	+
Inosine ^d	—	+	+	—	—	+	+	—
IMP	+	+	—	—	+	+	—	—
GMP	+	+	—	—	+	+	—	—
Unidentified ultraviolet-absorbers	2 ^f	2	0	0	2	1	0	0
Carbonyls								
Infrared	—	+	^g	^g	—	^g	^g	^g
Ultraviolet	—	+	+	+	—	+	+	+
Sulphydryl ^e	+	+	—	—	+	+	—	—
Biuret test	+	+	—	—	+	—	—	—
Electrophoresis	—	—	^g	^g	—	—	^g	^g
pH Reading	4.8	5.8	7.3	6.8	4.8	7.2	7.6	7.4

^a Determined by paper chromatography. See Table III for complete list of amino acids found.

^b Determined by *p*-dimethylaminobenzaldehyde test (Feigl, 1956).

^c Determined by absorbance of ultraviolet light at 2537 Å.

^d Further identified by Wood's AgNO₃-bromophenol blue test (Wood, 1955).

^e Determined by sodium nitroprusside test (Anson, 1941).

^f Number of different compounds found.

^g Not tested.

to the over-all chicken flavor. Further sensory tests of lactic acid combined with other identified components might clarify its role in the total flavor.

Tests for sulphydryl groups were positive in both white and dark *B* fractions, although more strongly so in white *B* than dark *B*. White *B* gave a positive biuret test. Glutamic acid, cysteine, and glycine were identified in both *B* fractions. These observations would occur if glutathione were present. This tripeptide has been discovered to be a precursor of chicken aroma by Mecchi *et al.* (1964), to improve the flavor of chicken broth by Kazeniak (1961), and to produce a chickenlike taste when combined with other precursors in the model system of Minor *et al.* (1966).

A greater number of different purine substances was found in the *B* fractions, although degradation products of adenylic acid occurred progressively in all fractions (Table II). Both white *B* and dark *B* contained inosine monophosphate (IMP), guanosine monophosphate (GMP), inosine, and one unidentified ultraviolet-absorbing compound; white *B* had a second unidentified ultraviolet-absorbing spot. Batzer *et al.* (1962) and Landmann and Batzer (1966) reported that IMP

was necessary for the development of meat aroma in beef. Minor *et al.* (1966) described cooked chicken broth from both breast and leg muscles as having "fairly strong MSG and 5'-nucleotide effects." In experiments in this laboratory to clarify the role of 5'-nucleotides in chicken flavor, Chow (1966) discovered that adding IMP to chicken muscle tissue did not add to the chicken flavor but rather intensified the meaty taste. The present study, as well as other investigations reviewed, bears out the probable importance of these components in the complexity of chicken flavor. Hypoxanthine and inosine have not been implicated in chicken flavor, and Kazeniak (1961) points out that both have a bitter taste. Chow (1966) reported that hypoxanthine was much more bitter than inosine at the same molar concentration in water; these compounds are not "meaty" or "brothy."

The pH value of 5.8 for white-meat fraction *B* is the same as that reported for raw breast-muscle slurry by Minor *et al.* (1966), but the 7.2 value for dark-meat *B* is higher than the 6.1 they report for raw leg-muscle slurry. This difference may partially account for the less pronounced chicken flavor in dark *B*, since Bouthilet

Table III. Amino Acids Identified in Fractions from White- and Dark-Meat Chicken Extract

	White Meat				Dark Meat			
	A	B	C	D	A	B	C	D
Amino acids ^a	9 ^e	16	6	0	14	11	2	0
Alanine	—	+	+	—	+	+	—	—
Arginine ^b	+	+	—	—	+	—	—	—
Aspartic acid	+	+	+	—	+	+	—	—
Cysteine	+	+	+	—	+	+	+	—
Glutamic acid	+	+	+	—	+	+	—	—
Glycine	+	+	—	—	+	+	—	—
Histidine	—	+	—	—	+	+	—	—
Isoleucine-leucine	+	+	+	—	+	—	—	—
Lysine	+	+	—	—	+	+	—	—
Methionine	—	+	—	—	—	+	—	—
Phenylalanine	—	—	—	—	—	—	—	—
Serine	—	+	—	—	+	+	—	—
Threonine	—	+	—	—	+	—	—	—
Taurine	+	+	—	—	+	+	+	—
Tryptophan ^c	—	+	+	—	+	+	—	—
Tyrosine ^d	—	+	—	—	—	—	—	—
Valine	+	+	—	—	+	—	—	—
Other ninhydrin-positive substances	1 ^e	3	0	0	2	1	0	0

^a Located with ninhydrin.

^b Verified by Sakaguchi reagent (Jepson and Smith, 1953).

^c Verified by Ehrlich's reagent (Smith, 1953).

^d Verified by 1-nitroso-2-naphthol test (Acher and Crocker, 1952).

^e Number of different compounds found.

(1949) found that the pH of chicken-meat extracts markedly influenced their flavor. In his studies, pH 5.8 brought about strong chicken, 6.2 mild chicken, 6.8 meaty and slightly chicken, 7.0 weak and meaty, and 8.0 sulfury flavors in the broth. The quantity of lactic acid present would influence the pH and, hence, the flavor of the fractions. Therefore, it may be important to determine quantitative data on lactic acid.

Because chicken extracts were subjected to dialysis and gel filtration (Sephadex G-25), components of the white- and dark-meat fractions would be expected to include no intact proteins but only small peptides and other compounds of molecular weights well under 5000. Attempts at electrophoretic separation indicated no proteins present in any of the fractions. Precursor molecules are small in size.

Observations of absorption characteristics in the infrared and the ultraviolet regions were made of certain fractions. Analysis of infrared spectra from white-meat fraction B showed a weak carbonyl band at 5.90 microns, very weak aliphatic bands at 3.3 to 3.4 microns, and a fairly strong hydroxyl band at 2.9 to 3.0 microns. Analysis of dark B was not made because of insufficient sample. Ultraviolet spectra of white B and dark B showed like curves with peaks at 245 m μ and almost no absorbance above 285 m μ . These curves were different in shape from those of the A, C, and D fractions. Interpretation indicated presence of an aliphatic system with conjugated double bonds and carbonyl compounds. Carbonyls have, of course, been implicated in chicken flavor by many investigators.

Results of these experiments indicate that the major components contributing to chicken flavor are water-soluble and of relatively small size. Qualitative tests demonstrated the presence in the flavorful fractions of sugars and lactic acid, amino acids, nucleotides, sulfhydryls, and carbonyls. This study, confirming and extending the results of other investigators, bears out the probable importance of these components in the complexity of chicken flavor. Eventually, continuing research will determine the critical components and their mode of interaction to develop the characteristic chicken flavor.

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