Chicken Flavor Studies

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Chemical analyses were made on light and dark chicken muscle samples to determine the effect of prolonged cooking (50 hours at 180° F.) on the concentrations of protein (nitrogen), ether extract (fat), ash, moisture, creatine, creatinine, cystine, methionine, sulfhydryl (glutathione), and sulfide (inorganic). Acetoin-diacetyl and inosinic acid determinations were made on the cooked broth. Glutathione, methionine, and 2,3-butanedione, known precursors of chicken volatiles, were combined with sulfide, ammonia, carbonyl, lactate, and phosphate precursors in a model system, and after heating gave a chickenlike flavor. Monosodium glutamate, disodium inosinate, and disodium guanylate were used as enhancers. Taurine imparted a serumy character to the product, changing it from a flavor resembling broth from breast meat to that of broth prepared from leg muscle. Chemical spot and organoleptic tests were used for tentative identification of the reaction products from the model system. Compounds identified included acetaldehyde, amine(s), thiol(s), organic disulfide(s), and hydrogen sulfide. These findings are discussed in relation to Strecker degradation of amino acids, the decomposition of glutathione and methionine, and reactions between amino acids and carbonyls as influenced by the presence of the active carbonyl, 2,3-butanedione, and a reducing atmosphere of hydrogen sulfide and nitrogen.

The role of hydrogen sulfide (H_2S) in the aroma and flavor of cooked chicken has been studied by Sadikov and coworkers (*28*), Bouthilet (*4*), Pippen and Eyring (*25*), Lineweaver and Pippen (*19*), and Kazeniac (*15*). The origin of hydrogen sulfide in heated chicken muscle was elucidated by Mecchi, Pippen, and Lineweaver (*22*) who determined the relative amounts derived from protein, glutathione, and sulfur-containing amino acids.

Functional group analysis, a solubility classification method, and gas chromatography were utilized by Minor and coworkers (*23*) to show the importance of sulfur compounds in the volatile fraction of cooked chicken. Mecchi, Pippen, and Lineweaver predicted that flavoring material may result from the reaction of hydrogen sulfide with some of the carbonyls found in chicken (*22*). Kazeniac (*15*) summarized the possible flavor relations of various compounds in chicken broths and tabulated them according to their effect(s) on taste, aroma, body, and mouth satisfaction. The compounds studied included amino acids, peptides, proteins, carbohydrates, lipids, inorganic salts, nonamino acid nitrogen compounds, sulfides, carbonyls, ammonia, phosphates, lactates, and inosinic acid. Hornstein and Crowe (*12*) suggested that ammonium lactate may be an important factor in meat flavor.

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The present study was made in two distinct parts. First, raw frozen muscle, cooked broth, and cooked freeze-dried meat-broth samples were analyzed for nitrogen, protein, ether extract (fat), ash, moisture, creatine, creatinine, cystine, methionine, sulfhydryl groups, inorganic sulfide, acetoin (3-hydroxy-2-butanone), diacetyl (2,3-butanedione), inosinic acid, and pH in order to determine the effects of prolonged cooking (50 hours at 180° F.) on the concentrations of these constituents. Then three experiments using model systems in attempts to synthesize chicken broth flavor were made: sulfide and lactate formation; sulfide and lactate formation and glutathione decomposition; and sulfide and lactate formation, and glutathione and methionine decomposition. The effect of taste enhancers—specifically, monosodium glutamate (MSG), disodium inosinate (DSI), and disodium guanylate (DSG)—and the influence of creatine and taurine on flavor were noted.

Materials and Methods

Reagents. The reagents were obtained from commercial sources.

Deionized, Triple-Distilled Water. Distilled water was redistilled prior to its passage through an ion-exchange column. After removal of ions, the water was distilled again to a specific conductance of 0.8 to 2.0 μohms. This prepared water, hereinafter referred to simply as water, was used for all water requirements.

Raw Frozen Muscle Samples. Six frozen heavy fowl carcasses (average weight 6 pounds each) were sawed into halves. Further sawing gave a crude separation of light from dark meat. Skin and kidney fat were removed and discarded. Bones, tendons, and veins were cut away, giving a clean separation of light and dark muscle. Light and dark muscle samples were ground separately, mixed with the respective juices, mixed and weighed as individual 100-gram portions, packaged under vacuum in 1-inch Cryovac casings, labeled, quick-frozen at −30° F., and stored at −10° F. until used.

Cooked Freeze-Dried Meat-Broth Slurry. A slurry consisting of 1 kg. of muscle (light or dark) was made up using 1.5 liters of hot water, placed in a three-necked 6-liter flask, and cooked under reflux for 50 hours at 180° F. The flask contents were transferred to a 12 × 12 × 2 inch stainless steel tray, covered with aluminum foil, cooled, and frozen overnight at −10° F. The aluminum foil covering was removed, and the tray contents were freeze-dried in a Stokes Model 2003 F-2 freeze-dryer in 36 hours at a tray temperature of 140° F. and a vacuum of 75 to 100 microns of Hg. The friable freeze-dried material was placed in Cryovac bags, vacuum-packed, and stored at −10° F. until used.

Model Systems. The apparatus and procedures of Folkard and Joyce (*6*) and Self, Rolley, and Joyce (*32*) were used for the three model experiments.

Model System 1. One milligram of sodium sulfide was placed in a 1 × 6 cm. reagent trap (*6*) containing 3 ml. of water, and 0.5 ml. of lactic acid was added. The odor was noted, then 1 mg. of carbonyl phosphate was added to provide a simple source of organic phosphate and carbonyl radicals. Then the

solution in trap 1 was heated by immersing it in a water bath at 180° F.

MODEL SYSTEM 2. The same procedure was followed, except that 1 mg. of glutathione and 0.1 ml. of 2,3-butanedione were added. The pH was then adjusted to 7.9 with ammonium hydroxide, using a Beckman Model G pH meter prior to heating and purging, as described earlier.

MODEL SYSTEM 3. One milligram of methionine was added to the reagents used in model system 2 in order to make the third model system. After adjusting the pH to 7.9, the same procedure was followed. To provide material for chemical tests and organoleptic evaluations based on the addition of MSG, 5′-nucleotide enhancers, creatine, and taurine, two repeat runs were made. The probable reaction products of this model system are hydrogen sulfide, carbonyl sulfide, sodium sulfide, sodium phosphate, sodium lactate, ammonium sulfide, ammonium lactate, cysteine, glutamic acid, glycine, acetaldehyde, 2,3-butanedione, acetoin, amine(s), methionine, methanethiol, dimethyl sulfide, and other organic sulfides.

Chemical Tests on Model System 3. The sodium hypoiodide test of Cheronis and Entrikin (*5*) was used to test for acetaldehyde, and a ferricyanide reagent spot plate test (*5*) was used for amine(s) on aliquots from traps 1 and 2. A few drops of borohydride solution as a test for organic disulfides(s) as described by Stahl and Siggia (*35*) were added to 1 ml. of the reaction products solutions in traps 1 and 2 of model system 3. A methylene blue test for sulfides with *p*-phenylenediamine reagent according to the procedure of Marbach and Doty (*20*) was used. Mercaptans were tested for by adding 1 ml. of ethanolic NaOH and a few drops of 1-chloro-2,4-dinitrobenzene (*6*, *32*) to 2 ml. of the solutions containing the reaction products from traps 1 and 2 of model system 3. A hydrogen sulfide test was made on the effluent stream from trap 3 using a filter paper moistened with lead acetate solution (*5*).

Organoleptic Tests. A trained panel of seven experienced tasters was used to evaluate the flavor in the model system and control samples of cooked muscle broth.

Quantitative Chemical Analysis of Muscle Samples. Raw-frozen and cooked, freeze-dried meat-broth slurry samples were taken as 100-gram paired aliquots. Determinations were made by the methods summarized in Table I with the following exceptions:

Tissue samples for creatine and creatinine determinations were prepared for colorimetric analysis by the water extraction procedure of the AOAC (*1*).

Inosinic acid was determined by a semiquantitative spectrophotometric method as described by Kazeniac (*15*). After 50 hours' cooking and distillation of the muscle-water slurry, the flask was cooled and the fibrous material allowed to settle. A 50-ml. aliquot of nearly clear broth was pipetted off. The slight haze remaining therein was removed by adding spectrophotometric grade chloroform (v./v.) and centrifuging at 10,000 r.p.m. for ½ hour. Centrifugation served to concentrate the haze at the aqueous-chloroform layer interface. A sample of clear supernatant broth was pipetted into a cuvette and the ultraviolet absorption was read at 250 mμ on a Beckman DU-2400 spectrophotometer equipped with a hydrogen lamp (*15*). Values were based on a standard curve for inosinic acid constructed by plotting known concentrations against absorbance.

Table I. Analytical Methods for Quantitatively Determining Selected Chemical Constituents in Raw Frozen Muscle and Cooked, Freeze-Dried Meat-Broth Samples

Constituent	Method
Nitrogen (protein)	Benne, Van Hall, and Pearson (*3*)
Ether extract (fat)	AOAC (*1*)
Ash	AOAC (*1*)
Moisture	AOAC (*1*)
Creatine	Peters (*24*)
Creatinine	Peters (*24*)
Cystine	Henderson and Snell (*9*)
Methionine	Henderson and Snell (*9*)
Sulfhydryl	Grunert and Phillips (*7*) as modified by Batzer and Doty (*2*)
Sulfide (inorganic)	Sands and coworkers (*29*)
Acetoin-diacetyl	Prill and Hammer (*27*) as modified by Stotz and Raborg (*36*) and Pippen, Eyring, and Nonaka (*26*)
Inosinic acid	Heaven, Holiday, and Johnson (*8*) and Kazeniac (*15*)

Results

Chemical Analyses. The concentration of nitrogen, protein, fat, ash, creatine, creatinine, methionine, and sulfhydryl in raw frozen muscle and cooked, freeze-dried meat-broth samples is summarized in Table II. Acetoin, diacetyl, and inosinic acid determinations were made on the cooked broth only, and the cooked, freeze-dried meat-broth samples were not analyzed for acetoin-diacetyl or inosinic acid.

A comparison of the values for raw frozen light and dark muscle shows some noteworthy differences. For example, protein is higher in light than dark muscle (74.2 *vs.* 68.3%); fat is lower (18.2 *vs.* 22.2%); ash is lower (4.3 *vs.* 7.4%); moisture is lower (72.7 *vs.* 75.0%). Acetoin in light muscle broth is higher (161.2 *vs.* 60.4 μg. per 100 ml.) and inosinic acid is also higher (3.3 *vs.* 2.9 μmoles per gram); and finally, the pH is lower (5.8 *vs.* 6.1).

Flavor Studies on Broth Samples. Results for the organoleptic evaluation of the muscle broth samples (Table III) indicate similarities and differences in leg and breast muscle flavor.

On making up the raw muscle slurries from the frozen dark muscle samples, a pronounced beef-like 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 coworkers (*10–14*). Chicken leg muscle also belongs in this category according to its organoleptic characteristics and the presence of heme compounds. The latter were proved present by extracting leg muscle samples with water at 30° F. overnight.

Table II. Chemical Components of Raw Frozen Muscle, Cooked Broth, and Cooked, Freeze-Dried Meat-Broth Slurry

	Breast Muscle		Leg Muscle	
	Rfm[a]	Cfd-Mbs[b]	Rfm[a]	Cfd-Mbs[b]
Nitrogen,[c] %	11.9	14.0	10.9	13.8
Protein,[c] %	74.2	87.5	68.3	86.3
Fat,[c] %	18.2	7.2	22.2	8.6
Ash,[c] %	4.3	1.8	7.4	2.4
H_2O, %	72.7	1.6	75.0	1.1
Creatine,[c] %	1.6	1.13	1.4	0.86
Creatinine,[c] %	1.4	0.98	1.4	0.74
Cystine,[c] mg./g.	9.0	8.5	8.1	8.0
Methionine,[c] mg./g.	23.4	27.8	22.2	24.6
Sulfhydryl,[c] p.p.m.	133	233	286	300
Sulfide[c] (inorganic), p.p.m.	<1	<1	<1	<1
Acetoin,[d] μg./100 ml.	161.2	...	60.4	...
Diacetyl,[d] μg./100 ml.	8.8	...	62.4	...
Inosinic acid,[d] μmoles/g.	3.3	...	2.9	...
pH[d]	5.8	6.2	6.1	6.3

[a] Raw frozen muscle.
[b] Cooked, freeze-dried meat-broth slurry.
[c] Values expressed on moisture-free basis.
[d] Determinations made on broth after cooking.

Table III. Organoleptic Results for Muscle Broth Samples after 50 Hours' Cooking Distillation at 180° F. under Nitrogen

Sample	pH[a] Slurry	pH[a] Broth	Odor and Flavor
Breast	5.8	6.2	Rich, mouth-filling, chicken, sweet, fatty, sulfide aroma. Mouth-coating, strong MSG, and 5′-nucleotide effects
Leg	6.1	6.3	Bland, sweet (resembling pork fat rendering aroma). Serumy, brothy taste with fairly strong MSG and 5′-nucleotide effects

[a] Values for pH taken on both raw muscle-water slurry and cooked broth at 23° C.

Absorption maxima of 425 mμ for heme compounds and 675 mμ for metmyoglobin were obtained in the ultraviolet range using a Beckman DU spectrophotometer.

Broth prepared from breast muscle exhibited typical strong and characteristic chicken aroma, characterized by sweet, oily, and sulfide flavor notes.

Flavor Studies on Model Systems. The organoleptic results from all three model systems are summarized in Table IV.

Enhancers. Addition of 1 mg. of MSG to 2 ml. of the reaction products in trap 1 of model system 3 improved the taste. The synergistic effect of meat flavor and MSG had been suggested by Sjöström, Cairncross, and Caul (*33*). A further improvement was noted when 50 μg. of a 50/50 mixture of DSI and DSG (nucleotides) was added following the MSG addition as suggested by Kuninaka, Kibi, and Sakaguchi (*17*). Despite the flavor improvement achieved by using these enhancers, the flavor of the reaction products from model system 3 still did not constitute a true chicken broth flavor when compared by the taste panel to control broth samples prepared from light and dark muscle.

Addition of 1 mg. of taurine as suggested by Kazeniac (*15*) to the reaction products of model system 3 plus enhancers imparted a serumy taste, connoting broth from dark muscle. No perceptible change was noted when 0.25 mg. of creatine was then added as used by Kazeniac (*15*). The organoleptic comparison of dark and light muscle broth samples with the three model systems showed that true chicken broth flavor was not obtained with the most complex of the model systems—model system 3—even with the addition of enhancers. However, the flavor of model system 3 resembled light meat chicken broth and was improved by the addition of enhancers.

Chemical Identifications in Model System 3. Using the chemical methods given previously, positive tests were obtained for acetaldehyde, amine(s), thiol(s), sulfide(s), organic disulfide(s), and hydrogen sulfide.

Table IV. Organoleptic Results for Three Model Systems

System	pH Initial	pH Final	Odor and Flavor
1	4.5	6.8	Egglike odor from hydrogen sulfide; addition of carbamyl phosphate and heating gave odor similar to chicken volatiles
2	7.9	5.4	Chicken odor, egglike taste
3	7.9	4.7	Connotation of breast meat broth odor. Strong sulfide, oily butter-like aroma. An incomplete breast meat broth taste

Discussion

The results show conclusively that heated aqueous solutions of three of the constituents of chicken muscle—glutathione, methionine, and 2,3-butanedione—produce a chicken-like flavor resembling that of light chicken muscle. Furthermore, the addition of enhancers, which are indigenous to chicken muscle—namely, MSG, DSI, and DSG—enriches the flavor of these solutions. Subsequent addition of taurine imparts a serumy taste reminiscent of broth from dark chicken muscle or beef.

Numerous known chemical factors indigenous to chicken muscle were omitted. These included fatty acid, carbohydrate, protein, and nonprotein factors including hemoglobin, which is indigenous to dark meat(s). Accordingly, complete and true chicken broth flavor(s) could not be anticipated.

The number and complexity of the reactions required to produce the reaction products formed in model system 3 are not explicable by Strecker degradation alone (*30*, *31*). It is probable that as a result of the reducing atmosphere prevailing throughout the reaction period due to constant liberation of hydrogen sulfide from glutathione (*22*), and despite the presence of the active carbonyl, 2,3-butanedione (*31*), little or no reaction occurred between the carbonyl and amino acid constituents as suggested by Speck (*34*).

Kendall, Mason, and McKenzie (*16*) and Mason (*21*) have reported on the structure of glutathione and its spontaneous decomposition in an aqueous media. By using model systems, Self, Rolley, and Joyce (*32*) obtained methanethiol from methionine and H_2S from cystine and cysteine in an aqueous medium containing the active carbonyl, dehydroascorbic acid. However, Self, Rolley, and Joyce (*32*) did not detect any organic sulfides or disulfides in their investigation. Sulfide(s) and disulfide(s) were tentatively identified in the present study together with hydrogen sulfide, thiol(s), amine(s), and acetaldehyde. The presence of thiol(s) may be attributable to the nitrogen- and hydrogen sulfide–reducing atmosphere. Sulfide, disulfide, and amine formation may be the result of using 2,3-butanedione as the active carbonyl in model system 3 together with a more complex medium than those employed by other workers.

Further quantitative work is indicated to verify the tentative identifications of reaction products resulting from heating glutathione, methionine, and 2,3-butanedione together in a model system. Addition of carbohydrates as suggested by Lilyblade and Peterson (*18*) together with suitable fatty acid additions to the compounds used in model system 3, plus enhancement with MSG and 5′-nucleotides after heating, may provide a flavor medium more nearly approximating that of light muscle broth from chicken.

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TOMATO FLAVOR

Formation of Volatile Sulfur Compounds in Processed Tomato Products

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The rate and amount of hydrogen sulfide and methyl sulfide produced in canned tomatoes and canned tomato juice were determined when these tomato products were stripped at various temperatures and pressures. The hydrogen sulfide was measured by a colorimetric technique involving the formation of methylene blue. The methyl sulfide was absorbed in concentrated sulfuric acid. The acid was then diluted, and the methyl sulfide in the headspace above it was determined by gas liquid chromatography. At 100° C. and atmospheric pressure, 150 p.p.b. hydrogen sulfide and 2 to 6 p.p.m. of methyl sulfide formed in an hour. Commercially canned tomatoes and juices contained 1.6 to 7.9 p.p.m. methyl sulfide. These amounts are far above their aroma thresholds in water, and probably both compounds modify the over-all aroma of processed tomato products.

Papers reporting on tomato volatiles (*3, 14, 20, 27, 28, 31, 32*) do not list any sulfur compounds, but they do show the complexity of the volatile fractions of raw and processed tomatoes. Chromatographic techniques indicate that there are at least three dozen compounds, and possibly several dozen more remain undetected. At least two dozen compounds including alcohols, aldehydes, ketones, esters, and several terpene-type compounds have been identified (*3, 14, 20, 27, 28, 31, 32*).

Sulfur compounds, although not yet shown in tomato products, have been reported in the volatiles of many other processed foods including vegetables (*7, 10, 13, 30*), citrus fruits (*17*), milk (*11*), eggs (*18*), chickens (*4, 25*), salmon (*1*), beef (*30*), coffee (*30*), tea (*30*), wine (*36*), and beer (*5*). Most of the vegetables (*30*) contained four or more volatile sulfur compounds. Potatoes may contain 10 such compounds, and onion volatiles showed eight sulfur compounds (*7, 13*).

The human nose can detect some of these sulfur compounds at extremely low concentrations. For example, the olfactory thresholds of methyl mercaptan and methyl sulfide in water are 0.02 and 0.33 p.p.b., respectively (*12*). Because of these very low thresholds, such volatile sulfur compounds appear to be important components of food aromas. For instance, one panel detected 5 p.p.b. of added hydrogen sulfide in the aroma of beer (*5*), and another panel detected 125 p.p.b. of the same compound added to chicken broth and believed that it intensified the broth aroma (*22*).

The strong odor of organic sulfides was detected in the cold traps (−78° and −196° C.) used to trap stripped volatiles from canned tomato juice. Gas chromatographic analysis showed that the trap odor might be caused by 3 to 4 p.p.m. methyl sulfide in the tomato juice. This concentration of methyl sulfide would be ten thousand times its odor threshold in water (*12*) and, therefore, probably quite important in the aroma of tomato products. This paper reports which volatile sulfur compounds and how much were present in some tomato products.

Experimental Procedure

Canned Tomatoes and Tomato Juice. The canned juice or tomatoes, listed by variety name in the tables and figures, were prepared at this laboratory. Ripe tomatoes for juice were carefully washed, trimmed, and put through a pulper with 0.033-inch screen. This cold break juice was deaerated and sealed in 202 × 204 cans under 27 inches of Hg vacuum. The cans were processed from zero time to 80 minutes in boiling water and immediately cooled in ice water.

Four varieties of tomatoes were canned. Whole tomatoes were heated in boiling water for 2 minutes, cooled in tap water, peeled, cored, and quartered. After mixing, the quartered tomatoes were canned in juice from the same tomatoes, vacuum sealed in 303 × 406 cans, processed for periods from zero time to 90 minutes in boiling water, and cooled in ice water. The headspace from canned juice and tomato samples was analyzed by GLC and some samples, processed 60 minutes, were used to determine the rate and amount of hydrogen sulfide and methyl sulfide production when stripped at boiling temperatures ranging from 30° to 100° C. under pressures of 30 to 760 mm. of Hg (Figures 2, 3, and 4 and Table I).

The canned tomato products analyzed for Table II were from retail markets or from processors.

Stripping Apparatus. The apparatus shown in Figure 1, with modifications as required, was used to strip the volatiles from tomato juice or canned tomatoes. Stripping was accomplished by boiling at pressures ranging from 30 to 760 mm. of Hg, with nitrogen bled through the tomato product at 60 to