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

L. J. MINOR, A. M. PEARSON, L. E. DAWSON, and B. S. SCHWEIGERT

Department of Food Science, Michigan State University, East Lansing, Mich.

Carbonyls, organic sulfur compounds, and hydrogen sulfide were isolated from the volatile fraction resulting when 4.5 kg. of ground chicken from White Leghorn pullets was oxidatively cooked in 5 liters of water for 13 hours at 102° C. and distilled under nitrogen for 10 hours at 102° C. A yield of 1.8 grams of carbonyl-2,4-dinitrophenylhydrazones was obtained. Carbonyl compounds that were identified by three or more methods included derivatives of diacetyl, acetone, methyl ethyl ketone, and normal aliphatic aldehydes containing two, three, four, five, six, and eight carbon atoms. One 2,4-dien-1-al was also tentatively identified. The presence of hydrogen sulfide, mercaptan(s), and organic disulfide(s) in the volatile fraction was demonstrated by separation and identification as their lead derivatives. Results indicate that carbonyl and sulfur compounds are of major importance in chicken flavor.

One of the most important attributes of chicken meat is its characteristic aroma and flavor, which develop during the cooking process. In summarizing the status of research dealing with chicken flavor, Lineweaver and Pippen (*9*) reported that essentially 100% of the volatile fraction from cooked chicken could be accounted for by nitrogen, sulfur, and carbonyl compounds, with the latter two classes of compounds playing the major role in development of flavor.

A classical report on the carbonyl components of the volatile fraction of chicken cooked under different conditions of oxidation was published in two articles by Pippen and coworkers (*24*) and Pippen and Nonaka (*21*). These authors demonstrated that the amount and kind of carbonyl constituents were variable and dependent upon the extent of oxidation during cooking. The importance of volatile sulfur—namely, hydrogen sulfide—in chicken flavor volatiles was stressed by Sadikov and coworkers (*25*) and Bouthilet (*1–4*), who postulated that its presence was due to an unstable precursor. Recently Mecchi, Pippen, and Lineweaver (*14*) reported that the presence of hydrogen sulfide in heated chicken is largely due to protein decomposition and can be related directly to the cystine content of the muscle. Total sulfur and sulfide sulfur in chicken broth distillate were determined by Pippen and Eyring (*19*), but mercaptans were not found. The importance of ammonium sulfide to the odor and flavor of chicken broth has been demonstrated by Kazeniac (*8*) by studying the effects of additives on the sulfide, ammonia, and diacetyl concentrations in cooked volatiles obtained from dialyzed light chicken meat or in chicken broth. In a recent study, Pippen and Nonaka (*22*) showed by gas chromatography that *n*-hexanal and *n*-2,4-decadienal are present in fresh chicken at low concentrations, but are undesirable at the higher concentrations indigenous to rancid chicken.

The purposes of the present study were to confirm the findings of Pippen and coworkers (*24*) with reference to characterization of the volatile carbonyl compounds of cooked chicken obtained by air entrainment and to determine the nature of sulfur compounds present in the volatile fraction.

Materials and Methods

A total of 9 pounds of ground chicken from seven White Leghorn pullets was prepared for cooking by the method described by Minor and coworkers (*16*).

Cooking and Distillation. The apparatus used for cooking and distillation has been described (*16*). After 4.5 kg. of ground chicken had been macerated with 5 liters of deionized-distilled water, prewarmed to 50° C., the mixture was introduced into the 12-liter flask and cooked under reflux at 102° C. for 13 hours. "Oxidation favoring conditions" were maintained by passing air through the system at a rate of 400 ml. per minute (*24*).

Collection of Volatile Compounds. A simple modification of the cooking and distillation apparatus was made by replacing the cold traps attached to the distillate condenser with a train of three 2-liter Erlenmeyer flasks made up as absorption traps using glass to glass fittings throughout. The first trap contained 1 liter of saturated lead acetate solution. The second trap contained 1 liter of 2,4-dinitrophenylhydrazine (2,4-DNP) solution consisting of 2 grams of 2,4-DNP per liter in 2*N* HCl. The third trap also contained 1 liter of saturated lead acetate solution to minimize the escape of sulfur-containing compounds.

After 13 hours of oxidative cooking at 102° C., the absorption traps were connected and volatiles were distilled from the slurry by purging the system with nitrogen gas at a flow rate of 200 ml. per minute for 10 hours, while maintaining the slurry at 102° C. Cold water was maintained in the reflux condenser to preclude steam distillation. Upon completion of the run, the 2,4-dinitrophenylhydrazones (2,4-DNPHS) and lead derivatives were filtered, washed with water, dried at 60° C. under 5 inches of vacuum, and stored over Drierite, in a desiccator for analysis.

Fractionation and Identification of 2,4-DNPHS. A gross separation of mono- and polycarbonyl components was made as suggested by Pippen and coworkers (*24*) according to the method of Malmberg (*11*), using hot alcohol and chloroform. The polycarbonyl residue was recrystallized from hot nitrobenzene and the crude monocarbonyl-2,4-DNPHS were separated by column chromatography.

Column Chromatography of Monocarbonyl-2,4-DNPHS. The monocarbonyl fraction was separated on eleven 35-mm.-O.D. glass columns packed with silicic acid–Celite to a height of 75 cm. by the method of Gordon and coworkers (*6*) as modified by Pippen and coworkers (*20*, *24*). Chloroform was used to dissolve the monocarbonyl-2,4-DNPH mixture and transfer it to the column. For development of the bands a solution consisting of 2% ethyl ether in 98% petroleum ether and having a boiling range of 50° to 60° C. was used. The concentration of ethyl ether in the de-

veloper was increased to 20% to elute the slower-moving bands. However, the last two bands were recovered from the adsorbent by extraction with chloroform after removal of the absorbent from the column. Corresponding fractions from the individual columns were combined and rechromatographed until bands either remained intact upon elution or were too small to recover. Fractions of sufficient size and purity were characterized by the methods of Pippen *et al.* (*24*) and Nonaka, Pippen, and Bailey (*18*). Smaller fractions were separated by paper chromatography by the procedure of Lynn, Steele, and Staple (*10*) using 45 × 4 cm. strips of Whatman No. 1 paper.

The mobility, R_h, was checked by means of a reference compound, heptanal-2,4-DNPH (*17*). Melting points were determined with a Thiele apparatus as modified by Hershberg (*7*) using a silicone-oil bath (*15*). Results were checked by precalibrating an electrically heated stage with a series of known authentic carbonyl-2,4-DNPHS (*23*), and crystallographic comparisons were made by the method of Pippen and coworkers (*24*). Direct ultraviolet spectrophotometric examinations of spots obtained by chromatography of 2,4-DNPHS were made according to Nonaka, Pippen, and Bailey (*18*). Infrared spectra of chloroform solutions of 2,4-DNPHS made up in KBr disks were obtained using the procedure of Pippen and coworkers (*24*).

A total of 20 authentic carbonyl-2,4-DNPHS was prepared for use in this study according to the method of Pippen and Nonaka (*23*). The purity of each was evaluated by melting point, ultraviolet, infrared, paper chromatography, and microscopy (*24*). These were then used as standards for identification of the unknowns.

Separation and Identification of Sulfur-Containing Volatiles. Sulfur compounds in the cooked volatile fraction were recovered as their lead derivatives. Since large amounts of black precipitate were formed, sufficient lead acetate reagent was added to assure an excess of reagent. The black precipitates were filtered, washed, and dried. Sulfur volatiles were released from 0.2-gram aliquots of dry precipitate by adding 2 ml. of 8*N* HCl in a 1 × 4 cm. reaction trap (*5*). Tests for disulfides and mercaptans were made using absorption trains and reagents as described by Folkard and Joyce (*5*). For mercaptans, absorption traps containing mercuric chloride solution (3% w./v.) and mercuric cyanide solutions (4% w./v.) were used in sequence. For disulfides, mercuric cyanide (4% w./v.) and mercuric chloride (3% w./v.) or mercuric acetate (4% w./v.) were used in sequence. Tests for hydrogen sulfide and methyl mercaptan were made by the method of Marbach and Doty (*12*) as modified by Martin and coworkers (*13*) using bismuth nitrate reagent and mercuric acetate solution in sequence.

Results and Discussion

A yield of 1.8 grams of crude carbonyl-2,4-DNPH precipitate was obtained from 4.5 kg. of ground chicken after 13 hours of oxidative cooking at 102° C. and 10 hours of distillation at 102° C. under "oxidation-inhibiting conditions." By solvent treatment as outlined earlier, 0.15 gram of polycarbonyl derivative(s) was obtained. A violet color, characteristic of bis-2,4-DNPHS having adjacent carbonyl groups, developed when an aliquot of washed precipitate was tested with alcoholic KOH (*17*), whereas a red color characteristic of monocarbonyls was obtained upon testing an aliquot of the filtrate. Tests on the polycarbonyl fraction including infrared melting point determinations, and microscopic comparisons of the crystals with those of authentic diacetyl-bis-2,4-DNPH (Table I) showed them to be identical. Other small bands, eluted from the columns after acetaldehyde-2,4-DNPH, gave a blue-violet color characteristic of polycarbonyls having adjacent carbonyl groups (*17, 24*).

Table I shows the results obtained by column chromatographic separation of the monocarbonyl-2,4-DNPH fraction. Paper chromatography used as a method of characterizing the components gave the results shown in Table II. Difficulty was experienced in separating fraction 5 (*n*-butanal and methyl ethyl ketone) by column chromatography (*6, 24*). Microscopic examination showed the purified crystals to be a mixture of *n*-butanal and methyl ethyl ketone. Separations were then made by paper chromatography using the method of Lynn, Steele, and Staple (*10*). After taking ultraviolet readings (*18*), the dried spots were washed repeatedly with deionized distilled water to remove the phenoxyethanol prior to chloroform extraction and subsequent alcohol recrystallization of the hydrazones. Infrared spectra of the hydrazones and melting points of the crystals then matched those of authentic derivatives of *n*-butanal and methyl ethyl ketone.

Eight monocarbonyl-2,4-DNPHS, including acetaldehyde, acetone, *n*-propanal, *n*-butanal, methyl ethyl ketone, *n*-pentanal, *n*-hexanal, and *n*-octanal, were identified by three or more criteria. In addition, one polycarbonyl (diacetyl) was identified and one monocarbonyl (2,4-dien-1-al) was tentatively identified to make a total of 10 compounds characterized. Two additional monocarbonyl fractions and one polycarbonyl fraction were separated, but could not be characterized. Thus the present study in a limited manner confirmed the earlier results of Pippen and coworkers (*24*), who identified 17 monocarbonyls and one polycarbonyl for a total of 18.

Sulfur Compounds. Sulfur derivatives from the cooked volatile fraction formed in prolific amounts as black precipitates in the reagent traps. Their formation was first noted within one-half hour after cooking-distillation at 102° C. began and continued unabated throughout the entire 23-hour cooking-distillation period. This same phenomenon was noted earlier by Yueh and Strong (*27*) in work on cooked beef volatiles and by Mecchi, Pippen, and Lineweaver (*14*) in the liberation of hydrogen sulfide from heated chicken muscle.

Mercaptan Test. A positive mercaptan test was obtained in the form of

Table I. Properties of Carbonyl-2,4-DNPHS from Cooked Chicken Volatiles

Fraction Number	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, D, 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, D, E
5	106–110	Mixture of methyl ethyl ketone and *n*-butanal	...	...	D, H, F
6	154–158	*n*-Propanal	155–157	154–158	A, D, E
7	124–126	Acetone	125–127	124–127	B, D, E
8	164–168	Acetaldehyde	168–169	164–168	A, E, F
9	94–95	Unknown	...	...	...
Polycarbonyl	...	Unknown	...	...	G
Polycarbonyl	*d* above 300	Diacetyl	*d* above 300	*d* above 300	E, F, G

A. Shows ultraviolet absorption characteristics of *n*-alkanal derivative.
B. Shows ultraviolet absorption characteristics of 2,2-alkanone derivative.
C. Shows ultraviolet absorption characteristics of 2,4-dien-1-al derivative.
D. Migration on paper chromatograms checked with authentic samples.
E. Infrared spectrum identical to that of authentic sample.
F. Microscopic examination of crystals with polarizing microscope showed that crystals were alike with respect to refractive indices and phase transformations.
G. Unknown exhibited blue-violet color in alkaline ethanol.
H. Infrared spectrum matched that of mixture of authentic *n*-butanal and methyl ethyl ketone. Separate crystals were identifiable microscopically.

Table II. Paper Chromatography and Ultraviolet Absorption Maxima of 2,4-DNPH Derivatives

Column Band No.	R_f Unknown, Cm.	R_f Known, Cm.	R_h[a] Unknown, Cm.	R_h[a] Known, Cm.	UV Absorption Maxima, mμ	Carbonyl Compound Indicated
...	...	29.0	...	1.00	369	*n*-Heptanal (reference compound)
Forerun	...	...	...	...	...	Unidentified
1	35	33.0	1.21	1.14	370	*n*-Octanal
2	12.8	...	0.44	...	399	2,4-Dien-1-al
3	25.1	26.0	0.87	0.90	370	*n*-Hexanal
4	21.0	22.0	0.73	0.76	370	*n*-Pentanal
5	16.9	15.5	0.58	0.54	370	*n*-Butanal
6	27.3	27.5	0.94	0.95	374	Methyl ethyl ketone
7	13.1	12.0	0.45	0.41	370	*n*-Propanal
8	13.2	13.5	0.46	0.47	373	Acetone
9	10.3	9.0	0.35	0.31	368	Acetaldehyde
10	...	...	...	...	393	Unidentified

[a] R_h equals distance moved (R_f) by unknown or known 2,4-DNPH/distance moved (R_f) by *n*-heptanal–2,4-DNPH.

a light yellow precipitate of 2,4-dinitrophenyl thioether(s) by the method of Folkard and Joyce (*5*).

Sulfide Test. A positive test for hydrogen sulfide was obtained using Marbach and Doty's (*12*) method as modified by Martin and coworkers (*13*).

Test for Organic Disulfides. A positive test for organic disulfides was obtained by the sodium chloride precipitation procedure of Folkard and Joyce (*5*). Reduction of the disulfides to mercaptans by treatment with sodium borohydride (*26*) resulted in confirmation of the identification by formation of the 2,4-dinitrophenyl thioether(s).

Several workers (*3, 8, 14, 19, 25*) have recognized and stressed the importance of hydrogen sulfide and ammonium sulfide in chicken flavor. In the present study, formation of sulfur derivatives occurred more rapidly and prolifically than carbonyl derivatives, which were not noted as 2,4-DNPHS until at least 2 hours after cooking-distillation began. It is possible that condensation and adsorption of nonsulfur volatiles may have occurred in the lead acetate trap, thus reducing the yield and retarding the formation of 2,4-DNPHS during the first 1 to 2 hours of heating. It is also possible that conditions in the lead acetate trap could have resulted in artifact formation due to the interaction of hydrogen sulfide, carbonyls, and other volatiles. This possibility was not investigated and has not been proved. However, it would appear that organic sulfur compounds are the first odorous components to be obtained in quantity in the volatile fraction from cooked chicken, with the exception of hydrogen sulfide and ammonia, which have low boiling points. These results suggest that sulfur compounds, such as hydrogen sulfide, organic sulfides and disulfides, and mercaptans are important to chicken flavor *per se* or as precursors of compounds which may be formed in the vapor and/or liquid phases as products of interactions.

Speculatively speaking, carbonyls may play dual roles, serving as either desirable or undesirable flavor components, depending upon their concentrations in chicken broth or in the vapor phase from cooked chicken as reported by a number of investigators (*8, 9, 14, 19, 22*). Mecchi, Pippen, and Lineweaver (*14*) have postulated that the flavor effects may be due to interactions between hydrogen sulfide and carbonyls. The beneficial flavor of ammonium sulfide formed as the result of interactions between hydrogen sulfide and ammonia was reported earlier by Kazeniac (*8*). Hence, it would appear that as protein and amino acid decomposition in heated muscle progresses because of longer cooking periods, higher concentrations of low molecular weight organic compounds containing oxygen, nitrogen, or sulfur become available, thus favoring interactions between components in the liquid and vapor phases. The resulting volatile mixture of odorous compounds from muscle and fat may be detectable to the human nose at extremely low concentrations and thus contribute to the flavor and aroma of cooked chicken.

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