relatively large amounts (~100 ppm) and gives an intense signal, whereas manganese is present at the lowest concentration of all eight metals (see Table I). All the precision values are considered to be in an acceptable range.

SUMMARY

It has been demonstrated that the hydrolysis procedure proposed for the determination of calcium in orange juice can be used also for the determination of seven other metals by flame atomic absorption spectrometry. Concentration values are compared for eight commercial brands produced and sold in Florida.

LITERATURE CITED

Instrumentation Laboratory, Inc., AA Procedures Manual, Lexington, Mass., 1972.
Isaac, R. A., Johnson, W. C., *J. Assoc. Off. Anal. Chem.* **58**, 433 (1975).
McHard, J. A., Winefordner, J. D., Attaway, J. A., *J. Agric. Food Chem.* **24**, 41 (1976).

Received for review February 12, 1976. Accepted June 18, 1976. This research was supported by the Florida Citrus Commission and by AFOSR-F44620-76-C-005.

Identification of Some Volatile Compounds in Cooked Chicken

Robert J. Horvat

A fraction isolated from boiling chicken broth by continuous steam distillation and pentane extraction was analyzed by gas–liquid chromatography/mass spectrometry (GLC/MS) with both packed and open tubular columns. Some of the compounds separated by gas–liquid chromatography (GLC) were trapped and identified by mass spectrometry and infrared analysis. In addition, headspace vapor above boiling chicken broth was analyzed for gaseous compounds by gas–solid (GSC) chromatography. Of the 53 compounds identified, 30 had not been previously identified in cooked chicken. The 53 included sulfur compounds, aldehydes, alcohols, amines, acids, 2-alkylfurans, ketones, hydrocarbons (cyclic and acyclic), alkylbenzenes, a terpene, and a nitrile.

A recent review by Wilson and Katz (1972) of compounds isolated from cooked chicken meat revealed that 136 volatile compounds had been identified up to the time of its publication. Janney and coworkers (1974) in a subsequent publication reported identification of an additional six volatile compounds from fried chicken. Also, Harkes and Begemann (1974) identified 11 previously unreported unsaturated aldehydes from chicken broth. In spite of the large number of compounds identified from cooked chicken, no single compound or mixture of compounds having a cooked chicken aroma has been found. However, no serious attempt has been made to determine the aroma of mixtures of these compounds at various concentrations. Apparently the volatiles of cooked chicken constitute an exceedingly complex mixture of organic compounds and require further study.

Since the yield of volatile material from cooked chicken meat is quite low, gas–liquid chromatography/mass spectrometry (GLC/MS) was chosen as the principal analytical technique to give the maximum amount of information for identification of the components.

EXPERIMENTAL SECTION

Isolation of Meat Volatiles. Processed ice-packed ready-to-cook frying chickens weighing 2.5–3 lb were obtained locally. They were immediately bagged in polyethylene and stored at 4 °C until used. Meat from the leg, breast, and thigh was separated from skin, fat, and bone and then cut into small pieces (about 1-cm^3 cubes). One chicken yielded about 400–450 g of meat. Meat (400 g) was placed in a 3-l. round-bottomed flask and 425 ml of distilled water added. The flask was connected to a Likens and Nickerson-type (1964) steam distillation, continuous pentane extraction apparatus. Pentane and chicken broth were boiled for 8 h. After the apparatus had cooled, the flask containing the pentane, 120 ml, was removed. The extract was maintained at 40 °C and was concentrated to about 0.5 ml by blowing a gentle stream of high purity nitrogen on its surface. The pentane extract was further concentrated to about 50 μl by allowing it to stand at room temperature. This procedure undoubtedly favored the concentration of higher boiling point compounds and the loss of lower boiling point compounds.

Broth for Headspace Analysis. One frying chicken was partially thawed and cut up as described in the section Isolation of Meat Volatiles. The meat and 425 ml of distilled water were placed in a 3-l. three-necked flask. The flask was equipped with a thermometer for measuring broth temperature, a water cooled condenser, and a silicone rubber septum for withdrawal of headspace samples. Meat was boiled for 2 h, then the temperature of the broth was reduced to 72 °C and held constant for a 2-h period, during which headspace samples were withdrawn for GSC analysis.

Analytical Methods. In this research, the approaches used in an attempt to identify the volatile compounds from cooked chicken meat were: analysis of headspace vapors of chicken broth by GLC; GLC separation of components of the pentane extract and collection of fractions for mass spectrometric and infrared analysis, and GLC/MS analysis by use of a 500 ft × 0.03 in. i.d. open tubular column, and a 500 ft × 0.02 in. open tubular column, each coated with a different stationary phase, and a 12 ft × $^1/_8$ in. packed

Animal Products Utilization and Marketing Research Laboratory, Richard B. Russell Research Center, U.S. Department of Agriculture, Agricultural Research Service, Athens, Georgia 30604.

Table I. Volatile Compounds of Chicken Broth Identified by Gas-Solid Chromatography of Headspace Gas

Compound	Retention time of unknown compd[c]	Retention time of authentic compds[c]
Hydrogen sulfide[a]	13 min 42 s	13 min 38 s
Dimethyl sulfide[b]	3 min 26 s	3 min 22 s

[a] Gas chromatographic analyses for hydrogen sulfide were performed at a column temperature of 45 °C. [b] Gas chromatographic analyses for dimethyl sulfide were performed at a column temperature of 155 °C. [c] Retention times were reproducible to ±5 s.

column. Each approach is discussed below.

GSC Analysis of Headspace Vapor. Headspace samples (1–5 ml) for GSC analysis were withdrawn with a gas-tight syringe through a silicone rubber septum from the flask containing chicken broth at 72 °C. Gas–solid chromatographic analyses were made with an Aerograph 1520 gas chromatograph equipped with a thermal conductivity detector. A 6 ft × $^1/_8$ in. glass column packed with Porapak Q, 50–60 mesh, was used (Cook and Ross, 1972). The parameters were as follows: detector current, 150 mA; injector, 180 °C; detector, 200 °C; and helium flow rate, 16 ml/min. The column temperature was 48 °C for hydrogen sulfide and 155 °C for dimethyl sulfide. These compounds were identified by comparison of their retention times with those of standards on the Porapak Q column (Table I). Retention times of compounds were reproducible to ±5 s.

GLC Separation of Pentane Concentrate. The pentane extract was separated and fractions collected by use of a Varian Aerograph Model 1200 gas chromatograph equipped with a 5 to 1 effluent splitter (exit port/flame ionization detector). Both detector and injector were maintained at 220 °C. Column flow was 25 ml of helium per min. A 7 ft × $^1/_8$ in. stainless steel column packed with 10% Carbowax 20M on Chromosorb W, 80–100 mesh, was used for separation of the pentane extract. The column was kept at 35 °C for 7 min and then programmed at 4 °C per min to 200 °C. Effluent corresponding to peaks was collected in standard melting point tubes which were cooled by dry ice. The tubes were examined under a magnifying glass for the liquid condensate, then cut into sections about 6 mm long and the mass spectra of their contents were determined by means of the direct insertion probe in a Du Pont 21-490B mass spectrometer. The conditions for mass spectral analysis were: ion source temperature, 150 °C; ionizing voltage, 70 eV; scanning rate, 200 s per decade; and ion source pressure, 4×10^{-8} Torr. Infrared analyses were made with a Perkin-Elmer Model 237B infrared spectrophotometer equipped with a Barnes Engineering Company Model 128 beam condenser and a 2-μl cell. In some cases there was not enough material for infrared analysis. Fractions were identified by comparison of their infrared and mass spectra with spectra of standard compounds. Also, retention times of the unknown compounds on the Carbowax 20M column were compared with those of standard compounds. The compounds identified are shown in Table II.

GLC/MS of the Pentane Concentrate with a Packed Column. Gas chromatographic–mass spectral analyses were made with a modified Varian Aerograph Model 1200 gas chromatograph interfaced by means of a glass jet separator with a Du Pont 21-490B mass spectrometer. Sixty percent of the column effluent was diverted to the jet separator and the remainder to the flame ionization detector of the gas chromatograph. Gas chromatographic conditions were: detector and injector maintained at 210 °C; helium flow at exit of column, 28 ml/min; and column programmed from 70 to 190 °C at 4 °C per min. A 12 ft × $^1/_8$ in. stainless steel column packed with 10% EGSS-X on Chromosorb W, 80–100 mesh, was used to separate the components of the pentane extract for mass spectroscopic analysis. A 1–2-μl sample of pentane concentrate was used for each GLC/MS analysis. Mass spectroscopic conditions were: glass jet separator temperature, 250 °C; ion source temperature, 150 °C; ionizing voltage, 70 eV; scan rate, 10 s per decade; and ion source pressure, 4×10^{-7} Torr. Compounds were identified by comparison of their mass spectra with those of known standards, and by comparison of their GLC retention times with those of known standards. A compound is generally considered to be identified when its mass spectrum and GLC retention time agree with those of a known standard. Compounds identified solely on the basis of a comparison of their mass spectra with standards in the literature are designated "tentatively identified".

Table II. Volatile Compounds of Chicken Broth Identified by Collection of Fractions from a 7 ft × $^1/_8$ in. Stainless Steel Column Packed with Carbowax 20M

Compound	Methods of identification[a]		
	Retention time	MS	Ir
2-Methylthiophene	+	+	+
Myristic acid	+	+	+
Palmitic acid	+	+	

[a] A plus sign indicates that values of any one of the three analytical methods used (GLC, MS, and ir) from an unknown and known compound were in essential agreement.

GLC/MS of the Pentane Concentrate with a Silicone Oil Coated Open Tubular Column. A Perkin-Elmer Model 900 gas chromatograph, interfaced by means of an effluent splitter with a Du Pont 21-490B mass spectrometer, was used. Forty percent of the effluent from the GC column was introduced directly into the ion source of the mass spectrometer and 60% to the chromatograph's flame ionization detector. The 500 ft × 0.03 in. i.d. open tubular column was coated with General Electric SF 96 (50) Silicone oil plus 1% by weight Igepal Co 880. For each GLC/MS analysis, 0.8–1.0 μl of concentrate was used. Conditions used for the gas chromatograph were: helium flow rate, 8 ml per min; injector, manifold and flame ionization detector at 200 °C; and column programming from 35 to 180 °C at a rate of 1.5 °C per min. Mass spectrometer conditions were: ion source temperature, 150 °C; scan rate, 10 s per decade; ionizing voltage, 70 eV; and ion source pressure, 4×10^{-7} Torr. The basis for the identification of compounds in the pentane extract was described above in the section GLC/MS of the Pentane Extract with a Packed Column. To determine whether any of the compounds identified in this phase of the research were artifacts in the pentane, 120 ml of pentane was concentrated to 50 μl by the procedure described in the Isolation of Meat Volatiles section. GLC/MS analysis of this concentrate established only the presence of isopentane, cyclopentane, 1-pentene, and pentane. Purity of the pentane used was greater than 98% based on GLC peak areas.

GLC/MS of the Pentane Concentrate with a Carbowax 20M Coated Open Tubular Column. Gas–liquid chromatographic/mass spectrometric analysis was made on the pentane concentrate by use of a 500 ft × 0.02 in. i.d. open tubular column coated with Carbowax 20M. The

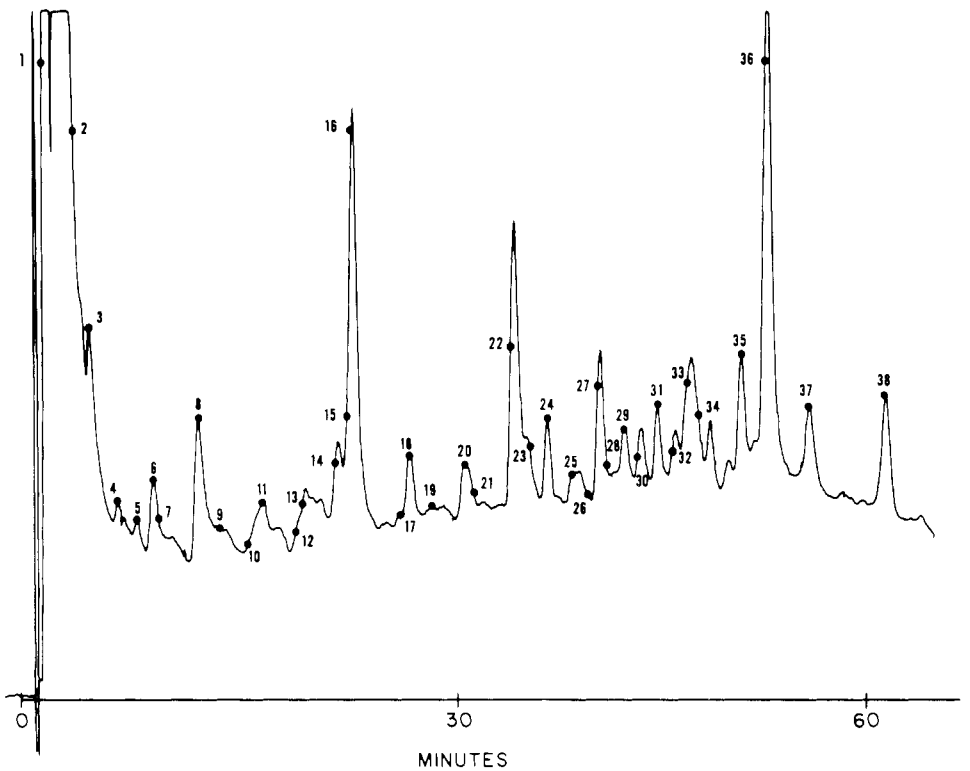

Figure 1. Chromatogram of pentane concentrate on 12 ft × 1/8 in. stainless steel column packed with 10% EGSS-X on Chromosorb W and programmed from 75 to 190 °C at 4 °C/min.

gas chromatographic and mass spectral conditions were the same as described in the section GLC/MS of Pentane Concentrate with a Silicone Coated Open Tubular Column, except that the column flow rate was 6.8 ml/min.

RESULTS AND DISCUSSION

Isolation of Meat Volatiles. In the judgment of several observers, the pentane extract, when removed from the Likens–Nickerson apparatus (1964), had a pleasant cooked chicken aroma. After concentration of the fraction, however, the odor became acrid.

GSC Analysis of Headspace Vapor. Gas–solid chromatographic analysis of headspace gas from chicken broth was included in these studies, because gases and other low boiling compounds are generally lost or their levels greatly reduced during concentration of volatiles as practiced here. Porapak Q solid support was used for analysis of the gaseous compounds from chicken broth since it has been established that this packing could separate a wide range of gases, and in addition it has been used successfully for analysis of hydrogen sulfide in the presence of water vapor (Cook and Ross, 1972). A glass column with on-column injection was used to minimize contact of the sample with hot metal surfaces which might react with sulfur compounds. However, only two compounds, hydrogen sulfide and dimethyl sulfide, were identified by a comparison of their retention times with those of known standards (Table I). Both of these compounds have been previously identified in cooked chicken meat (Wilson and Katz, 1972). Failure to find other low boiling compounds was probably due to the GC parameters and to the relative insensitivity of the thermal conductivity detector used.

GLC Separation of the Pentane Concentrate. From the fractions that were collected from the pentane concentrate 2-methylthiophene, myristic acid, and palmitic acid (Table II) were identified by comparison of their mass spectra and GLC retention times to those of authentic compounds. Although the two acids had not been previously identified in cooked chicken broth, their presence was not surprising, since both are constituents of chicken lipids. These lipids might have been hydrolyzed during the 8 h required for preparation of a broth extract.

Table III. Volatile Compounds of Chicken Broth Identified by GLC/MS with a 12 ft × 1/8 in. Stainless Steel Column Packed with 10% EGSS-X on Chromosorb W

GC peak	Compound	Retention time[a]	MS identification[b]
16	Undecyl cyanide	+	Positive
17	1-Dodecene	+	Positive
18	1-Tetradecene	+	Positive
20	1-Pentadecene	+	Positive
23	1-Hexadecene	+	Positive
24	2-Pentadecalone	+	Positive
27	Palmitoaldehyde	+	Tentative
38	1-Octadecene	+	Positive

[a] The plus sign indicates that retention times of known and unknown compounds are the same within the precision of these measurements. [b] Tentative refers to compounds which were identified solely on the basis of a comparison of their mass spectra with those recorded in the literature. Positive refers to compounds which were identified by comparison of their retention times and mass spectra to those of known compounds.

GLC/MS of the Pentane Concentrate with a Packed Column. A typical chromatogram (Figure 1) of the pentane concentrate showed 27 peaks. The numbered dots on the peaks in the GLC chromatograms represent data points where MS scans were obtained. Seven compounds (Table III), which had not been previously reported in cooked chicken broth, were identified. They were 1-dodecene, 1-tetradecene, 1-pentadecene, 1-hexadecene, 1-octadecene, 2-pentadecalone, and undecyl cyanide. Many of the spectra from this GLC/MS analysis could not be interpreted, as they represented extremely complex mixtures. The identification of compounds with

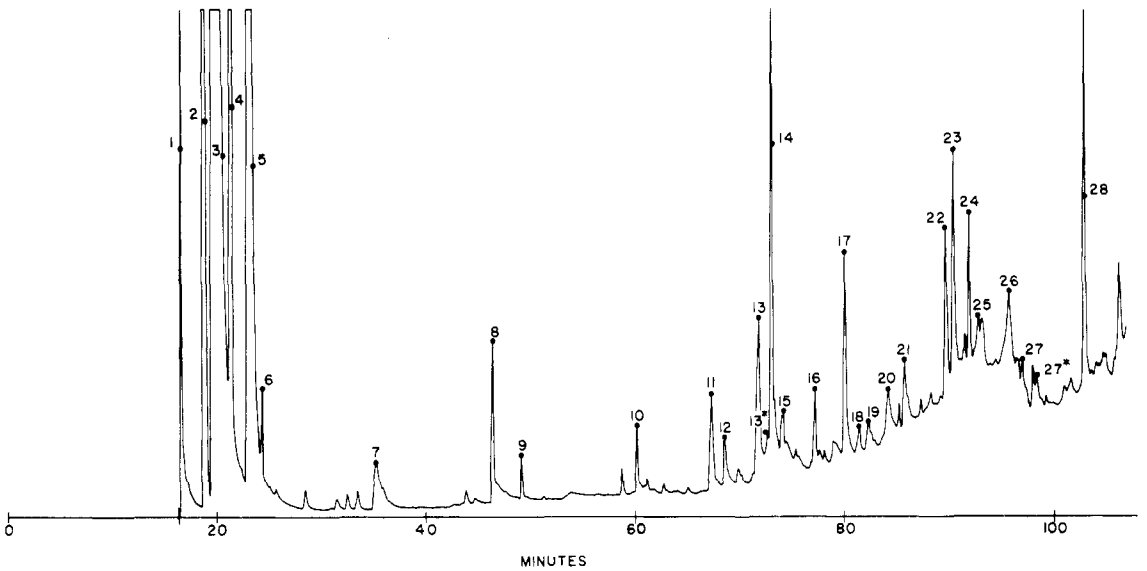

Figure 2. Chromatogram of pentane concentrate on stainless steel capillary (500 ft × 0.03 in.) coated with General Electric SF 96 (50) silicone oil and programmed from 35 to 180 °C at 1.5 °C/min.

molecular weights higher than those for compounds previously reported was possibly due to the GLC conditions used. Although nitriles have not been previously identified in cooked poultry or other cooked meats, there is no evidence that undecyl cyanide or other compounds identified are artifacts or contaminants.

GLC/MS of the Pentane Concentrate with a Silicone Oil Coated Open Tubular Column. About 170 peaks were obtained in the chromatogram of the concentrate from an analysis on a 500 ft × 0.03 in. open tubular column. Only that portion of the chromatogram where interpretable mass spectra were obtained is presented in Figure 2. The numbered dots on the GLC peaks represent data points (MS scans). Thirty-one compounds were identified; all eluted within 104 min (Table IV). After that time the mass spectral background became very intense and made interpretation of the spectra extremely difficult. The compounds 1-pentane, isopentane, and cyclopentane were shown to be artifacts in the pentane fraction by concentration of a 120-ml aliquot of pentane to 50 μl and analysis of this residue by GLC/MS techniques.

Data in Table IV indicate that many of the low molecular weight sulfur compounds previously identified in cooked chicken meat (Wilson and Katz, 1972) were absent. Failure to identify these compounds is probably due to their loss and/or reduction to levels below the limits of detection during concentration of the pentane extract. The fact that mercaptans are known to react with hot metal surfaces in the GLC/MS system could account for their loss. Other low molecular weight compounds were identified, including acetaldehyde and acetone. Some of the aldehydes reported by other investigators were identified, namely, acetaldehyde, hexanal, and heptanal. Failure to identify enals or dienals, which were previously reported, might be due to their loss through oxidation or other chemical reactions during sample preparation. Toluene and propylbenzene, previously reported by Nonaka et al. (1967), were identified in several fresh pentane concentrates. Gas–liquid chromatographic/mass spectrometric analysis of a pentane concentrate which had been stored 3 months in a refrigerator revealed the presence of the same alkylbenzene series reported by Nonaka et al. (1967). They were toluene, propylbenzene, butylbenzene, pentylbenzene, hexylbenzene, and heptylbenzene. Nonaka et al. (1967) reported that propyl- and butylbenzene formed from autoxidation of 2,4-decadienal and suggested that the other alkylbenzenes might originate from oxidation of other dienals. In addition, cyclopentanone, cyclohexane, and 1,2-dimethylcyclohexane were identified for the first time. The results of this phase of the research demonstrate the large number and wide variety of volatile compounds in cooked chicken.

Table IV. Volatile Compounds of Chicken Broth Identified by GLC/MS with a Silicone Coated 500 ft × 0.03 in. Open Tubular Column

GLC peak no.	Compound	Retention time[a]	MS identification
1	Acetaldehyde	+	Positive
2	Acetone and isopentane (artifact)	+	Positive
3	1-Pentene and pentane (artifacts)	+	Positive
4	Cyclopentane (artifact)	+	Positive
5	Hexane[b] and 1-hexene[b]	+	Positive
6	Cyclohexane[b]	+	Positive
8	2-Pentanone	+	Positive
9	3-Pentanone[b] and butanol	+	Positive
10	2,3-Dimethyl-2-pentene[b] and toluene	+	Tentative Positive
11	2-Methylthiophene	+	Positive
12	Cyclopentanone[b]	+	Positive
13	Pentanol and hexanal	+	Positive
13[c]	3-Methylheptane[b]	+	Positive
14	Isomer of isohexenal		Tentative
15	2-Heptanone	+	Positive
16	2-Methylpyrazine	+	Positive
18	Heptanal	+	Positive
19	1,2-Dimethylcyclohexane[b]	+	Positive
20	2-Methyl-3-heptanone[b]	+	Positive
23	Benzaldehyde	+	Positive
24	Dimethyl disulfide	+	Positive
25	3-Methyl-3-ethylhexane[b]	+	Positive
26	2-Pentylfuran		Tentative
27[c]	4-Nonyne[b]	+	Positive
28	Propylbenzene	+	Positive

[a] Plus sign indicates unknown compounds's retention time matches that of the known compound. [b] Compounds identified for the first time in cooked chicken meat. [c] Peaks that were identified after initial draft of manuscript.

GLC/MS of the Pentane Concentrate with a Carbowax 20M Coated Open Tubular Column. In this

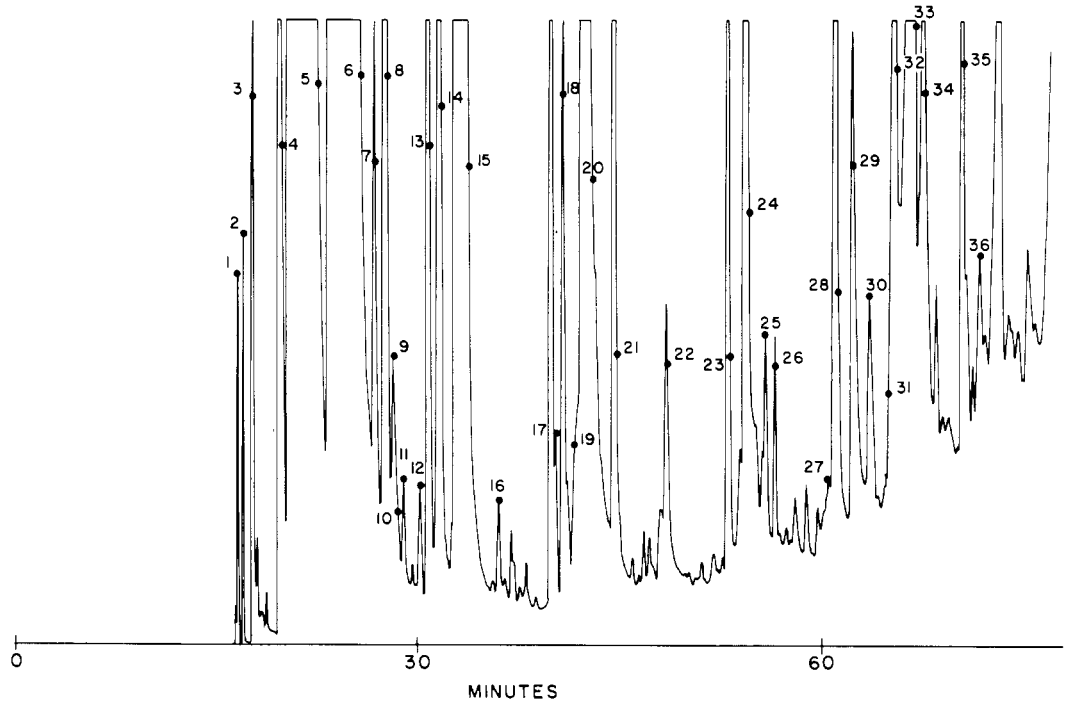

Figure 3. Chromatogram of pentane concentrate on stainless steel capillary (500 ft × 0.02 in.) coated with Carbowax 20M and programmed from 35 to 180 °C at 1.5 °C/min.

analysis of the concentrate, 25 compounds were identified and all were eluted within 72 min (Figure 3). Only that part of the chromatogram which yielded workable spectra is shown. The numbered dots on the GLC peaks represent data points (MS scans). After 72 min the spectra became very difficult to interpret due to high mass spectral background and incomplete resolution of the compounds being eluted from the gas chromatography column.

Several sulfur compounds were identified (Table V) and included 2-methylthiophene, 2-acetylthiophene, 2-propyl-5-isopentylthiophene, and 3,5-dimethyl-1,2,4-trithiolane. The last compound was identified for the first time in cooked chicken broth although it had been previously found in cooked beef (Herz and Chang, 1970). In addition, 2-methylpyridine was identified. Identification of α-terpineol confirmed the results of Wilson and Katz (1972) who had speculated that it was introduced in poultry through the food chain.

GENERAL COMMENTS

Of the 53 compounds identified in this study, 30 had not been previously identified in cooked chicken meat. The total number of compounds identified from cooked chicken meat is at present 183 (153 reported in the literature plus 30 identified in this study). However, only 45 of the 183 volatile compounds have been confirmed by one or more investigators. This situation may be explained on the basis of differences in both preparation and analyses of samples. Those differences indicate the inadequacy of any one analytical system to provide a complete analysis of the extremely complex mixture of volatile compounds in cooked chicken meat. A further step required to relate these previously identified compounds to chicken aroma would be the measurement of their threshold values and their concentration in the vapor phase over cooking chicken.

Table V. Volatile Compounds of Chicken Broth Identified by GLC/MS with a Carbowax 20M Coated 500 ft × 0.02 in. Open Tubular Column

GLC peak no.	Compound	Retention time[a]	MS identification
1	Dimethylamine[b]	+	Positive
2	Isopentane (artifact)	+	Positive
3	Pentane and	+	Positive
	1-pentene (artifacts)	+	Positive
4	2-Aminobutane[b]	+	Positive
5	Toluene and	+	Positive
	hexanal	+	Positive
6	2-Methythiophene	+	Positive
7	2-Heptanone	+	Positive
8	Heptanal and	+	Positive
	isomer of isohexanal		Tentative
9	2-Pentylfuran		Tentative
12	2-Octanone	+	Positive
14	2-Acetylthiophene[b]		Tentative
15	2-Methylpyridine[b]	+	Positive
	and 2-nonanone		Tentative
	and 4-propylheptane[b]		Tentative
18	2-Decanone		Tentative
22	1-Dodecene[b]	+	Positive
23	Isomer of 3,5-dimethyl-1,2,4-trithiolane[b]	+	Positive
24	Isomer of 3,5-dimethyl-1,2,4-trithiolane	+	Positive
26	2-Propyl-5-isopentyl-thiophene[b]		Tentative
27	α-Terpineol	+	Positive
29	*p*-Ethylbenzaldehyde[b]		Tentative
32	1-Methoxy-4-(1-propenyl)benzene[b]		Tentative
35	Oxygenated terpene (mass 152)		Tentative
36	4-*sec*-Butylphenol[b]		Tentative

[a] Plus sign indicates unknown compound's retention time matched that of an authentic sample. [b] Compounds identified for the first time in cooked chicken meat.

ACKNOWLEDGMENT

The author thanks A. A. Klose for discussions and suggestions during this investigation, Kathy Gibbons for help in the GLC/MS analysis, Robert E. Lovins and Gary Tolbert, Department of Biochemistry, University of Georgia, Athens, Ga., for the GLC/MS analysis with packed columns, and Ron Buttery, Western Regional

Research Center, U.S. Department of Agriculture, Berkeley, Calif., for help in interpretation of certain mass spectra.

LITERATURE CITED

Cook, W. G., Ross, R. A., *Anal. Chem.* **44**, 641 (1972).
Harkes, P. D., Begemann, W. J., *J. Am. Oil Chem. Soc.* **51**, 356 (1974).
Herz, K. O., Chang, S. S., *Adv. Food Res.* **18**, 1 (1970).
Janney, C. G., Hale, K. K., Higman, H. C., *Poultry Sci.* **53**, 1738 (1974).
Likens, S. T., Nickerson, G. B., *Am. Soc. Brew. Chem. Proc.*, 5 (1964).
Nonaka, M., Black, D. R., Pippen, E. L., *J. Agric. Food Chem.* **15**, 713 (1967).
Wilson, R. A., Katz, I., *J. Agric. Food Chem.* **20**, 741 (1972).

Received for review February 9, 1976. Accepted May 18, 1976. Reference to a company and/or product is only for identification and does not imply approval or recommendation of the product by the Department to the exclusion of others which may also be suitable.

Nature of Carbohydrates in Pulses

P. Srinivasa Rao

Four commonly consumed pulses, Bengalgram, greengram, redgram, and blackgram, were studied to ascertain the chemical nature of the carbohydrates contained in them. Total carbohydrates, starch, and soluble sugars were determined. The nature of starch with respect to amylose content and its degree of polymerization was found to be different in the four pulses tested. Carbohydrate tolerances in children fed Bengalgram and greengram were also found to be different. The significance of these in vivo studies on the utilization of carbohydrates is discussed in relation to the chemical nature of carbohydrates in these pulses.

In an attempt to explain the flatulence associated with ingestion of pulses, investigations were carried out to test the in vitro digestibility of carbohydrates (α-amylolysis) of commonly consumed pulses. The results of these studies revealed marked differences in the rate of in vitro α-amylolysis of pulses. Of the legumes tested, maximal differences were seen between Bengalgram (*Cicer arietinum*) and greengram (*Phaseolus areus*) (Srinivasa Rao, 1969). These studies were, therefore, extended to ascertain (1) whether the in vitro observations have any relevance to the in vivo situation and (2) whether the nature of the carbohydrates contained in these pulses is different. The results of these investigations are reported here.

EXPERIMENTAL SECTION

In Vivo Studies in Children. These studies were carried out on a group of eight children (six males and two females) between the ages of 3 and 4 years, using a crossover design. A test meal of 20 g of cooked Bengalgram or greengram dhal, mashed to give homogenous slurry, was given to children in the morning under fasting conditions. Four children received greengram while four others received Bengalgram. Capillary blood was collected by finger prick before the meal and at intervals of 30, 60, 120, and 180 min after the meal. After 1 week, the children received a meal of the second pulse. Greengram was fed to those children who had received Bengalgram earlier and vice versa. Fasting blood samples were collected before and after the meal as described earlier. Blood glucose was estimated by the ferricyanide method of Park and Johnson (1949).

Chemical Analysis. Chemical analyses were carried out on pooled decorticated samples of pulses collected from different sources. Bengalgram (*Cicer arietinum*), greengram (*Phaseolus areus*), redgram (*Cajanus cajan*), and blackgram (*Phaseolus mungo*) were finely ground and passed through a 60 mesh sieve prior to analysis.

The starch content was determined by the method of McCready et al. (1950) while total carbohydrate and soluble sugars were estimated according to methods described by Friedmann and coworkers (1967). Isolation of starch from pulses was carried out following the procedure of Badenhuizen (1964).

Determination of Amylose Content of Starch. Amylose content of isolated starches was determined by measuring iodine affinity potentiometrically according to the method of Schoch (1964). A preliminary calibration curve was obtained to relate the emf reading (in millivolts) to the amount of free iodine in solution under conditions identical with those employed in starch titration.

Titration of Isolated Pulse Starches. An accurately weighed amount of defatted starch (about 40 mg) from the pulse was transferred to a clean dry 250-ml beaker and 1 ml of water was added to suspend the sample. Five milliliters of 1 N KOH was added and the sample kept at 4 °C for 30 min with occasional stirring until clear. After neutralizing with HCl (Methyl Orange as indicator), 10 ml of 0.5 N KI solution was added. The contents were titrated against iodine solution and the emf noted. Values for free iodine were derived from the earlier calibration curve. The bound iodine was obtained by deducting free iodine values from the total iodine. The free iodine values for each sample were then plotted against bound iodine. The upper linear portion of the curve was extrapolated back to intersect the zero axis, and from this the amount of bound iodine was calculated. The percentage iodine affinity (which corresponds to the percentage amylose content) was derived as follows: % iodine affinity = (mg of bound iodine for the sample at zero intersect/sample weight in mg) × 100. One hundred milligrams of pure potato amylose in our experiments gave 18.9 mg of bound iodine. Hence the amylose content of the pulse starch sample was calculated from the relation: % amylose = (mg of bound

National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania, Hyderabad-500007, India.