Volatile Components of Roasted Peanuts. The Major Monocarbonyls and Some Noncarbonyl Components

M. E. MASON,[1] BOBBY JOHNSON,[1] AND MYNARD C. HAMMING[2]

Condensates obtained from roasted Spanish peanuts using high vacuum distillation and cryogenic trapping procedures possessed typical roasted peanut aroma. Analyses using gas chromatographic and qualitative chemical techniques revealed the presence of several aldehydes. Analysis of the total condensates and carbonyl compounds regenerated from their 2,4-dinitrophenylhydrazones using a combination gas chromatograph–mass spectrometer (GC–MS) and thin-layer chromatographic analysis of the 2,4-DNPH derivatives resulted in the positive identification of acetaldehyde, isobutyraldehyde, benzaldehyde, and phenylacetaldehyde, and tentative identification of 2- and 3-methylbutanal and 3-methyl-2-butanone. Ethyl acetate, toluene, and *N,N*-dimethylformamide were tentatively identified but their presence in the carbonyl regenerates remains unexplained.

The presence of carbonyl as well as other classes of compounds in roasted peanuts was revealed by Pickett and Holley (*7*). Recently, the presence of mono- and dicarbonyl compounds has been confirmed (*10*). Pattee, Beasley, and Singleton (*6*) found carbonyl compounds as well as ethanol and ethyl acetate to be volatile constituents of peanuts which had been treated to produce off-flavors. However, little has been done to identify the specific compounds associated with the typical flavor from roasted peanuts. The authors positively identified several of the volatile carbonyl compounds found in roasted Spanish peanuts. These identifications should serve to define the typical roasted flavor chemically. Brief comments are made concerning the possible contributions of some of the components to the total flavor.

Reagents and Materials

2,4-Dinitrophenylhydrazine, reagent grade, Eastman Kodak Co., Rochester, N. Y.

Gas-Chrom Q, 100 to 120 mesh, Applied Science Laboratories, Inc., P. O. Box 140, State College, Pa.

Carbowax 20M, Apiezon L, and Chromosorb W, 60 to 80 mesh, Varian Aerograph, 2700 Mitchell Drive, Walnut Creek, Calif.

Glycerol, U. S. P., heavy-grade, Pro cter & Gamble Co., Cincinnati, Ohio.

Phenylacetaldehyde, benzaldehyde, acetaldehyde, isovaleraldehyde, 3-methylbutanal, K and K Laboratories, Plainview, N. Y.

Methylene chloride, n_D^{20} 1.4238, Aldrich Chemical Co., Milwaukee 10, Wis., redistilled at 40° C.

Skellysolve B., Skelly Oil Co., P. O. Box 36, Kansas City, Mo.

Ethyl acetate, reagent grade, J. T. Baker Chemical Co., Phillipsburg, N. J.

Benzene, reagent grade, Fisher Scientific Co., Fair Lawn, N. J.

α-Ketoglutaric acid, Calbiochem, 3625 Medford St., Los Angeles, Calif.

Procedures

Preparation of Condensates from Roasted Peanuts. This procedure involved collecting volatile compounds, at pressures of about 10^{-4} mm. of Hg, from roasted Spanish peanuts ground to a fine slurry in glycerol and water. The glycerol was found free of carbonyl compounds by testing with the 2,4-dinitrophenylhydrazine reagent. Liquid nitrogen was used as the coolant.

In order to eliminate water for preparative gas chromatography, the aqueous condensates were extracted three times with 1/10 volumes of methylene chloride. About 3 ml. of condensate were obtained for each pound of peanuts extracted. The extracts were combined and, reduced to about one tenth their former volume on a rotary evaporator, and 200-μl. quantities separated by gas chromatography. In this procedure, the solvent peak made collection of the first five or more components impossible. However, the removal of the water which was required for chromatography on the combination gas chromatography–mass spectrometer (GC-MS) instrument was achieved. The aqueous condensates were chromatographed without previous extraction (Figure 1) using the Perkin-Elmer 800 gas chromatograph equipped with a flame ionization detector.

Preparation of 2,4-Dinitrophenylhydrazone (2,4-DNPH) Derivatives. To the thawed, aqueous condensates freshly removed from the liquid nitrogen traps were added 10 volumes of a saturated solution of 2,4-dinitrophenylhydrazine in 2*N* HCl. The precipitate

[1] Department of Biochemistry, Agricultural Experiment Station, Oklahoma State University, Stillwater, Okla.

[2] Continental Oil Co., Ponca City, Okla.

was collected by filtration and dried over anhydrous calcium sulfate in a vacuum desiccator.

Regeneration of Carbonyls from Their 2,4-DNPH Derivatives. Regenerations were performed according to the procedure of Ralls (*8*) with some modification of regeneration tubes (*4*). For gas chromatography, 6 to 40 mg. of the mixture of the 2,4-DNPH derivatives were weighed and triturated with three times their weight of α-ketoglutaric acid and the mixtures transferred to the regeneration tubes. Using a gas-tight syringe, carbonyl vapors were taken from the hot tube immediately after regeneration and injected into the gas chromatograph–mass spectrometer (GC–MS instrument).

Gas Chromatography. Relative retention times were obtained by gas chromatography of aliquots of the aqueous condensates on a Perkin-Elmer Model 800 gas chromatograph equipped with a dual hydrogen flame detector using a 20-foot × 1/8-inch o.d. (0.062-inch i.d.) stainless steel column packed with 20% w./w. Apiezon L on Chromosorb W, 60 to 80 mesh. Nitrogen was the carrier gas and flow rates of 35 to 40 ml. per minute were used; column temperatures were programmed at 6° C. per minute from 100° to 200° C. Retention times of the unknowns were normalized by setting that of butanal (internal standard) equal to 1.00.

Preparative separations of unknowns 18 and 21 (Figure 1) were made on a 20-foot × 1/4-inch (0.25-inch o.d., 0.16-inch i.d.) diameter column packed with the same material as the 1/8-inch column (0.12-inch o.d., 0.062-inch i.d.). An F and M Model 500 gas chromatograph provided with a four-filament hot-wire detector was the instrument used. For collection of eluted compounds, glass U-tubes were constructed so that the inlets could be inserted directly into the exit port of the thermal detector through a silicone rubber septum. The construction of these traps has been described (*5*).

Gas Chromatography–Mass Spectrometry. The combination gas chromatograph–mass spectrometer (GC–MS) instrument used was a prototype of the LKB 9000 mass spectrometer constructed in the laboratories of Ragnar Ryhage, Karolinska Institutet, Stockholm, Sweden. To obtain the gas chromatographic separations for mass spectral analyses, gas from regenerated 2,4-DNPH derivatives was introduced onto a 0.25-inch o.d. × 24-foot glass column packed with 5% w./w. Carbowax 20M on Gas-Chrom Q. Helium at a flow of 35 ml. per minute was used and temperatures used are specified elsewhere.

Mass spectra were taken at successive points along each major peak and at the apexes of the very minor peaks. In this way, the homogeneity or heterogeneity of peaks representing major components could be confirmed.

Thin-Layer Chromatography of 2,4-DNPH Derivatives. Procedures used for analytical and preparative thin-layer chromatography were those outlined by Dhont and De Rooy (*2*) and Ruffini (*9*). Solvent systems used were as follows: solvent system I, 5% ethyl acetate in benzene (v./v.); solvent system II, ethyl acetate and Skellysolve B, 1:2 (v./v.); solvent system III, benzene and Skellysolve B, 3:1 (v/v.). Visible spectral analyses on preparatively separated derivatives were performed on the Cary Model 14 spectrophotometer in the chloroform solvent in which they were eluted.

Results and Discussion

Figure 1 shows a typical gas chromatogram of the aqueous suspension (condensate) removed from roasted peanuts by vacuum distillation in the manner described above. No quantitative significance was attributed to this chromatogram because of the heterogeneity of the sample.

When this suspension was extracted with methylene chloride and the extract was chromatographed on the preparative column using the hot-wire detector, several carbonyl compounds were detected among the eluting components by bubbling the effluent gas through a saturated solution of 2,4-dinitrophenylhydrazine in 2*N* HCl. These included components labeled 1 to 5, 18, and 21. Components 1 to 5 possessed odors similar to aliphatic aldehydes while component 18 had a cinnamon-like odor. Component 21 had an odor reminiscent of oil of roses.

Subsequently, relative retentions (retention of 1-butanal = 1.00) of some of the common aliphatic aldehydes and of benzaldehyde (cinnamon-like) and phenylacetaldehyde (oil of roses) were determined. Component 1 corresponded to acetaldehyde which had an average relative retention time, RR_t, of 0.50, while component 3 corresponded to isobutyraldehyde which had an RR_t of 0.86; component 4 corresponded to 3-methylbutanal—RR_t of 1.37. Even though authentic 2-methylbutanal was not available, the compound was made in situ from a heated mixture of isoleucine and ninhydrin in citrate buffer, and a sample of the vapor was gas chromatographed using headspace sampling techniques. This aldehyde had an RR_t (1.41) slightly higher than that of 3-methylbutanal but the same as that of component 5. Authentic benzaldehyde had an average RR_t of 5.56, while that of phenylacetaldehyde was 6.40. These corresponded to the average RR_t of components 18 and 20 (Figure 1).

Relative retention values (R_f component/R_f 3-pentanone) from thin-layer chromatograms and wavelengths of maximum absorption (λ_{max}) for the 2,4-DNPH derivatives of isolated components and authentic standards are listed in Table I. λ_{max} data agreed well with published values (*3*). The data confirmed the presence of acetaldehyde, phenylacetaldehyde, and one or both of the isomeric methylbutanals. Isobutyraldehyde and a ketone (λ_{max} 363 mμ) migrated together in all solvents and the presence of the ketone was deduced from the λ_{max} of this mixture. However, a shoulder at about 356 mμ indicated the presence of the aldehyde in a somewhat lesser amount.

The conclusion that the ketone was 3-methyl-2-butanone was based on the agreement of the λ_{max} and relative retention of the unknown thin-layer spot with that of the derivative of the authentic compound (Table I). Also, 3-methyl-2-butanone had almost exactly the same gas chromatographic RR_t as 3-methylbutanal and would have eluted under the same peak as the isomeric methylbutanals (Figure 2).

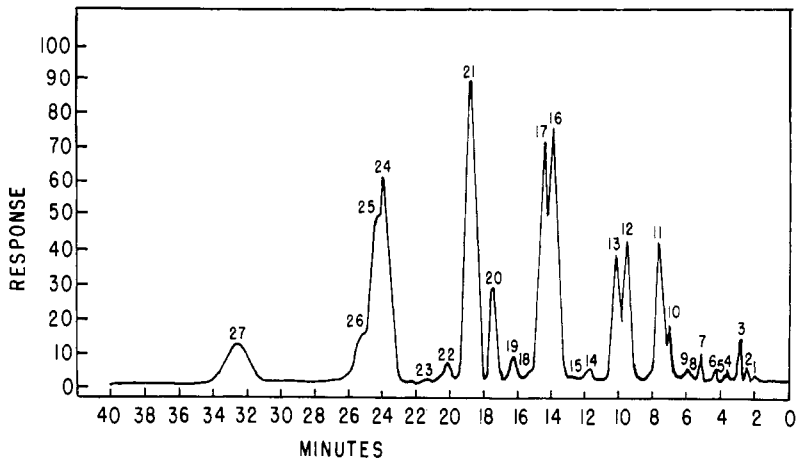

Figure 1. Gas chromatogram of 1 μl. of aqueous condensate from roasted Spanish peanuts

Table I. Relative Retentions (R_f Component/R_f 3-Pentanone) and Wavelengths of Maximum Absorption by Thin-Layer Chromatography of Isolated Components and Authentic Reference Standards

2,4-DNPH Derivatives	λ_{max}		Solvent I		Solvent II		Solvent III	
	Component	Standard	Component	Standard	Component	Standard	Component	Standard
Acetaldehyde	355	354	0.81	0.81	0.75	0.75	0.82	0.82
Isobutyraldehyde and	363 with	357		1.00		1.00		1.01
3-Methyl-2-butanone	shoulder at 356	363	1.00	1.02	1.01	1.01	1.03	1.00
3-Methylbutanal	358	358	1.06	1.03	1.08	1.06	1.01	1.02
Phenylacetaldehyde	355	355	0.99	1.00	0.99	1.00	0.83	0.80

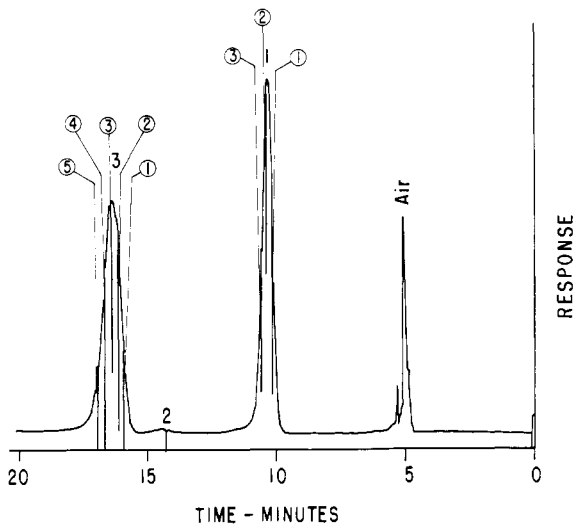

Figure 2. Gas chromatograph–mass spectrometer (GC–MS) strip chart obtained by chromatography of 250 μl. of vapors regenerated from 9 mg. of 2,4-dinitrophenylhydrazone derivatives

From the relative amounts of 2,4-DNPH derivatives present after separation by thin-layer chromatography, the isomeric methylbutanals and mixture of isobutyraldehyde and 3-methyl-2-butanone were estimated to be present in approximately equal amounts and collectively constituted most of the monocarbonyls. However, the amount of acetaldehyde derivative present was much larger than was predicted from the GC–MS analysis of the regenerated carbonyls. The amount of phenylacetaldehyde derivative was small and very small amounts of other monocarbonyl derivatives occasionally visible on the thin-layer chromatograms were probably due to benzaldehyde and other monocarbonyl compounds whose presence in minute amounts was indicated by mass spectral evidence. Biscarbonyl derivatives possessed very low retentions in the solvents used and their identifications will be the subject of another paper.

In order to gas chromatograph the monocarbonyl compounds free of biscarbonyls and other classes of compounds such as the pyrazines (*5*), they were regenerated from the mixture of 2,4-DNPH derivatives as described. Figure 2 shows a chromatogram from the GC–MS instrument obtained by injecting 250 μl. of vapors from 9 mg. of regenerated 2,4-DNPH derivatives; the column was operated at 70° C. Only three peaks other than air were obtained at the attenuations used and one of these (component 2) was extremely small. Thus, components 1 and 3 were by far the major monocarbonyl compounds present as determined by this procedure. Table II shows complete spectra of these

two major components, along with reference standard spectra taken at or near the apexes of the peaks; intensities less than 1% of the major fragment were omitted. Intensities of major ions in successive spectra taken of the two major peaks were included, also. The successive spectra were obtained at points marked 1, 2, 3, etc., which appear as discontinuities on the curves in Figure 2.

Successive spectra showed that component 1 was homogeneous and the spectrum at the apex corresponded to that of isobutyraldehyde confirming evidence obtained from thin-layer chromatography. Conversely, successive spectra showed that component 3 was heterogeneous, consisting of 2-methylbutanal but containing some other carbonyl compound (probably 3-methylbutanal) under the trailing edge of the peak. This was manifested by sharp increases in relative intensitites of *m/e* values of 27, 41, 44, and 58 in spectra 4 and 5 of component 3 while other fragment intensities were nearly the same as in the first three spectra. By consulting the reference standard spectra for 2-methyl- and 3-methylbutanal shown in Table II, the major differences in intensities of fragments, relative to an *m/e* of 57, were at *m/e* values of 27, 44, and 58. Thus, the increase of relative amounts of these fragments in spectra 4 and 5, especially *m/e* = 44, was probably due to the presence of some of the 3-methyl derivative. In a subsequent chromatogram, discussed later in the text, component 2 was found to be ethyl acetate.

The reference standards shown in Table II, with the exception of 2-methylbutanal, were obtained by analyzing commercially available compounds on the GC–MS instrument. That of 2-methylbutanal was from Dow uncertified spectra (*1*).

In order to obtain good spectra of minor monocarbonyls present, 40 mg. of the 2,4-DNPH derivatives

Table II. Mass Spectra of Components from GLC Separation of 250 μl. of Vapors from Regenerate of 9-Mg. 2,4-DNPH Derivatives

Relative Intensities

	Complete Spectra of Components and Standards					Significant Ions of Successive Spectra							
						Spectra of Component 1			Spectra of Component 3				
m/e	1	Isobutyraldehyde	3	2-Methylbutanal	3-Methylbutanal	1	2	3	1	2	3	4	5
15	1.5	4.4	1.5	1.5	5.4								
26	3.3	6.0	3.9	3.9	3.0								
27	39.6	48.5	29.2	26.9	35.5	39.6	38.8	39.5	23.4	29.2	31.5	37.3	33.9
28	11.3	19.5	13.0	8.9	1.1								
29	25.8	22.7	91.2	88.2	36.2	25.8	24.2	24.6	86.5	91.2	88.8	97.1	95.5
30			3.6	3.2	1.1								
31	1.0	1.8		1.5	1.5								
37	2.1	2.6											
38	3.3	5.5		1.9	4.4								
39	18.9	20.5	18.9	17.0	28.5	18.9	19.8	19.2					
40	2.5	4.1	3.3	2.2	5.8								
41	64.3	65.0	87.2	81.0	79.0	64.3	64.0	67.5	81.5	87.2	89.8	107.0	125.0
42	8.3	9.4	5.7	4.3	16.8								
43	100.0	100.0	15.7	10.0	73.5	100.0	100.0	100.0	15.9	15.7	20.5	36.5	54.5
44	4.6	8.0	7.5	1.3	100.0				4.5	7.5	17.0	42.2	67.5
45	0.5	2.2	1.8	3.2	15.5								
50			1.6	1.5	2.4								
51			1.8	1.6	2.3								
53	1.0	1.4	3.9	3.0	5.2								
55	1.5	2.4	8.9	8.5	5.4								
56			8.3	8.8	3.0								
57	2.9	4.9	100.0	100.0	23.5	2.9	2.9	2.6	100.0	100.0	100.0	100.0	100.0
58	1.0	4.6	72.5	65.0	51.8				71.5	72.5	75.5	85.4	94.0
59			3.3	2.6	3.5								
61					1.6								
67					1.6								
68					2.0								
69					2.2								
70					1.4								
71	1.0	1.2	6.4	4.5	21.2								
72	52.8	42.6			1.9	52.8	49.2	48.8					
73	2.6	5.8			4.4								
74			4.8	4.3									
85					2.5								
86			11.1	15.3	6.7				11.0	11.1	10.8	12.2	13.4
87			0.9	3.1	0.8								

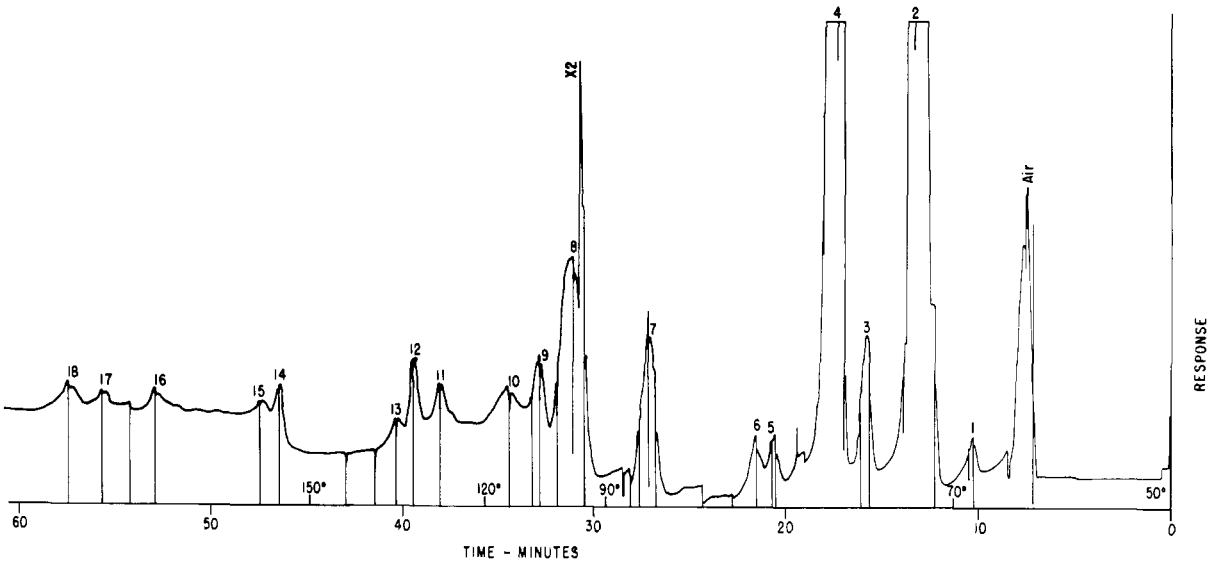

Figure 3. Gas chromatograph–mass spectrometer (GC–MS) strip chart obtained by chromatography of 5 ml. of vapors regenerated from 40 mg. of 2,4-dinitrophenylhydrazone derivatives

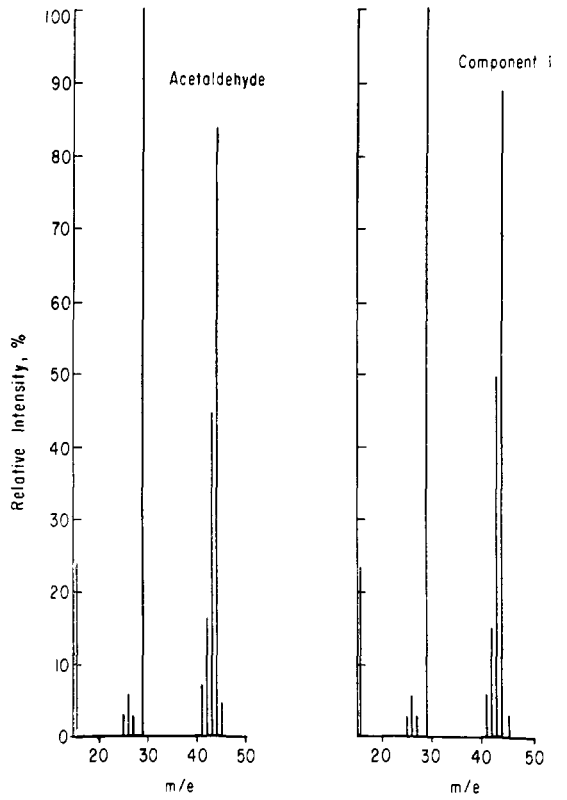

Figure 4. Mass spectra of unknown 1 (Figure 3) and acetaldehyde

were regenerated and 5 ml. of the vapors introduced onto the column attached to the GC–MS instrument. This represented about 100 times the amount of material used for the chromatogram shown in Figure 2. Figure 3 is the chromatogram obtained showing the number of components separated (arabic numerals) and the number of spectra taken on each peak (discontinuities). Again discontinuities on the curves, except for one point where an attenuation was made, marked the points at which successive spectra were taken. The temperature of the column was initially 50° C., but was raised abruptly at several points during the chromatogram as shown in the figure.

Components 2 and 4 of Figure 3 were found to be the same as 1 and 3 of Figure 2 (isobutyraldehyde and 2-methylbutanal mixed with 3-methylbutanal and 3-methyl-2-pentanone). Spectra of components 1, 3, 7, and 10 compared well with spectra of reference standards (Figures 4, 5, 6, and 7) obtained under similar conditions, except for that of toluene which was taken from API spectra. Component 1 proved to be acetaldehyde, which confirmed evidence obtained from thin-layer chromatography and visible spectral analysis.

Obviously, from the size of peaks in Figure 3, all monocarbonyls except isobutyraldehyde and the isomeric methylbutanals are minor constituents of the condensates, confirming conclusions drawn from thin-layer separations. Just how such compounds as ethyl acetate, toluene, and dimethylformamide could appear in a regeneration of carbonyl compounds was not immediately obvious. Since water was the only solvent used in making these derivatives, the most likely explanation was that these compounds were occluded in or adsorbed to the 2,4-DNPH derivatives in such a manner that they were not freed during the washing process.

This explanation seems likely because ethyl acetate has been reported as a constituent of volatiles from roasted peanuts by Pattee, Beasley, and Singleton (*6*). Other possibilities are that such compounds arose during the regeneration process or were impurities in the 2,4-DNPH reagent or α-ketoglutaric acid used in the regeneration; these seem less likely, however.

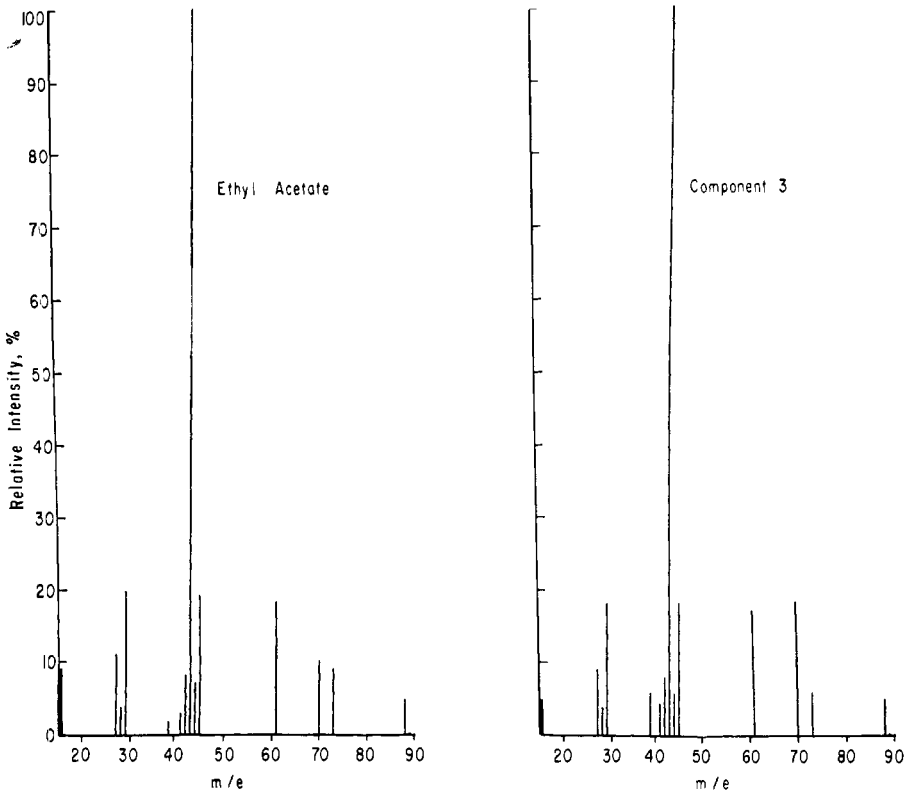

Figure 5. Mass spectra of unknown 3 (Figure 3) and ethyl acetate

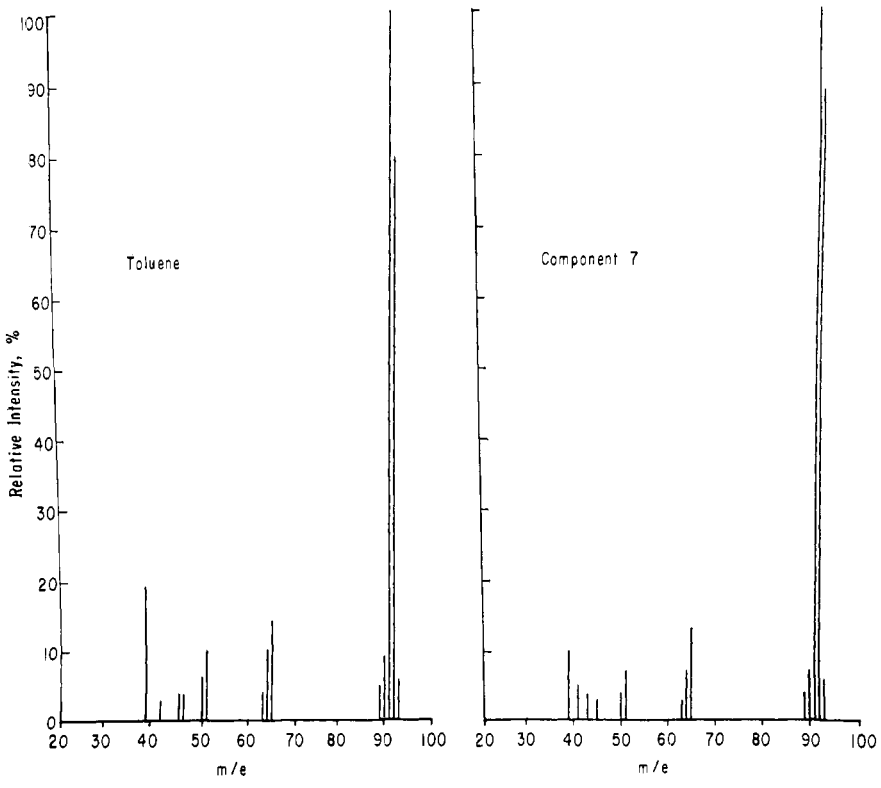

Figure 6. Mass spectra of unknown 7 (Figure 3) and toluene

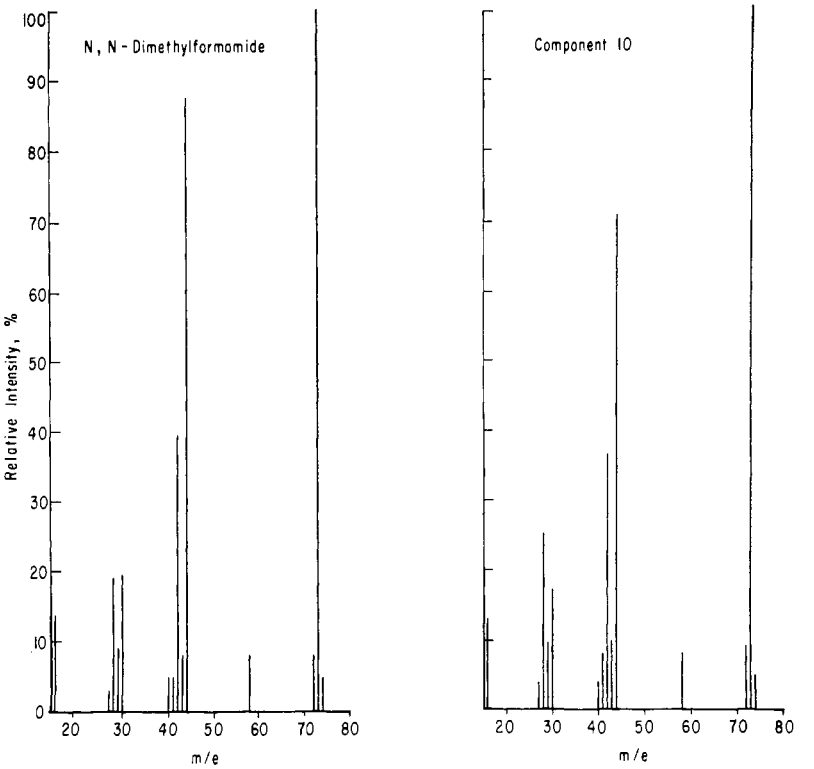

Figure 7. Mass spectra of unknown 10 (Figure 3) and *N,N*-dimethylformamide

Table III. Tentative Identities of Minor Components Shown in Figure 3 Based on Mass Spectral Data

Component	Tentative Identity
5	3-Methylfuran
6	Pentanal
8	Thiacyclohexane
9	2-Methylbutanal
11	3-Methylthia-cyclohexane
12	4-Methylthia-cyclohexane
13	Heptanal
14–18	Unknown

Some of the minor constituents may be of major importance for flavor—for example, the authors believe that phenylacetaldehyde will prove of considerable importance to the sweet bouquet of roasted peanut aroma.

Table III contains a listing of remaining components in Figure 3 for which tentative identities were obtained from the mass spectra. Background was relatively high in spectra of the latter five components (14 to 18 in Figure 3) and probable identities were not discernible.

Benzaldehyde and phenylacetaldehyde were not present in the regenerated vapors in sufficient amounts to obtain good mass spectra from the chromatographic peaks. Consequently, methylene chloride extracts of the condensate were chromatographed on the GC–MS instrument. Mass spectra obtained from components corresponding to peaks 18 and 21 of Figure 1 are listed numerically in Table IV along with reference standards obtained from commercially available chemicals. These data confirmed thin-layer and visible spectral evidence for the presence of phenylacetaldehyde.

Although objective determination of the contribution of these monocarbonyl compounds to typical roasted peanut flavor has not been possible yet, their odors and abundance suggest they must exert a very important contributory effect to over-all flavor. For instance, when phenylacetaldehyde was removed from the total condensate by gas chromatography, and the remaining components were recombined, the sweet background or bouquet of roasted peanut aroma was lost. When the low molecular weight aldehydes were removed, the harsh aroma usually associated with warm freshly roasted peanuts was lost.

In both cases, dramatic differences in intensity and type of aroma resulted. Since these were removed by gas chromatographic separation, possibly small amounts of other components were lost also which could have an effect on the aroma. However, compounds having typical roasted and nutty aromas were still present and the roasted aromas have been attributed to certain pyrazine compounds and their mixtures (*5*).

Table IV. Mass Spectra of Compounds Corresponding to Components 18 to 21 in Figure 1 and Reference Standards

	Relative Intensities			
m/e	Benzaldehyde standard	Component 18	Phenylacetaldehyde standard	Component 21
29	3.8	11.1		
37	4.0	3.8		
38			1.2	1.9
39	9.0	15.2	7.3	10.3
41			2.1	5.4
49	4.1	5.7		
50	23.5	28.7	2.5	3.4
51	44.8	42.2	4.5	6.6
52	15.8	12.0		
53	2.1	6.1		
61	2.0	3.1		
62	2.5	2.3	2.4	2.5
63	3.9	3.2	5.8	6.0
64			1.3	2.2
73	5.1			
74	13.1	25.3		
75	6.7	15.6		
76	7.5	7.5		
77	100.0	100.0		
78	26.8	19.0		
89			3.6	4.7
90			2.8	2.8
91			100.0	100.0
92			23.8	24.0
93			1.8	3.2
105	96.5	109.9		
106	96.8	109.9		
107	12.7	12.7		
120			26.5	27.0
121			2.6	4.2

The major monocarbonyls probably arose by Strecker degradation of corresponding free amino acids. Information which indicates this to be the case will be the subject of a future paper.

Acknowledgment

Purchase and maintenance of the GC–MS instrument were made possible through National Science Foundation Grant GB 3482. The authors are indebted to Ralph Matlock for furnishing peanuts of known grade and genetic background and to Rolf Hjalm for technical assistance with the mass spectrometer.

Literature Cited

(1) ASTM Committee E-14 File of Uncertified Mass Spectra, A. H. Struck, Chairman, Perkin-Elmer Corp., Norwalk, Conn. (Spectra data from Dow Chemical Co.).
(2) Dhont, J. H., De Rooy, C., *Analyst* **86,** 74 (1961).
(3) Jones, L. A., Holmes, J. C., Seligman, R. B., *Anal. Chem.* **28,** 191 (1956).
(4) Mason, M. E., Johnson, Bobby, Hamming, M. C., *Ibid.*, **37,** 760 (1965).
(5) Mason, M. E., Johnson, Bobby, Hamming, M. C., J. AGR. FOOD CHEM. **14,** 454 (1966).
(6) Pattee, H. E., Beasley, E. O., Singleton, J. A., *J. Food Sci.* **30,** 388 (1965).
(7) Pickett, T. A., Holley, K. T., *Ga. Expt. Sta. Tech. Bull.* **1,** 1952.
(8) Ralls, J. W., *Anal. Chem.* **32,** 332 (1960).
(9) Ruffini, G., *J. Chromatog.* **17,** 483 (1965).
(10) Young, C. T., Holley, K. T., *Ga. Expt. Sta. Tech. Bull. N.S.* **41,** 1965.

Received for review May 19, 1966. Accepted October 21, 1966. Division of Agricultural and Food Chemistry, 152nd Meeting, ACS, New York, N. Y., September 1966. Work supported by the Oklahoma Agricultural Experiment Station and Corn Products Institute of Nutrition.