Volatile Components of Roasted Peanuts: Basic Fraction

Bobby R. Johnson,[1] George R. Waller,* and Alma L. Burlingame[2]

Volatile components were isolated by vacuum degassing of the pressed oil from whole roasted peanuts. The aqueous vacuum distillate was separated into basic and neutral fractions by methylene chloride extraction at pH 0.5 and 8.5. The basic fraction was analyzed by combination gas chromatography–mass spectrometry utilizing both packed and open tubular columns in conjunction with both high- and low-resolution mass spectrometers. Some 19 alkylpyrazines are reported for the first time from roasted peanuts. Identifications were made by comparison of mass spectra with reference spectra. Confirmation of identities were made with relative retention indices and, in some cases, ir and uv.

Until 1966 very little was known about the specific identity of the volatile constituents of roasted peanuts. At that time this laboratory (Mason *et al.*, 1966) reported the identification of some low molecular weight pyrazines and a pyrrole isolated from roasted peanut aroma. Since then Mason *et al.* (1967) reported the major carbonyls of roasted peanut aroma and Brown *et al.* (1968) reported 12 acids and a few miscellaneous compounds. At present the most abundant volatile constituents have been reported but gas chromatographic analysis shows numerous less abundant compounds present in roasted peanut volatiles. The present study was directed specifically toward the identification of the less abundant components of roasted peanuts. Preliminary communications (Johnson and Waller, 1970; Johnson *et al.*, 1968) of part of this work have been presented. This paper is devoted to the basic fraction and a subsequent paper (Johnson *et al.*, 1971) describes the studies performed on the neutral fraction.

EXPERIMENTAL

Materials. Good quality, field dried, shelled Spanish peanuts (*Arachis hypogea*) were obtained from a local commercial seed supplier. Authentic samples of alkylpyrazines were obtained from reliable commercial sources or were gifts from the Corporate Research Department of the Coca-Cola Co., Firminich and Co., Research Division of Wyandotte Chemicals, and International Flavors & Fragrances, Inc.

Isolation of Volatiles. Whole roasted peanuts were pressed at 10,000 psi while still hot, and the oil was collected and passed through a vacuum degassing system (Figure 1). The system was operated at 3–5 $\times$ 10^{-2} Torr with the expansion bellows steam heated. The traps and cold finger were cooled to −196° C. In a typical isolation, oil from 3 lb of peanuts (approximately 400 ml of oil) was placed in a 1-l. flask (A) and slowly introduced into the evacuated foaming chamber (B). The oil was introduced to the heated bellows after foaming ceased at a rate of 1 drop per sec. As the oil passed over the bellows, the volatiles were distilled into the −196° C traps. The oil was collected in the bottom flask (C), recycled once through the complete system, starting with flask (A), and discarded. It was essentially free of the roasted peanut aroma after being subjected to this vacuum degassing process. The condensed volatiles were collected by isolating the system from the vacuum pumps and cryogenically pumping from traps 1 and 2 into the final collection tube. The condensate consisted of 0.3–0.5 ml of a milky aqueous suspension. Eight hours was required to complete the entire isolation procedure.

Fractionation of the Condensate. The condensate was fractionated into a basic and neutral–acidic fraction using a procedure similar to that previously described for fractionation of steam volatile constituents of roasted cocoa beans (van Praag *et al.*, 1968). The process involved diluting the condensate to 100 ml with deionized water, adding 10 g of sodium chloride and adjusting the pH to 0.5 by slowly adding concentrated hydrochloric acid. This solution was extracted five times with 5-ml volumes of methylene chloride (CH_2Cl_2) (Fisher Scientific SpectrAnalyzed, redistilled) to obtain the neutral–acidic fraction. The pH was readjusted to 8.5 and the solution extracted four times with 5-ml volumes of CH_2Cl_2 to obtain the basic fraction.

The CH_2Cl_2 extracts were stored at −18° C. Prior to analysis an appropriate aliquot was removed and the volume reduced about 100–150-fold under reduced pressure using a rotatory evaporator. The loss of pyrazines was minimal.

Glc Analysis of Basic Fraction. Preliminary gas chromatographic separations utilized a modified Barber-Colman Model 5000 (Waller, 1967) with a flame ionization detector containing a 21-ft $\times$ 0.25-in. o.d. coiled glass column packed with 5% Carbowax 20M on 100- to 120-mesh base-washed

Biochemistry Department, Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma 74074
[1] Department of Food Science, North Carolina State University, Raleigh, North Carolina 27607
[2] Space Sciences Laboratory, University of California, Berkeley, California 94720

Gas Chrom Q (w/w) (Auda *et al.*, 1967). The column was linearly programmed from 70° to 170° C at 3°/min and held with a helium flow of 40 ml/min. Improved separation of compounds was obtained with a 500- × 0.02-in. i.d. stainless steel capillary column coated with Carbowax 1540 containing 1% KOH. This column was also used on the Barber-Colman instrument and was operated by linear programming at 2°/min from 65 to 165° C and held with a helium flow of 2.8 ml/min.

Combination Gas Chromatography–Mass Spectrometry (gc–ms) Analysis. Initial gc–ms analyses were obtained on a 17-ft × 0.25-in. o.d. coiled glass column packed with 15% Carbowax 20M on base-washed firebrick 80–100 mesh (w/w). This column was operated in a prototype of the LKB 9000 gc–ms (Waller, 1967) under the following conditions: programmed linearly from 70 to 170° C at 3°/min, helium flow, 30 ml/min; ionization voltage, 70 eV; accelerating voltage, 3.5 kV; trap current, 49 μA; electron multiplier voltage, 0.7 to 2.1 kV; separator temperature, 220° C; scan speed, from *m/e* 2 to *m/e* 225 in 5 sec. Subsequent low-resolution mass spectral analyses utilized the 500-ft × 0.02-in. i.d. capillary column previously described. A postcolumn gas adder was constructed and used to introduce additional helium to the capillary column effluent immediately before it entered the jet separators of the mass spectrometer. This addition of extra carrier gas was necessary for optimum separator efficiency, which requires a gas flow rate of 20–30 ml/min. Both the column and the mass spectrometer were operated under the conditions previously specified for each.

Capillary Gas Chromatography–High-Resolution Mass Spectrometry (gc–hrms) Analysis. The 500-ft × 0.02-in. i.d. capillary column described above was employed. The column was operated isothermally at 70° C for 10 min and then programmed to 180° C at 2°/min and held. The helium flow was 1.5 ml/min. All of the column effluent was introduced directly into the ion source of a modified Associated Electrical Industries, Ltd. MS-902 high-resolution mass spectrometer. Complete high-resolution mass spectra were determined in real time, using an on-line digital computer for data acquisition (Burlingame, 1970). The adaptation between the capillary column and the MS-902 was accomplished by passing the last 6 ft of the column from an adjacent gas chromatography oven through a heated 0.25-in. copper tube to an on–off valve mounted near the ion source of the mass spectrometer. A heated all-glass line connected the valve and the ion source of the instrument. All points between the column and the ion source were maintained at 200° C to 215° C. A recorder monitored the total ion current output of the mass spectrometer to obtain a tracing of the compounds as they were eluted from the column. Alternatively the system described by Johnson *et al.* (1971) was used.

Separation of Samples for Infrared (ir) and Ultraviolet (uv) Spectroscopy. Preparative gas chromatographic separations utilized a Perkin-Elmer Model 801 gas chromatograph containing a 24-ft × 0.25-in. o.d. coiled glass column packed with 15% Carbowax 20M on 100 to 120 mesh (w/w) base-washed Gas Chrom Q and with the last foot of the column packed with 5% OV-17 on Gas Chrom Q to minimize column bleed. The basic fraction was separated into fractions on this column by linearly programming from 70° C to 170° C at 4°/min with a 60 ml/min flow of nitrogen. The instrument was equipped with a 4:1 postcolumn stream splitter allowing one part of column effluent to go the flame ionization detector and four parts to be collected in 3-mm o.d. glass traps. Repetitive 50 μl injections of a highly concentrated

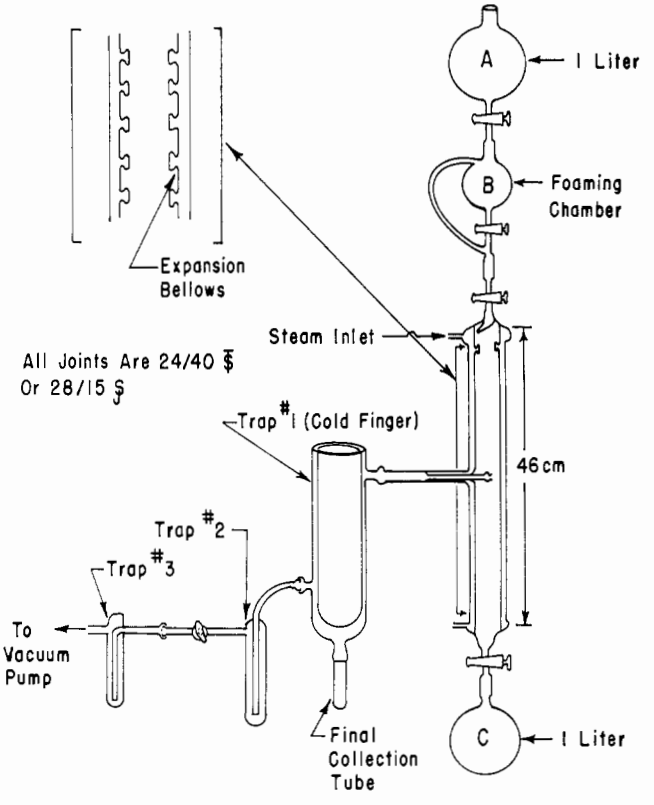


Figure 1. Vacuum degassing system

CH_2Cl_2 solution of the basic fraction were employed to obtain the compounds for ir and uv analysis.

Ir spectra were recorded with a Perkin-Elmer Model 457 double beam grating instrument using carbon disulfide solutions in a 10-μl sodium chloride microcell for the collected samples, while standards were determined on liquid films.

Uv spectra were recorded in 95% ethanol using a Bausch and Lomb Spectronic Model 505 recording spectrophotometer.

Glc Retention Time Indices (I_E). I_E values were calculated by linear interpolation of the retention time of the unknown between retention times of a series of methyl esters of normal carboxylic acids used as internal standards, similar to the method of van den Dool and Kratz (1963). The I_E value of each internal standard was arbitrarily given the value of the carbon number of the acid of the ester, thus the I_E of methyl heptanoate was 7.0. I_E values were obtained under temperature programming conditions utilizing the above described capillary column.

RESULTS AND DISCUSSION

The total volatile condensate obtained by vacuum degassing of roasted peanuts possessed an aroma of typical roasted peanuts. The basic fraction has a sweet "nutty" or "nut-like" aroma, not "peanutty," and was reminiscent of the odor of alkylpyrazines.

Figure 2 shows a typical total ion current tracing from a gc–ms analysis of the basis fraction. The peaks are labelled alphabetically from B through EE. Peak A is methylpyrazine and is not shown, since the valve to the ion source was closed until after it had been pumped away. Most of the major compounds were identified by this mode of analysis, *i.e.*, gc–low-resolution ms utilizing long 0.25-in. packed columns. However, analysis on a capillary column revealed additional compounds previously unresolved. A typical

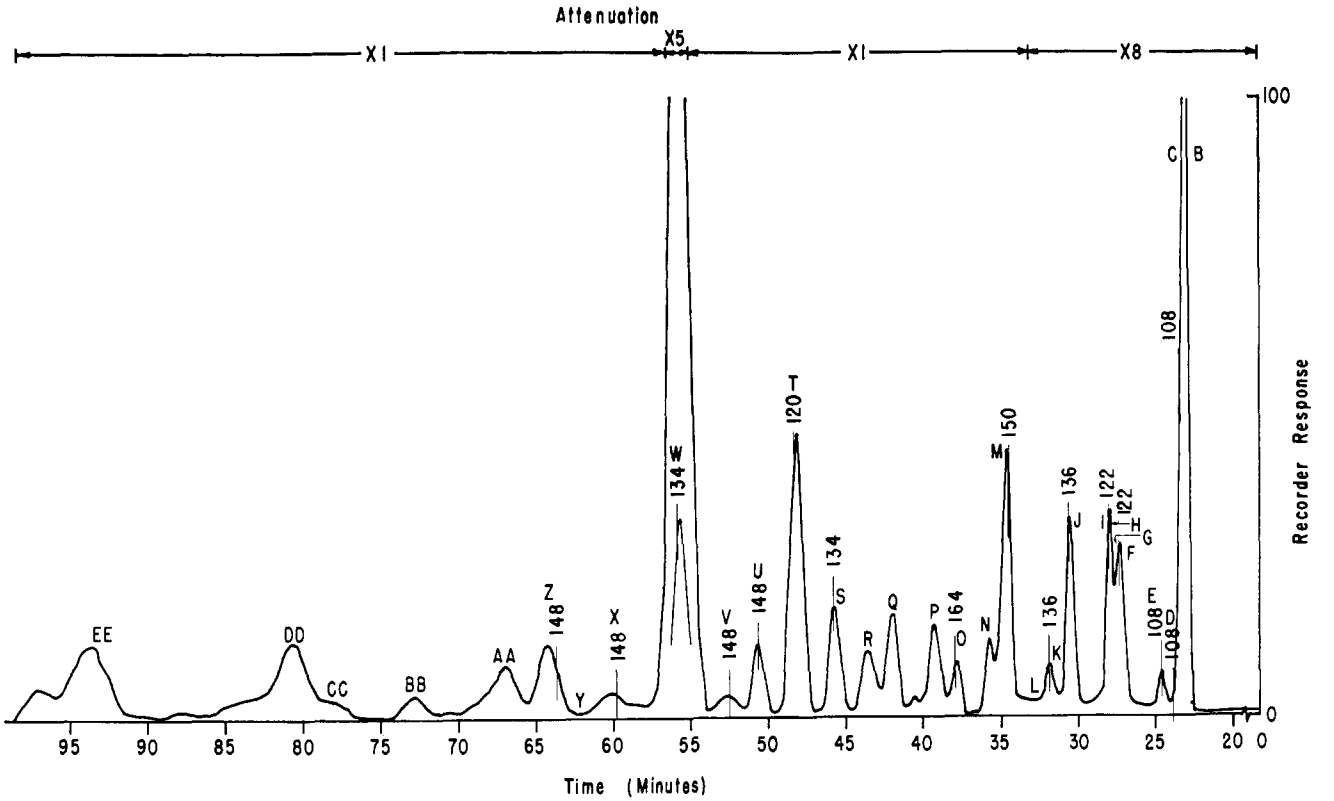


Figure 2. Typical gc–ms chromatogram of the basic fraction of roasted peanut volatiles. 5-μl injection of concentrated basic fraction of roasted peanut volatiles on the Carbowax 20M analytical column described in text. Temperature programmed from 70 to 170°C at 3° per min and held

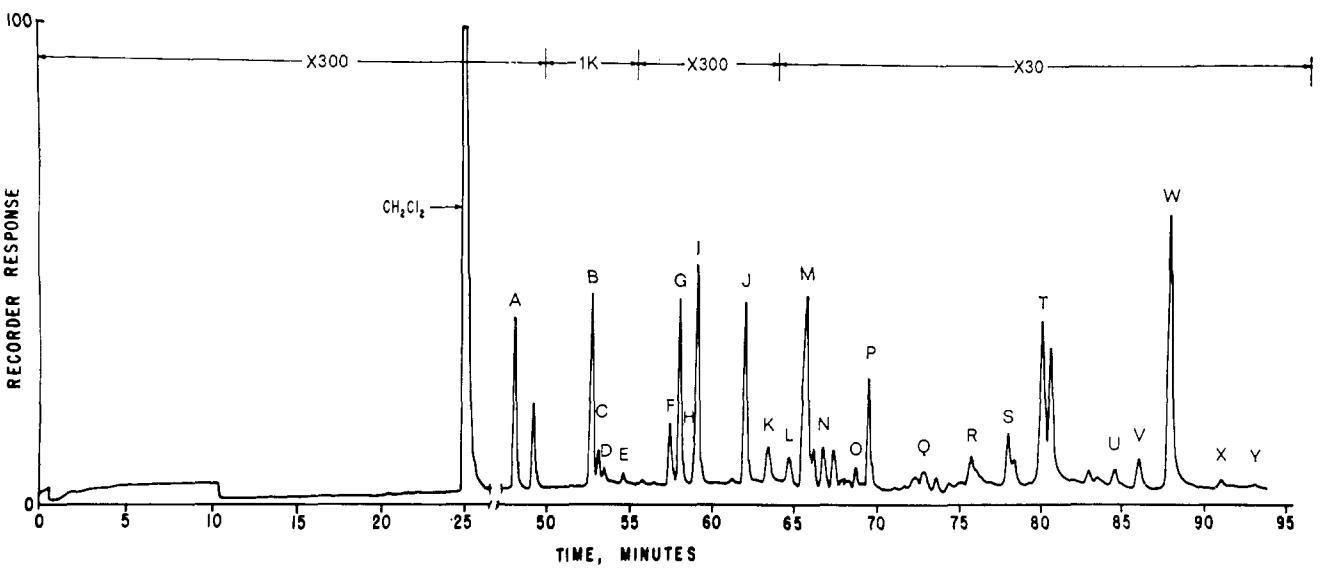


Figure 3. Temperature programmed capillary glc analysis of basic fraction. Column: 500-ft × 0.02-in. i.d. stainless steel capillary coated with Carbowax 1540 containing 1% KOH, programmed from 65 to 165° C at 2° per min and held. Helium flow of 2.8 ml per min. Peak numbering system is the same as for Figure 2 and Table I

capillary gc analysis of the basic fraction is shown in Figure 3. Capillary gc–ms analysis was employed in identifying these newly resolved components shown in Figure 3.

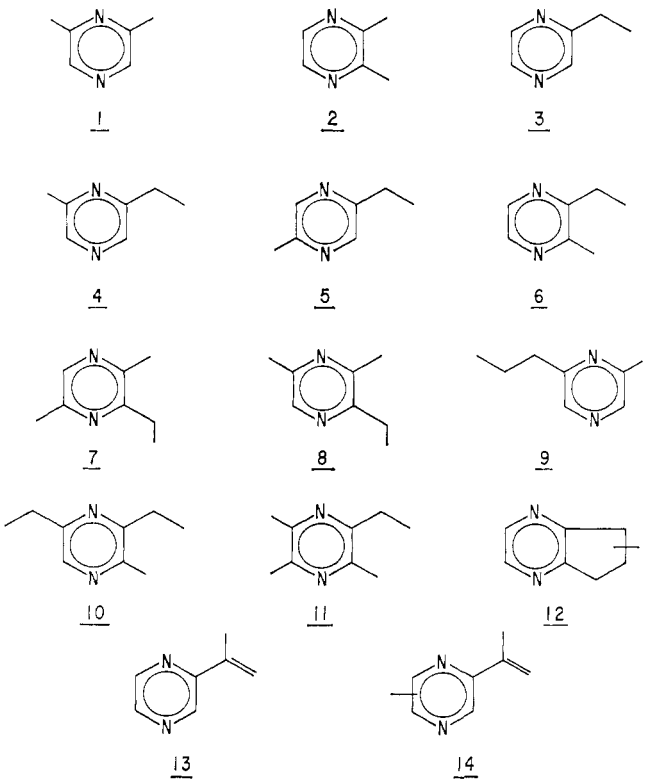
Compounds were identified by interpretation of their mass spectra and confirmed by comparison with mass spectra of authentic compounds obtained on the same instrument under similar operating conditions. Comparisons with literature spectra were made if authentic standards were not available. Identifications were further verified by ir and uv in some cases and by comparison of relative retention time indices (I_E) of standard compounds with those of the unknowns. The components identified are listed in Table I. I_E values for standard alkylpyrazines were in excellent agreement (+ or −0.02 I_E value) with the corresponding basic fraction components. In Figure 2 peaks A, B, and I were previously identified as 2-methylpyrazine, 2,5-dimethylpyrazine, and trimethylpyrazine (Mason *et al.*, 1966). Peaks C and E were identified as two isomers of alkylpyrazines with a molecular weight of 108, 2,6-dimethylpyrazine (**1**), and 2,3-dimethylpyrazine (**2**), respectively. Their mass spectra are in close agreement with

Table I. Summary of Pyrazines Identified in Basic Fraction of Roasted Peanut Volatiles

Compound	Mol wt	Component	Analyzed by: ms[a]	avs–ms[b]	ir[c]	nmr[d]	gc–rt[e]	uv	Identification[f]
2-Methyl-[g]	94	A	X			X	X		P
2,5-Dimethyl-[g]	108	B	X			X	X		P
2,6-Dimethyl-	108	C	X				X		P
2-Ethyl-	108	D	X	X			X		P
2,3-Dimethyl-	108	E	X				X		P
2-Ethyl-6-methyl-	122	F	X(hr)		X		X		P
2-Ethyl-5-methyl-	122	G	X		X		X		P
2-Ethyl-3-methyl-	122	H	X				X		T
Trimethyl-[g]	122	I	X			X	X		P
2,5-Dimethyl-3-ethyl-	136	J	X(hr)		X		X		P
2,6-Dimethyl-3-ethyl-	136	K	X		X		X		T
2-Methyl-6-propyl-	136	L	X						T
2,6-Diethyl-3-methyl-	150	M	X				X		T
2-Ethyl-3,5,6-trimethyl-	150	N	X				X		T
Alkylpyrazine	164	O	X						T
Ethylvinylpyrazine	134		X						T
Methyl-2,3-cyclopentane-	134	S	X(hr)						T
Alkylpyrazine	148		X						T
2-Isopropenyl-	120	T	X					X	T
Methyl isopropenyl-	134	W	X(hr)					X	T
Alkylpyrazine	148	X	X						T
Alkylpyrazine	148	Z	X						T

[a] Mass spectra (hr = high-resolution). [b] Accelerating voltage switching–mass spectra. [c] Infrared. [d] Nuclear magnetic resonance. [e] Gas chromatographic retention time. [f] P = positive, T = tentative. [g] Previously identified in roasted peanut volatiles.

mass spectra of authentic compounds (Bondarovich *et al.*, 1967). The retention indices for both agreed very closely with the corresponding standard compounds.



Peak D was unresolved from peaks B and C, but its presence was suspected from close examination of a number of rapid mass spectral scans over the entire peak. The mass spectra suggested the minute component to be ethylpyrazine.

A novel mass spectrometric technique was employed to aid in determining if peak D was actually ethylpyrazine. This method, as proposed by Sweeley *et al.* (1966) for determining the composition of unresolved or partially resolved mixtures in gas chromatographic effluents, involved the simultaneous recordings of the changing intensities of two selected *m/e* values during elution of the mixture from the chromatography column. This technique revealed the presence of a fragment ion strongly indicative of ethylpyrazine.

The occurrence of ethylpyrazine was later verified by resolving peak D on the capillary column (Figure 3). The alternating voltage accelerator technique could be of significant value in the analysis of complex aroma mixtures where resolution of all the components is nearly impossible. A mass spectrum of peak D obtained from capillary gc–ms analysis matches that of ethylpyrazine (**3**) (Bondarovich *et al.*, 1967).

Peak F was shown to be 2-ethyl-6-methypyrazine (**4**). The mass spectrum and retention index were compatible with the authentic compound (Bondarovich *et al.*, 1967). High-resolution mass spectra (obtained by direct gc–hrms analysis) showed the molecular formula to be $C_7H_{10}N_2$ and fragment compositions compatible with known paths of breakdown. Comparison of infrared data from unresolved peaks F and G, obtained prior to use of capillary analysis, with that of standard 2-ethyl-6-methylpyrazine and 2-ethyl-5-methylpyrazine showed bands in the mixture to be unique to each of the mentioned compounds. The mass spectrum of peak G was compatible with that of authentic 2-ethyl-5-methylpyrazine (**5**) (Bondarovich *et al.*, 1967) and the retention indices also agreed with this compound.

A mass spectrum of peak H was very similar to that of 2-ethyl-3-methylpyrazine (**6**) (Bondarovich *et al.*, 1967). With the additional confirmation of the retention index, peak H was tentatively identified as 2-ethyl-3-methylpyrazine, since the mass spectrum was a mixture of peak H and the preceding peak.

Peak J was identified as 2,5-dimethyl-3-ethylpyrazine (**7**) and its infrared spectrum agreed closely with that of the authentic compound. The mass spectrum is con-

sistent with that of the known compound (Goldman *et al.*, 1967) and the high-resolution mass spectrum showed the expected molecular composition of $C_8H_{12}N_2$ and provided consistent fragment ion compositions.

The mass spectrum of peak K was very similar to both authentic 2,6-dimethyl-3-ethylpyrazine (**8**) (Goldman *et al.*, 1967) and 2-ethyl-5,6-dimethylpyrazine (van Praag *et al.*, 1968). The retention indices agreed closely with the 2,6-dimethyl-3-ethyl isomer. Since the other isomer was unavailable for comparison, peak K was tentatively identified as 2,6-dimethyl-3-ethylpryazine.

Peak L was tentatively identified as 2-methyl-6-propylpyrazine (**9**). The mass spectrum (Goldman *et al.*, 1967) was quite characteristic of a propyl-substituted aromatic ring, as evidenced by the large M-28 at *m/e* 108 (base peak) and a lesser but significant M-15 at *m/e* 121.

The mass spectrum of peak M was very similar to that of 2,6-diethyl-3-methylpyrazine (**10**) (Bondarovich *et al.*, 1967). Retention indices support the mass spectral identification but the unavailability of a 2,5-diethyl-3-methyl isomer for comparison required peak M to be identified tentatively as 2,6-diethyl-3-methylpyrazine.

Peak N was found to be a mixture of two compounds. The mass spectrum showed two probable parent ions at *m/e* 150 and 154. The spectrum was compatible with that of 2-ethyl-3,5,6-trimethylpyrazine (**11**), as reported by van Praag *et al.* (1968). Retention indices suggested that one of the two compounds of peak N was tentatively identified as 2-ethyl-3,5,6-trimethylpyrazine.

The mass spectrum of peaks P, S, T, U, W, X, and Z indicates all to be pyrazines but with 2 hydrogens less than their corresponding saturated analogs, indicating either a double bond or ring side chain. High resolution mass spectra were obtained on all these peaks except T and X and confirmed their molecular formulas as containing 2 hydrogens less than saturated alkylpyrazines.

Preparative gas chromatographic separation and collection of components T and W permitted uv analysis of these two components. These data, together with uv, mass spectral, and gas chromatographic retention data of a number of standard pyrazines, permitted tentative structure assignments. In a preliminary communication (Johnson and Waller, 1970) peaks S, T, and W were tentatively reported as a methyl-2,3-cyclopentanepyrazine (**12**), isopropenylpyrazine (**13**), and a methylisopropenylpyrazine (**14**), respectively. However, Walradt *et al.* (1970) reported some identifications, which included those which correspond to these three compounds. They reported 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine, 6,7-dihydro-5*H*-cyclopentapyrazine, and 2-methyl-6,7-dihydro-5*H*-cyclopentapyrazine, which correspond to peaks S, T, and W, respectively.

Of various isolation techniques attempted, the authors found that vacuum degassing of the oil from freshly roasted peanuts yielded the most representative, qualitative volatile gc profile. In comparison with subsequent work by Walradt *et al.* (1970), well over 100 additional components were identified from volatiles obtained by a steam distillation technique.

This study has resulted in the identification of 19 alkylpyrazines not previously reported from the basic fraction of roasted peanut volatiles. Although no specific compound has been identified as possessing a characteristic roasted peanut aroma, the alkylpyrazines as a group seem to contribute substantially to the "nutty" character of typical roasted peanut aroma. It is of particular note that essentially all of the compounds identified thus far are also common constituents of other roasted food flavors, particularly those of coffee, cocoa, potato chips, and popcorn.

LITERATURE CITED

Auda, H., Juneja, H. R., Eisenbraun, E. J., Waller, G. R., Kays, W. R., Appel, H. H., *J. Amer. Chem. Soc.* **89,** 2476 (1967).

Bondarovich, H. A., Friedel, P., Krampl, V., Renner, J., Shephard, F. W., Gianturco, M. A., J. AGR. FOOD CHEM. **15,** 1093 (1967).

Brown, B. A., Konigsbacher, K. S., Ellison, F. E., Mann, G. E., *J. Food Sci.* **33,** 595 (1968).

Burlingame, A. L., Abstracts of Paper, ANAL 18, Canadian Institute of Chemistry–ACS Joint Conference, Toronto, Canada, May 1970.

Goldman, I. M., Seibl, J., Flament, Y., Gautschi, F., Winter, M., Willhalm, B., Stoll, M., *Helv. Chim. Acta* **50,** 694 (1967).

Johnson, B. R., Waller, G. R., Abstracts of Papers, 160th ACS National Meeting, Chicago, Ill., September 1970, AGFD 71.

Johnson, B. R., Waller, G. R., Foltz, R. L., J. AGR. FOOD CHEM. **19,** 1025 (1971).

Johnson, B. R., Waller, G. R., Mason, M. E., Abstracts of Papers, 156th ACS National Meeting, Atlantic City, N.J., September 1968, AGFD 115.

Mason, M. E., Johnson, B. R., Hamming, M., J. AGR. FOOD CHEM. **14,** 454 (1966).

Mason, M. E., Johnson, B. R., Hamming, M., J. AGR. FOOD CHEM. **15,** 760 (1967).

Sweeley, C. C., Elliott, W. H., Fries, I., Ryhage, R., *Anal. Chem.* **38,** 1549 (1966).

van den Dool, H., Kratz, P. D., *J. Chromatogr.* **11,** 463 (1963).

van Praag, M., Stein, H. S., Tibbetts, M. S., J. AGR. FOOD CHEM. **16,** 1005 (1968).

Waller, G. R., *Proc. Okla. Acad. Sci.* **47,** 271 (1967).

Walradt, J. P., Pittet, A. D., Kinlin, T. E., Sanderson, A., Abstracts of Papers, 160th ACS National Meeting, Chicago, Ill., September 1970, AGFD 70.

Received for review November 9, 1970. Accepted April 19, 1971. Journal Article No. 2133 of the Agricultural Experiment Station, Oklahoma State University, Stillwater, Okla. This research was supported in part by Grants from Best Foods Division of the Corn Products Corporation International and the National Science Foundation, Research Grant No. GB-7731.