Introductory Remarks: A Tribute to Walter G. Jennings

Walter G. Jennings recently retired from the University of California at Davis, where he had a distinguished career as a Professor in the Department of Food Science and Technology. As Dr. Jennings has been an effective leader in and active contributor to both the Analytical and Agricultural and Food Chemistry Divisions, a national meeting of the American Chemical Society (ACS) was considered a most appropriate place to hold a symposium in his honor.

At the September 1989 ACS meeting in Miami Beach, a symposium on "Analytical Methods in Agriculture and Food Chemistry—A Tribute to Walter G. Jennings" was held. Speakers were restricted to former students, postdoctoral students, and colleagues that have worked with Walter. This somewhat unusual restriction was appropriate because of his effectiveness as a teacher. He has trained many graduate students, postdoctoral students, and visiting scientists who value his role in their professional development.

Dr. Jennings has made many significant scientific contributions to flavor chemistry. He is particularly known for his role in the development and effective application of fused-silica capillary columns. The on-column injector is another chromatographic device that has benefited from his creative efforts.

This issue contains three papers that were presented at the symposium, which was cosponsored by the Agriculture and Food Chemistry and Analytical Chemistry Divisions.

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Characterization of Ham Flavor Using an Atomic Emission Detector†

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Volatile flavor compounds were isolated from a cured, precooked premium ham by using a Likens-Nickerson apparatus. Four individual 250-g ham samples were used to provide flavor isolates. These isolates were pooled and concentrated for extensive gas chromatographic analysis. Atomic emission detection (AED), flame ionization detection, flame photometric detection, nitrogen phosphorus detection, and gas chromatography–mass spectrometry were used to qualitatively determine specific constituents of the pooled solvent fraction. AED spectra proved useful in the selective detection of nitrogen-, oxygen-, and sulfur-containing compounds by comparison of the elemental profiles to the various GC chromatograms. More than 60 heteroatomic compounds were tentatively identified in this study.

INTRODUCTION

To date, there have been few studies on ham flavor. Much of the research concerning ham flavor was conducted before the development of the sophisticated instrumentation that we have today [e.g., Hornstein and Crowe (1960), Macy et al. (1964), and Ockerman et al. (1964)]. Now gas chromatography and mass spectrometry are ubiquitous tools in the flavor chemist's regime of analysis. Complex chromatograms, typical of meat and other food extracts, can be resolved with fused silica capillary columns and identified by using the table-top mass spectrometers and respective automated spectral library searching algorithms (Petitjean et al., 1983). In a recent study, Shen et al. (1988) reported 75 volatile compounds in a Jinhua ham (most famous of China), isolated by simultaneous distillation extraction and identified by GC–MS. These compounds included hydrocarbons, aldehydes, alcohols, ketones, esters, furans, phenols, and sulfur-containing compounds. In general, meat flavors have been noted for the heteroatomic constituents produced thermally via nonenzymatic browning reactions (MacLeod and Ames, 1986; Shahidi, 1989).

Because of the great number of possible chemical compounds found in foods and the limited ability of mass spectral data searches to discriminate between various possible structures, a complementary means of compound identification is essential (Petitjean et al., 1983). Therefore, often unknown volatiles are categorized in reference to the linear elution time of *n*-alkane hydrocarbons on polar and nonpolar GC columns, as discussed by Jennings and Shibamoto (1980). Another aid in the chromatographic analysis of flavor volatiles is the employment of selective detectors such as the NPD for nitrogen-containing compounds and the FPD for sulfur-containing compounds. The newly available atomic emission detector (AED) allows the

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simultaneous detection of up to four elements (depending upon the characteristic wavelength of emission) in a complex sample matrix (Szelewski and Wilson, 1988). Fox and Wylie (1989) have demonstrated that the sensitivity of the AED was adequate in the detection of nitrogen-, sulfur-, and oxygen-containing heteroatomic compounds in a cooked meat flavor isolate. The objective of this study was to analyze qualitatively the flavor volatiles from a processed premium ham by using the tradiational GC detectors and the AED for elemental profiles of carbon (C), nitrogen (N), and sulfur (S) and also the AED for oxygen (O).

MATERIALS AND METHODS

Sample Preparation. A 2-kg premium ham was purchased at a local supermarket and stored at 4 °C until sample preparation. All sample preparation took place within 24 h of purchase. The casing and outer 2-cm portion were trimmed from the ham to avoid contamination from the plastic shrink-wrap packaging and to avoid direct sampling of the smoke condensates deposited on the ham surface. Four individual 250-g portions of the remaining ham were each blended for 10-s segments intermittently for a total of 1 min with 500 mL of distilled water in a Waring Blendor. This slurry was then stirred for 10 min with a magnetic stirrer at low speed. The ham and water mixture was added to a 5-L round-bottom flask. An additional volume of 1.5 L of distilled water at 80 °C was added to the tissue mixture prior to heating, and then the sample flask was attached to a modified Likens-Nickerson apparatus. In a 100-mL round-bottom flask, 35 mL of chromatographic grade methylene chloride was added. Additionally, 10 mL of methylene chloride was added into the Likens-Nickerson solvent return loop. Both solvent and sample mixtures were heated to boiling with heating mantles and allowed to reflux for 1.5 h. This time was determined by previous trials to yield a solvent extract with a smoky, cured-ham aroma. After the mixture cooled to ambient temperature, the solvent was quantitatively recovered from both the collection flask and the solvent return loop. Recovered extracts were pooled and dried with anhydrous $MgSO_4$. Concentration to $^1/_{75}$ the combined fraction mass was achieved by gentle sparging with purified nitrogen gas for injection into the gas chromatograph. A control experiment or system/solvent blank was conducted by using only distilled water and solvent under the same conditions.

Gas Chromatography. A Hewlett-Packard (HP) Model 5890 gas chromatograph equipped with either a FID, AED, or mass selective detector (MSD) and a HP Model 5880 chromatograph equipped with a NPD or FPD were used. Separation was achieved on a 30 m × 0.25 mm i.d. × 1.0 μm film thickness fused silica capillary column (J&W Scientific; Folsom, CA), coated with cross-linked 5% phenylmethylsilicone (DB-5). All injections were performed under the same following GC conditions. The oven temperature was held at 40 °C for 1 min and then programmed at 5 °C/min up to 270 °C (14-min hold). The injector temperature was 275 °C for all instruments. Detector temperatures were 300, 275, 200, and 275 °C for the AED, FID, FPD, and NPD, respectively. Helium was used as the carrier gas at a column flow rate of 1.25 mL/min and 15 psi of head pressure. A splitless injection with a 45-s valve delay and 1 μL extract volume was injected each time. The data from the HP 5880 GC were recorded on a HP Level Four integrator, while the data from the HP 5890 GC were recorded by using the HP Chem Station software. Values reported were the average of two analyses. Linear retention indices of the volatile constituents were calculated from the spiked injection of *n*-alkanes (C_6–C_{26}) as references (Novák and Ruzicková, 1974).

Mass Spectrometry. Positive ion, electron impact mass spectrometry data were collected on a HP Model 5970 mass spectrometer. The capillary column was interfaced directly into the mass spectrometer operating at 70-eV ionization potential, with an ion source temperature of 220 °C and a scan threshold of 750, scanning from m/z 29 to 400 at 0.86 s/cycle. The mass spectra of the compounds identified were compared with those in the NBS/EPA and user-generated libraries by using the Chem Station data system.

Table I. AED Parameters

element	wavelength, nm	scavenger gas	makeup flow, mL/min
C	193.0	O_2/H_2	30
S	181.4	O_2/H_2	30
N	174.3	O_2/H_2	30
O	777.3	$H_2/N_2/CH_4$	30

spectrometer purge flow	nitrogen at 2 L/min
window purge flow rate, mL/min	40
solvent backflush used?	yes
transfer line temp, °C	300
cavity temp, °C	300
water temp., °C	65

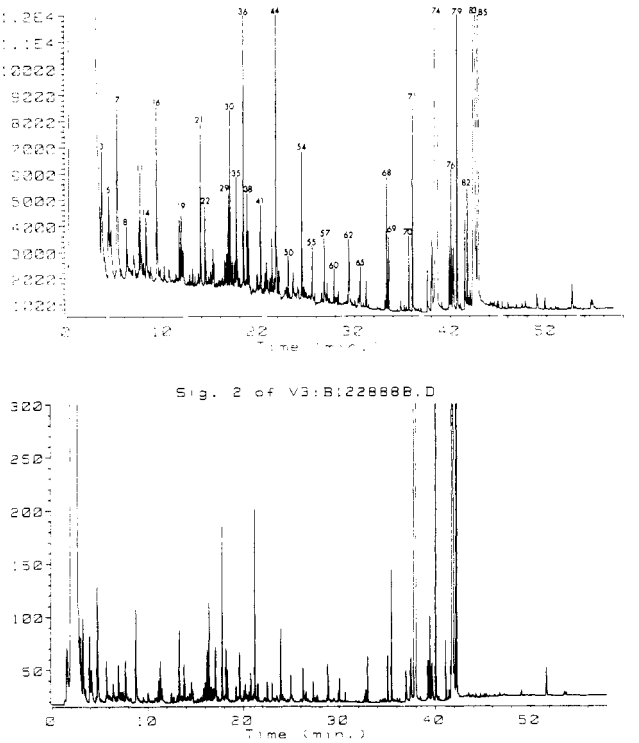

Figure 1. Chromatogram of the carbon-containing volatiles isolated from cured ham by using the AED (top) and the FID (bottom).

Atomic Emission Detection. A prototype HP 5921A atomic emission detector was used in the analysis of the elements C, N, O, and S. Due to the detectable range of the positionable diode array in the AED, C, S, and N were analyzed in the first injection, and O was analyzed in a succeeding injection. Conditions were selected based on those of Fox and Wylie (1989) and are given in Table I.

RESULTS AND DISCUSSION

A typical chromatogram of the volatile ham components obtained under the previously described conditions is shown in Figure 1. Chromatographic peaks from the NPD and FPD (Figures 2 and 3) were referenced to the carbon trace of the FID and the total ion count (TIC) of the MSD by corresponding retention times and relative chromatographic profiles. Selective profiles were scaled identically from 0 to 60 min, and the use of a light box was employed to assure peak assignment. However, such laborious matching was not necessary for the respective AED elemental profiles. As these chromatograms were obtained simultaneously on the same instrument, an overlay plot of the four elements (C, N, O, S) was constructed to scale from the system software. It should be noted that the overlay plot proved invaluable for

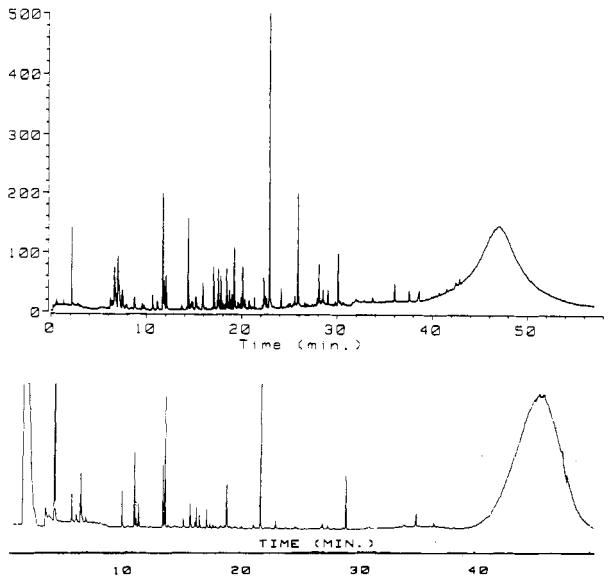

Figure 2. Chromatogram of the sulfur-containing volatiles isolated from cured ham by using the AED (top) and the FPD (bottom).

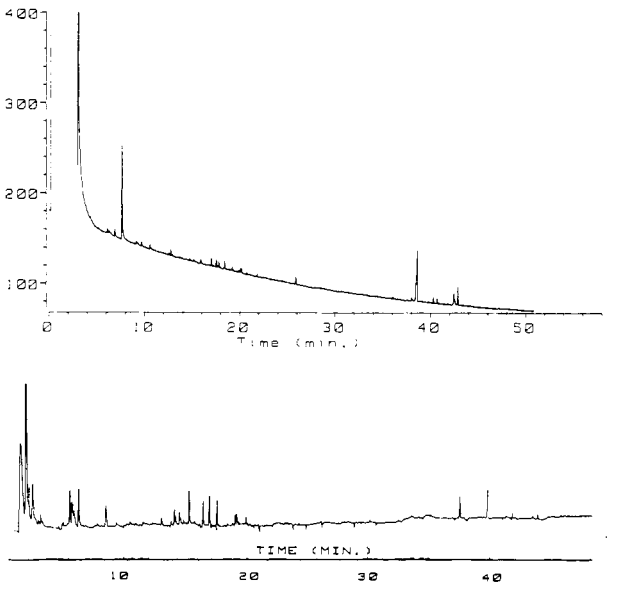

Figure 3. Chromatogram of the nitrogen-containing volatiles isolated from cured ham by using the AED (top) and the NPD (bottom).

compound identification and alleviated confusion of variability of compound retention times due to instrument or parametric deviations, in spite of the same operational conditions being used. Table II summarizes the volatile compounds found in the cured ham.

The baselines of all the AED chromatograms, except from the AED-S, show a large peak in the region of solvent elution. With the selective detectors one would not expect to observe a response to methylene chloride. With the NPD and the FPD this is a result of quenching due to the cooling of the detector due to a large amount of solvent eluting. Likewise, the negatively spiked peaks of the NPD chromatogram indicate quenching due to the elution of large isolate peaks. The AED-N and AED-O chromatograms show a large solvent-like peak as a result of the solvent backflush option in the AED parameters. As the backflush valve opens, a trace of air sweeps in and thus the detection of N_2 and O_2.

Some of the peaks observed by using selective detectors could not be attributed to a compound containing the element being monitored (Table II). The two explanations for this are either the detector is not absolutely selective or there is, in fact, a trace amount of another compound coeluting with the identified compound which does contain the element being monitored. We did not make an effeort to determine which explanation was correct.

Relative Sensitivity. Areas listed for each compound (Table II) are the absolute areas from integration. Values for the AED were generally greater than the respective value from the traditional detector. For the AED-C, the areas were 1–3 orders of magnitude greater than the FID values. Absolute areas were about 1–2 orders of magnitude greater for the AED-S than for the FPD, and the difference for the nitrogen-containing peaks was less pronounced with a difference of about 1 order of magnitude. This is not to suggest that the sensitivity of the AED is better than all traditional detectors outside this sampling. The apparent enhanced sensitivity of the AED remains confined to this example and the instrumentation employed.

Sulfur-Containing Compounds. Seven sulfur-containing compounds were tentatively identified in the Likens-Nickerson extract. Examination of the absolute areas of the AED-S and the FPD for these compounds reveals that the AED response is greater than the FPD response for all compounds. Furthermore, the AED/FPD response ratio ranges from 24 for 1-(methylthio)-propane to 193 for 2-methylthiazole. This variation may be due to the differences in sensitivity and/or response linearity of each detector. To test the linearity of both sulfur detectors, an experiment was conducted by using dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) at concentrations of 0.2 and 4 ng/μL. Considering the sulfur molar ratio for DMDS and DMTS, the AED area response for the DMTS was 1.12 times (which is the expected linear factor) greater than the DMDS within 3% at both concentrations. However, the FPD area response ratio for DMTS/DMDS was in error 18% and 15% of the predicted linear area response at the low and high concentrations, respectively. Thus, the AED is more linear in response to sulfur than the FPD for detecting sulfur-containing compounds.

In five instances a sulfur compound was detected by both the AED and the FPD, whereby a nonsulfurous compound was identified from the mass spectral information. Two of these compounds, 2-acetylfuran and guaiacol, were injected as reference compounds for confirmation of identity. Since both retention properties and mass spectra matched the unknowns, the unidentified sulfur responses are most probably due to the coelution of sulfur compounds below the MSD detection limit.

Noteworthy of the selectivity of the AED is the signal to noise ratio (SNR) of peak 50, dimethyl tetrasulfide. The mass spectral SNR is at the minimal detection limit of 3.5 and was not integrated automatically at the threshold used. However, the SNR of the FPD (153) and AED S-mode (218) of peak 50 demonstrates that both of these detectors are substantially more sensitive than the MSD. The AED was somewhat more sensitive than the FPD.

A compound of particular interest is elemental sulfur, peak 86 (41–52-min elution range). The large Gaussian-shaped peak in the FPD chromatogram was initially thought to be a baseline defect or a problematic FPD detector. When the concentrated system/solvent blank failed to produce the same effect, this suggested that the peak was perhaps from the ham extract. A similar elution response was also observed in the AED S-mode chromatogram. A mass spectral average of the 3-min range centered at 47 min provided the mass spectrum shown in

Table II. Volatile Compounds Identified in Cured Ham

peak compound	RI DB-5	selective area response AED-C	FID	AED-N	NPD	AED-S	FPD	AED-O
2 2-butanone	680	67264	952					
3 ethyl acetate[a]	706	254574	3603					4971
4 unknown	719	21714	289					
5 isovaleraldehyde[a]	737	244344	3252					1541
6 2-methyl-1-nitropropane	745	223289	757				534	414
7 2,3-pentanedione[a]	774	536591	5169					6250
8 1-(methylthio)propane[a]	812	99333	1595			1004	41	411
9 2,3,3-trimethylpentane	834		407	372		7800	102	
10 2-methylthiazole[a]	851	105532		3557	327	2118	11	510
11 3-methyl-2-buten-1-ol	854	117326	1399					1689
12 unknown	861	51911	570					
13 4-methyl-2,3-dihydrofuran	866	41929	493			1181		
14 hexanal[a]	876	135394	1819					452
15 methylpyrazine[a]	894	28042		216	25			
16 furfural[a]	911	365437	3744		243	588		3135
17 unknown	949	36885	547	166		537	45	
18 2-methyl-3-pentanethiol[a]	981	92407	764			7761	86	
19 3-methyl-2-cyclopenten-1-one[a]	987	117733	1451				12	
20 2-acetylfuran[a]	993	45972	357			2238	31	
21 benzaldehyde[a]	1047	231376	2681				72	1066
22 dimethyl trisulfide[a]	1059	128672				6103	150	460
23 phenol[a]	1068	63566	1399		35	1059		130
24 1-ethylcyclohexene	1080	16584	365					
25 unknown	1086	34756	765		115	630	10	
26 unknown	1092	157701	368					
27 unknown	1127	40129	565				7	
28 isomer of 25	1131	59595	815				24	
29 2-phenylacetaldehyde[a]	1137	128884	1727				16	532
30 *o*-cresol	1146	227536	2937	313	177	2231		822
31 unknown	1151	26432	433					
32 3,4,5-trimethyl-2-cyclopenten-1-one	1158	33285	325					
33 unknown	1161	10895	285					
34 acetophenone[a]	1163	61280	135			2399		
35 *m*-cresol	1168	188587	1746		240			759
36 guaiacol[a]	1187	474049	5453	388	194	2743	21	3368
37 *p*-cresol[a]	1198						6	446
38 unknown	1201	137316	1776					
39 2,6-dimethylphenol[a]	1205	76637	1032			901		266
40 2-methoxyphenol[a]	1234							
41 2-ethylphenol	1237	25231	610		72			
42 unknown	1241	12997					67	
43 2,3-dimethylphenol	1249	176496	2688	182				692
44 2-methoxybenzaldehyde	1263	24286	278		31			198
45 3,4-dimethylphenol	1271	66177	453			403		175
46 2,5-dimethylphenol	1282	33597	412					
47 1,4-dimethoxybenzene	1286	72790	906			622		466
48 4-methylguaiacol	1301	485846	5957					3291
49 3,4,5-trimethoxyphenol	1313	47559	659					
50 dimethyl tetrasulfide	1334		735			22217	169	
51 anisyl methyl ester	1348	57300	481					299
52 geraniol	1356	33116						
53 unknown	1356							
54 4-ethylguaiacol[a]	1397	205850	2555				9	1317
55 4-vinylguaiacol	1436	81915	641			582		493
56 methyl thiazoleethanol acetate	1441	1211		350		7718		
57 4-allylguaiacol[a]	1484	91094	896					526
58 unknown	1487							
59 5-propylguaiacol	1495	35717	267					151
60 unknown	1526	46601	581			424		
61 *cis*-isoeugenol	1541	18086	213				7	120
62 geranyl acetate	1586	78226	1820			366		
63 unknown						3436		
64 unknown	1632	15177	331					
65 unknown	1636	52392	731					
66 BHT	1658	36795	329					
67 unknown	1746							
68 unknown	1751	174009	392					
69 dodecanal[a]	1761	93939	1443					215
70 2-pentadecanone	1756	97711	1471					243
71 tetradecanal[a]	1874	277521	4139			1393	22	208
72 pentadecanal	1946	52857	1150		153	891	6	467
73 unknown	1969	178717	2497	151				258
74 hexadecanal[a]	1998	6028667	287129	1603		1459		7236
75 unknown	2059	99368	1843					
76 unknown	2069	194958	2779		152			375

Table II (Continued)

peak compound	RI DB-5	selective area response AED-C	FID	AED-N	NPD	AED-S	FPD	AED-O
77 9-octadecenal	2077	82315	2228					
78 4-methylpentadecan-2-one	2081	66203		227				
79 octadecanal[a]	2101	927242	13572	190				1490
80 dibutyl phthalate	2156	165151	1954					1013
81 2-octadecenal	2175	24305	369		33			
82 9,12-octadecadienal	2186	75440	1255					543
83 9,12-octadecadienol	2195	3431463	40345	251		108		6149
84 9,17-octadecadienol	2199	1099942	17741					5341
85 octadecandienol isomer	2217	2501999	55327	825		621		5727

[a] Compound confirmed by reference injection.

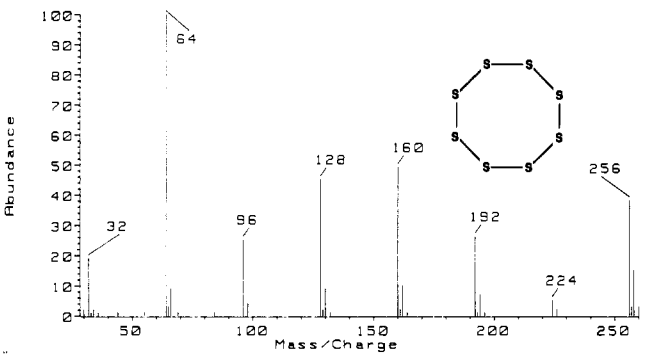

Figure 4. Mass spectrum of the cyclooctasulfur in cured ham.

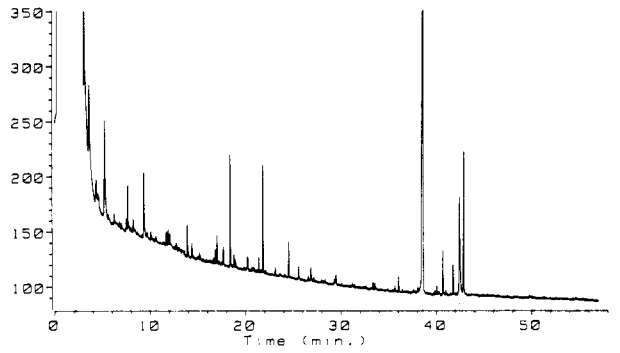

Figure 5. Chromatogram of the oxygen-containing volatiles isolated from cured ham by using the AED.

Figure 4. The presence of orthorhombic cyclooctasulfur, the thermodynamically stable allotropic form (Meyer, 1964), was confirmed by injection of elemental sulfur in methylene chloride. From a standard curve, it was estimated to be present at 1–2 ppb in the original ham sample. The poor chromatographic peak shape appears to be due to the nonvolatile nature of the compound. The source of the elemental sulfur in this ham sample is unknown.

Nitrogen-Containing Compounds. Only three N-containing compounds were identified in the cured-ham sample. Of these, 2-methylthiazole and 2-methylpyrazine were detected by both the AED and the NPD. The AED area responses exceeded those of the NPD (factors of 11 and 9, respectively). While an additional 11 peaks were detected by using the AED-N (Figure 3) and 13 by using the NPD (Figure 3), only 2 of these were detected by both detectors. As noted above, none of these compounds were identified by MS as containing nitrogen.

Oxygen-Containing Compounds. The oxygen chromatogram (Figure 5) from the AED is most useful in characterizing cured-ham flavor. Unlike many cooked meat flavors, ham lacks the numerous S- or N-containing heteroatomic compounds. Cured-ham flavor has been described as being smoky or cured. Sodium nitrite ($NaNO_2$) added to the cure solution has been considered responsible in part for the "cured" flavor in ham (MacDonald et al., 1980b; Mottram, 1984). However, Price and Greene (1978) concluded from results of a 13-member sensory panel that curing without $NaNO_2$ would still produce a ham with a cured flavor provided that NaCl was included in the formulation.

The majority of O-containing volatile compounds from this ham are also found in hardwood and softwood smoke condensate (Maga, 1987). Phenolic compounds are well noted for their smoky qualities. Maga and Fapojuwo (1986) have pointed out the contribution of carbonyls to smoke flavor. Eighteen phenolic compounds were identified and most probably constitute the smoky aroma of cured ham. Guaiacol and 4-methylguaiacol were the predominant phenolic compounds and most likely make a major contribution to the smoky character of cured ham. Guaiacol and 4-methylguaiacol have low odor thresholds (0.021 and 0.09 ppm) and low taste thresholds (0.013 and 0.065 ppm), respectively, in water (Wasserman, 1966). These two phenolics along with *o*-cresol, *m*-cresol, 4-ethylguaiacol, and 2,6-dimethylphenol constituted 72% of the phenolic content in the ham sample or 7.5% of the total volatile ham composition on the basis of the FID chromatogram. Short-chain aldehydes, usually formed via fat oxidation and indicators of off-flavor in meats (Reineccius, 1979), were minimal, probably due to the inhibition effect of nitrite (MacDonald et al., 1980a). However, long-chain aldehydes (C_{12}–C_{18}) dominated the chromatogram, contributing nearly 35% of the total FID area response. Several O-containing compounds tentatively identified by MS were not detected by the AED. Those compounds not detected were most likely below the detection level of the AED. Of the four elements profiled in the AED study, oxygen showed the lowest sensitivity and highest background noise. Although the sensitivity of the prototype AED in oxygen mode appeared to be lacking in comparison to the other elements studied, recent operational developments by the manufacturer have enhanced oxygen detection by a factor of 4–5.

CONCLUSIONS

The importance of using GC selective detectors with the GC–MS for the identification of unknown volatiles in a complex flavor isolate, such as ham, has been reaffirmed. The presence of heteroatomic compounds was more easily discerned with the aid of both the AED and the traditional detectors. For detection of C and S, the AED provied to be more sensitive than the FID or the FPD. Although the AED appeared slightly more sensitive than the NPD in nitrogen detection, the sensitivity comparison is not conclusive because too few N-containing peaks were present. As the cured-ham sample was dominated by O-containing compounds, the AED-O chromatogram was useful in their discrimination. Overall, the AED proved

valuable for the complex flavor analysis and greatly facilitated mass spectral identification.

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Registry No. BHT, 128-37-0; 2-butanone, 78-93-3; ethyl acetate, 141-78-6; isovaleraldehyde, 590-86-3; 2-methyl-1-nitropropane, 625-74-1; 2,3-pentanedione, 600-14-6; 1-(methylthio)propane, 3877-15-4; 2,3,3-trimethylpentane, 560-21-4; 2-methylthiazole, 3581-87-1; 3-methyl-2-buten-1-ol, 556-82-1; 4-methyl-2,3-dihydrofuran, 34314-83-5; hexanal, 66-25-1; methylpyrazine, 109-08-0; furfural, 98-01-1; 2-methyl-3-pentanethiol, 1639-04-9; 3-methyl-2-cyclopenten-1-one, 2758-18-1; 2-acetylfuran, 1192-62-7; benzaldehyde, 100-52-7; dimethyl trisulfide, 3658-80-8; phenol, 108-95-2; 1-ethylcyclohexene, 1453-24-3; 2-phenylacetaldehyde, 122-78-1; *o*-cresol, 95-48-7; 3,4,5-trimethyl-2-cyclopenten-1-one, 55683-21-1; acetophenone, 98-86-2; *m*-cresol, 108-39-4; guaiacol, 90-05-1; *p*-cresol, 106-44-5; 2,6-dimethylphenol, 576-26-1; 2-ethylphenol, 90-00-6; 2,3-dimethylphenol, 526-75-0; 2-methoxybenzaldehyde, 135-02-4; 3,4-dimethylphenol, 95-65-8; 2,5-dimethylphenol, 95-87-4; 1,4-dimethoxybenzene, 150-78-7; 4-methylguaiacol, 93-51-6; 3,4,5-trimethoxyphenol, 642-71-7; dimethyl tetrasulfide, 5756-24-1; anisyl methyl ester, 121-98-2; geraniol, 106-24-1; 4-ethylguaiacol, 2785-89-9; 4-vinylguaiacol, 7786-61-0; 4-allylguaiacol, 97-53-0; 5-propylguaiacol, 58539-27-8; *cis*-isoeugenol, 5912-86-7; geranyl acetate, 105-87-3; dodecanal, 112-54-9; 2-pentadecanone, 2345-28-0; tetradecanal, 124-25-4; pentadecanal, 2765-11-9; hexadecanal, 629-80-1; 9-octadecenal, 5090-41-5; 4-methylpentadecan-2-one, 129216-51-9; octadecanal, 638-66-4; dibutyl phthalate, 84-74-2; 2-octadecenal, 56554-96-2; 9,12-octadecadienal, 26537-70-2; 9,12-octadecadienol, 1577-52-2; 9,17-octadecadienol, 129216-52-0; octadecadienol, 12767-10-1; cyclooctasulfur, 10544-50-0.