Studies on Meat Flavor. 3. A Novel Method for Trapping Volatile Components from Uncured and Cured Pork

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Volatile components from uncured and cured pork were trapped onto a solid adsorbent (Florisil cartridge) and in an organic solvent (pentane) using the novel nitrogen purge-and-trap (NPT) technique. A total of 32 compounds not previously reported in the meat-flavor literature were identified. It was also evident that the meat-flavor concentrates prepared by the NPT method showed the presence of heterocyclic and phenolic constituents not detected in the aroma concentrates previously prepared by us using continuous steam distillation–extraction. Of the 26 compounds that were found only in the uncured-pork aroma concentrates, 2-methylhexanal, tetrahydro-*cis*-2,4-dimethylfuran, 3-propyl-1*H*-1,2,4-triazole, 4-methyl-1-decene, nonylcyclopropane, 5-propyldecane, 1,3-dimethoxybenzene, (1,1-dimethylethyl)-4-methoxyphenol, 3-amino-5,6-dimethyltriazolo[4,3-*a*]pyrazine, 2-butylphenol, and bis(2-ethylhexyl) phthalate were newly identified. In the present investigation only the two newly identified components 2-methylcyclopentanol and 2-butyl-2-octenal were identified uniquely in the aroma concentrates of cured pork.

INTRODUCTION

The complex composition of cooked meat flavor is possibly the result of a number of volatile components differing in chemical nature and varying in concentration. Meat from four common domestic species is generally consumed—beef, pork, lamb, and poultry. Each species has a characteristic and recognizable flavor of its own, and yet they have a basic element common in them. In each case the flavor is quickly recognized as "meaty". In addition, there is another type of flavor which is typical of cured meats, i.e., meat treated with nitrite and salt, as well as with other less important curing agents. Preslaughter factors and post-mortem handling conditions contribute to additional secondary effects. It is also believed that a single compound or a particular class of compounds alone cannot explain the complete flavor spectrum of cooked meat (Chang and Peterson, 1977).

A great deal of effort has been made in the past three decades to determine the chemical nature of meat flavor, and a number of outstanding reviews on the progress of meat flavor chemistry have been published (Gray et al., 1981; MacLeod and Seyyedain-Ardebili, 1981; Ramaswamy and Richards, 1982; Moody, 1983; Baines and Mlotkiewicz, 1984; Shahidi et al., 1986; Rhee, 1989). Odor descriptions of some of the compounds that may be contributing to the meat aroma have also been provided (Shahidi et al., 1986).

With the aid of gas chromatographic retention times and infrared spectroscopic analysis, Ockerman et al. (1964) identified six aldehydes, three ketones, five acids, and the bases ammonia and methylamine in the volatiles of dry-cured ham. They also mentioned the presence of hydrogen sulfide and other sulfides but failed to give a quantitative account of the identified components. Cross and Ziegler (1965) made an attempt to characterize the volatiles of uncured and cured ham. Though the qualitative composition was almost identical, there were distinct quantitative differences in the compositions of these samples. The differences were particularly evident for the lipid oxidation products pentanal and hexanal that were the major components of uncured ham but were either absent or present only in small traces in the cured-ham volatiles. They further proposed that the cured-meat flavor components were derived from nontriglyceride precursors and that carbonyl compounds were not responsible for cured-meat flavor.

Using a vacuum distillation system, Lillard and Ayres (1969) identified a total of 42 compounds in country-cured ham. Of these, 24 were aldehydes. Though they were successful in identifying certain components that were not detected in uncured ham, these compounds, however, had already been reported in the volatiles of cooked mutton, chicken, and beef (Hornstein and Crowe, 1960, 1963; Jacobson and Koehler, 1963; Minor et al., 1965). Piotrowski et al. (1970) reported that the precursors of the basic meaty aroma from cured ham were water-extractable. Gas chromatographic analyses of the volatiles developed on heating of ham diffusates were complex, and showed some qualitative differences. However, they also failed to identify any single component that could be responsible for the basic meaty aroma. Ho et al. (1983) isolated volatile flavor compounds from fried bacon and identified a large number of components that were probably derived during the smoking and frying operations. More recently, Berdague et al. (1991) have isolated the volatile components of dry-cured ham by vacuum distillation. A total of 76 compounds were identified, of which two heterocyclic nitrogen compounds and two lactones were identified in addition to a large number of carbonyls, hydrocarbons, and alcohols. Olfactory tests of the separated components indicated that some of the peaks with a strong smell associated with dry-cured ham remained unidentified.

In our previous attempts, we have provided quantitative information on the carbonyls and hydrocarbons present in the aroma concentrates of uncured and nitrite-cured pork isolated by the conventional steam distillation and continuous steam distillation–extraction (SDE) methods (Ramarathnam et al., 1991a). Using the SDE technique, we have also isolated, identified, and quantitated volatiles from uncured and nitrite-cured beef and chicken and provided a summary of those carbonyl components that

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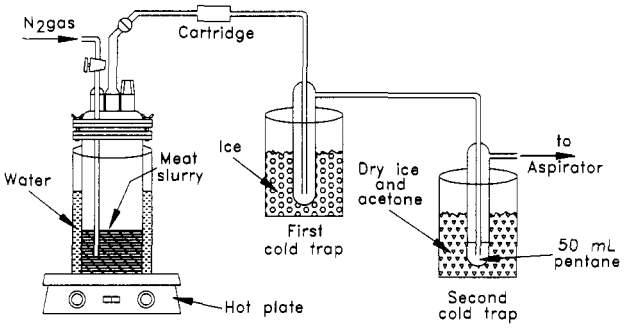

Figure 1. Schematic presentation of the nitrogen purge-and-trap (NPT) assembly.

may be responsible for the species differences (Ramarathnam et al., 1991b). Solid adsorbents such as Tenax have been successfully used for trapping heterocyclic constituents of roast beef, beef flavor concentrate, and beef meat powder (Vercellotti et al., 1987). With a similar approach and suitable modifications, we have continued our attempts to identify the key components that are responsible for the "cured-meat" aroma or the basic "meaty aroma" of cooked meat. We have isolated the volatiles from cured and uncured pork, using the nitrogen purge-and-trap (NPT) method, which is a milder technique than the ones used by us previously.

MATERIALS AND METHODS

Meat. Fresh pork loin was purchased from a local market and used immediately. After careful removal of the excess depot fat, the meat was deboned manually, cut into small pieces, and then ground twice using an Oster meat grinder (0.476-cm grind plate, Model 990-68).

Proximate Analysis. The fat content of cooked-meat samples was determined by the Soxhlet extraction method (AOAC, 1984) and their moisture content by oven drying at 102 ± 1 °C for a period of 18 h. The cooked pork in all experiments contained 69.7 ± 0.3% water and 10.8 ± 0.2% fat.

Reagents. Anhydrous sodium sulfate, sodium chloride, and sodium nitrite, all of analytical grade, and sodium ascorbate (USP grade) were purchased from BDH Chemicals (Toronto, ON). Sodium tripolyphosphate (food grade) was obtained from ERCO Industries, Ltd. (Toronto, ON), while *n*-pentane (spectral grade) was purchased from Caledon Laboratories Ltd. (Toronto, ON). Gas chromatographic standards hexanal (99%) and decanal (95%) were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI).

Cooking. Ground pork (250–400 g) was placed in a 2-L beaker. Distilled water was added so as to attain a meat-to-water ratio of 4:1 (w/w) (Ramarathnam et al., 1991a), and the contents were heated in a thermostated water bath, maintained at 85 °C, with intermittent stirring to facilitate uniform cooking. Heating was carried out until the meat slurry attained a constant temperature of 73 °C and held at that temperature for 10 min.

Curing of the ground meat was carried out simultaneously in another 2-L beaker by adding sodium chloride (2% w/w), sugar (1.5%, commercial sucrose), sodium ascorbate (0.05% w/w), sodium tripolyphosphate (0.3% w/w), and sodium nitrite (150 ppm). The cooked-meat (uncured and cured) samples were cooled to room temperature and stored in a refrigerator at 4 °C for 24 h. Prior to purging, distilled water was added to the cooked-meat samples (1:1 w/w), and the samples were thoroughly ground by using a Braun MR 30 hand blender.

Nitrogen Purge-and-Trap (NPT) Technique. A schematic representation of the NPT technique is illustrated in Figure 1. The apparatus comprised a 2-L extraction jar with a removable three-neck glass lid. One of the necks served as the inlet for the purging gas, oxygen-free nitrogen (Canadian Oxygen Ltd., Toronto, ON). The second neck supported the solid adsorbent, a Sep-Pak C_{18} Florisil cartridge (Waters Associates, Milford, MA), that was firmly attached to the glass tube with the help of Teflon tubing. The third neck was used as a safety outlet to prevent damage to the glassware in case of accidental increase in pressure due to accumulation of ice in the second cold trap. This neck was kept closed by use of a glass stopper that was fastened with a tape just sufficiently tight enough to prevent the loss of volatiles.

The cooked meat (250–400 g) was homogenized with 500 mL of distilled water using a Polytron homogenizer (Brinkmann Instruments, Model PT 10/35) until a free-flowing meat slurry was obtained. The slurry was placed in the extraction jar, where it was constantly maintained at 65 ± 5 °C, and stirred with the help of a magnetic hot plate/stirrer. A slow stream of oxygen-free nitrogen gas was passed through the meat slurry so as to purge the volatiles from the headspace. The effluent stream was made to pass through the Florisil cartridge, which was connected to a cold trap maintained at 4–5 °C with crushed ice, and finally through the second cold trap containing 50 mL of *n*-pentane maintained at −60 °C with the help of an acetone–dry ice mixture. The second cold trap was further connected to an aspirator. This mode of fractionation was designed so as to adsorb some of the less volatile components onto the Florisil cartridge, condense excess water and water-soluble components in the first cold trap, and absorb the volatile components, which were not adsorbed earlier onto the Florisil cartridge, in *n*-pentane in the second cold trap.

The volatiles were collected over a 10-h purging period. At the end of the experiment, the cartridge was flushed with *n*-pentane (2 × 5 mL) and the pentane extract dried over anhydrous sodium sulfate and concentrated by passing a slow stream of oxygen-free nitrogen to a final volume of around 250 μL. Aroma concentrates from the first cold trap were prepared in a similar way by extracting the components from the aqueous condensate using *n*-pentane (2 × 10 mL). The pentane extracts from the first cold trap and second cold trap were dried and concentrated as described above. A blank run with distilled water was carried out exactly as described above to ascertain whether any artifacts formed during the stages of trapping and elution of the volatiles.

Gas Chromatography–Mass Spectrometric (GC–MS) Analysis. A Hewlett-Packard Model HP 5880A gas chromatograph equipped with a DB-5 capillary column [0.13 mm (i.d.) × 30 m] and coupled to a Hewlett-Packard Model HP 5987A mass spectrometer was used. Analysis was carried out by using helium as the carrier gas, with the column temperature maintained initially at 30 °C for 2 min and then programmed from 30 to 280 °C at a rate of 10 °C/min, where it was held for 3 min. The source, injector, analyzer, and transfer-line temperatures were 200, 250, 300, and 300 °C, respectively. The ionization voltage applied was 70 eV. Mass spectra obtained were compared with those of known compounds in the NBS (now NIST) library by using an HP 1000E series computer. Kovats retention indices were calculated against C_7–C_{25} *n*-paraffins as references (Jennings and Shibamoto, 1980). The identification of the individual constituents was based on the Kovats retention indices and the MS data.

Quantitation of the Individual Components. Quantitative analysis of the individual constituents identified in the different fractions of the uncured- and cured-pork aroma concentrates was carried out by spiking the cured meat with hexanal (12.7 mg/mL) in *n*-pentane) and the uncured meat with decanal (11.1 mg/mL in *n*-pentane). Hexanal and decanal were used as the internal standards on the basis of our preliminary observations discussed elsewhere (Ramarathnam et al., 1991b). All samples were analyzed in duplicate. From the peak areas of different known concentrations of hexanal and decanal, the amount of individual constituents present in uncured and cured meat was calculated and expressed in terms of milligrams per kilogram of meat.

RESULTS AND DISCUSSION

Formation of Artifacts. Blank runs were made with distilled water to ascertain if the adsorption and elution steps contributed to the formation of undesirable constituents. Preliminary runs indicated that presence of trace amounts of volatile components eluted from the cartridge materials. Pretreatment of the cartridge with distilled *n*-pentane followed by drying before adsorption

Table I. Compounds Identified in the Aroma Concentrates of Pork, Isolated by the Nitrogen Purge-and-Trap Method

RT, min	compound	Kovats index[a]	cartridge,[c] mg/kg		1st trap,[c] mg/kg		2nd trap,[c] mg/kg	
			uncured	cured	uncured	cured	uncured	cured
3.59	2-methyl-3-hexanone[b]	734	–[d]	0.08	0.13	0.11	–	–
3.64	2,4-dimethylhexane	736	–	–	–	–	0.50	0.33
4.16	methylbenzene	763	0.90	1.20	2.15	1.48	1.92	2.20
4.45	2,2,4-trimethylhexane	777	0.80	1.04	1.34	0.42	1.57	1.88
4.65	hexanal	787	–	–	0.11	–	7.28	–
5.08	2,3,5-trimethylhexane	810	–	0.15	0.19	0.07	0.26	0.26
5.47	4-ethyl-1-methylhexane[b]	831	–	0.07	0.10	0.04	0.14	0.21
6.11	unidentified	866	–	–	0.25	–	–	–
6.18	2,2,5,5-tetramethylhexane[b]	870	–	0.08	–	0.11	0.14	0.15
6.28	2,2,4-trimethylheptane	875	–	0.17	0.22	0.23	0.24	0.36
6.39	unidentified	881	–	0.10	0.13	0.11	0.15	0.20
6.50	2-methylhexanal[b]	887	–	–	–	–	0.10	–
6.66	heptanal	896	–	–	0.07	0.06	0.50	0.15
7.04	3,3-dimethylpentane[b]	917	–	–	0.03	0.03	–	–
7.83	unidentified	961	–	–	0.05	–	0.11	0.08
8.23	7-octen-4-ol[b]	983	1.50	0.09	1.10	–	0.11	–
8.41	(*E*,*E*)-2,4-nonadienal	993	–	–	–	–	0.13	–
8.60	tetrahydro-*cis*-2,4-dimethylfuran[b]	1004	0.22	–	–	–	0.11	–
9.09	unidentified	1033	–	0.09	–	–	–	–
9.20	unidentified	1039	0.62	–	–	–	–	–
9.38	unidentified	1050	0.22	–	–	–	–	–
9.58	(*E*)-2-octenal	1062	0.25	–	–	–	–	–
9.84	octanol	1078	4.20	0.15	–	–	–	–
10.38	*trans*-1,2-dimethylcyclopentane[b]	1110	1.31	0.58	–	–	0.10	0.08
11.32	3-propyl-1*H*-1,2,4-triazole[b]	1172	0.25	–	–	–	–	–
11.74	1,1-dimethylcyclopentane	1199	0.70	–	–	–	–	–
11.94	2-methylundecane[b]	1213	0.15	0.12	–	–	–	–
12.02	2-methylcyclopentanol[b]	1217	–	0.17	–	–	–	–
12.20	decanal	1228	0.66	–	–	–	–	–
12.93	2-undecanone	1274	0.16	–	–	–	–	–
13.00	4-methyl-1-decene[b]	1278	0.18	–	–	–	–	–
13.11	nonylcyclopropane[b]	1285	1.16	–	–	–	–	–
13.23	1-nonen-3-ol	1293	0.20	–	–	–	–	–
13.50	5-propyldecane[b]	1311	1.52	–	–	–	–	–
13.76	(*E*,*E*)-2,4-decadienal	1330	0.18	–	–	–	–	–
14.15	1,3-dimethoxybenzene[b]	1358	–	–	0.06	–	–	–
14.40	2-undecenal	1376	0.24	–	–	–	–	–
14.58	2-butyl-2-octenal[b]	1389	–	2.33	–	–	–	–
14.88	2,3,5-trimethyldecane[b]	1411	0.55	0.21	–	–	–	–
15.01	dodecanal	1420	0.19	–	–	–	–	–
15.79	4-pentylbenzaldehyde	1476	0.21	–	–	–	–	–
15.93	(1,1-dimethylethyl)-4-methoxyphenol[b]	1486	–	–	0.09	–	–	–
16.00	(*E*)-9-octadecene	1491	0.21	–	–	–	–	–
16.21	pentadecane	1500	0.45	–	–	–	–	–
16.37	tridecanal	1518	0.33	–	–	–	–	–
17.49	hexadecane	1600	–	0.19	0.14	0.03	–	–
17.64	tetradecanal	1618	0.29	–	–	–	–	–
18.86	4-(2,2,3,3-tetramethylbutyl)phenol[b]	1725	0.43	0.13	0.28	0.08	0.12	0.08
18.95	4-nonylphenol[b]	1733	–	0.13	0.37	0.10	0.16	0.11
19.03	1,3-dihydro-2*H*-imidazo[4,5-*b*]pyridin-2-one[b]	1740	–	0.10	0.28	0.08	0.11	0.08
19.08	2,4,6-trimethylpyridine[b]	1744	–	–	0.11	0.03	–	–
19.14	4-(1-methylpropyl)phenol[b]	1750	–	–	0.16	0.06	–	–
19.24	3-amino-5,6-dimethyltriazolo[4,3-*a*]pyrazine[b]	1758	–	–	0.03	–	–	–
19.30	3-methyl-1,2-benzisothiazole[b]	1763	–	–	0.11	0.04	–	–
19.40	4-ethyl-2,6-dimethylpyridine[b]	1772	–	0.08	0.25	0.07	0.11	0.07
19.50	unidentified	1780	–	0.08	0.06	–	–	–
19.54	2-butylphenol[b]	1784	–	–	0.04	–	–	–
19.67	2,4-diphenyl-1*H*-pyrrole[b]	1790	–	–	0.07	0.04	–	–
20.04	hexadecanal	1830	1.75	1.06	–	–	–	–
20.69	(*E*)-5-octadecene[b]	1894	–	0.08	–	–	0.09	0.07
21.62	bis(2-methoxyethyl) phthalate[b]	1986	0.15	0.46	1.65	0.55	0.62	0.43
22.87	methyl 11,14-eicosadienoate[b]	2118	–	0.08	0.50	0.26	0.15	0.10
26.90	bis(2-ethylhexyl) phthalate[b]	2563	–	–	0.04	–	0.09	–

[a] Kovats indices calculated for the DB-5 capillary column of the GC–MS system. [b] Newly identified. [c] Average of two determinations. [d] –, not detected.

proved to be effective in the removal of such volatiles already present in the cartridge. Test samples obtained from the first and second cold traps during blank runs did not show the presence of any undesirable volatiles. In the preparation of the aroma concentrates for GC–MS analysis, prewashed cartridges were used.

Gas Chromatography–Mass Spectrometric (GC–MS) Analysis. The components identified in the three aroma concentrates of uncured and nitrite-cured pork prepared by the NPT method, as described above, are listed in Table I. In all, 63 compounds were detected in the different fractions of the aroma concentrates, 32 of them being identified and reported for the first time as meat-flavor constituents. It was observed that the aroma concentrates isolated from uncured and cured pork had 60 and 34 components, respectively. Of the total number

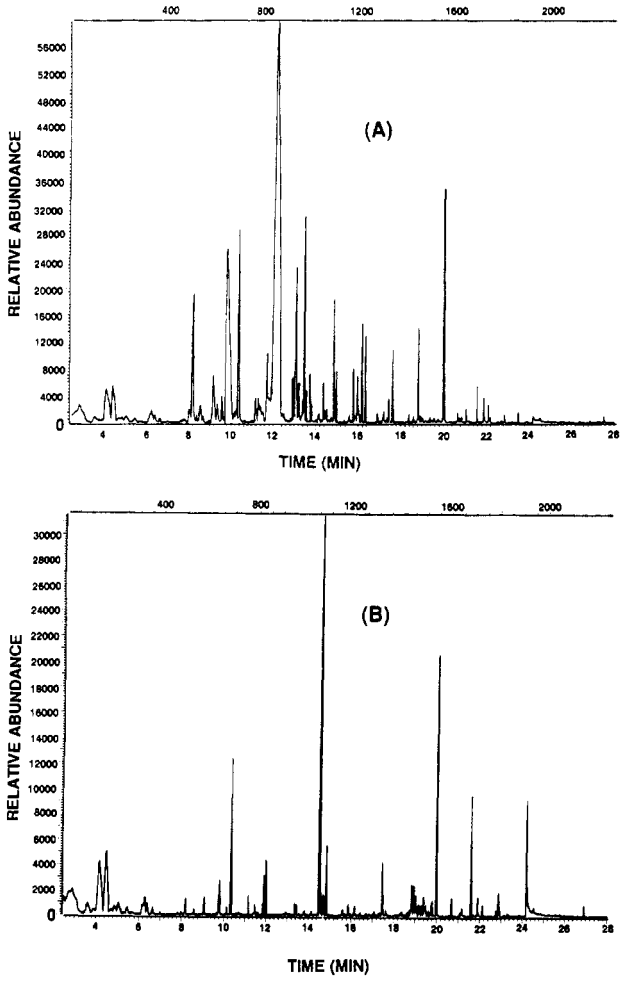

Figure 2. Total ion chromatograms (TICs) of (A) uncured-pork and (B) cured-pork flavor concentrates isolated by the NPT method (Sep-Pak C_{18} cartridge).

of components identified, 20 were hydrocarbons, 16 carbonyls, 4 alcohols, 5 phenols, 3 esters, and 8 heterocyclics. In our earlier investigation on pork (Ramarathnam et al., 1991a), we had reported that the uncured- and cured-pork aroma concentrates prepared by the SDE method resolved into 77 and 72 compounds, respectively. Of those, 50 were identified as hydrocarbons, 37 as carbonyls, 6 as carboxylic acids, and 2 as alcohols. The extent of variation in the qualitative composition of components identified in uncured- and cured-pork aroma concentrates prepared according to the NPT and SDE methods has clearly demonstrated the mild nature of the NPT method, which has prevented the formation of certain breakdown products.

Analysis of the Cartridge Fraction. The total ion chromatograms (TICs) of the separated constituents trapped onto the C_{18} Florisil cartridge are illustrated in Figure 2. The adsorption and collection of volatiles generated during the NPT method were done in such a way that the less volatile carbonyls and hydrocarbons, in the retention time range 8.00–20.00 min, would be adsorbed onto the Florisil cartridge. As expected, a wide variation in the qualitative and quantitative composition of the volatiles adsorbed onto the cartridge was observed between uncured and cured pork. Of the 41 compounds identified in this fraction, 12 were carbonyls, 17 hydrocarbons, 4 alcohols, 4 heterocyclic compounds, 2 esters, and 2 phenols.

The concentration of 7-octen-4-ol (RT 8.23 min) was more than 15 times higher in the uncured pork, where it was present to the extent of 1.50 mg/kg, while the concentration of this component in the cured pork was only 0.09 mg/kg. Secondary alcohols with hydroxyl groups in the 3-position have been implicated to be responsible for mushroom-like, grassy, and ethereal flavor notes (Persson and von Sydow, 1973; Peterson and Chang, 1982). This compound was also detected in the aroma fractions of uncured pork extracted from the first and second cold traps, while it was absent in the corresponding samples of cured pork. The concentration of octanol (RT 9.84 min) was also higher in the uncured pork (4.20 mg/kg) than in cured pork (0.15 mg/kg). This component was absent in the aroma fractions of both cured pork and uncured pork, isolated from the two cold traps.

A small group of volatiles in the retention time range 12.00–14.00 min was detected only in the uncured-pork aroma concentrate. These compounds included decanal (RT 12.20 min), 2-undecanone (RT 12.93 min), 4-methyl-1-decene (RT 13.00 min), nonylcyclopropane (RT 13.11 min), 1-nonen-3-ol (RT 13.23 min), 5-propyldecane (RT 13.50 min), (*E*,*E*)-2,4-decadienal (RT 13.76 min), and 2-undecenal (RT 14.40 min). Some of the other more volatile constituents that were detected only in the uncured-pork aroma concentrate were the heterocyclic compounds tetrahydro-*cis*-2,4-dimethylfuran (RT 8.60 min) and 3-propyl-1*H*-1,2,4-triazole (RT 11.32 min), the carbonyl compound (*E*)-2-octenal (RT 9.58 min), and the hydrocarbon 1,1-dimethylcyclopentane (RT 11.74 min).

Some of the other carbonyls and hydrocarbons already known in the meat-flavor literature but identified uniquely in the volatiles of uncured pork adsorbed onto the cartridge included dodecanal (RT 15.01 min), 4-pentylbenzaldehyde (RT 15.79 min), (*E*)-9-octadecene (RT 16.00 min), pentadecane (RT 16.21 min), tridecanal (RT 16.37 min), and tetradecenal (RT 17.64 min).

2-Methyl-3-hexanone (RT 3.59 min), 2,3,5-trimethylhexane (RT 5.08 min), 4-ethyl-1-methylhexane (RT 5.47 min), 2,2,5,5-tetramethylhexane (RT 6.18 min), 2,2,4-trimethylheptane (RT 6.28 min), 2-methylcyclopentanol (RT 12.02 min), 2-butyl-2-octenal (RT 14.58 min), hexadecane (RT 17.49 min), 4-nonylphenol (RT 18.95 min), 1,3-dihydro-2*H*-imidazo[4,5-*b*]pyridin-2-one (RT 19.03 min), 4-ethyl-2,6-dimethylpyridine (RT 19.40 min), (*E*)-5-octadecene (RT 20.69 min), and methyl 11,14-eicosadienoate (RT 22.87 min) were detected only in the aroma concentrate of cured pork.

Analysis of the First Cold Trap Extract. The first cold trap, maintained at 4–5 °C with crushed ice, was mainly used with the intention of condensing water vapor and the water-soluble components (Figure 1). The TICs of aroma concentrates of uncured and cured pork, prepared from the first cold trap, are shown in Figure 3. It was interesting to note that this fraction showed the presence of 10 hydrocarbons, only 2 carbonyls, 1 alcohol, all of the 5 phenols and 3 esters identified in this investigation, and 6 of the 8 heterocyclic components. Organoleptic evaluation of the contents of the first cold trap strongly indicated the presence of the components responsible for the desirable meaty aroma of cooked meat.

Except for the distinct difference in the content of 7-octen-4-ol (RT 8.23 min), the flavor profiles of the aroma concentrates of uncured and cured pork, prepared from the first cold trap, were qualitatively similar. Mass spectrometric analysis of the aroma concentrates showed that the aroma fraction trapped in this cold trap was richer in the heterocyclic constituents (Table I). Less volatile components that were present only in the uncured-meat aroma concentrate prepared from this fraction included 1,3-dimethoxybenzene (RT 14.15 min), (1,1-dimethyleth-

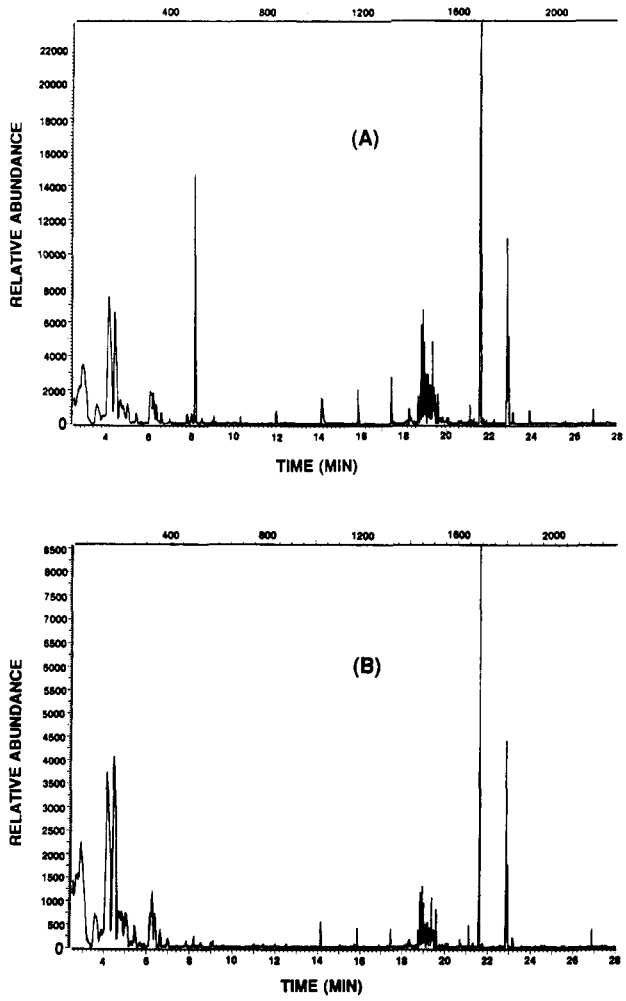

Figure 3. Total ion chromatograms (TICs) of (A) uncured-pork and (B) cured-pork flavor concentrates isolated by the NPT method (first cold trap).

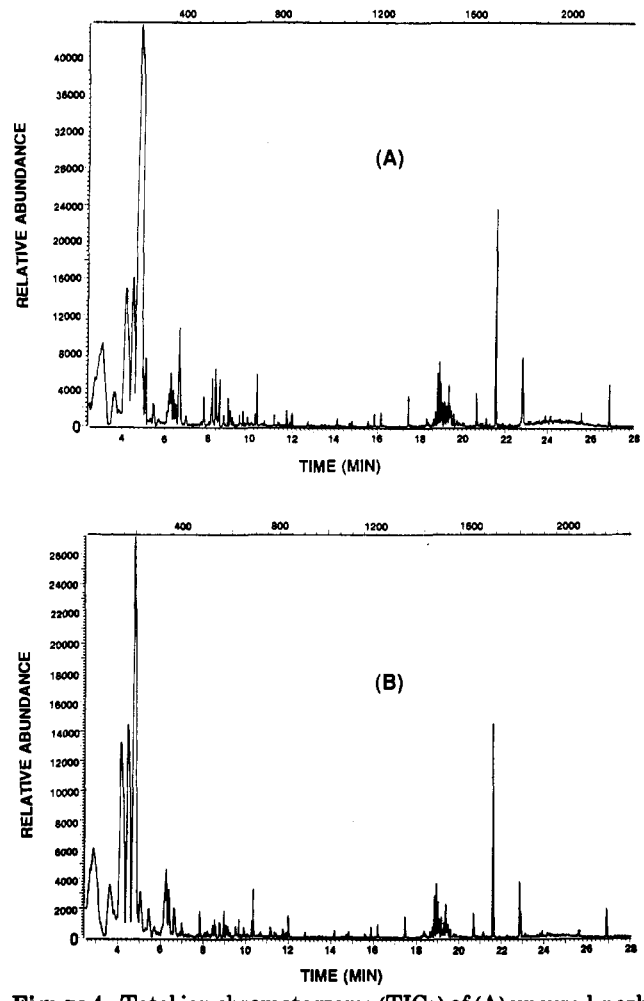

Figure 4. Total ion chromatograms (TICs) of (A) uncured-pork and (B) cured-pork flavor concentrates isolated by the NPT method (second cold trap).

yl)-4-methoxyphenol (RT 15.93 min), 3-amino-5,6-dimethyltriazolo[4,3-*a*]pyrazine (RT 19.24 min), 2-butylphenol (RT 19.54 min), and bis(2-ethylhexyl) phthalate (RT 26.90 min). 2,2,5,5-Tetramethylhexane (RT 6.18 min) was the only compound that was found to be present in small amounts (0.11 mg/kg) in the cured-pork sample and absent in the uncured pork. Of the 32 newly identified components, 20 were present in the condensates of the first cold trap. This clearly demonstrated the importance of this fraction that was essentially richer in less volatile heterocyclic constituents. It is possible that due to the selective trapping of heterocyclic and phenolic components in sufficient quantities in the first cold trap, the NPT method of collection of volatiles facilitated their identification with less interference from carbonyls and hydrocarbons that are usually present in very high concentrations.

Analysis of the Second Cold Trap Extract. The second cold trap, containing 50 mL of *n*-pentane maintained at −60 °C with acetone–dry ice mixture (Figure 1), was mainly used to trap the more volatile carbonyls and hydrocarbons that escaped adsorption onto the cartridge. The TICs of aroma concentrates of uncured and cured pork prepared from the second cold trap are shown in Figure 4. In all, 22 compounds were identified in this fraction; of these, 9 were hydrocarbons, 4 were carbonyls, 1 was an alcohol, 2 were phenols, 3 were heterocyclic compounds, and 3 were esters. The profiles of volatile compounds in the two extracts were qualitatively similar, though the concentration of the constituents of the aroma concentrate of uncured pork was slightly higher than those of cured pork. Hexanal (RT 4.65 min), the major lipid oxidation product, was present as a major constituent of uncured pork. This component was present at a level of 7.28 mg/kg in the aroma concentrate of uncured pork, while it was present in small traces in the cured-pork aroma concentrate. The other minor components that were detected only in the uncured pork, but either present in small traces or absent in cured pork, were 2-methylhexanal (RT 6.50 min), 7-octen-4-ol (RT 8.23 min) (*E*,*E*)-2,4-nonadienal (RT 8.41 min), tetrahydro-*cis*-2,4-dimethylfuran (RT 8.60 min), and bis(2-ethylhexyl) phthalate (RT 26.90 min).

All eight heterocyclic components identified and reported in this investigation are new to the literature on meat flavor currently available. Tetrahydro-*cis*-2,4-dimethylfuran (RT 8.60 min) may be a potent flavoring component, as closely related members of this group have been implicated in the literature to be responsible for meaty and sulfurous aroma notes (Solms, 1968; Ohloff, and Flament, 1978). Pyridine, pyrazine, and thiazole derivatives have been known to be responsible for roasted, fatty, nutty, and popcorn-like flavor notes (Pittet and Hruza, 1974; Chang and Peterson, 1977; Ohloff, and Flament, 1978). Thus, by combination in proper proportion among themselves or in the presence of other aromatic constituents, the heterocyclic compounds may be essential for the perception of the overall meaty flavor notes in cooked meat.

Hydrocarbons such as 1,1-dimethylcyclopentane (RT 11.74 min) and the newly identified compound 4-methyl-1-decene (RT 13.00 min) were found to be present uniquely in the uncured-pork aroma concentrates. In addition, certain carbonyls such as 2-methylhexanal (RT 6.50 min), (*E,E*)-2,4-nonadienal (RT 8.41 min), decanal (RT 12.20 min), 2-undecanone (RT 12.93 min), (*E,E*)-2,4-decadienal (RT 13.76 min), 2-undecenal (RT 14.40 min), and 4-pentylbenzaldehyde (RT 15.79 min) were also found only in the aroma concentrates of uncured pork. The newly identified compounds 2-methylcyclopentanol (RT 12.02 min) and 2-butyl-2-octenal (RT 14.58 min), in addition to those already reported by us in our earlier investigations (Ramarathnam et al., 1991a,b), seem to be unique components of the cured-meat aroma.

CONCLUSIONS

The objective of the present investigation was to isolate the volatiles from uncured and cured pork in three different fractions depending on their volatility and solubility. It was believed that this mode of collection would facilitate the identification of the key compounds responsible for the cured-meat aroma, or the basic meaty aroma of cooked meat, with less or no interference from constituents that are usually present in high concentration but makes less or no contribution to the flavor notes mentioned above. Using pork, under mild conditions of cooking and aroma extraction, we have reported the identification of 32 new constituents. The use of nitrogen in the purge-and-trap technique not only served as a carrier of aroma constituents but also provided an inert medium which prevented the oxidation of the cellular components such as the polyunsaturated fatty acids, sugars, and amino acids. In the previous investigations not only were the carbonyls and hydrocarbons identified in greater numbers, but quantitatively they were present in such high concentrations that identification of minor components, heterocyclic and phenolic constituents, became difficult. Using the novel nitrogen purge-and-trap method, we have solved this problem and succeeded in the identification of these constituents without any interference of the carbonyls and hydrocarbons. We have plans to extend this technique to the isolation and characterization of volatiles of uncured and cured beef and chicken. In the present investigation, though many new compounds not known before have been identified, it is not known as yet how many of them actually contribute to the meaty-aroma and species-specific flavor notes. A detailed sensory evaluation of the newly identified components will be attempted in the near future, and final confirmation of the "character impact" components will be made with the help of authentic or synthesized samples.

LITERATURE CITED

AOAC. *Official Methods of Analysis*; Association of Official Analytical Chemists: Arlington, VA, 1984; pp 431–443.

Baines, D. A.; Mlotkiewicz, J. A. The Chemistry of Meat Flavour. In *Recent Advances in the Chemistry of Meat*; Bailey, A. J., Ed.; Royal Society of Chemistry: London, 1984; Chapter 7.

Berdague, J.; Denoyer, C.; Quere, J. L.; Semon, E. Volatile Components of Dry-Cured Ham. *J. Agric. Food Chem.* **1991**, *39*, 1257–1261.

Chang, S. S.; Peterson, R. J. Symposium: The Basis of Quality in Muscle Foods. Recent Developments in The Flavor of Meat. *J. Food Sci.* **1977**, *42*, 298–305.

Cross, C. K.; Ziegler, P. A Comparison of the Volatile Fractions from Cured and Uncured Meats. *J. Food Sci.* **1965**, *30*, 610–614.

Gray, J. I.; MacDonald, B.; Pearson, A. M.; Morton, I. D. Role of Nitrite in Cured Meat Flavor: A Review. *J. Food Prot.* **1981**, *44*, 302–312.

Ho, C.; Lee, K.; Jin, Q. Z. Isolation and Identification of Volatile Flavor Compounds in Fried Bacon. *J. Agric. Food Chem.* **1983**, *31*, 336–341.

Hornstein, I.; Crowe, P. F. Flavor Studies on Beef and Pork. *J. Agric. Food Chem.* **1960**, *8*, 494–498.

Hornstein, I.; Crowe, P. F. Meat Flavor: Lamb. *J. Agric. Food Chem.* **1963**, *11*, 147–149.

Jacobson, M.; Koehler, H. H. Components of the Flavor of Lamb. *J. Agric. Food Chem.* **1963**, *11*, 336–339.

Jennings, W., Shibamoto, T., Eds. *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*; Academic Press: New York, 1980; pp 9–11.

Lillard, D. A.; Ayres, J. C. Flavor Compounds in Country Cured Hams. *Food Technol.* **1969**, *23*, 251–254.

MacLeod, G.; Seyyedain-Ardebili. Natural and Simulated Meat Flavors (with Particular Reference to Beef). *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *14*, 309–437.

Minor, L. J.; Pearson, A. M.; Dawson, L. E.; Schweigert, B. S. Separation and Identification of Carbonyl and Sulfur Compounds in the Volatile Fraction of Cooked Chicken. *J. Agric. Food Chem.* **1965**, *13*, 298–300.

Moody, W. G. Beef Flavor—A Review. *Food Technol.* **1983**, *37*, 227–232, 238.

Ockerman, H. W.; Blumer, T. N.; Craig, H. B. Volatile Chemical Compounds in Dry-Cured Hams. *J. Food Sci.* **1964**, *29*, 123–129.

Ohloff, G.; Flament, I. Heterocyclic Constituents of Meat Aroma. *Heterocycles* **1978**, *11*, 663–695.

Persson, T.; von Sydow, E. Aroma of Canned Beef. Gas Chromatographic and Mass Spectrometric Analysis of the Volatiles. *J. Food Sci.* **1973**, *38*, 377–385.

Peterson, R. J.; Chang, S. S. Identification of Volatile Flavor Compounds of Fresh, Frozen Beef Stew and a Comparison of These with Those of Canned Beef Stew. *J. Food Sci.* **1982**, *47*, 1444–1448, 1453.

Piotrowski, E. G.; Zaika, L. L.; Wasserman, A. E. Studies on Aroma of Cured Ham. *J. Food Sci.* **1970**, *35*, 321–325.

Pittet, A. O.; Hruza, D. E. Comparative Study of Flavor Properties of Thiazole Derivatives. *J. Agric. Food Chem.* **1974**, *22*, 264–269.

Ramarathnam, N.; Rubin, L. J.; Diosady, L. L. Studies on Meat Flavor. 1. Qualitative and Quantitative Differences in Uncured and Cured Pork. *J. Agric. Food Chem.* **1991a**, *39*, 344–350.

Ramarathnam, N.; Rubin, L. J.; Diosady, L. L. Studies on Meat Flavor. 2. A Quantitative Investigation of the Volatile Carbonyls and Hydrocarbons in Uncured and Cured Beef and Chicken. *J. Agric. Food Chem.* **1991b**, *39*, 1839–1847.

Ramaswamy, H. S.; Richards, J. F. Flavor of Poultry Meat—A Review. *Can. Inst. Food Sci. Technol. J.* **1982**, *15*, 7–18.

Rhee, K. S. Chemistry of Meat Flavor. In *Flavor Chemistry of Lipid Foods*; Min, D. B., Smouse, T. H., Eds.; AVI Publications: New York, 1989; Chapter 9.

Shahidi, F.; Rubin, L. J.; D'Souza, L. A. Meat Flavor Volatiles: A Review of the Composition, Techniques of Analysis, and Sensory Evaluation. *CRC Crit. Rev. Food Sci. Nutr.* **1986**, *24*, 141–243.

Solms, J. Flavor Substances and Aroma Substances in Meat. *Fleischwirtschaft* **1968**, *48*, 287–291.

Vercellotti, J. R.; Kuan, J. W.; Liu, R. H.; Legendre, M. G.; St. Angelo, A. J.; Dupuy, H. P. Analysis of Volatile Heteroatomic Meat Flavor Principles by Purge-and-Trap/Gas Chromatography-Mass Spectrometry. *J. Agric. Food Chem.* **1987**, *35*, 1030–1035.

Received for review March 2, 1993. Accepted March 8, 1993.