Studies on Meat Flavor. 4. Fractionation, Characterization, and Quantitation of Volatiles from Uncured and Cured Beef and Chicken

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Volatile components from uncured and cured beef and chicken were trapped onto a solid adsorbent (Florisil cartridge) and in an organic solvent (pentane) using the nitrogen purge-and-trap (NPT) technique. In addition to the 32 compounds newly identified in pork and reported by us in our previous investigation, we have identified 12 more new compounds in beef and chicken. Of these, the newly identified compounds 4-ethyl-1-methylhexane, 1,1,3-trimethylcyclohexane, α-pinene, 4-ethyl-1,2-dimethylbenzene, and 3,6-dimethylundecane were identified uniquely in beef. A total of seven components were exclusively identified in chicken and absent in pork and beef. These included the newly identified compounds 1,2-dibutylcyclopentane, 2,6-bis(1,1-dimethylethyl)-4-methylphenol, and 1,12-dodecanediol.

INTRODUCTION

Meat is widely consumed and highly desired by most people because of its nutritive value and acceptable sensory qualities. Though the flavor of meat is of great academic as well as practical importance, this important sensory quality of muscle foods is not yet completely understood. Raw meat has very little flavor, but cooked meat has a desirable flavor which depends on the temperature and method of cooking (Kramlich and Pearson, 1958). The "meaty" flavor is believed to originate from the lean and "species-specific" flavor from the fat tissue (Hornstein and Crowe, 1960; Hornstein et al., 1963). Several other factors, both preslaughter and post-mortem, such as the type of feed, storage and sanitation conditions, and processing methods influence the final flavor quality of the cooked meat.

The composition of the aroma generated during cooking of meat has been exhaustively studied and over 1000 components have been identified in the four commonly consumed meat species—beef, pork, poultry, and lamb (Herz and Chang, 1970; Bailey and Swain, 1973; Dwivedi, 1975; Chang and Peterson, 1977; Wasserman, 1979; 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). However, the search for individual components responsible for the meaty flavor notes distinctly identifiable in the cooked meat of all species and for the character-impact compounds responsible for the aroma notes specific for pork, beef, chicken, and lamb continues until this date.

The classes of compounds listed by Chang and Peterson (1977), which are probably important contributors to meaty aroma notes, included lactones, nonaromatic heterocyclic compounds, acyclic sulfur-containing compounds, and aromatic heterocyclic compounds. They concluded that aromatic and aliphatic hydrocarbons, carbonyl compounds, saturated alcohols, carboxylic acids, and esters probably do not contribute to the basic meaty flavor. Min et al. (1977) isolated volatile flavor compounds from roast beef and separated them into acidic, basic, and neutral fractions. They further reported that the neutral fraction of the roast beef volatiles was the only fraction presenting a pleasant beef-like aroma. In a subsequent study on the neutral fraction of roast beef, Min et al. (1979) suggested the importance of lactones, substituted aromatics, furans, and sulfur-containing compounds as contributors to roast beef flavor. Hsu et al. (1982) isolated volatiles from cooked beef and added a list of 67 new compounds, including 11 heterocyclics, to the literature on beef flavor. In their search for components responsible for meaty odor notes, MacLeod and Ames (1986a) trapped volatiles from roasted beef and succeeded in the identification of 2-methyl-3-(methylthio)furan.

In comparison with the number of papers published on the flavor of beef and pork, the literature available on the flavor of cooked poultry meat is rather limited. The first major study comes from Minor et al. (1965a,b), who studied the chemical constituents in the volatile fraction of cooked meat consisting of water slurries of both leg and breast muscles. They reported 29 compounds in the volatiles from leg muscle and 25 from breast muscle, belonging to major classes including carbonyls, acids, amines, mercaptans, esters, sulfides, and disulfides. Furthermore, Minor et al. (1965b) suggested that the meaty aroma of cooked chicken muscle could be due to sulfur compounds, while the "chickeny" aroma could be traceable to the presence of carbonyl compounds. They detected 13 sulfur compounds in the flavor volatiles of chicken. Nonaka et al. (1967) used capillary gas chromatography fractionation and mass spectral identification to study chicken volatiles. They tentatively identified over 225 compounds and confirmed the identity of 62 of them, of which 50 were new to the literature available on chicken flavor.

Hydrogen sulfide (H_2S) has been reported to be continuously produced during the cooking of chicken (Mecchi et al., 1964; Klose, 1965; Minor et al., 1965a). Pippen and Mecchi (1969) carried out further investigations of the role of H_2S in poultry flavor and obtained some quantitative results that led them to conclude that H_2S contributed directly to the flavor of poultry products. They also suggested the possibility of interactions between H_2S and the carbonyls, giving poultry a more stable flavor property. Investigations by other workers (Hobson-Frohock, 1970; Janney et al., 1974; Horvat, 1976) have led to the addition of some more sulfur compounds in poultry products.

Of the numerous volatiles isolated from cooked meat, heterocyclic compounds have been most frequently implicated to be responsible for the basic meaty flavors, although they are present in very minute amounts (Ohloff and Flament, 1978; Wasserman, 1979; Bordero et al., 1981;

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Katz, 1981; MacLeod and Seyyedain-Ardebili, 1981; Fors and Olofsson, 1986; Liu et al., 1987). Pyridines (Ho et al., 1983) and pyrazines (Watanabe and Sato, 1971) have been identified mainly in fried and roasted meat. Sulfur components are potent flavoring substances and have very low thresholds (Golovnja and Rothe, 1980). Cross and Ziegler (1965) demonstrated that when the volatile constituents from nitrite-treated and untreated ham, beef, or chicken were passed through a solution of 2,4-dinitrophenylhydrazine, the effluent stream in all of the systems had a characteristic "cured-ham" aroma. However, when the resulting volatiles were further passed through a solution of mercuric chloride, the pleasant cured-ham aroma, or the basic meaty aroma of cooked meat, was lost. This clearly illustrated the importance of sulfur compounds in meat flavor. Minute traces of these compounds can be highly aroma effective and even in small traces can contribute a great deal to the flavor of uncured and cured meat. However, as they are highly reactive, they interact readily with various organic substances in the meat system. Hence, isolation and identification of such minor components can prove to be extremely difficult (MacLeod and Ames, 1986b).

In our previous work, we have provided quantitative information on the carbonyls and hydrocarbons present in the aroma concentrates of uncured and nitrite-cured beef and chicken isolated by the continuous steam distillation–extraction (SDE) method (Ramarathnam et al., 1991b). We also provided a summary of those carbonyl components that may be responsible for the species differences. 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). In continuation of our attempts to identify the key components that are responsible for the cured-meat aroma or the basic meaty aroma of cooked meat, we isolated the volatiles from cured and uncured pork, using the nitrogen purge-and-trap (NPT) method, and reported the identification of 32 new compounds, of which 8 were heterocyclic in nature (Ramarathnam et al., 1993). We have extended this method to identify volatile components in the cooked meat of uncured and cured beef and chicken, the results of which are discussed in this paper.

MATERIALS AND METHODS

Meat. Fresh ground beef (lean meat from shoulder) and chicken breasts with skin on were purchased from a local market and used immediately. Care was taken to ensure that the meat bought from this outlet was made available within a day after its arrival from the slaughterhouse. Until its sale at the retail counter, the post-mortem temperature of the meat was maintained at 4 °C. The skin and excess fat in chicken were removed, the pieces were deboned manually, and the meat was then ground using an Oster meat grinder (0.476-cm grind plate, Model 990-68).

Proximate Analysis. The fat content of cooked-meat samples was determined according to 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 meats in all experiments contained 68.2 ± 0.05% water in beef and 73.2 ± 0.3% water in chicken, while the fat contents were 7.2 ± 0.3% and 2.5 ± 0.3% for beef and chicken (skin off), respectively.

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. The ground meat (250–400 g) was cooked according to the procedure already discussed in detail in our previous paper (Ramarathnam et al., 1993). 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 ground thoroughly by using a Braun MR 30 hand blender.

Nitrogen Purge-and-Trap (NPT) Technique. The assembly of the apparatus and the mode of collection of meat-flavor volatiles using the NPT method were discussed in our previous paper (Ramarathnam et al., 1993).

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 1st 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 two cold traps were separately dried and concentrated as described above.

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. Qualitative and quantitative analyses of the aroma concentrates of uncured and cured beef and chicken were carried out under the same analytical conditions mentioned in our previous paper (Ramarathnam et al., 1993).

RESULTS AND DISCUSSION

Gas Chromatography–Mass Spectrometric (GC–MS) Analysis. Blank runs made with distilled water indicated the presence of trace amounts of several volatile components which eluted from the cartridge materials. As published previously (Ramarathnam et al., 1993), pretreatment of the cartridge with distilled *n*-pentane followed by drying before adsorption proved to be effective in the removal of such volatiles already present in the cartridge. In the preparation of the aroma concentrates for GC–MS analysis, prewashed cartridges were used.

The components identified in the aroma concentrates of uncured and cured beef and chicken prepared according to the NPT method are listed in Table I. In all, 60 components were identified in the different fractions of the beef aroma concentrates, while the number of compounds identified in the different fractions of chicken aroma concentrates amounted to 52. Of the total number of compounds identified in uncured and cured beef, 32 were hydrocarbons, 8 carbonyls, 4 alcohols, 5 phenols, 4 esters, and 6 heterocyclic compounds. The corresponding figures for uncured and cured chicken were 21 hydrocarbons, 11 carbonyls, 4 alcohols, 6 phenols, 5 esters, and 5 heterocyclic compounds. Aroma concentrates isolated from uncured and cured pork by the NPT method had 60 and 34 components, respectively (Ramarathnam et al., 1993), of which 20 were hydrocarbons, 16 carbonyls, 4 alcohols, 5 phenols, 3 esters, and 8 heterocyclic compounds. In comparison with these results, irrespective of the species, nearly twice the number of carbonyls and hydrocarbons were identified in the aroma concentrates prepared by the continuous steam distillation–extraction (SDE) method (Ramarathnam et al., 1991a,b). This is clearly indicative of the mild nature of the NPT method that limits the breakdown of the volatile components and protects them from undergoing further oxidation due to the use of the inert nitrogen gas.

Analysis of the Cartridge Fraction. A wide variation in the qualitative and quantitative composition of the

volatiles adsorbed onto the Florisil cartridge was observed among uncured and cured beef and chicken. The concentration of 7-octen-4-ol (RT 8.23 min) was nearly 12 times higher in the uncured beef, where it was present to the extent of 2.84 mg/kg, while the concentration of this component in the cured beef was only 0.24 mg/kg. A similar ratio was also observed in the content of this compound in uncured and cured pork (Ramarathnam et al., 1993). This compound was detected only in the cartridge fraction of the aroma concentrate of uncured chicken, where it was present to the extent of 1.19 mg/kg, but absent in the cured-chicken aroma concentrate. The concentration of octanol (RT 9.84 min) was also higher in the uncured beef (4.69 mg/kg) than in cured pork (0.26 mg/kg). The values for the content of octanol in uncured and cured chicken were 3.07 and 0.07 mg/kg, respectively. This component was absent in the aroma fractions of both uncured and cured beef and chicken prepared from the two cold traps.

The volatile constituents that were detected only in the cartridge fraction of the uncured beef aroma concentrate were decanal (RT 12.20 min), nonylcyclopropane (RT 13.11 min), and 5-propyldecane (RT 13.50 min). These compounds were not detected in the aroma concentrates of uncured and cured beef prepared from the two cold traps. In addition to these differences, a number of components detected only in the cured-beef aroma concentrate of the cartridge fraction, but absent in the uncured beef, included 2-methyl-3-hexanone (RT 3.59 min), 4-ethyl-1-methylhexane (RT 5.47 min), 2,2,5,5-tetramethylhexane (RT 6.18 min), heptanal (RT 6.66 min), D-limonene (RT 9.12 min), 2,2,4,6,6-pentamethylheptane (RT 9.73 min), camphene (RT 11.85 min), 3,6-dimethylundecane (RT 11.91 min), 2-methylcyclopentanol (RT 12.02 min), 1,3-dimethoxybenzene (RT 14.15 min), 2-butyl-2-octenal (RT 14.58 min), 4-(2,2,3,3-tetramethylbutyl)phenol (RT 18.86 min), 4-nonylphenol (RT 18.95 min), 1,3-dihydro-2*H*-imidazo[4,5-*b*]-pyridin-2-one (RT 19.03 min), 2,4,6-trimethylpyridine (RT 19.08 min), 4-(1-methylpropyl)phenol (RT 19.14 min), 3-methyl-1,2-benzisothiazole (RT 19.30 min), 4-ethyl-2,6-dimethylpyridine (RT 19.40 min), 2-butylphenol (RT 19.54 min), (*E*)-5-octadecene (RT 20.69 min), methyl 11,14-eicosadienoate (RT 22.87 min), and bis(2-ethylhexyl) phthalate (RT 26.90 min). Of these, 2,2,4,6,6-pentamethylheptane (RT 9.73 min) is a newly identified compound which was also found in the second cold traps of uncured beef and uncured chicken. Two other newly identified components present only in beef volatiles were camphene (RT 11.85 min) and 3,6-dimethylundecane (RT 11.91 min).

Of the volatile components present in the cartridge fraction of the aroma concentrate of chicken, 4-ethylbenzaldehyde (RT 11.46 min), decanal (RT 12.02 min), nonylcyclopropane (RT 13.11 min), 5-propyldecane (RT 13.50 min), dodecanal (RT 15.01 min), tridecanal (RT 16.37 min), tetradecanal (RT 17.64 min), and 1,12-dodecanediol (RT 18.89 min) were identified only in the aroma concentrate of uncured chicken. Among these constituents, 4-ethylbenzaldehyde (RT 11.46 min) was unique to uncured chicken and was not identified in the aroma concentrates of beef and pork (Ramarathnam et al., 1993). As in the case of uncured pork (Ramarathnam et al., 1993), the hydrocarbons 1,1-dimethylcyclopentane (RT 11.74 min) and nonylcyclopropane (RT 13.11 min) and the carbonyls decanal (RT 12.20 min), dodecanal (RT 15.01 min), tridecanal (RT 16.37 min), and tetradecanal (RT 17.64 min) were identified only in the cartridge fraction of the uncured chicken, while the aroma concentrates from the two cold traps did not show their presence. The dihydric alcohol 1,12-dodecanediol (RT 18.89 min) was found to be present uniquely in the aroma concentrates of uncured chicken and cured chicken. It was not detected in the aroma concentrates of pork (Ramarathnam et al., 1993) and beef (Table I) and hence may be an important constituent for the formulation of the chicken aroma concentrate.

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. This fraction of beef aroma concentrate showed the presence of eight hydrocarbons, four carbonyls, one alcohol, three esters, and all five phenols and six heterocyclic constituents identified in beef in the present investigation. The corresponding figures for chicken were six hydrocarbons, four carbonyls, two alcohols, and five esters and five heterocyclic compounds, and four of the six phenolic constituents identified in the different fractions of chicken aroma. As observed in our previous investigation (Ramarathnam et al., 1993), 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 beef and chicken, 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 (data not shown). The volatile components that were present only in the uncured beef aroma concentrate prepared from this fraction included 2,4-dimethylhexane (RT 3.64 min), hexanal (RT 4.65 min), 4-ethyl-2-methylhexane (RT 5.50 min), 3,3-diethylpentane (RT 7.04 min), 7-octen-4-ol (RT 8.23 min), 2,4-dihydroxybenzaldehyde (RT 14.21 min), (1,1-dimethylethyl)-4-methoxyphenol (RT 15.93 min), 3-methyl-1,2-benzisothiazole (RT 19.30 min), 2-butylphenol (RT 19.54 min), and 2,4-diphenyl-1*H*-pyrrole (RT 19.61 min), while in the case of chicken the constituents that were present only in the uncured-chicken aroma concentrate of this fraction included 2,4-dimethylhexane (RT 3.64 min) and 7-octen-4-ol (RT 8.23 min). 4-Ethyl-2-methylhexane (RT 5.50 min) and 2,4-dihydroxybenzaldehyde (RT 14.21 min), the newly identified components, were detected uniquely in the uncured-beef aroma concentrate of the cartridge fraction at concentrations of 0.05 and 0.09 mg/kg, respectively. These compounds were also present in the aroma concentrates of uncured and cured chicken prepared from the two cold traps. (1,1-Dimethyl)-4-methoxyphenol (RT 15.93 min) was present at a level of 0.13 mg/kg in the uncured beef, while its concentrations in uncured pork and chicken were 0.09 (Ramarathnam et al., 1993) and 0.10 mg/kg. The concentration of this compound was less than half that in cured chicken (0.04 mg/kg). The component that was unique to cooked chicken and identified for the first time was 1,12-dodecanediol (RT 18.89 min). This compound was present in the uncured chicken to the extent of 0.43 (cartridge fraction) and 0.38 mg/kg (first cold trap), while in the cured-chicken aroma concentrate of the first cold trap the concentration of this compound was 0.18 mg/kg. Methyl 14-hydroxy-5-tetradecenoate (RT 22.98 min) has been identified in the aroma concentrates of uncured and cured beef and chicken for the first time. The concentrations of this compound in the uncured- and cured-beef aroma concentrate of the cartridge fraction were 0.21 and 0.07 mg/kg, respectively, while the corresponding values for

Table I. Total Content of Individual Compounds Identified in the Different Fractions of the Aroma Concentrates of Pork, Beef, and Chicken Isolated by the Nitrogen Purge-and-Trap Method

RT, min	compound	Kovats index[a]	pork,[c,d] mg/kg		beef,[c] mg/kg		chicken,[c] mg/kg	
			uncured	cured	uncured	cured	uncured	cured
3.59	2-methyl-3-hexanone[b]	734	0.13	0.19	0.13	0.46	0.03	0.09
3.64	2,4-dimethylhexane	736	0.50	0.33	0.82	0.76	0.38	0.67
4.16	methylbenzene	763	4.97	4.88	5.51	5.24	4.73	2.93
4.48	2,2,4-trimethylhexane	777	3.71	3.34	6.36	5.01	1.26	1.70
4.65	hexanal	787	7.39	–[e]	4.63	0.13	7.29	0.02
5.08	2,3,5-trimethylhexane	810	0.45	0.48	0.85	0.58	0.31	0.41
5.47	4-ethyl-1-methylhexane[b]	831	–	–	0.26	0.30	–	–
5.50	4-ethyl-2-methylhexane[b]	833	0.24	0.32	0.05	–	0.20	0.20
5.72	1,1,3-trimethylcyclohexane[b]	845	–	–	–	0.08	–	–
5.77	1,1,3,3-tetramethylcyclopentane[b]	847	–	–	0.10	–	–	0.06
6.00	1,2-dimethylbenzene	860	–	–	0.14	–	–	–
6.11	1,3-dimethylbenzene	866	0.25	–	0.26	0.48	–	–
6.18	2,2,5,5-tetramethylhexane[b]	870	0.14	0.34	0.24	0.31	–	–
6.28	2,2,4-trimethylheptane	875	0.46	0.76	1.44	1.07	0.44	0.44
6.39	unidentified	881	0.28	0.41	0.26	0.54	–	–
6.50	2-methylhexanal[b]	887	0.10	–	–	–	–	–
6.58	1,4-dimethylbenzene	891	–	–	0.13	–	–	–
6.66	heptanal	896	0.57	0.21	0.59	0.26	0.06	0.16
7.04	3,3-diethylpentane[b]	917	0.03	0.03	0.13	0.07	–	–
7.40	α-pinene[b]	937	–	–	0.08	–	–	–
7.83	unidentified	961	0.16	0.08	–	–	–	–
7.88	2,2,6-trimethyloctane	963	–	–	0.27	0.15	–	–
7.89	3-methyloctane	964	–	–	–	–	0.15	0.11
8.23	7-octen-4-ol[b]	983	2.71	0.09	3.72	0.24	1.51	–
8.41	(*E*,*E*)-2,4-nonadienal	993	0.13	–	–	–	–	–
8.50	1,3,5-trimethylbenzene	998	–	–	0.17	–	–	–
8.61	decane	1000	–	–	0.10	–	0.10	–
8.62	tetrahydro-*cis*-2,4-dimethylfuran[b]	1004	0.33	–	–	–	–	–
8.64	2-methylcyclopentanol	1006	–	–	0.07	–	–	–
8.81	1,4-dichlorobenzene	1016	–	–	0.11	0.07	0.09	–
9.02	2,2-diethyldecane	1028	–	–	0.21	0.11	–	–
9.03	2,2,6-trimethyloctane	1029	–	–	–	–	0.15	0.08
9.09	unidentified	1032	–	0.09	–	–	–	–
9.12	D-limonene	1034	–	0.05	0.15	0.24	–	0.05
9.20	unidentified	1039	0.62	–	–	–	–	–
9.38	unidentified	1050	0.22	–	–	–	–	–
9.58	(*E*)-2-octenal	1062	0.25	–	0.08	–	0.09	–
9.73	2,2,4,6,6-pentamethylheptane[b]	1070	–	–	0.10	0.12	0.08	–
9.84	octanol	1078	4.20	0.15	4.69	0.26	3.07	0.07
10.13	4-ethyl-1,2-dimethylbenzene[b]	1094	–	–	0.05	–	–	–
10.38	*trans*-1,2-dimethylcyclopentane[b]	1110	1.41	0.66	2.15	0.33	2.41	0.13
11.32	3-propyl-1*H*-1,2,4-triazole[b]	1172	0.25	–	–	–	–	–
11.46	4-ethylbenzaldehyde	1181	–	–	–	–	0.33	–
11.74	1,1-dimethylcyclopentane	1199	0.70	–	–	–	0.44	–
11.85	camphene[b]	1206	–	–	0.05	0.10	–	–
11.91	3,6-dimethylundecane[b]	1210	–	–	–	0.19	–	–
11.94	2-methylundecane[b]	1213	0.15	0.12	–	–	–	–
12.02	2-methylcyclopentanol[b]	1217	–	0.17	–	0.20	–	0.08
12.20	decanal	1228	0.66	–	0.04	–	1.02	–
12.93	2-undecanone	1274	0.16	–	–	–	–	–
13.00	4-methyl-1-decene[b]	1278	0.18	–	–	–	–	–
13.11	nonylcyclopropane[b]	1285	1.16	–	2.81	–	2.91	–
13.23	1-nonen-3-ol	1293	0.20	–	–	–	–	–
13.50	5-propyldecane[b]	1311	1.52	–	0.69	–	0.83	–
13.76	(*E*,*E*)-2,4-decadienal	1330	0.18	–	–	–	–	–
14.15	1,3-dimethoxybenzene[b]	1358	0.06	–	–	0.05	–	–
14.21	2,4-dihydroxybenzaldehyde[b]	1362	–	–	0.09	–	0.06	0.03
14.40	2-undecenal	1376	0.24	–	–	–	–	–
14.58	2-butyl-2-octenal[b]	1389	–	2.33	1.85	–	–	3.71
14.76	1,2-dibutylcyclopentane[b]	1402	–	–	–	–	–	0.13
14.88	2,3,5-trimethyldecane[b]	1411	0.55	0.21	0.94	0.39	0.51	0.11
15.01	dodecanal	1420	0.19	–	–	–	0.32	–
15.79	4-pentylbenzaldehyde	1476	0.21	–	–	–	–	–
15.93	(1,1-dimethylethyl)-4-methoxyphenol[b]	1486	0.09	–	0.13	–	0.10	0.04
16.00	(*E*)-9-octadecene	1491	0.21	–	–	–	–	–
16.21	pentadecane	1500	0.45	–	–	–	0.30	0.06
16.37	tridecanal	1518	0.33	–	–	–	0.46	–
16.49	2,6-bis(1,1-dimethylethyl)-4-methylphenol[b]	1527	–	–	–	–	–	0.41
17.46	hexadecane	1600	0.14	0.22	0.90	0.44	–	0.11
17.47	diethyl phthalate	1603	–	–	–	–	0.17	0.08
17.64	tetradecanal	1618	0.29	–	–	–	0.33	–
18.86	4-(2,2,3,3-tetramethylbutyl)phenol[b]	1725	0.83	0.29	0.20	0.41	–	–
18.89	1,12-dodecanediol[b]	1728	–	–	–	–	0.81	0.18
18.95	4-nonylphenol[b]	1733	0.53	0.34	0.67	0.53	0.50	0.25
19.03	1,3-dihydro-2*H*-imidazo[4,5-*b*]pyridin-2-one[b]	1740	0.39	0.26	0.88	0.39	0.37	0.17

Table I (Continued)

RT, min	compound	Kovats index[a]	pork,[c,d] mg/kg uncured	pork,[c,d] mg/kg cured	beef,[c] mg/kg uncured	beef,[c] mg/kg cured	chicken,[c] mg/kg uncured	chicken,[c] mg/kg cured
19.08	2,4,6-trimethylpyridine[b]	1744	0.11	0.03	0.56	0.15	–	–
19.14	4-(1-methylpropyl)phenol[b]	1750	0.16	0.06	0.22	0.19	0.16	0.08
19.24	3-amino-5,6-dimethyltriazolo[4,3-a]pyrazine[b]	1758	0.03	–	0.31	0.07	0.21	0.10
19.30	3-methyl-1,2-benzisothiazole[b]	1763	0.11	0.04	0.08	0.05	0.15	0.06
19.40	4-ethyl-2,6-dimethylpyridine[b]	1772	0.36	0.22	0.28	0.34	0.34	0.16
19.54	2-butylphenol[b]	1784	0.04	–	0.22	0.11	0.13	0.07
19.61	2,4-diphenyl-1*H*-pyrrole[b]	1790	0.07	0.04	0.09	–	0.05	0.03
20.04	hexadecanal	1831	1.75	1.06	0.83	0.27	1.69	0.52
20.69	(*E*)-5-octadecene[b]	1894	0.09	0.15	–	0.19	–	–
21.62	bis(2-methoxyethyl) phthalate[b]	1985	2.42	1.44	4.75	2.48	2.82	1.41
22.87	methyl 11,14-eicosadienoate[b]	2118	0.65	0.44	0.66	0.42	0.40	0.15
22.98	methyl 14-hydroxy-5-tetradecenoate[b]	2130	–	–	0.21	0.13	0.15	0.06
26.90	bis(2-ethylhexyl) phthalate[b]	2563	0.13	–	–	0.08	0.25	0.25

[a] Kovats indices calculated for the DB-5 capillary column of the GC–MS system. [b] Newly identified. [c] Average of two determinations. [d] Ramarathnam et al. (1993). [e] –, not detected.

Table II. Volatile Components Uniquely Identified in the Aroma Concentrates of Pork, Beef, and Chicken Isolated by the Nitrogen Purge-and-Trap Method

RT, min	compound	Kovats index[a]	content,[c] mg/kg uncured	content,[c] mg/kg cured
	Pork[d]			
6.50	2-methylhexanal[b]	887	0.10	–[e]
8.41	(*E,E*)-2,4-nonadienal	993	0.13	–
8.62	tetrahydro-*cis*-2,4-dimethylfuran[b]	1004	0.33	–
11.32	3-propyl-1*H*-1,2,4-triazole[b]	1172	0.25	–
11.94	2-methylundecane[b]	1213	0.15	0.12
12.93	2-undecanone[b]	1274	0.16	–
13.00	4-methyl-1-decene[b]	1278	0.18	–
13.23	1-nonen-3-ol	1293	0.20	–
13.76	(*E,E*)-2,4-decadienal	1330	0.18	–
14.40	2-undecenal	1376	0.24	–
15.79	4-pentylbenzaldehyde	1476	0.21	–
16.00	(*E*)-9-octadecene	1491	0.21	–
	Beef			
5.49	4-ethyl-1-methylhexane[b]	831	0.26	0.30
5.72	1,1,3-trimethylcyclohexane[b]	845	–	0.08
6.00	1,2-dimethylbenzene	860	0.14	–
6.58	1,4-dimethylbenzene	891	0.13	–
7.40	α-pinene[b]	937	0.08	–
7.88	2,2,6-trimethyloctane	963	0.27	0.15
8.50	1,3,5-trimethylbenzene	998	0.17	–
8.64	2-methylcyclopentanol	1006	0.07	–
9.02	2,2-diethyldecane	1028	0.21	0.11
10.13	4-ethyl-1,2-dimethylbenzene[b]	1094	0.05	–
11.85	camphene[b]	1206	0.05	0.10
11.91	3,6-dimethylundecane[b]	1210	–	0.19
	Chicken			
7.89	3-methyloctane	964	0.15	0.11
9.03	2,2,6-trimethyloctane	1029	0.15	0.08
11.46	4-ethylbenzaldehyde	1181	0.33	–
14.76	1,2-dibutylcyclopentane[b]	1402	–	0.13
16.49	2,6-bis(1,1-dimethylethyl)-4-methylphenol[b]	1527	–	0.41
17.47	diethyl phthalate	1603	0.17	0.08
18.89	1,12-dodecanediol[b]	1728	0.81	0.18

[a] Kovats indices calculated for the DB-5 capillary column of the GC–MS system. [b] Newly identified. [c] Average of two determinations. [d] Ramarathnam et al. (1993). [e] –, not detected.

uncured and cured chicken were 0.15 and 0.06 mg/kg, respectively. Unlike pork, the aroma concentrates of cured beef and cured chicken did not show any component that was uniquely identified in them in the first cold trap.

Analysis of the Second Cold Trap Extract. The second cold trap was essentially used to trap the more volatile carbonyls and hydrocarbons that escaped adsorption onto the cartridge. In all, 38 compounds of beef-aroma concentrates of this fraction were identified, while only half this number was identified in chicken-aroma concentrates. Of these, 26 hydrocarbons, 3 carbonyls, 1 alcohol, 2 phenols, 3 esters, and 2 heterocyclic compounds were identified in the beef volatiles. The chicken-aroma concentrates prepared from this fraction, however, contained 12 hydrocarbons, 3 carbonyls, and 2 esters. All of the heterocyclic and phenolic constituents identified in chicken were trapped in the first cold trap.

The profiles of volatile constituents in the extracts of uncured and cured beef and uncured and cured chicken were qualitatively similar, though the concentration of the constituents of the aroma concentrate of uncured meat was slightly higher than those of cured meat. Hexanal (RT 4.65 min), the major lipid oxidation product, was present as a major constituent of uncured beef, 4.63 mg/kg, while its concentration in uncured chicken was 7.25 mg/kg and was 7.28 mg/kg in the aroma concentrate of uncured pork (Ramarathnam et al., 1993). This compound was present in small traces in the cured meat of all three species. The other minor components that were detected only in the uncured beef and chicken, but either present in small traces or absent in the cured meat, were 1,1,3,3-tetramethylcyclopentane (RT 5.77 min), 1,2-dimethylbenzene (RT 6.00 min), 1,4-dimethylbenzene (RT 6.58 min), α-pinene (RT 7.40 min), 1,3,5-trimethylbenzene (RT 8.50 min), decane (RT 8.61 min), 2-methylcyclopentanol (RT 8.64 min), 1,4-dichlorobenzene (RT 8.81 min), D-limonene (RT 9.12 min), (*E*)-2-octenal (RT 9.58 min), 2,2,4,6,6-pentamethylheptane (RT 9.73 min), 4-ethyl-1,2-dimethylbenzene (RT 10.13 min), *trans*-1,2-dimethylcyclopentane (RT 10.38 min), and camphene (RT 11.85 min). Of these, 1,1,3,3-tetramethylcyclopentane (RT 5.77 min) and 2,2,4,6,6-pentamethylheptane (RT 9.73 min) were newly identified in the beef- and chicken-aroma concentrates, while α-pinene (RT 7.40 min) and 4-ethyl-1,2-dimethylbenzene (RT 10.13 min) were identified only in the aroma concentrates of uncured beef.

The newly identified compound 1,1,3-trimethylcyclohexane (RT 5.72 min) and 2,2,5,5-tetramethylhexane (RT 6.18 min) were not present in the uncured beef but were detected in the cured-beef aroma concentrate (Table I). In a similar comparison for the aroma concentrates of uncured and cured chicken, it was observed that the newly identified compound 1,1,3,3-tetramethylcyclopentane (RT 5.77 min) and heptanal (RT 6.66 min) were identified only in the cured product.

Table I also summarizes the total content of volatiles identified in the aroma concentrates of uncured and cured pork, beef, and chicken prepared by using the NPT method. A total of 88 compounds were detected in the three meat species. Of these, 40 were hydrocarbons, 18

Table III. Volatile Components Uniquely Identified in the Aroma Concentrates of Uncured and Cured Meat of Pork, Beef, and Chicken Isolated by the Nitrogen Purge-and-Trap Method

RT, min	compound	Kovats index[a]	pork,[c,d] mg/kg		beef,[c] mg/kg		chicken,[c] mg/kg	
			uncured	cured	uncured	cured	uncured	cured
			Uncured					
8.61	decane	1000	–[e]	–	0.10	–	0.10	–
9.58	(*E*)-2-octenal	1062	0.25	–	0.08	–	0.09	–
11.74	1,1-dimethylcyclopentane	1199	0.70	–	–	–	0.44	–
12.20	decanal	1228	0.66	–	0.04	–	1.02	–
13.11	nonylcyclopropane[b]	1285	1.16	–	2.81	–	2.91	–
13.50	5-propyldecane[b]	1311	1.52	–	0.69	–	0.83	–
15.01	dodecanal	1420	0.19	–	–	–	0.32	–
16.37	tridecanal	1518	0.33	–	–	–	0.46	–
17.64	tetradecanal	1618	0.29	–	–	–	0.33	–
			Cured					
12.02	2-methylcyclopentanol[b]	1217	–	0.17	–	0.20	–	0.08

[a] Kovats indices calculated for the DB-5 capillary column of the GC–MS system. [b] Newly identified. [c] Average of two determinations. [d] Ramarathnam et al. (1993). [e] –, not detected.

carbonyls, 6 alcohols, 6 phenols, 5 esters, 8 heterocyclic compounds, and 5 remained unidentified. The content of hexanal (RT 4.65 min, Table I), the major lipid oxidation product, was almost identical in the aroma concentrates of uncured pork (7.39 mg/kg) and chicken (7.29 mg/kg), while the concentration of this compound was 4.63 mg/kg in uncured beef. In the aroma concentrates prepared according to the SDE method, the content of hexanal was present nearly 1.5 times higher in all of the species (Ramarahanam et al., 1991a,b). This again supports our view that the NPT method, being milder in nature than the SDE method, limits the breakdown and oxidation of cellular components of meat such as the polyunsaturated fatty acids, sugars, and amino acids. In addition to the 32 components newly identified and reported in pork in our previous publication (Ramarathnam et al., 1993), we have reported here the identification of 12 more new compounds.

Compounds Responsible for Species Differences. A critical comparison of the composition of the different aroma concentrates of pork, beef, and chicken prepared according to the NPT method was also attempted. Except for the identification of the two heterocyclic compounds tetrahydro-*cis*-2,4-dimethylfuran (RT 8.62 min) and 3-propyl-1*H*-1,2,4-triazole (RT 11.32 min) in the aroma concentrates of pork, the compounds that have been found to be mainly responsible for the species differences included carbonyls and hydrocarbons. Table II highlights the list of compounds that have been uniquely identified in one species but absent in the other two. In the aroma concentrates of pork a total of 12 compounds were uniquely identified, of which 6 have been found to be new to the literature on meat flavor. These included 2-methylhexanal, tetrahydro-*cis*-2,4-dimethylfuran, 3-propyl-1*H*-1,2,4-triazole, 2-methylundecane, 2-undecanone, and 4-methyl-1-decene. Of the 12 compounds that were exclusively identified in the beef-aroma concentrates, 4-ethyl-1-methylhexane 1,1,3-trimethylcyclohexane, α-pinene, 4-ethyl-1,2-dimethylbenzene, and 3,6-dimethylundecane were newly identified. Seven compounds were found to be specific for chicken aroma and included three hydrocarbons, one aldehyde, one alcohol, one ester, and one phenol. Of these, 1,2-dibutylcyclopentane, 2,6-bis(1,1-dimethylethyl)-4-metylphenol, and 1,12-dodecanediol have been newly identified.

Table III documents a list of nine compounds that were found only in the aroma concentrates of uncured meat. Of these, five were aldehydes that included (*E*)-2-octenal, decanal, dodecanal, tridecanal, and tetradecanal. Among the four hydrocarbons identified, nonylcyclopropane (RT 13.11 min) and 5-propyldecane (RT 13.50 min) are newly reported. Of the 88 compounds identified in the different fractions of pork-, beef-, and chicken-aroma concentrates, only 2-methylcyclopentanol (RT 12.02 min) was found to be unique to the cured-meat aroma.

CONCLUSIONS

In continuation of our efforts to solve the mystery behind the chemical nature of the basic meat flavor, we have adopted a stepwise approach to tackle this problem. In the first stage, we compared the qualitative and quantitative composition of carbonyls and hydrocarbons present in the aroma concentrates of pork, beef, and chicken. As a second step we have attempted now to identify the key constituents responsible for the cured-meat aroma or the basic meaty aroma of cooked meat, which in our opinion should be essentially comprised of heterocyclic constituents. In the previous paper we reported the identification of 32 new constituents in the pork-aroma concentrates that were prepared using the nitrogen purge-and-trap method, under extremely mild conditions of cooking and aroma extraction. In the present investigation we are able to report the presence of 12 additional compounds that are new to the literature on meat flavor currently available. The nitrogen purge-and-trap method has been extremely useful in the fractionation of aroma concentrates into less volatile constituents that were adsorbed onto the cartridge, more volatile carbonyls and hydrocarbons that were trapped in the second cold trap maintained at –60 °C, and the heterocyclic and phenolic constituents in the first cold trap maintained at 4–5 °C. Interestingly, the first cold trap also had organoleptic properties reminiscent of the characteristic cured-meat aroma, or presumably the desirable meaty aroma note of cooked meat of all species. We intend to work on this fraction in depth and, if essential, use modes of separation and identification techniques other than the ones used currently. We strongly believe that this fraction has still some minor constituents that need to be characterized. Further, our aim is to use supercritical fluids such as carbon dioxide for the extraction of meat-flavor components. It is expected that many additional compounds not known so far may be identified in our future work. However, how many such newly identified components will actually contribute to the meaty aroma notes will be determined only by a detailed sensory evaluation. This aspect will be the main focus of our attention in the next phase of our research program.

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