Aroma Components of American Country Ham

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ABSTRACT: The aroma-active compounds of American country ham were investigated by using direct solvent extraction-solvent assisted flavor evaporation (DSE-SAFE), dynamic headspace dilution analysis (DHDA), gas chromatography-olfactometry (GCO), aroma extract dilution analysis (AEDA), and gas chromatography-mass spectrometry (GC-MS). The results indicated the involvement of numerous volatile constituents in the aroma of country ham. For DHDA, 38 compounds were identified as major odorants, among them, 1-octen-3-one, 2-acetyl-1-pyrroline, 1-nonen-3-one, decanal, and (E)-2-nonenal were the most predominant, having FD-factors ≥ 125 in all 3 hams examined, followed by 3-methylbutanal, 1-hexen-3-one, octanal, acetic acid, phenylacetaldehyde, and Furaneol™. For the DSE-SAFE method, the neutral/basic fraction was dominated by 1-octen-3-one, methional, guaiacol, (E)-4,5-epoxy-(E)-decenal, p-cresol as well as 3-methylbutanal, hexanal, 2-acetyl-1-pyrroline, phenylacetaldehyde, and γ-nonalactone. The acidic fraction contained mainly short-chain volatile acids (3-methylbutanoic acid, butanoic acid, hexanoic acid, and acetic acid) and Maillard reaction products (for example, 4-hydroxy-2,5-dimethyl-3(2H)-furanone). The above compounds identified were derived from lipid oxidation, amino acid degradation, and Maillard/Strecker and associated reactions. Both methods revealed the same nature of the aroma components of American country ham.

Keywords: American country ham, aroma-active compound, aroma extract dilution analysis, gas chromatography-olfactometry, solvent assisted flavor extraction

Introduction

Dry-cured ham is an important product worldwide. There are 3 ham belts in the world, including the southeastern United States, southern and central Europe, and southern China. Virginia, Tennessee, Kentucky, and North Carolina are major producing areas for American salt-cured country hams. The 9- to 12-mo fermentation period gives a final product with a distinctive and pleasant flavor—being very different from that of thermally processed meat products (Voltz and Harvell 1999; Arnold 2004).

A considerable amount of research has been conducted on dry-cured hams, mostly on European hams such as Iberian and Parma hams (Berdague and others 1991; Garcia and others 1991; Barbieri and others 1992; Ruiz and others 1998; Blank and others 2001; Carrapiso and others 2002a, 2002b; Belitz and others 2004). But to our knowledge, up to now, little information is available concerning either the general flavor compounds or the aroma-active components of dry-cured American country ham. Gas chromatography-olfactometry (GCO), including aroma extract dilution analysis (AEDA), has served as a very useful tool in flavor research for identifying and ranking the key odorants in various foods (Grosh 1993). The purpose of the present study was to identify and characterize the aroma-active compounds of American country ham by using gas chromatography-olfactometry (GCO), including both dynamic headspace dilution analysis (DHDA) and aroma extract dilution analysis (AEDA) techniques.

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Materials and Methods

Samples and chemicals

Hams. Hams 1, 2, and 3 (raw, unsmoked) were purchased from manufacturers located in North Carolina, Virginia, and Kentucky, respectively. The lean meat portions of the hams were cut into small pieces (approximately 1 cm^3), frozen in liquid nitrogen, and ground to fine powder.

Chemicals. Diethyl ether (anhydrous, 99.8%), sodium chloride (99%), sodium sulfate (anhydrous), sodium bicarbonate (99.7%), and hydrochloric acid (36.5%) were obtained from Fisher Scientific (Pittsburgh, Pa., U.S.A.). 2-Methyl-3-heptanone and 2-ethylbutanoic acid (internal standards for neutral/basic fraction and acidic fraction, respectively) were obtained from Aldrich Chemical Co. (St. Louis, Mo., U.S.A.). Authentic reference aroma compounds were also obtained from Aldrich.

Isolation of volatiles for instrumental analysis

Direct solvent extraction—SAFE. Ham powder (100 g) was placed in a Teflon bottle and extracted with 100, 75, and 75 mL of diethyl ether 3 times, respectively. The solvent-phase extracts were combined, concentrated using a Vigreux column to approximately 100 mL, then applied to a modified SAFE apparatus (ACE Glassware, Vineland, N.J., U.S.A.) for solvent extraction. The SAFE apparatus was similar to that described by Engel (Engel and others 1999), consisting of high vacuum pump, diffusion pump, receiving tube, and waste tube, operating at high vacuum (10^{-4} to 10^{-5} Torr) and very low temperature (–196 °C) to trap volatile substance and to avoid the production of artifact. Distillation was carried out for 2 h under vacuum. After distillation, the distillate was concentrated to about 30 mL by Vigreux column. The concentrated distillate was then extracted with aqueous $NaHCO_3$ (0.5 M, 3 × 30 mL). The solvent phase (upper phase) was concentrated by gentle nitrogen flow to about 10 mL, then dried over Na_2SO_4 (anhydrous) and

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concentrated again by gentle nitrogen flow to about 200 μL, which was a neutral/basic fraction for GC analysis. The acidic fraction was obtained as follows: the pH of aqueous phase (bottom phase) was adjusted to about 2.5 with hydrochloric acid (30% [w/w]), and then extracted with diethyl ether (3 × 20 mL). The extract was then concentrated by gentle nitrogen flow to about 10 mL, then dried over Na_2SO_4 (anhydrous) and concentrated again by gentle nitrogen flow to about 200 μL, also being ready to be applied to GC analysis.

Dynamic headspace sampling (DHS). Ham powder (1 g) was put into a purge-and-trap vessel (280 mL volume, SMS Co.). After equilibrating 5 min at 50 °C (water-bath circulation), the sample was purged with a nitrogen stream at a flow-rate of 50 mL/min for 25, 5, or 1 min, or at 10 mL/min for 1 min, respectively. Volatile compounds of the sample headspace were trapped onto a Tenax TA tube, which was placed onto the vessel. The Tenax tube was then dry purged for 20 min (TD controller, Gerstel, Mulheim Germany) to remove moisture.

Aroma extract dilution analysis (AEDA)

SAFE. Serial dilutions (1:3, 1:9, and so on) of each fraction were prepared in diethyl ether and analyzed by AEDA (Grosch 1993). GCO was conducted on an HP6890 GC (Agilent Technologies Inc.) equipped with FID and sniff port (DATU Technologies). Separations were performed on DB™-FFAP or DB™-5MS capillary columns (15 m × 0.32 mm i.d. × 0.5 (m film, J & W Scientific Folsom, Calif., U.S.A.) using helium as carrier gas at 2.2 mL/min. In order to minimize sample decomposition, each dilution (2 μL) was injected in the cool (38 °C) on-column mode. GCO was performed by 3 experienced panelists, and average $\log_3$FD factors were determined (Chung and Cadwallader 1994).

Dynamic headspace dilution analysis (DHDA). DHDA was performed on an Agilent 6890 GC equipped with a flame ionization detector (FID) and an olfactometry port (Gerstel). Aroma compounds from Tenax trap were thermally desorbed at 280 °C using a TDSA2 system (Gerstel) into a cryo-cooled (–150 °C) CIS inlet

Table 1—Predominant odorants in American country ham by AEDA (neutral/basic fraction).

Nr	Compound name	Odor property	R.I.		$\log_3$FD					
					Ham 1		Ham 2		Ham 3	
			DB-wax	DB-5	Outer	Inner	Outer	Inner	Outer	Inner
2	3-Methylbutanal	Dark chocolate-like	924	<700	3	2	3	3	3	4
39	Ethyl 2-methylbutanoate	Fruity	1070	—	ND[c]	ND	ND	ND	6	3
4	Hexanal	Cut-grass-like	1094	798	4	4	4	6	5	4
6	Unknown	Dark chocolate-like	1199		ND	4	3	1	3	3
9	1-Octen-3-one	Mushroom-like	1295	974	4	6	6	6	6	6
10	2-Acetyl-1-pyrroline[a]	Popcorn-like	1338	921	3	1	5	5	5	5
11	(*Z*)-1,5-Octadien-3-one[a,b]	Metallic	1363	992	<1	3	3	<1	3	2
16	Unknown	Milky	1445	1005	4	4	3	3	2	2
18	Methional	Cooked potato-like	1462	902	4	7	6	5	6	5
20	(*Z*)-2-Nonenal[a]	Hay-like, stale	1514	1142	3	3	5	3	5	3
21	(*E*)-2-Nonenal	Hay-like, stale	1538	1170	<1	3	1	3	1	ND
22	(*E,Z*)-2,6-Nonadienal[a,b]	Cucumber-like	1595	1154	1	0	3	2	3	1
24	Phenylacetaldehyde	Rosy	1654	1040	5	6	5	5	4	4
29	2-Acetyl-2-thiazoline	Popcorn-like	1772	1099	2	4	3	3	2	4
30	(*E,E*)-2,4-Decadienal	Fatty, fried	1820	1320	3	3	4	4	3	3
31	Guaiacol	Smoky	1865	1081	5	7	7	7	6	7
32	2-Phenylethanol[b]	Wine-like, floral	1905	1113	2	4	5	6	6	6
40	γ-Octalactone[b]	Coconut-like	1916	1268	2	ND	3	ND	5	2
41	4-Methylguaiacol	Smoky	1931		ND	2	5	5	ND	ND
34	(*E*)-4,5-Epoxy-(E)-decenal[a,b]	Flour-like, unripe	2017	1377	4	5	6	6	7	5
36	γ-Nonalactone[b]	Peachy	2039	1357	5	1	6	5	7	4
37	p-Cresol (4-methylphenol)	Fecal, bandaid	2078		4	7	5	5	6	6
38	γ-Decalactone[b]	Lactone-like	2103		ND	ND	0	ND	6	4
42	2-Aminoacetophenone[b]	Tortilla-like	2212		ND	4	4	ND	1	1
43	2,6-Dimethoxyl phenol	Smoky	2255		ND	ND	4	1	5	2
44	(*Z*)-6-Dodecene-γ-lactone[b]	Coconut-like	2392		ND	ND	4	ND	1	0
45	δ-Decalactone[a,b]	Peachy	2164		4	ND	4	ND	6	4

[a]Compounds tentatively identified by GCO, comparing their RIs and odor properties reference standards.
[b]Compounds not previously identified as aroma-active constituents of dried-cured hams (6 to 7).
[c]Not detected.

Table 2—Predominant odorants in American country ham by AEDA (acidic fraction).

Nr	Compound name	Odor property	R.I.	$\log_3$FD					
				Ham 1		Ham 2		Ham 3	
			DB-wax	Outer	Inner	Outer	Inner	Outer	Inner
1	Acetic acid	Vinegar-like, sour	1446	3	4	3	3	2	<1
2	2-Methylpropanoic acid	Fecal, cheesy	1553	ND	ND	1	1	1	ND
3	Butanoic acid	Fecal, cheesy	1623	6	4	4	3	3	<1
4	3-Methylbutanoic acid	Cheesy, fecal	1663	7	6	6	7	5	6
5	Pentanoic acid	Fecal, rancid	1718	ND	ND	ND	ND	3	ND
6	Hexanoic acid	Sour	1835	1	4	3	2	ND	2
7	Furaneol	Burnt sugar-like	2026	3	ND	5	4	ND	ND
8	Octanoic acid	Sour, waxy	2037	4	2	3	3	3	4
9	Phenylacetic acid	Rosy	2564	6	3	4	4	3	4

(Gerstel). Injection was splitless (inlet heating rate of 12 °C/min to 260 °C). Two different capillary columns were used for identifying the aroma compounds: Stabilwax-DA (15 cm × 0.32 mm i.d. × 0.5 (m film, Restek, Bellefonte, Pa., U.S.A.) and DB-5 (15 cm × 0.32 mm i.d. × 0.5 (m film, J & W Scientific). The effluent from GC was split 1:1 (v/v) between the FID and the sniffing port. GC oven program was 35 °C for 5 min, then ramped at 10 °C/min to 225 °C, and held at 225 °C for 15 min. GCO was performed by 3 trained panelists.

Identification

LRI and odor quality. The identification of volatile compounds was done by matching odor descriptions and linear retention indices (LRI) with reference compounds on both polar and nonpolar column. n-Alkanes (C_7 to C_{22} for DB-5 column and C_6 to $C_{26,28,30}$ for Stabilwax column) were analyzed under the same conditions to calculate LRIs.

Gas chromatography-mass spectrometry (GC-MS). GC-MS analysis of SAFE extracts was performed on an HP 6890 GC (Hewlett-Packard, Foster City, Calif., U.S.A.) coupled with an HP 5973 mass spectrometer (Hewlett-Packard) equipped with Stabilwax-DA column (30 m × 0.2 mm i.d. × 0.25 (m film) by direct on-column injection (1 L). GC oven program was 35 °C for 5 min, then ramped at 4 °C/min to 225 °C, and held at 225 °C for 20 min. Electron-impact mass spectra were generated at 70 eV with m/z scan range being 30 to 300.

DHS-GC-MS analysis was performed as described previously except that a TDS2 (Gerstel) system was installed. The thermodesorption system operated at the same condition as that of DHS-GC-O. The GC oven program was the same with that of SAFE extract-GC-MS.

Quantification of major volatile compounds

Both headspace (DHS) and solvent extraction (SAFE) methods were applied for quantification. For the DSE-SAFE-GC-MS method, 10 μL 2-methyl-3-heptanone (1.42 μg/μL in methanol) and 20 μL 2-ethylbutanoic acid (2.56 μg/μL in methanol) were also added to the samples at the same time as internal standards. For the DHS-GC-MS method, 1 μL 2-methyl-3-heptanone (1.42 μg/μL in methanol) and 5 μL 2-ethylbutanoic acid (2.56 μg/μL in methanol) were added to the purge-and-trap vessel as internal standards just before equilibrating the samples. The concentration for each compound was calculated as follows:

Table 3—Predominant odorants in American country hams flavor by DHDA-GC/O.

Nr	Compound name	Odor property	R.I.		FD factors[a]					
					Ham 1[b]		Ham 2[b]		Ham 3[b]	
			DB-wax	DB-5	Outer[c]	Inner[c]	Outer	Inner	Outer	Inner
1	Methanethiol[d]	Rotten, sulfurous	600 to 900		5	5	25	25	5	5
2	3-Methylbutanal[d]	Dark chocolate	914	<700	25	25	25	125	25	25
3	2,3-Butanedione[d]	Buttery	982		5	5	125	125	25	5
4	Hexanal[d]	Green, cut-grass	1079	796	25	25	125	125	5	5
5	1-Hexen-3-one[e]	Pungent, plastic, water bottle	1123	774	5	125	125	125	25	125
6	Unknown	Dark chocolate	1195		5	25	25	125	5	5
7	(Z)-4-Heptenal[e]	Rancid, crabby	1233	898	25	25	5	25	5	5
8	Octanal[d]	Green, orange peel	1285	999	25	25	125	125	25	25
9	1-Octen-3-one[e]	Mushroom	1295	978	125	125	125	125	125	125
10	2-Acetyl-1-pyrroline[e]	Popcorn	1338	921	125	125	125	125	125	125
11	(Z)-1,5-Octadien-3-one[e]	Metallic	1363	988	25	5	5	25	5	5
12	Dimethyl trisulfide[d]	Garlic, cooked cabbage	1380	967	25	25	25	25	25	25
13	Nonanal[d]	Stale, green, orange	1384	1108	125	5	5	25	125	125
14	1-Nonen-3-one[e]	Mushroom	1404		125	125	25	125	125	125
15	(E)-2-Octenal[d]	Raw peanut	1441		25	5	125	125	5	5
16	Unknown	Milky	1445		25	25	25	25	5	25
17	Acetic acid[d]	Vinegar, sour	1451		25	125	25	25	25	125
18	Methional[d]	Potato	1462	907	25	25	25	25	5	5
19	Decanal[d]	Orange, green	1498	1203	125	125	125	25	125	125
20	(Z)-2-Nonenal[e]	Hay, stale	1514	1148	25	25	125	25	25	25
21	(E)-2-Nonenal[d]	Hay, stale	1538	1162	125	125	125	125	125	125
22	(E,Z)-2,6-Nonadienal[e]	cucumber	1596	1155	25	25	25	25	5	5
23	Butyric acid[d]	Fecal, cheesy	1623		25	5	125	25	25	5
24	Phenylacetaldehyde[d]	Rosy	1654	1046	25	125	125	25	25	125
25	Isovaleric acid[d]	Cheesy, fecal	1663	872	25	25	125	25	25	25
26	2-Methyl-(3-methyldithio)furan[e]	Meaty, vitamin	1679	1173	25	25	5	5	1	1
27	(E,E)-2,4-Nonadienal[d]	Fatty, fried	1715	1202	25	25	25	25	25	25
28	(E)-2-Undecenal[e]	Cilantro	1756		125	5	5	25	125	25
29	2-Acetyl-2-thiazoline[d]	Popcorn	1772	1099	5	5	5	25	5	5
30	(E,E)-2,4-Decadienal[d]	Fatty, fried	1820	1320	25	25	25	25	25	25
31	Guaiacol[d]	Smoky	1865	1089	25	25	25	25	25	25
32	2-Phenylethanol[d]	Wine, floral	1917	1113	5	1	25	25	25	25
33	4-Methylguaiacol[d]	Smoky, mushroom	1931		1	25	1	25	1	25
34	(E)-4,5-Epoxy-(E)-decenal[e]	Flour, unripe	2017	1286	25	25	1	25	25	25
35	Furaneol[e]	Burnt sugar	2031	1087	125	5	125	25	125	25
36	γ-Decalactone[d]	Peachy	2039	1360	25	5	5	25	25	5
37	p-Cresol[d]	Fecal, bandaid	2082	1085	25	25	25	25	25	25
38	δ-Decalactone[d]	Peachy	2162	1467	1	5	125	25	125	25

[a] 1-g ham powder, nitrogen stream purging at 50 mL/min for 25, 5, 1 min respectively, the FD factors were 1, 5, 25 respectively; 1-g ham powder, nitrogen stream purging at 10 mL/min for 1 min, the FD factor was 125.
[b] The origins of hams 1, 2, and 3 are North Carolina, Virginia, and Kentucky, respectively.
[c] Outer, inner referred to the outer layers, inner layers of the hams.
[d] Compounds were tentatively identified by RI value, odor properties, and MS.
[e] Compounds were tentatively identified by GCO, comparing their RIs and odor properties with referenced RIs and odor qualities.

$$Concn_i = Concn_{IS} g Area_i / Area_{IS}$$

where $Concn_i$ was the concentration of an odorant; $Concn_{IS}$· was the concentration of internal standard; $Area_i$ was the area of an odorant on chromagram; $Area_{IS}$ was the area of internal standard on chromagram. The quantitative results are shown in Table 4 to 6.

Results and Discussion

AEDA

Twenty-seven compounds with average log_3FD-factor of $\geq$ 2 (Table 1) were identified as predominant odorants in neutral/basic fractions. Compounds with the odors of dark chocolate-like, cut-grass-like, mushroom-like, popcorn-like, cooked potato-like, hay-like/stale, rosy, fatty/fried, smoky, wine-like/floral, flour-like/unripe, peachy, and fecal/bandaid had high average log_3FD-factor of $\geq$ 3. These included 3-methylbutanal, hexanal, 1-octen-3-one, 2-acetyl-1-pyrroline, methional, (Z)-2-nonenal, phenylacetaldehyde, (E,E)-2,4-decadienal, guaiacol, 2-phenylethanol, (E)-4,5-epoxy-(E)-decenal, γ-nonalactone, and p-cresol. The rest of compounds listed in Table 1 were also important, but at slightly lower intensities or lower occurrences (not being detected in all 3 hams). Some compounds even had high log_3FD-factor of 6 in particular ham, such as ethyl 2-methylbutanoate, γ-octalactone, γ-decalactone, δ-decalactone, and so on, indicating their importance to the overall aroma of the corresponding hams. Smoky-smelling compounds of guaiacol, 4-methylguaiacol, and 2,6-dimethoxyphenol may gain these notes from storing with smoked hams together. The detection of these phenolic and lactone compounds was due to the advantage of SAFE techniques, which is suitable for extracting polar and high boiling-point compounds from complex food matrix. Compounds with average log_3FD-factor of $\leq$ 1 were considered to make only minor contributions to the overall aroma, and are not listed in the table. In the acidic fraction, 3-methylbutanoic acid was the most potent odorant, followed by butanoic acid, phenylacetic acid, and acetic acid (Table 2).

To our knowledge, 1-octen-3-one and methional were positively identified for a 1st time as aroma-active compounds from dried-cured ham by the GC-MS method. These compound were tentatively identified from Parma ham (Blank and others 2001), and Iberian ham (Carrapiso and others 2002a, 2002b) by comparison of LRIs and odor qualities with reference compounds, because their concentrations were too low to be detected by GC-MS to obtain unequivocal mass spectra. But in the American country hams examined, the concentration of 1-octen-3-one was 8 to 9 ng/g for ham 1 (from North Carolina) and 9 to 34 ng/g for ham 2 (from Virginia). Because of its low threshold (0.005 ng/L in water), its OAV (odor activity value) was high, being 1662 to 1680 for ham 1 and 1800 to 6800 for ham 2. The concentration of methional was 42 to 54 ng/g for ham 1, 23 to 33 ng/g for ham 2, and 13 to 14 ng/g for ham 3 (from Kentucky). Also due to its low threshold (0.2 ng/L in water), its OAV was fairly high, being 65 to 270 for all the 3 hams. These concentrations were high enough for them to be detected by GC-MS; therefore clear mass spectra were obtained (Agilent Technologies Inc. 2006; Vu 2007).

Being similar to above, compounds (nr 11, 22, 32, 34, 38) were also 1st time identified as aroma-active constituents of solvent extracts of hams. Besides, ethyl 2-methylbutanoate (nr 39, fruity), γ-octalactone (nr 40, coconut-like), 2-aminoacetophenone (nr 42, tortilla-like), (*Z*)-6-dodecene-γ-lactone (nr 44, coconut-like), and δ-decalactone (nr 45, peachy) were also detected from dried-cured ham for the 1st time. These aroma components contributed a pleasant fruity smell to the overall note of American country ham.

Important odorants such as 3-methylbutanal, hexanal, 1-octen-3-one, 2acetyl-1-pyrroline, methional, (Z)-2-nonenal, phenylacetaldehyde, guaiacol, 2-phenylethanol, (E)-4,5-epoxy-(E)-decenal, γ-nonalactone, and p-cresol were identified from both headspace and solvent extract of the American country ham. Among them, 1-octen-3-one (mushroom-like) and 2-acetyl-1-pyrroline (popcorn-like) were the most potent odorants in both portions (inner and outer layer) of the hams.

DHDA-GCO

Thirty-eight compounds were identified as major odorants (Table 3), among them, 1-octen-3-one (mushroom-like), 2-acetyl-1

Table 4—Concentration of predominant odorants in American country hams SAFE samples (neutral/basic fraction).

		Ham 1				Ham 2				Ham 3			
	Threshold	Outer		Inner		Outer		Inner		Outer		Inner	
Odorant	(ppb in water)	Concn (ppb)	OAV	Concn (ppb)	OAV	Concn (ppb)	OAV	Concn (ppb)	OAV	Concn (ppb)	OAV	Concn (ppb)	OAV
3-methylbutanal	0.2	—	—	—	—	—	—	197	985	—	—	—	—
2,3-butanedione	4	—	—	—	—	—	—	181	45	—	—	212	53
hexanal	4.5	430	96	287	64	655	146	1410	313	631	140	97	22
1-octen-3-one	0.005	8	1662	9	1800	34	6800	9	1800	—	NA	—	NA
(E)-2-heptenal	13	Trace	NA	Trace	NA	Trace	NA	86	7	Trace	NA	7	0
nonanal	1	Trace	NA	Trace	NA	Trace	NA	205	205	67	67	Trace	NA
1-octen-3-ol	1	69	69	Trace	NA	217	217	271	271	12	NA	27	27
methional	0.2	42	210	54	270	33	165	23	115	13	65	14	70
(E)-2-nonenal	0.15	Trace	NA	26	173	125	833	91	607	40	267	36	240
phenylacetaldahyde	4	164	41	180	45	270	68	277	69	39	10	45	11
(E,E)-2,4-nonadienal	0.09	60	667	18	200	115	1278	64	711	22	244	37	411
(E,E)-2,4-decadienal	0.07	56	800	24	343	314	4486	452	6457	25	357	24	343
guaiacol	2.5	50	20	11	4	188	75	64	26	49	20	32	13
2-phenylethanol	1000	Trace	NA	Trace	NA	481	0	641	1	294	0	555	1
4-methylguaiacol	—	—	—	—	—	—	—	—	—	9	—	44	—
γ-octalactone	7	187	0	Trace	NA	42	0	106	0	1	0	8	0
γ-nonalactone	30	75	0	17	0	83	0	175	0	9	0	11	0
p-cresol	55	108	2	66	1	132	2	74	1	27	0	27	0
δ-decalactone	100	Trace	NA	Trace	NA	13	0	10	0	Trace	NA	1	0

-pyrroline (popcorn-like), 1-nonen-3-one (mushroom-like), decanal (orange/green), and (E)-2-nonenal (hay-like/stale) were predominant odorants, having FD-factors $\geq$ 125 in all 3 hams examined. But due to their low concentrations in the headspace of hams, among them, only decanal was positively identified by the GC-MS method. This result agreed with the work of Carrapiso and others (2002a) to some extent, which stated that 1-octen-one was a secondary potent mushroom-smelling compound of Iberian ham headspace with a medium intensity of DF 20. But in our study, this compound showed its highest FD factor of 125, being one of the most potent compounds of American country ham.

At slightly lower intensities, 3-methylbutanal (dark chocolate-like), 1-hexen-3-one (plastic/water bottle-like), octanal (green/orange peel-like), acetic acid (vinegar-like/sour), phenylacetaldehyde (rosy), and Furaneol (burnt sugar-like) were also considered key aroma components. Remaining compounds in Table 3 may also be important in the overall aroma of the hams. The DHDA aroma profiles of the 3 ham samples were similar, with Virginia country ham having a slightly stronger overall aroma.

1-Nonen-3-one is also a key odorant in the headspace of American country ham, having a very intense mushroom-like odor. It was tentatively identified by the comparison of its LRI with that of the reference compound. This compound was reported in the flavor of frankfurter sausage (Chevance and Farmer 1999), also being tentatively identified by LRI and odor quality, but it was not detected in other hams such as Parma and Iberian hams (Blank and others 2001; Carrapiso and others 2002a, 2002b).

2-Methyl-(3-methyldithio)furan was tentatively identified by the comparison of its LRI with that of the reference compound. Although it had been found in cooked beef (MacLeod and Jennifer 1986; Gasser and Grosch 1988) and frankfurters (Chevance and Farmer 1999), it was identified for the 1st time from hams by us, possessing a vitamin/sulfurous odor character, which contributed considerably to the overall aroma profile of American country hams.

There were 2 unknown compounds in the ham headspace, nr 6 and 16 in Table 3. Number 6 compound had a strong odor of dark chocolate, with an LRI being 1195 on DB-Wax column. Compound nr 16 (LRI being 1445 on DB-Wax column) had a very interesting smell of fresh milk, rather potent in ham flavor. These compounds were also picked up by SAFE-GCO. The compounds mentioned previously were unique compared to the aroma components of other hams. Because of their important contribution to American country ham, their further identification was needed.

In this study, several compounds were reported for the 1st time as aroma-active constituents of dried-cured hams, such as (Z)-1,5-octadien-3-one (nr 11), 1-nonen-3-one (nr 14), (E,Z)-2,6-nonadienal (nr 22), 2-methyl-(3-methyldithio)furan (nr 26), (E)-2-undecenal (nr 28), 2-phenylethanol (nr 32), (E)-4,5-epoxy-(E)-decenal (nr 34), γ-nonalactone (nr 36), and δ-decalactone (nr 38). They possessed a wide region of odors of metallic,

Table 5—Concentration of predominant odorants in American country hams SAFE samples (acidic fraction).

		Ham 1				Ham 2				Ham 3			
	Threshold	Outer		Inner		Outer		Inner		Outer		Inner	
Odorant	(ppb in water)	Conc (ppb)	OAV	Conc (ppb)	OAV	Conc (ppb)	OAV	Conc (ppb)	OAV	Conc (ppb)	OAV	Conc (ppb)	OAV
Acetic acid	22000	4292	0	2724	0	5051	0	740	0	1066	0	216	0
2-Methylpropanoic acid	3400	3444	1	2236	1	3091	1	2528	1	2549	1	1719	1
Butanoic acid	240	2640	11	2101	9	234	1	1907	8	1441	6	433	2
3-Methylbutanoic acid	250	6185	25	4343	17	5760	23	4258	17	4697	19	7000	28
Pentanoic acid	2100	1530	1	709	0	1169	1	1916	1	275	0	228	0
Hexanoic acid	3000	3659	1	3966	1	3066	1	4063	1	1336	0	1389	0
Octanoic acid	3000	455	0	Trace	NA	438	0	541	0	373	0	252	0
Benzeneacetic acid	1000	1143	1	74	0	2131	2	819	1	1385	1	2225	2

Table 6—Concentration of predominant odorants in American country hams headspace.

		Ham 1				Ham 2				Ham 3			
	Threshold	Outer		Inner		Outer		Inner		Outer		Inner	
Odorant	(ppb in water)	Conc (ppb)	OAV	Conc (ppb)	OAV	Conc (ppb)	OAV	Conc (ppb)	OAV	Conc (ppb)	OAV	Conc (ppb)	OAV
3-Methylbutanal	0.2	577	2883	288	1440	245	1225	222	1110	660	3300	130	650
2,3-Butanedione	4	132	33	59	15	195	49	183	46	99	25	39	10
Hexanal	4.5	1465	325	1014	225	2859	635	1293	287	56	12	130	29
Octanal	0.7	Trace	NA	250	357	58	83	141	201	62	89	Trace	NA
Dimethyl trisulfide	0.01	Trace	NA	19	1900	Trace	NA	Trace	NA	Trace	NA	Trace	NA
Nonanal	1	271	271	571	571	112	112	206	206	179	179	83	83
1-Octen-3-ol	1	Trace	NA	324	324	312	312	516	516	80	80	113	113
Acetic acid	22000	79	0	Trace	NA	208	0	126	0	176	0	Trace	NA
Decanal	0.1	67	670	43	430	Trace	NA	Trace	NA	112	1120	29	290
Butanoic acid	240	Trace	NA	Trace	NA	Trace	NA	Trace	NA	53	0	Trace	NA
Phenylacetaldahyde	4	56	14	53	13	32	8	25	6	Trace	NA	Trace	NA
3-Methylbutanoic acid	250	Trace	NA	Trace	NA	Trace	NA	31	0	86	0	21	0
(E,E)-2,4-Nonadienal	0.09	Trace	NA	Trace	NA	21	233	29	322	Trace	NA	Trace	NA
(E,Z)-2,4-Decadienal		Trace	NA	Trace	NA	16	NA	11	NA	Trace	NA	Trace	NA
(E,E)-2,4-Decadienal	0.07	—	—	—	—	—	—	—	—	—	—	13	186
Guaiacol	2.5	30	12	Trace	NA	10	4	3	1	58	23	Trace	NA
2-Phenylethanol	1000	7	0	Trace	NA	40	0	52	0	768	1	39	0
γ-Nonalactone	30	Trace	NA	Trace	NA	14	NA	14	NA	Trace	NA	Trace	NA
p-Cresol	55	3	0	Trace	NA	8	0	3	0	Trace	NA	Trace	NA

mushroom-like, cucumber-like, vitamin-like/meaty, cilantro-like, wine-like/floral, flour-like/unripe, peachy, and peachy, respectively. Because of their fairly high FD factors, they were also considered as important aroma components of American country ham. They had not been identified previously as aroma-active constituents of other dried-cured hams. These compounds were most likely derived from lipid auto-oxidation except 2-phenylethanol (probably derived from phenylalanine), 2-methyl-(3-methyldithio)furan (Mottram 1991; MacLeod 1998; Chen and Ho 1998; Blank 1998; Frankel 2006), and more, lactones were also products of amino acid degradation (Tressel 1978).

Quantification

Quantitative data were consistent fairly with the AEDA and DHDA results (Table 4 to 6). Odor activity values (OAVs) were calculated for each positively identified compound. Most compounds were present at levels above their thresholds and their OAVs agreed with their FD factors; among them, some agreed well. For example, in Table 6, the concentration of 3-methylbutanal in outer portion of ham 3 was 660 ng/g, accounting for its highest FD factor of 125 among the 6 portions of the 3 hams examined. The concentrations of hexanal in outer and inner portions of ham 2 were 2859 and 1293 ng/g, giving their highest FD factor of 125. In the case of solvent extraction, similar things happened: the concentration of hexanal in inner portion of ham 2 was 1410 ng/g, being the highest among the 6 portions of the 3 hams examined, exhibiting its highest $\log_3$FD-factor of 6, comparing to other ham portions. The concentrations of methional in outer and inner portions of ham 2 were higher than those of portions of other 2 hams, so their $\log_3$FD-factors were greater. But the FD factors of some compounds did not agree very well with their concentration; this might be due to the problem of coelution with other compounds.

The results of GCO, AEDA, and DHDA indicated that in the involvement of numerous volatile constituents in the aroma of country ham, no single odorant was responsible for the overall American country ham note, being the same situation as that of Parma ham (Blank and others 2001) and Iberian ham (Carrapiso and others 2002a, 2002b).

The results also showed that there was no big difference between the outer and inner portions of the hams examined. In the case of ham headspace, the FD factors of both outer and inner portions of the 5 predominant odorants (1-octen-3-one, 2-acetyl-1-pyrroline, 1-nonen-3-one, decanal, and (E)-2-nonenal) were all the same, being 125. Only the FD factors of acetic acid, butanoic acid, and phenylacetaldehyde were slightly different between the 2 different portions (Table 3). As shown in Table 6, although the concentrations of 3-methylbutanal, 2,3-butanedione, and hexanal in outer portions were higher than those of inner ones, the rest compounds did not follow this rule. In the case of ham solvent extract, a similar thing happened, still no great difference between the 2 portions. In general, from FD factors to concentrations of odorants, no obvious evidence proved that the outer portions were more aromatic than the inner ones, and vice versa.

Lipid oxidation played an important role in ham flavor formation. Hexanal,(Z)-4-heptenal, octanal,1-octen-3-one,(Z)-1,5-octadien-3-one, nonanal,1-nonen-3-one,decanal,(Z)-2-nonenal (E)-2-nonenal,(E,Z)-2,6-nonadienal,(E,E)-2,4-nonadienal,(E)-2-undecenal, (E,E)-2,4-decadienal,(E)-4,5-epoxy-(E)-decenal, various lactones, and shot-chain fatty acid were derived from lipid oxidation.

Due to their detection and abundances in ham flavor such as 3-methylbutanal, 2-acetyl-1-pyrroline, methional, phenylacetaldehyde, p-cresol, Maillard/Strecker and associated reactions (amino acid degradation) were essential in the flavor formation of American country ham as these compounds were structurally related to isoleucine, proline, methionine, phenylalanine. Maillard reaction also accounted for the formation of furaneol (4-hydroxy-2,5-dimethyl-3(2H)-furanone), an important odorant (FDs were relative high) having a caramel-like note, fairly important in the flavor of American country ham, which was also reported 2nd time from dry-cured hams, firstly identified in Parma ham with a medium odor intensity of 1 to 2 (Berdague and others 1991; Barbieri and others 1992; Flores and others 1998; Blank and others 2001; Carrapiso and others 2002a).

The presence of ester such as ethyl 2-methylbutanoate suggested that esterization of ethanol with short-chain fatty acid was another pathway of the formation of ham flavor. Thus, catabolism of amino acids, lipids, and carbohydrates could lead to the formation of important odorants of American country ham. By careful controlling the reaction conditions in ham production and storage, these key aroma-active compounds mentioned previously may be formed properly, contributing desire flavor profile, and may serve as useful quality markers leading to improvements in ham production and storage practices.

Conclusions

Compared to the odor-active compounds of Parma and Iberian dry-cured hams (Blank and others 2001; Carrapiso and others 2002a), more odor-active compounds were identified from American country ham, and their characterization was determined. A better knowledge of the roles involving ham flavor formation is helpful for the understanding the nature of the unique flavor of American country ham. Further study on investigating factors that affect the flavor profile of American country ham may be useful in product quality control.

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