Volatile Components of Dry-Cured Ham

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The volatile components of dry-cured ham were isolated by vacuum distillation and analyzed by gas chromatography–mass spectrometry. The structures of 76 molecules were identified by mass spectrometry, cochromatography, or measured Kovats indices. The compounds identified may come from the catabolism of the main constituent parts of the meat (glucids, lipids and protids) during the curing of the hams, from the pig feed, or from technological processes. The volatile compounds extracted from 50 g of ham according to the technique described correspond to a chromatographical surface equivalent to 0.12 mg of the dodecane internal standard, or approximately 2.4 ppm of the weight of the sample. Flavor tests showed the existence of several aromatic molecules of which some, still unidentified, had the characteristic odor of salted products.

INTRODUCTION

Dry-cured ham is a nonsmoked product manufactured according to basic principles: stabilization through a drop in water activity and elaboration of the flavor through maturation. The different steps in the manufacturing processes are described in detail by Frentz (1982). French production of dry-cured hams increased considerably from 13 500 metric tons in 1970 to 39 000 metric tons in 1988. During this period of rapid growth in production the evolution both of the raw material used and of technological processes contributed to bringing about substantial modifications in the quality of the product and in particular in its taste. Very little research has been carried out on the flavor of dry-cured ham. There are the American works of Ockerman et al. (1964) and Lillard and Ayres (1969) on country-style hams, Italian works of Giolitti et al. (1971) on Italian and Parma type hams, and the Spanish research of Garcia et al. (1991) into Iberian hams. These authors have shown the presence of carbonyl compounds (alkanals, alk-2-enals, alk-2,4-dienals, and ketones), alcohols, fatty acids, and sulfurous molecules or various alkanes (Garcia et al., 1991). The aim of this study is to extend and update the identification of the volatile compounds of dry-cured ham and to carry out flavor tests to determine the molecules that are responsible for its aroma. It constitutes a preliminary stage leading to more technological research into the flavor of dry ham.

MATERIALS AND METHODS

Materials. For the purpose of this study we used four dry unsmoked hams of similar appearance and quality which were bought in four French industrial units of production. The hams had a potential shelf life of 7–9 months and had been deboned and wrapped in a polyethylene film at the time of purchase. With a view to subsequent extraction, a 25-g slice was cut from the center of each ham, perpendicular to the cavity of the bone. A 1-cm perimeter was cut away from each of the four slices which were then placed in a polyethylene bag, deep frozen to −30 °C, and ground in a domestic blender. Immediately after this operation, 50 g of ham was placed in a flask to extract the volatile compounds.

Isolation of Volatiles. The volatile constituents from hams were isolated by vacuum distillation and cold trapped in glass containers which were cooled by liquid nitrogen. The isolation involved two steps. In the first step, reduced pressure pumping (10^{-1} Pa) made it possible, in 5 h, to obtain virtually all the water contained in the ham as well as the more volatile substances according to the method described in detail by Dumont and Adda (1970). In the second step, the ham (now in a vacuum and deep frozen) was placed in a flask containing a cold trap as described by Forss and Holloway (1967) or Dumont and Adda (1972). In the absence of water in the sample it was thus possible to reach very low residual pressures (10^{-4} Pa), making it possible to extract in 4 h a high proportion of the less volatile substances. The flask was maintained at approximately 30 °C for the duration of the whole experiment.

After each extraction stage the contents of the traps were carefully rinsed with distilled water. All rinsing fractions were grouped together and brought to pH 9 by sodium hydroxide (*N*/10) to fix the majority of carboxylic acids in the form of soap in the aqueous fraction. The neutral fraction of the volatile compounds was then extracted in an erlenmeyer, with magnetic stirring with 3 × 10 mL of bidistilled dichloromethane.

Measured amounts of dodecane (1 mL, 2/100 m/v) were added to the organic extract just before final concentration to 150 μL with a Kuderna column (Kuderna-Danish). The dodecane made it possible to check the efficiency of the concentration stage.

Capillary Gas–Liquid Chromatography–Mass Spectrometry (GC–MS) Analysis. A Nermag R10-10c quadrupole GC–MS system, directly coupled to a Girdel 31 gas chromatograph equipped with a split–splitless injector, was used. Separation was performed with a J&W Scientific fused silica capillary column (60 m × 0.32 mm i.d.) coated with DB5 (film thickness 1 μm). Carrier gas was helium (velocity, 35 cm s^{-1}), and the oven was programmed from 40 to 240 °C at a rate of 3 °C min^{-1}. Splitless injection was used, and Kovats indices were calculated after the method of Tranchant (1982) and compared with available literature data. The mass spectra were measured by electron impact at 70 eV.

Flavor Tests. The sensory characteristics of the different compounds chromatographed were measured by olfactory tests through a dividing system (SGE OSS-2) installed at the exit of the chromatographic column. Chromatographic separation was carried out under the same conditions as for the identification of molecules by GC–MS coupling with a column of 30 m instead of 60 m. To obtain a direct comparison with the mass spectrometry results, flame ionization detection (FID) of compounds was carried out at the same time as the olfactory tests. The division relationship FID/olfaction was 20/80. Three assessors took part in the flavor tests.

RESULTS AND DISCUSSION

In dry-cured ham, dry matter represented between 55 and 60% and the proportions in lipids and proteins were about 7 and 70%, respectively. The aromatic compounds

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Table I

no.[a]	peak no.[b]	chemical name	Kovats index[c]	reliability of ID[d]	rel area[e]	F[f]
		Hydrocarbons: *n*-Alkanes				
1	21	*n*-nonane	900	A	0.87	
2	27	*n*-decane	1000	A	1.05	4
3	36	*n*-undecane	1100	A	0.42	4
4	42	*n*-dodecane (internal standard)	1200			
5	47	*n*-tridecane	1300	A	0.14	4
6	54	*n*-pentadecane	1500	A	0.17	4
7	60	*n*-heptadecane	1700	A	<0.10	4
8	62	*n*-octadecane	1800	A	0.20	4
9	74	*n*-heneicosane	2100	A	0.14	4
		Hydrocarbons: Methyl-Branched Alkanes				
10	8	?-trimethylpentane	757	D	0.26	
11	9	?-trimethylpentane	766	D	0.20	
12	10	4-methylheptane	767	B	0.25	
13	29	?-methyldecane	1022	D	0.23	
14	33	3-methyldecane	1073	B	0.22	
15	45	?-dimethylundecane	1280	D	0.13	
		Hydrocarbons: Aromatics				
16	11	toluene	771		7.52	
17	16	ethylbenzene	856	B	2.66	
18	18	1,3-dimethylbenzene (*m*-xylene)	867	B	0.61	
19	20	1,2-dimethylbenzene (*o*-xylene)	892	B	0.42	
20	26	1,2,4-trimethylbenzene (pseudocumene)	990	B	0.35	
		Terpenoids				
21	64	farnesol	1834	C	0.40	
		Aldehydes: Aliphatics, Aromatics				
22	3	3-methylbutanal (isovaleraldehyde)		C	1.76	1, 3, 4
23	14	hexanal	798	A	3.06	2, 4
24	28	octanal	1004	A	0.14	2
25	37	nonanal	1099	A	4.88	2, 4
26	39	2-nonenal	1158	B	<0.10	2, 4
27	43	decanal	1205	A	1.68	2, 4
28	46	undecanal	1296	A	0.12	2
29	52	dodecanal	1398	A	<0.10	2
30	59	tetradecanal	1610	A	0.13	
31	61	pentadecanal	1710	A	0.22	
32	63	hexadecanal	1811	A	6.11	4
33	67	?-octadecenal	1913	D	0.17	
34	72	9-octadecenal	1993	C	1.11	
35	73	octadecanal	2017	C	0.44	
36	31	benzeneacetaldehyde	1055	B	1.21	4
		Ketones: Aliphatics				
37	1	butan-2-one		C	3.61	1, 2, 4
38	4	3-methylbutan-2-one		C	1.43	4
39	5	3-hydroxybutan-2-one (acetoin)	721	B	2.92	4
40	19	heptan-2-one	889	B	0.23	4
41	25	octan-2-one	989	B	0.37	
42	53	*trans*-geranylacetone	1452	C	0.23	
		Aliphatic Alcohols				
43	6	3-methylbutan-1-ol	743	B	3.10	4
44	7	2-methylbutan-1-ol	748	B	0.78	4
45	12	pentan-1-ol	766	B	0.85	4
46	17	hexan-1-ol	862	B	8.69	2, 4
47	23	1-octen-3-ol	978	A	0.82	4
48	34	octan-1-ol	1079	B	0.46	2
49	38	phenyl-2-ethanol	1114	B	1.37	4
		Carboxylic Acids				
50	24	hexanoic acid		A	0.28	
51	35	heptanoic acid	1083	B	0.10	
52	40	octanoic acid	1177	B	0.80	
53	44	nonanoic acid	1273	B	1.68	
54	56	undecanoic acid	1561	B	0.17	
55	65	pentadecanoic acid	1851	C	0.24	4
56	69	9-hexadecenoic acid	1938	C	0.75	
57	70	hexadecanoic acid	1957	C	1.40	
58	75	9,12-octadecadienoic acid (linoleic acid)		C	0.62	
59	76	9-octadecenoic acid (oleic acid)		C	0.14	
		Aliphatic Esters				
60	68	hexadecanoic acid, methyl ester	1916	B	0.10	

Table I (Continued)

no.[a]	peak no.[b]	chemical name	Kovats index[c]	reliability of ID[d]	rel area[e]	F[f]
		Lactones				
61	32	γ-hexalactone	1064	B	0.24	4
62	49	γ-nonalactone	1372	B	0.68	4
		Nitrogen Compounds				
63	22	?-dimethylpyrazine	911	D	0.31	
64	30	1-methyl-2-pyrrolidinone	1042	C	4.87	
		Chloride Compounds				
65	2	trichloromethane (contaminant of solvent)		A	5.82	
66	15	tetrachloroethene (contaminant of solvent)	808	B	0.13	
67	48	unidentified contaminant of solvent	1332		0.60	
68	50	2-chloronaphthalene (contaminant, used in laboratory)	1379	A	0.11	
69	51	1-chloronaphthalene (contaminant, used in laboratory)	1382	A	2.22	
		Others				
70	13	2,4,6-trimethyl-1,3,5-trioxane	776	C	1.40	
71	41	butoxyethoxyethan-1-ol	1184	C	0.10	
72	55	BHT	1513	B	1.50	4
73	57	1,2-benzenedicarboxylic acid, ? alkyl ester	1593	D	0.40	
74	58	1,2-benzenedicarboxylic acid, ? alkyl ester	1595	D	0.12	
75	66	1,2-benzenedicarboxylic acid, ? alkyl ester	1863	D	0.33	4
76	71	1,2-benzenedicarboxylic acid, ? alkyl ester	1958	D	0.50	4

[a] Current number of compounds. [b] Peak number in Figure 1. [c] Kovats indices calculated for the DB5 capillary column of the GC–MS system. [d] The reliability of the identification or structural proposal is indicated by the following symbols: A, mass spectrum and retention time identical with those of an authentic sample; B, mass spectrum and Kovats index in agreement with the corresponding literature data; C, mass spectrum consistent with spectra found in the literature; D, tentative identification by mass spectrum, e.g., position of methyl branching unknown. [e] Relative percentage of total peak area (solvent and internal standard excluded). [f] Already identified in: 1, Ockerman et al. (1964); 2, Lillard and Ayres (1969); 3, Giolitti et al. (1971); 4, Garcia et al. (1991).

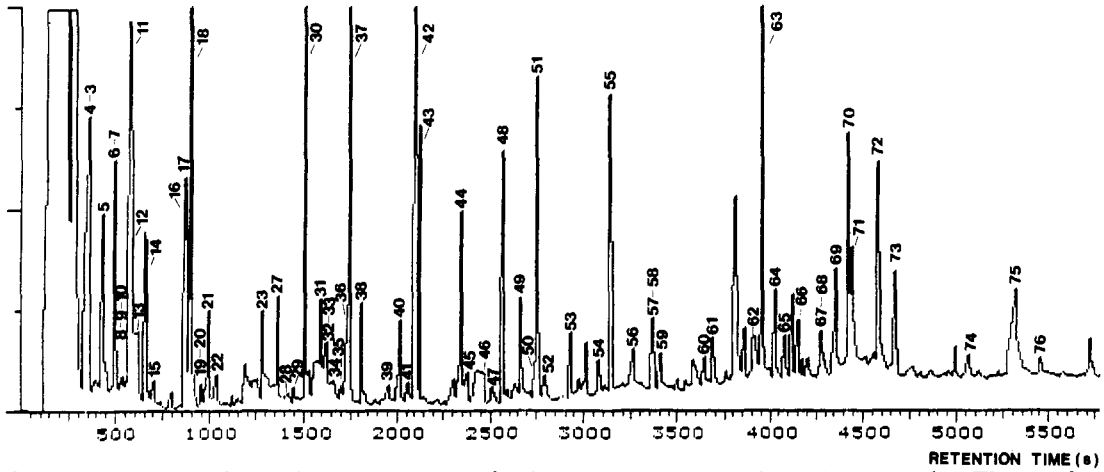

Figure 1. Gas chromatogram obtained from an equiponderal mixture of the four hams in the study. The numbers mark such compounds identified. For more information, see Table I and Materials and Methods.

may thus be dissolved in water or in lipids or even adsorbed and retained in the protein structure of the meat. The vacuum distillation used enabled us to obtain extracts which smelled of ham, containing compounds of varied volatility for which the molecular weight was between 72 (butan-2-one) and 282 (oleic acid). However, current headspace analyses should permit the identification of the most volatile compounds (sulfur in particular) which are not lost during removal of dichloromethane. Isolated compounds correspond to a total mass of 0.12 mg of dodecane, or 2.4 ppm of the mass of the ham. Theses low contents can probably be explained by high retention of aromatic molecules in the lipidic and proteic phases, since it is impossible to deodorize ham through the distillation method used.

A chromatogram obtained by plotting the total ion current observed during a GC–MS run is given in Figure 1. Of a total of 100 individual peaks detected, 76 peaks were definitely or tentatively identified (Table I). These 76 peaks represent about 88% of the chromatogram surface (excluding solvant and dodecane).

A study of the different chemical families identified shows, in order of presentation in the table, the presence of hydrocarbons (20), aldehydes (15), ketones (6), alcohols (8), carboxylic acids (10), lactones (2), an ester, and a pyrazine, all of which constitute the main part of the identified components liable to come from the ham.

Among the hydrocarbons, eight *n*-alkanes, six branched alkanes, and five aromatic compounds were detected. Alkanes constitute a low percentage of the relative chromatogram area (<4.38%). According to Loury (1972), the *n*-alkanes may come from the autoxidation of the lipids. Because of the great structural diversity of the branched alkanes, it was not possible to identify their structure with only mass spectrometry in electron impact. Branched alkanes are molecules frequently found in fresh meats (Shahidi et al., 1986) which may come from the oxidation of branched fatty acids naturally present in low quantities in animal tissues or perhaps from the nonsaponifiable fraction of vegetable products used in pig feed (Van Straten, 1977; Rembold et al., 1989). In the mixture of the four hams (Figure 1), the aromatic hydrocarbons

represent an important class (total relative area, 6.56%), and one should note the presence of major amounts of toluene and ethylbenzene chromatographic peaks; those of xylene and pseudocumene are much less significant. All these components have already been identified by Wittkowski (1989) in smoke aromas used to flavor processed meat. Separate analysis of the four hams and the absence of aromatic hydrocarbons in other tests on French or Spanish dried hams (Garcia et al., 1991) confirmed the contamination of one of the hams analyzed by aromas from smoking.

The chemical family with the highest proportion in the extract is the aldehydes (total relative area 26.23% of which 25.02% is just for aliphatic aldehydes). The alkanals or alkenals with more than six carbon atoms are typical products of lipid oxidation. Indeed, the autoxidative mechanisms of the free fatty acids lead to the complete inferior homologous series of aldehydes (Loury, 1972; Paquette et al., 1985). Carbon short-chain components with a methyl branch such as 3-methylbutanal (or, for alcohols, 2- or 3-methylbutan-1-ol and, for ketones, 3-methylbutan-2-one) have a high degree of volatility. According to Hertz and Chang (1970), there is no typical source for these compounds since they may not only represent traces of living cell activity but also originate from lipolysis, sugar catabolism, or meat proteolysis. Phenylacetaldehyde and phenyl-2-ethanol are amino acid catabolism indicators (phenylalanine in particular).

Ketones (total relative area 8.79%) are represented essentially by methyl ketones and by *trans*-geranylacetone. Methyl ketones are the products of β-keto acid decarboxylation or of saturated fatty acid β-oxidation (Lehninger, 1981).

Alcohols (total relative area 16.07%) are best represented in the extract by hexanol, 3-methylbutanol, and 1-octen-3-ol.

The free carboxylic acids (total relative area 6.18%) come from the hydrolysis of the triglycerides and phospholipids of the ham.

The γ-lactones are the products of dehydration and cyclization of the γ-hydroxyacids that are known to be present in animal fats (Shahidi et al., 1986).

The only two nitrogenous compounds of the extract are a cyclic amide, 1-methyl-2-pyrrolidinone or γ-butyrolactame (derivative of the cyclization of the γ-amino acids), and a dimethylpyrazine. According to Baines and Mlotkiewicz (1983), only 5 pyrazines have been identified in red pork, compared with 48 and 14 for beef and mutton, respectively. The presence of these molecules in meat products is mainly linked to reactions occurring during the cooking process as is shown by their wide diversity in the volatile compounds of fried bacon (Ho et al., 1983).

BHT might originate in pig feed because of its incidence on the pig's lipidic metabolism and its antioxygen properties. The addition of BHT to animal feed has already been studied by Pascal and Desmoulins (1973). According to legislation its residual quantity should not be higher than 100 ppm.

The origin of the chloride compounds is set out in Table I. The phthalates are contaminants that probably come from the polyethylene films used during experimental storing of the hams in a vacuum. 2,4,6-Trimethyl-1,3,5-trioxane or paraldehyde is a product of the condensation of the acetaldehyde (Arnaud, 1973); its origin is unknown as is that of the butoxethoxyethan-1-ol.

Several identified molecules show sensory features during the olfactory tests at the exit of the chromatographic column. Certain aldehydes like hexanal, nonanal, or 2-nonenal smell of tallow, fat, or oil, thus probably contributing to the rancid component of the aroma of ham. 3-Methylbutanal (rancid, sweaty, pungent) and benzeneacetaldehyde (harsh, hawthorn) also have dinstinctive odors. Among the ketones, 3-hydroxybutan-2-one and also butane- and pentane-2,3-dione identified by Garcia et al. (1991) have a strong smell of butter. Other ketones have olfactive characteristics: heptan-2-one (spicy, blue cheese), octan-2-one (green, herbaceous), *trans*-geranylacetone (parsley). The alcohols with the most distinctive smells are 1-octen-3-ol (mushroom), octan-1-ol (sharp, fatty, waxy), phenylethanol (rose), and farnesol (musklike). γ-Nonalactone (musk, coconut, licorice), pyrazine (potatoes), 1-methyl-2-pyrrolidinone (penetrant, undefined) also have strong smells.

However, several molecules with a very weak chromatographic signal are associated with floral smells or smells which are characteristic of the product. Thus, several small nonidentified peaks, for which the Kovats indices are between those of pyrazine (911) and 1-octen-3-ol (978), are linked with strong smells from salting dried sausage skin or fermented meat. Because of their aromatic characteristics, identification of these molecules will be the subject of our next studies.

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