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Characterization of the Most Odor-Active Compounds of Iberian Ham Headspace

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Gas chromatography−olfactometry (GC-O) based on detection frequency (DF) was used to characterize the most odor-active compounds from the headspace of Iberian ham. Twenty-eight odorants were identified by GC-O on two capillary columns, including aldehydes (11), sulfur-containing compounds (7), ketones (5), nitrogen-containing compounds (2), esters (2), and an alcohol. Among them, the highest odor potencies (DF values) were found for 2-methyl-3-furanthiol, 2-heptanone, 3-methylbutanal, methanethiol, hexanal, hydrogen sulfide, 1-penten-3-one, 2-methylpropanal, ethyl 2-methylbutyrate, and (*E*)-2-hexenal. Nine of the 28 most odor-active compounds were identified for the first time as aroma components of dry-cured ham, including hydrogen sulfide, 1-penten-3-one, (*Z*)-3-hexenal, 1-octen-3-one, and the meaty-smelling compounds 2-methyl-3-furanthiol, 2-furfurylthiol, 3-mercapto-2-pentanone, 2-acetyl-1-pyrroline, and 2-propionyl-1-pyrroline.

KEYWORDS: Odor-active compounds; Iberian ham; dry-cured meat; olfactometry; purge and trap

INTRODUCTION

The characteristic aroma of meat products greatly contributes to their overall acceptance (*1*). This fact is especially important in Iberian ham, an expensive Spanish dry-cured ham with a prolonged curing process and excellent consumer acceptance. To date, a considerable amount of research has been devoted to the study of the volatile compounds of dry-cured hams (cf. review by Flores et al., *2*), and a large number of volatile compounds have been identified. Most research focused on ham volatile compounds has been limited to the study of the most abundant compounds (hydrocarbons, aldehydes, ketones, and alcohols) without elucidating their contribution to dry-cured ham aroma. However, it is well accepted that only a limited number of volatile compounds actually contribute to the overall food aroma (*3*). In addition, it is known that some powerful odorants found in meat systems exist at concentrations too low to allow their identification by the usual gas chromatography−mass spectrometry (GC-MS) procedures (*4*). Therefore, a different approach is required to understand ham aroma and identify its odor-active compounds.

Gas chromatography−olfactometry (GC-O) is a useful and powerful tool to research food aroma (*5*). Several olfactometry techniques are available to identify and rank the odorants, such as Charm analysis and aroma extract dilution analysis (AEDA) (cf. review by Shieberle, *3*), Osme analysis (*6*), and detection frequency (*7*). Whereas the odorants of cooked meats have been widely investigated by using GC-O (*8*−*10*), there is a lack of research about the odor-active compounds of dry-cured meat products. Although the odorants from fermented-dried sausages (*11*) and dry-cured ham (*2*, *12*) have already been investigated, they have not been ranked according to their relative odor potency or their possible contribution to ham aroma. In addition, Iberian ham odorants have never been researched.

Therefore, the purpose of the present work was to identify and characterize the most odor-active compounds of dry-cured ham, specifically of Iberian ham.

MATERIALS AND METHODS

Samples. Twenty-seven Iberian hams processed according to the traditional method (*13*) were analyzed. A piece of biceps femoris muscle was taken, vacuum-packaged, frozen, and kept at −80 °C until required (6 months).

Chemicals. The reference compounds of the odorants listed in **Table 1** were obtained from Sigma and Aldrich (Steinhein, Germany). Standard solutions were prepared at a concentration of 5 μL mL^{-1} of reference compounds in hexane or dichloromethane (HPLC grade).

Isolation of Volatile Compounds. Before analysis, visible fat and the surface of each sample (0.5 cm) were removed. Frozen samples were then minced and blended, and 6 g was placed into a flask for the volatile compound extraction. The isolation was carried out using an automatic HP G1900A purge and trap concentrator (Hewlett-Packard). The sample headspace was swept onto the Tenax/silica gel/charcoal trap using a helium stream of 40 mL/min. Conditions were as follows: trap temperature during purge, −20 °C; sample temperature, 50 °C; preheat time, 5 min; purge time, 30 min. The volatile compounds were desorbed by heating the trap at 220 °C and were immediately injected into the gas chromatograph (GC). The transfer line to the GC was held at 210 °C, and the trap heating was kept at 220 °C for 2 min.

GC-O. *GC-O Conditions.* GC-O was performed using an HP 5890 series II chromatograph (Hewlett-Packard) equipped with a flame ionization detector (FID) and a sniffing port ODO-1 (SGE, Ringwood, Australia) (without control of humidified air temperature). The effluent from the capillary column was split 1:1 (v/v) between the FID and the

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Table 1. Most Odor-Active Compounds of Iberian Ham Headspace

no.[a]	LRI[b] HP-5	LRI[b] HP-FFAP	odorant	descriptors[c]	DF[d]	ref[e]
1	<500	<800	hydrogen sulfide[h]	boiled or rotten eggs, sewage	43	
2	<500	<800	methanethiol[h]	rotten eggs, meat or fish, cheesy	45	32
3	521		unknown	cured, rancid, apple-like	5	
4	558	834	2-methylpropanal[f]	toasted, fruity, pungent	30	12
5	590	991	2,3-butanedione[f]	sweety, caramel-like, vanilla-like	4	30
6	614		unknown	fruity, toasted	5	
7	656	931	3-methylbutanal[f]	fruity, almond-like, toasted	45	31
8	668	905	2-methylbutanal[f]	rancid, almond-like, toasted	3	31
9	678	1034	1-penten-3-one[f]	rotten, sewer-like, fruity	39	
10	700	944	2-pentanone[f]	green, fruity, tropical fruit-like	3	30
11	715	972	pentanal[f]	nutty, toasted, fruity	5	30
12	761	972	ethyl 2-methylpropanoate[g]	fruity, toasted, pungent	7	2
13	791		unknown	cured, nutty, almond-like	6	
14	803	1082/1146	hexanal[f]/(*Z*)-3-hexenal[i]	green, fruity, acorn-like	43	30/–
15	857	1053/1224	ethyl 2- methylbutyrate[g]/(*E*)-2-hexenal[f]	fruity, apple-like, strawberry-like	26	31/30
16	882	1335/1172	2-methyl-3-furanthiol[g]/2-heptanone[f]	cured ham-like, toasted, nutty	49	–/31
17	907	1200/1363	heptanal[f]/3-mercapto-2-pentanone[i]	cured ham-like, toasted, sewage, fatty, fruity	6	30/–
18	914	1471/1443	methional[f]/2-furfurylthiol[g]	boiled meat-like, cured ham-like, potato-like	9	31/–
19	922	1348	2-acetyl-1-pyrroline[i]	overheated meat-like, cured ham-like, roasted	20	
20	940		(*E*)-2-heptenal[f]	almond-like, fruity, fried food-like	7	30
21	954		dimethyl trisulfide[h]	rotten egg-like, burnt	3	17
22	991	1301/1395	1-octen-3-one[g]/1-octen-3-ol[f]	mushroom-like, dirty, dust	20	–/31
23	1023	1417/1289	2-propionyl-1-pyrroline[i]/octanal[f]	stew-like, boiled meat-like, rancid	25	–/30
24	1044	1410	(*E*)-2-octenal[f]	fruity, rancid, tropical fruit-like	6	30

[a] Odors are presented in order of elution on the HP-5 column. [b] LRI values: linear retention indices (LRI) are given on two different polarity capillary columns, when applicable. [c] Odor quality perceived at the sniffing port. [d] Detection frequency (DF) determined in Iberian ham using an HP-5 column. Analyses were performed by nine sniffers. [e] Earlier reported in the literature as volatile compound in Iberian ham. [f] Identified by comparing it with the reference compounds on the basis of the following criteria: MS spectra, LRI on two stationary phases (when applicable), and odor quality as well as odor intensity perceived at the sniffing port. [g] MS signals were too weak; compound was identified by comparing it with the reference compound on the basis of the remaining criteria. [h] Compound was identified by comparing it with literature data on the basis of the following criteria: MS spectra, LRI on two stationary phases (when applicable), and odor quality as well as odor intensity perceived at the sniffing port. [i] MS signals were too weak; the compound was identified by comparing it with literature data on the basis of the remaining criteria.

sniffing port using two deactivated but uncoated fused silica capillaries (50 cm × 0.32 mm). HP-5 (50 m × 0.32 mm i.d., film thickness = 1.05 μm, Hewlett-Packard) and HP-FFAP (30 m × 0.32 mm i.d., film thickness = 0.25 μm, Hewlett-Packard) fused capillary columns were used. The injector and detector were maintained at 230 and 250 °C, respectively. After the splitless injection, oven conditions were as follows: 35 °C for 5 min, 10 °C min^{-1} to 150 °C, 20 °C min^{-1} to 250 °C, 250 °C for 10 min. Humidified air was added in the sniffing port at 500 mL min^{-1}.

Odor Detection Frequency. The detection frequency method (*7*) was applied to identify and rank the odorants according to their odor potencies. A panel of nine assessors was selected among people experienced in sensory analysis and was trained in GC-O using reference compound solutions and the volatile compounds isolated by purge and trap from Iberian ham samples. During GC-O, sniffers were asked to give a description of each perceived odor, even if they did not recognize it. They were also asked about its length and its intensity in order to aid the odorant identifications. Two replicates of each sample (27 × 2) were performed on the nonpolar column (HP-5) to calculate detection frequency data and were randomly smelled by the assessors (six times per assessor). Data from the nine sniffers were analyzed, and the detection frequency (DF) of odors having the same linear retention index (LRI) and a similar description was calculated as the number of times they were smelled. Each odor peak was considered only if it was smelled by at least three assessors.

Nine additional replicates were used to aid the confirmation of odorant identities by using the polar column (HP-FFAP).

Identification. *LRI and Odor Quality*. The identification of volatile compounds was performed by matching odor descriptions (odor quality and intensity) and LRI on the two columns with those of reference compounds under the same conditions or with odor description and LRI previously reported (*9*, *14*, *15*). Solutions of hydrocarbons (C_5−C_{18} for HP-5 and C_5−C_{25} for HP-FFAP) were analyzed in the same conditions to calculate LRI.

GC-MS. GC-MS analysis was performed on an HP 5890 series II chromatograph (Hewlett-Packard) coupled to an HP 5971A mass spectrometer (Hewlett-Packard) and equipped with one of the two capillary columns described above. Oven conditions were similar to those applied for GC-O. Mass spectra were generated by electronic impact at 70 eV, with a multiplier voltage of 1675 V. Data were collected at a rate of 1 scan s^{-1} over the *m*/*z* range 30−300. The transfer line to the mass spectrometer was maintained at 280 °C. Compounds were identified by comparison of mass spectra and LRI with those of reference compounds or with mass spectra contained in the Wiley and the NIST/EPA/NIH mass spectral libraries and LRI previously reported (*9*, *14*, *15*).

RESULTS AND DISCUSSION

The most odor-active compounds of the headspace of Iberian ham were characterized by application of GC-O based on the detection frequency method. Despite the differences in volatile compounds when the same ham types were compared (*16*), the number of Iberian hams analyzed (27 × 2) ensures that the odorants identified and their DF are representative of Iberian ham aroma. Twenty-four odor-active regions were smelled by at least three assessors on the HP-5 column. Odor-active regions are given in order of elution on this column in **Table 1**. A great variety of odor qualities, such as fruity, cheesy, mushroom-like, or nutty, were perceived, and even four regions were described as cured ham-like smelling peaks (16−19). The detection frequency chromatogram is shown in **Figure 1**. The DF data revealed the highest frequencies for peaks 16 (cured ham-like, toasted), 7 (fruity, almond-like), 2 (rotten egg-like), 14 (green, fruity), 1 (boiled eggs), 9 (rotten, sewer-like), and 4 (toasted, fruity). Somewhat lower frequencies were found for peaks 15 (fruity, apple-like), 23 (stew-like, boiled meat-like), 19 (overheated meat-like), and 22 (mushroom-like).

Identification of the odorants revealed that some of the odor-active regions on the HP-5 column were made up of more than

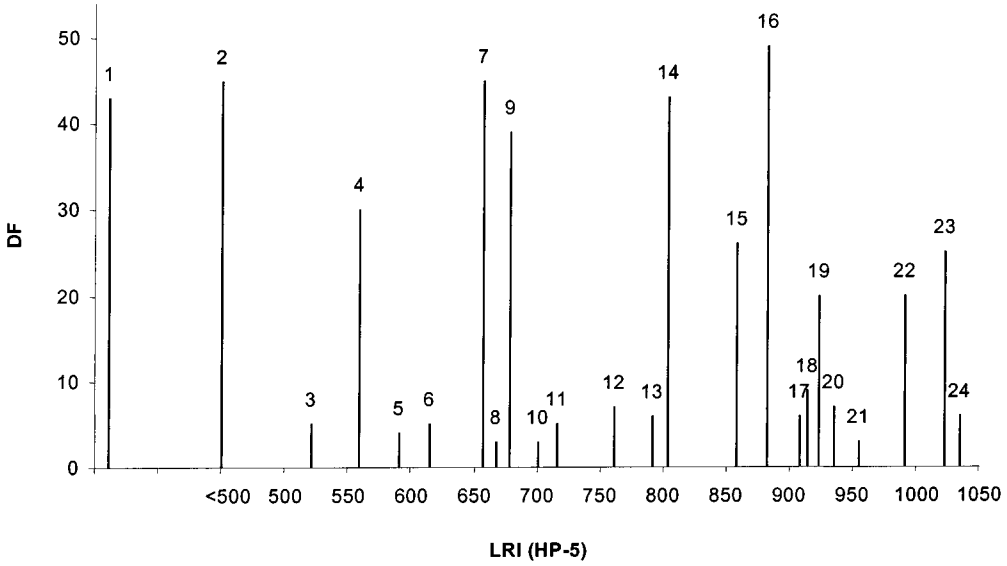

Figure 1. Detection frequency chromatogram obtained by applying the detection frequency method on Iberian ham headspace. Numbers correspond to **Table 1**.

one odorant. Most odor-active regions were correctly separated on the HP-5 column, and no evidence of coelutions was found during the GC-MS analysis on this column. They were found only when the second column was used to confirm identities. This confirms that the use of a second column is advisable to avoid assigning odor description of coelutions to abundant compounds with clear MS spectra.

The identities of 16 of the compounds listed have been established by comparison of LRI on two columns, odor quality, and mass spectral data with those of reference compounds, and probable identities have been suggested for a further 12 compounds (**Table 1**). Among the odor-active regions with the highest DF, most odorants readily identified by MS have already been reported as volatile constituents in dry-cured ham (methanethiol, 2-methylpropanal, 3-methylbutanal, hexanal, 2-heptanone, and 1-octen-3-ol). Their contribution to dry-cured ham seems to be clear; in fact, some of them have been related to sensory characteristics of ham aroma, that is, 3-methylbutanal (nutty, chessy, *17*) or lipid oxidation products (rancidity, *18*).

Besides the potent odor-active compounds already reported as volatile compounds, a total of nine further odorants were found for the first time as dry-cured ham constituents. Among them, some meaty-smelling compounds and unsaturated ketones are odorants showing high odor frequencies, although in most cases no clear signal was displayed by the FID and MS detectors. The low concentrations of the newly identified compounds and their coelution with more abundant compounds, such as hydrocarbons and aldehydes, probably explain why these low odor threshold compounds have not been reported in previous investigations on dry-cured ham volatiles, because they were mainly performed without the application of GC-O. This fact confirms that LRI with odor quality and intensity is a useful tool to identify, in particular, odor-active compounds with extremely low odor threshold values, as was indicated by Buettner and Schieberle (*19*). This approach allowed the identification of the potent meaty-smelling odorants 2-methyl-3-furanthiol (**16**), 2-furfurylthiol (**18**), 3-mercapto-2-pentanone (**17**), 2-acetyl-1-pyrroline (**19**), and 2-propionyl-1-pyrroline (**23**), despite the fact that it was not possible to obtain an unequivocal mass spectrum. To date, these compounds have never been identified either in dry-cured hams or in Iberian ham, although they have been found in cooked meats (*8*−*10*).

2-Methyl-3-furanthiol (2-MFT) and 2-furfurylthiol (2-FF) are some of the most potent odorants found in food, as their odor threshold value in air is 0.0025 ng/L (*20*). They usually appear at very low concentrations (*10*) and yield no clear mass spectra when the usual GC-MS techniques are applied (*4*). The identifications were based on two analytical criteria (LRI on the HP-5 column and LRI on the HP-FFAP column) and two sensory criteria (odor quality and intensity). In this work it was not possible to obtain any trace of 2-MFT by GC(HP-5)-MS because of its coelution with other compounds, but using GC-(HP-FFAP)-MS the main ion was found with an LRI similar to that of the reference compound. The odor quality of the 2-MFT-containing peak in the HP-FFAP column was similar to that of the reference compound, but different in the HP-5 column, where it appeared in coelution with 2-heptanone and 1-hexanol, two high odor threshold value compounds which probably increased the DF and enhanced the meaty quality of this peak. The high DF value for this odor-active region shows 2-MFT to be an outstanding contributor to ham aroma. Although this compound has not been previously identified in dry-cured ham and meats, its presence could be expected because of the high concentration of precursors in pork meat such as thiamin (*21*) and conditions of meat during the curing process, such as acid pH and low A_w values, where 2-MFT is preferentially formed (*22*, *23*). For the other sulfur-substituted furan no trace was found by MS. According to its LRI on the two columns and the odor quality and odor intensity, we proposed an identification as 2-furfurylthiol (peak 18). This thiol has been previously found in a large variety of cooked meats (*8*).

3-Mercapto-2-pentanone (peak 17) was tentatively identified on the basis of the odor LRI on the two capillary columns. This compound has already been identified in cooked meats (*9*, *10*) and shows a low odor threshold value [0.045−0.18 ng/L in air (*14*)]. In any case, it seems to contribute to a lesser extent to ham aroma.

The meaty-smelling compounds in peaks 19 and 23 were tentatively identified as 2-acetyl-1-pyrroline (2-AP) and 2-propionyl-1-pyrroline (2-PP) by comparing their LRI and odor descriptions on two capillary columns with literature data (*14*). Both compounds have low odor threshold values [0.02 ng/L (*24*) and 0.02 ng/L (*25*), respectively] and possess intense roast odors (*14*, *26*). 2-AP has been found in cooked meats (*9*, *10*)

and recently in fermented dried sausages (*11*). Its formation has been related to Maillard-type reaction under thermal treatments (*27*) and also to the amount of yeast in the baking process (*28*) and fungal growth on the surface of fermented dried sausage (*11*). Therefore, fungal growth could explain the presence of 2-AP in Iberian ham. With regard to 2-PP (peak 23), its presence in foodstuffs as an odorant seems to be infrequent. 2-PP has been identified in only freshly popped corn and roasted skin of fried chicken (cf. review by Hofmann and Schieberle, *26*).

Hydrogen sulfide (**1**) was also identified for the first time in dry-cured ham aroma. It is an intermediate in Maillard reactions that yield meaty-smelling compounds (*23*) and has been found in cooked meats (*10*). Because of its large DF, this compound seems to be an important contributor to ham aroma.

Among the carbonyl compounds newly identified, 1-penten-3-one (peak 9) was the most potent odorant. Its low odor threshold value [0.73 μg/L in oil (*15*)] is one of the lowest found for this type of compound. 1-Penten-3-one has been reported as a constituent of olive oil (*15*), but not in cooked meats. Its presence in Iberian ham could be due to the high oleic acid content of Iberian pigs (*29*).

(*Z*)-3-Hexenal (peak 14) was tentatively identified on the basis of the LRI of the odor on the HP-FFAP column and odor quality. Its coelution with hexanal on the HP-5 column and its low concentration hindered a more complete identification. Its odor threshold value is also low [0.09−0.36 ng/L (*14*)], and it has been also identified in olive oil (*15*).

A procedure similar to those for identifying 2-MFT (LRI and MS identification only with the main ion on the HP-FFAP column) allowed the identification of 1-octen-3-one as the most potent mushroom-smelling compound of Iberian ham headspace. Previous studies on dry-cured ham have identified 1-octen-3-ol, but not 1-octen-3-one, which shows a similar LRI on the HP-5 capillary column (peak 22). However, the LRI of the main mushroom-smelling region on the HP-FFAP column matched the LRI of 1-octen-3-one, despite only the main ion being found by MS on this column. Otherwise, only a weak odor matching 1-octen-3-ol LRI was detected.

Although the representativeness of the isolated compounds was not checked, headspace techniques are believed to yield representative odorant isolations as they extract the volatile compounds that surround the food (and therefore which are perceived by the olfactory system) (*7*). The purge temperature strongly affects the compounds extracted, and temperatures >80 °C generate significant changes in the volatile compound profile (*33*). A previous work on Iberian ham volatile compounds showed similar results for samples heated at 40 or 60 °C for 20 min (*32*). Therefore, although the purge temperature applied to isolate the odor-active compounds was higher than eating temperature, results could be considered as representative of Iberian ham headspace. In addition, it is accepted that the detection frequency method generates results similar to those found with other olfactometric methods (*34*, *35*).

The number of volatile compounds newly identified, including some of the most potent odorants, indicates the difficulty in studying the aroma composition of dry-cured ham, and specifically of Iberian ham, by applying only the usual GC-MS procedures. None of the numerous and abundant hydrocarbons (neither aliphatic nor aromatic hydrocarbons) reported in previous studies performed on dry-cured hams were found to be potent odorants. Although most of the odor-active compounds identified have already been found in dry-cured ham, all of the meaty-smelling compounds included in **Table 1** were tentatively identified for the first time.

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