Characterization by Volatile Compounds of Microbial Deep Spoilage in Iberian Dry-Cured Ham

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Abstract: In the present study, volatile compounds of spoiled dry-cured Iberian ham with deep spoilage or "bone taint" were analyzed and correlated with level of spoilage and the microorganisms detected. Volatile compounds extracted by a solid phase micro-extraction technique were assayed by gas chromatography/mass spectrometry. The spoiled hams were evaluated sensorially, and the correlations among volatile compounds, spoilage level, and microbial counts were studied. The spoiled hams had higher concentrations of hydrocarbons, alcohols, acids, esters, pyrazines, sulfur compounds, and other minor volatile compounds than unspoiled hams. The sensorial analysis showed that the spoilage level of hams correlated with several volatile compounds, most of them associated with Gram-positive catalase positive cocci and *Enterobacteriaceae* counts. Cyclic compounds such as cyclohexanone, some ethers, and pyrazines should be considered as indicators to monitor incipient microbial deep spoilage in the elaboration of this meat product.

Keywords: Iberian dry-cured ham, microbial deep spoilage, volatile compounds

Introduction

Iberian dry-cured ham is a traditional meat product obtained after 24 mo of ripening. During the 1st periods of processing, salting and slow drying are combined with a low temperature to reduce the risk of pathogenic bacteria and microbial spoilage. The most important cause of bacterial spoilage in Iberian and other kinds of dry-cured ham is called "bone taint" or "deep spoilage." This alteration occurs most commonly in the large muscle masses adjacent to the bone structures, and is characterized by a pasty texture and foul-smelling odor. The microbial growth results in the generation of peptides, amino acids, amines, ammonia, sulfides, alcohols, aldehydes, ketones, and organic acids with unpleasant, and unacceptable off-flavors (García and others 2000). The off-odors and flavors that develop during the ripening process in spoiled hams have been described as slightly acid to putrid (Córdoba and others 2001), leading to the rejection of the whole piece. However, they could be slight and overlooked in the piece, remaining undetected until consumption.

The prevalent microbial groups in spoiled Iberian dry-cured hams are Gram-negative bacteria and Gram-positive catalase-positive cocci (GPCP), but lactic acid bacteria (LAB) have also been reported at low levels (García and others 2000; Martín and others 2008). Several studies have considered the *Enterobacteriaceae* group to be the bacterial population responsible for spoilage of different kinds of dry-cured hams, including Iberian dry-cured ham (Marín and others 1996; Miranda and others 1998; Paarup and others 1999; García and others 2000). In addition, a synergy between non-enteric Gram-negative bacteria (NEGN) and GPCP has also been observed in microbial deep spoilage of Iberian dry-cured ham (Martín and others 2008).

To detect this microbial alteration, it is necessary to know if there are indicator compounds that can be used to determine incipient deep spoilage, and also in the quality control to detect slight and overlooked spoilage. Volatiles are suitable compounds for such a purpose since their non-destructive direct extraction from the ham is possible using headspace SPME (Ruiz and others 2001). Several volatile compounds have been associated with bacterial spoilage of such protein foodstuffs as prawns and cold smoked salmon (Chinivasagam and others 1998; Joffraud and others 2001; Jónsdóttir and others 2008). However, little is known about volatile compounds that are associated with the deep microbial spoilage of dry-cured hams.

The objective of this study was to identify volatile compounds that could be used as indicators of deep spoilage in Iberian dry-cured ham for use in the quality control of dry-cured meat products. For this purpose, volatile compounds determined in Iberian dry-cured hams with signs of microbial deep spoilage were correlated with level of spoilage and the microorganisms detected.

Materials and Methods

Sample collection

The study included 30 spoiled Iberian hams, taken from the ripening process when they showed initial sings of spoilage (off-odor). In all of the spoiled hams the alteration had been overlooked in the internal muscle (*biceps femoris*). The off-odor of these hams was first detected after internal puncture with a thin bone. Total of 3 unspoiled hams without internal off-odor ripened under the same conditions as the spoiled hams were sampled as controls.

Samples (15 g approx.) were aseptically collected with a 2.5-cm diameter sterile metal cork borer from approximately the geometric centre of the ham, near to the coxofemoral joint (Martín and others 2004). The innermost part of the cylinder consisting of *biceps femoris* as the basis muscle was selected for microbial analysis. The adjacent zone of this muscle (50 g approximately) was taken for the volatile compound and sensorial analyses.

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Microbiological analysis

For microbiological enumeration and identification, 10 g of the internal sample of ham were homogenized in 90 mL sterile 0.1% peptone in a Stomacher (Lab Blender, Model 4001, Seward Medical, London, UK) for 30 s. Appropriate dilutions were made with 0.1% peptone broth, and 1 mL aliquots were plated onto the culture media under the following conditions: mesophilic aerobic bacteria on plate count agar (PCA, Oxoid, Unipath, Basingstoke, U.K.) for 72 h at 30 °C; *Enterobacteriaceae* on Violet Red Bile Glucose Agar (VRBGA, Oxoid) for 24 h at 37 °C; lactic acid bacteria, LAB, on MRS Agar (Oxoid) in anaerobic conditions (Anaerocult A system, Merck, Darmstadt, Germany) for 72 h at 30 °C in jars; Gram-positive catalase-positive cocci on Mannitol Salt Agar (MSA, Oxoid) after 72 h at 37 °C; sulfite reducing clostridia on Sulfite-Polymyxin-Sulfadiazine (SPS) agar incubated anaerobically for 72 h at 37 °C; intestinal enterococci on Slanetz and Bartley agar (S&B, Oxoid) for 24 h at 37 °C; yeasts and moulds on Malt Extract Agar (MEA, Oxoid) for 4 d at 25 °C.

Samples with counts lower than 2 log CFU g^{-1} were discarded for microbiological analysis since the role attributable to these low counts in the microbial alteration of the Iberian ham is really scarcely appreciable. For the samples with counts higher than 2 log cfu g^{-1}, the colonies were selected according to their morphology from plates that had counts of between 30 and 300 colonies (2 to 5 colonies for each plate), and were then subcultured on the same medium on which they had been isolated. Each isolate was examined for colony and cell morphology under a microscope, and in the case of bacteria was tested for its Gram reaction. Complementarily, catalase, oxidase, and urease activities, and glucose and lactose fermentation, were tested to characterize the colonies at the microbial group level.

Volatile compound extraction

Frozen samples were minced and 1 g was weighed into a 10 mL headspace vial (Hewlett-Packard, Palo Alto, Calif., U.S.A.) and sealed with a PTFE butyl septum (Perkin-Elmer, Foster City, Calif., U.S.A.) in an aluminium cap. Volatile compounds were extracted by solid phase microextraction (SPME) (Ruiz and others 1998) with a 10 mm long, 100 μm thick fibre coated with carboxen/polydimethylsiloxane (Supelco, Bellefonte, Pa., U.S.A.). Prior to collection of volatiles, the fibre was preconditioned at 220 °C for 50 min at the GC injection port. It was then inserted into the headspace vial for 30 min at 40 °C in a water bath.

Gas chromatography/mass spectrometry (GC/MS) analyses

GC/MS analyses were performed using an Agilent 6890 GC–5973 MS system (Agilent Technologies, Little Falls, Del., U.S.A.). A 5% phenyl–95% polydimethylsiloxane column (30 m × 0.32 mm ID, 1.05 μm film thickness, Hewlett-Packard) was used for the separation of volatile compounds. The carrier gas was helium. The injection port was in a splitless mode. The SPME fiber was kept at 220 °C in the injection port throughout the chromatographic run. The temperature program was isothermal for 15 min at 35 °C, increased to 150 °C at 4 °C min^{-1}, and then to 250 °C at 20 °C min^{-1}. To calculate the Kovats index of the compounds, n-alkanes (R-8769, Sigma Chemical Co., St. Louis, Mo., U.S.A.) were run under the same conditions. The GC/MS transfer line temperature was 280 °C. The mass spectrometer was operated in the electron impact mode, with an electron energy of 70 eV, a multiplier voltage of 1650 V, and a rate of 1 scan s^{-1} over a range of m/z 40 to 300 for data collection. The NIST/EPA/NIH mass spectrum library and Kovats indeces were used to identify the volatile compounds.

Sensorial analysis

Total of 18 panelists trained with different samples of spoiled and unspoiled hams were asked to characterize the spoilage level of the samples. For that, 2 g of each spoiled ham sample was minced into 50-mL Falcon tubes, and a ranking analysis was performed according to international standard methods (ISO 2006). The only sensory descriptor used was "anomalous odor." During each session, 6 spoiled hams were presented in randomized order to the panelists who judged the spoilage level, ordering the samples from lesser to greater anomalous odor using a numbered scale (from 1 to 6 points). Each sample was evaluated in duplicate with a total of 10 sessions.

Statistical methods

Statistical analysis of the data was carried out using SPSS for Windows, 10.0. (SPSS Inc., Chicago, Ill., U.S.A.). Mean values were calculated for the area percentages of volatile compounds. Volatile compound values were studied by one-way analysis of variance (ANOVA). The relationships between volatile compound values, microbial counts and spoilage level of hams were evaluated by Pearson correlation coefficients.

Results and Discussion

Counts of the different microbial groups

Counts of the microorganism groups in spoiled and unspoiled hams are presented in Table 1. Most of the strains isolated from the culture media PCA were non-enteric Gram-negative bacteria (NEGN) according to their cell morphology, and catalase and oxidase activities. Some 90% of spoiled hams presented counts greater than 2 log CFU g^{-1} whereas in the control hams the counts were always less than 2 log CFU g^{-1}. This microbial group has been described as predominant in spoiled Iberian dry-cured ham (Martín and others 2008).

Table 1–Microbial prevalence and counts in the Iberian dry-cured ham batches studied.

Culture medium	Microorganism group[a]	Spoiled hams		Unspoiled hams	
		%[b]	log ufc g^{-1}[c]	%	log ufc g^{-1}
PCA	**NEGN**	90	2.0 to 8.3 (5.4)	0	<2
MSA	**GPCP**	56.7	2.0 to 6.0 (3.0)	66.7	2.1 to 2.5 (2.4)
VRBGA	**Enterobacteriaceae**	26.7	2.0 to 6.0 (2.9)	0	<2
MRS	**LAB**	26.7	2.0 to 4.9 (3.5)	0	<2
SPS	——	0	<2	0	<2
S&B	——	0	<2	0	<2

[a]NEGN = non-enteric Gram-negative bacteria; GPCP = Gram-positive catalase-positive cocci; LAB = lactic acid bacteria.
[b]Incidence per batch.
[c]Range of counts (mean of counts).

Gram-positive catalase-positive cocci (GPCP) were the only microbial group isolated from MSA agar. The counts were less than 3 log CFU g^{-1} in unspoiled hams, whereas the spoiled hams had maximum counts of 6 log CFU g^{-1}. These microorganisms have been identified as predominant during most of the ripening time in the normal processing of different types of dry-cured hams (Huerta and others 1988; Rodríguez and others 1994; Losantos and others 2000). In unspoiled Iberian hams, GPCP counts of 4 log CFU g^{-1} have been found in deep tissues at the end of the post-salting period (Rodríguez and others 1994). Regarding the *Enterobacteriaceae* counts obtained in VRBGA medium, 26.7% of the spoiled hams had counts greater than 2 log CFU g^{-1}, whereas in the control hams the counts were lower than 2 log CFU g^{-1}. This microbial group has also been reported at levels of 2 to 6 log CFU g^{-1} in spoiled Iberian dry-cured hams (García and others 2000; Losantos and others 2000), so that these microorganisms can be attributed a major role in spoilage.

MRS Agar showed counts greater than 2 log CFU g^{-1} of LAB in 26.7% of the spoiled hams, but with counts always below 5 log CFU g^{-1} (Table 1). LAB counts of around 2 log CFU g^{-1} have been found in spoiled and unspoiled Iberian dry-cured ham (García and others 2000). Finally, counts in SPS and S&B culture media were irrelevant, with levels below 2 log CFU g^{-1} in both spoiled and unspoiled hams. Microorganisms growing in these culture media were therefore discarded from further consideration for this survey.

Volatile compounds

Over 70 volatile compounds were identified in the samples (Table 2), including aliphatic, branched, and aromatic

Table 2–Volatile compounds from unspoiled and spoiled Iberian dry-cured hams.

Peak number	Compounds	ID[a]	% Area Unspoiled hams	% Area Spoiled hams	P[b]
Hydrocarbons			*3.98*	*12.00*	++
5	Hexane	A	> 0.01	0.23	
21	Toluene	A	> 0.01	1.99	++
24	Octane	A	1.00	0.06	++
33	m-xylene	A	> 0.01	2.68	+
38	o-xylene	A	> 0.01	0.10	
41	Nonane	A	> 0.01	0.65	+
48	Branched hydrocarbon	C	> 0.01	0.48	++
51	Branched hydrocarbon	C	> 0.01	0.18	
54	Decane	A	> 0.01	0.21	
58	d-Limonene	A	0.95	0.76	
62	Undecane	A	> 0.01	0.06	
67	Dodecane	A	> 0.01	0.17	
69	1,3-bis (1,1-Dimethylethyl) benzene	B	1.50	0.73	
71	Tetradecane	A	> 0.01	1.78	+
73	Pentadecane	A	0.38	0.73	
74	Hexadecane	A	> 0.01	0.92	+
75	Heptadecane	A	> 0.01	0.28	
Alcohols			*3.90*	*21.25*	++
1	Ethanol	A	.....		
3	1-Propanol	A	> 0.01	0.19	
10	1-Butanol	A	> 0.01	0.08	
15	3-Methyl butanol	A	1.48	0.33	+
17	2-Methyl butanol	A	1.27	0.86	
20	1,2-Propanodiol	B	> 0.01	0.03	
22	1-Pentanol	A	> 0.01	2.56	
27	2,3-Butanodiol	A	> 0.01	5.40	+
28	R 2,3-Butanodiol	A	> 0.01	4.24	+
34	1,3 Butanodiol	A	> 0.01	6.54	++
36	Hexanol	A	1.04	0.63	
49	1-Octen-3-ol	A	> 0.01	0.16	+
65	Phenyl ethyl alcohol	B	> 0.01	0.24	
Aldehydes			*36.15*	*18.31*	+
2	2-Methyl propanal	A	> 0.01	1.03	+
8	3-Methyl butanal	A	8.88	4.05	
9	2-Methyl butanal	A	5.90	1.28	+++
12	Pentanal	A	1.92	1.20	
25	Hexanal	A	13.85	3.23	+
43	Heptanal	A	2.64	0.17	+++
46	Benzaldehyde	A	> 0.01	0.18	+
59	Benzene acetaldehyde	A	0.18	6.87	+
64	Nonanal	A	2.75	0.30	+++
Ketones			*7.15*	*1.45*	++
4	2-Butanone	A	3.02	0.18	+++
11	2-Pentanone	A	> 0.01	0.55	
18	3-Hydroxy-2-butanone (Acetoin)	A	1.54	0.10	+
19	3-Methyl-2-pentanone	A	0.92	0.10	
39	Cyclohexanone	A	> 0.01	0.15	+
50	2-Methyl-3-octanone	A	> 0.01	0.03	
53	6-Methyl-5-hepten-2-one	A	1.62	0.34	++
Acids			*1.24*	*6.07*	+
13	Acetic acid	A	1.09	1.97	
23	Propanoic acid	A	> 0.01	0.06	
29	Butanoic acid	A	0.09	2.08	+
35	Pentanoic acid	A	> 0.01	0.33	
37	3-Methyl butanoic acid	A	> 0.01	0.51	
40	2-Methyl hexanoic acid	B	> 0.01	0.15	
42	2-Methyl butanoic acid	A	> 0.01	0.97	
Esters			*49.42*	*34.35*	
14	Ethyl propanoate	A	> 0.01	2.18	
26	Ethyl butanoate	A	21.82	7.85	++
30	Ethyl-2-hydroxy propanoate	A	> 0.01	1.42	
31	Ethyl 2-methyl butanoate	A	1.74	3.46	
32	Ethyl 3-methyl butanoate	A	1.91	5.91	+
44	Ethyl pentanoate	A	2.10	0.54	+
55	Ethyl hexanoate	A	21.13	7.19	++
63	Ethyl heptanoate	A	> 0.01	0.42	+
68	Ethyl octanoate	A	0.65	1.67	
70	Ethyl nonanoate	A	> 0.01	1.09	
72	Ethyl decanoate	A	> 0.01	2.63	
Pyrazines			0.05	*1.52*	+
45	2.6-Dimethyl pyrazine	A	> 0.01	0.05	
56	Trimethyl pyrazine	B	> 0.01	0.58	
61	Tetramethyl pyrazine	B	> 0.01	>0.01	
66	2,3,5-Trimethyl 6-ethyl pyrazine	B	> 0.01	0.01	
Other compounds			*0.07*	*5.20*	+++
7	Trimethyl amine	B	> 0.01	0.03	
16	Dimethyl disulfide	A	> 0.01	0.25	
47	Dimethyl trisulfide	A	> 0.01	0.31	+
52	2-Pentyl furane	A	> 0.01	0.27	
57	Ethanol 2,2 (ethoxy-ethoxy)	B	> 0.01	4.33	+++
60	5-Ethyl dihydro 2(3H)-furanone	B	> 0.01	0.01	

[a]Reliability of identification: A: mass spectrum and Kovats index. B: mass spectrum.
[b]*P* values: + ($P < 0.1$); ++ ($P < 0.05$); +++ ($P < 0.001$).

hydrocarbons, aldehydes, furans, carboxylic acids, alcohols, aromatic alcohols, ketones, pyrazines, esters, ethers, and sulfur compounds. Most compounds identified have been reported in unspoiled Iberian dry-cured ham (Ruiz and others 1998, 1999; Martín and others 2006), although most of the aforementioned volatile groups showed relevant differences between control and spoiled batches (Table 2).

Hydrocarbons were 3.98% of the total area of volatile compounds in the control hams, whereas in the spoiled hams they were 12%. The increase of linear and branched hydrocarbons in spoiled hams, mainly of shorter chain length hydrocarbons, can be attributed to a high microbial lipolytic activity which plays a decisive role in increasing free fatty acids, favouring lipid oxidation and both linear and branched hydrocarbon formation (Berdagué and others 1991; Ruiz and others 1999; Martín and others 2006). Toluene and m-xylene were the most abundant hydrocarbons in the spoiled hams. The origin of these benzene compounds in Iberian dry-cured ham has been attributed to the pig's feed (Buscailhon and others 1993; Ruiz and others 1999), although aromatic hydrocarbons have been observed to increase during the ripening time in dry-cured meat products and in culture media inoculated with microorganisms (Martín and others 2003; Benito and others 2004; Andrade and others 2009). Indeed, the amounts of these compounds vary in dry-cured ham samples obtained from different locations and inoculated with different microorganisms (Martín and others 2006).

Table 2 also lists the alcohols (3.90% of total area in the control batch and 21.25% in the spoiled batch). Ethanol was not considered due to its use for sample sterilization. 3-methyl butanol had its greatest percentage in the control batch, while butane-1,3-diol, butane-2,3-diol, and (R)-butane-2,3-diol had higher levels in the spoiled batch. Butane-1,3-diol and butane-2,3-diol have also been found in unspoiled dry-cured ham, but in low amounts (García and others 2000). The higher content of the above compounds in the spoiled samples may be related to the activity of several microorganisms. Indeed, increases of butane-1,3-diol and butane-2,3-diol have been reported in the spoilage of protein foodstuffs, including dry-cured ham (García and others 2000; Joffraud and others 2001).

The linear and branched carbonyl compounds had, in general, higher percentages in the control batch (Table 2). Hexanal, a carbonyl that arises from the oxidation of n-6 fatty acids, was the main such compound (13.85%). Similar or higher percentages to these have been reported in Iberian dry-cured ham (García and others 1991; Ruiz and others 1999). Branched aldehydes, such as 3-methyl butanal and 2-methyl butanal, have been associated with nutty, cheesy, and salty notes in Parma ham (Hinrichsen and Pedersen 1995), and correlate with the flavor of cured hams (Careri and others 1993). The importance of these compounds to overall flavor has been described in Iberian dry-cured ham (Ruiz and others 1999). On the contrary, cyclic and aromatic carbonyls such as benzaldehyde, benzeneacetaldehyde, and cyclohexanone were found at higher percentages in the spoiled hams. In particular, benzeneacetaldehyde has been described as a relevant odorant in spoiled dry-cured Iberian ham (Carrapiso and others 2010). Some of these cyclic compounds have been detected in unspoiled dry-cured ham (Ruiz and others 1999; Martín and others 2006), but at a lower proportion.

With respect to the acids, these were at higher percentages in the spoiled batch (6.07% of total area). The higher amounts of acids in spoiled than in unspoiled hams can be explained by the microbial fermentation of free amino acids via Stickland reactions which result in the production of acetic and butanoic acids, found in high amounts in the spoiled ham batch (Table 2). These acid compounds, together with 3-methyl and 2-methyl butanoic acids, have been reported as predominant in spoiled dry-cured ham (García and others 2000).

Total of 11 ester compounds were found most of them previously reported in dry-cured ham (Table 2). Esters are formed by esterification of carboxylic acids and alcohols and they have fruity notes, especially those formed from short-chain acids, whereas long-chain acids have a slight fatty odor. Ethyl butanoate had its greatest percentage in the control batch, while ethyl 2-methyl butanoate and ethyl 3-methyl butanoate had higher percentages in the spoiled batch, as did their acid precursors. The ester 2-methyl butanoate had been identified among the most odor-active compounds found in spoiled Iberian dry-cured ham (Carrapiso and others 2010). Microbial sterase activity could be a possible origin for their formation during the ripening of dry-cured ham (Sabio and others 1998; García and others 2000).

Other volatile compounds such as pyrazines, volatile amines, sulfur compounds, ethers, and furans were found (Table 2). Most of them were at higher percentages in the spoiled batch than in the control batch. The microbial proteolytic activity in spoiled hams may contribute to synthesis of these compounds by the increase that has been reported in free amino acids (Martín and others 2008). Pyrazines can be formed from amino acids through either Maillard reactions (Belizt and Grosch 1999) or microbial metabolism (Janssens and others 1992; Tressl and others 1993; Magan and Evans 2000). Indeed, inoculation of pork loins with a highly proteolytic mould strain leads to an increase of pyrazines (Martín and others 2003). With respect to sulfur compounds, there are many associated with the production of methanethiol by microbial activity (Spinnler and others 2001), and are referred to as causing unpleasant odors in meat products, including dry-cured ham (Ruiz and others 1999; García and others 2000). The higher percentages of these compounds in the spoiled hams may be related to the off-flavor of this alteration. Ethanol 2,2-(ethoxyethoxy) represented 4.33% of the total area of volatile compounds in spoiled ham (Table 2). Ether compounds have been found during ripening of pork meat inoculated with proteolytic microorganisms (Martín and others 2003; Benito and others 2005).

Sensorial analysis and relationship with volatile compounds

The spoiled hams were scored by the panelists at between 1.9 and 5.6 points. In the study of correlations between the sensorial analysis and volatile compounds, a total of 16 volatile compounds (4 pyrazines, 3 hydrocarbons, 3 carbonyls, 2 esters, 2 acids, 1 ether, and 1 alcohol) showed significant positive correlation with the spoilage level of the Iberian dry-cured hams studied (Table 3). Although most of these volatile compounds have been found in unspoiled Iberian dry cured ham, in inappropriate proportions they may contribute to the generation of off-flavor. Ethyl esters of C_2-C_8 fatty acids, ketones, acids, and pyrazines have been associated with bacterial spoilage of protein foods, including dry-cured ham (Chinivasagam and others 1998; García and others 2000; Jónsdóttir and others 2008; Carrapiso and others 2010).

Most of these volatile compounds related with spoilage level correlated with high counts of GPCP or *Enterobacteriaceae* (Table 3). The growth of GPCP favoured high pyrazine concentrations in the spoiled hams, showing this microbial group to be a relevant contributor to the generation of pyrazine precursors. Indeed, GPCP together with *Enterobacteriaceae* have been observed to

Table 3–Volatile compounds positively correlated with spoilage level in spoiled Iberian dry-cured ham.

Volatile compounds	Correlation with spoilage level	Correlations with microbial counts[a]			
		NEGN	Ent	GPCP	LAB
Hydrocarbons					
Toluene	0.417*			0.398*	
Nonane	0.305[x]		0.573**		
1,3 bis (1,1 Dimethyl ethyl) benzene	0.421*			0.645**	
Alcohols					
1,2-Propanodiol	0.304[x]			0.389*	
Carbonyls					
2-Methyl propanal	0.308[x]				
Benzaldehyde	0.388[x]				
Cyclohexanone	0.546**			0.546**	
Acids					
Acetic acid	0.398*		0.542**		
2-Methyl butanoic acid	0.300[x]		0.710**		
Esters					
Ethyl 2-methyl butanoate	0.439*		0.483*		
Ethyl 3-methyl butanoate	0.479*				
Ethers					
Ethanol 2,2 (ethoxy-ethoxy)	0.601**			0.514**	
Pyrazines					
2,6-Dimethyl pyrazine	0.565**			0.590**	
Trimethyl pyrazine	0.616**			0.734**	
Tetramethyl pyrazine	0.463*			0.638**	
2,3,5,-Trimethyl pyrazine	0.366[x]			0.528**	

**Correlation is significant at the 0.01 level.
*Correlation is significant at the 0.05 level.
[x]Correlation is significant at the 0.1 level.
[a]Ent: *Enterobacteriaceae*; GPCP: Gram-positive catalase-positive; NEGN: Non-enteric Gram-negative bacteria; LAB: Lactic acid bacteria.

be the predominant microorganisms involved in the degradation of myofibrillar proteins and in the generation of free amino acids in the spoilage of Iberian dry-cured ham (Martín and others 2008). The amounts of 1,2 propanodiol, ethanol 2,2 (ethoxy-ethoxy), and cyclic compounds such as toluene and 1,3 bis (1,1-dimethyl ethyl) benzene, and cyclohexanone also showed a positive correlation with high counts of GPCP. The counts of *Enterobacteriaceae* correlated positively with increases in ethyl 2-methyl butanoate, 2-methy butanoic acid, and acetic acid. This finding is consistent with reports that acetic acid and other organic acids are produced by *Enterobacteriaceae* in cured meat products (García and others 2000; Martín and others 2008). In addition, the counts of this microbial group showed a correlation with the percentage of nonane, derived from fatty acid degradation in the spoiled hams. *Enterobacteriaceae* have often been reported to be free fatty acid producers from lipase and esterase activities in cured meat products (Hinrichsen and others 1994).

Conclusions

There is evidence that microbial deep spoilage of Iberian dry-cured hams markedly alters the volatile profile when compared with unspoiled hams. Some of these volatile compounds such as pyrazines, ethanol 2,2 (ethoxy-ethoxy), and cyclohexanone show a high correlation with spoilage level. Thus, these compounds should be considered as indicators to monitor incipient deep spoilage and to detect overlooked alterations throughout the quality control process of Iberian dry-cured ham and similar dry-cured meat products.

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