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Contribution of a selected fungal population to the volatile compounds on dry-cured ham

Alberto Martín [1], Juan J. Córdoba, Emilio Aranda [1], M. Guía Córdoba [1], Miguel A. Asensio *

Higiene de los Alimentos, Facultad de Veterinaria, Universidad de Extremadura, Avda. de la Universidad s/n, 10071-Cáceres, Spain

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Abstract

Dry-cured ham is obtained after several months of ripening. Different fungi strive on the surface, including toxigenic molds. Proteolysis and lipolysis by the endogenous and microbial enzymes seem to play a decisive role in the generation of flavor precursors in dry-cured meat products. In addition, fungi show a positive impact on the volatile compounds of ripened pork loins. However, the contribution of the fungal population to flavor formation in dry-cured ham remains unclear. One selected strain each of *Penicillium chrysogenum* and *Debaryomyces hansenii* was inoculated as starter cultures on dry-cured ham. Volatile compounds extracted by solid phase micro-extraction technique were analyzed by gas chromatography/mass spectrometry. A trained panel evaluated flavor and texture of fully ripened hams. The wild fungal population on non-inoculated control hams correlates with higher levels of short chain aliphatic carboxylic acids and their esters, branched carbonyls, branched alcohols, and some sulfur compounds, particularly at the outer muscle. Conversely, *P. chrysogenum* and *D. hansenii* seem to be responsible for higher levels of long chain aliphatic and branched hydrocarbons, furanones, long chain carboxylic acids and their esters. The very limited impact of *P. chrysogenum* on pyrazines in inoculated hams can be due to the activity of the yeast. Lower levels for some of the more volatile linear carbonyls at the ham surface suggest an anti-oxidant effect by micro-organisms. The differences in volatile compounds did not show a neat impact on flavor in the sensorial analysis. Nonetheless, inoculated hams got a better overall acceptability, which has to be attributed to their improved texture. The lower toughness of inoculated hams is a direct consequence of an early settling of a highly proteolytic mold. Thus, the use of selected fungi as starter cultures may be useful to obtain high-quality and safe dry-cured ham.

Keywords: *Penicillium chrysogenum*; *Debaryomyces hansenii*; Volatile compounds; Dry-cured ham

1. Introduction

Dry-cured ham is a traditional meat product obtained after several months of ripening. During this time enzymatic and chemical reactions are involved in the development of flavor. Proteolysis and lipolysis constitute the main biochemical reactions in the generation of flavor precursors, where the endogenous enzymes play the most important role (Toldrá, 1998). The contribution of the fungal population and their enzymes to proteolysis in minced dry-cured meat products, such as dry-cured sausages, is widely known (Díaz et al., 1997; Zapelena et al., 1999; Bruna et al., 2002, 2003; Sunesen and Stahnke, 2003). In these meat products, methyl aldehydes, methyl ketones, and other volatile compounds have been attributed to staphylococci (Stahnke, 1995; Montel et al., 1996; Sunesen and Stahnke, 2003). However, the role of microbial population on flavor formation in dry-cured ham, where the surface/volume ratio is much lower, remains unclear. *Micrococcus*, *Staphylococcus*, yeasts, and molds strive for several months on the surface of different kinds of dry-cured ham (Raczynski et al., 1978; Langlois et al., 1982; Monte et al., 1986; Huerta et al., 1987; Molina et al., 1990; Carrascosa et al., 1992).

Strains of *Debaryomyces hansenii* and molds isolated from Iberian dry-cured ham showed high proteolytic activity against myofibrillar proteins when inoculated on raw pork (Rodríguez et al., 1998; Martín et al., 2001). In addition, a starter culture from dry-cured ham including *Penicillium chrysogenum* Pg222 and *D. hansenii* Dh345 promoted protein hydrolysis at the external muscle in dry-cured ham (Martín et al., 2004). The inoculation of

* Corresponding author. Tel.: +34 927 257124; fax: +34 927 257110.
E-mail address: masensio@unex.es (M.A. Asensio).
[1] Present address: Nutrición y Bromatología, Escuela de Ingenierías Agrarias, Universidad de Extremadura, Badajoz, Spain.

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Table 1
Volatile compounds from dry-cured hams using solid phase micro-extraction (SPME) GC/MS

Compound	Peak number [a]	Reliability of identification [b]
Aliphatic hydrocarbons		
Hexane	14	A
Heptane	27	A
Octane	45	A
Decane	76	A
Limonene [c]	85	A
Undecane	103	A
Dodecane [c]	121	A
Tridecane	130	A
Bicyclohexyl [c]	132	B
Alpha terpinene [c]	134	B
Tetradecane [c]	139	A
Pentadecane	143	A
Cyclododecane [c]	144	B
Hexadecane [c]	148	A
Heptadecane	151	A
Octadecane [c]	153	A
Branched hydrocarbons		
2-Methylpentane	11	B
3-Methylpentane	12	B
Methyl cyclopentane [c]	120	A
Cyclohexane [c]	22	A
Branched cyclohexane [c]	26	C
2,3-Dimethyl 1,3-pentadiene [c]	29	B
4-Propylheptane	66	B
Branched hydrocarbon A	68	C
3-Ethyl 3-heptene [c]	80	C
Branched hydrocarbon B	81	C
2,2,4,6,6-Pentamethyl heptane [c]	82	C
2,3,8-Trimethyl decane	87	C
Branched hydrocarbon C [c]	90	C
5-Methyldecane [c]	91	A
2-Methyldecane [c]	92	A
4-Methyldecane [c]	94	C
3-Methyldecane	95	A
Branched hydrocarbon D [c]	99	C
1-ethyl 2-methyl cis cyclohexane [c]	102	C
Branched hydrocarbon E [c]	106	C
9-Methyl (E) 3-undecene [c]	107	C
Pentyl cyclohexane	111	A
5-Methyl undecane	113	A
4-Methyl undecane [c]	114	A
2-Methyl undecane [c]	116	A
3-Methyl undecane	117	A
2,6-Dimethyl undecane	123	B
Hexylcyclohexane	125	A
2-Methyl dodecane	127	A
Branched hydrocarbon F [c]	131	C
Branched hydrocarbon G [c]	135	C
4(1,1-Dimethyl ethyl) cyclohexane [C]	137	B
Aromatic hydrocarbons		
Methylbenzene	41	A
Ethylbenzene	52	A
p-Xylene	55	B
m-Xylene	55	B
o-Xylene	59	A
1,2,3-Trimethyl benzene	77	A
Decahydro trans naphthalene	96	B
Decahydro *cis* naphthalene [c]	108	B

Table 1 (*continued*)

Compound	Peak number [a]	Reliability of identification [b]
Aromatic hydrocarbons		
Decahydro 2-methyl naphthalene	110	B
Decahydro *cis–syn* 2-methyl naphthal. [c]	112	B
Decahydro 2-methyl naphthalene [c]	118	C
Decahydro 1,6-methyl naphthalene [c]	119	C
1,4-bis(1,1-Dimethylethyl) bezene	128	B
1,1-Biphenyl [c]	140	B
Nonyl benzene [c]	147	B
Aldehydes		
2-Methylpropanal	9	A
Butanal	16	A
2-Methylbutanal	21	A
3-Methylbutanal	23	A
Pentanal	28	A
Hexanal	46	A
Heptanal	60	A
3-Methylthiopropanal [c]	61	A
2-Heptenal (Z)	67	A
Benzaldehyde	69	A
Octanal	78	A
Bencenacetaldehyde	89	A
Nonanal	105	A
Decanal	122	A
Hexadecanal	154	A
Carboxylic acids		
Acetic acid	15	A
2-Methyl propanoic acid	40	A
Butanoic acid	43	A
3-Methyl butanoic acid	49	A
2-Methyl butanoic acid	51	A
Pentanoic acid	56	A
Hexanoic acid	70	A
Octanoic acid	115	A
Nonanoic acid	126	A
Decanoic acid	133	A
Tetradecanoic acid	152	A
Hexadecanoic acid	157	A
Aliphalic alcohols		
Ethanol	2	B
2-Propanol	3	A
1-Propanol	10	B
2-Methyl 3-buten-2-ol [c]	17	A
2-Methyl 1-propanol	19	A
1-Penten-3-ol	24	A
3-Methyl 3-buten-1-ol	34	B
3-Methyl 1-butanol	35	A
2-Methyl 1-butanol	36	A
1-Pentanol	42	A
1-Hexanol	54	A
1-Octen-3-ol	71	A
2-Ethyl hexanol [c]	83	A
Linalool [c]	104	A
Aromatic alcohols		
3-Ethyl phenol [c]	64	A
2-Methyl phenol	97	B/C
Ethanol benzene	109	B
Bis(1,1-dimethyl ethyl) phenol [c]	145	B
2,6-bis(1,1-Dimethyl ethyl) 4-methyl phenol	146	B

(continued on next page)

Table 1 (*continued*)

Compound	Peak number [a]	Reliability of identification [b]
Ketones		
2-Propanone	4	B
2,3-Butanedione (diacetyl)	13	B
2-Pentanone	25	A
2-Butanol-3-one (acetoin)	31	A
4-Methyl 2-pentanone	37	B
2-Hexanone	44	A
2-Heptanone	58	A
2,3-Octanedione	72	A
2-Octanone	73	A
3-Octen-2-one	86	A
Dimethyl 2-cyclopeten-1-one [c]	88	B
2-Nonanone	100	A
6,10-Dimethyl (Z) 5,9-undecadien-2-one [c]	141	B
Furans		
2-Ethylfuran [c]	30	A
2-Pentylfuran	74	A
5-Ethyl dihydro-2(3H)-furanone	93	B
5-Butyl dihydro-2(3H)-furanone	129	B
5-Pentyl dihydro-2(3H)-furanone	136	B
Pyrazines		
2-Methylpyrazine	48	B
2,5-Dimethylpyrazine	62	A
Trimethylpyrazine	79	B
Sulfur compounds		
Methanethiol [c]	1	B
Dimethyl sulfide	5	B
Thiourea	8	B
Dimethyl disulfide	38	A
Metane sulfonilbis [c]	63	B
Dithio *s*-methyl carbonic acid [c]	98	B
Benzene Isothiocyanate [c]	124	B
Benzene 1,1-thiobis	150	B
Ester compounds		
Methyl acetate	6	B
Ethyl acetate	18	A
Methyl butanoate	33	B
Butyl acetate	47	A
Ethyl 1-methylbutanoate	50	B
Ethyl 3-methylbutanoate	52	A
Methyl heptanoate [c]	65	B
Ethyl hexanoate	75	A
Ethyl 1-ethylhexanoate	84	A
Ethyl heptanoate [c]	101	A
Ethyl octanoate	120	A
Ethyl decanoate [c]	138	B
Dimethyl 1,2 benzenecarboxylate [c]	142	B
Diethyl benzenecarboxylate	149	B
Bis(dimethyl) 1,2-benzenedicarboxylate [c]	155	B
Other compounds		
Dichloromethane	7	B
n-Methylene ethenamine [c]	32	A
Pyridine	39	A
Hexanenitrile [c]	57	A

these two strains on pork loins showed a positive impact on the volatile compounds: it decreased those derived from lipid oxidation, such as linear aldehydes and alcohols, while increased those derived from the amino acids catabolism, such as branched aldehydes and pyrazines (Martín et al., 2003). Some of these changes on branched aldehydes have been attributed to the microbial population in Parma ham (Hinrichsen and Pedersen, 1995).

Given that it is not possible to keep dry-cured hams as sterile control for several months without interfering with the ripening process, the contribution of the tested strains on the volatile compounds, sensory characteristics, and acceptability has to be tested against hams with a wild microbial population.

The aim of this work has been to investigate the impact of one selected non-toxigenic strain each of *P. chrysogenum* and *D. hansenii* on the volatile compounds and the sensorial characteristics of dry-cured ham.

2. Materials and methods

2.1. Experimental design

Hams from commercial crossbred pigs of 90–120 kg were dry-cured following a traditional process. After pH checking and salting, the hams were divided into two batches. One of them was inoculated with *P. chrysogenum* Pg222 and *D. hansenii* Dh345, while the other one was kept as a naturally contaminated control (non-inoculated). The procedures to obtain the inoculums, inoculate the hams, and collect samples have been those described by Martín et al. (2004). Sampling from 5 hams per batch was performed after 6 and 12 months of ripening. The initial tissue was divided into deep (from *Biceps femoris*) and superficial (from adductor) samples. Mold count from the ham surface after 6 months of ripening reached 5.1 log c.f.u./cm^2 on inoculated hams, but just 2.0 log c.f.u./cm^2 on control samples, while yeasts reached 8.1 log c.f.u./cm^2 on inoculated and 4.1 log c.f.u./cm^2 on control batches. However, at the end of the ripening process, both batches showed counts close to 10^6 c.f.u./cm^2 for moulds and 10^5–10^6 c.f.u./cm^2 for yeasts. Over 90% of the latter were *D. hansenii* in both batches. Moulds were dominated by *P. chrysogenum* in the inoculated batch, while *Cladosporium* spp. and *Penicillium* spp. were the main isolates from the non-inoculated batch, with none of them characterized as *P. chrysogenum*.

2.2. Extraction of volatile compounds

Deep and superficial samples were vacuum-packaged and stored at −80 °C until analysis. Frozen samples were minced and 1 g was weighed into a 10 ml headspace vial (Hewlett-Packard, Palo Alto, CA, USA) and sealed with a PTFE butyl septum (Perkin-Elmer, Foster City, CA, USA) in an aluminum cap.

Notes to Table 1:

[a] Peak number in chromatogram of dry-cured hams.

[b] The reliability of the identification or structural proposal is indicated by the following symbols: A: mass spectrum and retention time identical to those of an authentic sample. B: mass spectrum consistent with spectra found in NIST, EPA, NDH library. C: tentative identification by mass spectrum.

[c] The components occurred in small or trace amounts.

Table 2
Selected volatile hydrocarbons of dry-cured hams (arbitrary area units)

Compound	6 months of ripening time				12 months of ripening time				P [a]		
	Adductor		*B. femoris*		Adductor		*B. femoris*		loc	inoc	time
	Non-inoc [b]	Inoc	Non-inoc	Inoc	Non-inoc	Inoc	Non-inoc	Inoc			
Aliphatic hydrocarbons											
Hexane	$12^{2,c}$	7^{2}	35^{2}	$282^{1,2}$	127^{2}	208^{2}	521^{1}	55^{2}	+++ [d]		+++
Cyclohexane	2^{3}	$10^{2,3}$	5^{3}	5^{3}	$46^{1,2}$	$11^{2,3}$	65^{1}	$15^{2,3}$		+++	+++
Heptane	26^{2}	11^{2}	16^{2}	20^{2}	164^{1}	68^{2}	n.d.[2, e]	n.d.[2]	+++		+++
Octane	2^{2}	1^{2}	n.d.[2]	n.d.[2]	31^{1}	30^{1}	n.d.[2]	n.d.[2]	+++		+++
Decane	48	50	21	41	135	316	13	16			
Undecane	$4^{2,3}$	$6^{2,3}$	6^{2}	$6^{2,3}$	$4^{2,3}$	13^{1}	1^{3}	8^{2}		+++	
Tridecane	$4^{1,2}$	7^{1}	$1^{1,2}$	$3^{1,2}$	$4^{1,2}$	$6^{1,2}$	1^{2}	$5^{1,2}$	+++	+++	
Pentadecane	6^{2}	4^{2}	5^{2}	4^{2}	5^{2}	9^{1}	n.d.[3]	10^{1}	++	+++	++
Heptadecane	n.d.	n.d.	n.d.	n.d.	n.d.	20	16	26			+++
Branched hydrocarbons											
2-Methyl pentane	0.3^{2}	6^{2}	1^{2}	$47^{1,2}$	$53^{1,2}$	4^{2}	106^{1}	14^{2}	++		+++
3-Methyl pentane	n.d.[2]	1^{2}	0.4^{2}	18^{2}	$29^{1,2}$	7^{2}	61^{1}	3^{2}		++	+++
4-Propyl heptane	3	10	7	3	17	14	8	11			++
Branched hydrocarbon A	1^{2}	14^{2}	11^{2}	n.d.[2]	17^{2}	11^{2}	85^{1}	24^{2}	+++	+++	+++
2,3,8-Trimethyl decane	n.d.[3]	n.d.[3]	n.d.[3]	n.d.[3]	16^{1}	16^{1}	10^{2}	11^{2}	+++		+++
2-Methyl decane	3^{3}	$6^{2,3}$	2^{3}	3^{3}	$9^{1,2}$	12^{1}	$6^{2,3}$	$6^{2,3}$	+++	+++	
Branched hydrocarbon B	n.d.[3]	n.d.[3]	n.d.[3]	n.d.[3]	8^{2}	10^{2}	7^{2}	16^{1}		+++	+++
3-Methyl decane	9^{1}	9^{1}	$6^{1,2}$	$5^{1,2}$	$8^{1,2}$	$7^{1,2}$	4^{2}	9^{1}	+++		
Pentyl cyclohexane	$4^{1,2}$	7^{1}	$3^{1,2}$	$3^{1,2}$	n.d.[2]	8^{1}	1^{2}	$5^{1,2}$	++	+++	
5-Methyl undecane	$4^{1,2}$	7^{1}	$3^{1,2}$	$3^{1,2}$	1^{2}	7^{1}	0.4^{2}	$4^{1,2}$	+++	+++	
3-Methyl undecane	$5^{1,2}$	$8^{1,2}$	$6^{1,2}$	$7^{1,2}$	$4^{1,2}$	10^{1}	2^{2}	$8^{1,2}$		+++	
2,6-Dimethyl undecane	$7^{1,2,3}$	13^{1}	$5^{2,3}$	$6^{1,2,3}$	$5^{2,3}$	$10^{1,2}$	1^{3}	$5^{1,2,3}$	+++	+++	
Hexyl cyclohexane	$8^{1,2}$	12^{1}	$5^{2,3}$	^{62,3}	$3^{2,3}$	$6^{2,3}$	1^{3}	$6^{2,3}$	+++	+++	+++
2-Methyl dodecane	$6^{1,2}$	9^{1}	$5^{1,2}$	$6^{1,2}$	$1^{1,2}$	$6^{1,2}$	n.d.[2]	$5^{1,2}$		+++	+++
Aromatic hydrocarbons											
Methyl benzene	39^{2}	42^{2}	34^{2}	42^{2}	47^{2}	100^{1}	$78^{1,2}$	$74^{1,2}$			+++
Ethyl benzene	3^{2}	8^{2}	5^{2}	81^{1}	14^{2}	5^{2}	15^{2}	8^{2}	++		
p-Xylene/*m*-Xylene	10^{2}	28^{2}	20^{2}	256^{1}	46^{2}	17^{2}	71^{2}	30^{2}	++		
o-Xylene	5^{2}	7^{2}	6^{2}	23^{1}	$14^{1,2}$	$16^{1,2}$	$9^{1,2}$	$13^{1,2}$		++	
1,2,3-Trimethyl benzene	$9^{2,3}$	$11^{2,3}$	5^{3}	$10^{2,3}$	17^{1}	$13^{1,2}$	8^{2}	$10^{2,3}$	+++		+++
Decahydro trans naphtalene	5	8	5	6	8	7	5	5	+++		
Decahydro 2-methyl naphtalene	$6^{1,2}$	$9^{1,2}$	$3^{1,2}$	$6^{1,2}$	$6^{1,2}$	15^{1}	n.d.[2]	$3^{1,2}$	+++	++	
1,4 bis(1,1-Dimethylethyl)benzene	9^{1}	$8^{1,2}$	$5^{2,3}$	$5^{2,3}$	n.d.[4]	$4^{2,3}$	n.d.[4]	$2^{3,4}$	+++	++	+++

[a] P_{loc}: P value due to location of muscle sampled. P_{inoc}: P value due to micro-organisms inoculated. P_{time}: P value due to ripening time.
[b] Non-inoc: non-inoculated batch. Inoc: inoculated batch.
[c] Values with different numbers as superscript are significantly different ($P<0.05$).
[d] P values: ++ ($P<0.01$), +++ ($P<0.001$).
[e] n.d.: not detected.

Volatile compounds were extracted by solid phase micro-extraction technique (SPME) (Ruiz et al., 1998) with a 10 mm long, 100 μm thick fiber coated with carboxen-poly-dimethylsiloxane (Supelco, Bellefonte, PA, USA). Prior to collection of volatiles, the fiber was preconditioned at 220 °C for 50 min at the GC injection port. The SPME fiber was inserted into the headspace vial for 30 min at 40 °C in a water bath.

2.3. Gas chromatography/mass spectrometry (GC/MS) analyses

GC/MS analyses were performed using a Hewlett-Packard 5890 S II gas chromatograph coupled to a Hewlett-Packard 5971A ion-trap mass spectrometer. A 5% phenyl–95% poly-dimethylsiloxane column (50 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 in the injection port at 220 °C during the whole chromatographic run. The temperature program was isothermal for 15 min at 35 °C, next 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, USA) 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 40–300 for data collection. The NIST/EPA/NIH mass spectral library and Kovats indexes were used to identify the volatile compounds.

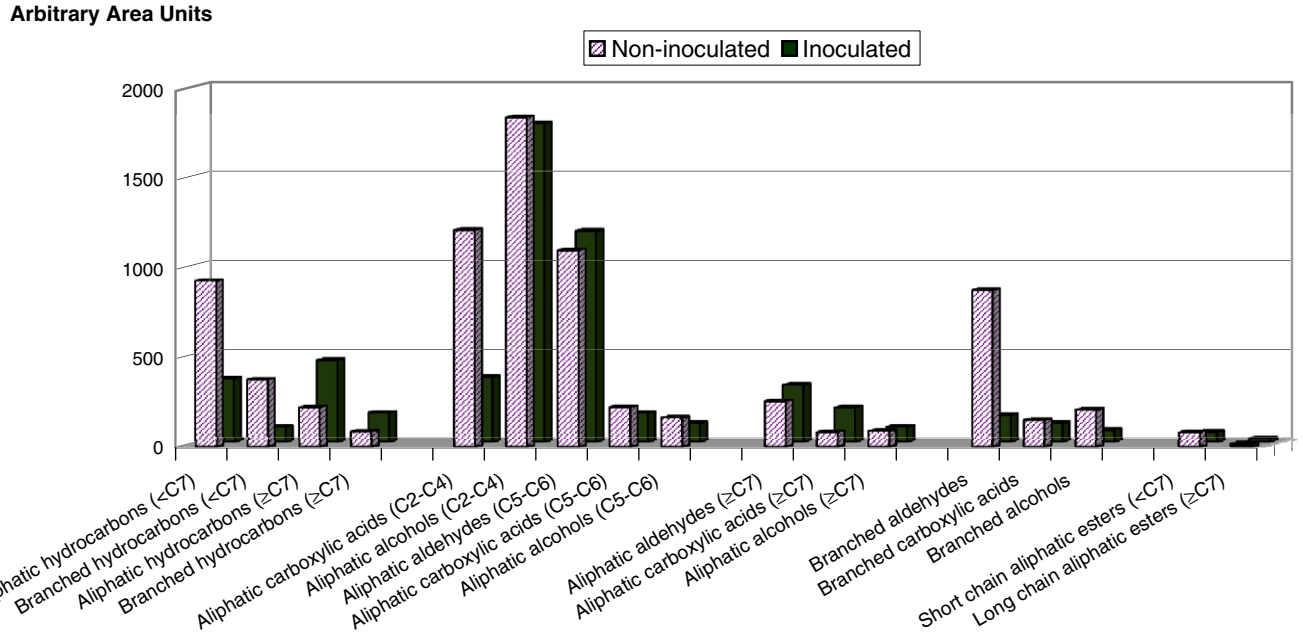

Fig. 1. Effect of inoculation on selected groups of volatile compounds showing significant differences at 12 months or ripening time. Bars represent the accumulated area from both outer and deep samples for control (non-inoculated) and Inoculated hams.

2.4. Sensory analysis

Fully ripened hams were sliced and subjected to a blind sensory evaluation by an 18-member panel selected and trained under ISO standards (ISO, 1993). Fifteen parameters related to flavor and texture were assessed using a structured line scale with intensity descriptors at the end points (1, low, 10, high). Samples were three-digit coded and the order of serving was determined by random permutation. Two panel replications were carried out on each sample. The response to each indicator was determined as the mean value of the panelist responses. In an additional hedonic test, the panel evaluated the samples for overall acceptability.

2.5. Statistical analysis

The effect of inoculation, sample location, and processing time on different groups of volatile compounds was evaluated by analysis of variance, and means were separated by Tukey's honest significant difference test using a SPSS software package (version 11.5.1) for Windows (SPSS, Chicago, USA).

3. Results and discussions

3.1. Volatile compounds

Over 150 volatile compounds were identified in all samples taken (Table 1), including aliphatic, branched, and aromatic hydrocarbons, aldehydes, furans, carboxylic acids, alcohols, aromatic alcohols, ketones, pyrazines, esters, and sulfur compounds. In some batches, several of the above-mentioned volatile compounds were detected at very low levels. Most of the identified compounds have been also reported in different kinds of dry-cured ham (Berdagué et al., 1991; García et al., 1991; Barbieri et al., 1992; López et al., 1992; Buscailhon et al., 1993; Hinrichsen and Pedersen, 1995; Flores et al., 1998; Ruiz et al., 1998, 1999).

Hydrocarbons were approximately 10–19% of total area from volatile compounds. Most aliphatic and branched hydrocarbons showed maximum levels at the last sampling time (Table 2). Those of shorter chain length (hexane, cyclohexane, heptane, 2-methyl pentane, 3-methyl pentane) reached higher values ($P<0.05$) in non-inoculated controls (Fig. 1). The increases in all these compounds can be partially attributed to lipid oxidation, which plays a decisive role on both linear and branched hydrocarbon formation in dry-cured ham (Berdagué et al., 1991; García et al., 1991; Ruiz et al., 1999). Thus, a higher antioxidant activity from inoculated micro-organisms can be inferred, perhaps just as a consequence of an early settling obtained on the ham surface (Martín et al., 2004). On the other hand, inoculation with *P. chrysogenum* and *D. hansenii*, particularly the high ratio of *P. chrysogenum,* seems to be related with higher levels ($P_{inoc}<0.001$) for some of the higher molecular weight aliphatic and branched hydrocarbons (Fig. 1). Those showing statistically significant higher values at the outer muscle included undecane, pentadecane, 5-methyl undecane, and 3-methyl undecane (Table 2). Methyl hydrocarbons can be synthesized by molds as secondary degradation products from triglycerides, as it has been demonstrated for a methyl branched alkene (Jacobsen and Hinrichsen, 1997). Thus, micro-organisms may contribute to the differences in *n*-alkanes found in dry-cured ham (Tejeda et al., 2001). Regardless of their origin, aliphatic and branched hydrocarbons have been considered as non-contributors to meat flavor (Shahidi et al., 1986), and are not amongst the most odor-active compounds described for dry-cured ham (Carrapiso et

Table 3
Selected volatile aldehydes, carboxylic acids, alcohols, and ketones of dry-cured hams (arbitrary area units)

Compound	6 months of ripening time				12 months of ripening time				P [a]		
	Adductor		*B. femoris*		Adductor		*B. femoris*		loc	inoc	time
	Non-inoc [b]	Inoc	Non-inoc	Inoc	Non-inoc	Inoc	Non-inoc	Inoc			
Aldehydes											
2-Methyl propanal	$3^{3,4,c}$	$5^{3,4}$	$2^{3,4}$	1^{4}	31^{1}	15^{2}	8^{3}	$6^{3,4}$	+++ [d]	+++	+++
Butanal	26	42	10	1	6	70	17	17			
3-Methyl butanal	33^{2}	21^{2}	16^{2}	14^{2}	568^{1}	54^{2}	98^{2}	41^{2}	+++	+++	+++
2-Methyl butanal	$19^{2,3}$	$15^{2,3}$	7^{3}	8^{3}	$69^{1,2}$	$17^{2,3}$	98^{1}	$22^{2,3}$		+++	+++
Pentanal	$26^{2,3,4}$	$16^{3,4}$	65^{2}	$47^{2,3}$	n.d.^{4,e}	n.d.^{4}	117^{1}	136^{1}	+++		+++
Hexanal	$228^{2,3}$	141^{4}	809^{1}	$550^{1,2}$	$276^{2,3}$	121^{4}	700^{1}	920^{1}	+++		
Heptanal	$25^{2,3}$	19^{3}	$39^{1,2,3}$	$30^{1,2,3}$	$40^{1,2,3}$	$20^{2,3}$	$43^{1,2}$	51^{1}	+++		+++
Benzaldehyde	11	19	20	19	28	38	18	21			++
Octanal	$21^{1,2}$	$17^{1,2}$	13^{2}	$15^{1,2}$	$17^{1,2}$	13^{2}	$19^{1,2}$	24^{1}			
Nonanal	96^{1}	$84^{1,2}$	$45^{1,2}$	$47^{1,2}$	$57^{1,2}$	$58^{1,2}$	33^{2}	$90^{1,2}$	++		
Decanal	$9^{1,2}$	14^{1}	$7^{2,3}$	$5^{2,3}$	$8^{2,3}$	$7^{2,3}$	2^{3}	$6^{2,3}$	+++		+++
Hexadecanal	50^{1}	48^{1}	27^{2}	22^{2}	22^{2}	$13^{2,3}$	n.d.^{3}	$14^{2,3}$	+++		+++
2-Heptenal (Z)	2	6	21	6	15	6	n.d.	18			
Benzeneacetaldehyde	3	4	2	2	3	2	2	3	++		
Carboxylic acids											
Acetic acid	101^{2}	82^{2}	70^{2}	52^{2}	792^{1}	94^{2}	81^{2}	167^{2}			++
2-Methyl propanoic acid	n.d.^{4}	1^{4}	n.d.^{4}	n.d.^{4}	$26^{1,2}$	13^{3}	32^{1}	$17^{2,3}$		+++	+++
Butanoic acid	$56^{2,3}$	40^{3}	$73^{2,3}$	41	$140^{1,2}$	34^{3}	194^{1}	$71^{2,3}$		+++	+++
3-Methyl butanoic acid	3^{2}	9^{2}	$19^{1,2}$	4^{2}	42^{1}	$27^{1,2}$	$22^{1,2}$	$22^{1,2}$			+++
2-Methyl butanoic acid	3^{3}	$5^{2,3}$	1^{3}	2^{3}	16^{1}	17^{1}	$12^{1,2}$	$13^{1,2}$			+++
Pentanoic acid	n.d.^{2}	n.d.^{2}	n.d.^{2}	n.d.^{2}	n.d.^{2}	n.d.^{2}	7^{1}	5^{1}	+++		+++
Hexanoic acid	$48^{2,3}$	22^{3}	$85^{2,3}$	$46^{2,3}$	$61^{2,3}$	$23^{2,3}$	155^{1}	139^{1}	+++	+++	+++
Octanoic acid	2^{3}	$6^{2,3}$	$6^{2,3}$	3^{3}	1^{3}	10^{2}	$6^{2,3}$	24^{1}	+++	+++	+++
Nonanoic acid	$1^{2,3}$	$2^{2,3}$	0.5^{3}	0.4^{3}	$2^{2,3}$	$4^{1,2}$	$1^{2,3}$	6^{1}		+++	+++
Decanoic acid	$4^{3,4}$	$6^{3,4}$	17^{1}	$9^{2,3}$	$3^{3,4}$	2^{4}	$3^{3,4}$	$14^{1,2}$	+++		+++
Tetradecanoic acid	$28^{1,2}$	$33^{1,2}$	37^{1}	$12^{1,2,3}$	$9^{2,3}$	n.d.^{3}	n.d.^{3}	$30^{1,2}$			+++
Hexadecanoic acid	$14^{1,2}$	$46^{1,2}$	$29^{1,2}$	73^{1}	$58^{1,2}$	$72^{1,2}$	n.d.^{2}	$33^{1,2}$		+++	
Alcohols											
Ethanol	$243^{1,2}$	$238^{1,2}$	$241^{1,2}$	208^{2}	$459^{1,2}$	552^{1}	$493^{1,2}$	$476^{1,2}$			+++
2-Propanol	$232^{4,5}$	$257^{4,5}$	$192^{4,5}$	93^{5}	644^{2}	430^{3}	797^{1}	$297^{3,4}$		+++	+++
1-Propanol	$3^{3,4}$	$5^{3,4}$	1^{4}	1^{4}	$13^{1,2}$	15^{1}	$8^{2,3}$	$6^{3,4}$	+++		+++
2-Methyl 1-propanol	n.d.^{2}	2^{2}	n.d.^{2}	n.d.^{2}	37^{1}	n.d.^{2}	n.d.^{2}	n.d.^{2}	+++	+++	+++
1-Penten-3-ol	6^{2}	8^{2}	8^{2}	5^{2}	5^{2}	3^{2}	17^{1}	7^{2}	+++	++	
3-Methyl 1-butanol	11^{2}	11^{2}	8^{2}	1^{2}	86^{1}	14^{2}	32^{2}	9^{2}	+++	+++	+++
2-Methyl 1-butanol	8^{2}	5^{2}	n.d.^{2}	n.d.^{2}	41^{1}	45^{2}	12^{2}	3^{2}	+++	+++	+++
1-Pentanol	$26^{1,2}$	$28^{1,2}$	22^{2}	17^{2}	$30^{1,2}$	$37^{1,2}$	52^{1}	$27^{1,2}$			+++
1-Hexanol	$19^{1,2}$	$22^{1,2}$	$32^{1,2}$	$21^{1,2}$	$22^{1,2}$	13^{2}	37^{1}	$23^{1,2}$	++		
1-Octen-3-ol	$25^{3,4,5}$	13^{5}	$48^{1,2,3}$	$27^{3,4,5}$	$29^{3,4,5}$	$36^{2,3,4}$	60^{1}	$52^{1,2}$	+++	++	+++
2-Methyl phenol	5^{3}	$7^{2,3}$	5^{3}	5^{3}	$11^{1,2}$	12^{1}	5^{3}	$10^{1,2,3}$	+++	++	+++
Ethanol benzene	4^{2}	5^{2}	3^{2}	2^{2}	27^{1}	5^{2}	n.d.^{2}	2^{2}	+++	+++	++
4-Methyl 2,6 bis (1,1 dimethyl ethyl) phenol	10^{1}	$6^{1,2,3}$	$5^{1,2,3}$	$3^{1,2,3}$	$8^{1,2}$	$2^{2,3}$	n.d.^{3}	$1^{2,3}$	+++	++	++
Ketones											
2-Propanone	$431^{1,2}$	$257^{2,3}$	$288^{2,3}$	216^{3}	$276^{2,3}$	$430^{1,2}$	531^{1}	$445^{1,2}$			+++
2,3-Butanedione (diacetyl)	2^{2}	$4^{1,2}$	$4^{1,2}$	$4^{1,2}$	$8^{1,2}$	$6^{1,2}$	16^{1}	3^{2}			++
2-Pentanone	$40^{1,2}$	32^{2}	79^{1}	30^{2}	23^{2}	26^{2}	$54^{1,2}$	$52^{1,2}$	+++		
2-Butanol-3-one (acetoin)	17^{2}	22^{2}	29^{2}	26^{2}	39^{2}	11^{2}	90^{1}	28^{2}	+++	+++	+++
2-Hexanone	$21^{1,2}$	$21^{1,2}$	$28^{1,2}$	$27^{1,2}$	n.d.^{2}	$32^{1,2}$	69^{1}	$48^{1,2}$	++		
2-Heptanone	17^{2}	20^{2}	61^{1}	$32^{1,2}$	7^{2}	16^{2}	$30^{1,2}$	$45^{1,2}$	+++		
2,3-Octanedione	32	15	13	13	19	66	8	15			
2-Octanone	6^{2}	7^{2}	$12^{1,2}$	$8^{1,2}$	7^{2}	$12^{1,2}$	$9^{1,2}$	13^{1}	+++		
3-Octen-2-one	7^{2}	12^{1}	$10^{1,2}$	$8^{1,2}$	n.d.^{3}	1^{3}	n.d.^{3}	1^{3}			+++
2-Nonanone	$3^{2,3}$	5^{2}	$3^{2,3}$	$3^{2,3}$	8^{1}	10^{1}	n.d.^{3}	$6^{1,2}$	+++	+++	+++

[a] P_{loc}: P value due to location of muscle sampled. P_{inoc}: P value due to micro-organisms inoculated. P_{time}: P value due to ripening time.
[b] Non-Inoc: non-inoculated batch. Inoc: inoculated batch.
[c] Values with different numbers as superscript are significantly different ($P<0.05$).
[d] P values: ++ ($P<0.01$), +++ ($P<0.001$).
[e] n.d.: not detected.

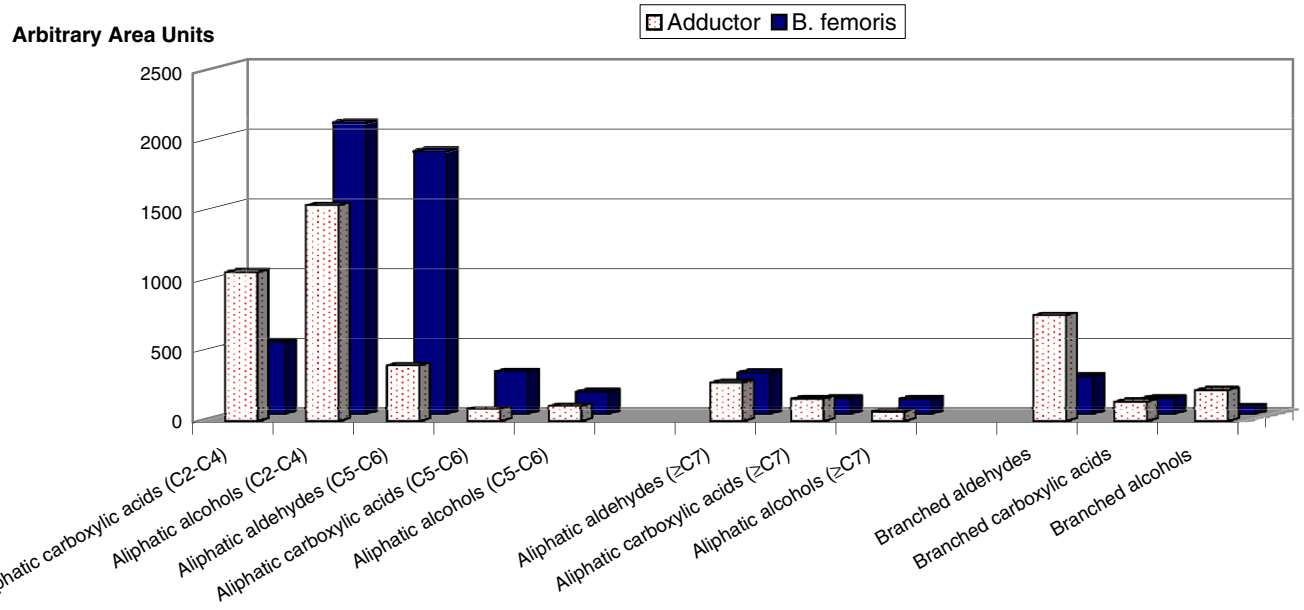

Fig. 2. Effect of localization on selected groups of volatile compounds showing significant differences at 12 months of ripening time. Bars represent the accumulated area from both non-inoculated and inoculated hams for outer (adductor) and deep (*B. femoris*) samples.

al., 2002). Aromatic hydrocarbons, responsible for smoke, phenolic-like odors in dry-cured ham (Flores et al., 1997), do not correlate well with inoculation. Only methyl benzene and 1,4 bis(1,1-dimethylethyl) benzene showed significantly higher contents in inoculated than in control fully ripened hams (Table 2).

Table 3 shows the aldehydes (11–36% of total area), carboxylic acids (8–11%), alcohols (13–30%) and ketones (8–21%). At 6 months of ripening, statistically significant differences between batches for all these compounds were scarce, but consistent with the observations at the end of processing. Those compounds showing significantly higher levels at the outer muscle (i.e.: decanal, hexadecanal, and 3-octen-2-one) reached maximum values in inoculated hams, while those showing higher levels at *B. femoris* (i.e.: hexanal, decanoic acid, 2-pentanone, and 2-heptanone) reached the maximum in non-inoculated hams (Table 3). After 12 months of ripening time, differences related to inoculation were found both at adductor and *B. femoris*. Linear carbonyls and alcohols, which are the most abundant volatile compounds described in dry-cured ham, have been attributed mainly to oxidative decomposition of lipids (García et al., 1991; López et al., 1992; Ruiz et al., 1999). However, outer samples reached lower levels ($P_{loc}<0.05–0.001$) for the more characteristic linear carbonyl compounds with five or six carbon atoms (Fig. 2), including pentanal, hexanal, 2-pentanone, 2-hexanone, pentanoic, and hexanoic acids (Table 3). These results suggest an anti-oxidant effect at the ham surface by the microbial population. In inoculated hams, it can be related to the decrease in lipid oxidation products originated by *P. chrysogenum* on dry-cured pork (Martín et al., 2003), as well as the high ratio of this mould as compared with non-inoculated hams (Martín et al., 2004). On the other hand, short chain carboxylic acids (2 or 4 carbon atoms) showed significant differences at the outer samples (Table 3), reaching higher levels in non-inoculated than in inoculated hams (Fig. 1). Compounds such as acetic or propanoic acids may derive from beta-oxidation of saturated fatty acids (Montel et al., 1998). Given that short chain fatty acids impair an unpleasant odor to dry-cured ham, a negative contribution to flavor of the wild fungal population can be inferred. Carboxylic acids over seven carbon atoms showed higher levels in inoculated than in non-inoculated hams (Fig. 1). A higher lipolytic activity of the inoculated micro-organisms would explain these results. However, the inoculated micro-organisms showed only a poor lipolytic activity (Núñez et al., 1998). In addition, the inoculation of lipolytic micro-organisms would lead to increases in free fatty acids mainly at the outer muscle, while no significant increase in these compounds was observed from adductor samples for the last six months of ripening (Table 3). Conversely, decanoic and tetradecanoic acids showed significant decreases in deep samples for the last six months of ripening time just in non-inoculated hams (Table 3). For this, a higher beta-oxidation activity by the wild fungal population on non-inoculated hams would better explain both the increase of short chain free fatty acids at the outer muscle and the decrease of long chain free fatty acids at the deep muscle.

Branched carbonyls and alcohols detected in fully ripened hams included aldehydes (2-methyl propanal, 3-methyl butanal, and 2-methyl butanal), acids (2-methyl propionic, 3-methyl butanoic, and 2-methyl butanoic), and alcohols (2-methyl propanol, 3-methyl butanol, and 2-methyl butanol) (Table 3). Non-inoculated controls usually showed higher values than their counterpart samples from inoculated hams (Fig. 1). Similarly, outer samples usually showed higher values than deep samples from the same batch (Fig. 2). The branched aldehydes may be formed from valine, leucine, and isoleucine by non-enzymatic Strecker degradation (García et al., 1991;

Table 4
Selected furans, pyrazines, sulfur compounds and esters of dry-cured hams (arbitrary area units)

Compound	6 months of ripening time				12 months of ripening time				P [a]		
	Adductor		*B. femoris*		Adductor		*B. femoris*		loc	inoc	time
	Non-Inoc [b]	Inoc	Non-Inoc	Inoc	Non-Inoc	Inoc	Non-Inoc	Inoc			
Furans											
2-Pentyl furan	6^{2} [c]	6^{2}	5^{2}	5^{2}	13^{1}	12^{1}	5^{2}	6^{2}	+++ [d]		+++
5-Ethyl dihydro-2(3H)-furanone	$6^{2,3}$	$8^{1,2}$	$6^{2,3}$	$8^{1,2,3}$	$6^{2,3}$	$7^{1,2,3}$	5^{3}	10^{1}		+++	
5-Butyl dihydro-2(3H)-furanone	$8^{1,2}$	13^{1}	$9^{1,2}$	$8^{1,2}$	n.d.^{3}	7^{2}	1^{3}	$8^{1,2}$		+++	+++
5-Pentyl dihydro-2(3H)-furanone	$3^{2,3}$	$2^{2,3}$	$5^{1,2}$	$4^{1,2,3}$	$4^{1,2,3}$	2^{3}	$3^{2,3}$	7^{1}	+++		
Pyrazines											
2-Methyl pyrazine	n.d. [e]	2	n.d.	n.d.	5	4	n.d.	0.4	++		
2,5-Dimethyl pyrazine	6^{2}	6^{2}	4^{2}	4^{2}	20^{1}	$12^{1,2}$	5^{2}	$9^{1,2}$	++		+++
Trimethyl pyrazine	2	2	1	1	n.d.	4	n.d.	1		++	
Sulfur compounds											
Dimethyl sulfide	3^{2}	4^{2}	4^{2}	1^{2}	14^{1}	n.d.^{2}	n.d.^{2}	1^{2}	++	++	
Carbon disulfide/Thiourea	231	325	32	31	44	60	22	20	++		++
Dimethyl disulfide	1^{2}	5^{2}	n.d.^{2}	n.d.^{2}	28^{1}	6^{2}	37^{1}	8^{2}		+++	+++
benzene thiobis	n.d.^{3}	n.d.^{3}	n.d.^{3}	n.d.^{3}	n.d.^{3}	10^{2}	$14^{1,2}$	17^{1}	+++	++	+++
Ester compunds											
Methyl acetate	n.d.^{2}	n.d.^{2}	n.d.^{2}	12^{1}	7^{1}	n.d.^{2}	10^{1}	0.1^{2}	+++		
Ethyl acetate	3	4	n.d.	1	6	15	13	8			+++
Methyl butanoate	5^{2}	n.d.^{2}	n.d.^{2}	n.d.^{2}	6^{2}	n.d.^{2}	29^{1}	4^{2}	+++	+++	+++
Butyl acetate	n.d.^{2}	n.d.^{2}	n.d.^{2}	15^{1}	n.d.^{2}	5^{2}	n.d.^{2}	n.d.^{2}		++	
Ethyl 2-methylbutanoate	2	4	6	1	n.d.	5	2	3			
Ethyl 3-methylbutanoate	2^{2}	n.d.^{2}	n.d.^{2}	3^{2}	14^{1}	11^{1}	14^{1}	$8^{1,2}$			+++
Ethyl hexanoate	n.d.^{2}	n.d.^{2}	n.d.^{2}	n.d.^{2}	n.d.^{2}	n.d.^{2}	$12^{1,2}$	27^{1}	+++		+++
Ethyl 2-methylhexanoate	3^{2}	$5^{1,2}$	$5^{1,2}$	4^{2}	$5^{1,2}$	7^{1}	4^{2}	8^{1}		+++	+++
Ethyl octanoate	4^{3}	$7^{2,3}$	5^{3}	5^{3}	$7^{2,3}$	$10^{1,2}$	6^{3}	12^{1}		+++	+++
Diethyl benzenedicarboxylate	21^{1}	15^{1}	21^{1}	15^{1}	n.d.^{2}	24^{1}	n.d.^{2}	24^{1}		+++	+++
Bis (dimethyl) 1,2-benzenedicarboxylate	12^{1}	136^{1}	27^{1}	16^{1}	14^{1}	12^{1}	n.d.^{2}	19^{1}			
Miscellaneous compounds											
Dichoromethane	5	16	9	1	8	n.d.	2	9			
Pyridine	43	6	12	62	8	12	9	6			

[a] P_{loc}: P value due to location of muscle sampled. P_{inoc}: P value due to micro-organisms inoculated. P_{time}: P value due to ripening time.
[b] Non-Inoc: non-inoculated batch. Inoc: inoculated batch.
[c] Values with different numbers as superscript are significantly different ($P<0.05$).
[d] P values: ++ ($P<0.01$), +++ ($P<0.001$).
[e] n.d.: not detected.

Barbieri et al., 1992; Ventanas et al., 1992), whilst the branched alcohols and acids can derive, respectively, from reduction and oxidation of the branched aldehydes. On the other hand, branched aldehydes can be also formed in meat products through deamination and decarboxylation by bacteria (Hinrichsen and Pedersen, 1995) and molds (Jacobsen and Hinrichsen, 1997). The significant differences in branched carbonyls and alcohols due to inoculation ($P_{inoc}<0.001$) point at micro-organisms playing a decisive role on their formation in dry-cured ham. The higher contents in control samples could be related to the ability of some fungi to degrade or absorb methyl branches aldehydes (Jacobsen and Hinrichsen, 1997). However, in a similar assay on dry-cured pork loins, *P. chrysogenum* Pg222, either alone or combined with *D. hansenii*, increased methyl-branched carbonyls in relation to sterile controls and samples inoculated only with *D. hansenii* (Martín et al., 2003). Given that branched aldehydes are also amongst the most odor-active compounds described for dry-cured ham (Carrapiso et al., 2002), the wild microbial population on control hams exerted a higher impact than the inoculated strains on some of these important flavor compounds. The rather negligible difference found in yeasts for both batches (Martín et al., 2004) points at the wild molds present on non-inoculated hams as the responsible for the higher levels of branched aldehydes.

From the six furans found in the samples (Table 1), only 2-pentyl furan increased in outer muscle with ripening time. These compounds are commonly found in dry-cured hams (Ruiz et al., 1999), but are not among those of intense flavor (Carrapiso et al., 2002). Furans are typically described as auto-oxidation products (Belizt and Grosch, 1999), and none of the micro-organisms used in this work affected furan formation in dry-cured pork loins (Martín et al., 2003). Furanones were found at similar levels in all batches, except for the lower levels in non-inoculated fully ripened hams, particularly at deep samples (Table 4). Furanones can be induced by Maillard reactions as sugar degradation products (Zabetakis et al., 1999). In addition,

Table 5
Sensory characteristics (from 0, low to 10, high) of control (non-inoculated) and inoculated dry-cured hams

	Non-inoculated	Inoculated	*P*
Fat Color			
Yellow	3.5±3.05	2.8±2.65	0.019
Pink	4.9±3.23	4.1±3.02	0.011
Meat Color			
Red	5.6±2.96	5.1±3.14	0.139
Texture			
Toughness	5.7±2.89	4.7±3.05	0.004
Dryness	4.9±3.04	3.6±2.98	0.001
Stringiness	5.9±3.16	5.2±3.13	0.058
Juiciness	5.2±2.91	5.7±3.08	0.091
Taste			
Salty	5.3±3.21	5.1±2.65	0.507
Sweet	3.5±3.09	3.9±3.24	0.210
Bitter	3.2±3.24	3.2±3.20	0.924
Flavor			
Cured	5.0±3.28	5.1±2.98	0.791
Rancid	3.4±3.38	3.2±3.13	0.484
Spicy	4.0±3.59	3.7±3.60	0.419
Moldy	2.4±3.10	2.3±3.00	0.878
Toasted	3.9±3.32	2.7±2.92	0.001

these compounds are produced by yeast, probably from a Maillard intermediate (Hayashida et al., 1999), and lactic acid bacteria (Hayashida et al., 2001). An enzymatic formation pathway has been also proposed as the main source for furanones in biological systems (Hauck et al., 2003). However, no significant increase was detected for the last 6 months of ripening. Similarly, furanones did not increase in dry-cured sterile pork loins (Martín et al., 2003). Due to the attractive flavor and low odor thresholds of some of these compounds (Schwab and Roscher, 1997), any positive effect of the selected organisms on furanones would be of interest for dry-cured meat products.

Pyrazines showed only low levels, except for 2,5-dimethyl pyrazine at the outer muscle, particularly in non-inoculated hams (Table 4). In addition, trimethyl pyrazine levels correlate to the inoculated micro-organisms ($P_{inoc}<0.01$). Similarly, *P. chrysogenum* Pg222 led to higher values of several pyrazines in dry-cured pork loins (Martín et al., 2003), that were related to increased levels in free amino acids (Martín et al., 2002). However, the increase of free amino acids in inoculated hams (Martín et al., 2004) did not lead to higher levels of pyrazines (Table 4). These results do not support a direct relationship between amino acids content and pyrazines formation through Maillard reactions in dry-cured ham, but rather a decisive role of the microbial population. On the other hand, the positive effect of *P. chrysogenum* on pyrazines in pork loins was restricted by the simultaneous inoculation with *D. hansenii* (Martín et al., 2003). Given that yeasts were always present at high numbers in these hams (Martín et al., 2004), the presence of *D. hansenii* could explain the negligible contribution of the mold on pyrazines in inoculated hams (Table 4). Taking into account that these compounds impart a pleasant aroma to dry-cured ham (Flores et al., 1997), the role of molds in their synthesis deserves further investigation to enhance pyrazines formation.

Sulfur compounds reaching statistically significant differences, i.e. dimethyl sulfide and dimethyl disulfide, showed higher levels in non-inoculated hams, particularly at the outer muscle (Table 4). These compounds can be generated without microbial contribution. Dimethyl sulfide is produced by spontaneous degradation of *S*-methylmethionine, and dimethyl disulfide has been detected at low levels in non-inoculated potato dextrose broth (Spinnler et al., 2001). On the other hand, some yeasts produced dimethyl sulfide by enzymatic biosynthesis in potato dextrose broth supplemented with L-methionine or *S*-methylmethionine (Spinnler et al., 2001). However, neither *P. chrysogenum* Pg222 nor *D. hansenii* Dh345 increased any of these compounds during controlled ripening of pork loins (Martín et al., 2003). Thus, the higher formation of sulfur compounds in non-inoculated hams has to be attributed to the wild microbial population. In the light of the unpleasant aroma of dimethyl disulfide (Flores et al., 1998), the microbial population on dry-cured ham should be controlled to prevent producers of sulfur compounds from being present in the hams.

Ester compounds did not reach high values, with some of them detected only at trace levels (Table 1). The more volatile esters showing statistically significant differences at the end of processing (i.e. methyl acetate and methyl butanoate) reached higher values in non-inoculated control hams, while those of longer fatty acids reached maximum values in inoculated hams (Table 4). Such differences correlate well with the concentration of their acid precursors (Table 3). Given that these compounds do not differ much from outer to deep samples, the micro-organisms on the surface do not show a strong esterase activity. In spite that esters have fruity notes, only a low impact on flavor can be expected due to the low levels found in fully ripened hams (Table 4).

Other compounds, such as dichloromethane and pyridine, have been detected. Chloride compounds have been related to pesticide residues or laboratory contamination (Flores et al., 1997; Ruiz et al., 1999).

3.2. Sensory evaluation

The sensory evaluation revealed some significant differences related to inoculation, including color and texture attributes (Table 5). On the other hand, the differences observed in volatile compounds did not showed a neat impact on flavor. Only a lower mark for “toasted” flavor was obtained from inoculated hams, which can be explained by the lower content of volatile compounds such as 1-penten-ol (Flores et al., 1998). Nonetheless, the overall acceptability mark for inoculated hams (5.7) was significantly higher ($P=0.043$) than that for control hams (4.7). This has to be attributed to their improved texture (Table 5), given that this trait has a strong impact on overall acceptability of dry-cured ham (Ruiz et al., 2002). The softer texture of inoculated hams is a direct consequence of the mold inoculation. An early settling of molds on hams prevents an

excessive drying at the surface (Lücke, 1986), thus lowering dryness. In addition, the increased proteolysis by the inoculated organisms (Martín et al., 2004) will contribute to lower toughness.

In conclusion, the inoculated micro-organisms do not remarkably alter the volatile profile when compared to the wild fungal population. The lower levels for some of the main odor-active volatile compounds were not detected by panelists. In addition, the selected fungal population improved texture, leading to better overall acceptability for inoculated hams. Therefore, the use of selected non-toxigenic starter cultures, such as *P. chrysogenum* Pg222 and *D. hansenii* Dh345, may be useful to obtain high-quality and safe dry-cured ham.

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