Note: Effect of Crossbreeding and Rearing System on Iberian Ham Volatile Compounds

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Several volatile compounds from Iberian ham trapped from the headspace were analysed to research the effect of crossbreeding (Iberian vs. Iberian × Duroc 50% pigs) and rearing system (Montanera system vs. Pienso system) on them. Thirty-four Iberian hams grouped following a factorial treatment structure were analysed. A two-way analysis of variance with interaction was applied to the data from 46 volatile compounds identified. The effect of crossbreeding was negligible on the studied volatile compounds, which was in agreement with the effect on fatty acid percentages of subcutaneous fat. On the contrary, the effect of rearing system was significant in most of the fatty acid percentages used to classify the hams into the current commercial grades of Iberian ham (16:0, 18:0 and 18:1, $p < 0.001$). However, a slight effect on the volatile compounds was found, and only a limited number of compounds were significantly affected. Results for the effect of crossbreeding and rearing system on headspace volatile compounds agreed with the sensory data for odour of hams.

Key Words: Iberian ham, volatile compounds, crossbreeding, rearing system

INTRODUCTION

Iberian ham is an aged (over 450 days) dry-cured meat made from Iberian pigs. Crossbreeds of Iberian with Duroc pigs are usually used to improve some productive characteristics of animals; in fact, up to Iberian × Duroc 50% pigs are currently allowed to produce Iberian hams (Ministerio de Presidencia, 2001) under the assumption that ham sensory characteristics are not modified. This assumption was recently confirmed by the lack of significant effect of crossbreeding on odour and flavour characteristics of Iberian ham (Carrapiso et al., 2003).

Volatile compounds are the main contributors to flavour (Mottram, 1998), an outstanding characteristic of Iberian ham closely related to acceptability (Ruiz et al., 2002). According to the sensory data, a significant effect of crossbreeding on the Iberian ham volatile compounds should not be expected. However, the limited data available showed high differences in some compounds (Antequera et al., 1996), including some compounds identified among the most active odorants of Iberian ham (Carrapiso et al., 2002b). These large differences clearly disagree with the assumption of the lack of effect of crossbreeding, and therefore differences should be re-examined.

Otherwise, pigs used to produce Iberian ham can be fattened using different rearing systems, which determine its commercial grade (Ministerio de Presidencia, 2001) and its price. Differences related to rearing systems are caused by a number of factors such as indoors or outdoors conditions, feed type and composition, feed availability or growing rate. Hams from pigs reared outdoors with acorns and pasture (Montanera system) are more expensive than those from pigs reared indoors with concentrate feedings (Pienso system).

The effect of rearing system on Iberian ham volatile compounds has already been studied but contradictory results have been reported (López et al., 1992; Martín et al., 1998; Cava et al., 2000). Differences in results are probably related to the different analytical techniques applied, but also to other factors not usually detailed which influence the sensory characteristics, the lipid composition or the lipid oxidation susceptibility, such as the Montanera feeding period length (Carrapiso et al., 2002a), the concentrate feeding composition (Cava et al., 2000) or the subcutaneous fatty acid percentages (they are the current analytical data to determinate Iberian ham commercial grade).

The purpose of this work was to investigate the effect of crossbreeding and rearing system on volatile compounds of dry-cured Iberian hams extracted by using a purge and trap equipment.

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MATERIALS AND METHODS

Samples

Thirty-four dry-cured Iberian hams grouped following a factorial treatment structure were used. Eighteen hams were obtained from pigs (11 Iberian pigs and seven Iberian × Duroc 50% pigs) reared in a 30 ha extension land during the fattening period (about 50 days) in which acorns and pasture were available (Montanera system). The other sixteen hams were obtained from pigs (10 Iberian pigs and six Iberian × Duroc 50% pigs) reared in a 6 ha extension land with a concentrate feeding (Pienso system). Details of the diets of pigs and its composition were previously published (Andrés et al., 2001).

Weights at the end of the fattening period were as follows (mean ± standard error): 139.3 ± 4.3 kg (Montanera system, Iberian pigs), 136.8 ± 4.4 kg (Montanera system, Iberian × Duroc pigs), 153 ± 2.7 kg (Pienso system, Iberian pigs) and 155.5 ± 2.7 kg (Pienso system, Iberian × Duroc pigs). Animals were stunned and slaughtered in a commercial abattoir. Legs were removed from the carcass and their weights (mean ± standard error) were: 7.8 ± 0.2 kg (Montanera system, Iberian pigs), 8.4 ± 0.1 kg (Montanera system, Iberian × Duroc pigs), 8.6 ± 0.2 kg (Pienso system, Iberian pigs) and 8.7 ± 0.2 kg (Pienso system, Iberian × Duroc pigs). One leg of each animal was processed into dry-cured ham in the same industry following the traditional way (García et al., 1996). Hams were allotted in three groups according to weight and introduced in piles of salt. The salting and post-salting stages (at 0–3 °C and 80–90% relative humidity) lasted for 4–6 months (depending on ham weight) to avoid microbial spoilage. Then, the hams were ripened for 15 months in a cellar at 10–27 °C and 58–80% relative humidity.

A piece of the biceps femoris muscle from each dry-cured ham was taken, vacuum-packaged, frozen and kept at −80 °C until use.

Percentages of Fatty Acids used for Commercial Grading

Relative fatty acid percentages of C16 : 0, C18 : 0, C18 : 1 and 18 : 2, the fatty acids currently used for classifying hams (Ministerio de la Presidencia, 2000), were calculated from data obtained according to the procedure described by Carrapiso et al. (2000). Carrapiso et al. (2003) reported a complete list of subcutaneous fatty acid percentages.

Analysis 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, then 6 g were placed into a flask for the volatile compound extraction. The sample order was randomised. The extraction was carried out using an HP G1900A purge and trap concentrator (Hewlett-Packard). The sample headspace was swept onto a 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 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.

Gas Chromatography-Mass Spectrometry (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 an HP-5 capillary column (50 m × 0.32 mm i.d., film thickness = 1.05 μm, Hewlett-Packard). The injector and detector were maintained at 230 and 250 °C respectively. After injection, oven conditions were as follows: 35 °C for 5 min, 10 °C/min to 150 °C, 20 °C/min to 250 °C, 250 °C for 10 min. A solution of *n*-alkanes (C_5–C_{18}) was analyzed under the same conditions to calculate lineal retention indices (LRI). Mass spectra were generated by electronic impact with an electron energy of 70 eV, and a multiplier voltage of 1675 V. Data were collected at a rate of 1 scan/s 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 analysed under the same conditions, or tentatively identified by comparison with mass spectra comprised in the Wiley and the NIST/EPA/NIH mass spectrum libraries and LRI previously reported (Kondjoyan and Berdagué, 1996; Rychlik et al., 1998). The reference compounds used (indicated in Table 2) were obtained from Sigma and Aldrich (Steinheim, Germany). Standard solutions were prepared at a concentration of 5 μL/mL of reference compounds in hexane (HPLC grade).

Data Analysis

Statistical analyses were performed on data of compounds identified in nearly all the samples of at least one group. A two-way analysis of variance with interaction by the general linear model procedure was performed to compare means. Factor analysis (using principal components analysis as method for factor extraction) was applied to evaluate the relationships among the hams of each group (Hair et al., 1998); criterion for variable inclusion was to reach at least $p < 0.2$ for a treatment in the analysis of variance, excluding contaminants. Statistic analyses were performed by means of the SPSS version 10.0.

RESULTS AND DISCUSSION

Data from the fatty acid analysis of the subcutaneous fat showed a lack of influence of crossbreeding (Table 1); therefore, crossbreeding at 50% did not affect the commercial grade of hams. On the contrary, rearing system significantly affected the fatty acids used for classification; in fact, according to subcutaneous fatty acid data, hams belonged to different commercial grades.

Data from 46 volatile compounds (the compounds identified in most samples of at least one group) underwent statistical treatment (Table 2). Twenty-eight of these compounds were identified by comparing mass spectra and LRI with those of reference compounds, and a further 18 compounds were tentatively identified by comparing mass spectra and LRI with literature data. Most of them were lipid-oxidation products (hydrocarbons, aldehydes and ketones).

Effect of Crossbreeding

Volatile compounds of Iberian ham were not significantly affected by crossbreeding. Significant differences appeared only for toluene, a usual environmental contaminant. The lack of a significant effect of crossbreeding on the volatile compounds matched the absence of sensory differences in the same hams for the sensory characteristics most related to volatile compounds, odour and flavour (Carrapiso et al., 2003) and also with the assumption about the lack of differences in the characteristics of dry-cured hams caused by crossbreeding up to 50% with Duroc pigs (Ministerio de la Presidencia, 2001). The lack of differences could be attributed to the genetic similarity between Iberian and Iberian × Duroc pigs at 50%, which was reflected in an intramuscular lipid composition very similar (Andrés et al., 2001). Results agreed with a previous work that reported a limited influence of crossbreeding (between a number of very different breeds) on the headspace volatile compounds and flavour of French dry-cured ham (Berdagué et al., 1993). Another work focused on the effect of crossbreeding between two different breeds (a selected and a non-selected breed) on some muscle characteristics also showed limited differences (Coutron-Gambotti et al., 1998).

Effect of Rearing System

The effect of rearing system on the volatile compounds was slight. Significant differences between hams from pigs reared in the Montanera system or in the Pienso system were found in the coelution of carbon dioxide and hydrogen sulphide, in heptan-2-one and 2-pentylfuran (Table 2). These compounds were not included in previous works focused on the effect of rearing system on the volatile constituents of dry-cured ham.

The coelution of carbon dioxide and hydrogen sulphide was larger in the Montanera group than in the Pienso group. Carbon dioxide was the main constituent of the coelution; it arose from the degradation of organic compounds but does not contribute to dry-cured ham aroma (Flores et al., 1997; Carrapiso et al., 2002b), therefore differences might be of little repercussion on ham odour. Hydrogen sulphide, formed from the breakdown of sulphur-containing compounds and contributor of Iberian ham aroma (Carrapiso et al., 2002b), was a minor component of the same chromatographic peak. However, because of its coelution, it was not possible to know the effect of rearing system on this compound; further research is required to check the actual effect of rearing system on this minor compound.

Heptan-2-one was more abundant in hams from pigs reared in the Pienso system than in the Montanera system. Heptan-2-one contributes to the odour of some types of dry-cured hams (Berdagué et al., 1993) including Iberian ham (Carrapiso et al., 2002b); therefore the significant differences (Table 2) could be related to differences in the sensory characteristics.

2-Pentylfuran abundance was larger in Pienso than in Montanera and significantly different. 2-Pentylfuran arises from reactions such as the breakdown of linoleate hydroperoxides or interaction of deca-2,4-dienal with cysteine (Zhang and Ho, 1989; Shahidi, 2000). 2-Pentylfuran did not contribute to the odour of French (Berdagué et al., 1993), Parma (Blank et al., 2001) and Iberian ham (Carrapiso et al., 2002b), although has been reported as contributor of Serrano ham odour (Flores

Table 1. Effect of crossbreeding and rearing system on the relative percentages of subcutaneous fatty acids used for classifying purposes (means and standard error of the mean, SEM).

	Montanera (Relative Percentages)		Pienso (Relative Percentages)			P^a		
	Iberian	Iberian × Duroc	Iberian	Iberian × Duroc	SEM	Breed	Rearing	$b \times r$
16 : 0	23.1	22.4	26.3	26.1	0.4	0.534	<0.001	0.684
18 : 0	9.9	9.6	11.4	12.9	0.3	0.066	<0.001	0.011
18 : 1	58.7	59.1	53.9	52.5	0.6	0.493	<0.001	0.247
18 : 2	8.3	8.9	8.4	8.5	0.1	0.195	0.525	0.355

[a]Two-ways analysis of variance, effects were considered significant at a level of 5%.

Table 2. Effect of crossbreeding and rearing system on the headspace volatile compounds of Iberian ham (mean and SEM and significance levels from a two-ways analysis of variance).

		Units of Area $\times 10^{-3}$							
		Montanera		Pienso			p[a]		
	LRI[b]	Iberian	Ib. × Duroc	Iberian	Ib. × Duroc	SEM	Breed	Rearing	b × r
CO_2[f]/hydrogen sulphide[f]	<500	472.7	258.3	157.2	184.0	48.5	0.315	0.042	0.199
Acetaldehyde[d]	<500	9.0	11.1	9.5	6.8	0.9	0.880	0.338	0.211
Methanethiol[f]	<500	18.1	19.2	17.3	19.9	1.3	0.505	0.988	0.783
Ethanol[d]	<500	149.9	158.7	173.4	161.5	15.0	0.961	0.686	0.749
Propan-2-one[d]2-propanol[f]/pentane[d]	<500	169.3	231.4	180.2	229.1	15.2	0.085	0.892	0.834
2-Methylpropanal[c]	554	24.9	21.6	24.5	30.3	1.8	0.747	0.273	0.227
Hexane[c]/butanal[e]	595	28.8	13.7	17.4	49.8	5.7	0.449	0.283	0.045
Chloroform[c]	621	61.0	24.1	150.3	135.2	25.5	0.619	0.062	0.834
3-Methylbutanal[c]	657	95.0	80.3	98.0	101.0	5.6	0.625	0.321	0.459
2-Methylbutanal[c]	667	62.4	52.4	60.6	65.6	3.6	0.742	0.458	0.327
Pentan-2-one[c]	689	58.2	51.5	64.7	58.0	2.9	0.272	0.291	0.997
Pentanal[c]/heptane[c]	700	61.3	49.6	60.1	56.8	4.1	0.401	0.733	0.635
3-Methylbutan-1-ol[c]	738	2.4	3.0	3.4	3.6	0.3	0.560	0.211	0.764
2-Methylbutan-1-ol[c]	745	1.6	1.2	1.2	1.2	0.1	0.464	0.438	0.581
Dimethyl disulphide[e]	753	4.2	5.2	5.9	5.7	0.4	0.699	0.213	0.493
Pentan-1-ol[c]	770	5.6	6.2	6.6	8.3	0.5	0.286	0.151	0.604
Toluene[c]	774	362.3	966.5	434.9	1004.3	122.5	0.022	0.821	0.943
Hexan-2-one[e]	790	4.9	3.1	4.3	4.1	0.4	0.226	0.826	0.360
Hexanal[c]/octane[c]	803	293.4	236.5	306.0	309.8	18.9	0.506	0.287	0.449
Hexan-1-ol[c]	867	11.1	12.7	12.9	16.6	0.9	0.174	0.136	0.574
Heptan-2-one[c]	889	13.2	9.9	15.8	17.1	1.2	0.676	0.048	0.334
Heptanal[c]/nonane[c]	900	34.1	33.2	26.0	31.5	4.0	0.787	0.566	0.708
1,4-Dimethylbenzene[c]	877	2.6	0.1	4.3	1.5	0.9	0.144	0.386	0.923
2-Pinene[e]	948	1.7	1.8	1.1	2.4	0.2	0.162	0.988	0.297
Benzaldehyde[e]	976	1.3	1.3	1.3	1.6	0.1	0.654	0.670	0.582
Oct-1-en-3-ol[c]	981	1.7	1.6	1.8	2.7	0.2	0.274	0.135	0.210
2-Octanone[e]	992	2.8	2.4	2.6	3.5	0.4	0.804	0.572	0.452
2-Pentylfuran[e]	996	0.3	0.6	1.2	0.9	0.1	0.917	0.039	0.270
Octanal[c]/decane[c]	1002	67.2	44.4	36.6	52.8	6.2	0.798	0.383	0.131
2-Ethylhexan-1-ol[e]	1031	20.7	14.8	8.8	18.4	2.8	0.753	0.475	0.192
Octan-1-ol[e]	1073	4.6	3.5	3.8	6.4	0.7	0.592	0.472	0.224
Nonan-2-one[e]	1094	1.8	1.4	2.1	2.4	0.3	0.962	0.316	0.521
Undecane[c]	1100	8.4	3.2	2.1	16.0	2.4	0.374	0.498	0.056
Nonanal[c]	1111	40.2	37.2	39.8	51.4	3.7	0.581	0.380	0.352
(*E*)-non-2-enal[c]	1172	3.5	2.5	1.8	2.1	0.6	0.774	0.418	0.608
Dodecane[c]	1200	5.4	1.5	2.1	30.9	5.2	0.245	0.224	0.130
Decanal[c]	1211	8.9	5.9	8.1	10.6	1.1	0.902	0.419	0.254
Tetradecane[c]	1400	8.6	7.8	5.8	17.7	2.2	0.232	0.437	0.171

[a]Effects were considered significant at a level of 5%; [b]Linear retention indices (LRI) on an HP-5 column; [c]The compound was identified by comparing it with the reference compound MS spectrum and LRI; [d]compound identified only by comparing it with the MS spectrum of the reference compound; [e]The compound was identified by comparing it with MS spectrum and LRI form literature; [f] compound identified only by comparing it with the MS spectrum from literature data.

et al., 1997). Therefore, changes in this compound are not necessarily related to Iberian ham odour differences.

A principal component analysis was performed using the variables most affected by crossbreeding and rearing system ($p < 0.2$, excluding contaminants). One variable (1,4-dimethylbenzene) was rejected because of its poor inclusion in the model (Kaiser-Meyer-Olkin measure of sampling adequacy: 0.150). Samples showed a slight grouping according to rearing system (Figure 1), within the group Pienso data had a higher dispersion than those from the Montanera group. No grouping according to crossbreeding was found.

The slight effect of rearing system on the volatile compound profile was consistent with the sensory data of the same hams, which showed no significant effect on odour (odour intensity and Montanera-ham typical odour) and only a significant effect on half of the flavour traits, despite the great effect on texture (Carrapiso et al., 2003) and despite of the large differences in the subcutaneous fatty acid percentages (Table 1) that showed the two groups of hams. It should be noted that compounds extracted by purge and trap would be more related to odour than to flavour because non-volatile compounds and high boiling point compounds were not extracted, and samples were not

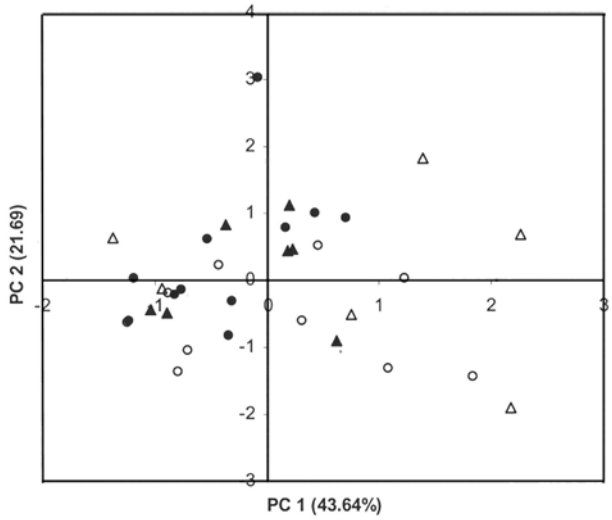

Figure 1. Projection of the samples onto the space defined by the two first principal components.Groups: (●) hams from Iberian pigs reared in the Montanera system; (▲) hams from Iberian x Duroc pigs reared in the Montanera system; (○) hams from Iberian pigs reared in the Pienso system; (△) hams from Iberian x Duroc pigs reared in the Pienso system.

chewed; it is probably that the differences in flavour were caused by compounds not included in Table 2.

The slight differences in volatile compounds (and also in odour and flavour) could be related to the short Montanera fattening period (about 50 days) of the Montanera pigs. This fact could lead to obtain hams from Montanera pigs without the expected odour and flavour differences from those of hams from Pienso pigs. In fact, Montanera fattening period length influenced odour, flavour and odorants of Iberian hams (Carrapiso et al., 2002a) and a period of at least 60 days is required to produce the best Iberian ham commercial grade (Ministerio de la Presidencia, 2000). The slight differences in the volatile compounds also agreed with data from Parma ham, which showed limited differences among the volatile compounds of hams from pigs fattened with different concentrate feedings (Pastorelli et al., 2003).

In summary, results from the present work showed no effect of crossbreeding (Iberian vs. Iberian × Duroc 50% pigs) and a slight effect of rearing system on volatile compounds when pigs are reared in Montanera for a short fattening period and indoor reared pigs are fed with a usual concentrate feeding. Results were consistent with odour and flavour characteristics of hams.

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