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A tentative characterization of white dry-cured hams from Teruel (Spain) by SPME-GC

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Abstract

Forty-six volatile compounds were identified and quantified in four parts (subcutaneous fat, and *biceps femoris*, *semitendinosus* and *semimembranosus* muscles) of 41 samples from Spain, France and Italy. The Univariate Brown–Forsythe test was used to determine the volatile compounds from each part of the ham that can distinguish the hams of Teruel from Iberian and white hams. Stepwise linear discriminant analysis was used in-tandem to refine the most discriminating volatile compounds. Six compounds (2-propanone, butanol, 3-methylbutanol, 3-methylbutanal, hexanal and limonene) were able to distinguish the dry-cured hams from Teruel, Iberian hams, and French and Spanish white hams simultaneously. MDS was also applied to the volatiles selected by SLDA. Information on the series of volatiles and individual compounds is also displayed.

Keywords: Dry-cured hams; Iberian hams; White hams; Volatiles; Chemometrics

1. Introduction

Dry-cured ham is a traditional foodstuff whose curing process is, in some cases, characteristic of a particular geographical origin and hence, sometimes, regulated by a protected designation of origin (PDO). These protected hams show specific sensory characteristics that might be related to sensory attributes of aroma, flavour and texture. The aroma is a determinant of consumer acceptability and is due to the presence of volatile compounds, most of them produced by lipolysis and proteolysis (Toldrá, 1998) during the post-mortem process (Flores, Grimm, Toldrá, & Spanier, 1997) although the aroma is also markedly affected by the pig breeding and feeding. Therefore, a better understanding of the dry-cured ham aroma should include the identification and quantification of its volatiles.

Several studies have reported information on the volatile composition of various kinds of dry-cured hams, which are very different in their aroma, such as Corsican, Iberian and Parma hams (Bolzoni, Barbieri, & Virgili, 1996; Flores et al., 1997; López et al., 1992; Pastorelli et al., 2003; Sabio, Vidal-Aragón, Bernalte, & Gata, 1998; Sánchez-Peña, Luna, García-González, & Aparicio, 2005; Timón, Ventanas, Carrapiso, Jurado, & García, 2001). These studies, however, were not focussed on the characterization of the hams by their geographical origin or by any specific PDO. This kind of characterisation requires not only selection of samples that represent the entire geographical zone, but also a comparison with the hams from other geographical origins, pig crossbreeding and pig feeding.

This work analyses the volatile profile of the white hams produced in the Spanish province of Teruel. The production of hams of Teruel is increasing, year by year, and it might represent 10% of the overall Spanish production of dry-cured hams (24 millions) in the

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next future; a great part of this production is protected by PDO "Jamón de Teruel" (BOA, 1993). Thus, the sensory and chemical profiles of these hams should be homogeneous and different, in any aspect, from other hams. Concerning the aroma, however, some of the published papers have pointed out the variability of the quantified volatiles, which may be due to the fact that the hams are not homogeneous products and the muscles and subcutaneous fat of the samples can differ. To avoid the characterization being affected by the lack of homogeneity, each volatile was independently quantified in four well-known ham locations: *biceps femoris, semimembranosus* and s*emitendinosus* muscles, and subcutaneous fat (Sánchez-Peña et al., 2005). The amount of each volatile produced at each location, the contribution of the volatiles quantified at each location to the ham sensory perception, and the volatiles that contribute to the characterisation of the white hams from Teruel were evaluted in comparison with other hams, such as the white hams from Spain and France (with similar curing process and pig breeding and feeding) or the Iberian hams which are also produced in Spain but whose pig breeding and feedings are quite different.

2. Materials and methods

2.1. Ham samples

Table 1 shows the codes of the samples, their geographical origin, breeds and maturation times of forty-one hams, seven of them being from the Spanish province of Teruel (four from PDO "Jamón de Teruel" and three non-PDO). The samples were selected to represent the kinds of hams described in Table 1 and, in particular, those cured inside the province of Teruel. All the hams were processed by local manufacturers using the traditional method of each geographical origin (Flores & Toldrá, 1993; Sabio et al., 1998). The samples were stored in vacuum plastic bags at −5 °C until they were required for the analytical studies.

2.2. Sample preparation

A sample of approximately 350 g of the part located along and behind the femur was collected from each ham. A cylindrical stainless steel tool, specially designed for ham sampling, was used to extract approximately 5 g along the sample thickness. The samples were collected from *biceps femoris* (BF), *semimembranosus* (SM) and *semitendinosus* (ST) muscles and subcutaneous fat (SF). The top and bottom layers of the cylindrical samples were removed to avoid any possible alteration of the initial quality of the hams. Three grammes of the rest were minced to increase the interface between the ham and the vapour phase during the concentration step.

2.3. Concentration of volatile compounds

Three grammes of the minced hams were placed in 20 ml glass vials, tightly capped with a PTFE septum, and left for 10 min at 40 °C to allow equilibration of the volatiles in the headspace. The septum covering each vial was then pierced with a solid-phase micro-extraction (SPME) (Gianelli, Flores, & Toldrá, 2002) needle and the fibre exposed to the headspace for 180 min. When the process was completed, the fibre was inserted into the injector port of the GC. The temperature and time were automatically controlled in a Combipal (CTC Analytics AG, Zwingen, Switzerland) by the software Workstation v.5.5.2 (Varian, Walnut Creek, CA).

The SPME fibre was purchased from Supelco (Bellfonte, PA) and was endowed with Stable Flex stationary phase (50/30 μm film thickness) of divinylbenzene/Carboxen/Poly-dimethylsiloxane (DVB/CAR/PDMS). The fibre was preconditioned at 270 °C for 60 min in the GC injector port, according to supplier instructions.

2.4. Reagents

All the volatile compounds described in the work were fully identified using standards purchased from Sigma–Aldrich (St. Louis, MO).

2.5. Gas-chromatography

The volatile compounds adsorbed by the fibre were desorbed in the injection port of the GC for 5 min at 260 °C with the purge valve off (splitless mode). The compounds were separated in a DB-WAX column (J&W Scientific, Folsom, CA; 60 m × 0.25 mm id × 0.25 μm film thickness) installed on a Varian 3900 gas chromatograph (Varian, Walnut Creek, CA) with a flame ionisation detector. The carrier gas was hydrogen. The oven temperature was held at 40 °C for 4 min and programmed to rise at 1 °C/min to a temperature of 91 °C, and then to rise at 10 °C/min to a final temperature of 201 °C, where it was held for 10 min to eliminate the memory effect. Each sample was analysed in three replicates.

The content of each volatile compound was calculated from the FID area and expressed as area units. A solution of 4-methyl-2-pentanol (3.2 mg/kg) was used as external standard in order to standardise the results of all the analyses. Thus, the quantitative result (mg/kg) of each volatile compound was computed by relating the peak intensity of the volatile compound to the intensity of external standard, and taking into account the sample weight. Table 2 shows the volatile quantified and identified by standards.

Table 1
Geographical origin, kind of pig breed and ham maturation of the coded samples

Code	Geographical origin (city, region, country)	Breed	Maturation (months)
F1	Yssingeaux, Auvergne, France	White[e]	12
F2	Solignac sur Loire, Auvergne, France	White[e]	Unknown
F3	St Maurice de Lignon, Auvergne, France	White[e]	6–8
F4	Clermont-Ferrand, Auvergne, France	White[e]	Unknown
F5	Ussel, Auvergne, France	White[e]	>7
F6	Parlan, Auvergne, France	White[e]	16–18
F7	Aurillac, Auvergne, France	White[e]	7–9
F8	Condat, Auvergne, France	White[e]	8.5–10.5
F9	Rignac, Aveyron, France	White[e]	12
F10	Lacaune, Lacaune, France	White[e]	15
F11	Murat sur Vèbre, Lacaune, France	White[e]	6–9
F12	Lacaune, Lacaune, France	White[e]	7–10
F13	Bordères sur Eches, Bayonne, France	Gasconne	18–24
F14	Les Aldudes, Bayonne, France	Basque	16–18
F15	Baraqueville, Aveyron, France	White[b]	12
F16	Espeyrox, Lot, France	White[b]	12
F17	Unknown, Bayonne, France	White[e]	Unknown
F18	Unknown, unknown, France	White[b]	7
F19	Aosta, Valle d'Aosta, Italy	White	Unknown
F20	Aosta, Valle d'Aosta, Italy	White	Unknown
T1	Teruel, Teruel, Spain	White[a]	16–18
T2	El Poyo del Cid, Teruel, Spain	White[a]	16–18
T3	Calamocha, Teruel, Spain	White[a]	19–20
T4	Formiche Alto, Teruel, Spain	White[b]	10–12
T5	Calamocha, Teruel, Spain	White[b]	16–18
T6	Formiche Alto, Teruel, Spain	White[a]	16–18
T7	El Poyo del Cid, Teruel, Spain	White[b]	13–15
S1	Sant Boi de Llobregat, Barcelona, Spain[f]	White[b]	<10
S2	Unknown, Unknown, Spain	White[b]	10–12
S3	Murcia, Murcia, Spain	White[b]	<10
S4	Unknown, Unknown, Spain	White[b]	13–15
S5	Unknown, unknown, Spain	White[b]	<10
S6	Guadix, Granada, Spain[f]	White[b]	16–18
I1	Ledrada, Salamanca, Spain	Iberian[c]	>20
I2	El Repilado, Huelva, Spain	Iberian[c]	19–20
I3	Espiel, Córdoba, Spain	Iberian[c]	>20
I4	Unknown, Extremadura, Spain	Iberian[d]	19–20
I5	Zalamea la Real, Huelva, Spain	Iberian[d]	19–20
I6	Guijuelo, Salamanca, Spain	Iberian[d]	19–20
I7	Unknown, Huelva, Spain	Iberian[d]	19–20
I8	Unknown, Huelva, Spain	Iberian[c]	19–20

[a] Possible crossbreeding: (Duroc or Landrace) × (Landrace or Large white or Landrace × Large white) (BOA, 1993).
[b] No legislation on the crossbreeding.
[c] 100% Iberian pig or Iberian × Duroc-Jersey with a minimum of 75% Iberian pig (BOE, 1986; BOE, 1995).
[d] 100% Iberian pig or Iberian × (Duroc or Duroc-Jersey) (BOE, 2001).
[e] Possibly (Large white × French Landrace) × (Piétrain × Large white).
[f] TSG (Traditional Speciality Guaranteed) Serrano.

2.6. Statistical analysis

Univariate and multivariate algorithms have been used, by means of Statistica (Statsoft, Tulsa OK) version 6.0. The Brown–Forsythe test was used to perform the univariate analysis as it gives quite accurate error rates, even when the underlying distributions for the raw scores deviate significantly from the normal distribution (Olejnik & Algina, 1987). This first screening of the volatiles was followed by the multivariate procedure of stepwise linear discriminant analysis (SLDA) to reduce the number of volatiles without loss of information. SLDA was applied under the strictest conditions to avoid the possibility of hyper-optimistic results. The criterion for the selection of variables (volatiles) was the *F*-to-enter value obtained from the *F*-distribution table ($F > 0.95$), taking into account the number of groups and the number of samples from the smallest group. Multidimensional scaling (MDS), an unsupervised procedure, was used with the Ward's method as amalgamation rule and city-block as distance measure. Tolerance was fixed at 10^{-3}. MDS was applied with the volatile compounds selected by SLDA to validate the classification result.

Table 2
List of the chemical compounds identified by standards

Volatile compound	RRT	Minimum	Maximum
Hexane	0.16	8.91×10^{-3}	0.60
Heptane	0.17	22.2×10^{-3}	3.25
Octane	0.20	14.4×10^{-3}	6.35
2-Propanone	0.21	21.5×10^{-3}	7.74
2-Butanone	0.27	13.5×10^{-3}	2.43
3-Methylbutanal	0.29	tr	1.53
2-Propanol	0.31	tr	0.72
Ethanol	0.32	7.98×10^{-3}	12.6
2-Ethyl furane	0.34	tr	4.10
2-Pentanone + 3-pentanone	0.38	0.72×10^{-3}	4.17
2,3-Butanodione	0.39	tr	4.48
α-Pinene	0.46	0.72×10^{-3}	0.41
Methyl benzene	0.51	24.7×10^{-3}	0.44
2-Methyl-3-buten-2-ol	0.53	0.55×10^{-3}	0.40
Methyl disulfide	0.60	0.69×10^{-3}	1.10
Butyl acetate	0.61	0.46×10^{-3}	0.28
Hexanal	0.64	25.2×10^{-3}	8.32
2-Methyl propanol	0.69	0.65×10^{-3}	0.43
2-Butanol	0.75	0.40×10^{-3}	0.33
Ethyl benzene	0.78	1.25×10^{-3}	0.74
Butanol	0.90	tr	4.30
2-Heptanone	1.05	tr	7.34
Heptanal	1.06	tr	9.78
Limonene	1.09	tr	5.66
3-Methylbutanol	1.21	16.4×10^{-3}	30.5
2-Pentyl furane	1.31	tr	0.95
3-Octanone + octen-3-one	1.43	tr	1.11
Pentanol	1.46	17.6×10^{-3}	2.19
(*E*,*E*)-2,4-Decadienal	1.59	0.55×10^{-3}	6.18
2-Octanone	1.61	tr	7.97
Octanal	1.63	tr	2.46
(*E*)-2-Heptenal	1.84	tr	4.02
2-Heptanol	1.89	tr	1.59
Hexanol	2.09	20.2×10^{-3}	5.40
2-Nonanone	2.30	tr	4.34
Nonanal	2.33	tr	2.38
(*E*)-2-Octenal	2.55	0.59×10^{-3}	0.30
1-Octen-3-ol	2.76	tr	2.54
Decanal	3.02	tr	0.42
Benzaldehyde	3.11	tr	0.61
(*E*)-2-Nonenal	3.22	0.59×10^{-3}	0.18
Octanol	3.47	tr	0.54
Butanoic acid	3.94	10.4×10^{-3}	1.17
Nonanol	4.13	tr	76.4×10^{-3}
Isobutyric acid	4.14	0.10	6.47
Hexanoic acid	4.35	9.38×10^{-3}	11.1

Maximum and minimum concentration (mg/kg) of the volatile compounds taking into account the four locations (SF, BF, SM, ST) of all the ham samples.
Note: RRT, relative retention time with respect to the external standard. Tentative concentrations were calculated by relating the peak area of the volatiles to the external standard (4-methyl-2-pentanol).

3. Results and discussion

The volatiles compounds of white hams from Teruel and of other geographical origins and breeds of swine were analysed by SPME-GC. The analyses of the volatile compounds were carried out in four locations of the hams (BF, SM and ST muscles and SF) in order to avoid the heterogeneity of the hams so much as possible. Forty-six volatile compounds were identified and quantified in each one of the ham locations of the samples (Table 2). In order to make the interpretation of the results easier, the data from these volatiles were initially clustered into four series of compounds: ketones, hydrocarbons, aldehydes and alcohols. Table 3 shows the values quantified in the four locations of the white hams from Teruel, the French white hams, other Spanish white hams and Iberian hams.

The first conclusion from Table 3 is that the total amount of ketones determined in the muscles does not contribute to distinguishing the kind of hams since non-significant differences between groups were found. Only the amount of volatiles quantified in the SF may distinguish the French hams from the other hams, although the muscles have the highest concentrations of ketones. Most of the quantified ketones were methyl-ketones (2-propanone, 2-butanone, 2-heptanone, 2-octanone, 2-nonanone) and they are formed by a chemical process or by micro-organisms if the microbial population is high (Pastorelli et al., 2003). The major compound was 2-propanone. The high concentration of this compound has been extensively reported (Buscailhon, Berdagué, & Monin, 1993; Dirinck, Van Opstaele, & Vandendriessche, 1997; Timón et al., 2001) and its concentration seems to depend on the food material and the processing technique (Flores et al., 1997). However, the concentration varies markedly depending on the ham location. The highest concentrations were detected in the *semitendinosus* and *biceps femoris* muscles (40–54%, depending on the kind of ham) and the lowest in the subcutaneous fat (15–27%). This compound, however, does not seem to contribute to the ham aroma. Two ketones (2-heptanone and 2-nonanone) contribute to "blue cheese" sensory attribute (Creuly, Laroche, & Gros, 1992), and a great intensity of this sensory perception is a symptom of bad quality hams. Concerning the other two methyl-ketones, 2-butanone may contribute to the sensory perception "ethereal" and 2-octanone has been characterised by the sensory attribute "green herbaceous" (Berdagué, Denoyer, Le Quéré, & Semon, 1991).

The second observation is the great amount of volatile compounds quantified in the Iberian hams, which are significantly higher than those in the other hams (i.e. the hams from Teruel). The contents of alcohols and aldehydes allowed the Iberian hams to be distinguished from the hams from Teruel and the other white hams. The maximum concentration of alcohols was measured in BF and ST muscles of the Iberian hams. 3-Methyl butanol was the most abundant alcohol, and its concentration may be due to the activity of the micro-organisms on its precursor 3-methylbutanal (Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004) produced by Strecker degradation of amino acids during the pro-

Table 3
Total concentration (mg/kg) of the volatiles clustered in four chemical classes at the different locations of hams

Compounds	Location	White hams from Teruel	French white hams	Spanish white hams	Iberian hams
Ketones	*Biceps femoris*	5.55 ± 1.09	5.66 ± 0.52	6.26 ± 1.10	5.35 ± 0.91
	semimembranosus	5.94 ± 1.02	4.94 ± 0.28	7.23 ± 0.79	4.53 ± 0.62
	semitendinosus	7.23 ± 1.35	6.17 ± 0.57	7.05 ± 0.20	5.08 ± 0.91
	Subcutaneous fat	6.47 ± 1.72	3.07 ± 0.28	5.61 ± 0.97	4.01 ± 0.44
	Total	25.2 ± 3.74	19.85 ± 1.07	26.2 ± 2.50	19.0 ± 2.72
Hydrocarbons	*Biceps femoris*	0.99 ± 0.23	0.86 ± 0.10	0.98 ± 0.13	1.54 ± 0.29
	semimembranosus	1.41 ± 0.63	1.50 ± 0.31	2.87 ± 1.53	3.20 ± 0.77
	semitendinosus	1.20 ± 0.19	1.00 ± 0.13	0.76 ± 0.07	1.56 ± 0.30
	Subcutaneous fat	1.54 ± 0.42	1.14 ± 0.19	2.24 ± 0.53	2.16 ± 0.33
	Total	5.14 ± 0.99	4.50 ± 0.43	6.84 ± 1.37	8.46 ± 1.30
Aldehydes	*Biceps femoris*	2.95 ± 1.46	2.33 ± 0.93	3.22 ± 0.90	2.18 ± 0.80
	semimembranosus	2.07 ± 0.50	2.09 ± 0.51	2.33 ± 0.59	3.90 ± 1.12
	semitendinosus	1.61 ± 0.19	1.75 ± 0.50	3.26 ± 0.63	2.13 ± 0.63
	Subcutaneous fat	3.25 ± 0.59	4.10 ± 0.64	2.76 ± 0.54	7.42 ± 0.77
	Total	9.88 ± 2.12	10.3 ± 2.06	11.6 ± 2.08	15.6 ± 2.44
Alcohols	*Biceps femoris*	4.70 ± 0.45	4.85 ± 0.42	6.52 ± 1.69	18.4 ± 3.32
	semimembranosus	5.03 ± 0.45	5.58 ± 0.57	5.73 ± 1.36	6.76 ± 1.72
	semitendinosus	4.34 ± 0.73	4.44 ± 0.39	4.13 ± 1.10	13.7 ± 3.20
	Subcutaneous fat	4.25 ± 0.79	3.30 ± 0.31	3.98 ± 0.54	7.42 ± 1.23
	Total	18.3 ± 2.04	18.2 ± 1.37	20.3 ± 3.69	46.2 ± 8.07
Total	*Biceps femoris*	16.0 ± 1.57	16.4 ± 1.26	19.7 ± 2.76	29.3 ± 2.92
	semimembranosus	15.8 ± 1.26	16.8 ± 1.21	20.4 ± 2.15	19.8 ± 2.59
	semitendinosus	16.0 ± 1.79	16.8 ± 1.31	17.8 ± 0.81	24.0 ± 3.34
	Subcutaneous fat	17.5 ± 2.64	14.5 ± 0.85	17.1 ± 1.12	24.0 ± 1.94

Note: Tentative concentrations were calculated by relating the peak area of the volatiles to the external standard. The given values are the mean and standard error of the mean. Total means the sum of all the quantified volatiles.

teolysis. This alcohol was more than 69% of the total alcohols in the BF muscle of the Iberian hams, and less than 50% in the white hams. It was also the most abundant in the other locations of the Iberian (49–60%) and white (37–48%) hams with the exception of the subcutaneous fat in which hexanol was the major compound (29–36% for all the hams). Lipids constitute 89.7% of the subcutaneous fat (Coutron-Gamboti & Gandemer, 1999), and hence the proteolysis mechanism is not an important producer of the volatiles from this location. This explains why alcohols produced by lipid oxidation, mainly hexanol, are concentrated in SF. Alcohols contribute to ham aroma, with fatty, woody and herbaceous notes (García & Timón, 2001), such as 3-methylbutanol, that has been characterized as a green aroma.

With respect to the aldehydes, the total concentration in the white hams was lower than in the Iberian hams. The highest differences were found in the SF and SM locations. Hexanal was the most abundant aldehyde in the Iberian hams, in accordance with García et al. (1991), and especially in the SF location where it was 64.2% of the total concentration of aldehydes. Hexanal is formed by the oxidation of either esterified or free linoleic acid, and the concentration of this acid was, obviously, higher in the subcutaneous fat (López et al., 1992). Aldehydes, on the other hand, play an important role in the ham aroma because of their low odour thresholds. Hexanal contributes to the characteristic odour of Iberian dry-cured hams in conjunction with other volatiles, such as 3-methylbutanal, that accounts for more than 25% of the total aldehydes in the muscle ST of the Iberian hams. 3-Methylbutanal has been characterized as the nutty and salty sensory notes (Hinrichsen & Pedersen, 1995).

Therefore, alcohols and aldehydes, specifically 3-methylbutanal, may be key compounds in the characterization of the hams from Teruel as against the Iberian hams. The individual contribution of each volatile for characterizing the Iberian vs. the white hams from Teruel was studied by means of the Brown–Forsythe test for homogeneity of variances ($p < 0.05$). This mathematical procedure selected six aldehydes, four alcohols, three ketones, two acids, and a terpene (Table 4). The selection of the compounds is due to the pig breeds and feedings; the hams from Teruel are exclusively fed with fodders that usually contain maize while the Iberian pigs are fed with acorns. Higher concentrations of the aldehydes and alcohols in the Iberian hams allowed us to foresee that the Iberian hams would be characterised by the sensory attributes: fatty and green herbaceous, due to the alcohols, and nutty, salty, floral and "cured ham", due to the aldehydes (Table 4). Literature reports have characterised Iberian hams by the sensory perceptions "dry fruit" or "acorn aroma" and "cured odour" (García &

Table 4
Volatile compounds, with their sensory characterics, that show significant differences ($p = 0.05$ by the Brown–Forsythe test) on comparing the hams from Teruel with each one of the other kinds of hams

Chemical compound	Sensory characteristic	Teruel white hams	Iberian hams	Spanish white hams	French white hams
Heptane	Alkane	ST:0.165 ± 0.033		ST:0.134 ± 0.038	
2-Propanone	–	SF:1.47 ± 0.222			SF:1.00 ± 0.046
3-Methylbutanal	Nutty, salty	SF:0.074 ± 0.022	SF:0.142 ± 0.021		
		SM:0.208 ± 0.086	SM:0.629 ± 0.143		
		ST:0.084 ± 0.018	ST:0.315 ± 0.079		
2-Propanol	Slightly buttery taste	BF:0.150 ± 0.077			BF:0.076 ± 0.013
		SM:0.181 ± 0.102			SM:0.065 ± 0.015
2,3-Butanodione	Buttery	BF:0.769 ± 0.620	BF:0.094 ± 0.072		SF:0.294 ± 0.061
		SF:0.064 ± 0.062	SF:0.652 ± 0.249		
		SM:0.694 ± 0.567	SM:0.317 ± 0.169		
Methyl benzene	Strong	BF:0.189 ± 0.047			BF:0.111 ± 0.015
		SF:0.210 ± 0.044			SF:0.108 ± 0.012
		SM:0.207 ± 0.030			SM:0.135 ± 0.013
		ST:0.163 ± 0.020			ST:0.110 ± 0.011
Hexanal	Green, grassy, rancid	SF:1.167 ± 0.383	SF:4.46 ± 0.783		
Ethyl benzene	–	BF:0.060 ± 0.011			BF:0.182 ± 0.027
Butanol	Medicinal, fruit	BF:0.630 ± 0.611	SF:0.126 ± 0.023		BF:0.023 ± 0.009
		SF:0.036 ± 0.011	SM:0.262 ± 0.051		SF:0.014 ± 0.004
		SM:0.026 ± 0.011			
2-Heptanone	Spicy, blue cheese	SM:1.32 ± 0.382	SM:0.817 ± 0.369	ST:2.028 ± 0.276	
		ST:1.89 ± 0.194	ST:1.04 ± 0.484		
Heptanal	Cured ham-like, toasted, oily, fatty	SF:0.513 ± 0.196	SF:0.745 ± 0.094		
Limonene	Lemon, wood	ST:0.480 ± 0.099	ST:1.16 ± 0.264	ST:0.168 ± 0.044	ST:0.043 ± 0.021
3-Methylbutanol	Green	BF:1.89 ± 0.505	BF:14.141 ± 4.055		
		ST:1.65 ± 0.303		ST:0.830 ± 0.334	
2-Octanone	Green herbaceous	BF:0.611 ± 0.360	BF:0.153 ± 0.034		
		SM:0.097 ± 0.043		SM:0.397 ± 0.119	
Octanal	Green, fresh	ST:0.071 ± 0.028		ST:1.087 ± 0.470	
Nonanal	Rancid, fatty	SF:0.151 ± 0.051	SF:0.387 ± 0.053		
1-Octen-3-ol	Mushroom	BF:0.650 ± 0.114	BF:0.369 ± 0.123		
Decanal	Penetrating, sweet, floral, citrus	SF:0.026 ± 0.010	SF:0.049 ± 0.007		
Benzaldehyde	Almond	SM:0.117 ± 0.26	SM:0.221 ± 0.043		
		ST:0.078 ± 0.023	ST:0.171 ± 0.036		
Octanol	Sharp, fatty	BF:0.050 ± 0.017	BF:0.153 ± 0.026		
		SF:0.125 ± 0.051	SF:0.0352 ± 0.047		
		SM:0.067 ± 0.017	SM:0.104 ± 0.018		
		ST:0.071 ± 0.027	ST:0.170 ± 0.035		
Butanoic acid	Fatty, cheese	BF:0.151 ± 0.033	BF:0.227 ± 0.072		
Hexanoic acid	Fatty, cheese, sweaty	SF:0.766 ± 0.355	SF:0.248 ± 0.077		

The values correspond to the volatile concentration (expressed as mean and standard deviation of the mean) determined in the ham locations.
Note: SF, subcutaneous fat; BF, *biceps femoris* muscle; SM, *semimembranosus* mucle; ST, *semitendinosus* muscle. Tentative concentrations (mg/kg) were calculated by relating the peak area of the volatiles to the external standard (4-methyl-2-pentanol).

Carrapiso, 2001; Ruiz, García, Muriel, Andrés, & Ventanas, 2002).

A problem arose on comparing the white hams from Teruel with the other white hams. Six compounds were detected by the Brown–Forsythe test on comparing the white hams from Teruel with the other Spanish white hams (Table 4). Five of these volatiles were quantified in the ST muscle (heptane, 2-heptanone, limonene, 3-methylbutanol, octanal), and one in the SM muscle (2-octanone) (Table 4). The major differences were assigned to the hydrocarbon limonene and the aldehyde octanal quantified in the ST muscle. Limonene was also the most abundant hydrocarbon in the Iberian hams but, among white hams, the highest concentrations of limonene were found in the hams from Teruel, whereas the minimum concentration was clearly observed in the French hams (Fig. 1). The presence of limonene in the hams has been associated with the pig feeding

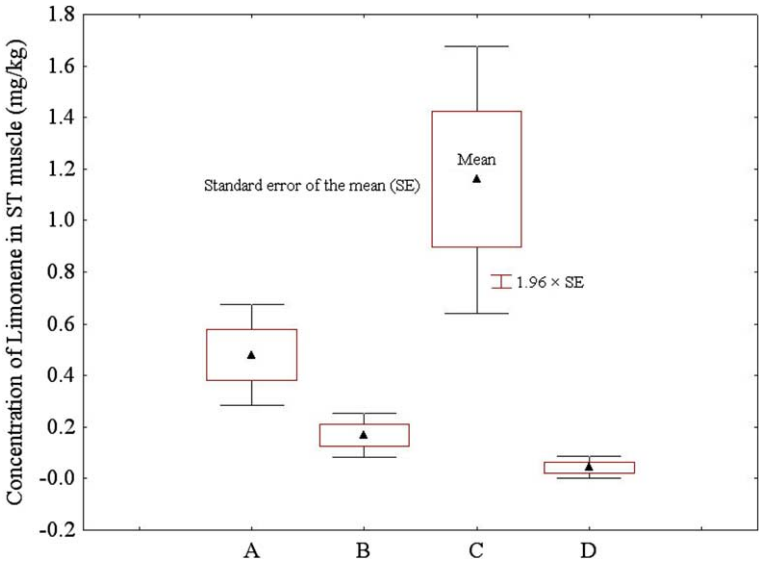

Fig. 1. Values of limonene quantified in the *semitendinosus* muscle of the hams. Categorization of the results by the Box–Whiskers plot. *Note*: A, White hams from Teruel; B, Spanish white hams; C, Iberian hams; D, French white hams.

(Buscailhon et al., 1993; Sabio et al., 1998). This may explain the highest values of limonene in the Iberian hams (Iberian pigs are fed with acorns) but it may also indicate that the white pig feeding might also vary as the fodder composition is diverse. On the other hand, octanal arises from unsaturated fatty acid oxidation (Timón et al., 2001) – oleic acid (Antequera & Martín, 2001) – whose concentration also depends on the pig feeding (i.e, acorn and maize). This compound, together with heptanal and nonanal, shows a decreasing trend with the ripening time (Martín, Córdoba, Benito, Aranda, & Asensio, 2003), which is shorter in French hams and longer in Iberian hams.

Seven volatiles, from different parts of the hams, were useful for distinguishing the white hams of Teruel from the French white hams, probably due to their diverse breeds (Table 1) and, perhaps, feedings. The highest differences were found in butanol and limonene (Fig. 1), together with 2-propanol and 2,3-butanodione (Table 4). The linear alcohol, butanol, seems to be formed from chemical breakdown of the mirastoleic acid (Flores et al., 1997) rather than microbiological metabolism (Coutron-Gamboti & Gandemer, 1999), and its concentration was higher in the white hams from Teruel. The concentration of methyl benzene was higher in all the locations of the hams from Teruel; the differences in the concentration of this compound between French and Spanish hams were already pointed out by Sánchez-Peña et al. (2005).

The univariate statistical procedure was useful for removing those volatile compounds, quantified in a particular ham location, that were unable to distinguish those categories of hams. The next step was to reduce the number of variables, already selected by the Brown–Forsythe test, by means of multivariate statistical procedures. The supervised statistical procedure of stepwise linear discriminant analysis (SLDA) was then applied. The compounds butanol, hexanal, limonene, 2-propanone, 3-methyl-butanol and 3-methylbutanal were selected by SLDA (F-to-Enter 5.50; F-distribution = 0.95) to discriminate the three kind of hams (the white hams from Teruel, the other white hams Spanish and French, and the Iberian hams) simultaneously. Limonene and hexanal correspond to their quantification in the *semitendinosus* muscle, butanol and 2-propanone correspond to the subcutaneous fat and 3-methylbutanal and 3-methylbutanol to the *biceps femoris* muscle, the most discriminating volatiles being limonene and 3-methylbutanol. 3-Methylbutanol contributes to the "green" sensory perception while 3-methylbutanal is responsible for the "nutty" sensory attribute (Hinrichsen & Pedersen, 1995), and its high concentration in the Iberian ham seems to explain its acceptability by the consumers (Ruiz, Ventanas, Cava, Andrés, & García, 1999).

All the hams were correctly classified with the exception of a ham (S1, TSG Serrano) cured in Catalonia that was classified inside the group of the white hams from Teruel (Fig. 2) according to the confidence ellipse at $p = 0.85$. This was the only false positive and there were no false negatives. Fig. 2 also shows a tendency to cluster the hams protected by the designation of origin "Jamón de Teruel" (T1, T2, T3, T6) in comparison with a greater dispersion of the other hams from Teruel (T4, T5, T7). It is possible that the control carried out by the PDO organization has homogenized the ham quality, the curing processing and the pig crossbreeding (BOA, 1993).

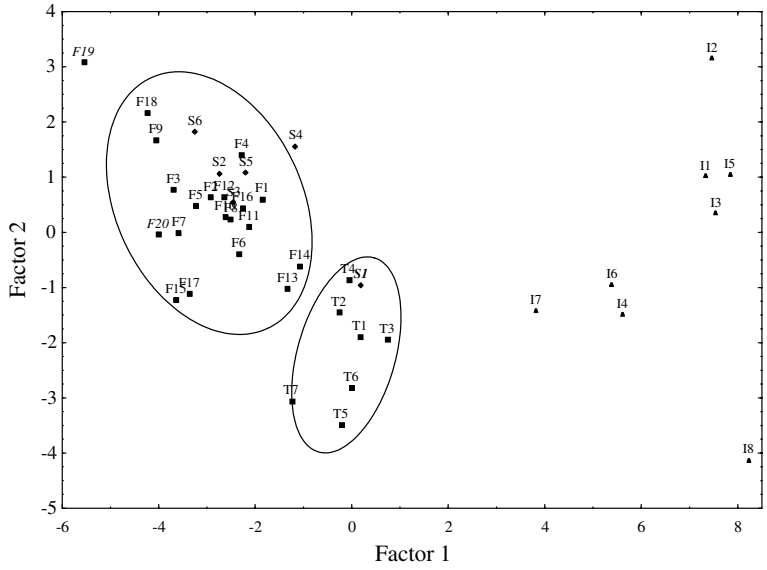

Fig. 2. Results of stepwise linear discriminant analysis (SLDA) classifying white hams from Teruel versus the other kind of hams. The confidence ellipses correspond to hams from Teruel ($p = 0.85$) and French hams ($p = 0.95$). Table 1 shows the information of the coded samples.

Fig. 2 also shows that Iberian hams sold by large producers or hyper markers (14, I6, 17) are quite separate from the Iberian hams produced by PDO (I1, I2, I3, I8). A possible explanation might be the objective of the large producers to sell hams with a standard sensory quality. Furthermore, the PDO strict regulations guarantee that Iberian hams are from 100% Iberian pigs or a crossbreeding with a minimum 75% of Iberian pig. On the other hand, the sample coded 12 corresponds to a ham produced in a geographical origin whose hams have a particular sensory perception that is very cherished by consumers. On the other hand, there is no explanation for the place in the plot of the sample coded 18 although there are diverse lines of the Iberian breed (Retinto, Entrepelado, Lampiño, Torbiscal) that might influence the volatile composition. The confidence ellipse ($p = 0.95$) of the French white hams includes all the other Spanish white hams, with the exception of S4, that is sold by a small producer, and a ham from Aosta (F19) although the other ham from Italy (F20) is inside the confidence ellipse. It is remarkable that the hams from Bayonne seem to be slightly different from the French hams, probably due to the autochthonous pig breeds of this geographical zone (Gasconne

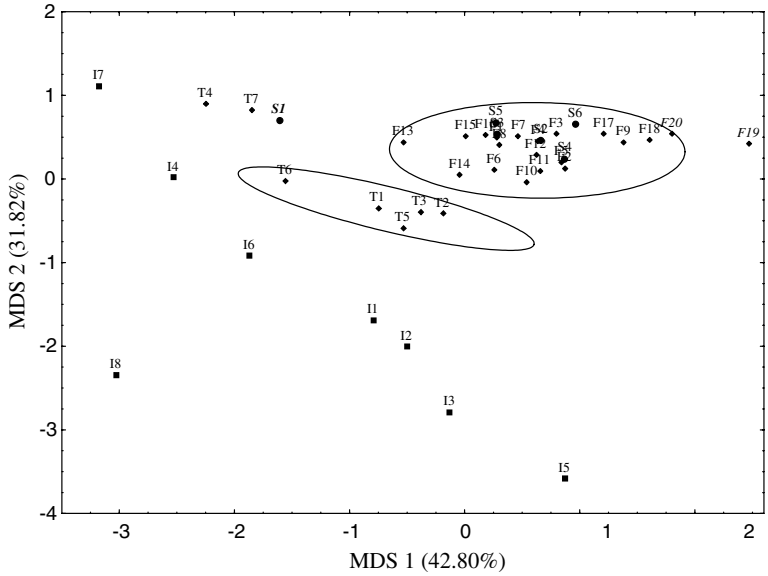

Fig. 3. Results of multidimensional scaling (MDS) with the variables selected by SLDA: 2-propanone, butanol, 3-methylbutanol, 3-methylbutanal, hexanal and limonene. The confidence ellipses correspond to hams from PDO "Jamón de Teruel" ($p = 0.80$) and French hams ($p = 0.95$). Values within parentheses are the explained variance. Table 1 shows the information of the coded samples.

and Basque). These pig breeds are different from the classical white breed although they have been crossed with Large White.

The cluster of the French white hams is more homogeneous, with independence of the geographical origin, possibly due to the maturation time; the shorter the ripening time, the smaller is the total amount of volatiles. Thus, the French hams were cured for less than 12 months with the exception of two hams from Bayonne (F13 and F14) and one from Auvergne (F6) while, in comparison, Iberian hams were cured for more of 18 months, and the hams from Teruel were cured for a time between 10 and 18 months.

The supervised statistical procedure of SLDA allowed us to distinguish the white hams of Teruel from the other kinds of hams and showed that the differences between Iberian hams and white hams are not the current challenge but the differences between white hams. The MDS procedure was then applied to the volatile compounds selected by SLDA to get a model based on an unsupervised procedure and, hence, to verify the results attained by SLDA. Fig. 3 shows the differences between the white hams from Teruel and the other kinds of hams with the first two dimensions. The three groups (hams from Teruel, Iberian hams and white hams) are neatly displayed in the plot although the group of hams from Teruel is closer to the other white hams than is shown in Fig. 2. The confidence ellipses of Fig. 3 correspond to the hams from PDO, "Jamón de Teruel" ($p = 0.80$) and French white hams ($p = 0.95$).

In conclusion, the white dry-cured hams from Teruel were characterized by only six volatile compounds (2-propanone, butanol, 3-methylbutanol, 3-methylbutanal, hexanal and limonene). The supervised and non-supervised statistical analyses proved that these compounds were able to distinguish hams from Teruel from other white hams and Iberian hams.

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