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Volatile hydrocarbon profile of Iberian dry-cured hams. A possible tool for authentication of hams according to the fattening diet

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ABSTRACT

The aims of this work were to carry out a comprehensive study of the volatile hydrocarbons of 34 Iberian dry-cured hams and to evaluate the efficiency of these compounds for discriminating hams according to the fattening system: "Montanera" (B) and "Cebo" (C). The samples of hams were obtained by mincing the *semimembranosus* and *semitendinosus* muscles from slices of dry-cured ham. The analyses were carried out by gas chromatography–mass spectrometry with a polar capillary column and after a previous extraction by Purge and Trap method. Forty-three volatile hydrocarbons were identified, 26 of them for the first time in Iberian dry-cured ham. Only five compounds showed significant differences between the two types of hams. Among the 33 volatile hydrocarbons, 22 of them allowed a complete discrimination of the two groups of hams according the fattening system.

1. Introduction

The Iberian dry-cured ham is a meat product manufactured following a traditional method in several regions of Spain. It has an extraordinary acceptance by consumers due to its sensory quality (aroma, flavor and texture), which depends on the ripening conditions [1–3] and factors that affects the raw meat characteristics, such as rearing system, mainly during the fattening period, age of animals and pig genotype [4–7]. Nevertheless, the factor that determines the ham prices in the market is the fattening diet of animals. According to this, three different types of dry-cured ham from Iberian pig are labelled: "*Montanera*" (fed only on acorns and pasture, usually known as montanera), "*Recebo*" (fed on acorns, pasture and formulated feed) and "*Cebo*" (fed on concentrate feed). In an attempt to differentiate the hams according to the types of fattening system, several compounds have been used like triacylglycerols [8,9], long chain hydrocarbons [10,11] or fatty acids [12], but no attempts have been conducted on short chain volatile hydrocarbon compounds. Due to their low impact on ham flavor, scarce information can be found on the literature on these compounds.

Several authors [13–15] have identified a large number of volatile compounds such as aldehydes, ketones, aliphatic hydrocarbons, aromatic hydrocarbons, alcohols, carboxylic acids, esters and lactones in the Iberian dry-cured hams. It has been postulated that these compounds arise from numerous chemical or enzymatic reactions such as lipolysis, chemical or enzymatic oxidation, proteolysis, Strecker degradation and Maillard reactions [16–20]. Most of these studies on volatile compounds have been carried out with the aim of characterizing them or describe their contribution to the flavor of dry-cured hams. Only an attempt to explore the utility of these compounds as classifying factor for the fattening diet has been carried out, but in loins not in hams [21].

The aims of this work were to carry out an exhaustive study of the volatile compound fraction of 34 Iberian ham samples and to explore the utility of these compounds, mainly short chain hydrocarbons, as discriminating factors for the fattening diet system. The volatile hydrocarbons were analysed by gas chromatography–mass spectrometry (GC–MS) after a previous concentration by Purge and Tramp, one of the most useful analytical methods to determine volatile compounds. By using them as chemical descriptors, pattern recognition (PR) techniques, such as principal component analysis (PCA) and cluster analysis (CA), were applied to discriminate between the "*Montanera*" and "*Cebo*" fattening diets.

2. Experimental

2.1. Ham samples

A total of 34 samples of dry-cured hams from castrated male 14-month-old pure Iberian pigs and processed in an industry for

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Table 1
Analyzed Iberian dry-cured ham slice.

Code	Fattening	Zone	CAMPAIGN
1C	*Cebo*	MÁLAGA	2004–2005
2C	*Cebo*	HUELVA	2004–2005
3C	*Cebo*	CADIZ	2004–2005
4C	*Cebo*	SEVILLA	2004–2005
5C	*Cebo*	SEVILLA	2004–2005
6C	*Cebo*	SEVILLA	2004–2005
7C	*Cebo*	SEVILLA	2004–2005
8C	*Cebo*	SEVILLA	2004–2005
9C	*Cebo*	BADAJOZ	2004–2005
10C	*Cebo*	HUELVA	2004–2005
11C	*Cebo*	CADIZ	2004–2005
12C	*Cebo*	CADIZ	2004–2005
1B	*Montanera*	HUELVA	2003–2004
2B	*Montanera*	HUELVA	2003–2004
3B	*Montanera*	SEVILLA	2003–2004
4B	*Montanera*	SEVILLA	2003–2004
5B	*Montanera*	HUELVA	2004–2005
6B	*Montanera*	SEVILLA	2004–2005
7B	*Montanera*	SEVILLA	2004–2005
8B	*Montanera*	HUELVA	2004–2005
9B	*Montanera*	HUELVA	2004–2005
10B	*Montanera*	HUELVA	2004–2005
11B	*Montanera*	HUELVA	2005–2006
12B	*Montanera*	MÁLAGA	2005–2006
13B	*Montanera*	CADIZ	2005–2006
14B	*Montanera*	MÁLAGA	2005–2006
15B	*Montanera*	MÁLAGA	2005–2006
16B	*Montanera*	CÓRDOBA	2005–2006
17B	*Montanera*	CÓRDOBA	2005–2006
18B	*Montanera*	CADIZ	2005–2006
19B	*Montanera*	BADAJOZ	2005–2006
20B	*Montanera*	HUELVA	2005–2006
21B	*Montanera*	HUELVA	2005–2006
22B	*Montanera*	BADAJOZ	2005–2006

24 months, were used: 23 corresponding to animals with a fattening diet based exclusively on acorn (*Quercus ilex, Q. suber and Q. faginea*) and pasture for 90 days prior to slaughter, usually called "*Montanera*" (B) and 12 corresponding to animals fed commercial feed and pasture in an extensive system, usually called "*Cebo*" (C). They were kindly provided by the Designation of Origin "Los Pedroches". Table 1 shows the identification code assigned to each sample. The animals were classified in the two different groups by the veterinary inspector.

Some slices were cut parallel to the femur and to different depths from each ham. Each slice contained *semimembranosus* and *semitendinosus* muscles. The samples were stored in vacuum plastic bags at −18 °C until they were required for the analytical studies.

In each slice, the *semimembranosus* and *semitendinosus* muscles were trimmer by removing the adipose tissue. The proportion of both muscles in the sample for analysis was the same than in the slice. The muscles were minced and mixed to increase the interface between the sample and the striping gas during the concentration step.

2.2. Volatile compounds analysis

The volatile compounds were isolated from 3 g of minced sample by the dynamic headspace technique and adsorbed on a Tenax trap, using a Purge and Trap Concentrator apparatus Tekmar velocity XPT (Thousand Oaks, CA, USA), based on the method described by Sabio et al. [5]. The purge conditions were:

- Sample temperature: 45 °C
- Tenax trap temperature: 35 °C
- Purge flow: 350 mL min^{-1} of nitrogen.
- Purge time: 14 min.

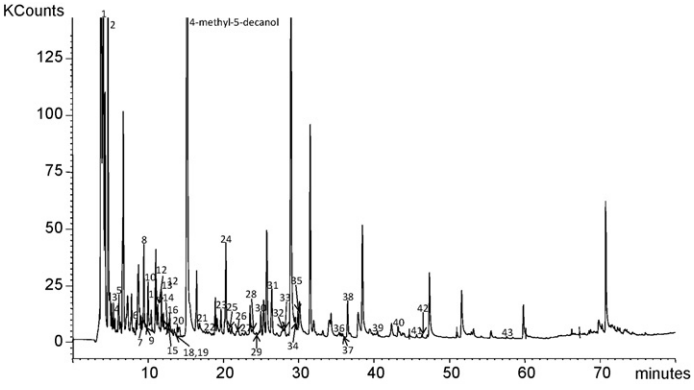

Fig. 1. Chromatograms of the volatile compounds profile of Iberian ham slice samples: (A) "*Montanera*" and (B) "*Cebo*" fattening diet; peaks identification: see Table 2.

After the purge time, the volatile compounds were desorbed by heating, the Tenax trap at 225 °C for 1 min, and sent throw of transfer line (kept at 150 °C) into the chromatograph injector.

The GC-ion-trap-MS analyses were performed using a Varian 3800 gas chromatograph coupled to a Saturno 2000 ion trap mass spectrometer (Varian, Palo Alto, CA, USA). The system was equipped

Table 2
Volatile hydrocarbons species identified by the GC–MS methods. Identification based on library and standards analysis (see Fig. 1).

Peak	Volatile hydrocarbons	Identification	T_{RR}	M$^+$
1	3-Methyl-hexane	L	0.27	100
2	2,4-Dimethyl-heptane	L	0.31	128
3	1,2-Diethyl-cyclobutane	L	0.36	112
4	2-Octene	L/KI	0.37	112
5	Nonane	L/KI	0.41	128
6	Butyl-cyclopentane	L	0.56	126
7	2,2,5,5-Tetramethyl-hexane	L	0.61	142
8	Dodecane	L	0.62	170
9	2,2,5-Trimethyl-hexane	L	0.64	128
10	2,3,5,8-Tetramethyl-decane	L	0.67	198
11	4-Carene	L/S	0.69	136
12	4-Methyl-1-decene	L	0.74	154
13	Methyl-benzene	L/S	0.77	92
14	2,4,6-Trimethyl-heptane	L	0.82	142
15	Diisoamilene	L	0.84	140
16	7-Methyl-pentadecane	L	0.85	226
17	2,2,3-Trimethyl-nonane	L	0.87	170
18	5-(1-Methyl-propyl)-nonane	L	0.89	184
19	Germacrane B	L	0.90	210
20	Heptyl-cyclohexane	L	0.92	182
21	2,6-Dimethyl-undecane	L	1.11	184
22	1-Ethyl-1-methyl-cyclohexane	L	1.19	126
23	p-Xylene	L/S/KI	1.30	106
24	m-Xylene	L/KI	1.34	106
25	Decahydro-*cis*-naphtalene	L	1.37	138
26	3-Methyl-5-undecene	L	1.43	168
27	4-Methyl-1-undecene	L	1.51	168
28	2-Methyl-decahydronaphtalene	L	1.57	152
29	Octyl-cyclohexane	L	1.61	196
30	o-Xylene	L/S/KI	1.65	106
31	Limonene	L/S/KI	1.74	136
32	Propyl-benzene	L	1.83	120
33	Decahydro-*trans*-naphtalene	L	1.85	138
34	1,3,5-Trimethyl-benzene	L	1.94	120
35	1-Ethyl-4-methyl-benzene	L	1.97	120
36	1-Methyl-3-(1-methyl-ethyl)-benzene	L	2.33	134
37	2-Ethenyl-cyclohexane	L	2.38	124
38	1,2,4-Trimethyl-benzene	L	2.41	120
39	Butenyl ciclohexene	L	2.66	136
40	1,2,3-Trimethyl-benzene	L	2.85	120
41	4-Ethyl-1,2-dimethyl-benzene	L	3.01	134
42	2-Ethyl-1,3-dimethyl-benzene	L	3.06	134
43	*cis*-1,2,3,4-Tetramethyl-cyclopentane	L	3.79	126

L: Library; S: Standard; KI: Kovats Index; T_{RR}: means relative retention time.

Table 3
Volatile hydrocarbon profile of Iberian dry-cured ham samples.

Volatile hydrocarbons	*"Montanera"*				*"Cebo"*			
	Mean	S.D.	Max	Min	Mean	S.D.	Max	Min
n-alkanes								
Nonane	0.36	0.28	0.91	0.00	0.25	0.15	0.45	0.05
Dodecane[a]	2.65	3.42	10.10	0.00	0.40	0.28	0.89	0.00
Branched alkanes								
3-Methyl-hexane	4.64	4.90	23.46	0.56	4.17	1.14	5.93	2.37
2,4-Dimethyl-heptane[a]	4.65	3.11	11.85	0.89	7.04	3.53	14.28	0.86
2,2,5,5-Tetramethyl-hexane[b]	0.12	0.28	1.12	0.00	0.48	0.42	1.15	0.00
2,2,5-Trimethyl-hexane	0.84	1.14	3.20	0.00	0.21	0.25	0.70	0.00
2,3,5,8-Tetramethyl-decane	1.78	1.74	5.38	0.05	0.98	0.94	2.67	0.00
2,4,6-Trimethyl-heptane[a]	0.60	0.54	1.77	0.00	0.23	0.19	0.54	0.02
7-Methyl-pentadecane	0.53	0.59	1.83	0.00	0.38	0.47	1.28	0.00
2,2,3-Trimethyl-nonane	0.31	0.41	1.51	0.00	0.25	0.35	0.98	0.00
5-(1-Methyl-propyl)-nonane	0.15	0.17	0.52	0.00	0.10	0.14	0.39	0.00
2,6-Dimethyl-undecane	0.11	0.12	0.41	0.00	0.05	0.08	0.24	0.00
n-alkenes								
2-Octene[a]	0.21	0.14	0.49	0.00	0.11	0.08	0.25	0.01
Branched alkenes								
4-Methyl-1-decene	1.64	1.58	4.83	0.06	0.90	0.66	2.30	0.10
3-Methyl-5-undecene	0.12	0.18	0.58	0.00	0.14	0.19	0.68	0.00
4-Methyl-1-undecene	0.18	0.21	0.93	0.00	0.26	0.30	1.08	0.03
Cyclic								
1,2-Diethyl-cyclobutane	0.17	0.10	0.41	0.00	0.35	0.56	2.06	0.05
Butyl-cyclopentane	0.16	0.20	0.93	0.00	0.15	0.23	0.87	0.00
Heptyl-cyclohexane	0.12	0.26	1.16	0.00	0.29	0.35	0.97	0.00
1-Ethyl-1-methyl-cyclohexane	0.08	0.16	0.73	0.00	0.10	0.14	0.46	0.00
Octyl-cyclohexane	0.06	0.15	0.64	0.00	0.18	0.31	0.99	0.00
2-Ethenyl-cyclohexane	0.03	0.07	0.29	0.00	0.02	0.02	0.05	0.00
Butenyl-cyclohexene	0.03	0.04	0.17	0.00	0.02	0.03	0.09	0.00
cis-1,2,3,4-Tetramethyl-cyclopentane	0.14	0.30	1.25	0.00	0.07	0.12	0.31	0.00
Terpenic								
Limonene	0.49	1.14	5.41	0.00	1.12	1.39	4.09	0.03
4-Carene	0.88	0.95	2.59	0.00	0.36	0.27	1.00	0.06
Germacrane B[a]	0.35	0.30	1.05	0.00	0.11	0.13	0.31	0.00
Aromatic								
Methyl-benzene	2.10	3.07	13.42	0.34	0.43	0.23	0.96	0.14
Diisoamilene	0.34	0.41	1.67	0.00	0.24	0.22	0.67	0.00
p-Xylene	0.30	0.20	0.79	0.00	0.30	0.25	0.76	0.05
m-Xylene	0.61	0.72	3.15	0.00	0.37	0.32	1.15	0.00
Decahydro-*cis*-naphtalene	0.31	0.50	2.08	0.00	0.04	0.06	0.18	0.00
2-Methyl-decahydronaphtalene	0.10	0.19	0.70	0.00	0.08	0.23	0.81	0.00
o-Xylene	0.14	0.16	0.60	0.00	0.33	0.38	1.16	0.00
Propyl-benzene	0.11	0.15	0.73	0.00	0.09	0.14	0.40	0.00
Decahydro-*trans*-naphtalene	0.05	0.08	0.24	0.00	0.11	0.15	0.37	0.00
1,3,5-Trimethyl-benzene	0.03	0.07	0.30	0.00	0.09	0.14	0.43	0.00
1-Ethyl-4-methyl-benzene	0.05	0.13	0.61	0.00	0.03	0.09	0.30	0.00
1-Methyl-3-(1-methyl-ethyl)-benzene	0.04	0.09	0.45	0.00	0.07	0.08	0.23	0.00
1,2,4-Trimethyl-benzene	0.15	0.20	1.00	0.00	0.12	0.08	0.30	0.05
1,2,3-Trimethyl-benzene	0.11	0.31	1.50	0.00	0.02	0.02	0.07	0.00
4-Ethyl-1,2-dimethyl-benzene	0.02	0.04	0.16	0.00	0.00	0.01	0.04	0.00
2-Ethyl-1,3-dimethyl-benzene	0.01	0.02	0.07	0.00	0.00	0.01	0.04	0.00

[a] For $p < 0.05$.
[b] For $p < 0.01$.

with a 1079 injector operating in full scan mode from 50 to 600 amu at 1 scan/sec for identification purpose. The column used was a Supelcowax™-10 (SUPELCO, Bellefonte, PA, USA) fused silica capillary column (60 m long × 0.25 mm i.d. × 0.25 μm film thickness). The GC conditions included hydrogen as carrier gas at 1.6 mL min^{-1} in constant flow mode. The oven temperature was held at 40 °C for 14 min and then rise to 91 °C at 1 °C min^{-1}, and then to 201 °C at 10 °C min^{-1}, and then to 220 °C at 5 °C min^{-1} where it was held for 20 min. Split injection mode was used with a ratio of 1:5. The injector temperature was kept at 250 °C.

The MS operating conditions were the following: ion source and transfer line temperatures were 200 and 290 °C, respectively. The electron energy was 70 eV a resolution of 1 and the emission current 250 μA; dwell time and inter-channel delay was 0.08 s and 0.02 s respectively. For GC-ion trap-MS. Varian MS Workstation version 6.3 software was used for data acquisition and processing of the results.

2.3. Identification and quantification of the volatile hydrocarbons

The tentatively assignment of the chromatographic peaks was done comparing the spectra with those from NIST (National Institute of Standards and Technology) and WILEY libraries and verified by standards purchased from Sigma–Aldrich and Fluka (S. Louis, MO). 4-Methyl-5-decanol was used as a reference to calculate the relative retention time, because it appears in all samples with high intensity at a mean retention time of 15.176 min. The peak area of the analyte was used as an analytical signal. The quantification of

Table 4
Result of the stepwise discriminant analysis. The "fattening diet" was considered as grouping factor and a priori classification probability was the same for all groups.

Hydrocarbons	*"Cebo"*	*"Bellota"*	*F* to remove (1.7)	*p*-Level
1-Methyl-3-(1-methyl-ethyl)-benzene	356.11	−965.049	47.52927	0.000233
2,2,5,5-Tetramethyl-hexane	85.52	−159.745	35.19516	0.000580
1-Ethyl-1-methyl-cyclohexane	−210.12	720.041	34.43237	0.000619
Germacrane B	136.10	−326.380	28.84545	0.001041
4-Carene	−64.48	205.367	26.35709	0.001348
Methyl-benzene	−40.40	64.780	25.34871	0.001505
Diisoamilene	109.91	−259.790	24.13942	0.001727
1,2,3-Trimethyl-benzene	263.22	−412.432	17.20099	0.004309
3-Methyl-5-undecene	−147.12	272.834	13.30091	0.008210
2-Ethyl-1,3-dimethyl-benzene	−540.86	3061.251	13.26689	0.008261
4-Ethyl-1,2-dimethyl-benzene	−1363.82	1704.067	12.70310	0.009168
1,2-Diethyl-cyclobutane	28.18	−63.702	12.26509	0.009964
Decahydro-*trans*-naphtalene	154.90	−328.288	11.01231	0.012792
4-Methyl-1-decene	−25.62	63.261	8.30598	0.023584
2,6-Dimethyl-undecane	108.43	−379.957	7.16414	0.031695
4-Methyl-1-undecene	46.31	−248.269	5.87993	0.045765
Constant	−40.71	−119.365		

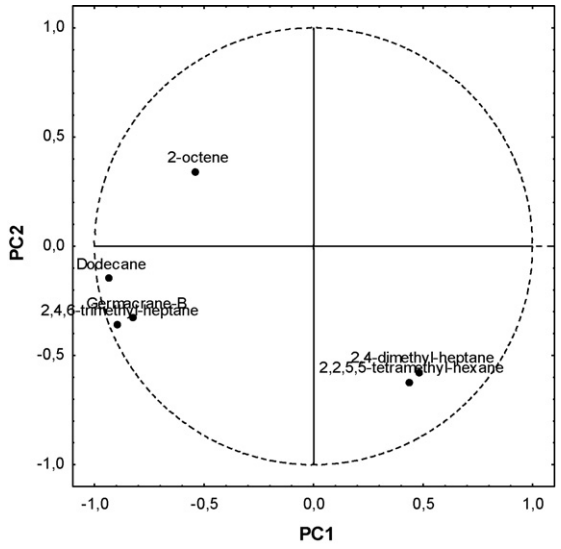

Fig. 2. Loadings plot for the first PCs.

individual volatile compounds was carried out by evaluating the corresponding relative percentage according to the normalization area procedure, assuming an equal factor response for any species.

2.4. Data analysis

The volatile hydrocarbons identified were considered as chemical descriptors. A data matrix whose rows are the samples and whose columns are the variables was built. Each element of this matrix x_{ij} corresponds to the content of the volatile compound j for the sample i. Statistical analysis based on pattern recognition (PR) techniques were used, including principal component analysis (PCA) and Linear discriminant analysis (LDA). The PR calculations were made by using the statistical package CSS: STATISTICA from Stafsoft™ (Tulsa, OK, USA).

3. Results and discussion

3.1. Volatile hydrocarbons profile of ham

The high polarity of the capillary column, has allowed obtaining a good resolution and 43 volatile hydrocarbons have been identified by GC–MS (Fig. 1). The relative retention time and molecular ion of the corresponding peaks are shown in Table 2.

In the group of linear and branched hydrocarbons, 2,4-dimethyl-heptane, 2-octene, 2,2,5,5-tetramethyl-hexane, 2,2,5-trimethyl-hexane, 2,3,5,8-tetramethyl-decane, 4-methyl-1-decene, 2,4,6-trimethyl-heptane, diisoamilene, 7-methyl-pentadecane, 2,2,3-trimethyl-nonane, 5-(1-methyl-propyl)-nonane and 3-methyl-5-undecene are observed for the first time in the volatile fraction of Iberian ham. Other hydrocarbons have been already described by several authors in the Iberian ham, such as 3-methyl-hexane [2,22], nonane [2,3,5,14,15], dodecane [3,5,15], 2,6-dimethyl-undecane [7] and 4-methyl-undecene [15].

The limonene is the only cyclic hydrocarbons that has been previously described by other authors [2,3,5,7,15,23,24,25]. All the other cyclic hydrocarbons: 1,2-diethyl-cyclobutane, butyl-cyclopentane, germacrane B, heptyl-cyclohexane, 1-ethyl-1-methyl-cyclohexane, octyl-cyclohexane, 2-ethenyl-cyclohexane, butenyl-cyclohexene and *cis*-1,2,3,4-tetramethyl-cyclopentane compounds have been identified for the first time in the present work. On the other hand, the 2-carene and the 3-carene were identified by Sabio et al. [5], but we have not detected them. In the present study we have identified the 4-carene, which has not been described previously.

Most of the aromatic hydrocarbons have been previously described by other authors. Methyl-benzene was described by Ruiz et al. [2,25], Sabio et al. [5], Sánchez-Peña et al. [23], Andrés et al. [3], Timón et al. [15], García-González et al. [24] and Ruiz et al. [25]; the p-xylene by Ruiz et al. [2,25], Andrés et al. [3] and López et al. [14]; m-xylene by Ruiz et al. [2,25] and Ramírez and Cava [7]; o-xylene by Ruiz et al. [2], Andrés et al. [3] and López et al. [14]; propyl-benzene by Ruiz et al. [2] and Timón et al. [15]; 1,3,5-trimethyl-benzene, 1-ethyl-4-methyl-benzene and 1,2,4-trimethyl-benzene by Ruiz et al. [2]; and 1-methyl-3-(1-methyl-ethyl)-benzene and 1,2,4-trimethyl-benzene were described by López et al. [14]. Decahydro-*cis*-naphtalene, decahydro-*trans*-naphtalene, 2-methyl-decahydronaphtalene and 2-ethyl-1,3-dimethyl-benzene have been described for the first time in this work.

Besides, ethyl-benzene, styrene, (1-methyl-propyl)-benzene, 1-propenyl-benzene and 1-methyl-4-(1-methyl-ethenyl)-benzene compounds were detected. Several authors have described ethyl-benzene [3,5,14,23,24] and styrene [3,14,22] in the volatile fractions from Iberian ham. About the others, they have not been described at the literature. The aromatic compounds, ethyl-benzene and styrene, have been described as contamination from plastic packaging of food [26]. That is why these compounds have been removed from the volatile profile in our study.

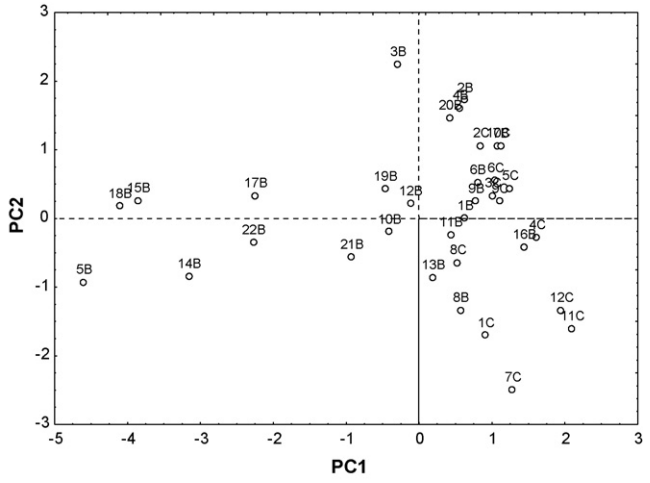

Fig. 3. Scores plot for the first PCs.

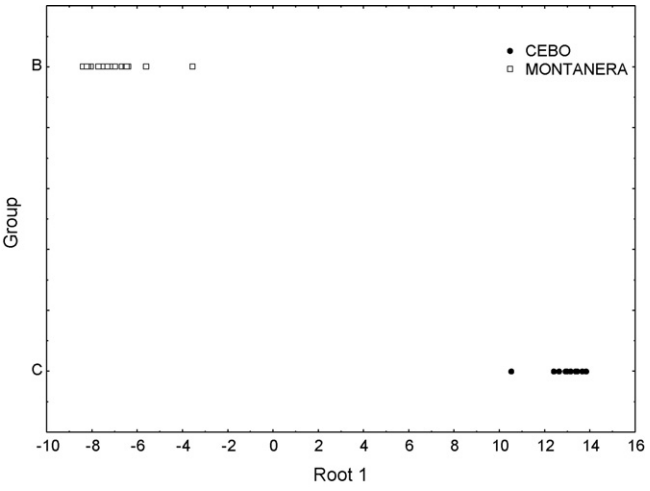

Fig. 4. Scatterplot of the canonical scores corresponding to *"Montanera"* and *"Cebo"*.

The different hydrocarbons identified in the volatile fraction from "*Montanera*" and "*Cebo*" samples are shown in Table 3, together with mean values, standard deviation (S.D.), maximum and minimum values.

It can be observed that the major hydrocarbon for the two types of fattening systems is the 2,4-dimethyl-heptane, but it was in a higher relative percentage in "*Cebo*". Only five compounds showed significant differences between the two groups of hams Cebo and Montanera (Table 3). 2,4-dimethyl-heptane ($p < 0.05$), 2-octene ($p < 0.05$), 2,2,5,5-tetramethyl-hexane ($p < 0.01$), dodecane ($p < 0.05$), 2,4,6-trimethyl-heptane ($p < 0.05$) and germacrane B ($p < 0.05$).

3.2. PCA-based display methods and discriminant analysis

Using the hydrocarbons with significant differences listed above, a PCA was performed. PC1 and PC2 explained up to 69.39% of the total variance, being 51.03% explained by PC1 and 18.36% by PC2 (Fig. 2). Accordingly, the scores plot obtained by selecting the variables most contributing to PC1 (2,4-dimethyl-heptane, 2-octene, 2,2,5,5-tetramethyl-hexane, dodecane, 2,4,6-trimethyl-heptane and germacrane B) are depicted in Fig. 3 that shows a fair separation between "*Montanera*" (B) and "*Cebo*" (C) samples. As it can be observed, all the "*Cebo*" samples are at the positive side of PC1, while half of the "*Montanera*" samples appear at PC1 < 1. Therefore, PCA offered a poor separation of the samples according to the fattening diet.

To achieve a better separation of the groups according to fattening diets a linear discriminant analysis (LDA) was carried out. The 43 hydrocarbons were included as variables considering "a priori" equal probability for a sample to be in one group independently of the group size. A tolerance of 0.001 was set to eliminate the variables that provided redundant information with those already included in the model. The classification functions together with F to remove p values for each volatile hydrocarbon ($p < 0.05$) are shown in Table 4.

3.3. Classification of samples

Fig. 4 shows the case discrimination, grouped by fattening diet, according to the first canonical variable or square roots obtained from the classification functions for the two types of fattening diets. In this figure, a complete separation between the two groups can be observed.

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