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A tentative characterization of volatile compounds from Iberian Dry-Cured Ham according to different anatomical locations. A detailed study

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RESUMEN

Una tentativa de caracterización de los compuestos volátiles del jamón curado Ibérico según su localización anatómica. Un estudio detallado.

El objetivo de este trabajo fue llevar a cabo una caracterización de la fracción volátil de 23 jamones curados de cerdo ibérico mediante GC-ion-trap-MS. Se analizaron dos zonas diferentes de las lonchas tomadas paralelas al fémur de cada jamón, Grasa subcutánea y magro. El análisis se realizó mediante cromatografía gaseosa en columna capilar de alta polaridad con detección de espectrometría de masas y extracción previa de la fracción volátil mediante purga y trampa. Un total de 109 compuestos volátiles fueron identificados, veintiocho de ellos descritos por primera vez en Jamón ibérico curado (isopropanol, 4-methyl-5-decanol, 2-butyl-1-octanol, 2-etoxy-ethanol, 2-ethyl-phenol, 2-hexen-1-ol, 3,5-octadien-2-ol, 2-decen-1-ol, 5-ethylcyclopent-1-enecarboxaldehyde, 2,4-heptadienal, 6-nonenal, cyclopentanone, 3-ethenyl-cyclohexanone, 2-methyl-cyclopentanone, 6-octen-2-one, 5-methyl-2-(1-methyl-ethyl)-cyclopentanone, 3,5-octadien-2-one, 2-hydroxymethyl-2,3,3-trimethyl-oxirane, 2-ethyl-hexyl 2-propenoate, 1-methoxy-pentane, 2,3-dihydrofurane, 2-D-2-pentadecyl-1,3-dioxolane, hexyl octyl eter, eucalyptol, di-(3-methyl-buthyl) eter, piperidine, isopropylamine and 2-ethenyl-pyridine).

PALABRAS CLAVE: Compuestos volátiles – GC-MS – Jamón Ibérico – Músculo – Purga y trampa – Tejido adiposo.

SUMMARY

A tentative characterization of volatile compounds from Iberian Dry-Cured Ham according to different anatomical locations. A detailed study.

The aim of this work was to carry out a characterization of the volatile fraction of 23 Iberian dry-cured hams by GC-ion-trap-MS. Two different locations –subcutaneous fat and muscle- from the slices taken parallel to the femur from each ham were analyzed. The analyses were done by Gas Chromatography-Mass Spectrometry with a polar capillary column and after a previous extraction using the Purge and Trap method. A total of 109 volatile compounds were identified, twenty-eight of which for the first time in Iberian dry-cured ham (isopropanol, 4-methyl-5-decanol, 2-butyl-1-octanol, 2-etoxy-ethanol, 2-ethyl-phenol, 2-hexen-1-ol, 3,5-octadien-2-ol, 2-decen-1-ol, 5-ethylcyclopent-1-enecarboxaldehyde, 2,4-heptadienal, 6-nonenal, cyclopentanone, 3-ethenyl-cyclohexanone, 2-methyl-cyclopentanone, 6-octen-2-one, 5-methyl-2-(1-methyl-ethyl)-cyclopentanone, 3,5-octadien-2-one, 2-hydroxymethyl-2,3,3-trimethyl-oxirane, 2-ethyl-hexyl 2-propenoate, 1-methoxy-pentane, 2,3-dihydrofurane, 2-D-2-pentadecyl-1,3-dioxolane, hexyl octyl eter, eucalyptol, di-(3-methyl-buthyl) eter, piperidine, isopropylamine and 2-ethenyl-pyridine).

KEY-WORDS: Adipose tissue – GC-MS – Iberian dry-cured ham – Muscle – Purge and trap – Volatile compounds.

1. INTRODUCTION

The elaboration of Iberian dry-cured ham is a long process of ripening (about 2-3 years). During this time, the lipids undergo a great transformation. Several studies have been carried out to see the principal changes that occur in the lipid fraction during the dry-cured processing of Iberian ham, although these studies have been done with intramuscular fat. Reactions of hydrolysis and oxidation, which produce the degradation of the lipid fraction from adipose tissue, take place during this process (López *et al*., 1992; Coutron-Gambotti y Gandemer, 1999). Hydrolysis mainly affects the triacylglycerols and diacylglycerols, and to a lesser affect, the monoacylglycerols and phospholipids (Narváez-Rivas *et al*., 2007). On the other hand, oxidation affects the fatty acids, producing oxidized compounds that have a short life; some of them are volatile and responsible for the characteristic flavor of dry-cured ham (Antequera *et al*., 1992).

The flavor of Iberian dry-cured ham is critical to consumer acceptance and the aroma is perhaps the most important quality parameter. It has been postulated that chemical or enzymatic reactions such as lipolysis, chemical or enzymatic oxidation, proteolysis, Strecker degradation and Maillard reactions are the origin of volatile compounds (Toldrá, 1998; Toldrá *et al*., 2009).

Due to the chemical composition of the fat in Iberian dry-cured ham, different factors like feed, breed, etc, will be able to produce changes in it (Narváez-Rivas *et al*., 2009). We could suppose that lipid alteration in composition of volatile compounds should be produced as a consequence of these factors. There are several scientific studies that prove this in the literature (Gandemer, 2009). Some studies have shown that volatile compounds can be affected by feed (López *et al*., 1992) and the conditions of dry-cured processing, such as its duration, salt content and temperature conditions (Ruiz *et al*., 1999; Andrés

et al., 2007). In addition to these factors, the genotype and sex of the animals have an effect on these volatile compounds (Ramírez and Cava, 2007).

There are many volatile compounds in Iberian dry-cured ham that have been characterized by different authors (aldehyde, ketone, alcohol, alkane, esters, etc). Several volatile compounds, like hexanal and heptanal, are always present. However, there are others, such as limonene, that has been associated with feed (Luna *et al*., 2006). Also, many compounds show values that are significantly different, depending on the animals´ feed (López *et al*., 1992).

The determination of volatile compounds has been made in samples taken from the muscles of any piece (slices), like *Biceps femoris, semitendinosus* or *semimembranosus* (García *et al*., 1991; López *et al*., 1992; Sabio *et al*., 1998; Timón *et al*., 1998; Luna *et al*., 2006; Andrés *et al*., 2007; Ramírez y Cava, 2007; García-González *et al*., 2008). Like in the evolution of glyceride compounds, the volatile compound fraction of subcutaneous and intermuscular fat has been poorly studied or characterized (Timón *et al*., 2001).

The aims of this work were to carry out a characterization of the volatile fraction of 23 Iberian dry-cured hams by GC-ion-trap-MS. Two different locations, subcutaneous fat and muscle, from the slices taken parallel to the femur from each ham have been analyzed.

2. MATERIALS AND METHODS

2.1. Ham samples

Twenty-three samples of dry-cured hams from castrated males, fourteen months old, pure Iberian and processed in an industry for 24 months, were analyzed. The animals received a fattening diet based exclusively on acorn (*Quercus ilex, Q. suber and Q. faginea*) and pasture for 90 days prior to slaughter. They were kindly provided by the Designation of Origin "Los Pedroches". All the animals included in the study were reared extensively.

The slices were taken parallel to the femur and at different depths in each ham. Each slice contained adipose tissue, *semimembranosus* and *semitendinosus* muscles. The samples were stored in vacuum plastic bags at –18°C until they were needed for the analytical studies.

In each slice, the *semimembranosus* and *semitendinosus* muscles were selected, minced and mixed, in order to increase the interface between the sample and the striping gas during the concentration step. The same treatment was applied to the adipose tissue from these slices.

2.2. Volatile compounds analysis

The volatile compounds were isolated from three grams of sample prepared as indicated above 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*., 1998. The purge conditions were as follows: sample temperature, 45°C; Tenax trap temperature, 35°C; purge flow, 350 mL min^{-1} of nitrogen and purge time, 14 min. After the purge time, the volatile compounds were desorbed, holding the Tenax trap at 225°C for 1 min, and sent through of transfer line (kept at 150°C) into the chromatographic 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 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^{m}-10 (SUPELCO, Bellefonte, PA, USA) fused silica capillary column (60 m long x 0.25 mm i.d x 0.25 m 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 programmed to rise 1°C min^{-1} to a temperature of 91°C, and then to rise at 10°C min^{-1} to a temperature of 201°C, and then to rise at 5°C min^{-1} to a final temperature of 220°C 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 as follows: 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 were 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 of the volatile compounds

The tentative assignment of the chromatographic peaks was done comparing the spectra with those from NIST (National Institute of Standards and Technology) and WILEY libraries and some of them were verified by standards purchased from Sigma-Aldrich and Fluka (S. Louis, MO). The peak area of the analyte was used as an analytical signal. The quantification of 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.

3. RESULTS AND DISCUSSION

Figure 1 shows a representative chromatogram of the total volatile compounds obtained under the conditions described above. A total of 109 volatile compounds have been identified by GC-MS. The assignation of each peak with its corresponding compound was carried out using NIST and WILEY libraries, and in some cases using standards.

Due to the high number of identified volatile compound, they have been classified into seven

groups according to their functional group (Tables 1-7). The different groups of volatile compounds identified are shown in each table together with the peak number, mean values and Standard Deviation (S.D.).

In order to explore whether there were significant differences between both anatomical locations, main effects analysis of variance (ANOVA) was performed according to the general lineal model procedure.

3.1. Hydrocarbons

This is the most numerous group present in the volatile fraction of slices from Iberian dry-cured ham. Table 1 show all the hydrocarbons identified in these samples. It can be observed that all of them are in the two different sample locations, adipose tissue and muscle, except octyl-cyclohexane, which appears only in the muscle.

Among the linear and branched hydrocarbons, twelve of these volatile compounds have been identified in this type of sample for the first time by Narváez-Rivas *et al*. (2010). One of them, 2-octene, is linear. However, the rest of these compounds are branched, being 2,4-dimethyl-heptane; 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. Other volatile hydrocarbons detected have been previously identified by other authors in the Iberian ham. These compounds are: 3-methyl-hexane, nonane, dodecane, 2,6-dimethyl-undecane and 4-methyl-undecane (López *et al*., 1992; Sabio *et al*., 1998; Timón *et al*., 1998; Ruiz *et al*., 1999; Andrés *et al*., 2002; Ramírez *et al*., 2007; Andrade *et al*., 2009).

In the group of cyclic hydrocarbons, 1,2-diethyl-cyclobutane, butyl-cyclopentane, germacrane B, heptyl-cyclohexane, octyl-cyclohexane, 2-etenyl-cyclohexane, butenyl-cyclohexene and *cis*-1,2,3,4-tetramethyl-cyclopentane compounds were described in volatile fraction from Iberian ham for the first time by Narváez-Rivas *et al*. (2010). On the other hand, the limonene is the only one that has been previously described by other authors (Ruiz *et al*., 1998; Sabio *et al*., 1998; Timón *et al*., 1998; Ruiz *et al*., 1999; Andrés Sáchez-Peña *et al*., 2005; Ramírez *et al*., 2007; García-González *et al*., 2008). In 1998, Sabio *et al*. identified 2-carene and 3-carene. However, 4-carene has not been detected by others before this research.

Most of compounds belonging to the aromatic hydrocarbon fraction have been previously described, such as: methyl-benzene, p-xylene, m-xylene, o-xylene, propyl-benzene, 1-methyl-3-(1-methyl-ethyl)-benzene, 1,2,4-trimethyl-benzene, 1,3,5-trimethyl-benzene or 1-ethyl-4-methyl-benzene (López *et al*., 1992; Ruiz *et al*., 1998; Ruiz *et al*., 1999; Sabio *et al*., 1998; Timón *et al*., 1998; Ruiz *et al*., 1999; Sáchez-Peña *et al*., 2005; Ramírez *et al*., 2007; García-González *et al*., 2008). Nevertheless, decahydro-*cis*-naphtalene, decahydro-*trans*-naphtalene, 2-methyl-decahydronaphtalene and 2-ethyl-1,3-dimethyl-benzene were detected recently for the first time in the volatile fraction from Iberian dry-cured ham (Narváez-Rivas *et al*., 2010).

In this research work, ethyl-benzene, styrene, (1-methyl-propyl)-benzene, 1-propenyl-benzene and 1-methyl-4-(1-methyl-ethenyl)-benzene, have also been detected. However, they are not included as they could be contaminants from the plastic packing (Reineccius, 2006).

Table 1 shows the mean values for the hydrocarbons identified. The 2,4-dimethyl-heptane was the major hydrocarbon in the subcutaneous fat and muscle., with the percentage in adipose tissue being higher than that found in the muscle, (11.22% and 4.65% respectively). Regarding anatomical location, only significant differences in 3-methyl-hexane ($p<0.01$), 4-methyl-1-decene ($p<0.05$), diisoamilene ($p<0.05$), 2,4-dimethyl-heptane ($p<0.01$), 2,4,6-trimethyl-heptane ($p<0.01$), Decahydro-*cis*-naphtalene ($p<0.05$), 3-methyl-5-undecene ($p<0.05$) and germacrane B($p<0.01$) were detected.

3.2. Alcohols

Alcohols do not usually contribute to aroma, but if they have high concentrations (ppm) or are unsaturated alcohols, they can usually be smelled. In Table 2, all alcohols detected in the volatile fraction of the samples are shown. They were detected in both locations, with the exception of 2-etoxi-ethanol, which was not detected in the adipose tissue.

Other authors have detected and identified 2-ethyl -1-hexanol, 2-pentanol, 1-butanol, 1-penten-3-ol, 1-pentanol, 1-hexanol and 1-octen-3-ol (López *et al*., 1992; Ruíz *et al*., 1998; Timón *et al*., 1998; Sabio *et al*., 1998; Ruiz *et al*., 1999; Carrapiso *et al*., 2002; Andrés *et al*., 2002; Sánchez-Peña *et al*., 2005; Ramírez *et al*., 2007; García-González *et al*., 2008). Isopropanol, 4-methyl-5-decanol, 2-butyl-1-octanol, 2-etoxi-ethanol, 2-hexen-1-ol, 3,5-octadien-2-ol and 2-decen-1-ol have been identified for the first time in this research work. 2-Ethyl-phenol has never been detected, although some authors have detected an ethtyl-phenol without specifying the isomer (Sabio *et al*., 1998). Also, the presence of 3-ethyl-phenol has been reported (Ruiz *et al*., 1999).

In this group, the 4-methyl-5-decanol was the major alcohol in the subcutaneous fat and muscle, the percentage found in adipose tissue being higher than that found in the muscle. In addition, 4-methyl-5-decanol was the major compound among the volatiles identified in this work. This result is did not agree with a previous report where the major compounds found were hexanal, octanal or hexanoic acid (López *et al*., 1992; Sabio *et al*.,1998; Ramírez *et al*., 2007; García-González *et al*., 2008). Finally, significant differences for the alcohols due to anatomical location have not been found.

3.3. Aldehydes

In Table 3, the detected aldehydes in the volatile fraction from Iberian dry-cured ham are shown.

Table 1
Means expressed as % and standard deviation (S.D.) of Volatile hydrocarbons detected by GC-MS technique of Iberian dry-cured ham from different anatomical locations

Peak	Volatile compounds	Adipose tissue		Muscle	
		Mean	S.D.	Mean	S.D.
2	3-methyl-hexane[b]	9.22	5.30	4.64	4.90
4	2,4-dimethyl-heptane[b]	11.22	8.21	4.65	3.11
7	1,2-diethyl-cyclobutane	0.18	0.18	0.17	0.10
8	2-octene	0.18	0.23	0.21	0.14
9	Nonane	0.26	0.27	0.36	0.28
16	Buthyl cyclopentane	1.29	3.64	0.16	0.20
21	2,2,5,5-tetramethyl-hexane	0.57	1.27	0.12	0.28
22	Dodecane	1.51	2.36	2.65	3.42
23	2,2,5-trimethyl-hexane	0.50	0.79	0.84	1.14
24	2,3,5,8-tetramethyl-decane	0.89	1.24	1.78	1.74
25	4-carene	0.47	0.67	0.88	0.95
27	4-methyl-1-decene[a]	0.81	1.02	1.64	1.58
28	Methyl-benzene	2.43	4.57	2.10	3.07
30	2,4,6-trimethyl-heptane[b]	0.21	0.23	0.60	0.54
31	Diisoamilene[a]	0.13	0.20	0.34	0.41
32	7-methyl-pentadecane	0.32	0.42	0.53	0.59
33	2,2,3-trimethyl-nonane	0.20	0.30	0.31	0.41
34	5-(1-methyl-prophyl)-nonane	0.08	0.10	0.15	0.17
35	Germacrane B[b]	0.13	0.16	0.35	0.30
36	Hepthyl-cyclohexane	0.11	0.20	0.12	0.26
42	2,6-dimethyl-undecane	0.10	0.16	0.11	0.12
44	1-ethyl-1-methyl-cyclohexane	0.03	0.04	0.08	0.16
46	p-xylene	0.19	0.25	0.30	0.20
48	m-xylene	0.61	0.88	0.61	0.72
49	Decahydro-*cis*-naphtalene[a]	0.07	0.09	0.31	0.50
50	3-methyl-5-undecene[a]	0.06	0.11	0.12	0.18
52	4-methyl-undecene	0.05	0.09	0.18	0.21
54	2-methyl-decahydronaphtalene	0.04	0.09	0.10	0.19
55	Octyl-cyclohexane	0.00	0.01	0.06	0.15
57	o-xylene	0.10	0.11	0.14	0.16
60	Limonene	0.47	1.72	0.49	1.14
63	Propyl-benzene	0.06	0.07	0.11	0.15
64	Decahydro-*trans*-naphtalene	0.03	0.06	0.05	0.08
68	1,3,5-trimethyl-benzene	0.04	0.06	0.03	0.07
69	1-ethyl-4-methyl-benzene	0.03	0.04	0.05	0.13
75	1-methyl-3-(1-methyl-ethyl)-benzene	0.07	0.26	0.04	0.09
77	2-ethenyl-cyclohexane	0.02	0.02	0.03	0.07
78	1,2,4-trimethyl-benzene	0.10	0.07	0.15	0.20
84	Butenyl cyclohexene	0.04	0.04	0.03	0.04
87	1,2,3-trimethyl-benzene	0.05	0.05	0.11	0.31
91	4-ethyl-1,2-dimethyl-benzene	0.01	0.02	0.02	0.04
92	2-ethyl-1,3-dimethyl-benzene	0.01	0.01	0.01	0.02
100	*cis*-1,2,3,4-tetramethyl-cyclopentane	0.05	0.12	0.14	0.30

[a]p<0,05; [b]p<0,01

Table 2
Means expressed as % and standard deviation (S.D.) of Volatile alcohols detected by GC-MS technique of Iberian dry-cured ham from different anatomical locations

Peak	Volatile compounds	Adipose tissue		Muscle	
		Mean	S.D.	Mean	S.D.
13	Isopropanol*	1.02	1.83	1.64	2.20
20	2-ethyl-1-hexanol	0.38	1.04	0.10	0.27
39	4-methyl-5-decanol*	23.86	15.78	19.30	13.93
45	2-pentanol	0.22	0.22	0.21	0.38
51	1-butanol	0.27	0.28	0.24	0.32
53	1-penten-3-ol	0.36	0.33	0.57	0.59
62	2-butyl-1-octanol*	0.08	0.14	0.14	0.17
70	2-etoxy-etanol*	0.00	0.01	0.01	0.03
71	2-ethyl-phenol*	0.28	0.60	0.25	0.47
74	1-pentanol	0.77	0.63	1.42	1.98
93	1-hexanol	0.90	0.97	1.59	1.85
96	2-hexen-1-ol*	0.07	0.23	0.08	0.15
97	3,5-octadien-2-ol*	0.11	0.09	0.15	0.17
102	1-octen-3-ol	0.54	0.27	0.64	0.39
105	2-decen-1-ol*	0.02	0.02	0.02	0.02

*Compound detected for the first time in the volatile fraction of Iberian dry-cured ham.

Table 3
Means expressed as % and standard deviation (S.D.) of Volatile aldehydes detected by GC-MS technique of Iberian dry-cured ham from different anatomical locations

Peak	Volatile compounds	Adipose tissue		Muscle	
		Mean	S.D.	Mean	S.D.
11	2-methyl-butanalc	0.57	0.53	2.19	1.54
12	3-mettyl-butanala	4.86	2.50	7.78	4.99
18	Pentanal	4.57	4.06	3.43	2.47
40	Hexanal	1.60	4.10	0.43	0.62
59	Heptanala	2.17	1.12	1.39	0.97
67	2-hexenal	0.05	0.09	0.01	0.01
81	Octanal	1.70	0.85	1.45	0.76
86	2-heptenal	0.21	0.18	0.27	0.14
89	2,4-decadienal	0.04	0.03	0.07	0.09
95	Nonanalb	0.82	0.47	1.37	0.78
98	5-ethylcyclopent-1-enecarboxaldehyde*	0.21	0.11	0.21	0.13
99	2-octenal	0.11	0.10	0.16	0.11
103	2-decenal	0.14	0.12	0.18	0.14
104	2,4-heptadienal*	0.02	0.03	0.02	0.03
109	6-nonenal*	0.01	0.01	0.02	0.02

*Compound detected for the first time in the volatile fraction of Iberian dry-cured ham. [a]p<0,05; [b]p<0,01; [c]p<0,00001.

All of them were found in the adipose tissue and muscle.

The aldehydes 2-methyl-butanal, 3-methyl-butanal, pentanal, hexanal, heptanal, octanal, nonanal, 2-hexenalm, 2-heptenal, 2,4-decadienal, 2-octenal and 2-decenal have been previously identified in the Iberian dry-cured ham by several authors (López *et al.*, 1992; Sabio *et al.*, 1998; Timón *et al.*,1998; Ruíz *et al.*, 1998; Ruiz *et al.*, 1999; Carrapiso *et al.*, 2002; Andrés *et al.*, 2002; Sánchez-Peña *et al.*, 2005; Andrés *et al.*, 2007; Ramírez *et al.*, 2007; García-González *et al.*, 2008; Andrade *et al.*, 2009). On the other hand, 5-ethylcyclopent-1-enecarboxaldehyde, 2,4-heptadienal and 6-nonenal have never been described in this type of sample. Although the presence of 6-nonenal has not been

Table 4
Means expressed as % and standard deviation (S.D.) of Volatile ketones detected by GC-MS technique of Iberian dry-cured ham from different anatomical locations

Peak	Volatile compounds	Adipose tissue		Muscle	
		Mean	S.D.	Mean	S.D.
10	2-butanone	0.28	0.34	0.39	0.25
17	2-pentanone	0.66	0.91	0.99	1.08
19	5-ethyl-dihydro-2(3H)-furanone	0.20	0.59	0.85	2.50
26	1-penten-3-one	0.12	0.17	0.18	0.20
56	Cyclopentanone*	0.10	0.19	0.09	0.15
58	2-heptanone	1.93	3.55	1.54	2.21
76	3-ethenyl-cyclohexanone*	0.02	0.02	0.06	0.08
79	2-methyl-cyclopentanone*	0.05	0.05	0.06	0.05
80	2-octanone	0.69	1.13	0.51	0.57
83	4-octen-3-one[a]	0.03	0.04	0.06	0.04
85	3-ethyl-cyclopentanone	0.05	0.06	0.07	0.09
88	6-octen-2-one*	0.06	0.07	0.10	0.10
90	6-methyl-5-hepten-2-one[b]	0.03	0.03	0.07	0.05
94	2-nonanone	0.42	0.76	0.75	1.28
101	5-methyl-2-(1-methyl-ethyl)-cyclopentanone*[a]	0.02	0.08	0.08	0.09
107	3,5-octadien-2-one*	0.03	0.04	0.05	0.08

*Compound detected for the first time in the volatile fraction of Iberian dry-cured ham. [a]p<0,05; [b]p<0,01.

previously reported, 2-nonenal has been detected by Sabio *et al*. (1998), Ruíz *et al*. (1998), and Andrés *et al*. (2002). This last compound could be a contaminant from plastic packing used after suffering high temperatures (Reineccius, 2006).

The major aldehyde in both anatomical locations was 3-methyl-butanal, showing a mean value in the muscle higher than that found in the subcutaneous fat, (7.78% and 4.86% respectively). On the other hand, significant differences between muscle and adipose tissue were found for 2-methyl-butanal (p<0.00001;), 3-methyl-butanal (p<0.05), heptanal (p<0.05) and nonanal (p<0.01).

3.4. Ketones

Table 4 shows the different ketones identified in the volatile fraction of the samples. It can be observed that all of them were detected in the two different locations studied. Several authors have identified some kenotes in Iberian ham samples, like 2-butanone, 2-pentanone, 5-ethyl-dihydro-2(3H)-furanone, 1-penten-3-one, 2-heptanone, 2-octanone, 4-octen-3-one, 6-methyl-5-hepten-2-one, and 2-nonanone (López *et al*., 1992; Ruíz *et al*., 1998; Timón *et al*.,1998; Sabio *et al*., 1998; Ruiz *et al*., 1999; Carrapiso *et al*., 2002; Andrés *et al*., 2002; Sánchez-Peña *et al*., 2005; Ramírez *et al*., 2007; Andrés *et al*., 2007; García-González *et al*., 2008; Andrade *et al*., 2009). Other kenones indentified, like cyclopentanone, 3-ethenyl-cyclohexanone, 2-methyl-cyclopentanone, 6-octen-2-one, 5-methyl-2-(1-methyl-ethyl)-cyclopentanone and 3,5-octadien-2-one, have never been described in the volatile fraction of Iberian ham.

In the ketone group, 2-heptanone shows the greatest value in both anatomical locations. It can observed that the mean value of 2-heptanone was slightly higher in the subcutaneous fat (1.93%) than in the muscle (1.54%). However, significant differences between locations were only found for 4-octen-3-one (p<0.05), la 6-methyl-5-hepten-2-one (p<0.01) and cyclopentanone (p<0.05).

3.5. Esters and Ethers

Different esters and ethers identified in the volatile fraction of the samples are shown in table 5, where the presence of all compounds can be observed in the adipose tissue and muscle, except eucalyptol which was not detected in the adipose tissue.

Only four of these compounds, 2-ethyl-furane, 2-n-butyl-furane, 2-pentyl-furane and ethyl hexanoate, have previously been identified among the volatiles of Iberian dry-cured ham (López *et a*l., 1992; Sabio *et al*., 1998; Ruíz *et al*., 1998; Ruiz *et al*., 1999; Andrés *et al*., 2002; Andrés *et al*., 2007; García-González *et al*., 2008). The rest of compounds have been identified for the first time in this research work.

In this fraction, the compound with the highest mean value is the 2-penthyl-furane for subcutaneous fat (2.82%) and the 2-ethyl-furane in muscle (3.45%). Significant differences between the subcutaneous fat and the muscle were found for 2-hydroxymethyl-2,3,3-trimethyl-oxirane (p<0.00001), 1-methoxy-pentane

Table 5
Means expressed as % and standard deviation (S.D.) of Volatile esters and ethers detected by GC-MS technique of Iberian dry-cured ham from different anatomical locations

Peak	Volatile compounds	Adipose tissue		Muscle	
		Mean	S.D.	Mean	S.D.
5	2-hydroxymethyl-2,3,3-trimethyl-oxirane*[d]	0.25	0.22	1.01	0.65
6	2-ethyl-hexyl 2-propenoate*	0.22	0.34	0.20	0.20
14	1-methoxy-pentane*[a]	0.29	0.29	1.65	2.82
15	2-ethyl-furane	0.91	0.96	3.45	8.69
29	2,3-dihydrofurane*	0.60	0.80	1.00	1.19
41	2-D-2-pentadecyl-1,3-dioxolane*	0.17	0.20	0.20	0.30
43	Hexyl octyl ether*[b]	0.03	0.05	0.29	0.41
47	2-n-buthyl-furane	0.17	0.11	0.32	0.57
61	Eucalyptol*	0.00	0.01	0.05	0.09
66	Di-(3-methyl-buthyl) ether*[a]	1.72	1.70	3.28	2.47
72	2-penthyl-furane[c]	2.82	1.40	1.39	0.84
73	Ethyl hexanoate	0.26	0.31	0.24	0.47

*Compound detected for the first time in the volatile fraction of Iberian dry-cured ham. [a]p<0,05; [b]p<0,01; [c]p<0,001; [d]p<0,00001

Table 6
Means expressed as % and standard deviation (S.D.) of Volatile nitrogen compounds detected by GC-MS technique of Iberian dry-cured ham from different anatomical location

Peak	Volatile compounds	Adipose tissue		Muscle	
		Mean	S.D.	Mean	S.D.
1	Piperidine*	7.18	10.81	7.51	13.77
65	N,N-dimethyl-2-buthoxi-Isopropylamine*	0.01	0.02	0.03	0.06
82	Hexanenitrile	0.12	0.11	0.11	0.12
106	2-ethenyl-pyridine*[a]	0.13	0.06	0.26	0.15
108	1H-pyrrole	0.00	0.01	0.01	0.01

*Compound detected for the first time in the volatile fraction of Iberian dry-cured ham. [a]p<0,001.

Table 7
Means expressed as % and standard deviation (S.D.) of other volatile compounds detected by GC-MS technique of Iberian dry-cured ham from different anatomical locations

Peak	Volatile compounds	Adipose tissue		Muscle	
		Mean	S.D.	Mean	S.D.
3	Carbon disulfide	1.35	2.72	1.55	2.20
37	Butanoic acid	0.05	0.12	0.03	0.12
38	Dimethyl disulfide[a]	0.07	0.09	0.26	0.23

[a]p<0,01.

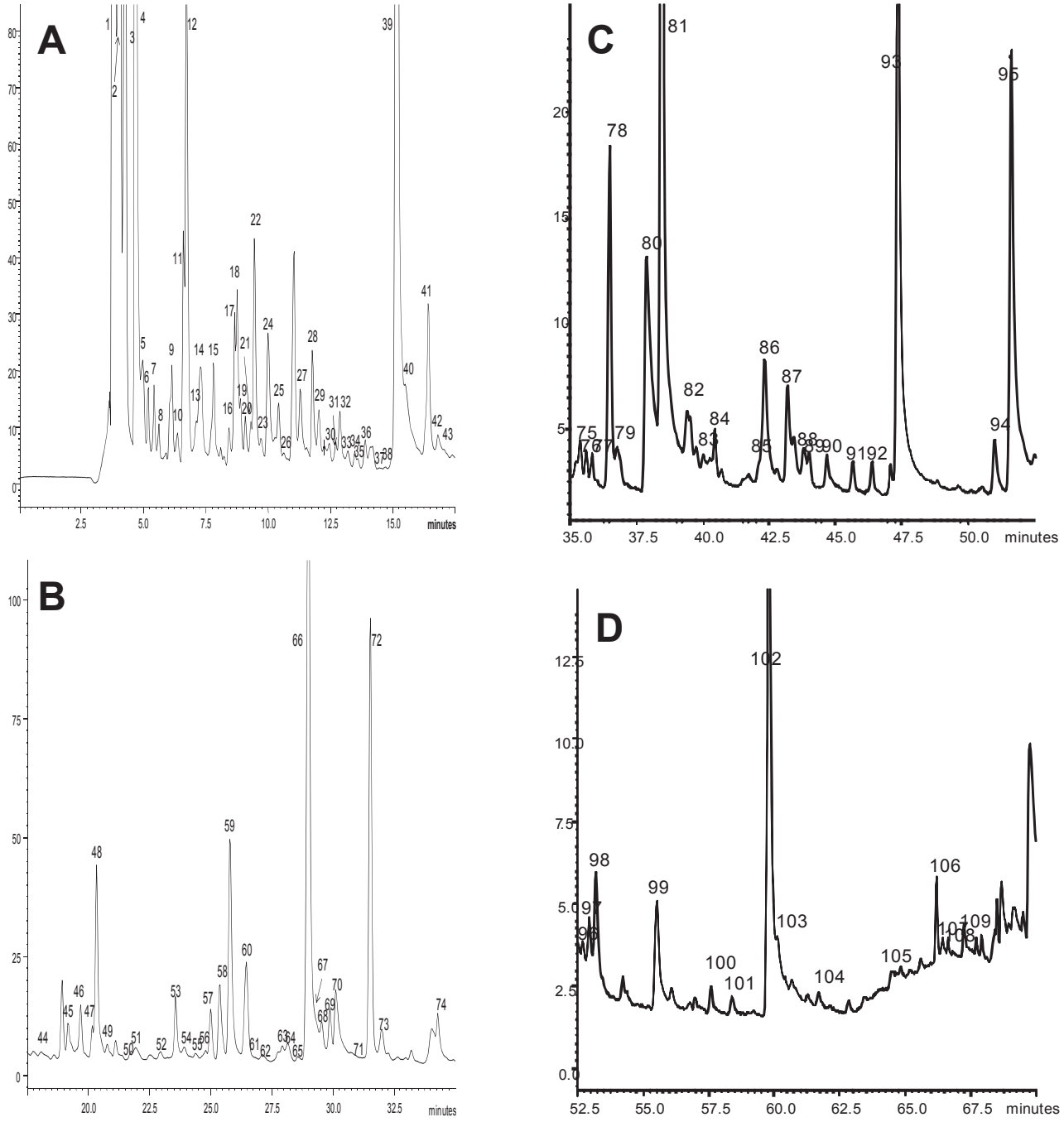

Figure 1.
GC-ion-trap-MS chromatogram in full scan mode of the total volatile compound fraction from Iberian dry-cured ham: A, from 0.0 to 17.5 minutes; B, from 17.5 to 35.0 minutes; C, from 35.0 to 52.5 minutes and D, from 52.5 to 70.0 minutes.

($p<0.05$), Hexyl octyl ether ($p<0.01$), eucalyptol ($p<0.05$), di-(3-methyl-buthyl) ether ($p<0.05$) and 2-penthyl-furane ($p<0.001$).

3.6. Nitrogen compounds

In Table 6 the different nitrogen compounds identified in this type of sample are shown. 1H-pyrrole was not identified in the adipose tissue. The rest of the nitrogen compounds were detected in both locations.

Hexanenitrile has been previously described in the fraction of volatile compounds of the Iberian ham by Sabio *et al*. (1998) and by Ruiz *et al*. (1999). Sabio *et al*. (1998) also identified 1H-pirrole in this type of sample. Nevertheless, other nitrogen compounds like piperidine, N,N-dimethyl-2-butoxi-isopropylamine and 2-etenyl-piridine have been described in this research work for the first time.

Piperidine is the compound with the highst mean value for both anatomical locations. Table 6

shows that only 2-ethenyl-pyridine has significant differences ($p<0.001$) between locations.

3.7. Others compounds

Other compounds identified in the volatile fraction of the samples are shown in Table 7. They were detected in the adipose tissue and muscle, as can be observed. All these compounds, carbon disulfide, butanoic acid and dimethyl disulfide have been described as volatile compounds of the Iberian ham by other authors (López *et al*., 1992; Timón *et al*., 1998; Ruíz *et al*., 1998; Sabio *et al*., 1998; Ruíz *et al*., 1999; Andrés *et al*., 2002; Sánchez-Peña *et al*., 2005; Ramírez *et al*., 2007; García-González *et al*., 2008; Andrade *et al*., 2009)

In this fraction, carbon disulfide had the major mean value although significant differences between anatomical locations was only found for dimethyl disulfide ($p<0.01$).

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