Volatile Compounds in Supercritical Carbon Dioxide Extracts of Iberian Ham

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The aim of the present study was to obtain aroma extracts from Iberian ham using supercritical carbon dioxide as the extraction technique of volatile compounds. After extraction, it was concluded that larger numbers and higher concentrations of volatile compounds were achieved at 40 °C temperature and at 0.50 g/mL density (91 atm pressure). Identification of volatile compounds using mass spectrometry yielded 87 and 35 compounds in the extracts obtained at 40 °C (91 atm) and 60 °C (129 atm), respectively. All substances belong to different chemical classes (aldehydes, ketones, alcohols, aliphatic hydrocarbons, aromatic hydrocarbons, carboxylic acids, lactones, esters), which have already been studied by other authors in Iberian ham. However, 41 of the identified compounds have never been found in this product. Perhaps this is due to the particular solvent selectivity of supercritical carbon dioxide for extracting substances such as hydrocarbons, ketones, and alcohols.

Keywords: Volatiles; supercritical fluid extraction (SFE); Iberian ham

INTRODUCTION

The Iberian ham is an uncooked, salted, and dried meat product of high quality produced following a traditional method in southwestern Spain from Iberian pigs (Ventanas et al., 1992).

The aroma of Iberian ham is one of the most important characteristics that influences the acceptance of this product by consumers. The study of ham flavor is very interesting for understanding the pathways leading to the formation of odor compounds during ripening as well as for controlling this process. Knowledge of these compounds can be also important for the food industry, especially the food aroma industry.

Different techniques have been employed so far to study aroma compounds, including liquid-liquid extraction, simultaneous extraction-distillation, headspace, or purge and trap, with satisfactory results obtained in products such as fruits (Ulrich et al., 1995), cheeses (Neeter et al., 1996), and wines (García-Jares et al., 1995) as well as meats and dry-cured products (Berdagué et al., 1991; Novelli et al., 1995, 1996; Mateo and Zumalacarregui, 1996; Ang and Liu, 1996; Sun et al., 1996; Bolzoni et al., 1996; Sabio et al., 1998). Recently, the use of supercritical carbon dioxide extraction of volatile compounds has increased markedly, being applied in vegetables (Bartley and Foley, 1994; Blanch et al., 1995) and in meat products (King et al., 1993; Merkle and Larick, 1994; Taylor and Larick, 1995).

Some researches have reported that flavor compounds obtained by supercritical carbon dioxide extraction showed a great similarity to the original source and a higher quality than those obtained from other methods (Merkle and Larick, 1994). The analysis of thermally

unstable samples is also possible using this technique, which allows compounds belonging to different chemical classes to be isolated through slight variations in fluid density (Majors, 1991).

The aim of the present study was to obtain aroma extracts from Iberian ham by supercritical carbon dioxide, studying the effect of different extraction conditions on the presence and concentration of volatile compounds.

MATERIALS AND METHODS

Samples were taken from biceps femoris muscle from five high-quality (according to trademark) dry-cured hams. They were vacuum-packaged in oxygen-impermeable bags, frozen, and kept at $-80~^{\circ}\text{C}$ until analysis.

A Hewlett-Packard 7680A supercritical fluid extractor was used. This apparatus was controlled by an HP Chemstation using Windows software.

Samples were ground, and 3.5 g was transferred into a standard 7 mL thimble provided by Hewlett-Packard. After equilibration for 2 min, the extraction was made for 15 min. The experiments were carried out at conditions of density and temperature of supercritical fluid reported by the bibliography in meat products which caused six different pressures; therefore, the extracts were obtained at 77 atm, 40 °C, and 0.25 g/mL density; 91 atm, 40 °C, and 0.5 g/mL; 93 atm, 60 °C, and 0.25 g/mL; 129 atm, 60 °C, and 0.5 g/mL; 134 atm, 40 °C, and 0.75 g/mL; and 218 atm, 60 °C, and 0.75 g/mL. The packing of the trap was small stainless steel balls. The temperatures of the nozzle and the trap were 45 and 30 °C, respectively. The flow of carbon dioxide was 2 mL/min. After extraction, the analytes contained in the trap were removed with acetone and collected in standard vials.

The different extracts were assessed by odor identification test, using 15 judges trained to evaluate sensorial characteristics of Iberian ham. Sensory evaluation was done by smelling a piece of paper that had been previously dipped into the extract and waiting for the evaporation of the solvent.

The chromatography was accomplished in a Hewlett-Packard 5890 Series II apparatus equipped with a flame ionization

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detector (240 °C) and a split—splitless injector (230 °C). A 50 m \times 0.32 mm i.d. HP-5 (cross-linked 5% phenyl methyl silicone) column with a 0.52 μm film thickness was used for the separation. The column temperature was programmed from 35 °C at a rate of 10 °C/min to 200 °C and held at this temperature for 20 min, and then the temperature was increased at 5 °C/min to 230 °C and held for 50 min. Helium was used as carrier gas with inlet pressure of 10 psi.

To achieve a suitable sensitivity of the volatile compounds, eight extractions were performed of each sample (8 \times 3.5 g) and the obtained extracts were put together, prior to the GC analysis.

Identification of volatiles was carried out in a Hewlett-Packard 5890 Series II apparatus coupled to a mass selective detector (Hewlett-Packard 5971A) with electron ionization at 1756 V. The mass detector temperature was set at 180 °C. The column, the temperature program, and other relevant GC conditions were the same as described for GC-FID. Kovats indices (Kovats, 1965) were calculated and compared with available literature data. Compounds were tentatively identified by comparison of spectra with those of the Wiley and NIST/EPA/NIH libraries.

RESULTS AND DISCUSSION

Only the odor of extracts obtained at 91 atm (40 °C and 0.5 g/mL density) and 129 atm (60 °C and 0.5 g/mL density) were identified as "ham odor" by most of the judges (nine). In this way, these extracts were selected to perform the chromatography. This analysis revealed that both the number and the area of peaks were very small when only the thimble capacity (3.5 g) was used for making the extractions (Figure 1a). For this reason, a larger number of thimbles was utilized to increase sample amount. The maximum number of peaks was achieved with 28 g of sample (Figure 1b).

Figure 2 shows typical chromatograms obtained from 28 g of Iberian ham using supercritical carbon dioxide extraction at 91 and 129 atm. The mean area and the frequency of peaks resulting from the hams studied by GC-FID are shown in Table 1. It was possible to detect 80 and 30 compounds when the extraction was performed at 91 and 129 atm, respectively. At 91 atm >80% of the compounds were extracted in all or most of the hams studied, whereas at 129 atm only 50% of the compounds were repeated with maximum frequency and even at lower concentrations than the first condition (Table 1). It can be observed that low pressure and temperature allowed volatile compounds to be separated easily from the sample. Probably, high temperature and pressure could modify the matrix of the ham and may cause reactions among components of the matrix and volatile compounds, hindering supercritical fluid penetration. Also, these conditions could alter the volatile compounds, eventually diminishing the success of the extraction process. Furthermore, these conditions, due to a higher pressure, increase the solubility of fatty acids and triglycerides, which can suppress the extraction of volatiles (Merkle and Larick, 1994). Taking into account that these compounds are present in great amounts in Iberian ham (Antequera et al., 1992), it is possible that volatile extraction decreases. These results are in agreement with those shown by Gere and Derrico (1994), who found that the number and concentration of volatile compounds increase when extraction pressure decreases, that is, at lower temperature and constant density. However, it should be noted that some compounds (peaks 1, 15, 16, 30, and 31 of Table 1) were extracted at a higher concentration at 129 atm compared with the concentrations extracted at 91 atm. This may

be because these compounds are highly polar and they can be firmly retained by the matrix and require more extreme pressure and temperature conditions to be released (Luque de Castro et al., 1993), thus accounting for the larger amounts extracted at 129 atm.

It is worth noting that the quantity of compounds obtained in this work was higher than those obtained by other authors from Iberian dry-cured ham using different methods of extraction, even when larger sample amounts were employed. In this sense, 77 compounds were identified by García et al. (1991) using 100 g of sample and 64 compounds were identified by López et al. (1992) using 50 g. However, the number of compounds is low compared with those obtained from other types of matrix by other authors (Bartley and Foley, 1994; Kim et al., 1995; Blanch et al., 1995; Toumala and Kallio, 1996), because the extraction could be hindered by the interaction of volatile compounds with the ham matrix. This one retains the volatiles and may even hamper carbon dioxide penetration.

By means of gas chromatography/mass spectrometry, in all, 89 volatile compounds were identified from extracts of Iberian ham, 87 of which were obtained at 91 atm and 35 at 129 atm (Table 2). These compounds belong to the following chemical classes: 14 aldehydes, 13 ketones, 9 alcohols, 28 aliphatic hydrocarbons, 6 aromatic hydrocarbons, 12 carboxylic acids, 3 esters, 1 lactone, and 3 that belong to other classes. Some of the compounds found in this work have been already described in Iberian ham and their origins identified (García et al., 1991; López et al., 1992; Ruiz, 1996). Nevertheless, other compounds identified have not been reported before. These compounds are specified in Table 2.

The high percentage of carboxylic acids found in both types of extract (32% in those obtained at 91 atm and 29% at 129 atm) was expected given the large amount of intramuscular fat in Iberian ham. The highest number and concentration of these compounds were obtained at 91 atm. These results were not in agreement with those of Merkle and Larick (1994), who reported that triglyceride and fatty acid extraction improved with higher fluid pressure. However, it is remarkable that fatty acids obtained in our study have shorter chains than those identified by the authors previously mentioned (tetradecanoic, pentadecanoic, hexadecanoic, and octadecanoic). These compounds should be present in a greater amount in extracts obtained at 129 atm but, due to their high molecular weight and low volatility, it was imposible to detect them by chromatography because they might not be volatilized in the injector or might be retained in the column due to an insufficient elution time.

On the other hand, the elevated presence of carboxylic acids in extracts might make difficult the identification of other compounds. Therefore, hexanal, elsewhere reported as the most abundant aldehyde (García et al., 1991; Ruiz, 1996), was not the predominant compound observed here. This may be because its retention time is very similar to that of butanoic acid, giving rise to possible coelution and thus hiding the real results. Something similar might account for the failure to identify 1-octen-3-ol in the extracts, always reported as a volatile compound of dry-cured ham. Kondjoyan and Berdagué (1996) suggest that the Kovats index of this alcohol, and consequently its retention time, is very similar to that of hexanoic acid.

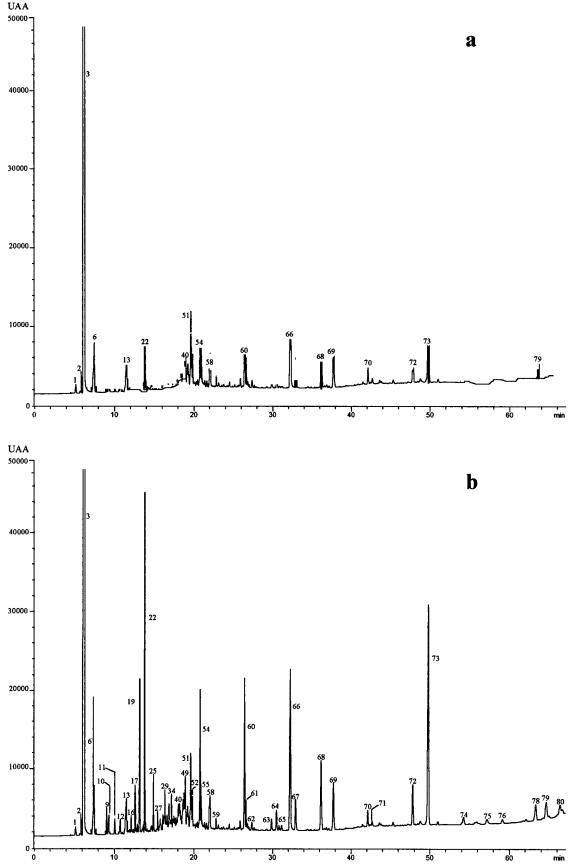


Figure 1. GC/FID chromatograms of Iberian ham compounds obtained by supercritical carbon dioxide from (a) 3.5~g of sample and (b) 28~g of sample. UAA = units of area.

However, other compounds with great aromatic importance were detected in the extracts, especially in those obtained at 91 atm. Hydrocarbons, including

aliphatic and aromatic hydrocarbons, were the dominant volatile compounds (Table 2). This confirms the observations of Rizvi (1986) and King et al. (1993) that

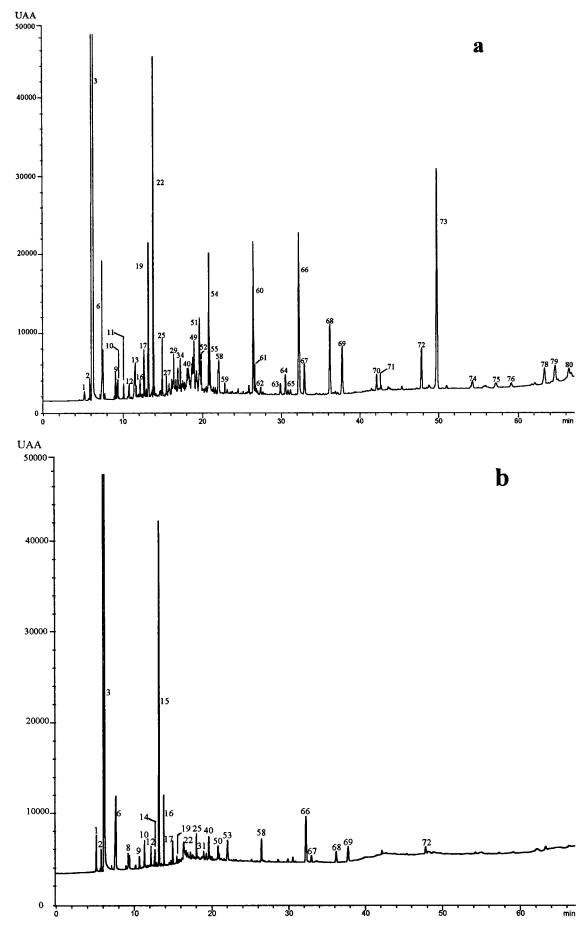


Figure 2. GC/FID chromatograms of Iberian ham compounds obtained by supercritical carbon dioxide at (a) 40 $^{\circ}$ C and 0.50 g/mL (91 atm) and (b) 60 $^{\circ}$ C and 0.50 g/mL (129 atm). UAA = units of area.

Table 1. Compounds Obtained by GC-FID in 40 $^{\circ}$ C-0.50 g/mL (91 atm) and 60 $^{\circ}$ C-0.50 g/mL (129 atm) Supercritical Fluid Extracts of Iberian Hams

		40 °C-0.5	60 g/mL (91 atm)	60 °C-0.50 g/mL	60 °C-0.50 g/mL (129 atm)	
peak	retention time	frequency ^a	area ^b	$area^b$	frequency	
1	5.18	5	1.02 ± 0.08	1.18 ± 0.33	5	
2	5.84	5 5	0.74 ± 0.21	0.74 ± 0.17	5 5 5	
3 4	6.13 6.56	5 4	6742 ± 192.40	3728.04 ± 866.24	5	
5	7.11	5	$\begin{array}{c} 0.13 \pm 0.02 \\ 0.20 \pm 0.04 \end{array}$			
6	7.51	4 3	8.24 ± 1.35	3.46 ± 1.63	4	
7	7.73	3	0.25 ± 0.05			
8 9	8.92 9.06	3 5 4	$\begin{array}{c} 0.26 \pm 0.09 \\ 0.91 \pm 0.59 \end{array}$	0.57 ± 0.18	3	
10	9.35	4	1.63 ± 0.64	0.77 ± 0.13 0.75 ± 0.11	4	
11	10.07	5	0.86 ± 0.45			
12	10.78	5	1.68 ± 0.39	0.69 ± 0.23	5	
13 14	11.52 11.65	4 4	$egin{array}{l} 4.93 \pm 2.19 \ 1.09 \pm 0.56 \end{array}$	0.69 ± 0.68	3	
15	11.89	4	0.68 ± 0.71	5.67 ± 5.25	3	
16	12.14	5 5	0.99 ± 0.34	1.29 ± 1.16	3 5	
17	12.63	5	3.06 ± 0.73	0.74 ± 0.36	5	
18 19	12.92 13.20	5 5	$egin{array}{l} 0.73 \pm 0.28 \ 1.40 \pm 10.31 \end{array}$	0.97 ± 6.64	5	
20	13.54	4	0.31 ± 0.06	0.97 ± 0.04	J	
21	13.63	4 5	0.35 ± 0.10			
22	13.81	5	20.09 ± 10.68	3.16 ± 3.15	5	
23 24	13.94 14.59	4	$egin{array}{l} 0.31 \pm 0.15 \ 0.45 \pm 0.13 \end{array}$			
25	14.93	5	2.78 ± 0.13	1.30 ± 0.44	5	
26	15.45	3	0.98 ± 0.53	0.32 ± 0.07	5 3	
27	15.77	5	1.71 ± 0.81			
28 29	16.13 16.29	3	$egin{array}{c} 0.87 \pm 0.33 \ 3.17 \pm 1.49 \end{array}$			
30	16.38	3 5 3 5 3 4	2.59 ± 1.71	2.82 ± 1.01	3	
31	16.61	4	0.89 ± 0.11	4.90 ± 8.35	3 5 3	
32	16.89	4 5 3 5 5 5 3 5 4	1.81 ± 1.09	0.75 ± 0.29	3	
33 34	17.09 17.22	3 5	$egin{array}{l} 1.59 \pm 0.55 \ 1.69 \pm 0.68 \end{array}$			
35	17.39	5	0.61 ± 0.34			
36	17.51	5	0.87 ± 0.55			
37	17.67	3	0.63 ± 0.40			
38 39	17.79 17.89	5 1	$egin{array}{l} 0.64 \pm 0.26 \ 0.75 \pm 0.15 \end{array}$			
40	18.09	3	2.16 ± 1.23	0.73 ± 0.39	4	
41	18.14	3	1.17 ± 0.31		_	
42	18.21	5	1.85 ± 0.74			
43 44	18.31 18.63	5 1	$egin{array}{l} 0.96 \pm 0.45 \ 1.94 \pm 1.11 \end{array}$			
45	18.77	3	3.66 ± 1.88			
46	18.86	3 5 5 4 3 3 4 5 5	3.11 ± 0.51			
47	18.94	4	3.46 ± 1.43	0.93 ± 0.61	3	
48 49	19.12 19.24	5 5	$\begin{array}{c} 1.12 \pm 1.11 \\ 2.21 \pm 1.48 \end{array}$			
50	19.62	4	6.52 ± 3.80	1.82 ± 1.43	4	
51	19.72	$\overline{4}$	2.30 ± 1.98			
52	19.83	5	1.49 ± 0.76	0.61 ± 0.30	3	
53 54	$20.79 \\ 20.94$	4 4 4	$\begin{array}{c} 8.48 \pm 3.05 \\ 2.58 \pm 2.62 \end{array}$	1.49 ± 0.98	3	
55	21.06	4	1.43 ± 0.86			
56	21.38	4	0.45 ± 0.14			
57	21.62	4 5	0.70 ± 0.16	1 90 + 0.25	E	
58 59	22.06 22.82	5 3	$\begin{array}{c} 3.65 \pm 0.30 \\ 0.61 \pm 0.37 \end{array}$	1.80 ± 0.35	5	
60	26.45	3 4 4 5 5 5	11.83 ± 3.58	2.71 ± 1.38	3	
61	26.68	4	0.72 ± 0.24			
62	27.37	5	0.60 ± 0.18			
63 64	29.89 30.52	5 5	$\begin{array}{c} 1.15 \pm 0.39 \\ 2.02 \pm 0.67 \end{array}$			
65	30.86		0.62 ± 0.05			
66	32.26	5	17.19 ± 9.11	4.49 ± 2.59	5	
67 68	32.96	4 5 5 5 5 5 4	4.04 ± 0.71	0.92 ± 0.40	5 3 3	
68 69	36.19 37.76	5 5	$8.73 \pm 2.55 \ 4.85 \pm 1.72$	$\begin{array}{c} 2.10 \pm 1.01 \\ 1.94 \pm 0.55 \end{array}$	3	
70	42.14	5	1.54 ± 0.54	1.04 ± 0.00	J	
71	42.68	4	0.79 ± 0.16			
72	47.82	5	5.30 ± 1.29	1.32 ± 0.69	3	
73 74	49.78 54.24	5 5	$21.56\pm18.15\ 1.54\pm0.41$			
74 75	57.29	3	1.14 ± 0.41 1.14 ± 0.37			
76	59.12	5 5 3 4 3 5 4 5	0.85 ± 0.17			
77	61.74	3	2.56 ± 1.82			
78 79	63.29 64.62	5 1	$8.94 \pm 6.97 \ 9.97 \pm 9.30$			
13	U4.U&	4	$\sigma.\sigma \iota \perp \sigma.\sigma U$			

 $[^]a$ Occurrence in the five samples. b Expressed as units of area/e4. Each value is the average (means \pm SD) from Iberian pig ham indicated at the frequency.

Table 2. Compounds Identified by GC/MS in 40 $^{\circ}$ C-0.50 g/mL (91 atm) and 60 $^{\circ}$ C-0.50 g/mL (129 atm) Supercritical Fluid Extracts of Iberian Hams

	_			rea ^c
KI^a	compound	reliability ^b	40 °C-0.50 g/mL (91 atm)	60 °C-0.50 g/mL (129 atm)
	aldehydes			
607	butanal	c	23.45 ± 2.34	10.42 ± 1.66
662 677	3-methylbutanal 2-methylbutanal	c	118.48 ± 5.87	$71.42 \pm 20.03 \\ 32.70 \pm 10.78$
683	pentanal	c c	54.17 ± 2.75	32.70 ± 10.78 11.12 ± 8.96
794	hexanal	c	80.12 ± 13.46	11.12 ± 0.00
905	heptanal	c	96.72 ± 5.55	
961	benzaldehyde	b	53.39 ± 1.88	
1008	octanal	c	366.13 ± 67.9	
1074	benzeneacetaldehyde d	b	362.58 ± 200.53	
1110	nonanal	С	266.99 ± 7.85	
1208	decanal	c	98.09 ± 10.63	
1218 1317	2,4-nonadienal 2,4-decadienal	c c	$\begin{array}{c} 12.87 \pm 7.42 \\ 98.32 \pm 7.82 \end{array}$	
1399	dodecanal	c	129.10 ± 7.57	
1000	ketones	C	120.10 ± 7.07	
674	1-hydroxy- 2 -propanone ^{d}	c	99.50 ± 25.67	71.85 ± 18.45
716	3-hydroxy-2-butanone	c	121.62 ± 13.57	58.54 ± 1.52
750	3-penten-2-one	c	45.73 ± 29.34	33.98 ± 2.34
804	4-methyl-3-penten-2-one ^d	b	633.79 ± 124.48	369.76 ± 29.37
845	3-hexen-2-one ^d	C	34.23 ± 1.83	60.07 ± 5.55
851 877	4-hydroxy- 4 -methyl- 2 -pentanone ^{d} 4 -heptanone	b c	$680.33 \pm 250.37 \ 29.80 \pm 71.23$	$98.95 \pm 97.13 \ 18.43 \pm 22.88$
894	2-heptanone	c	55.72 ± 10.04	16.43 ± 22.66
913	cyclohexanone	c	119.78 ± 38.11	
943	4-methyl- 2 -heptanone ^{d}	b	18.56 ± 1.46	
1016	5-hydroxy-3-methyl-3-hexanone ^d	b	252.75 ± 42.62	
1146	3-nonen- 2 -one ^{d}	c	64.23 ± 60.28	55.26 ± 2.28
1233	3-decen- 2 -one ^{d}	c	99.40 ± 9.09	
704	alcohols		00 77 1 0 17	
784	1,3-butanediol	С	88.75 ± 2.47	
784 821	$2,3$ -butanediol 4 -methyl- 2 -pentanol d	c b	$\begin{array}{c} 203.17 \pm 18.53 \\ 18.65 \pm 1.09 \end{array}$	296.02 ± 100.45
871	1-hexanol	C	48.79 ± 0.56	1413.89 ± 20.56
997	3-methyl- 4 -heptanol ^{d}	b	72.74 ± 6.32	1410.00 ± 20.00
1033	2-ethyl-1-hexanol ^d	c	288.05 ± 19.06	
1051	$benze^{g}$	b	200.42 ± 18.47	
1139	benzeneethanol d	b	1109.25 ± 77.63	88.83 ± 3.64
1579	dodecanol	c	36.12 ± 2.54	
000	aliphatic hydrocarbons		00.40 + 40.00	
600	hexane	a	89.48 ± 10.02	
700 800	heptane octane	a a	$61.14 \pm 8.53 \ 77.98 \pm 5.28$	
900	nonane	a	77.36 ± 3.26 73.76 ± 4.79	80.87 ± 15.65
1000	decane	a	101.05 ± 13.57	00.07 ± 10.00
1024	2 -methyldecane d	c	71.24 ± 25.31	87.99 ± 3.58
1047	limonene	c	140.35 ± 23.65	
1057	5-methyldecane ^d	c	142.80 ± 8.04	102.63 ± 19.73
1085	4-methyl-1-undecene ^d	b	106.05 ± 45.78	
1100	undecane 1-dodecene ^d	a	103.83 ± 31.88	284.82 ± 32.75
1192 1200	dodecane	c a	$\begin{array}{c} 1058.41 \pm 149.84 \\ 128.42 \pm 11.32 \end{array}$	184.06 ± 10.87
1203	2-dodecene ^{d}	c C	126.42 ± 11.32 107.46 ± 65.38	
1211	3-dodecene ^d	c	57.44 ± 4.12	
1257	5-methyldodecane ^d	c	106.70 ± 18.29	
1389	1 -tetradecene d	c	1804.44 ± 78.43	107.29 ± 8.14
1400	tetradecane	a	200.01 ± 20.87	
1403	2-tetradecene ^d	c	75.57 ± 23.65	
1407	2-ethyl-1-dodecene ^d	b	43.84 ± 3.88	
1416	3-tetradecene ^{d}	С	112.91 ± 10.02	
1500 1593	pentadecane 1-hexadecene ^d	a	$\begin{array}{c} 96.69 \pm 10.48 \\ 1215.24 \pm 50.72 \end{array}$	122.64 ± 14.86
1600	hexadecane	c a	1213.24 ± 30.72 180.31 ± 8.43	1&&.U4 ± 14.0U
1604	3-hexadecene ^d	a C	47.04 ± 5.79	
1694	$cyclododecane^d$	c	132.66 ± 123.42	
1700	heptadecane	a	83.48 ± 5.31	
1795	1 -octadecene d	c	508.62 ± 29.04	
1800	octadecane	a	31.15 ± 4.23	
	aromatic hydrocarbons		000 000 100 000	A- A
778	toluene	c	379.77 ± 18.26	85.67 ± 32.56
971	propylbenzene	C b	442.28 ± 50.49	
1095 1106	4-ethyl-1,2-dimethylbenzene ^d 1-ethyl-3,5-dimethylbenzene ^d	b b	421.82 ± 14.67 353.47 ± 13.32	
1100			353.47 ± 13.32	
1146	1,3,4,5-tetramethylbenzene ^d	b	311.15 ± 200.12	

			$area^c$		
KI^a	compound	${\rm reliability}^b$	40 °C-0.50 g/mL (91 atm)	60 °C-0.50 g/mL (129 atm)	
	carboxylic acids				
622	acetic acid	c	408.54 ± 20.22	675.14 ± 20.54	
702	propanoic acid d	c	52.08 ± 6.98	55.28 ± 40.87	
792	butanoic acid	c	180.73 ± 12.87	124.56 ± 12.75	
881	pentanoic acid	c	123.93 ± 16.36		
981	ĥexanoic acid	c	881.52 ± 35.27	147.23 ± 13.65	
1069	heptanoic acid	c	718.28 ± 36.92	34.65 ± 10.32	
1172	octanoic acid	c	1335.42 ± 176.92	122.62 ± 36.48	
1180	benzoic acid d	c	134.70 ± 16.97		
1243	nonanoic acid d	c	1039.95 ± 500.65	88.45 ± 4.59	
1349	$\operatorname{decanoic}\operatorname{acid}^d$	c	2260.76 ± 1236.9	35.85 ± 25.61	
1558	dodecanoic acid d	c	608.05 ± 13.13	38.79 ± 7.24	
1767	$tetradecanoic\ acid^d$	c	1701.54 ± 773.72	55.86 ± 1.09	
	esters				
767	2 -methylpropanoic acid d	c	281.25 ± 35.72		
839	2-methylbutanoic acid	c	56.99 ± 2.34		
861	3-methylbutanoic acid d	c	227.35 ± 45.87		
	lactones				
927	butyrolactone	c	408.98 ± 20.25		
	others				
629	chloroform	c	2995.35 ± 124.98	52.21 ± 4.12	
788	3 -methylbutamine d	b		34.21 ± 1.23	
1617	benzenedicarboxylic acid d	b	921.98 ± 36.81		

 a Kovats indices calculated for DB-5 capillary column of the GC/MS system. b The reliability of the identification or structural proposal is indicated by the following symbols: a, mass spectrum, Kovats index, and retention time identical to those of standard compounds; b, mass spectrum consistent with spectra found in the literature; c, mass spectrum and Kovats index in agreement with the corresponding literature data. c Expressed as units of area/e6. Each value is the average (means \pm SD) from five Iberian pig hams. d Identified compounds not found in Iberian ham by other authors.

supercritical carbon dioxide is very selective for extracting these substances. Some of these compounds such as *n*-alkenes, branched hydrocarbons, and alkylbenzenes have not been found in Iberian ham, but King et al. (1993) and Merkle and Larick (1994) have isolated them from raw beef and beef fat, respectively.

The concentration of aldehydes, ketones, and alcohols was lower than that of hydrocarbons. These are more polar and thus would be less soluble in supercritical carbon dioxide (Taylor and Larick, 1995). The presence of some of these compounds in Iberian ham has not been previously reported; this may be due to the particular solvent selectivity of this fluid. However, 2-ethyl-1-hexanol was identified in fried bacon by Ho et al. (1983) and in raw beef by King et al. (1993).

It is remarkable that 2-methylbutanal was present in extracts obtained at 129 atm but not in those obtained at 91 atm. This result is peculiar because this aldehyde together with 3-methylbutanal is usually isolated from Iberian ham (García et al., 1991; Ruiz, 1996). It is possible that the 3-methylbutanal peak, which presented a high concentration in the 91 atm extracts, included both compounds but the identification by GC/MS may have been defective because their retention times and mass spectra are very similar.

In conclusion, SFE combined with GC/MS has been shown to be successful in the analysis of Iberian ham flavor. Several new compounds not previously reported by other authors have been tentatively identified in this study; this may be due to the particular solvent selectivity of the supercritical carbon dioxide. Also, this technique may be ideal to obtain aroma extracts of ham, which smell of original product and, thus, to determine the contribution of compounds included in these extracts to the special aroma of Iberian ham. Volatile extraction was more efficient at 91 atm (40 °C–0.50 g/mL) than at 129 atm (60 °C–0.50 g/mL), that is, at a lower extraction pressure.

LITERATURE CITED

Ang, C. Y. W.; Liu, F. Static headspace capillary GC method for determining changes of volatiles from heated chicken breast meat. J. Muscle Foods 1996, 7, 131–138.

Antequera, T.; López-Bote, C.; Córdoba, J. J.; García, C.;
Asensio, M. A.; Ventanas, J.; García-Regueiro, J. A.; Díaz,
I. Lipid oxidative changes in the processing of Iberian pig hams. Food Chem. 1992, 45, 105-110.

Bartley, J. P.; Foley, P. Supercritical fluid extraction of Australian-grown ginger. *J. Sci. Food Agric.* **1994**, *66*, 365–371

Berdagué, J. L.; Denoyer, C.; Le Quéré, J. L.; Semon, E. Volatile components of dry cured ham. *J. Agric. Food Chem.* **1991**, *39*, 1257–1261.

Blanch, G. P.; Reglero, G.; Herraiz, M. Analysis of wine aroma by off-line and on-line supercritical fluid extraction-gas chromatography. *J. Agric. Food Chem.* **1995**, *43*, 1251–1258.

Bolzoni, L.; Barbieri, G.; Virgili, R. Changes in volatile compounds of Parma ham during maturation. *Meat Sci.* **1996**, *43*, 301–310.

García, C.; Berdagué, J. L.; Antequera, T.; López-Bote, C.; Córdoba, J. J.; Ventanas, J. Volatile components of dry cured Iberian ham. *Food Technol.* **1991**, *23*, 251–254.

García-Jares, C. M.; García-Martín, M. S.; Carro-Merino, N.; Cela-Torrijos, R. GC-MS identification of volatiles components of Galician (Northwestern Spain) white wines. Application to differentiate Rias Baixas wines from wines produced in nearby geographical regions. *J. Sci. Food Agric.* **1995**, *69*, 175–184.

Gere, D. R.; Derrico, E. M. SFE theory to practice—first principles and method development, part I. *LC-GC Int.* **1994**, *7*, 325–331.

Ho, C. T.; Lee, K. N.; Jin, Q. Z. Isolation and identification of volatile flavor compounds in fried bacon. *J. Agric. Food Chem.* 1983, 31, 336–342.

Kim, S. M.; Wu, C. M.; Kobayashi, A.; Kubota, K.; Okumura, J. Volatile compounds in stir-fried garlic. *J. Agric. Food Chem.* **1995**, *43*, 2951–2955.

King, M. F.; Hamilton, B. L.; Matthews, M. A.; Rule, D. C.; Field, R. A. Isolation and identification of volatiles and

- condensable material in raw beef with supercritical carbon dioxide extraction. *J. Agric. Food Chem.* **1993**, *41*, 1974–1981.
- Kondjoyan, N.; Berdagué, J. L. A Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds, Laboratoire Flaveur, INRA de Theix: Saint Genes Champanelle. 1996.
- Kovats, E. Gas chromatographic characterization of organic substances in the retention index system. In *Advances in Chromatography*; Giddings, J. C., Keller, R. A., Eds.; Dekker: New York, 1965; pp 229–232.
- López, M. O.; De la Hoz, L.; Cambero, M. I.; Gallardo, E.; Reglero, G.; Ordóñez, J. A. Volatile compounds of dry hams from Iberian pigs. *Meat Sci.* 1992, 31, 267–277.
- Luque de Castro, M. D.; Valcárcel, M.; Tena, M. T. Extracción con Fluidos Supercríticos en el Proceso Analítico; Reverté, S.A.: Barcelona, 1993.
- Majors, R. E. Supercritical fluid extraction—an introduction. *LC–GC Int.* **1991**, *4*, 10–17.
- Mateo, J.; Zumalacarregui, J. M. Volatile compounds in chorizo and their changes during ripening. *Meat Sci.* **1996**, *44*, 255–273.
- Merkle, J. A.; Larick, D. K. Conditions for extraction and concentration of beef fat volatiles with supercritical carbon dioxide. *J. Food Sci.* **1994**, *59*, 478–483.
- Neeter, R.; Jong, C. de; Teisman, H. G. J.; Ellen, G. Flavour research of cheese using the purge and trap technique. *Voedings Middelen Technol.* **1996**, *29*, 12–13.
- Novelli, E.; Gandemer, G.; Meynier, A.; Zanardi, E.; Chizzolini, R. *Composizione dell'aroma di due prodotti di salumeria:* mortadella e salame Milano; Atti del congresso "grassi e qualita" della carni: Parma, June 24, 1995.
- Novelli, E.; Gandemer, G.; Meynier, A.; Zanardi, E.; Chizzolini, R. Flavour compounds isolated from salami Milano and mortadella; Annali della Focolta di Medicina Veterinaria: Parma, 1996; pp 177–198.

- Rizvi, S. S. H.; Daniels, J. A.; Benado, A. L.; Zollweg, J. A. Supercritical fluid extraction: operating principles and food applications. *Food Technol.* 1986, July, 57–64.
- Ruiz, J. Estudio de parámetros sensoriales y físico-químicos implicados en la calidad del jamón Ibérico. Tesis Doctoral, Universidad de Extremadura, 1996.
- Sabio, E.; Vidal-Aragón, M. C.; Bernalte, M. J.; Gata, J. L. Volatile compounds present in six types of dry-cured ham from south European countries. *Food Chem.* **1998**, *61*, 493– 503
- Sun, J. J.; Chen, S. T.; Zhou, R. B. Flavour components of beef. J. Zhengzhou Grain College 1996, 16, 7-19.
- Taylor, D. L.; Larick, D. K. Investigations into the effect of supercritical carbon dioxide extraction on the fatty acid and volatile profiles of cooked chicken. *J. Agric. Food Chem.* 1995, 43, 2369–2374.
- Toumala, T.; Kallio, H. Identification of free fatty acids and some other volatile flavour compounds from Swiss cheese using on-line supercritical fluid extraction-gas chromatography. *Z. Lebensm.-Unters. -Forsch.* **1996**, *203*, 236–240.
- Ulrich, D.; Eunert, S.; Hoberg, E.; Rapp, A. Analysis of strawberry aroma by solid-phase microextraction. *Dtsch. Lebensm.-Rundsch.* **1995**, *91*, 339–351.
- Ventanas, J.; Córdoba, J. J.; Antequera, T.; García, C.; López-Bote, C.; Asensio, M. A. Hydrolysis and Maillard reaction during the ripening of Iberian ham. J. Food Sci. 1992, 57, 813–815.

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