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Chemical and structural changes in lipids during the ripening of Teruel dry-cured ham

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Abstract

The aim of this paper is to investigate the chemical and microstructural changes in intramuscular and subcutaneous fat during the processing of Teruel dry-cured ham by gas chromatography and electron microscopy techniques. This paper will contribute to the specific characterisation of a product included in the European Union list of special quality products, and provides a new perspective for the identification of changes related to flavour development. There seems to be a relationship between the degradation of phospholipids and the increase in the free fatty acid content, especially polyunsaturated fatty acids. The microstructural changes of the adipose tissue during the process would explain the availability of the fat to the lipolitic enzymes and the contribution to the typical flavour and taste of cured ham.

Keywords: Dry-cured ham; Gas chromatography; Cryo-SEM; SEM; Lipolysis

1. Introduction

Nowadays, in Spain there are four "Protected Designations of Origin (PDO)" which are identified in order to protect extra-quality hams obtained in their respective production areas: "Guijuelo", "Teruel", "Dehesa de Extremadura" and "Huelva" cured ham. "Protected Designation of Origin Teruel" ("PDO Teruel"), is a high quality meat product processed using the traditional dry-curing procedure. Since 1997, this product has been included in the European Union list of special quality products.

One of the main biochemical changes that takes place during the process is the degradation of the lipid fraction (Baldini, Palmia, Pezzani, & Lambertini, 1993). Lipolysis is one of the main degradation mechanisms affecting lipids during the processing of dry-cured ham (Andrés, Cava, Martín, Ventanas, & Ruiz, 2005; Martín, Antequera, Córdoba, Timón, & Ventanas, 1998). The development of the sensory characteristics that typify this ham is closely connected with the hydrolysis and oxidation phenomena in the fat (Flores, Bermell, Nieto, & Miralles, 1985). The quality of meat and meat-based products is closely related to its microstructure (Silva, Orcutt, Forrest, Bracker, & Judge, 1993; Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995). The microstructural study of cured ham would provide valuable information about the phenomena that take place during processing. Furthermore, in the specific case of those foods protected by Designation of Origin, knowing the food's microstructure may be useful for characterising it, as well as for defining and optimising the processes involved.

There has been very little investigation into the structural changes that take place in muscle tissue during the processing of meat products such as cured ham. However, the microstructure of meat during the postmortem storage has been studied by several authors in the last years; so Boyer-Berri and Greaser (1998) studied the effect of

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postmortem storage on the *Z*-line region of titin in bovine muscle; and Sotelo et al. (2004) studied the microstructural changes in rabbit meat wrapped with *Pteridium aquilinum* fern during postmortem storage. Other authors such as Monin et al. (1997) have studied the structural changes in dry-cured hams (Bayonne hams) during processing and effects of dehairing technique, but there are not studies about microstructural changes in the adipose tissue during the cured ham manufacturing process.

This study follows the main chemical and microstructural changes which occur in the intramuscular fat of the *biceps femoris* muscle and in the subcutaneous fat throughout the processing of "PDO Teruel" cured ham. It is presented as a contribution to the specific characterisation of a product considered to be of special quality by the European Union, the "PDO Teruel" cured ham.

2. Materials and methods

Hams were obtained from white-breed pigs (mother, Landrace × Large White; father, Duroc) of 8–9 kg live weight. There were processed to dry-cured ham "PDO Teruel" in the traditional way. Sampling was carried out in triplicate taking into account the more relevant steps of the process:

- "Raw ham" from the green stage: The hams were kept after slaughter for 4 days at 0–4 °C and then sampling was carried out.
- "Salted ham" from the salting: The hams were covered with salt for a maximum of 14 days at 5–6 °C and 90% relative humidity. Sampling was carried out at the end of the salting.
- "Post-salted ham" from the post-salting: The hams were brushed to get rid of the salt left on their surfaces. Then the hams were taken to a chamber for 45–90 days, at 3–6 °C and 80–90% relative humidity. Sampling was carried out at the end of the step.
- "Half-cured ham" from the ripening: The hams were kept under environmental conditions for 6–8 months in ripening rooms. Half-cured hams were samples taken at the half of the ripening (3–4 months).
- "Cured ham": Final product after 6–8 months of ripening.

The process takes place along 12 months.

2.1. Fatty acid analysis

At each step of the process under study, triple samples were taken from subcutaneous fat and from the *biceps femoris* muscle so the lipids could be studied. On each ham, the subcutaneous fat with no skin was trimmed off the ham to be analysed. To study intramuscular fat, a homologous sample of all the ham muscle was obtained.

Total lipids were extracted in duplicate from 5 g muscle for the intramuscular fat study and a 2 g sample for the subcutaneous fat study by means of a modification of the chloroform/methanol (2/1; v/v) method of Folch, Lees, and Sloane-Stanley (1957). Nonadecanoic acid (C19:0) was added as an internal standard. After methylation of the fatty acids with NaOH/MeOH followed by HCl/MeOH, the fatty acid methyl esters (FAME) were analysed by gas chromatography (HP 6890) using a CP-Sil88 column for FAME (100 m × 250 μm × 0.2 μm) (Chrompack, The Netherlands) as described by Raes et al. (2004). The conditions were: injector: 250 °C; detector: 280 °C, H_2 as carrier gas; temperature program: 150 °C for 2 min, followed by an increase of 1 °C/min to 158 °C, then held at 158 °C for 22 min, followed by an increase of 1 °C/min to 200 °C. Peaks were identified by comparing the retention times with those of the corresponding standards (Sigma, Belgium; Nu-Chek Prep., Mn, USA).

The separation of total muscle lipids into phospholipids (PL), free fatty acid (FFA) monoglycerides, diglycerides and triglycerides (TG) fractions was carried out as described by Raes, De Smet, Balcaen, Claeys, and Demeyer (2003), using thin layer chromatography. Fatty acid methyl esters (FAME) from the different fractions were analysed by gas chromatography applying the same conditions used for total lipids.

2.2. Cryo scanning electron microscopy (Cryo-SEM)

Biceps femoris muscle and subcutaneous fat were excised from the hams and cubes (3 mm^3) were cut with a stainless steel cutter from the central zone in the muscle and from the subcutaneous fat. Cubes were immersed in slush Nitrogen (−210 °C), and were transferred to a cryo-trans (CT 15000 C of Oxford Instruments, Oxford, England) linked to a scanning electron microscope JEOL JSM 5410 (Jeol Tokio, Japan), operating at a temperature below −130 °C. Samples were cryo fractured at −180 °C and etched at −90 °C. The observations in the microscope were carried out at 15 kV and at a working distance of 15 mm.

2.3. Scanning electron microscopy (SEM)

For the SEM study, the samples were cut into cubes (3 mm^3), with a stainless steel cutter. The samples obtained were fixed in a solution of 2.5% glutaraldehyde in 0.1 M (pH 7.3) phosphate buffer for 24 h at 4 °C, dehydrated in a series of 10%, 20%, 40%, 60%, 80% and 100% ethanol (ethanol/water, V/V) every 20 min, rinsed in acetone and ultradehydrated by critical point with CO_2 (1100 psi, 31.5 °C) in a POLARON E3000 instrument (Watford, United Kingdom). Then they were gold-coated using POLARON E6100 Equipment (10^{-4} mbar, 20 mA, 80 s) and observed in a Jeol JSM 6300 Scanning Electron Microscope (Jeol, Tokio, Japan) at 15 kV and a working distance of 15 mm.

3. Results and discussion

3.1. Changes in lipids of the intramuscular fat

The chemical changes undergone by the fat components throughout the "PDO Teruel" ham curing process greatly influence the product's final characteristics. Lipid content in *biceps femoris* was 13.29 g fat/100 g (DM).

Table 1 shows the evolution of the lipid fractions: triglycerides (TG), diglycerides, monoglycerides, phospholipids (PL) and free fatty acids (FFA), throughout the manufacturing process. The results are expressed as a percentage of each fraction in the total fraction.

In the intramuscular fat of the raw ham, TG are the majority (74.85%). PL also have an important presence (21.37%). The presence of FFA in raw ham only makes up 2.62% and the monoglyceride and diglyceride fractions are present in very small quantities.

TG did not show remarkable changes throughout the process of "PDO Teruel" cured ham, whereas the amounts of PL and FFA vary greatly throughout the process. The quantity of phospholipids drops sharply from 21.37% in raw ham to 2.54% in "PDO Teruel" dry-cured ham. This is due to the fact that the PL are very unstable and have high levels of unsaturated fatty acids, which are the most notably affected by the hydrolysis reactions prior to oxidation. The degradation of this fraction must play and important role in developing the flavour of the cured ham. These results agree with those obtained by other authors for Serrano ham (Flores et al., 1985).

The amount of FFA does not change greatly during the first steps of the process up to post-salting, while the pieces are kept at low temperatures. There is, however, a considerable increase during the ripening stage and up to the end of the process, where the presence of FFA in the *biceps femoris* reaches a level of 18.64%. These results agree with Buscailhon, Gandemer, and Monin (1994), who describe similar changes in the intramuscular lipids during the processing of French dry-cured ham.

These results would support the hypothesis that the FFA generated during the process mainly come from PL hydrolysis. Same conclusion has been reached in other papers on the changes in content and composition of fatty acids in meat during different processes, such as the curing ham (Buscailhon et al., 1994; Flores, Bermell, Nieto, & Costell, 1987) or the storage of fresh meat (Sharma, Kowale, & Joshi, 1982).

3.1.1. Total fraction

Table 2 shows the composition of fatty acids in the total lipids fraction of the *biceps femoris* intramuscular fat. Monounsaturated fatty acids (MUFA) and saturated ones were the main fatty acids found in the total lipid fraction (45.99% and 35.06%, respectively). Oleic acid and palmitic acid predominate (41.5% and 22.67%, respectively), and polyunsaturated fatty acids (PUFA) contained essentially linoleic acid, which accounts for 10.52% of the total.

The reduction in the percentage of PUFA throughout the process up to half-ripening stage is especially notable; so, it drops from 15.54% in raw ham to 11.73%. These fatty acids are those that undergo a more marked change in the "PDO Teruel" ham curing process. These results are similar to those obtained by other authors studying the composition of fatty acids in pork muscle (Leseigneur-Meynier & Gandemer, 1991) and in cured ham (Buscailhon et al., 1994; Flores et al., 1987; Martín, Córdoba, Ventanas, & Antequera, 1999). In pork meat fat, there is a high percentage of fatty acids belonging to the families $n-6$ and $n-3$, such as linoleic acid ($n-6$), linolenic acid ($n-3$), docosahexenoic ($n-3$) or eicosapentenoic ($n-3$). In the last few years, these fatty acids have been found to be of nutritional importance, and, as such, it is important to point out that about 75% of the original PUFA are still to be found in the final product. In addition, "PDO Teruel" has a high percentage of oleic acid (44.69%), the properties of which have also been proven (Mata, Alonso, & Mata, 2002). The results of total fraction are similar to those obtained by other investigators in pork muscle (Leseigneur-Meynier et al., 1991) and in cured ham (Martín et al., 1999).

3.1.2. Triglycerides fraction

Regarding TG of the intramuscular fat in the *biceps femoris* from raw ham (Table 3), MUFA are the most abundant (55.46%), followed by the saturated (37.28%) and the PUFA (6.74%). The highest percentage of fatty acid in this fraction is that of oleic acid (49.87 %), followed by palmitic acid (24.78%). Linoleic acid is the PUFA which is the majority one in this fraction, representing 4.87% of the total fatty acids in this fraction. There is fairly little change in the fatty acids in this fraction during processing.

Table 1
Evolution of the lipid fractions of intramuscular fat in the *biceps femoris* muscle of raw ham, salted ham, post-salted ham, half-cured ham and "PDO Teruel" cured ham, throughout manufacturing process (g fatty acids in each fraction/100 g total fatty acids in total fraction)

Fractions	*biceps femoris*				
	Raw ham	Salted ham	Post-salted ham	Half-cured ham	Cured ham
Triglycerides	*74.85*	83.44	91.09	88.82	*76.29*
Diglycerides	0.82	1.01	0.43	0.81	2.07
Monoglycerides	0.34	0.31	0.22	0.13	0.47
Phospholipids	***21.37***	13.58	7.21	4.79	***2.54***
Free fatty acids	***2.62***	1.65	1.04	5.45	***18.64***

Table 2
Changes in fatty acid composition of total lipids of the intramuscular fat during processing (g/100 g of total fatty acids)

Fatty acids	Total lipids				
	Raw ham	Salted ham	Post-salted ham	Half-cured ham	Cured ham
C14:0	1.26 ± 0.04	1.06 ± 0.04	1.13 ± 0.001	1.13 ± 0.001	1.27 ± 0.02
C16:0	*22.67* ± 0.39	22.87 ± 0.09	22.61 ± 0.04	22.76 ± 0.05	*22.90* ± 0.14
C18:0	10.54 ± 0.48	10.83 ± 0.15	12.15 ± 0.04	11.89 ± 0.06	11.07 ± 0.08
Others[a]	0.60 ± 0.03	0.50 ± 0.06	0.57 ± 0.004	0.66 ± 0.03	0.56 ± 0.01
Total saturated	***35.06*** ± 0.92	35.26 ± 0.21	36.46 ± 0.08	36.44 ± 0.08	***35.82*** ± 0.26
C16:1	3.63 ± 0.10	3.16 ± 0.03	3.30 ± 0.05	2.85 ± 0.03	3.62 ± 0.01
C18:1	*41.45* ± 0.21	44.69 ± 0.50	46.80 ± 0.22	46.46 ± 0.11	44.69 ± 0.18
Others[b]	0.92 ± 0.07	1.03 ± 0.02	0.96 ± 0.01	0.88 ± 0.02	0.69 ± 0.02
Total monounsaturated	***45.99*** ± 0.30	48.88 ± 0.55	51.06 ± 0.29	50.20 ± 0.12	***48.99*** ± 0.20
C18:2 ($n-6$)	*10.52* ± 0.21	10.07 ± 0.38	7.14 ± 0.06	9.03 ± 0.22	9.76 ± 0.11
C20:4 ($n-6$)	1.83 ± 1.59	1.50 ± 0.63	1.35 ± 0.08	0.80 ± 0.50	1.63 ± 0.01
Others[c]	1.25 ± 0.03	1.08 ± 0.04	0.91 ± 0.005	0.92 ± 0.01	1.09 ± 0.03
Total $n-6$	13.61 ± 1.50	12.65 ± 0.78	9.40 ± 0.01	10.75 ± 0.27	12.48 ± 0.15
C18:3 ($n-3$)	0.48 ± 0.01	0.43 ± 0.08	0.35 ± 0.01	0.52 ± 0.02	0.42 ± 0.002
C20:5 ($n-3$)	0.14 ± 0.02	0.08 ± 0.001	0.06 ± 0.003	0.06 ± 0.001	0.08 ± 0.003
C22:5 ($n-3$)	0.34 ± 0.06	0.20 ± 0.09	0.17 ± 0.002	0.19 ± 0.004	0.27 ± 0.01
Others[d]	0.97 ± 1.61	0.18 ± 0.01	0.04 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
Total $n-3$	1.93 ± 1.69	0.88 ± 0.16	0.62 ± 0.02	0.98 ± 0.03	0.98 ± 0.00
Total polyunsaturated	***15.54*** ± 0.32	13.53 ± 0.78	10.02 ± 0.02	11.73 ± 0.24	***13.46*** ± 0.15

[a] C12:0, C15:0, C17:0.
[b] C14:1, C17:1.
[c] C18:3 ($n-6$), C20:2 ($n-6$), C22:4 ($n-6$).
[d] C20:3 ($n-3$), C22:6 ($n-3$).

Table 3
Changes in fatty acid composition of triacylglycerides of the intramuscular fat during processing (g/100 g of total fatty acids)

Fatty acids	Triglyceride fraction				
	Raw ham	Salted ham	Post-salted ham	Half-cured ham	Cured ham
C14:0	1.69 ± 0.06	1.21 ± 0.03	1.27 ± 0.03	1.21 ± 0.02	1.43 ± 0.04
C16:0	*24.78* ± 0.32	23.55 ± 0.09	23.93 ± 0.02	23.38 ± 0.08	*24.49* ± 0.51
C18:0	10.24 ± 0.21	10.83 ± 0.07	11.55 ± 0.51	11.83 ± 0.01	11.10 ± 0.20
Others[a]	0.58 ± 0.08	0.58 ± 0.07	0.59 ± 0.09	0.62 ± 0.04	0.50 ± 0.04
Total saturated	***37.28*** ± 0.66	36.16 ± 0.25	37.34 ± 0.12	37.05 ± 0.03	***37.51*** ± 0.78
C16:1	4.64 ± 0.12	3.74 ± 0.32	3.66 ± 0.07	3.00 ± 0.07	4.04 ± 0.46
C18:1	*49.87* ± 0.30	49.93 ± 0.71	50.49 ± 0.09	49.32 ± 0.07	*49.59* ± 1.38
Others[b]	0.95 ± 0.03	1.07 ± 0.09	0.96 ± 0.10	1.05 ± 0.02	0.95 ± 0.09
Total monounsaturated	***55.46*** ± 0.44	54.74 ± 0.93	55.11 ± 0.54	53.38 ± 0.12	***54.58*** ± 1.76
C18:2 ($n-6$)	*4.87* ± 0.10	6.28 ± 0.33	4.45 ± 0.02	7.05 ± 0.09	*5.78* ± 0.50
C20:4 ($n-6$)	0.21 ± 0.02	0.12 ± 0.10	0.20 ± 0.04	0.23 ± 0.01	0.15 ± 0.14
Others[c]	0.41 ± 0.00	0.57 ± 0.09	0.54 ± 0.07	0.67 ± 0.01	0.62 ± 0.10
Total $n-6$	5.50 ± 0.12	6.97 ± 0.38	5.19 ± 0.04	7.94 ± 0.10	6.55 ± 0.75
C18:3 ($n-3$)	0.43 ± 0.02	0.48 ± 0.05	0.34 ± 0.12	0.52 ± 0.004	0.40 ± 0.16
C20:5 ($n-3$)	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.09	0.00 ± 0.00	0.00 ± 0.00
C22:5 ($n-3$)	0.00 ± 0.00	0.04 ± 0.03	0.05 ± 0.01	0.08 ± 0.001	0.06 ± 0.08
Others[d]	0.82 ± 0.16	0.72 ± 0.44	0.85 ± 0.02	0.07 ± 0.005	0.14 ± 0.10
Total $n-3$	1.25 ± 0.18	1.24 ± 0.46	1.24 ± 0.01	0.68 ± 0.001	0.60 ± 0.14
Total polyunsaturated	***6.74*** ± 0.30	8.21 ± 0.83	6.43 ± 0.03	8.62 ± 0.09	***7.15*** ± 0.89
Polyunsaturated/total	0.07	0.08	0.06	0.09	0.07

[a] C12:0, C15:0, C17:0.
[b] C17:1, C20:1.
[c] C18:3 ($n-6$), C20:2 ($n-6$), C20:3 ($n-6$), C22:4 ($n-6$).
[d] C20:3 ($n-3$), C22:6 ($n-3$).

3.1.3. Phospholipid fraction

The saturated fatty acids (SFA) represent 25.09% and the MUFA 16.22% of the total fatty acids in the PL fraction of the intramuscular fat of the raw ham (oleic acid 15.21%) (Table 4). PUFA are the most abundant (42.75%) fatty acids in this fraction. Linoleic acid predom-

Table 4
Changes in fatty acid composition of phospholipid of the intramuscular fat during processing (g/100 g of total fatty acids)

Fatty acids	Phospholipid fraction				
	Raw ham	Salted ham	Post-salted ham	Half-cured ham	Cured ham
C14:0	0.14 ± 0.03	0.29 ± 0.28	0.35 ± 0.24	0.13 ± 0.01	0.31 ± 0.44
C16:0	*12.46* ± 0.29	17.13 ± 3.36	15.31 ± 0.51	17.50 ± 4.89	15.47 ± 3.11
C18:0	*11.61* ± 0.22	12.79 ± 0.42	9.32 ± 1.28	12.61 ± 0.04	9.92 ± 1.18
Others[a]	0.88 ± 0.18	0.37 ± 0.40	0.00 ± 0.00	0.34 ± 0.48	0.00 ± 0.00
Total saturated	***25.09*** ± 0.73	30.58 ± 4.46	24.98 ± 2.04	30.58 ± 5.43	***25.70*** ± 4.72
C16:1	0.71 ± 0.01	0.37 ± 0.42	0.00 ± 0.00	0.60 ± 0.33	0.00 ± 0.00
C18:1	*15.21* ± 0.29	16.80 ± 3.86	15.01 ± 0.82	14.72 ± 1.70	18.53 ± 4.64
Others[b]	0.29 ± 0.02	1.42 ± 0.76	1.32 ± 0.94	1.53 ± 1.37	0.63 ± 0.89
Total monounsaturated	***16.22*** ± 0.33	18.59 ± 5.04	16.33 ± 1.76	16.86 ± 3.40	***19.16*** ± 5.53
C18:2 ($n-6$)	*27.39* ± 0.93	28.71 ± 1.44	32.14 ± 0.52	30.17 ± 1.62	32.48 ± 2.22
C20:4 ($n-6$)	11.68 ± 0.08	5.67 ± 5.02	7.06 ± 0.83	0.03 ± 0.04	6.79 ± 0.35
Others[c]	3.68 ± 0.26	2.61 ± 0.60	0.00 ± 0.00	2.88 ± 0.72	0.51 ± 0.73
Total $n-6$	42.75 ± 1.27	36.99 ± 7.06	39.20 ± 1.35	33.08 ± 2.38	39.78 ± 3.30
C18:3 ($n-3$)	0.67 ± 0.03	0.65 ± 0.10	0.00 ± 0.00	0.47 ± 0.09	0.00 ± 0.00
C20:5 ($n-3$)	0.47 ± 0.01	0.55 ± 0.10	0.00 ± 0.00	0.43 ± 0.02	0.00 ± 0.00
C22:5 ($n-3$)	1.26 ± 0.03	1.24 ± 0.11	0.00 ± 0.00	0.56 ± 0.79	0.00 ± 0.00
Others[d]	0.00 ± 0.00	3.64 ± 6.31	0.00 ± 0.00	10.22 ± 0.99	0.00 ± 0.00
Total $n-3$	2.39 ± 0.07	6.08 ± 6.61	0.00 ± 0.00	11.69 ± 1.90	0.00 ± 0.00
Total polyunsaturated	***42.75*** ± 1.27	40.63 ± 13.36	39.20 ± 1.35	43.30 ± 3.37	***39.78*** ± 3.30
Polyunsaturated/total	0.18	0.13	0.09	0.14	0.09

[a] C12:0, C15:0, C17:0.
[b] C17:1, C20:1.
[c] C18:3 ($n-6$), C20:2 ($n-6$), C20:3 ($n-6$), C22:4 ($n-6$).
[d] C20:3 ($n-3$), C22:6 ($n-3$).

inates with 27.39% of the total fatty acids. MUFA and PUFA are the most affected by the curing process in this fraction, MUFA increase since 16.22% in raw ham to 19.16% in cured ham, and PUFA decrease since 42.75% to 39.78%. It is well known that PUFA are especially sensitive to oxidation and degradation phenomena both by enzymatic and chemical oxidation, which generate a great variety of volatile compounds (Coutron-Gambotti & Gandemer, 1999; Toldrá, 1998). The degradation of the components of the PL fraction plays an important role in the development of the flavour of cured ham. These results agree with those obtained by other authors for Serrano ham (Flores et al., 1985).

3.1.4. Free fatty acid fraction

In the FFA fraction of the intramuscular fat of the raw ham (Table 5), SFA (36.16%) and polyunsaturated ones (37.76%) are present in similar amounts. Of the SFA, the predominant one is palmitic acid (24.62%) followed by stearic acid (10.54%). Oleic acid represents 25.30% of the total of analysed fatty acids, whereas linoleic acid (27.20%) is predominant in the polyunsaturated ones. The predominant fatty acids at the end of the "PDO Teruel" curing process are linoleic (29.89%), oleic (20.64%), palmitic (19.73%) and stearic (12.20%). On the other hand, it can been observed that in the FFA fraction, the proportion of PUFA increases from 37.76% in raw ham to 44.18% at the end of the process. Taking into account that the biggest decrease of PUFA takes place in the PL fraction, it may be considered that the PUFA analysed in the FFA fraction during processing come principally from PL fraction by hydrolysis. The results are similar to those obtained by other investigators in cured ham (Martín et al., 1999).

3.2. Changes in fatty acids of subcutaneous fat

Table 6 shows the fatty acid composition of the total lipid fraction of the fat associated to the *biceps femoris* muscle or subcutaneous fat.

SFA make up 37.54% of the subcutaneous fat. The composition of fatty acids present in the subcutaneous fat in raw ham is mainly characterised by a high percentage of MUFA, specifically oleic acid (46.88%). The relative percentage of PUFA is 10.39% in the subcutaneous fat, whereas in the intramuscular fat it is 15.54%. These results agree with others authors who have studied the lipolysis and oxidation in subcutaneous adipose tissue during dry-cured processing (Coutron-Gambotti et al., 1999). The SFA composition of the subcutaneous fat throughout the process is highly stable. The MUFA, however, suffer an important drop, from 50.70% in raw ham to 42.98% in the final product, where the sharpest fall is oleic acid, from 46.88% in raw ham to 40.11% in cured ham. This decrease coincides with a parallel increase in the PUFA, from 10.39% in raw ham to 17.17% in the final product.

Table 5
Changes in free fatty acid composition of the intramuscular fat during processing (g/100 g of total fatty acids)

Fatty acids	Free fatty acid fraction				
	Raw ham	Salted ham	Post-salted ham	Half-cured ham	Cured ham
C14:0	0.86 ± 0.15	1.04 ± 0.19	1.24 ± 0.31	0.64 ± 0.11	0.47 ± 0.01
C16:0	*24.62* ± 0.45	24.23 ± 1.47	27.30 ± 1.70	20.31 ± 0.72	*19.73* ± 0.66
C18:0	*10.54* ± 0.14	12.71 ± 2.02	15.18 ± 0.59	14.04 ± 0.10	*12.20* ± 0.16
Others[a]	0.15 ± 0.21	0.79 ± 0.70	1.35 ± 0.10	0.63 ± 0.06	0.64 ± 0.03
Total saturated	***36.16*** ± 0.66	38.78 ± 3.83	45.07 ± 2.70	35.61 ± 0.99	***33.05*** ± 0.84
C16:1	0.55 ± 0.77	1.44 ± 0.34	1.31 ± 0.24	1.29 ± 0.08	1.02 ± 0.06
C18:1	*25.30* ± 1.06	26.91 ± 1.21	28.40 ± 1.11	29.16 ± 0.18	*20.64* ± 0.50
Others[b]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total monounsaturated	***25.85*** ± 0.28	28.35 ± 1.54	29.71 ± 1.35	30.45 ± 0.10	***21.66*** ± 0.56
C18:2 ($n-6$)	*27.20* ± 0.13	23.32 ± 1.50	17.69 ± 0.77	23.38 ± 0.79	*29.89* ± 0.38
C20:4 ($n-6$)	7.87 ± 1.23	6.74 ± 0.50	6.04 ± 0.31	7.19 ± 0.81	8.37 ± 0.19
Others[c]	0.70 ± 0.99	0.28 ± 0.49	0.00 ± 0.00	1.11 ± 0.28	3.13 ± 0.01
Total $n-6$	35.77 ± 0.36	30.34 ± 2.00	23.73 ± 1.09	31.68 ± 0.26	41.39 ± 0.21
C18:3 ($n-3$)	2.00 ± 0.33	1.44 ± 0.46	1.43 ± 0.17	0.84 ± 0.05	0.70 ± 0.08
C20:5 ($n-3$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.75 ± 0.05	0.41 ± 0.04
C22:5 ($n-3$)	0.00 ± 0.00	0.95 ± 1.64	0.00 ± 0.00	0.53 ± 0.74	1.31 ± 0.03
Others[d]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.37 ± 0.06
Total $n-3$	2.00 ± 0.33	2.38 ± 1.20	1.43 ± 0.17	2.11 ± 0.85	2.79 ± 0.16
Total polyunsaturated	***37.76*** ± 0.69	32.72 ± 2.54	25.16 ± 1.26	33.79 ± 1.11	***44.18*** ± 0.05
Polyunsaturated/total	0.38	0.33	0.25	0.34	0.45

[a] C12:0, C15:0, C17:0.
[b] C17:1, C20:1.
[c] C18:3 ($n-6$), C20:2 ($n-6$), C20:3 ($n-6$), C22:4 ($n-6$).
[d] C20:3 ($n-3$), C22:6 ($n-3$).

Table 6
Fatty acid composition (g fatty acid/100 g total fatty acids) of the subcutaneous fat during processing

Fatty acids	Total lipids				
	Raw ham	Salted ham	Post-salted ham	Half-cured ham	Cured ham
C14:0	1.67 ± 0.10	1.20 ± 0.004	1.26 ± 0.05	1.27 ± 0.13	1.32 ± 0.03
C16:0	24.78 ± 0.04	22.97 ± 0.04	23.18 ± 0.53	24.90 ± 3.45	23.31 ± 0.15
C18:0	10.40 ± 0.57	12.16 ± 0.45	11.64 ± 0.36	12.51 ± 1.43	10.63 ± 0.60
Others[a]	0.69 ± 0.03	0.86 ± 0.09	1.04 ± 0.02	1.59 ± 0.21	0.84 ± 0.01
Total saturated	***37.54*** ± 0.40	37.19 ± 0.50	37.12 ± 0.96	40.27 ± 5.22	***36.09*** ± 0.73
C16:1	3.50 ± 0.38	2.15 ± 0.06	2.54 ± 0.01	2.61 ± 0.35	2.51 ± 0.07
C18:1	*46.88* ± 0.16	43.69 ± 1.57	44.58 ± 1.05	42.93 ± 6.39	*40.11* ± 3.53
Others[b]	0.32 ± 0.00	0.27 ± 0.00	0.59 ± 0.02	0.28 ± 0.04	0.36 ± 0.01
Total monounsaturated	***50.70*** ± 0.22	46.10 ± 1.63	47.70 ± 1.01	45.82 ± 6.00	***42.98*** ± 3.61
C18:2 ($n-6$)	8.69 ± 0.11	12.56 ± 0.35	11.60 ± 0.06	10.68 ± 0.95	14.85 ± 0.41
Others[c]	0.90 ± 0.02	1.10 ± 0.07	1.02 ± 0.01	0.97 ± 0.02	1.11 ± 0.01
Total $n-6$	9.58 ± 0.08	13.65 ± 0.28	12.62 ± 0.05	11.65 ± 0.93	15.97 ± 0.42
C18:3 ($n-3$)	0.70 ± 0.003	0.97 ± 0.02	0.90 ± 0.01	1.18 ± 0.23	1.06 ± 0.02
Others[d]	0.11 ± 0.01	0.18 ± 0.002	0.13 ± 0.001	0.15 ± 0.03	0.14 ± 0.002
Total $n-3$	0.81 ± 0.00	1.15 ± 0.03	1.03 ± 0.01	1.33 ± 0.26	1.20 ± 0.02
Total polyunsaturated	***10.39*** ± 0.08	14.80 ± 0.31	13.65 ± 0.04	12.98 ± 1.19	***17.17*** ± 0.44

[a] C12:0, C15:0, C17:0.
[b] C17:1, C20:1.
[c] C18:3 ($n-6$), C20:2 ($n-6$), C20:3 ($n-6$), C22:4 ($n-6$).
[d] C20:3 ($n-3$), C20:5 ($n-3$), C22:5 ($n-3$), C22:6 ($n-3$).

3.3. Microstructural changes in the intramuscular fat

The microstructural changes that take place in the intramuscular and subcutaneous fat of the "PDO Teruel" dry-cured ham during the process provide relevant information in order to understand the chemical changes that take place during the process and that have been studied in this paper. The modifications observed in the microstructure of the intramuscular adipose tissue during the process of curing "PDO Teruel" ham would explain the fat's availability to

the lipolitic enzymes which would be active throughout the whole process working on the fat released from the adipocytes.

Fig. 1 shows the intramuscular fat observed by Cryo-SEM. In raw ham (Fig. 1A), the adipocytes, surrounded by a membrane and full of fat in the amorphous state, are situated in the perimysial connective tissue zone. In salted ham (Fig. 1B) it is possible to observe how some fat has already been released from the adipocytes, and by coalescence forms small areas of free fat. The released fat is now situated in the intercellular spaces. At the end of the process, in the dry-cured ham (Fig. 1C), the fat and the products of lipolysis are free in the ham and infiltrating the muscular tissue cells, where the adipocytes were originally situated. The phospholipid degradation from the membranes could also explain the increase in the free fatty acid fraction observed at the end of the process in the chemical analysis. The liberated fat makes an effective contribution to the typical flavour and taste of cured ham (Antequera et al., 1992; Motilva, Toldrá, Nieto, & Flores, 1993; Toldrá, Flores, & Sanz, 1997; Toldrá, 1998).

Fig. 2 shows a transverse section of the *biceps femoris* of raw ham, salted ham and cured ham, observed by SEM. The intramuscular fat of the raw ham observed by SEM (Fig. 2A) is made up of spherical adipocytes surrounded by a membrane and very closely joined together, among which can be seen perimysial connective tissue fibres. In salted ham (Fig. 2B) small areas of fat released from the adipocytes can be observed and, in dry-cured ham (Fig. 2C), this fat occupies large areas in the zones where the original adipose tissue was situated. In Fig. 2D there is a detail of fat droplets situated inside the muscle tissue of the dry-cured ham. Due to the fact that osmium tetroxide is used as a fixer and that water is eliminated from the samples prior to observation, it must be pointed out that the SEM technique makes it easier to observe the fat released from the adipocytes throughout the process, which coalesces and infiltrates the muscle cells.

3.4. Microstructural changes in the subcutaneous fat

In Fig. 3 the subcutaneous fat of raw ham, salted ham and "PDO Teruel" cured ham is observed by Cryo-SEM. This layer of adipose tissue acts as a genuine barrier between the muscle of *biceps femoris* and the ham's surface. Fig. 3A shows how the polygonal adipocytes fit together perfectly and are joined by fine fibres of connective tissue (Fig. 3B). The interior of the adipocytes is full of fat in an amorphous state. Fig. 3C shows the structure of the subcutaneous fat of salted ham; during the process the saline solution penetrates into the tissue, and infiltrates the cells which are seen to be separated by large intercellular spaces full of saline solution. The typical eutectic artefact can be observed in these spaces, indicating the presence of solutes, which, in this case, come from the saline solution. Fig. 3D shows a transversal cut in the subcutaneous fat of dry-cured ham. The cells now make up a compact tissue due to the product's water loss during the final stages of the process (post-salting and ripening); free fat has spread among the subcutaneous adipose tissue cells.

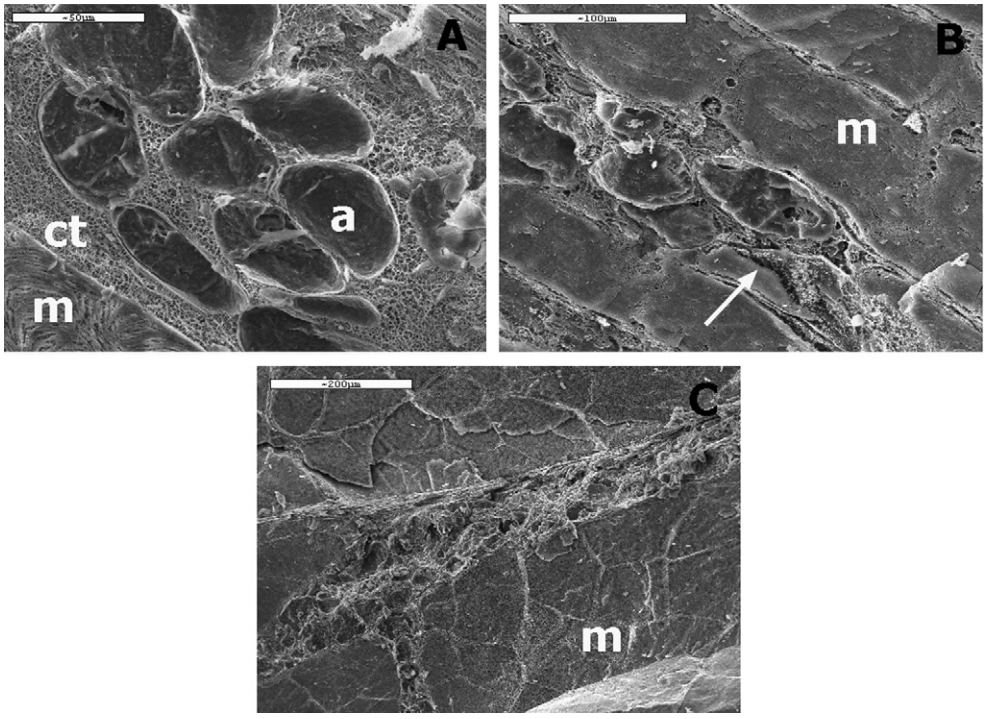

Fig. 1. Transversal section of intramuscular fat in *biceps femoris* of raw ham (A), salted ham (B) and "PDO Teruel" dry-cured ham (C) observed by Cryo-SEM. (a, adipocytes; ct, connective tissue; m, muscular tissue; arrow, fat released from the adipocytes).

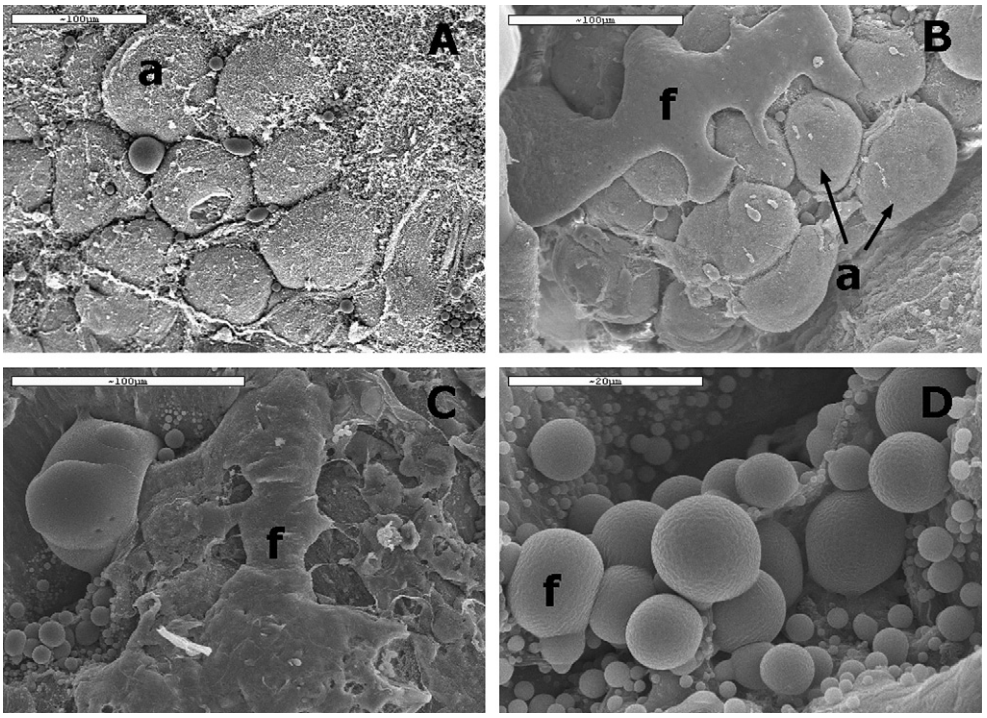

Fig. 2. Transversal section of intramuscular fat in *biceps femoris* of raw ham (A), salted ham (B) and "PDO Teruel" dry-cured ham (C and D) observed by SEM. (f, free fat released from the adipocytes; a, adipocytes).

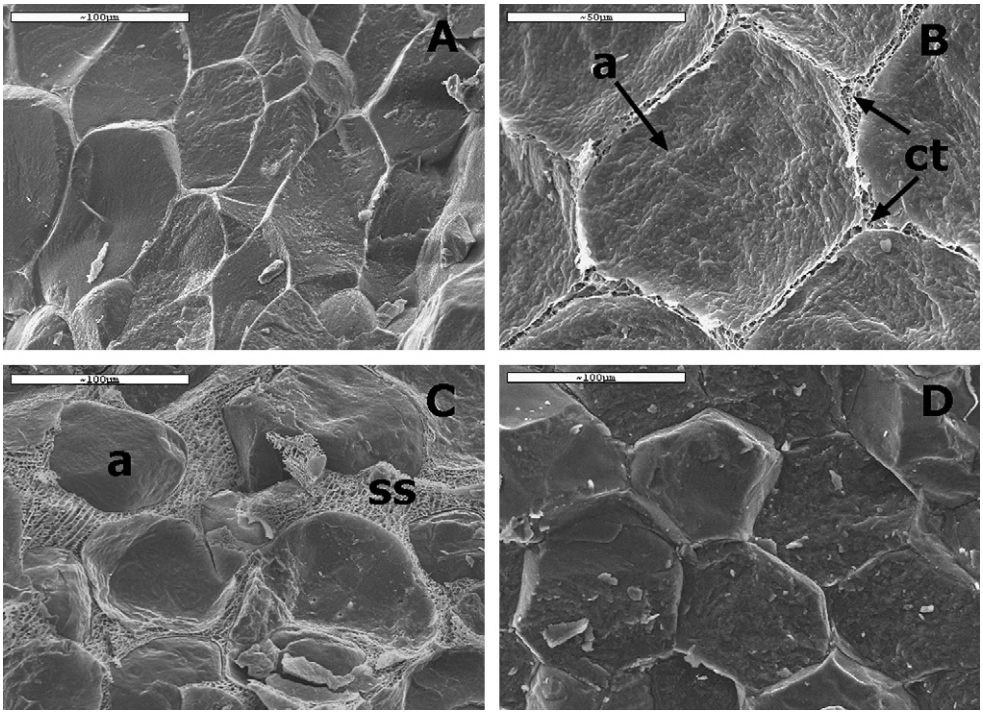

Fig. 3. Transversal section of the subcutaneous fat of raw ham (A and B), salted ham (C) and "PDO Teruel" cured ham (D) observed by Cryo-SEM. (a, adipocytes; ct, connective tissue; ss, saline solution).

When the subcutaneous fat of raw ham is observed by SEM (Fig. 4), it can be seen that this tissue is structured by groups of rounded adipose cells surrounded by connective tissue (Fig. 4A). Fig. 4B shows the subcutaneous fat of salted ham. The membranes of some of the adipocytes are degraded, and free fat can be seen.

The subcutaneous adipose tissue of dry-cured ham (Fig. 4C and D) shows large areas of plasmolysed adipose

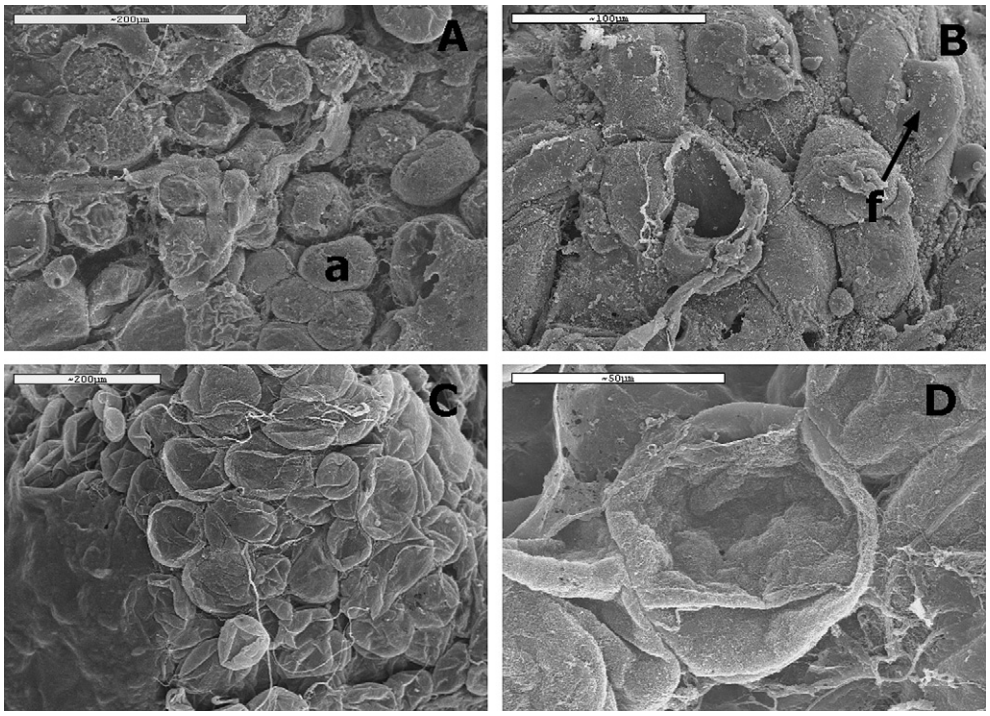

Fig. 4. Transversal section of the subcutaneous fat of raw ham (A), salted ham (B), and "PDO Teruel" cured ham (C and D) observed by SEM. (a, adipocytes; f, free fat).

cells if compared to the subcutaneous fat of raw ham. The degradation of the adipocytes membranes throughout the process allows the fat be released from the cells. In the "PDO Teruel" cured ham, the liberated fat stays in the adipose cells. The structural changes in the adipose tissue throughout the process would explain the ability for lipase activity and the changes in the consistence and fluidity of the fat, the juiciness and the perception of the typical flavour of dry-cured ham.

4. Conclusions

The chemical changes which occur in fat during the curing process of "PDO Teruel" ham, affects the components of the intramuscular adipose tissue and agree with the microstructural modifications of this tissue.

During the 365 days of processing, the fatty acid composition of phospholipids decreases significantly; however, the free fatty acid content increases. There seems to be a relationship between the degradation of phospholipids and the increase in the free fatty acid content, especially polyunsaturated fatty acids.

The intramuscular fat of the raw ham is made up of spherical adipocytes surrounded by a membrane and very closely joined together. In salted ham small areas of fat released from the adipocytes can be observed and in dry-cured ham, this fat occupies large areas in the zones where the original adipose tissue were situated.

The microstructural changes of the adipose tissue during the process would explain the availability of the fat to the lipolitic enzymes and the contribution to its texture, the juiciness and the perception of the typical flavour and taste of dry-cured ham.

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