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Subcutaneous and intermuscular fat characterisation of dry-cured Iberian hams

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Abstract

The fatty acid composition of the triacylglycerols and free fatty acids of subcutaneous (internal and superficial layers) and intermuscular fat and the contribution of these fatty acids to the formation of volatile compounds were determined in dry-cured Iberian ham. The profile of the fatty acids and volatile compounds showed that lipolytic and oxidative processes occur more intensively in subcutaneous than intermuscular fat, however, few differences were found compared to those found in ham lean.
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Keywords: Iberian dry-cured ham; Subcutaneous and intermuscular fat; Fatty acids; Volatile compounds

1. Introduction

Subcutaneous, intermuscular and intramuscular fat are important components of dry-cured ham, which vary in quantity and quality according to genotype, feeding system and ripening process.

Iberian pigs are characterised by a high level of intramuscular fat and their hams also have a large subcutaneous layer (Mayoral et al., 1999). This fat is rich in monounsaturated and polyunsaturated fatty acids (MUFA and PUFA) in the hams of pigs raised on an extensive system based on free availability of acorns and pasture (“montanera”; Flores, Biron, Izquierdo & Nieto, 1988).

This fact has both sensorial and nutritional implications. The nutritional aspect is related to the fatty acid composition since it is known that PUFA- and MUFA-rich diets decrease cholesterol levels in blood and are related to a low incidence of cardiovascular diseases (Mattson & Grundy, 1985). On the other hand, the fat influences the sensory quality of the ham. During dry-curing the lipids are subjected to both lipolytic and oxidation processes. Lipolysis produces free fatty acids that can undergo oxidation and give rise to volatile compounds that are responsible for the ham’s characteristic flavour (García, Berdagué, Antequera, López-Bote, Córdoba & Ventanas, 1991).

Previous studies have shown that lipolytic changes occur specifically in unsaturated triacylglycerols and oxidative reactions in unsaturated fatty acids (Coutron-Gambotti & Gandermer, 1999). Therefore, differences in volatile patterns are influenced by the fatty acid composition of the lipids which is related to the composition of the diet. Pig feeding in extensive systems based on acorns and pasture gives rise to hams with a unique and intense flavour that has not been found in any other type of dry-cured ham (García, Ventanas, Antequera, Ruiz, Cava & Álvarez, 1996). These hams are the most valuable and command the highest prices in the market.

Several studies have looked at the characteristics of the fat from Iberian hams but they have focused on the intramuscular fat (Cava, Ruiz, Ventanas & Antequera, 1999). However, subcutaneous and intermuscular fat has not been studied although such fats represent a large amount of the product and are eaten by consumers.

The aim of this work was to characterise the subcutaneous (superficial and internal layer) and intermuscular fat of dry-cured Iberian hams by studying the fatty acid composition of the triacylglycerols and the free fatty acid fractions of the lipids and the

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contribution these fatty acids make to the formation of the aroma.

2. Materials and methods

2.1. Samples

Subcutaneous (internal and superficial layer) and intermuscular fat (easily accessible on the *Biceps femoris* muscle; Fig. 1) of 10 hams from Iberian pigs, produced traditionally (Fig. 2; García et al., 1991), were analysed. The pigs were fed on a traditional extensive system based on acorns and pasture during the fattening period (last eight weeks prior to slaughter). These hams were graded "high quality" in the market.

2.2. Fatty acid determination

Total lipids from 10 g of internal and superficial layer, which constitute subcutaneous tissue, and intermuscular fat were extracted with a chloroform:methanol mixture (1:2) by the method of Bligh and Dyer (1959). Fats obtained (0.1 g) were fractionated into neutral lipids and free fatty acids on NH_2-aminopropil minicolumns as described by García-Regueiro, Gilbert and Díaz (1994). The phospholipid fraction was not analysed in this study because it is present in insignificant amounts in the subcutaneous and intermuscular fat.

Fatty acid methyl esters of the triacylglycerol and free fatty acid fractions were prepared by acidic trans-sterification in the presence of sulphuric acid and were analysed (0.1 μl) by gas chromatography using a Hewlett Packard 5890A system, equipped with an on-column injector and a flame-ionization detector (FID). A capillary column (30-m length; 0.53-mm internal diameter) coated with FFAP-TPA stationary phase (1-μm thickness) was used to separate the fatty acids. The temperature of the column was maintained at 225°C for the whole run (30 min). The temperature of the injector and detector was 230°C. The carrier gas (nitrogen) had a flow rate of 2.6 ml/min.

Identification of the fatty acids was by comparison of the retention times with those of reference compounds (Sigma). Amounts of fatty acids from the two fractions (triacylglycerols and free fatty acids) were expressed as a percentage of the total area.

The effect of the location (internal and superficial layer of the subcutaneous fat and intermuscular fat) on the triacylglycerol and free fatty acid compositions of

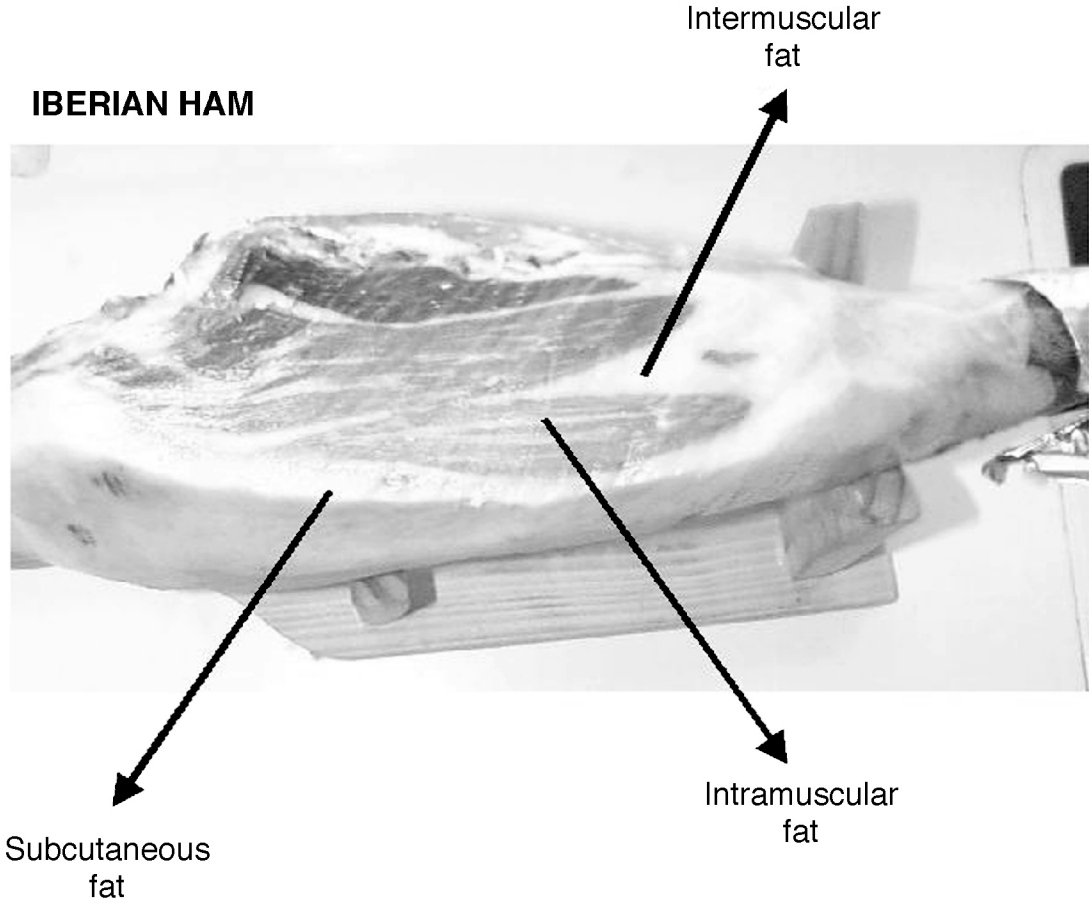

Fig. 1. Sliced dry-cured Iberian ham showing the subcutaneous, intermuscular and intramuscular fat.

the fat was assessed by analysis of variance using the general linear model of SAS, significant means were compared by a Tukey test at $P \leqslant 0.05$.

2.3. Volatile compound determination

The isolation of volatile compounds by the dynamic headspace technique was carried out with an Automatic System Hewlett-Packard G1900A Purge and Trap Concentrator. The fat sample (8 g) was held at 40°C for 10 min, and subsequently, for 30 min at 35°C while the volatile substances were purged with purified helium and adsorbed on a Tenax trap held at −20°C by carbon dioxide. The compounds were thermally desorbed onto the gas chromatograph (Hewlett Packard 5890 series II) by heating at 225°C for 2 min. The transfer line temperature was 200°C.

Separation of volatiles was performed on a 5% Phenyl-Methyl Silicone (DB-5) bonded phase fused silica capillary column (50 m×0.32 mm i.d., film thickness 1.05 μm). Oven program was: 35°C, 10 min; 7°C min^{-1} to 150°C; 20°C min^{-1} to 250°C and holding at this temperature for 5 min. The transfer line temperature to the mass spectrometer was 280°C.

Identification of volatiles was carried out in a mass selective detector (Hewlett Packard 5971A) with electron ionization at 1756 V. The mass detector temperature was set at 280°C. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST/EPA/NIH and Wiley libraries and by comparison of Kovats indices with those reported in the literature by Acree and Arn (1997) and Kondjoyan and Berdagué (1996).

3. Results and discussion

3.1. Triacylglycerol composition

The fatty acid contents of the triacylglycerols of subcutaneous and intermuscular fat in the hams agrees with other researchers studying Iberian pigs fed on acorns and pasture (Flores et al., 1988). The most abundant fatty acid is oleic acid, followed by palmitic acid; stearic and linoleic acids are also present in relatively large proportions (Table 1). These results are similar to those found in the intramuscular fat of these same hams (Cava et al., 1999; De la Hoz, López, Hierro, Cambero & Ordóñez, 1996). Fat composition is influenced by the pig's diet; fatty acids from the food accumulate in the fat of animals with little change (Miller, Shackelford, Hayden & Reagan, 1990) and as shown above, they remain in the ham after processing.

Despite the fact that compositions of the subcutaneous and intermuscular fat are similar (Jaturasitha, Kreuzer, Lange & Köhler, 1996), some differences can be observed in the fatty acid profile of the triacylglycerols (Table 1), not only between the two types of fat but also in the different layers of the subcutaneous fat. The largest quantities of PUFA are in triacylglycerols well below the surface (intermuscular and internal subcutaneous fat) suggesting these polyunsaturated triacylglycerols

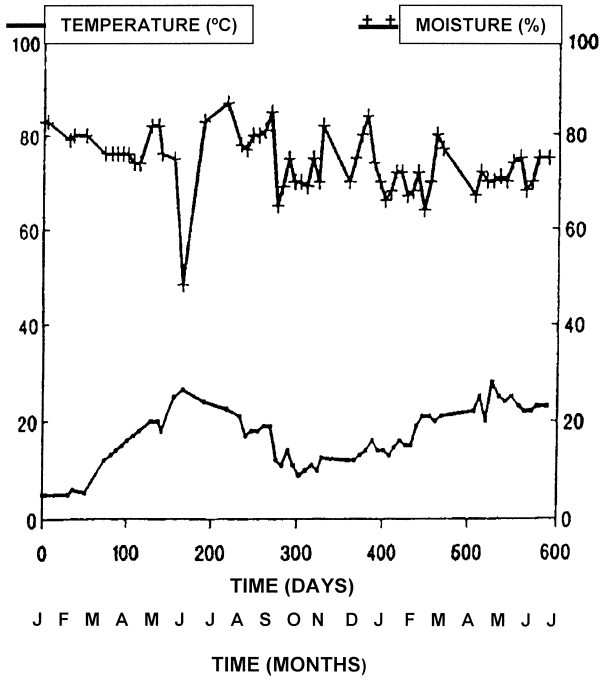

Fig. 2. Temperatures and relative humidity processes during Iberian ham processing.

Table 1
Fatty acid composition (expressed as percentage) of triacylglycerols of the subcutaneous and intermuscular fat from dry-cured Iberian hams[a]

Fatty acid	Location		
	Subcutaneous fat		Intermuscular fat
	Superficial layer	Internal layer	
12:0 Lauric	0.11±0.03	0.12±0.02	0.07±0.00
14:0 Myristic	1.44±0.09	1.25±0.14	1.27±0.03
16:0 Palmitic	23.82±0.52	23.74±0.80	22.92±0.28
16:1 Palmitoleic	3.39±0.30	3.84±0.26	3.39±0.13
18:0 Stearic	9.09±1.03	9.25±0.41	7.45±0.84
18:1 Oleic	53.97±0.84a,b	52.42±0.81b	54.51±0.66a
18:2 Linoleic	7.66±0.24b	8.78±0.25a,b	9.41±0.38a
18:3 Linolenic	0.34±0.03b	0.45±0.06b	0.65±0.03a
20:0 Araquidic	0.12±0.02	0.10±0.02	0.22±
20:4 Arachidonic	0.05±0.02	0.04±0.01	0.11±
ΣSFA	34.58±0.76	34.46±0.70	31.93±
ΣMUFA	57.36±0.72	56.27±0.65	57.90±
ΣPUFA	8.05±0.25b	9.27±0.26b	10.17±a

[a] Means with different letters (a,b) indicate significant differences among locations ($P < 0.05$, Tukey's test). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

have been less exposed to conditions which favour lipolysis, such as temperature (Martín, Córdoba, Ventanas & Antequera, 1999). This result does not agree with those found in raw subcutaneous fat, where the superficial layers are more unsaturated than the internal layers (Irie & Sakimoto, 1992). Nevertheless, oleic acid is present in slightly larger quantities in the superficial layer, suggesting less degradation of the triacylglycerols containing this fatty acid compared with the polyunsaturated triacylglycerols. Thus, the polyunsaturated triacylglycerols could be more susceptible to lipolysis, and decrease more during curing (Díaz & García-Regueiro, 1991). Obviously, if these triacylglycerols have been degraded their fatty acids will be present in smaller quantities, especially in the superficial layer of subcutaneous fat, where the polyunsaturated triacylglycerols are found in larger quantities.

Coutron-Gambotti and Gandemer (1999), suggest that unsaturated fatty acids are susceptible to lipases in the water–oil interface where these fatty acids are liquid at 14–18°C during curing. Therefore, the largest quantities of these unsaturated triacylglicerols in the superficial layer of subcutaneous fat would lead to more intense lipolysis which would result in a greater decrease of the fatty acids that constitute these triacylglycerols.

3.2. Free fatty acid composition

The free fatty acid profiles of subcutaneous and intermuscular fat should support the trend of triacylglycerol degradation, and at the same time, reflect the autooxidation of these free fatty acids (Table 2). The smaller PUFA contents in the superficial layer of the subcutaneous fat suggests that, despite the intense lipolysis in this layer, these fatty acids are more susceptible to oxidation, due to greater exposure to oxygen and light (Antequera et al., 1992), than those in the internal subcutaneous and intermuscular fat. However, palmitic and palmitoleic acids are present in higher concentrations in the superficial layer which is expected since the MUFA are more resistant to oxidation. Saturated fatty acids do not oxidise (Gray & Pearson, 1987), and thus these fatty acids disappear very slowly if at all.

3.3. Volatile compound analysis

Volatile concentrations are higher in the subcutaneous fat than in the intermuscular fat (Table 3) because their formation involves oxidation processes which are greatest at the surface. This is supported by the lower concentration of polyunsaturated free fatty acids in the subcutaneous fat (Table 2).

The most abundant aldehydes were hexanal, pentanal, heptanal and octanal, which arise from unsaturated fatty acid oxidation (Grosch, 1987); therefore, they are present in higher concentrations in the superficial subcutaneous fat (Table 3). The most prevalent ketone (propanone) has a similar origin (Larick, Turner, Schoenherr, Coffey & Pilkington, 1992). Aldehydes with a different origin to that outlined above are present in smaller quantities (2- and 3-methylbutanal; Ruiz, Ventanas, Cava, Andrés & García, 1999).

The most abundant alcohol, 2-propanol, is found in higher concentrations in the internal layer of the subcutaneous fat (Table 3). This alcohol can arise from amino acids by means of Strecker degradation reactions (Flores, Spanier & Toldrá, 1998); therefore, it is present in the lean and in the fat adjacent to the lean.

Similar concentrations of hydrocarbons are found in the internal and superficial layers of the subcutaneous fat because branched alkanes, aromatic hydrocarbons and terpenes are associated with the unsaponifiable fraction of the diet (Ruiz et al., 1999), which is deposited in the animal fat without change in both the superficial and internal layers.

It is seen that volatile concentrations begin to decrease in the superficial layer of subcutaneous fat as the chain size increases (Table 3). For example, the aldehydes nonanal, 2-nonenal, decanal, 2-decenal, 2-undecenal are present in higher concentrations in the interior of ham. The same occurs with the alcohols 1-heptanol, 1-octanol and 1-octen-3-ol and with ketones 2-heptanone, 2-octanone, 2-nonanone and 2-decanone. This could be because degradation of these compounds in the superficial layer of the subcutaneous fat increases compared to those in the internal fat due to oxidation.

Table 2
Fatty acid composition (expressed as percentage) of free fatty acids of the subcutaneous and intermuscular fat from dry-cured Iberian hams[a]

Fatty acid	Location		
	Subcutaneous fat		Intermuscular fat
	Superficial layer	Internal layer	
12:0 Lauric	0.10±0.04	0.09±0.03	0.13±0.01
14:0 Myristic	2.42±0.23	2.57±0.32	2.20±0.08
16:0 Palmitic	31.50±1.23a	29.97±1.69b	28.62±0.78b
16:1 Palmitoleic	4.52±0.38a	5.06±0.48a	3.65±0.15b
18:0 Stearic	7.76±0.65	7.43±0.51	7.84±0.22
18:1 Oleic	46.93±1.27	45.54±1.85	46.77±0.67
18:2 Linoleic	6.63±0.45b	9.14±0.35a,b	9.60±0.53a
18:3 Linolenic	0.11±0.05b	0.16±0.06b	0.72±0.06a
20:0 Araquidic	0.04±0.03	0.02±0.02	0.34±0.10
20:4 Arachidonic	0.02±0.01	0.01±0.01	0.12±0.01
ΣSFA	41.82±1.64	40.09±1.57	39.13±0.91
ΣMUFA	51.44±1.31	50.60±1.42	50.42±0.75
ΣPUFA	6.74±0.43b	9.31±0.36a	10.45±0.58a

[a] Means with different letters (a,b) indicate significant differences among locations ($P < 0.05$, Tukey's test). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 3
Volatile compounds found in the headspace (expressed as units of area/total area) of the subcutaneous and intermuscular fat from dry-cured Iberian hams

Volatile compounds	Reliability[a]	Location		
		Subcutaneous fat		Intermuscular fat
		Superficial layer	Internal layer	
Hydrocarbons				
2-Methylpentane	a	40.81	54.53	
Hexane	a	1127.94	855.98	
3-Metylene heptane	b	4.24	1.16	
Toluene	a	376.47	917.60	64.21
2-Octene	b	1.28		
m-Xilene	a	14.96	19.40	2.65
p-Xilene	a	32.83	30.74	6.19
Nonane + Ethenylbenzene	a,b	7.33		
β-Pinene	a	4.16	5.91	2.02
1-Ethyl 4-Methylbenzene	b		6.65	6.76
Decane	a	9.59		
Ethenylcyclohexane	b			34.90
Limonene	a	8.07	4.09	7.25
Dodecane	a			22.20
Tetradecane	a			1.80
Total		1627.68	1896.06	147.98
Aldehydes				
Butanal	a		134.98	111.11
3-Methylbutanal	a	81.18	45.51	14.62
2-Methylbutanal	a	33.84	51.60	14.87
Pentanal	a	459.84	88.40	24.85
3-Methyl-2butenal	b	4.24	1.16	
Hexanal	a	841.51	395.75	73.19
2,4-Octadienal	a	7.76	3.61	
Heptanal	a	35.71	25.17	7.26
2-Heptenal	b		5.10	
2,4-Nonadienal	a	24.75	24.38	3.67
Octanal	a	28.26	14.83	1.80
Benzeneacetaldehyde	b		9.08	9.06
2-Octenal	b	6.21	18.57	
Nonanal	a	13.05	15.92	9.34
2-Nonenal	b	28.66	42.96	29.65
Decanal	a			156.44
2-Decenal	b	50.60	85.14	82.00
2-Undecenal	b	60.39	67.68	14.85
Dodecanal	a			1.50
Total		1676.01	1029.84	554.20
Alcohols				
Etanol	b	27.90	17.98	
2-Propanol	a	874.98	1490.03	80.81
1-Penten-3-ol	b			16.59
3-Methyl-3-buten-2-ol	b			20.19
3-Methylbutanol	a	90.81	22.37	10.26
2-Methylbutanol	a	4.24	1.16	
1-Pentanol	a	6.56		
1-Hexanol	a	12.91		3.27
1-Heptanol	a		12.33	2.03
1-Octen-3-ol	a	11.43	13.44	6.26
1-Octanol	a		38.16	
1-Undecanol	b			57.85
Total		1028.84	1595.47	197.25

(*continued on next page*)

Table 3 (*continued*)

Volatile compounds	Reliability[a]	Location		
		Subcutaneous fat		Intermuscular fat
		Superficial layer	Internal layer	
Ketones				
2-Propanone	a	193.00	57.05	78.28
2-Pentanone	a	12.98	30.65	21.45
2,3-Pentanedione	b	21.17	13.48	10.30
2-Hexanone	a	17.72	13.62	6.03
2-Heptanone	a	11.47	15.50	7.02
2,3-Octanedione + 3-Octanone	b,b	11.43	13.44	6.26
3,5-Heptanedione	b	3.12	8.07	3.45
2-Octanone	a			5.14
3-Octen-2-one	b	4.34		
3,5-Octadien-2-one	b	2.18	7.26	
2-Nonanone	a			5.47
2-Decanone	a			22.20
Total		277.41	159.07	165.6
Total overall		4609.94	4680.49	1065.03

[a] Reliability of identification: a, mass spectrum and KI in agreement with the literature; b, mass spectrum consistent with spectrum in NS and Wiley libraries.

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