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Meat Science 51 (1999) 129–134

MEAT SCIENCE

Changes in intramuscular lipids during ripening of Iberian dry-cured ham

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Received 6 April 1998; received in revised form 12 June 1998; accepted 21 June 1998

Abstract

Thirty-one thighs were obtained from Iberian pigs fattened with acorns and were processed during 22 months in the traditional dry-curing process. Lipolysis affecting intramuscular fat during the processing of Iberian dry-cured ham has been analyzed by studying the changes of glycerides, phospholipids and free fatty acids in lipids from *Biceps femoris* muscle. Little change affected the fatty acid composition of glycerides during processing. A double-phased increase in the acidity values and a decrease in the quantity of fatty acids of phospholipids during the processing were observed. There seems to be a relationship between the extension of the lipolysis taking place during the maturing and the processing conditions and raw material used.

1. Introduction

Iberian dry-cured ham is a typical product of high sensorial quality produced in the southwest of Spain. Iberian pigs are produced of extensive mode, with feeding based on acorns and grass during the last three months of life, leading to very heavy pigs (140–160 kg liveweight). Due to the raw material used and the prolonged technological processing, Iberian hams have taste and flavour characteristics not to be found in any other type of dry-cured ham. Many changes occur in lipids during processing, such as lipolysis. This phenomenon is very important since it constitutes the prior step to free fatty acid auto-oxidation, which gives rise to numerous volatile compounds, that are responsible to a large extent for the dry-cured ham's characteristic flavour (García et al., 1991; López et al., 1992; Bolzoni et al., 1996; Flores et al., 1997). In addition, the importance of lipolysis is also due to their different fractions, in particular the phospholipid fraction, as fatty acids content of the phospholipids have been correlated with differences in flavour characteristics (Larick and Turner, 1990). Several workers have investigated the fatty acid composition in intramuscular fat during processing of Italian (Manfredini et al., 1991), 'French type' (Buscailhon et al., 1994) and Serrano (Flores et al., 1987; Motilva et al., 1994) dry-cured hams. For Iberian ham, the compostition of apolar and polar lipid fractions from intramuscular fat has been reported in both raw ham (Ordóñez et al., 1996; Cava et al., 1997) and dry-cured ham (De la Hoz et al., 1996), as well as the evolution of total free fatty acids during the ripening (Antequera et al., 1993a). In the present work, the changes affecting the fatty acid composition of the three fractions (glycerides, phospholipids and free fatty acids) of intramuscular fat during the processing of Iberian dry-cured ham were investigated.

2. Material and methods

2.1. Processing and sampling of hams

Thirty-one thighs were obtained from Iberian pigs (160 kg liveweight) fattened extensively with acorns from *Quercus ilex* and *Quercus suber,* and were processed in a local industry for 22 months as follows: hams were rubbed with salt, containing about 1% potassium nitrate, and placed in piles of salt at low temperature (4°C) and high relative humidity (83%) for 1 day kg^{-1} of weight. After washing to remove salt from the surface, the hams were hung at 4°C and relative

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0309-1740/98/$—see front matter
PII: S0309-1740(98)00109-0

humidity of 80% for 9 weeks, then they were taken to a dryer at temperatures varying from 4 to 27°C and a relative humidity ranging from 70 to 43% for 17 weeks. Next, the hams were left to mature for 15 months in a cellar at temperatures ranging from 10–27°C and relative humidity of 58–80%. The enviromental conditions (temperature and relative humidity) were taken throughout the whole period of maturing (Fig. 1). The steps included in the sampling procedure, the number of hams removed for testing at each stage and the number of days from the beginning of the processing were as follows:

1. Green state ($n=5$), 0 days (**GR**).
2. End of salting–postsalting ($n=5$), 76 days (**PS**).
3. End of drying ($n=6$), 197 days (**DR**).
4. Four months of cellar ($n=6$), 314 days (**4C**).
5. Eight months of cellar ($n=5$), 456 days (**8C**).
6. Fully matured ham ($n=4$), 665 days (**FM**).

Samples of *Biceps femoris* muscle of each ham were removed and kept at −80°C until analysed.

2.2. Chemical analyses

2.2.1. Lipid extraction

Lipids were extracted from samples with a chloroform: methanol mixture (2:1) by the method of Folch et al. (1957).

2.2.2. Acidity

The determination of percentage of oleic acid was carried out by the method recommended by the AOAC (1984).

2.2.3. Fractionation of lipids

Total intramuscular lipids were fractionated into neutral lipid, free fatty acids and phospholipids on NH_2-aminopropyl minicolumns according to the method described by García-Regueiro et al. (1994). Fatty acid composition of each fraction was determined by gas liquid chromatography of methyl esters prepared in acidic conditions. Fatty acids of each fraction were quantified using heptadecanoic acid as internal standard. The analysis was carried out using a Hewlett–Packard-5890A system, equipped with an on-column injector and a flame-ionization detector. A capillary column (30 m length; 0.53 mm internal diameter) coated with FFAP-TPA stationary phase (1 μm thickness) was used. The temperature of the column was maintained at 220°C for all the run (30 min). The temperature of the injector was 230°C, and the temperature of the detector was 240°C. The carrier gas (nitrogen) flow rate was 15 ml/min. Identification of fatty acids was performed by comparison of the retention times with those of known fatty acids. Amounts of fatty acids from the three fractions (glycerides, free fatty acids and phospholipids) were expressed as mg fatty acid/g of intramuscular lipid.

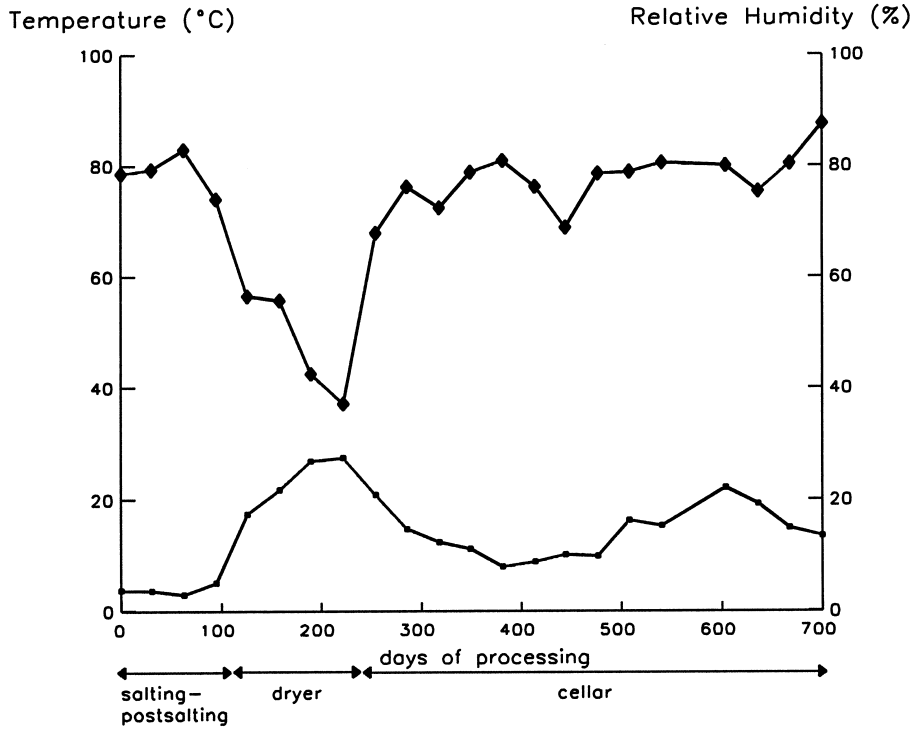

Fig. 1. Changes of enviromental temperature (bottom) and relative humidity (top) during the processing of Iberian dry-cured ham.

2.2.4. Statistical analysis

The effect of time and different muscle on changes of lipid characteristic was analyzed by variance analysis. When variance analysis indicated a significant effect at $p < 0.05$, the means were separated using the Bonferroni test. The statistical analyses were performed using STATGRAPHICS 5.0

3. Results and discussion

Table 1 shows the composition (expressed as mg g^{-1} of intramuscular fat) in fatty acids of the three fractions, glycerides, phopholipids and free fatty acids in raw ham, before processing. Glycerides represented a large amount, 92.9% of the lipid content. This large content of glycerides, compared with values given for White pigs by Buscailhon et al. (1994) reported as 75%, is related to the high lipid content of muscle from Iberian pig, which was 20% DM in the raw hams studied in the present work. In this fraction the monounsaturated fatty acids were the most abundant followed by the saturated fatty acids. Oleic (C18:1) and palmitic (C16:1) acids were the main fatty acid of the glycerides. Polyunsaturated fatty acids contained mainly linoleic acid (C18:2). These fatty acid profiles are in agreement to those reported both in pig muscle (Leseigneur-Meynier and Gandemer, 1991) and in dry-cured ham (Flores et al., 1987; Buscailhon et al., 1994). However, in Iberian pig it is remarkable the high oleic acid percentage (56.4%) and the low linoleic acid percentage (5.7%) found in this fraction, that is not shown in any other kind of raw ham, this difference being possibly responsible for the typical sensorial characteristics of Iberian ham. Before processing, the most abundant fatty acid in phospholipids fraction (which accounted for 2.1% of the lipid content) was linoleic acid (C18:2) followed by oleic acid (C18:1). Polyunsaturated fatty acids accounted for a much higher percentage (36.1%) than in the former fraction (6.6%). The high level of arachidonic acid (C20:4) found in this phospholipid fraction (which represented 10.8%) is noteworthy. In phospholipids, saturated fatty acids were the most abundant. These results are in agreement with other authors for 'Serrano' dry-cured ham (Flores et al., 1987) and Iberian ham (Cava et al., 1997). The content of free fatty acids at the beginning of the process represented 5% of the lipid content, which revealed that some lypolysis had already taken placed at this stage. In this sense, it has been described by Girard et al. (1986) that the slaughter conditions could influence the degree of glycerides hydrolysis due to activation of the triglyceride lipase by adrenalin released. This could influence the composition which showed a large quantity of monounsaturated fatty acid, the most abundant fatty acids in neutral lipids fraction, as it is in free fatty acid fraction. Flores et al. (1987) also reported this high quantity of monounsaturated fatty acids, although they found even a higher percentage of total free fatty acids (11%) in raw ham. In the free fatty acid fraction, the oleic acid (C18:1) was the most abundant fatty acid followed by the linoleic acid (C18:2). These results differ from those reported by Buscailhon et al. (1994) and Motilva et al. (1994), who found the linoleic acid the most abundant. Nevertheless, this difference could be related to the difference in animals used in the experiments.

Table 1
Fatty acid composition (expressed as mg fatty acid g^{-1} intramuscular fat) and percentage of each fraction of the lipid content before processing

	Free fatty acids	Glycerides	Phospholipids
C12	2.58 ± 0.10	2.81 ± 0.10	0.76 ± 0.03
C14	1.00 ± 0.08	7.09 ± 0.61	0.29 ± 0.02
C16	2.48 ± 0.23	104.37 ± 1.85	1.52 ± 0.13
C18	2.27 ± 0.17	44.50 ± 7.17	1.61 ± 0.14
C20	0.97 ± 0.06	2.25 ± 0.21	0.28 ± 0.01
Σ Saturated	9.30 ± 0.61	161.02 ± 6.09	4.46 ± 0.29
C16:1	1.12 ± 0.07	17.09 ± 1.11	0.14 ± 0.01
C18:1	10.19 ± 0.82	271.89 ± 31.35	2.42 ± 0.16
Σ Monounsaturated	11.31 ± 0.86	288.98 ± 25.24	2.56 ± 0.17
C18:2	3.54 ± 0.28	27.37 ± 3.10	2.55 ± 0.24
C18:3	0.80 ± 0.07	2.67 ± 0.36	0.22 ± 0.02
C20:4	0.96 ± 0.07	1.78 ± 0.23	1.19 ± 0.12
Σ Polyunsaturated	5.30 ± 0.30	31.82 ± 2.68	3.96 ± 0.34
% of the fraction	4.99	92.89	2.17

The evolution of the acidity degree (total content of free fatty acids) during the ripening process of Iberian ham is given in Fig. 2. A double-phased increase of the acidity values in the studied muscle (*Biceps femoris*) was observed. Two significant increases ($p < 0.05$) took place during dryer stage (from 4.75 ± 0.68 to 7.72 ± 0.33% oleic) and in the last months of cellar, (up to 13.25 ± 0.75% oleic), which is in relation to the higher temperature at these periods of maturing (Fig. 1). The second increase which occurred while the hams were in the cellar, was lower than the one in the drying stage (the dairy rise was 0.014 and 0.03, respectively). This difference could be related to the remarkable oxidative processes taking place while the hams are in the cellar: these prevail over lipolysis processes. Possibly, although it has not been reported in Iberian ham, a decrease of the lipolytic activity enzyme on progresive dessication and salt content at the end stages could also occur.

The acidity values found in this study were a little higher than those obtained by other authors in Iberian ham (Antequera et al., 1993b). These lower contents can probably be explained by the shorter time of processing in the latter study (588 days) compared with our work (665 days). Astiasarán et al. (1991) also showed, in Iberian ham, a lower quantity of total free fatty acids. The latter authors propose that either lipolysis is less marked in Iberian ham or this type of ham has a

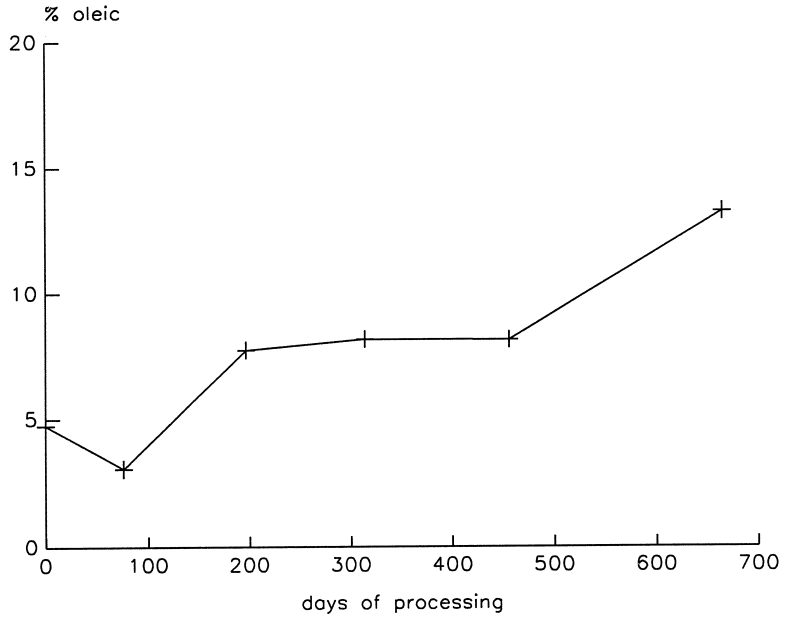

Fig. 2. Evolution of acidity degree expressed as % oleic acid in *Biceps femoris* muscle during the ripening of Iberian dry-cured ham.

bigger formation of carbonil compounds from free fatty acids.

Table 2 shows the composition (expressed as mg g^{-1} of intramuscular fat) in fatty acids of the three fractions, free fatty acids, glycerides and phopholipids in dry-cured ham, at the end of processing. Fig. 3a shows the tendency of free fatty acids grouped as saturated, monounsatured and polyunsaturated during the different stages of processing. The saturated free fatty acids (C12, C14, C16 and C18) showed a similar evolution to the acidity index. As expected, these saturated fatty acids were the most stable, showing an increase during the maturing, which was significant ($p < 0.05$) during the postsalting and drying stages. The evolution of the monounsaturated (C16:1 and C18:1) and polyunsaturated (C18:2, C18:3 and C20:4) free fatty acids showed a decrease chiefly during late ripening stages, that was statistically significant ($p < 0.05$) for polyunsaturated fatty acids. This fact must be attributed to oxidative reactions.

Table 2
Fatty acid composition (expressed as mg fatty acid g^{-1} intramuscular fat) and percentage of each fraction of the lipid content in dry-cured ham

	Free fatty acids	Glycerides	Phospholipids
C12	2.56 ± 0.26	3.00 ± 0.22	0.77 ± 0.08
C14	1.35 ± 0.20	9.57 ± 0.36	0.27 ± 0.04
C16	7.56 ± 0.15	144.83 ± 7.82	0.13 ± 0.08
C18	2.85 ± 0.34	54.29 ± 5.53	0.45 ± 0.06
C20	0.95 ± 0.09	2.26 ± 0.07	0.28 ± 0.03
Σ Saturated	15.27 ± 0.62	213.95 ± 3.20	1.90 ± 0.29
C16:1	0.86 ± 0.04	25.74 ± 4.51	0.10 ± 0.02
C18:1	9.21 ± 0.05	325.23 ± 7.41	1.25 ± 0.23
Σ Monounsaturated	10.07 ± 0.72	350.97 ± 6.88	1.35 ± 0.25
C18:2	1.80 ± 0.30	35.79 ± 3.73	0.13 ± 0.01
C18:3	0.53 ± 0.04	3.05 ± 0.53	0.16 ± 0.01
C20:4	0.55 ± 0.06	3.43 ± 0.88	0.15 ± 0.01
Σ Polyunsaturated	2.88 ± 0.39	42.27 ± 2.90	0.44 ± 0.01
% of the fraction	4.42	95.01	0.58

The content of total free fatty acids at the end of the processing (Table 2) was lower than that found in Iberian ham by Antequera et al. (1993a), this is probably due to the hams being studied under different circumstances, which implies differences in ripening conditions. We are in agreement with Buscailhon et al. (1994) and Motilva et al. (1994), who studied lipolysis in ham from White pigs, observing that lipolysis is deeper during early stages of maturing, between 0 and 5 months of processing. Buscailhon et al. (1994) also found that the saturated free fatty acid proportion rose markedly and polyunsaturated free fatty acid decreased, however, our results differ from the latter authors because they reported that monounsaturated free fatty acid remained unchanged.

In relation to neutral lipid fraction (Fig. 3b), it is observed that the quantity of fatty acids of glycerides did not vary during maturing ($p < 0.05$) (Tables 1 and 2). These results are in agreement with those reported in dry-cured ham from White pigs, by Flores et al. (1987) and Buscailhon et al. (1994). The evolution and the composition of saturated, monounsaturated and polyunsaturated fatty acid from glycerides were dissimilar to those found in the free fatty acid fraction. This fact reflects that the fraction of glycerides was not an important source of free fatty acids. This hypothesis was also proposed by other authors in White pig hams (Flores et al., 1987; Buscailhon et al., 1994). However, Antequera et al. (1993a), with samples corresponding to

finished Iberian hams, and Díaz and García-Regueiro (1991) in White pig hams, found that certain triglycerides decreased markedly, mainly in the early processsing stages.

A decrease in the quantity of fatty acids of phospholipids during the processing was observed (Fig. 3c). The decrease was statistically significant for linoleic (C18:2), arachidonic (C20:4), oleic (C18:1), palmitic (C16) and estearic (C18) acids ($p < 0.05$). This observation support the idea that free fatty acids come basically from phospholipids, in agreement with other authors who found that the most changes in lipid affect this polar fraction (Igene et al., 1981; Flores et al., 1985; Buscailhon et al., 1994; Huertas, 1990). During the early stages of processing the highest decrease in fatty acids of phospholipids was observed. Later, while the hams were in the cellar stage the decrease was less pronounced.

A reduction of 66% in the total quantity of fatty acid of phospholipids was observed comparing fatty acids in this fraction in raw ham (Table 1) to values found in dry-cured ham (Table 2). The results showed a different behaviour depending on the type of fatty acid: the polyunsaturated fatty acids were released in the highest quantity (88%), followed by saturated (58%) and monounsaturated (46%) fatty acid. Such a marked decrease should be reflected in the increase of free fatty acid in a large extend. However, it did not occur possibly due to suceptibility to oxidation, especially for polyunsaturated fatty acid, as the saturated fatty acids were the only ones that increased during the hams processing. Other authors who studied lipolysis in White pig hams did not find a selective degradation depending on the type of fatty acid (Flores et al., 1987).

The changes observed in fatty acids suggest that there is a relationship between factors, such as processing conditions and raw material, and lipolysis for dry-cured ham. Fatty acids released during processing in Iberian ham come mainly from phospholipid fraction, as a marked decrease in these fatty acids was reported.

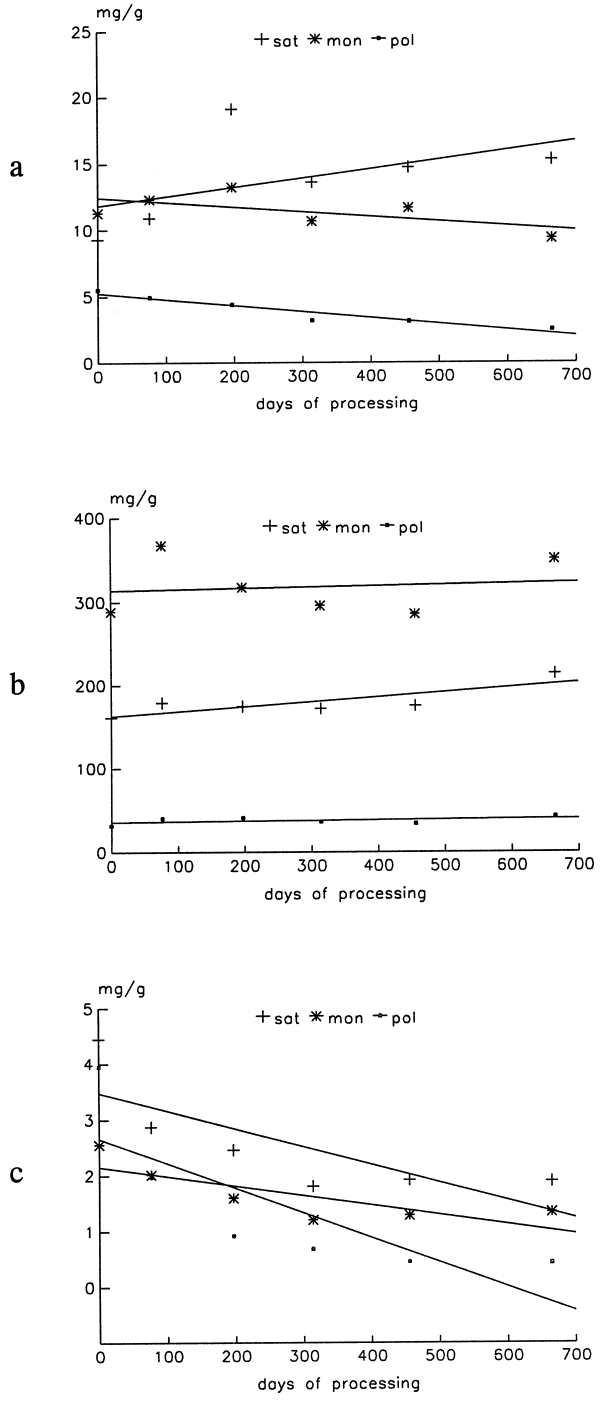

Fig. 3. Tendency in saturated (sat), monounsaturated (mon) and polyunsaturated (pol) fatty acids of the free fatty acids (a), glycerides (b) and phospholipids (c) fractions of intramuscular fat from *Biceps femoris* muscles during the processing of Iberian dry-cured ham, expressed as mg fatty acid g^{-1} i.m. fat.

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