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Lipid oxidative changes in the processing of Iberian pig hams

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Attempts to identify the compounds responsible for the particular flavour of Iberian pig ham indicate that many derive from lipid oxidation. In the present work, the evolution of the degree of acidity, the peroxide value and the content of certain aldehydes was followed to assess the extent of the lipid oxidation in the *Biceps femoris* and *Semimembranosus* muscles of Iberian pig hams. Lipolysis occurs continuously throughout the process, being especially intense immediately after salting and during drying. The peroxide value was higher after salting and in the first stage in the cellar. Aldehyde content rose continuously in the first stages, but the sharpest rise took place before drying. Saturated aldehydes were more abundant than unsaturated. During the last stage, autoxidation seems to be considerably reduced.

INTRODUCTION

The ham from Iberian pigs is a meat product of high organoleptic quality with a first-rate consumer acceptance. The extensive system of feeding of these pigs based on acorns and grass, the prolonged technological processing of the hams covering 18–24 months, and the specific climatological conditions give the final product organoleptic characteristics not to be found in any other type of cured ham. Any attempt to shorten the cycle has been rejected up to now, because of the negative effect on the quality of the final product.

Because of the characteristic high level of marbling in Iberian pig hams (around 10–13% intramuscular fat), the properties of this product are due, at least in part, to the hydrolytic and oxidative changes that take place in the lipid fraction during processing (Flores *et al.*, 1988; López-Bote *et al.*, 1990). These changes are mainly responsible for the formation of volatile carbonyls in the final product (Alford *et al.*, 1971; Demeyer *et al.*, 1974; León Crespo *et al.*, 1985; Lois *et al.*, 1987). Oxidative attack of the unsaturated fatty acids leads to the formation of important volatile compounds, which have been related by several workers to quality flavour (Forss, 1972; Halvarson, 1973; Anderson, 1980).

The most abundant of the many compounds identified in the volatile fraction are the carbonyls (Mottram *et al.*, 1984; García *et al.*, 1991), which, in some cases, as in country-style hams, have a higher concentration when longer ageing periods are used (Ockerman *et al.*, 1964).

Amongst the carbonyls, the aldehydes have very-low-threshold olfactory concentrations, and therefore constitute an important group of natural flavours. These components are formed during the processing, mainly by oxidation of unsaturated fatty acids in enzymatic and non-enzymatic reactions (Ohloff, 1973; Tressl *et al.*, 1981). In the present work, the extent of the lipid oxidation during the processing of Iberian hams was studied. The degree of acidity as an overall measure of the formation of free fatty acids (FFA) was

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determined, as was the peroxide value (PV) to assess the formation of primary oxidation products, and the evolution of the content of certain aldehydes as final products during the maturation process of the Iberian pig ham.

MATERIALS AND METHODS

Selection of the hams

Hams were selected from Iberian pigs that were grown extensively in pastures with acorns (*Quercus suber* and *Q. ilex*) as their basic feed until they achieved a final weight of about 140 kg. After conventional slaughter and refrigeration for 48 h, 25 thighs of homogeneous characteristics (pH and weight) were sorted out.

Processing of the hams

The selected hams were thoroughly rubbed with sea salt, containing about 1% nitrate, and were buried in a pile of salt at 0–4°C for 15 days.

The hams were processed in the traditional way: during the first period, low temperature was combined with high relative humidity to allow diffusion of the salt into the hams. Next, the temperature was moderately raised up to 30°C and the relative humidity progressively lowered down to 40% to achieve adequate drying of the thighs. Then, the hams were left to mature for 12 additional months under environmental conditions in a cellar (temperature range of 10–25°C and relative humidity of 70–80%). Changes of temperature and relative humidity (RH) during the whole process are shown in Fig. 1.

Sampling procedure

The lot was divided into seven groups of hams according to the sampling protocol. The number of days from the beginning of the processing, and the number of hams taken at every stage, were as follows:

Green state	(G)	0 days, $n = 5$
Salted	(S)	15 days, $n = 2$
Post-salting 1	(PS1)	75 days, $n = 2$
Post-salting 2	(PS2)	120 days, $n = 2$
Dryer	(D)	168 days, $n = 4$
Half cellar	(HC)	360 days, $n = 5$
Fully aged	(FA)	588 days, $n = 5$

Samples were taken from the *Biceps femoris* and *Semimembranosus* muscles, ground for about 5s and kept at −80°C until analysed.

Analytical methods

Lipid extraction

Lipids were extracted from samples with a chloroform : methanol mixture (2:1) using the method of Bligh and Dyer (1959).

Acidity

The determination of total FFA was carried out by the method recommended by the AOAC (1984*a*).

Peroxide value (PV)

The method recommended by the AOAC (1984*b*) was followed for the determination of PV.

Volatile aldehydes

The aldehydes from the hams were converted into 2,4-dinitro-phenylhydrazone (2,4-DNP) derivatives and separated into individual compounds by high-performance liquid chromatography (HPLC) according the

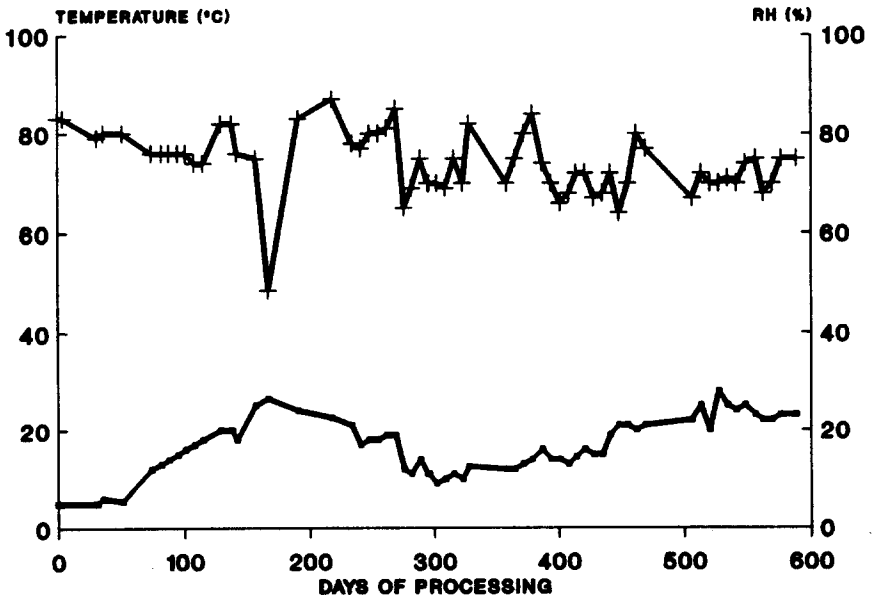

Fig. 1. Changes of environmental temperature (bottom) and RH (top) during the processing of Iberian pig hams.

method of Reindl and Stan (1982). For this, a sample of 5–10 g of meat was homogenized with 50 ml of cold ethanol in a Sorwall-omnimixer with glass mount. The homogenate was immediately centrifuged under refrigeration (0°C) at 900*g* for 10 min. The supernatant was distilled under vacuum (2·5–4 kPa) in a rotary evaporator at a water-bath temperature of 50°C. The condenser was cooled to 0°C. After the first distillation, 50 ml of water was added and a second distillation performed with a higher water-bath temperature of 65°C.

The distillate receiver was filled with 45 ml of 2,4-DNP solution. Upon termination of the distillation, the 2,4-DNP solutions were combined in a 250-ml Erlenmeyer flask and kept for 12 h at room temperature in the dark. To this solution, 50 ml of *n*-hexane was added and the mixture was stirred for 2 h. Then, the hexane phase was separated and washed twice with 10 ml of water. The solvent was removed at 30°C using a rotatory evaporator. The dry residue was dissolved in 2 ml of acetonitrile : water:tetrahydrofuran (75 : 24 : 1) for HPLC analysis.

The 2,4-DNP derivatives were separated in an LKB HPLC with Supecosil LC 18 column (5 μm, 250 × 4·6 mm) with isocratic elution of 1 ml/min. The eluent was acetonitrile : water : tetrahydrofuran (75 : 24 : 1) and the temperature in the column was 40°C. Detection took place at 360 nm.

Statistical analysis

Statistical analysis of the data was carried out by analysis of variance and the Tukey test.

RESULTS AND DISCUSSION

The total content of FFA (expressed as a percentage of oleic acid) is shown in Table 1. Although lipolysis occurs continuously throughout the curing process, if the length of every sampling stage is considered, there seems to be two phases of greater intensity: the first occurring immediately after salting and the second in the dryer. The initial acidity values are similar to those reported by Domínguez and Zumalacárregui (1991) in the maturing of 'chorizo' (peppered pork sausage) and greater than those found by Arnau (1988) in the subcutaneous fat in Landrace × Large White pig hams. During the cellar stages, the FFA content continues to rise gradually. Worthy of note is the greater degree of acidity presented by the *Semimembranosus* muscle throughout the curing process in comparison to the *Biceps femoris*, the increase in FFA being 8·75 and 5·10%, respectively. This difference could be related to the higher lipolytic activity in the *Semimembranosus* muscle, possibly because of lipolytic micrococci and yeast, whose concentration in this muscle has been reported to be higher (Carrascosa *et al.*, 1989; Huerta *et al.*, 1988).

The evolution of the oxidative processes during maturation is shown by PV value (Table 2). Although, according to these data, autoxidation seems to be single-phased during the stabilization period (up to the dryer), with maximum intensity after salting, there is likely to be an unobserved peak at the beginning of the PS2 stage, which is probably even of higher intensity, judging by the massive formation of aldehydes at this stage (as will be discussed later, see Fig. 3). It appears that a remarkable increase in peroxides could occur during PS2, but, due to their extreme reactivity, their formation and destruction might be so quick that they have not been detected. Peroxides have a transitory nature and are primary products, intermediate in the formation of hydroxyl and carbonyl compounds (Gray, 1978). By the time of sampling, a decline in the peroxides might have occurred, giving way to carbonyl and other breakdown products. Again, a great quantity of peroxides is found during the HC stage ($P < 0.05$), but these, once more, decline considerably during the last stage ($P < 0.05$). Flores *et al.* (1988) observed an evolution of PV similar to that of the present in the internal

Table 1. Means and standard errors of FFA concentration in *Semimembranosus* and *Biceps femoris* muscles during the ripening of Iberian pig hams

Stages	*Semimembranosus* (% oleic acid)	*Biceps femoris* (% oleic acid)
G	1.80 ± 0.42^{a}	1.64 ± 0.29^{a}
S	2.26 ± 0.38^{a}	$2.00 \pm 0.14^{a,b}$
PS1	$5.80 \pm 0.73^{b,c}$	$3.98 \pm 0.24^{b,c}$
PS2	5.13 ± 0.78^{c}	$4.21 \pm 0.58^{b,c}$
D	$7.85 \pm 1.25^{b,*}$	$4.65 \pm 0.85^{c,d,**}$
HC	$9.97 \pm 0.81^{d,*}$	$6.22 \pm 0.55^{d,e,**}$
FA	$10.75 \pm 0.76^{d,*}$	$6.74 \pm 0.27^{e,**}$

$^{a-e}$ Means with different letters in the same column are significantly different ($P < 0.05$).
*, ** Means with different numbers of asterisks in the same row are significantly different ($P < 0.05$).

Table 2. Means and standard errors of PV in *Semimembranosus* and *Biceps femoris* muscles during the ripening of Iberian pig ham

Stages	Semimembranosus (meq O_2 per kg fat)	Biceps femoris (meq O_2 per kg fat)
G	0.81 ± 0.38^{a}	1.05 ± 0.45^{a}
S	6.77 ± 0.40^{b}	$2.31 \pm 0.70^{a,b}$
PS1	$9.51 \pm 0.71^{b,c}$	$7.09 \pm 1.19^{b,c}$
PS2	$8.99 \pm 0.14^{b,c}$	$6.96 \pm 0.55^{b,c}$
D	$12.54 \pm 0.88^{c,*}$	$6.34 \pm 1.21^{b,c,**}$
HC	$41.92 \pm 4.44^{d,*}$	$28.72 \pm 2.81^{e,**}$
FA	$27.57 \pm 0.71^{c,*}$	$9.22 \pm 0.47^{c,**}$

$^{a-e}$ Means with different letters in the same column are significantly different ($P < 0.05$).
*, ** Means with different numbers of asterisks in the same row are significantly different ($P < 0.05$).

subcutaneous adipose tissue and in the lean of dry-cured hams during either slow or rapid processing. Also, Cerise *et al.* (1973) found similar behaviour during the maturing of pork salami sausages, there being a rise during the first days of the processing that was followed by a sharp fall, which they considered to be due to the interaction of peroxides with proteins. In the present experiment, this behaviour is similar for the two muscles studied, although there appears to be a greater oxidation in the *Semimembranosus* muscle than in the *Biceps femoris* ($P < 0.05$) probably due to its more external situation.

When studying the composition and evolution of aldehydes during maturation of the Iberian pig hams, the following have been identified: hexanal, heptanal, octanal, nonanal, 2-nonenal 2,4-nonadienal and 2,4-decadienal (Fig. 2).

The composition and evolution of aldehydes during the processing of Iberian pig hams are shown in Fig. 3. It can be seen that the saturated aldehydes were always the commonest in the two muscles studied. Among them, the most abundant were hexanal and nonanal, the main secondary products of the oxidation of oleic and linoleic acid, respectively. These aldehydes had a very similar evolution during processing, with a moderate increase in the first stages (S and PS1) and a sharp rise in PS2, with statistically significant differences with respect to the previous stages ($P < 0.05$).

The aldehyde contents diminish considerably in the next stage (D), while PV was observed not to increase significantly. This marked descent in all the aldehydes studied may be due to the high temperature reached in D, thus favouring reactions amongst the aldehydes themselves and their degradation. Also, reactions of aldehydes with amino acids (especially the basic ones), which are actively released at this stage from muscular proteins (Córdoba, 1990), can be considered of importance in the decreasing of the aldehyde concentration. The reactivity of the amino acids is favoured by the physico-chemical conditions in the ham (pH around 6·06, and water activity 0·88 and 0·92 in the *Semimembranosus* and *Biceps femoris* muscles, respectively) (López-Bote *et al.*, 1990). On the other hand, certain amino acids have been shown to have an antioxidant capacity, which may be of importance for the decreased values of the peroxides formerly seen, although this ability is very dependent on their concentration, the pH, the presence of metals, etc. (Erikson, 1987).

A high amount of aldehydes has been found again at HC, which accords with the peroxide formation. On studying the individual behaviour of these compounds, it was observed that, while the commonest aldehydes were also hexanal and nonanal, their values never reached levels as high as in PS2.

At HC, hexanal and 2,4-decadienal levels rise, presumably through oxidative degradation of linoleic acid.

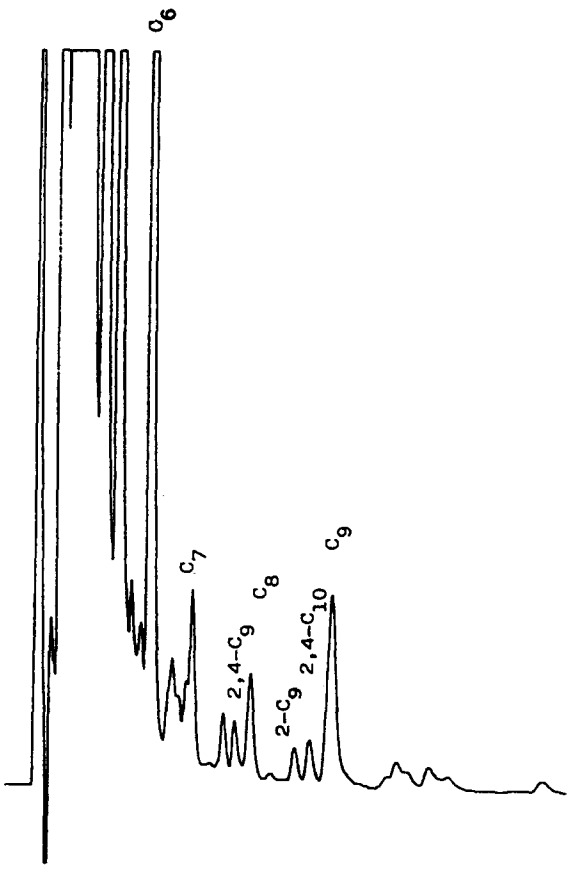

Fig. 2. Chromatogram of volatile aldehydes from Iberian pig hams, determined as 2,4-dinitrophenylhydrazones using reversed-phase HPLC.

Nonanal also rises, though to a lesser degree, so that oleic acid may also be undergoing degradative processes but with less intensity.

Although the differences between muscles are only significant in specific cases, generally the *Semimembranosus* muscles have higher PV and slightly higher aldehyde levels, possibly as a consequence of the greater lipolysis presented by surface muscles than deeper muscles. Also, since it has a more external situation, it might be more exposed to the action of oxygen and microbial lipases. However, it can be observed that, in the final product, the hexanal contents approach 2000 ng/g in the *Biceps femoris*, while considerably lower values are found in the *Semimembranosus* muscle (590 ng/g) This observation has also been noted by other workers, who consider it to be the result of the action of diverse microorganisms that provoke the diminution of certain carbonyl compounds (Harris & Lindsay, 1972; Misock *et al.*, 1979).

Heptanal and octanal have an evolution similar to hexanal, although the small quantities found make their oscillations less notable. Both octanal and heptanal originate in the homolysis of the hydroperoxides formed during oleate degradation, followed by cleavage (β-scission) of the fatty-acid chain adjacent to the alkoxy radical (Ladikos & Lougovois, 1990).

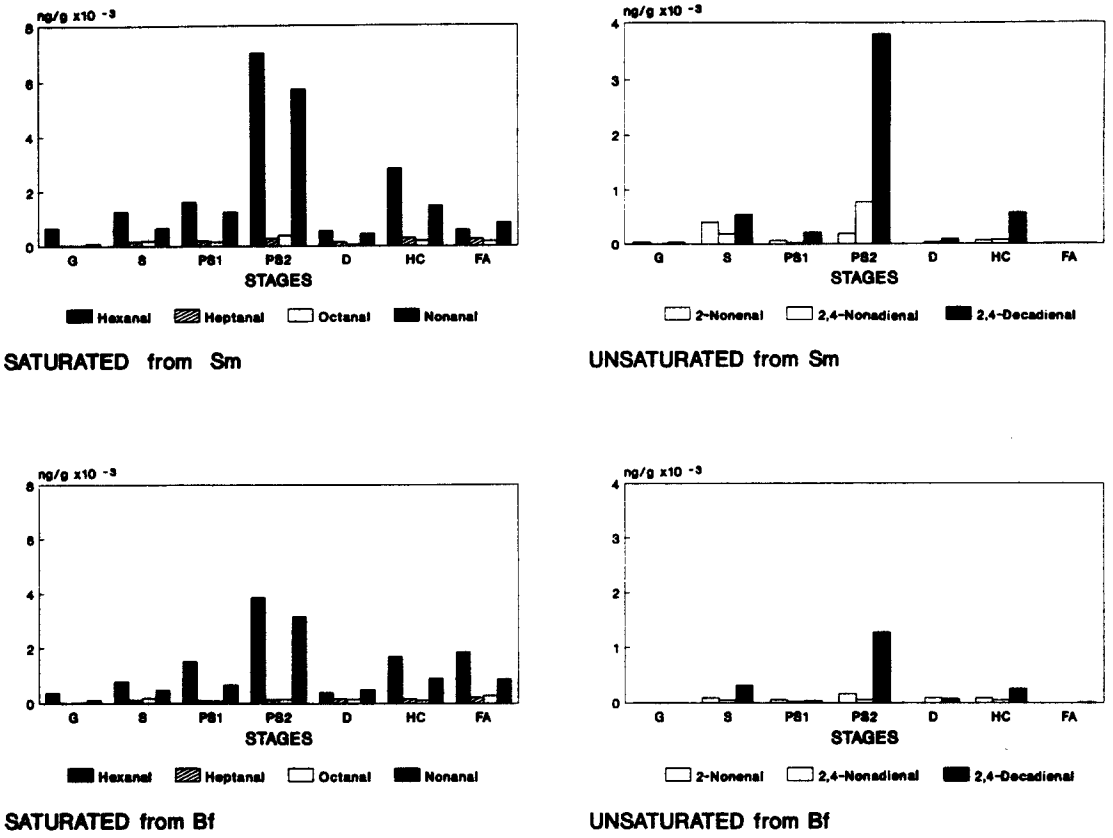

Fig. 3. Saturated and unsaturated volatile aldehydes found in *Semimembranosus* (Sm) and *Biceps femoris* (Bf) muscles at stages during the ripening of Iberian pig hams.

According to Badings (1970), the increase in octanal is very important for the development of flavour.

Unsaturated aldehydes are found through all the stages under study in amounts far below those of saturated aldehydes. Since there is no reason to believe that they are not produced through fatty-acid oxidation, this lower concentration may be due to their higher facility to degrade via oxidation. The commonest of the unsaturated aldehydes is always 2,4-decadienal which degrades with great facility to hexanal (Belitz & Grosch, 1988). This same tendency for degradation could explain the oscillations observed in its evolution, which follow a very similar pattern in the two muscles studied. In both cases, the unsaturated aldehydes have almost disappeared in the final product.

From the results, it is also remarkable that autoxidation of fatty acids seems to be considerably reduced in the last stage, and so the progress of lipolysis (Table 1) led to the rise of the degree of acidity in the final product.

García *et al.* (1991), in a work on volatiles from samples corresponding to finished Iberian pig hams, found the proportion of aldehydes to be the highest, and particularly noteworthy was the high level of hexanal, which is the major component not only among the aldehydes but also among the other volatiles, thus corroborating the intense oxidation that the lipids undergo.

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