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Proteolytic and lipolytic modifications during the manufacture of dry-cured lacón, a Spanish traditional meat product: Effect of some additives

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Abstract

The extractability of sarcoplasmic and myofibrillar proteins, the myofibrillar proteins and their degradation products, classical nitrogen fractions, free amino acids, acidity of the fat, and free fatty acids were determined throughout the manufacturing process of dry-cured lacón, a traditional dry-salted and ripened meat product made in the northwest of Spain from the foreleg of the pig, following a similar technological process to that of dry-cured ham. The effect of the use of additives (glucose, sodium nitrite, sodium nitrate, sodium ascorbate and sodium citrate) on the proteolytic and lipolytic changes was also studied.

Throughout the manufacture, approximately 87% of the sarcoplasmic proteins and 91% of the myofibrillar proteins became insoluble. There was a significant ($p < 0.05$) decrease of the myosin heavy chain, actin, and myosin light chains 1, 2 and 3, and also a significant ($p < 0.05$) increase in the components generated as a result of the degradation of these myofibrillar proteins. The content of the different nitrogen fractions and of the free amino acids indicated that protein degradation during the manufacture of dry-cured lacón is only moderate. Data on the acidity of fat and of free fatty acids also indicated that lipolysis in dry-cured lacón is lower than in hams. The use of additives did not significantly influence the protein and lipid degradation, which occur throughout the manufacturing process.

Keywords: Dry-cured lacón; Protein extractability; Proteolysis; Lipolysis; Additives; Cured meat-products

1. Introduction

Dry-cured lacón is a traditional raw-cured meat product made in the northwest of Spain, from the foreleg of the pig, using similar manufacturing processes to those used in the production of dry-cured ham.

Dry-cured lacón is a product that is well appreciated in the areas where it is produced, but at present there are some problems that prevent its wider distribution. Such problems include the heterogeneity of the product and questionable organoleptic quality of some product. These deficiencies are partly due to a lack of knowledge about the biochemical and microbiological phenomena that take place throughout the manufacturing process and that are responsible for the organoleptic quality of the final product.

Studies carried to date on dry-cured lacón have basically concerned the biochemical characterisation of the final product (Marra, Salgado, Prieto, & Carballo, 1999; Veiga, Cobos, Ros, & Díaz, 2003) and investigation of the biochemical (Lorenzo, Prieto, Carballo, & Franco, 2003) and microbiological (Vilar, García Fontán, Prieto, Tornadijo, & Carballo, 2000) changes that take place during the manufacturing process, with sampling and study points established only at the end of each stage (salting, post-salting and drying–ripening). Although these studies have improved the knowledge of the manufacturing process of this meat product, their conclusions do not provide effective suggestions to improve the quality of this product.

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The product has traditionally been manufactured using only coarse salt, without any other additives. Recently, the industry has started to use some common additives, with the aim of improving the appearance and quality of the final product (development of the typical colour of cured meats and inhibition of growth of mould on the surface). The effects of these additives on the modifications that occur throughout the manufacturing process have not been studied in depth.

During the manufacture of raw-cured meat products made from whole cuts, different physicochemical and chemical changes take place, of which protein and lipid degradation are the most important and are those that have the greatest effects on the organoleptic characteristics of the final products.

Muscle proteins undergo changes in solubility and are also degraded, particularly by the enzymes naturally present in the muscle. This process firstly generates large-sized peptides, which are in turn degraded to oligopeptides, and then to free amino acids that are catabolised by deamination, decarboxylation and transamination reactions, giving rise to different compounds, such as ammonia, ketoacids, amines, methyl ketones, aldehydes, etc. (Toldrá & Flores, 1998; Antequera & Martín, 2001). Some of these compounds, together with those originating from the degradation of nucleotides, are of interest because of their contribution to the aroma and taste of the matured products.

The lipids (triglycerides and phospholipids), are also hydrolysed by enzymes to form free fatty acids, which under the action of different catalytic agents (light, Fe, NaCl, high temperatures) can also undergo oxidative processes and generate hydroperoxides, which are then degraded to give rise to secondary products of oxidation (aldehydes, ketones, hydrocarbons, alcohols, lactones and esters), which also contribute to the taste and aroma of the matured products (Toldrá & Flores, 1998; Gandemer, 2002).

The aim of the present study, which forms part of a wider study on the microbiological and biochemical changes that take place during the manufacture of dry-cured lacón, was to obtain information about the proteolytic and lipolytic changes that occur during the manufacturing process, as well as to study the effects of some additives on these changes.

2. Materials and methods

2.1. Samples

Six batches of dry-cured lacón were manufactured in three different factories, each batch consisted of nine lacón pieces from the same slaughter-house. Fresh pieces weighing around 4 kg were used. In three batches (one per factory), raw pieces were salted with coarse salt, forming piles alternating between pieces and salt. The pieces remained in the pile four days (a day per kg of weight); the temperature of the salting room was between 2 and 5 °C and the relative humidity between 80 and 90%. After the salting stage, the pieces were taken from the pile, brushed, washed, and transferred to a post-salting room where they stayed for 14 d at 2–5 °C and around 85–90% relative humidity. After the post-salting stage, the pieces were transferred to a room at 12 °C and 74–78% relative humidity, where a drying–ripening took place for 84 d. In the other three batches (one per factory), before the salting process each piece was rubbed with a mixture composed of glucose (8 g), sodium nitrite (E250) (500 mg), sodium nitrate (E251) (700 mg), sodium ascorbate (E301) (2000 mg), and sodium citrate (E331) (400 mg). In these batches, the salting, post-salting and drying–ripening were carried out in the same way as in the batches manufactured without additives.

In each batch, samples were taken from fresh pieces, after the end of the salting stage, after 7 and 14 d of post-salting, and after 7, 14, 28, 56, and 84 d of drying–ripening. Each sample consisted of one whole lacón piece. Samples were transported to the laboratory under refrigeration conditions (below 4 °C) and analysed on arrival. Once in the laboratory, the entire pieces were skinned and boned, and finally minced in a high-capacity mincer.

2.2. Analytical methods

Moisture, NaCl content, pH and a_w were determined in duplicate in each sample, using the methods cited by Marra et al. (1999). Total nitrogen (TN), total non-protein nitrogen (NPN), α-aminoacidic nitrogen (NH_2–N), and total basic volatile nitrogen (TBVN) were quantified also using the methods cited by Marra et al. (1999). Sarcoplasmic and myofibrillar proteins were extracted and quantified using the methods cited by García, Díaz, and Zumalacárregui (1997). All the nitrogen fractions were determined in quadruplicate.

The identification of the myofibrillar proteins and their degradation products was carried out using PAGE techniques, following the procedure described by Johansson, Berdagué, Larsson, Tran, and Borch (1994). Gels were stained using Phast System® equipment (Amersham Pharmacia Biotech, Piscataway, NJ). The identification was carried out using myosin, β-galactosidase, phosphorylase B, bovine serumalbumin, ovalbumin, carbonic anhydrase, trypsin inhibitor and lysozyme (Bio-Rad Laboratories, Hercules, CA) as standards. Quantification was carried out using an Image Master® scanner equipped with the software package 1D PRIME software, version 3.01. Results of each band were expressed as a percentage of the total optical density. All electrophoresis analyses were performed in duplicate.

The extraction of free amino acids was performed, as described by Alonso, Álvarez, and Zapico (1994). The identification and quantification of amino acids were carried out by HPLC, using the conditions described by Alonso et al. (1994) with some minor modifications. The liquid chromatography equipment consisted of a Waters 2690 separation module (Waters, Milford, MA), a UV/Visible

Waters 996 photodiode array detector, and a Millennium 2010 Waters computer program. The column used was a reversed phase C18 Ultrasphere 5-ODS, 4.6 × 250 mm from Beckman (San Ramón, CA). The temperature of the column was controlled to 50 ± 1 °C with a column heater (Spectra Physics 8792). The wavelength of the detector was at 254 nm. Standards of the 22 different amino acids were supplied by Sigma Chemical Co. (St Louis, MO).

Extraction of fat was performed according to Folch, Lees, and Stanley (1957). The values of acidity of the fat were determined using the Spanish Official Standard UNE 50.011 (Presidencia del Gobierno, 1977). Free fatty acids were separated from the triglycerides in polypropylene columns packed with NH_2-aminopropyl, following the procedure described by Antequera et al. (1994). The procedure described by Schlenk and Gellerman (1960) with some modifications was followed for the methylation of the free fatty acids. The identification and quantification of the free fatty acids was performed by gas chromatography using a Trace GC (Thermo Finnigan, Austin, TX) chromatograph, equipped with a split/splitless AI 3000 Autoinjector and a flame ionisation detector. The separation of the different fatty acids was carried out using an Innowax column: 30 m long, 25 mm ID, 0.25 μm film thickness (Agilent Technologies, Palo Alto, CA). The temperature of the detector was 250 °C and that of the injector 230 °C. The gases used were air (350 ml/min), hydrogen (335 ml/min) and helium (carrier gas) (30 ml/min).

A standard from Sigma Chemical Co. that contained the methyl esters of the following fatty acids was used: butyric (C4); caproic (C6); caprylic (C8); capric (C10); undecanoic (C11); lauric (C12); tridecanoic (C13); myristic (C14); myristoleic (C14:1); pentadecanoic (C15); *cis*-10-pentadecenoic (C15:1); palmitic (C16); palmitoleic (C16:1); margaric (C17); *cis*-10-heptadecenoic (C17:1); stearic (C18); oleic (C18:1 *cis*); elaidic (C18:1 *trans*); linoleic (C18:2); linolelaidic (C18:2 *trans*); linolenic (C18:3); arachidic (C20); *cis*-11-eicosenoic (C20:1); *cis*-11, 14 eicosadienoic (C20:2); *cis*-11, 14, 17-eicosatrienoic (C20:3); arachidonic (C20:4); heneicosanoic (C21); behenic (C22); erucic (C22:1); *cis*-13, 16 docosadienoic (C22:2); *cis*-4, 7, 10, 13, 16, 19-docosahexaenoic (C22:6); tricosanoic (C23); lignoceric (C24); and nervonic (C24:1). This standard contained between 2 and 4% of each one of these fatty acids.

All the samples and standards were injected at least in duplicate. Repeatability tests were performed by injecting a standard and a sample consecutively six times in a day. Reproducibility tests were also carried out by injecting the standard and the sample twice a day for three days under the same experimental conditions. Significant differences ($p < 0.05$) were not found between the results obtained in these tests.

2.3. Statistical analysis

In order to study the effect of the additives at each sampling time and the effect of ripening, analysis of variance was performed, with a confidence interval of 95% ($p < 0.05$). Means were compared by the least squares difference test, using the computer programme Statistica 5.1 for Windows (Statsoft Inc, 1996, Tulsa, OK).

3. Results and discussion

The changes in the extractability of the sarcoplasmic and myofibrillar proteins during the manufacture of dry-cured lacón processed without and with additives are shown in Fig. 1.

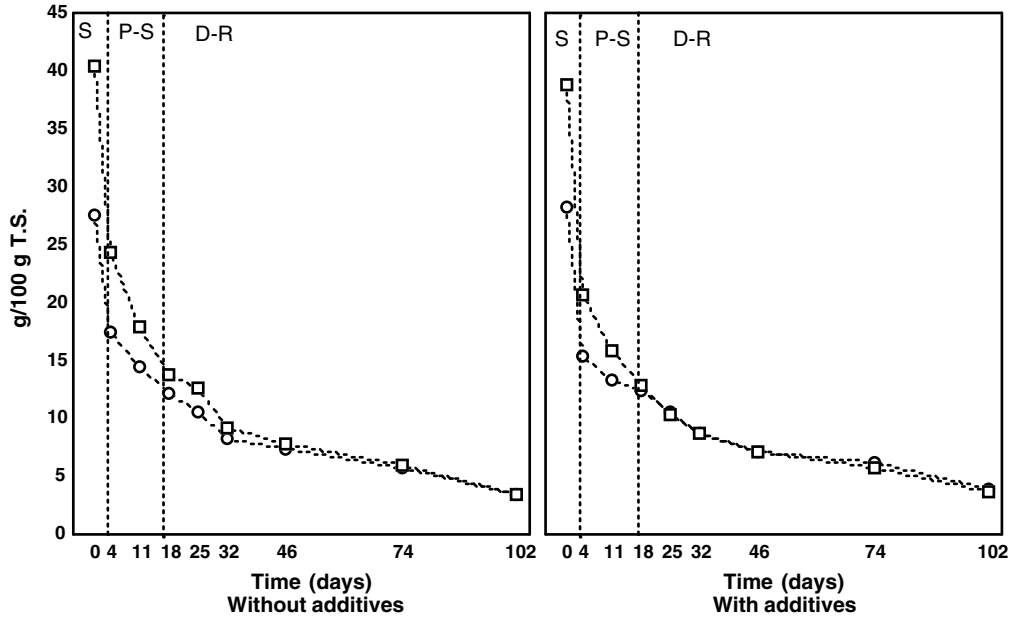

Fig. 1. Evolution of the extractibility of the sarcoplasmic (○- -○) and myofibrillar (□- -□) proteins during the manufacture of dry-cured lacón made without and with additives. Plotted values are the average of three batches. S = Salting stage; P-S = Post-salting stage; D–R = Drying–ripening stage.

The extractibility of the sarcoplasmic proteins decreased from 27.6 and 28.2 g/100 g of total solids (TS) to 3.50 and 3.83 g/100 g of TS, in the batches processed without and with additives, respectively, which indicates that approximately 87% of the sarcoplasmic proteins became insoluble during the manufacturing process. Other authors (De Prado, 1988; Córdoba et al., 1994; García et al., 1997) have observed percentages of insolubility of 75–80% in different raw-cured meat products made from whole pieces. The myofibrillar proteins suffered a greater degree in insolubility than the sarcoplasmic proteins, and their extractability decreased during manufacturing from 40.4 and 38.9 g/100 g of TS to 3.55 and 3.64 g/100 g of TS in the batches made without and with additives, respectively; this indicates that approximately 91% of the myofibrillar proteins became insoluble. The loss of solubility was therefore higher in the myofibrillar than in the sarcoplasmic proteins, which is consistent with the findings reported by other authors for different raw-cured meat products made from whole pieces (Astiasarán et al., 1988; De Prado, 1988; Córdoba et al., 1994; García et al., 1997).

As proteolysis was not very intense in the raw-cured lacón judging from the non-protein nitrogen values obtained, it appears that the protein insolubility is due to denaturation. Denaturation is probably caused by the NaCl and in fact, the highest loss of protein solubility was observed after the salting process, although the synergistic action of salt with other factors such as dehydration, which are quite marked during manufacturing (see Table 1), may also be important.

The changes in the contents of the myofibrillar proteins and their degradation products (expressed as % of the total optical density) during the manufacturing of lacón with and without additives, are shown in Tables 2 and 3, respectively.

In accordance with the Rf values obtained from the electropherograms of the myofibrillar proteins separated by SDS-PAGE in the lacón samples, and with the molecular weights indicated by several authors for the myofibrillar proteins (Porzio, Pearson, & Cornforth, 1979; Porzio et al., 1979; Greaser, Yates, Krzywicki, & Roelke, 1983; Robson & Huiatt, 1983), we were able to identify the following proteins with the corresponding molecular weights (kDa): 200 (myosin of heavy chain), 170 (myomesin), 55 (desmin), 45 (actin), 25 (myosin LC1), 18 (myosin LC2) and 15 (myosin LC3). Apart from these bands, other bands corresponding to molecular weights of 116,000 Da, 66,000 Da and 30,000–40,000 Da, that could not be identified, were observed.

The progressive disappearance of the myosin heavy chain (200 KDa) during the manufacturing process was evident and may be due to denaturation and reduction in solubility of the myosin, which therefore would not appear in the extraction buffer or in the electropherograms. It is also possible that the myosin is degraded to peptides of smaller molecular weight during the ripening stage. The progressive disappearance of the myosin heavy chain has also been observed in other raw-cured meat products, such as Iberian

Table 1
Values of some physicochemical parameters during the manufacture of dry-cured lacón made without and with additives (average values ± standard deviations of three batches of each type)

	Fresh piece	After salting	Post-salting (days)		Drying–ripening (days)				
			7	14	7	14	28	56	84
Without additives									
T. S.[1]	29.7 ± 6.23^{a}	36.5 ± 5.72^{b}	37.0 ± 5.16^{b}	40.1 ± 3.44^{bcd}	39.4 ± 2.08^{bc}	43.9 ± 4.40^{cd}	46.0 ± 2.92^{de}	51.1 ± 2.97^{e}	58.0 ± 2.81^{f}
NaCl[2]	0.47 ± 0.02^{a}	9.27 ± 1.82^{b}	13.1 ± 1.70^{b}	13.3 ± 2.00^{b}	14.3 ± 1.02^{b}	12.8 ± 3.38^{b}	14.3 ± 3.79^{b}	13.9 ± 4.19^{b}	13.1 ± 4.90^{b}
pH	6.36 ± 0.27^{a}	6.17 ± 0.22^{a}	6.22 ± 0.06^{a}	6.24 ± 0.21^{a}	6.16 ± 0.16^{a}	6.34 ± 0.24^{a}	6.25 ± 0.07^{a}	6.25 ± 0.09^{a}	6.40 ± 0.22^{a}
a_w	0.997 ± 0.003^{a}	0.968 ± 0.003^{ab}	0.962 ± 0.003^{ab}	0.951 ± 0.015^{ab}	0.947 ± 0.014^{bc}	0.944 ± 0.016^{bc}	0.930 ± 0.025^{bc}	0.900 ± 0.059^{cd}	0.876 ± 0.075^{d}
With additives									
T.S.[1]	28.7 ± 4.25^{a}	40.2 ± 2.61^{b}	40.5 ± 3.53^{b}	38.7 ± 2.25^{b}	42.2 ± 5.21^{bc}	44.1 ± 4.74^{bc}	48.0 ± 3.23^{cd}	50.8 ± 3.50^{de}	55.0 ± 2.86^{e}
NaCl[2]	0.45 ± 0.03^{a}	8.33 ± 1.45^{b}	11.1 ± 1.59^{b}	11.3 ± 2.84^{b}	13.3 ± 2.49^{b}	13.4 ± 3.50^{b}	12.6 ± 4.87^{b}	12.4 ± 5.63^{b}	13.1 ± 5.58^{b}
pH	6.58 ± 0.10^{a}	6.02 ± 0.10^{bc}	6.32 ± 0.21^{abc}	6.23 ± 0.31^{bc}	6.20 ± 0.19^{bc}	6.13 ± 0.27^{bc}	6.26 ± 0.07^{bc}	6.38 ± 0.12^{c}	6.30 ± 0.23^{abc}
a_w	0.996 ± 0.001^{a}	0.966 ± 0.011^{ab}	0.956 ± 0.016^{abc}	0.953 ± 0.024^{abc}	0.945 ± 0.023^{ef}	0.940 ± 0.022^{bc}	0.928 ± 0.037^{bcd}	0.910 ± 0.034^{cd}	0.885 ± 0.032^{d}

[a–f]Values in the same row (corresponding to the same physicochemical parameter) not followed by a common letter differ significantly ($p < 0.05$). *Values which were significantly different ($p < 0.05$) when batches made without additives were compared with those made with additives.

[1] Total solids (Expressed as g/100 g).

[2] Expressed as g/100 g of total solids.

Table 2
Evolution of the myofibrillar proteins and their degradation products (expressed as % of the total optical density) during the manufacture of dry-cured lacón made without additives (mean ± standard deviation of three batches)

Molecular weight (KDa)	Fresh piece	After salting	Post-salting (days)		Drying–ripening (days)				
			7	14	7	14	28	56	84
200	11.6 ± 0.52^{a}	10.6 ± 0.43^{a}	9.27 ± 0.81^{b}	7.53 ± 0.12^{c}	6.74 ± 0.45^{d}	$5.61 \pm 0.64^{d*}$	4.90 ± 0.34^{de}	4.11 ± 0.30^{ef}	3.38 ± 0.62^{f}
170	7.32 ± 0.10^{a}	8.17 ± 0.45^{ab}	8.69 ± 0.63^{ab}	9.60 ± 0.58^{bc}	10.8 ± 0.85^{cd}	11.3 ± 0.90^{cd}	11.5 ± 0.50^{d}	11.7 ± 1.18^{d}	12.1 ± 1.27^{d}
116	5.89 ± 0.87^{a}	7.07 ± 1.29^{ab}	8.29 ± 0.86^{bc}	8.99 ± 0.88^{cd}	9.10 ± 1.15^{cd}	10.2 ± 0.49^{de}	11.3 ± 0.90^{ef}	12.2 ± 0.18^{fg}	13.0 ± 0.96^{g}
66	6.14 ± 0.19^{a}	7.39 ± 0.52^{b}	9.26 ± 0.43^{c}	9.86 ± 1.05^{c}	11.7 ± 1.20^{d}	12.9 ± 0.18^{e}	13.7 ± 0.07^{ef}	14.1 ± 0.08^{f}	14.5 ± 0.88^{f}
55	9.52 ± 0.10^{a}	9.66 ± 0.96^{a}	9.64 ± 0.07^{a}	10.1 ± 0.18^{a}	9.48 ± 0.08^{a}	$8.78 \pm 0.74^{a*}$	9.15 ± 0.16^{a}	9.85 ± 0.37^{a}	9.75 ± 0.55^{a}
45	14.9 ± 0.85^{a}	14.6 ± 1.05^{a}	13.8 ± 1.24^{a}	$12.2 \pm 0.26^{b*}$	$11.6 \pm 0.01^{bc*}$	11.4 ± 0.18^{bc}	11.6 ± 0.41^{bc}	10.8 ± 0.17^{c}	10.8 ± 0.21^{c}
30-40	13.3 ± 0.73^{a}	13.6 ± 1.64^{ad}	14.4 ± 1.73^{abd}	16.7 ± 2.63^{bcd}	17.4 ± 2.80^{bcd}	17.7 ± 1.95^{cd}	17.0 ± 0.61^{bd}	16.3 ± 0.10^{d}	16.8 ± 0.88^{b}
25	10.9 ± 0.91^{a}	9.18 ± 0.69^{b}	8.63 ± 0.44^{bc}	8.20 ± 0.69^{bcd}	7.80 ± 0.09^{cde}	7.08 ± 0.38^{def}	6.61 ± 0.28^{ef}	6.63 ± 0.10^{ef}	6.37 ± 0.47^{f}
18	10.9 ± 0.49^{a}	10.9 ± 0.95^{a}	9.65 ± 0.49^{ab}	9.13 ± 0.18^{bc}	8.33 ± 0.52^{cd}	7.96 ± 0.62^{cd}	7.97 ± 0.46^{cd}	7.88 ± 0.52^{cd}	7.69 ± 0.37^{d}
15	9.80 ± 0.13^{a}	8.85 ± 0.84^{b}	8.31 ± 0.49^{bc}	7.75 ± 0.71^{cd}	7.28 ± 0.40^{de}	7.08 ± 0.17^{def}	$6.63 \pm 0.10^{ef*}$	$6.39 \pm 0.27^{fg*}$	$5.59 \pm 0.13^{g*}$

[a–g]Values in the same row (corresponding to the same molecular weight) not followed by a common letter differ significantly ($p < 0.05$).
* Values which were significantly different ($p < 0.05$) when batches made without additives were compared with those made with additives.

Table 3
Evolution of the myofibrillar proteins and their degradation products (expressed as % of the total optical density) during the manufacture of dry-cured lacón made with additives (mean ± standard deviation of three batches)

Molecular weight (KDa)	Fresh piece	After salting	Post-salting (days)		Drying–ripening (days)				
			7	14	7	14	28	56	84
200	11.4 ± 0.54^{a}	10.3 ± 0.88^{b}	8.85 ± 0.59^{c}	8.31 ± 0.33^{cd}	7.44 ± 0.16^{de}	6.74 ± 0.19^{e}	5.35 ± 0.38^{f}	4.81 ± 0.10^{fg}	3.93 ± 0.07^{g}
170	6.97 ± 0.12^{a}	7.68 ± 0.15^{ab}	8.45 ± 0.51^{ab}	8.73 ± 0.60^{abc}	9.32 ± 0.67^{bc}	10.4 ± 0.08^{cd}	11.9 ± 1.08^{de}	13.1 ± 1.66^{e}	13.0 ± 1.74^{e}
116	6.33 ± 0.40^{a}	7.09 ± 0.86^{a}	8.02 ± 1.45^{ab}	8.98 ± 0.21^{bc}	9.93 ± 0.24^{c}	10.7 ± 0.33^{cd}	11.8 ± 0.30^{de}	12.9 ± 0.28^{ef}	13.9 ± 1.53^{f}
66	6.34 ± 0.15^{a}	7.29 ± 0.41^{a}	8.49 ± 0.56^{b}	9.36 ± 0.30^{b}	10.7 ± 0.12^{c}	11.9 ± 0.01^{d}	13.0 ± 0.05^{de}	13.4 ± 0.11^{ef}	14.4 ± 0.74^{f}
55	9.33 ± 0.13^{a}	10.1 ± 0.35^{a}	10.2 ± 1.07^{a}	10.0 ± 0.71^{a}	10.6 ± 1.53^{a}	10.2 ± 0.50^{a}	9.95 ± 0.26^{a}	9.59 ± 0.85^{a}	9.83 ± 0.11^{a}
45	15.1 ± 0.16^{ab}	15.4 ± 0.30^{a}	14.7 ± 0.32^{ab}	14.3 ± 0.80^{bc}	13.3 ± 0.16^{c}	12.1 ± 0.40^{d}	11.3 ± 0.25^{de}	11.0 ± 0.13^{de}	10.9 ± 0.38^{e}
30-40	14.0 ± 1.62^{a}	13.8 ± 0.65^{a}	15.4 ± 0.22^{a}	15.2 ± 0.11^{a}	15.1 ± 1.22^{a}	15.5 ± 0.95^{a}	15.7 ± 1.32^{a}	15.9 ± 1.39^{a}	15.6 ± 1.41^{a}
25	10.2 ± 1.48^{a}	9.63 ± 1.46^{ab}	8.79 ± 0.49^{bc}	8.49 ± 0.15^{bc}	7.99 ± 0.70^{cd}	7.70 ± 0.36^{cd}	7.53 ± 0.05^{cd}	6.68 ± 0.34^{d}	6.72 ± 0.18^{d}
18	10.9 ± 0.42^{a}	10.2 ± 1.35^{ab}	9.40 ± 0.05^{bc}	8.83 ± 0.71^{cd}	8.34 ± 0.76^{cde}	8.06 ± 0.65^{de}	7.71 ± 0.18^{de}	7.34 ± 0.69^{e}	7.07 ± 0.56^{e}
15	9.51 ± 0.04^{a}	8.65 ± 0.20^{a}	7.74 ± 0.06^{b}	7.77 ± 0.38^{b}	7.32 ± 0.31^{bc}	6.74 ± 0.13^{c}	5.72 ± 0.67^{d}	5.30 ± 0.71^{de}	4.71 ± 0.32^{e}

[a–f]Values in the same row (corresponding to the same molecular weight) not followed by a common letter differ significantly ($p < 0.05$).

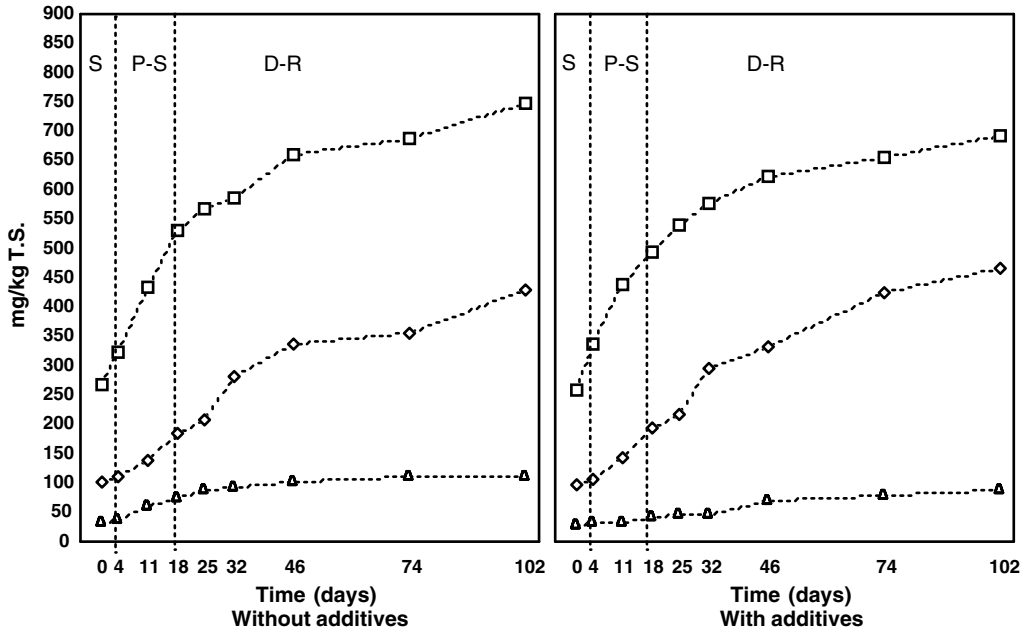

Fig. 2. Evolution of the non-protein nitrogen (□- -□), α-amino acid nitrogen (◇- -◇) and total basic volatile nitrogen (△- -△) during the manufacture of dry-cured lacón made without and with additives. Plotted values are the average of three batches. S = Salting stage; P-S = Post-salting stage; D–R = Drying–ripening stage.

hams (De Prado, 1988; Córdoba, 1990), hams from white pig (Toldrá, Miralles, & Flores, 1992b; Toldrá, Rico, & Flores, 1993), Spanish cecina (García et al., 1997) and in fermented sausages (Verplaetse, Debosschere, & Demeyer, 1989; García de Fernando & Fox, 1991).

During the ripening of dry-cured lacón, there was a significant increase ($p < 0.05$) in the 66, 116 and 170 kDa components. The appearance of components of molecular weight of between 50 and 150 kDa has been observed in electropherograms in many studies and are thought to originate mainly as a result of degradation of myosin (Verplaetse et al., 1989; Toldrá et al., 1992b, 1993), although some of these components may also originate from other myofibrillar proteins that are present at much lower concentrations (titin, α-actin, etc.).

The appearance of these components is probably due to the effect of muscle proteolytic enzymes on the muscle fibres, as suggested by the results of several *in vitro* studies in which the actions of calpain and of B, D, H and L cathepsins on the isolated myofibrillar proteins have been investigated (Schwartz & Bird, 1977; Matsukura, Okitani, Nishimura, & Katoh, 1981; Koohmaraie, Kennick, Elgasim, & Anglemier, 1984; Ouali et al., 1987).

We did not observe any increase in desmin (molecular weight 55 kDa) during the manufacture of dry-cured lacón. However, some authors have observed a gradual increase in the corresponding band during the manufacture of Spanish cecina (García et al., 1997) and during the ripening of sausage (Verplaetse et al., 1989).

There was a slight but significant ($p < 0.05$) decrease in the band corresponding to actin (molecular weight of 45 kDa) during the manufacture of dry-cured lacón processed without and with additives. Studies on the natural maturation of meat and those carried out to determine the effects of different proteases on isolated muscle fibres have shown that actin suffers a lower degree of proteolysis than most other myofibrillar proteins. The present results are consistent with those of García de Fernando and Fox (1991), who observed a decrease in the presence of actin (presumably due to proteolytic processes) during the ripening of sausages, although it did not disappear altogether.

In the present study, slight oscillations of two bands of molecular weights between 30 and 40 kDa were observed; these bands were in many cases difficult to quantify separately, since they appear very close together in the electropherograms and, in many cases, diffuse. These components, which are basically degradation products of actin and myosin, have also been quantified by other authors during the ripening of raw-cured hams (De Prado, 1998; Córdoba, 1990; Toldrá et al., 1993). Troponin T (37 kDa) and tropomyosin (35 kDa) also have similar molecular weights and may appear within these two bands blended with the previously mentioned degradation products.

Finally, we detected three bands of approximate molecular weights of 25, 18 and 15 kDa which appear to correspond to myosin light chains 1, 2 and 3, respectively. These three bands underwent a significant ($p < 0.05$) decrease in both types of manufacturing process (without and with additives). This is consistent with the observations of Toldrá et al. (1992b, 1993), who described a decrease in these three bands during the manufacture of raw-cured ham, and even their disappearance by the end of the process in some cases.

Table 4
Evolution of the free amino acids (expressed as mg/100 g of total solids) during the manufacture of dry-cured lacón made without additives (mean ± standard deviation of three batches)

	Fresh piece	After salting	Post-salting (days)		Drying–ripening (days)				
			7	14	7	14	28	56	84
Asp	7.05 ± 0.08^{a}	9.30 ± 0.88^{a}	14.7 ± 4.48^{ab}	20.8 ± 2.08^{bc}	26.4 ± 2.00^{c}	27.0 ± 1.98^{c}	39.1 ± 7.66^{d}	46.4 ± 9.92^{d}	53.1 ± 9.36^{e}
Glu	15.2 ± 3.15^{a}	44.8 ± 20.0^{ab}	92.1 ± 31.6^{bc}	116 ± 27.4^{c}	182 ± 35.4^{d}	205 ± 40.1^{d}	228 ± 23.9^{de}	264 ± 16.5^{e}	282 ± 12.5^{f}
Asn	8.96 ± 6.13^{a}	30.2 ± 4.38^{a}	59.2 ± 18.9^{b}	83.0 ± 14.5^{bc}	106 ± 6.86^{c}	119 ± 4.35^{d}	141 ± 3.90^{e}	148 ± 5.85^{f}	153 ± 18.9^{f}
Ser	24.8 ± 2.28^{a}	37.3 ± 2.40^{a}	96.0 ± 3.37^{b}	104 ± 1.27^{b}	124 ± 19.8^{c}	155 ± 9.50^{d}	170 ± 3.86^{de}	182 ± 10.1^{ef}	193 ± 8.89^{f}
Gln	44.4 ± 14.0^{a}	68.8 ± 18.1^{ab}	93.6 ± 25.4^{bc}	106 ± 26.6^{bc}	128 ± 38.8^{cd}	137 ± 41.4^{cd}	160 ± 48.0^{de}	190 ± 28.9^{e}	201 ± 17.9^{e}
Gly	23.8 ± 4.05^{a}	37.9 ± 0.97^{ab}	44.1 ± 3.46^{abc}	55.2 ± 4.68^{bc}	71.6 ± 11.7^{cd}	85.5 ± 19.2^{de}	103 ± 19.1^{ef}	110 ± 14.8^{ef}	$125 \pm 14.1^{f*}$
His	16.0 ± 3.23^{a}	20.9 ± 0.99^{a}	31.7 ± 2.64^{b}	36.8 ± 2.96^{b}	44.0 ± 3.19^{c}	47.1 ± 4.44^{c}	$55.0 \pm 5.24^{d*}$	67.6 ± 5.31^{e}	75.6 ± 3.39^{f}
Tau	38.2 ± 4.13^{a}	52.4 ± 1.76^{b}	68.9 ± 1.49^{c}	74.7 ± 2.60^{d}	83.6 ± 3.76^{e}	91.3 ± 1.23^{f}	96.9 ± 3.53^{g}	106 ± 3.16^{h}	118 ± 2.16^{i}
Gaba	12.7 ± 2.64^{a}	46.6 ± 2.01^{b}	58.2 ± 2.79^{bc}	62.8 ± 3.19^{c}	103 ± 3.09^{d}	142 ± 16.7^{e}	163 ± 8.63^{f}	194 ± 9.17^{g}	224 ± 9.07^{h}
Arg	25.8 ± 0.94^{a}	34.4 ± 1.31^{ab}	44.8 ± 0.27^{bc}	53.0 ± 2.85^{cd}	60.9 ± 3.59^{de}	66.0 ± 7.28^{e}	81.2 ± 8.92^{f}	93.5 ± 2.63^{g}	99.0 ± 3.64^{g}
Thr	17.5 ± 4.69^{a}	25.9 ± 3.52^{ab}	31.8 ± 4.65^{abc}	40.1 ± 3.94^{bcd}	49.6 ± 9.60^{cde}	52.8 ± 9.21^{def}	67.2 ± 2.50^{ef}	70.4 ± 4.49^{f}	$57.2 \pm 34.8^{def*}$
Ala	36.8 ± 1.45^{a}	43.0 ± 1.67^{a}	56.9 ± 1.42^{b}	65.4 ± 1.70^{bc}	68.2 ± 0.98^{cd}	75.3 ± 4.37^{d}	104 ± 7.50^{e}	133 ± 5.26^{f}	$165 \pm 15.8^{g*}$
Pro	25.8 ± 11.4^{a}	32.5 ± 13.1^{ab}	47.8 ± 2.51^{bc}	54.5 ± 3.80^{cd}	67.1 ± 3.22^{d}	96.2 ± 4.13^{e}	108 ± 0.66^{e}	140 ± 17.2^{f}	177 ± 17.6^{g}
Tyr	13.1 ± 3.62^{a}	17.8 ± 3.03^{a}	24.0 ± 1.30^{a}	43.0 ± 2.54^{b}	57.0 ± 5.65^{bc}	60.5 ± 6.68^{c}	71.3 ± 5.53^{cd}	76.8 ± 5.89^{d}	82.0 ± 8.32^{d}
Val	15.6 ± 4.28^{a}	23.6 ± 3.82^{ab}	31.6 ± 3.53^{b}	$52.1 \pm 6.11^{c*}$	57.8 ± 7.69^{cd}	66.4 ± 7.42^{d}	79.9 ± 10.5^{e}	83.7 ± 6.60^{e}	87.1 ± 7.39^{e}
Met	4.79 ± 0.80^{a}	13.8 ± 4.11^{ab}	20.0 ± 8.06^{bc}	21.7 ± 9.54^{bcd}	23.3 ± 6.11^{bcd}	23.5 ± 5.18^{bcd}	26.0 ± 4.98^{cd}	24.1 ± 4.28^{cd}	30.2 ± 3.93^{d}
Ile	14.9 ± 1.54^{a}	17.8 ± 2.64^{a}	24.9 ± 2.57^{a}	53.4 ± 15.0^{b}	67.2 ± 12.4^{bc}	73.7 ± 9.17^{c}	83.7 ± 4.84^{cd}	97.4 ± 9.83^{de}	106 ± 5.46^{e}
Leu	25.3 ± 3.87^{a}	31.2 ± 4.88^{ab}	35.4 ± 0.82^{ab}	52.6 ± 4.55^{bc}	60.5 ± 18.1^{c}	83.8 ± 12.8^{d}	92.2 ± 11.1^{de}	93.8 ± 18.2^{de}	110 ± 16.8^{e}
Phe	12.9 ± 1.34^{a}	24.6 ± 2.13^{ab}	35.6 ± 11.1^{bc}	46.5 ± 15.1^{cd}	52.5 ± 16.1^{d}	58.5 ± 11.9^{de}	74.2 ± 5.83^{ef}	76.5 ± 5.73^{f}	89.6 ± 8.05^{f}
Trp	3.31 ± 1.44^{a}	10.2 ± 6.60^{ab}	12.5 ± 5.76^{b}	12.9 ± 0.82^{b}	15.8 ± 1.54^{bc}	17.3 ± 5.39^{bcd}	21.6 ± 3.21^{cd}	23.4 ± 7.86^{d}	22.5 ± 6.64^{cd}
Lys	26.4 ± 13.0^{a}	54.3 ± 2.38^{a}	104 ± 3.63^{ab}	178 ± 38.4^{bc}	227 ± 70.3^{cd}	284 ± 55.3^{de}	322 ± 50.9^{e}	350 ± 42.5^{e}	430 ± 49.0^{f}
Total	413 ± 8.52^{a}	677 ± 49.6^{b}	1028 ± 107^{c}	1333 ± 130^{d}	1675 ± 134^{e}	1965 ± 162^{f}	2285 ± 140^{g}	2570 ± 105^{h}	2881 ± 118^{i}

[a–i]Values in the same row (corresponding to the same amino acid) not followed by a common letter differ significantly ($p < 0.05$).
* Values which were significantly different ($p < 0.05$) when batches made without additives were compared with those made with additives.

The use of additives does not significantly influence the degrading processes undergone by the myofibrillar proteins.

The contents of the different nitrogen fractions during the manufacture of the batches made without and with additives are shown in Fig. 2. In the present study there was a significant ($p < 0.05$) increase in the total NPN during the manufacturing process, and on the 84th day of the drying–ripening stage, this nitrogen fraction represented on average 9.31 and 7.69% of the total nitrogen for the batches made without and with additives, respectively.

The values obtained at the end of the manufacturing process are similar to those reported for ham (Astiasarán et al., 1988; Martín, Córdoba, Antequera, Timón, & Ventanas, 1998) and slightly lower than those reported by García, Díaz, and Zumalacárregui (1998) for Spanish cecina. However much higher NPN values (20–25% of the total nitro-

Table 5
Evolution of the free amino acids (expressed as mg/100 g of total solids) during the manufacture of dry-cured lacón made with additives (mean ± standard deviation of three batches)

	Fresh piece	After salting	Post-salting (days)		Drying–ripening (days)				
			7	14	7	14	28	56	84
Asp	7.17 ± 0.85^{a}	11.6 ± 3.77^{ab}	16.6 ± 6.86^{abc}	18.2 ± 7.44^{bc}	20.9 ± 3.84^{bc}	25.9 ± 10.8^{cd}	34.6 ± 8.36^{d}	47.0 ± 10.6^{e}	55.3 ± 7.82^{e}
Glu	15.5 ± 3.06^{a}	51.7 ± 21.4^{ab}	78.8 ± 31.6^{b}	130 ± 45.9^{c}	177 ± 37.1^{cd}	197 ± 31.1^{d}	217 ± 40.2^{de}	233 ± 34.1^{de}	255 ± 45.6^{e}
Asn	12.4 ± 7.35^{a}	43.2 ± 14.3^{b}	70.3 ± 26.0^{bc}	80.3 ± 35.4^{cd}	101 ± 16.7^{de}	120 ± 15.9^{ef}	136 ± 16.2^{fg}	146 ± 22.1^{fg}	162 ± 16.6^{g}
Ser	25.6 ± 3.63^{a}	34.9 ± 1.98^{a}	93.4 ± 3.40^{b}	101 ± 5.27^{b}	130 ± 23.9^{c}	137 ± 21.8^{c}	162 ± 9.90^{d}	181 ± 16.3^{de}	194 ± 20.0^{e}
Gln	39.2 ± 3.75^{a}	66.9 ± 16.1^{ab}	87.2 ± 8.42^{bc}	116 ± 23.7^{cd}	127 ± 31.6^{cd}	155 ± 26.0^{de}	178 ± 28.4^{ef}	208 ± 47.3^{f}	209 ± 31.3^{f}
Gly	25.4 ± 3.47^{a}	36.1 ± 1.50^{ab}	47.1 ± 3.23^{ab}	60.0 ± 11.4^{bc}	75.8 ± 9.75^{cd}	89.1 ± 12.2^{de}	106 ± 14.71^{ef}	117 ± 15.0^{f}	92.3 ± 54.7^{de}
His	16.9 ± 3.65^{a}	21.9 ± 2.12^{a}	32.1 ± 2.24^{b}	39.9 ± 0.74^{c}	46.1 ± 3.81^{d}	52.8 ± 2.43^{e}	63.8 ± 4.49^{f}	70.1 ± 2.86^{g}	75.8 ± 3.97^{h}
Tau	35.1 ± 2.14^{a}	54.8 ± 0.67^{b}	66.8 ± 3.10^{c}	75.2 ± 3.65^{d}	84.8 ± 3.28^{e}	93.4 ± 2.00^{f}	98.0 ± 0.95^{g}	103 ± 0.83^{h}	119 ± 0.77^{i}
Gaba	12.2 ± 2.84^{a}	49.2 ± 3.97^{b}	56.1 ± 4.26^{b}	71.0 ± 9.82^{c}	98.7 ± 4.86^{d}	146 ± 3.11^{e}	175 ± 21.1^{f}	197 ± 2.76^{g}	228 ± 10.8^{h}
Arg	27.8 ± 2.45^{a}	38.7 ± 6.34^{ab}	48.1 ± 8.97^{bc}	57.5 ± 5.42^{cd}	67.7 ± 8.26^{de}	74.1 ± 7.21^{ef}	80.7 ± 7.62^{fg}	91.3 ± 5.19^{gh}	92.8 ± 16.7^{h}
Thr	20.8 ± 6.27^{a}	31.7 ± 4.55^{ab}	31.9 ± 5.01^{ab}	40.8 ± 4.49^{bc}	50.4 ± 2.55^{bcd}	58.8 ± 4.21^{cd}	62.3 ± 0.78^{d}	68.3 ± 4.61^{d}	90.7 ± 30.1^{e}
Ala	35.8 ± 3.07^{a}	43.5 ± 1.58^{a}	58.9 ± 1.18^{b}	62.3 ± 1.44^{b}	67.3 ± 2.88^{b}	77.6 ± 1.92^{c}	104 ± 1.69^{d}	129 ± 2.43^{e}	153 ± 15.2^{f}
Pro	29.1 ± 12.90^{a}	42.7 ± 0.46^{ab}	43.7 ± 1.13^{ab}	53.0 ± 5.16^{bc}	66.7 ± 10.7^{c}	96.8 ± 9.54^{d}	110 ± 3.07^{d}	132 ± 10.2^{e}	174 ± 26.1^{f}
Tyr	14.2 ± 3.25^{a}	18.5 ± 6.94^{a}	25.5 ± 1.62^{a}	44.6 ± 22.1^{b}	52.5 ± 15.4^{bc}	64.1 ± 16.96^{cd}	69.3 ± 9.13^{de}	75.3 ± 9.46^{de}	82.8 ± 10.6^{e}
Val	14.1 ± 4.00^{a}	21.6 ± 4.44^{ab}	27.0 ± 2.24^{b}	40.2 ± 8.44^{c}	57.2 ± 9.02^{d}	63.4 ± 4.85^{d}	65.8 ± 4.87^{de}	75.5 ± 4.58^{ef}	83.5 ± 2.96^{f}
Met	5.93 ± 0.29^{a}	13.1 ± 1.58^{a}	15.0 ± 3.30^{ab}	24.6 ± 8.83^{bc}	26.2 ± 5.25^{c}	29.8 ± 9.72^{c}	26.5 ± 8.16^{c}	28.2 ± 5.10^{c}	33.1 ± 5.69^{c}
Ile	15.5 ± 1.62^{a}	21.2 ± 3.26^{a}	27.5 ± 3.82^{a}	50.2 ± 21.5^{b}	63.7 ± 13.1^{bc}	80.0 ± 24.2^{cd}	87.8 ± 13.2^{d}	97.2 ± 17.7^{de}	112 ± 6.88^{e}
Leu	23.0 ± 72.4^{a}	30.2 ± 2.14^{a}	37.3 ± 1.70^{ab}	54.0 ± 16.7^{bc}	74.2 ± 24.4^{cd}	84.4 ± 12.1^{de}	95.1 ± 14.7^{de}	100 ± 10.1^{e}	123 ± 25.5^{f}
Phe	15.3 ± 53.2^{a}	23.9 ± 4.66^{ab}	29.6 ± 5.37^{ab}	38.6 ± 8.47^{bc}	54.3 ± 10.3^{cd}	64.3 ± 14.9^{de}	72.5 ± 11.2^{ef}	83.8 ± 13.5^{fg}	94.9 ± 7.81^{g}
Trp	3.92 ± 1.53^{a}	6.09 ± 1.34^{ab}	8.74 ± 1.55^{abc}	12.6 ± 3.95^{bc}	14.5 ± 3.18^{ce}	15.7 ± 2.49^{cde}	22.5 ± 4.03^{def}	21.1 ± 4.97^{ef}	24.8 ± 4.23^{f}
Lys	25.2 ± 4.90^{a}	49.1 ± 7.77^{ab}	109 ± 2.56^{bc}	150 ± 36.5^{cd}	218 ± 46.7^{de}	291 ± 50.8^{ef}	368 ± 82.6^{f}	387 ± 79.2^{fg}	463 ± 70.9^{g}
Total	420 ± 12.8^{a}	710 ± 28.2^{b}	1010 ± 60.4^{c}	1319 ± 205^{d}	1673 ± 137^{e}	2016 ± 190^{f}	2336 ± 169^{g}	2590 ± 202^{h}	2917 ± 178^{i}

[a–i]Values in the same row (corresponding to the same amino acid) not followed by a common letter differ significantly ($p < 0.05$).

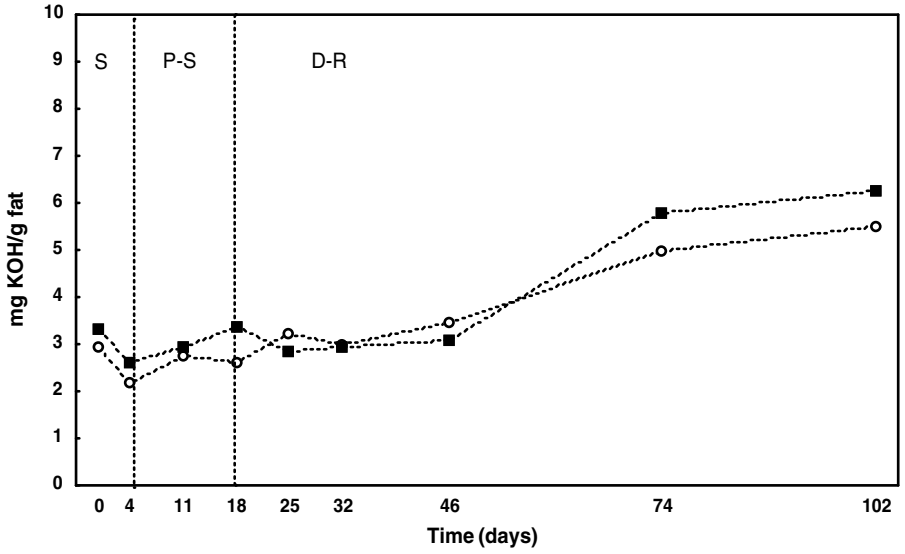

Fig. 3. Evolution of the acidity value of the fat during the manufacture of dry-cured lacón made without (○- -○) and with (■- -■) additives. Plotted values are the average of three batches. S = Salting stage; P-S = Post-salting stage; D–R = Drying–ripening stage.

gen) than those found in the present study have been reported for different types of hams (De Prado, 1988; Córdoba, 1990; Buscailhon, Monin, Cornet, & Bousset, 1994b).

Of the two nitrogen fractions studied in the NPN, the α-amino acid nitrogen fraction experienced the greatest increase during the manufacturing process, and at the end of the drying–ripening stage represented 57.8% of the total NPN in the batches made without additives, and 67% of the total NPN in the batches made with additives. These values are similar to those reported by other authors (Córdoba, 1990; García et al., 1998; Martín et al., 1998) for different raw-cured meat products made from whole pieces.

The total basic volatile nitrogen increased from average initial values of 28.7–30.9 mg/100 g of TS in the fresh pieces to values of 110 and 85.6 mg/100 g of TS at the end of the drying–ripening stage for the batches made without and with additives, respectively. These final values are low, compared to values reported for other meat products made from whole pieces, which varied between 50 and 240 mg/100 g of TS (De Prado, 1988; Córdoba, 1990; Ventanas et al., 1992; García et al., 1998; Martín et al., 1998). There was a noteworthy increase (significant at $p < 0.05$) in the TBVN content during the drying–ripening stage, coinciding with the highest temperatures reached in the manufacturing process. The final contents found in the present study reached values of 17% and 13% of the total NPN for the batches made without and with additives, respectively, and these percentages were higher than those reported by other authors (Martín et al., 1998). However, Buscailhon et al. (1994b) reported higher TBVN percentages for *biceps femoris* muscle in ham than those reported in the present study.

The present data allow us to conclude that protein degradation during the manufacture of dry-cured lacón is only moderate, and that the use of additives does not significantly affect the degree of proteolysis in this meat product. The high NaCl contents, the low environmental temperatures, and the intense dehydration suffered by the pieces during the manufacturing process (see Table 1) appear to be the cause of the reduced protein degradation observed in the dry-cured lacón, when compared with other raw-cured meat products made from whole pieces such as ham and Spanish cecina.

The changes in the content of free amino acids during manufacture of the batches made without and with additives are shown in Tables 4 and 5, respectively. The main free amino acid in the fresh pieces was glutamine, followed by taurine and alanine, which is similar to the observations made by Schivazappa, Saccani, Virgili, and Soresi-Bordini (1995) in fresh ham pieces. There was an important increase

Table 6
Evolution of the free fatty acids (expressed as mg/g of fat) during the manufacture of dry-cured lacón made without additives (mean ± standard deviation of three batches)

	Fresh piece	After salting	Post-salting (days)		Drying–ripening (days)				
			7	14	7	14	28	56	84
C10	N.D.	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.01^{a}	0.02 ± 0.03^{a}	0.02 ± 0.04^{ab}	$0.05 \pm 0.04^{b*}$
C12	N.D.	N.D.	0.01 ± 0.01^{a}	0.04 ± 0.06^{ab}	0.09 ± 0.08^{ab}	0.19 ± 0.16^{ab}	0.24 ± 0.21^{ab}	0.30 ± 0.15^{b}	0.85 ± 0.40^{c}
C14	1.01 ± 0.78^{a}	1.27 ± 0.95^{ab}	1.36 ± 0.94^{abc}	1.52 ± 0.88^{abc}	1.66 ± 0.94^{abc}	1.77 ± 1.04^{abcd}	2.18 ± 0.82^{bcd}	2.44 ± 0.60^{cd}	2.84 ± 0.47^{d}
C15	0.69 ± 0.05^{a}	0.73 ± 0.01^{ab}	0.77 ± 0.03^{ab}	0.82 ± 0.12^{ab}	0.88 ± 0.14^{ab}	0.94 ± 0.11^{abc}	$0.98 \pm 0.14^{abc*}$	$1.07 \pm 0.19^{bc*}$	$1.31 \pm 0.23^{c*}$
C16	2.31 ± 0.97^{a}	2.70 ± 1.15^{a}	3.33 ± 0.94^{ab}	3.65 ± 1.31^{ab}	4.28 ± 1.74^{ab}	4.92 ± 2.19^{bc}	6.62 ± 0.67^{cd}	7.57 ± 0.34^{de}	8.80 ± 0.98^{e}
C16:1	0.26 ± 0.16^{a}	0.45 ± 0.15^{ab}	0.94 ± 0.23^{ab}	1.45 ± 0.67^{b}	1.64 ± 0.70^{abc}	1.77 ± 0.85^{abc}	2.67 ± 0.61^{cd}	3.70 ± 0.89^{d}	4.12 ± 0.28^{e}
C17	0.02 ± 0.02^{a}	0.08 ± 0.01^{a}	0.13 ± 0.15^{a}	0.15 ± 0.14^{a}	0.19 ± 0.19^{a}	0.36 ± 0.28^{ab}	0.48 ± 0.41^{ab}	0.70 ± 0.72^{b}	0.83 ± 0.79^{b}
C17:1	N.D.	N.D.	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.03 ± 0.02^{ab}	0.06 ± 0.02^{abc}	0.08 ± 0.03^{bc}	0.10 ± 0.03^{c}	$0.16 \pm 0.03^{d*}$
C18	5.48 ± 1.78^{a}	5.58 ± 1.73^{ab}	7.46 ± 0.84^{abc}	8.81 ± 1.34^{abcd}	9.42 ± 1.82^{bcd}	10.9 ± 2.68^{cd}	12.6 ± 1.98^{de}	16.0 ± 3.73^{e}	17.8 ± 3.97^{f}
C18:1	10.9 ± 6.98^{a}	14.0 ± 7.13^{ab}	16.6 ± 4.59^{ab}	21.3 ± 4.99^{b}	31.6 ± 4.65^{c}	34.2 ± 3.39^{c}	39.0 ± 2.48^{cd}	57.9 ± 14.23^{e}	70.4 ± 22.74^{e}
C18:2	8.60 ± 1.91^{a}	9.54 ± 1.45^{a}	13.3 ± 2.37^{ab}	16.2 ± 5.06^{b}	17.7 ± 4.80^{bc}	21.3 ± 3.78^{c}	21.9 ± 2.43^{cd}	22.6 ± 1.77^{cd}	26.6 ± 2.18^{d}
C18:3	0.02 ± 0.02^{a}	0.05 ± 0.04^{ab}	0.13 ± 0.11^{ab}	0.17 ± 0.15^{ab}	0.22 ± 0.15^{ab}	0.28 ± 0.21^{ab}	0.49 ± 0.22^{abc}	0.61 ± 0.24^{bc}	$1.06 \pm 0.45^{c*}$
C20	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
C20:1	1.27 ± 1.11^{a}	1.33 ± 1.16^{a}	1.93 ± 0.25^{ab}	1.98 ± 0.21^{ab}	2.08 ± 0.23^{ab}	2.13 ± 0.25^{ab}	2.22 ± 0.34^{ab}	2.54 ± 0.59^{ab}	2.97 ± 0.72^{b}
C20:2	0.73 ± 0.63^{a}	0.73 ± 0.64^{a}	0.82 ± 0.71^{a}	1.28 ± 0.07^{ab}	1.35 ± 0.05^{ab}	1.45 ± 0.07^{ab}	1.65 ± 0.07^{ab}	1.78 ± 0.14^{bc}	2.70 ± 0.97^{c}
C20:4	1.94 ± 1.69^{a}	2.77 ± 0.38^{ab}	2.87 ± 0.41^{ab}	2.93 ± 0.38^{ab}	3.00 ± 0.36^{ab}	3.06 ± 0.40^{ab}	3.25 ± 0.49^{ab}	3.67 ± 0.99^{b}	4.03 ± 0.92^{b}
C22	N.D.	N.D.	1.77 ± 3.06^{a}	3.23 ± 2.87^{a}	3.40 ± 2.97^{a}	3.49 ± 3.04^{a}	3.55 ± 3.08^{a}	3.57 ± 3.10^{a}	4.13 ± 3.58^{a}
C22:1	0.87 ± 0.15^{a}	0.95 ± 0.13^{ab}	0.97 ± 0.11^{ab}	1.00 ± 0.13^{ab}	1.05 ± 0.17^{ab}	1.14 ± 0.29^{ab}	1.17 ± 0.29^{ab}	1.31 ± 0.45^{bc}	1.61 ± 0.76^{c}
C24	5.28 ± 0.81^{a}	5.73 ± 0.86^{a}	6.03 ± 0.59^{a}	6.26 ± 0.79^{a}	6.35 ± 0.67^{a}	6.43 ± 0.65^{a}	6.67 ± 1.02^{a}	6.66 ± 0.94^{a}	7.25 ± 1.19^{a}
Total	39.4 ± 7.60^{a}	45.9 ± 9.10^{ab}	58.4 ± 8.30^{bc}	70.8 ± 8.33^{cd}	85.0 ± 10.6^{de}	94.5 ± 14.1^{ef}	106 ± 10.16^{f}	133 ± 24.0^{fg}	157 ± 31.8^{g}
S	12.7 ± 1.77^{a}	13.8 ± 1.85^{a}	18.5 ± 4.49^{ab}	22.3 ± 5.01^{bc}	23.6 ± 5.97^{bc}	25.9 ± 7.42^{bc}	29.4 ± 6.14^{cd}	34.5 ± 8.75^{de}	39.1 ± 8.64^{e}
MU	15.3 ± 7.68^{a}	18.9 ± 8.24^{ab}	22.8 ± 5.66^{ab}	28.0 ± 6.09^{b}	39.1 ± 5.31^{c}	42.5 ± 4.12^{cd}	49.1 ± 2.73^{d}	69.4 ± 15.0^{e}	83.9 ± 24.7^{f}
PU	11.3 ± 2.08^{a}	13.1 ± 0.96^{ab}	17.1 ± 2.45^{bc}	20.6 ± 5.15^{cd}	22.3 ± 4.71^{cde}	26.1 ± 4.26^{def}	27.3 ± 3.17^{ef}	28.7 ± 2.90^{f}	34.4 ± 2.21^{g}
U	26.6 ± 5.83^{a}	32.0 ± 7.28^{ab}	40.0 ± 3.81^{bc}	48.5 ± 3.45^{c}	61.6 ± 4.83^{d}	68.6 ± 6.64^{de}	76.4 ± 4.07^{ef}	98.1 ± 17.1^{g}	118 ± 26.3^{g}
S/U	0.48 ± 0.05^{a}	0.44 ± 0.04^{ab}	0.46 ± 0.06^{ab}	0.46 ± 0.08^{ab}	0.38 ± 0.07^{bc}	0.37 ± 0.07^{bc}	0.38 ± 0.06^{bc}	0.35 ± 0.07^{c}	0.34 ± 0.08^{c}

S: sum of saturated fatty acids; MU: sum of monounsaturated fatty acids; PU: sum of polyunsaturated fatty acids. U: sum of unsaturated fatty acids; S/U: ratio of saturated/unsaturated fatty acids. N.D. = Not detected.

[a–g] Values in the same row (corresponding to the same fatty acid or fatty acid group) not followed by a common letter differ significantly ($p < 0.05$).

[*] Values which were significantly different ($p < 0.05$) when batches made without additives were compared with those made with additives.

in the free amino acid contents during the manufacture of dry-cured lacón, consistent with the changes in the α-amino nitrogen content.

The average total free amino acid content increased significantly ($p < 0.05$) from 413–420 mg/100 g of TS in the fresh pieces to 2881 and 2917 mg/100 g of TS at the end of the drying–ripening stage for the batches made without and with additives, respectively. These final contents were lower than those reported by other authors (Antequera et al., 1994; Buscailhon et al., 1994b; Schivazappa et al., 1995; Monin et al., 1997; Ruíz et al., 1999; Martín, Antequera, Ventanas, Benítez-Donoso, & Córdoba, 2001; Virgili, Saccani, Gabba, Tanzi, & Soresi Bordini, 2007) for cured ham.

Approximately 10% of the total increase in the free amino acid contents during the manufacturing process occurred during the salting stage, 25% in the post-salting stage and 63% during the drying–ripening stage. In the present study, therefore, the increase in free amino acid content was observed over the entire manufacturing period.

The increase in the individual free amino acids observed during the process was consistent with the increase in the total free amino acid content. This increase differed in the different amino acids: taurine, threonine and arginine suffered the least increase; aspartic acid, serine, glutamine, glycine, histidine, alanine, proline, tyrosine, valine, methionine, leucine, isoleucine, phenylalanine and tryptophan underwent moderate increases; and glutamic acid, asparagine, α-aminobutyric acid and lysine underwent the greatest increases. According to De Prado (1988), in addition to the effects of the breed, feeding and age at slaughtering of the pigs, the differences in the amino acids freed during ripening may be due to the different technological systems used in the manufacture of the product. Schivazappa et al. (1995) reported that the quantity and quality of the free amino acids freed depends on the activity of the muscle aminopeptidases, cathepsins, and peptidases and on the sodium chloride content and water activity values, which affect the enzymatic activity.

At the end of the manufacturing process of dry-cured lacón, the main free amino acids in the pieces made both without and with additives were lysine, followed by glutamic acid, γ-aminobutyric acid, glutamine and serine. This free amino acid profile basically coincides with those observed by different authors for cured ham (Toldrá et al., 1992a; Careri et al., 1993; Antequera et al., 1994; Buscailhon et al., 1994b; Monin et al., 1997; Virgili, Parolari, Soresi Bordini, & Schivazappa, 1999; Ruíz et al., 1999; Martín et al., 2001; Virgili et al., 2007).

The concentration of each free amino acid in the six batches of lacón studied, in many cases exceed established values of threshold of perception (Haefeli & Glaser,

Table 7
Evolution of the free fatty acids (expressed as mg/g of fat) during the manufacture of dry-cured lacón made with additives (mean ± standard deviation of three batches)

	Fresh piece	After salting	Post-salting (days)		Drying–ripening (days)				
			7	14	7	14	28	56	84
C10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
C12	N.D.	N.D.	N.D.	N.D.	N.D.	0.08 ± 0.14^{a}	0.16 ± 0.27^{a}	0.23 ± 0.40^{a}	0.94 ± 0.10^{b}
C14	0.89 ± 0.65^{a}	1.11 ± 0.60^{ab}	1.28 ± 0.59^{ab}	1.44 ± 0.39^{abc}	1.57 ± 0.31^{abc}	1.80 ± 0.33^{abcd}	2.20 ± 0.57^{bcd}	2.53 ± 0.29^{cd}	2.83 ± 0.06^{d}
C15	0.89 ± 0.06^{a}	0.92 ± 0.06^{a}	0.96 ± 0.05^{a}	1.05 ± 0.16^{ab}	1.11 ± 0.25^{abc}	1.16 ± 0.23^{abc}	1.36 ± 0.44^{bc}	1.47 ± 0.36^{cd}	1.81 ± 0.51^{d}
C16	1.96 ± 0.98^{a}	2.36 ± 1.21^{ab}	3.01 ± 1.25^{ab}	3.45 ± 1.34^{abc}	4.43 ± 0.92^{bc}	5.19 ± 0.93^{cd}	6.58 ± 1.81^{de}	7.57 ± 1.47^{e}	8.57 ± 1.57^{e}
C16:1	0.43 ± 0.35^{a}	0.51 ± 0.38^{a}	0.62 ± 0.35^{a}	0.87 ± 0.43^{a}	1.21 ± 0.83^{a}	1.29 ± 0.82^{a}	2.83 ± 1.40^{b}	3.77 ± 0.97^{b}	4.47 ± 0.86^{c}
C17	0.05 ± 0.03^{a}	0.07 ± 0.03^{a}	0.12 ± 0.09^{a}	0.18 ± 0.13^{a}	0.20 ± 0.14^{a}	0.26 ± 0.17^{a}	0.34 ± 0.14^{a}	0.41 ± 0.16^{a}	0.52 ± 0.20^{a}
C17:1	0.01 ± 0.01^{a}	0.03 ± 0.02^{ab}	0.03 ± 0.03^{ab}	0.04 ± 0.04^{abc}	0.06 ± 0.03^{abcd}	0.07 ± 0.03^{bcd}	0.09 ± 0.04^{cd}	0.11 ± 0.03^{d}	0.24 ± 0.08^{e}
C18	6.66 ± 1.64^{a}	7.47 ± 0.87^{ab}	7.67 ± 0.76^{ab}	8.30 ± 1.02^{ab}	8.63 ± 0.85^{ab}	10.7 ± 1.30^{bc}	13.0 ± 2.51^{cd}	16.7 ± 2.58^{de}	20.4 ± 4.99^{e}
C18:1	11.9 ± 6.34^{a}	16.4 ± 1.42^{ab}	18.5 ± 2.49^{ab}	20.4 ± 3.03^{b}	23.5 ± 3.61^{b}	32.0 ± 6.32^{c}	40.8 ± 3.93^{d}	74.5 ± 7.04^{e}	85.3 ± 4.71^{e}
C18:2	8.30 ± 1.28^{a}	12.1 ± 2.76^{ab}	15.3 ± 2.32^{bc}	15.9 ± 2.29^{bc}	16.6 ± 1.45^{bcd}	17.6 ± 0.58^{cd}	21.5 ± 3.72^{de}	25.7 ± 5.08^{ef}	26.9 ± 3.52^{f}
C18:3	0.02 ± 0.03^{a}	0.10 ± 0.11^{a}	0.10 ± 0.12^{a}	0.13 ± 0.13^{a}	0.14 ± 0.16^{a}	0.19 ± 0.16^{ab}	0.26 ± 0.24^{ab}	0.76 ± 0.76^{b}	1.75 ± 1.05^{c}
C20	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
C20:1	0.70 ± 1.21^{a}	0.71 ± 1.23^{a}	1.44 ± 1.24^{ab}	1.45 ± 1.25^{ab}	1.47 ± 1.27^{ab}	2.15 ± 0.12^{b}	2.38 ± 0.20^{b}	2.58 ± 0.09^{bc}	3.98 ± 1.28^{c}
C20:2	N.D.	N.D.	0.40 ± 0.69^{a}	0.66 ± 1.14^{a}	1.18 ± 1.08^{ab}	1.76 ± 0.36^{b}	1.89 ± 0.33^{b}	2.11 ± 0.41^{b}	3.39 ± 0.53^{c}
C20:4	N.D.	N.D.	2.05 ± 1.77^{a}	2.08 ± 1.80^{a}	2.10 ± 1.82^{a}	3.24 ± 0.08^{ab}	3.31 ± 0.15^{ab}	3.47 ± 0.19^{ab}	3.94 ± 0.41^{b}
C22	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.03 ± 3.51^{a}
C22:1	1.03 ± 0.01^{a}	1.05 ± 0.03^{a}	1.06 ± 0.02^{a}	1.07 ± 0.02^{a}	1.07 ± 0.02^{a}	1.08 ± 0.01^{a}	1.08 ± 0.01^{a}	1.16 ± 0.09^{a}	1.28 ± 0.22^{a}
C24	5.20 ± 2.10^{a}	5.33 ± 2.02^{a}	5.78 ± 1.91^{a}	6.19 ± 2.02^{a}	6.44 ± 2.08^{a}	6.65 ± 2.14^{a}	6.81 ± 2.06^{a}	7.12 ± 2.34^{a}	7.39 ± 2.12^{a}
Total	38.1 ± 10.4^{a}	48.2 ± 7.06^{ab}	58.3 ± 5.64^{bc}	63.2 ± 5.50^{bc}	69.8 ± 5.83^{c}	85.2 ± 6.93^{d}	105 ± 8.67^{e}	150 ± 3.66^{f}	176 ± 10.8^{g}
S	14.1 ± 3.71^{a}	15.4 ± 3.02^{a}	16.4 ± 2.56^{a}	18.0 ± 1.76^{a}	19.2 ± 1.81^{ab}	22.0 ± 1.30^{ab}	26.7 ± 3.42^{bc}	32.2 ± 4.19^{c}	40.4 ± 5.67^{d}
MU	15.6 ± 7.94^{a}	20.6 ± 3.85^{ab}	24.0 ± 4.40^{abc}	26.4 ± 4.92^{bc}	30.5 ± 5.66^{c}	40.5 ± 5.37^{d}	50.9 ± 4.20^{e}	85.9 ± 5.49^{f}	99.3 ± 4.54^{g}
PU	8.32 ± 1.31^{a}	12.2 ± 2.86^{a}	17.9 ± 3.61^{b}	19.0 ± 3.78^{b}	20.1 ± 3.44^{b}	22.8 ± 1.16^{bc}	26.9 ± 3.60^{cd}	32.0 ± 4.77^{de}	36.0 ± 4.38^{e}
U	23.9 ± 6.65^{a}	32.8 ± 4.84^{ab}	41.9 ± 3.63^{bc}	45.2 ± 4.11^{c}	50.6 ± 4.07^{c}	63.3 ± 6.38^{d}	77.8 ± 7.64^{e}	118 ± 1.94^{f}	135 ± 5.38^{g}
S/U	0.59 ± 0.03^{a}	0.47 ± 0.08^{b}	0.39 ± 0.04^{bc}	0.40 ± 0.03^{bc}	0.38 ± 0.01^{bc}	0.35 ± 0.03^{c}	0.34 ± 0.06^{c}	0.27 ± 0.04^{c}	0.30 ± 0.03^{c}

S: sum of saturated fatty acids; MU: sum of monounsaturated fatty acids; PU: sum of polyunsaturated fatty acids. U: sum of unsaturated fatty acids; S/U: ratio of saturated/unsaturated fatty acids. N.D. = Not detected.

[a–g]Values in the same row (corresponding to the same fatty acid or fatty acid group) not followed by a common letter differ significantly ($p < 0.05$).

1990). These amino acids are therefore expected to affect the flavour of the dry-cured lacón. Leucine and valine may cause a bitter flavour, and alanine a sweet flavour. The quantity of glutamic acid required to produce the typical umami flavour is 120 ppm (Maga, 1983). Considering that the concentrations of this amino acid in the six studied batches were much higher than the mentioned value, glutamic acid must therefore contribute to the flavour of dry-cured lacón. The free amino acid content and profile were not significantly affected by the use of additives.

The changes in the fat acidity during the manufacture of the batches made without and with additives are shown in Fig. 3. Overall, the values of the fat acidity, which are indicative of the degree of fat hydrolysis and of the free fatty acid content, increased during the manufacturing process in all the batches studied. The fat acidity values decreased during salting and then remained practically constant during the post-salting stage. This is consistent with the data reported by Flores, Biron, Izquierdo, and Nieto (1988) for ham, which indicate that the free fatty acids remain practically constant during the post-salting stage. During the drying–ripening stage, the fat acidity values increased gradually, until reaching final values of 5.50 ± 2.30 and 6.24 ± 2.68 mg of KOH/g of fat, in the batches made without and with additives, respectively. This significant increase ($p < 0.05$) may be due to the environmental humidity and temperature at this stage (12 °C and RH 74–78%), which may favour lipolysis (Balderas et al., 1993).

The fat acidity values obtained at the end of both types of manufacturing process (without and with additives) were lower than those reported by other authors for ham (Astiasarán et al., 1988; Flores et al., 1988; Antequera et al., 1993, 1994; Balderas et al., 1993). These results allow us to conclude that dry-cured lacón undergoes a lower degree of lipolysis during its manufacture than hams. During drying and maturation, hams are probably subjected to environmental conditions that are more appropriate for the development of the activity of tissue lipolytic enzymes, which are the main cause of the hydrolytic phenomena that the fat suffers during the manufacture of raw-cured meat products made from whole pieces.

The changes in the content of the different free fatty acids during the manufacture of the batches processed without and with additives are shown in Tables 6 and 7, respectively. The total average content of free fatty acids increased significantly ($p < 0.05$), from 38–39 mg/g of fat in the fresh pieces to 157 and 176 mg/g of fat at the end of the drying–ripening stage for the batches processed without and with additives, respectively.

The main free fatty acids in the fresh pieces were oleic (C18:1), followed by linoleic (C18:2), stearic (C18), lignoceric (C24) and palmitic (C16). This free fatty acid profile is consistent with that reported by Veiga et al. (2003) for dry-cured lacón and also with those reported by different authors for ham (Antequera et al., 1993, 1994; Buscailhon, Gandemer, & Monin, 1994a; Martín, Córdoba, Ventanas, & Antequera, 1999; Timón, Martín, Petrón, Jurado, & García, 2002).

During the manufacture of dry-cured lacón, there was an increase in all of the free fatty acids, with oleic (C18:1) and linoleic (C18:2) being the main fatty acids released. The greatest increase in levels of these fatty acids took place during the drying–ripening stage, which is consistent with the findings of Antequera et al. (1994) in Iberian ham. During the manufacturing process, linoleic (C18:2) and arachidonic (C20:4) acids underwent a significant increase ($p < 0.05$), which may be due to release of these fatty acids from the phospholipid fraction, in which linoleic is the main fatty acid and arachidonic is present at high concentrations. Increases in linoleic and arachidonic acid contents have also been described in ham (Flores, Nieto, Bermell & Alberola, 1987; Antequera, 1990; Díaz, 1993), although some authors (Martín et al., 1999) have observed a decrease in the contents of these acids.

The final amount of each individual fatty acid should be the result of the balance between its release from glycerides and phospholipids and its oxidative degradation. The main free fatty acid at the end of the manufacturing process was oleic (C18:1), followed by linoleic (C18:2), stearic (C18) and palmitic acid (C16). This free fatty acid profile is similar to that described by other authors for ham (Flores et al., 1987; Buscailhon et al., 1994a; Antequera et al., 1993, 1994; Coutron-Gambotti, Gandemer, Rousset, Maestrini, & Carabianca, 1999; Martín et al., 1999) and dry-cured lacón (Veiga et al., 2003). The final free fatty acid contents (157 and 176 mg/g of fat in the batches made without and with additives, respectively) were similar to those reported by Díaz (1993) for the *biceps femoris* muscle of ham, although the same author reported higher final values in *semimembranosus* muscle. The free fatty acid content and profile were not significantly affected by the use of additives.

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