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Chemical and Structural Changes in Dry-cured Hams (Bayonne Hams) during Processing and Effects of the Dehairing Technique

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ABSTRACT

Pigs of similar genetic backgrounds and feeding regimes were slaughtered in two abattoirs, one carrying out dehairing by scalding and the other by singeing. One ham from each of 80 carcasses was retained. Sixteen fresh hams (8 from each dehairing technique) were used for analysis while 64 hams were processed into dry-cured ham. Sixteen hams (8 from each dehairing technique) were taken for analysis at end of salting (day 14), end of rest (day 78), mid-processing (day 127) and end of processing (day 251). During processing, the water content of all muscles decreased while the salt content increased. The salt concentration in muscle water tended to equalize in all muscles. The nitrogen content of desalted dry matter (i.e. dry muscle tissue) decreased in both Biceps femoris *and* Semimembranosus. *The content of every free amino acid increased with time, except for taurine and glutamine. Electrophoresis of the low ionic strength-soluble fractions showed all protein bands decreased during processing. Electrophoresis of the myofibrillar fractions indicated changes in all bands except actin (42 kDa). These changes were more marked in the* Semimembranosus *than the* Biceps femoris *in the earlier processing steps. Ultrastructural changes were more marked in* Semimembranosus *than* Biceps femoris. *Hardness and chewiness increased in both muscles during the first half of processing then returned to values close to the initial ones in* Semimembranosus *but changed little in* Biceps femoris. *The scalded hams lost more weight than the singed ones during processing. The salt content was higher in scalded hams. Water-soluble nitrogen and NPN were higher in singed hams at the end of processing. The scalded hams were saltier and pungent. They had more pronounced aromas of dry ham, rancidity and hazelnut, and less aroma of fresh meat. Their texture was drier and less mellow. © 1997 Elsevier Science Ltd*

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INTRODUCTION

There have been a number of publications concerning the chemical changes occuring in the dry-cured ham during processing (for recent reviews, see Buscailhon and Monin, 1994*a*,*b*; Hortos Bahi, 1995). However little is known about ultrastructural changes even though these are considered of prime importance regarding texture changes in raw meat during ageing (Ouali, 1990). The ultrastructural changes are related to proteolysis, which is influenced by salt concentration and water activity (Sarraga *et al.*, 1989; Sarraga, 1992; Toldra, 1992).

Monin *et al.* (1995*a*) have shown that dehairing pig carcasses by singeing has some advantages over dehairing by scalding. The carcasses were heavier and there was evidence that meat quality was favourably affected by singeing. The dehairing technique affects the state of the skin, the hair being pulled out with the follicles in scalding while the follicles and some parts of the hair are left in the skin after singeing. This could influence the kinetics of salt penetration and water extraction during dry-curing of hams, and in turn the compositional, ultrastructural and sensory quality of the product.

The present study investigated the changes in some compositional and ultrastructural parameters during processing of dry-cured hams from pigs dehaired by singeing or scalding and the effect of dehairing technique on texture and sensory quality.

MATERIALS AND METHODS

Processing of hams and sampling

One hundred and twenty crossbred pigs of similar genetic background and feeding regimes were slaughtered in two abattoirs, one dehaired the pig carcasses by scalding and the other by singeing. Two days after slaughter, the hams were sent at chill temperature to the processing plant (Salaisons Pyrénéennes, Tarbes, France). Three days after slaughter, they were sorted according to weight and pH. pH was measured directly in muscle tissue using a portable pH meter equipped with a glass electrode. Eighty hams were split into five groups. Each group constituted 16 hams (eight from each dehairing technique), of comparable weights (on average 8·4 kg) and pH (on average 5·6). Sixteen hams (eight from each dehairing technique) were kept for chemical, microscopic and rheological analyses. The 64 remaining hams were processed. They were covered with dry salt containing 0·5% potassium nitrate, placed on platforms and held for one week at 4°C. Excess salt was removed by brushing, the hams salted again and held for a further week at 4°C. After washing to remove salt from the surface, they were left on platforms at 4°C at a relative humidity (RH) varying cyclically between 65 and 85% for 9 weeks (resting period). The hams were then hung and held at 20–22°C for 4 days and finally seasoned for 6 months at 12–15°C and 75–80% RH. At mid-seasoning, the cut surface was covered with a mixture of pig fat and spices (greasing) to slow down drying.

Sixteen hams (eight from each dehairing technique) were taken for analysis at the following times: end of salting (day 14), end of rest (day 78), mid-processing (day 127) and end of processing (day 251). All were used for chemical analysis. Eight from each group (four from each dehairing technique) were used for protein electrophoresis and electron microscopy. Rheological analyses were carried out at days 127 and 251. Moreover, the 16 hams at day 251 were submitted to sensory analysis.

A 4 cm thick slice was taken from each ham at the level of the middle of the femur for chemical and microscopic analysis, [Fig. 1(a)]. Pieces about 1 cm^3 were taken from the *Semimembranosus* and *Biceps femoris* muscles and from the cover fat at the *Biceps femoris*

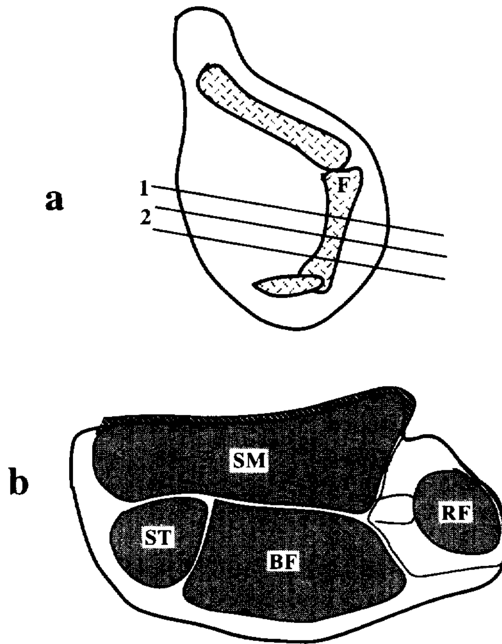

Fig. 1. Muscle sampling. (a) location of the slices taken for chemical analysis (1) and sensory analysis (2); F: femur bone; (b) slice before sampling of muscles. Muscles: SM: *Semimembranosus*; ST: *Semitendinosus*; BF: *Biceps femoris*; RF: *Rectus femoris*. The hatched part of the *Semimembranosus* was trimmed before analysis.

and processed for electron microscopy [Fig. 1(b)]. The outer edge (approximately 0·3–0·5 cm thick) of the *Semimembranosus* was cut, as it corresponded to the muscle part damaged during salting, washing and greasing. The *Semimembranosus*, *Biceps femoris*, *Semitendinosus* and *Rectus femoris* were separated from the slice. The muscles were minced individually in a domestic mincer. Mince from all four muscles was analysed for water and salt content. Mince from *Semimembranosus* and *Biceps femoris* was used for determination of total nitrogen, soluble nitrogen, non-protein nitrogen (NPN) and free amino acids, and also for protein electrophoresis.

At 0, 127 and 251 days, a further 6 cm thick slice was cut from the proximal side [Fig. 1(a)] and the rheological analyses performed on the *Semimembranosus*, *Biceps femoris* and *Rectus femoris*. Sensory analysis was carried out on the group processed for 251 days, using the part of the *Biceps femoris* remaining after sampling for chemical and rheological analyses.

Chemical analyses

Water content

About 2 g of muscle were dried at 104°C for 48 hr. The water content was calculated as the difference between the weights before and after drying.

Salt content

10 g of muscle were boiled for 1 hr in 85 ml of distilled water. The mixture was chilled and 2 ml of ferrocyanide (15%) and 2 ml of zinc acetate (30%) added for deproteinization.

The solution was then brought to 100 ml with distilled water and filtered. The chloride content was determined in the filtrate with Ag electrode (Chloridometre S100, Bioarrow, France). Salt content was calculated as [Cl]×58·4.

Total protein
Nitrogen was determined by the Kjeldhal procedure using about 300 mg of muscle, by the colorimetric method of Ferrari (1960) adapted to a continuous flow analyzer.

Protein solubility
One gram of tissue was homogenized in 50 ml of distilled water using a Polytron homogenizer. The homogenate was left for 2 hr at 4°C, centrifuged and the pellet kept for electrophoresis. The supernatant (water-soluble nitrogen fraction) was filtered through glass wool. Half was kept for subsequent nitrogen determination and electrophoresis, while the other half was mixed with 1 volume of 20% trichloroacetic acid. The extracts were left overnight at 4°C, and then centrifuged. The supernatant was filtered through paper and used to determine non-protein nitrogen (NPN). Nitrogen was determined in the fractions as described above. Soluble nitrogen and NPN were expressed as percent total nitrogen.

Free amino acids
One g of minced muscle was homogenized in 10 ml of water in an Ultra Turrax at maximum speed for 2×30 s. The homogenate was centrifuged at 40000 g for 1 hr at 4°C and the supernatant filtered on glass wool. Trifluoroacetic acid (TFA) was added to a final concentration of 10%. Proteins were removed by filtration through a 0·22 μm membrane. The supernatants were derivatized with phenylisothiocyanate as described by Bidlingmeyer *et al.* (1987).

The derivatized amino acids and dipeptides, anserine and carnosine were separated by HPLC at 37°C on a 30 cm long C18 column. Components were eluted by a linear and binary gradient (first eluent: sodium acetate 0·07 M, pH 6·5; second eluent: water 40%, acetonitrile 60%; flow rate 1 ml min^{-1}). The compounds were detected at 254 nm. Free amino acids were expressed in μmol per g of nitrogen.

Electrophoresis

Two grams of muscle were homogenized in 20 ml of (NaCl 150 mM; KCl 25 mM; MgCl2 3 mM; EDTA 4 mM; pH 6·5). The mixture was kept for 30 min at 4°C then centrifuged at 10 000 g for 10 min. The supernatant (low ionic strength-soluble fraction) was kept at −20°C prior to electrophoresis. The pellet was washed (homogenization + centrifugation) twice with (KCl 5 mM; mercaptoethanol 5 mM; EDTA 1 mM; pH 6·5) and once with (KCl 5 mM; mercaptoethanol 5 mM; pH 6·5), then kept at −20°C for electrophoresis (myofibrillar fraction). SDS-PAGE electrophoresis was carried out on both fractions using polyacrylamide gels (stacking: 7·5% acrylamide, pH 6·8; resolving: 12·5% acrylamide, pH 8·8). The gels were fixed in (5% acetic acid; 30% ethanol; 65% water), before staining with Coomassie Blue (0·12% in the fixation solution). The gels were photographed using a video camera. Images were computed using the NIH.Image.1·54 programme for MacIntosh computer.

Microscopy

Small blocks of muscle or subcutaneous fat (1–2 mm^3) were fixed in 2% glutaraldehyde in cacodylate buffer (0·1 M) at room temperature for 0·5 h and then at 4°C for 4 hr. Post-fixation was performed in 1% osmium tetroxide in 0·1 M cacodylate for 1 hr. The fixed samples were rinsed in distilled water, and dehydrated through an ascending series of

ethanol solutions before being embedded in epoxy resin. Ultrathin sections (80–90 nm) of muscle tissue and thin sections (300 nm) of fat tissue were cut using an ultramicrotome (Ultracut E from Reichert) and collected on copper grids. Sections were stained with uranyl acetate and lead citrate. The micrographs of muscle tissue were made using a Philips EM 400 electron microscope at an accelerating voltage of 80 KV. Fat tissue was analysed with an optical microscope.

Rheological analyses

A sample 13 mm thick, 40 mm wide and parallel to the fibers was cut from the *Biceps femoris* and *Semimembranosus* muscles to evaluate texture. A Texturometer (Zenken, Tokio, Japan), designed to simulate human chewing, was used to record the following parameters: hardness (kg), springiness (cm), adhesiveness and cohesiveness (specific units of the equipment). Chewiness represents the work needed to reduce the bolus to dimensions such that it can be swallowed. It was computed as hardness×springiness×cohesiveness.

Sensory analysis

Thirteen assessors were trained in four sessions on taste recognition. During these sessions, they tasted various ham muscles used in the present study to define the attributes of the sensory profile used. These attributes were: appearance: redness, marbled, bright; taste: salty, acid, bitter, pungent; flavour: dry-cured ham, rancid, fresh meat, fresh fat, butter, hazelnut, mushroom; texture: dry, mellow.

For analysis, the *Biceps femoris* muscle was cut in 2 mm thick slices and packed under aluminium foil and samples from a scalded ham and a singed ham were given simultaneously. The samples were taken randomly from each dehairing group. The panelists scored the attributes on non-structured scales of 40 points displayed on the screen of an individual screen-keyboard set (FIZZ Minitel system).

Statistical analysis

The data were analyzed by analysis of variance and linear regression, using the Statview II (SE + Graphics) package (Feldman *et al.*, 1987). Significance of differences was tested using least significant difference.

RESULTS

Weight changes

The weight losses during processing are shown in Fig. 2. The scalded hams lost more weight from day 14 to day 127 ($p < 0.01$ at all steps). Between days 127 and 251 scalded hams lost slightly less than singed hams (8·4% vs 9·3%). The difference in weight loss between the dehairing techniques (35·1% in singed vs 38·6% in scalded) was not significant at the end of processing.

Compositional traits

Water and salt contents

During processing, the water content of all muscles decreased (Table 1). The decrease was faster in *Semimembranosus* and *Rectus femoris* which were not covered with skin and

subcutaneous fat. Relative to desalted wet matter, which better expresses the loss of water by the muscle tissue, the variation between muscles was slightly higher (results not detailed). Dehairing technique significantly affected the water content at the end of processing. At day 251, the water content of the *Biceps femoris*, *Rectus femoris* and *Semitendinosus* was higher in singed than in scalded hams by about 3% ($p < 0.05$).

The salt content increased rapidly in the *Semimembranosus* and *Rectus femoris* which were in direct contact with salt during salting (Table 2). In all muscles except *Semimembranosus* the concentration of salt by wet matter continued to increase to the end of the processing. However, in the last stages, this increase was due mainly to desiccation, as shown by comparison between data expressed relative to either wet or dry matter (Table 2). In the *Rectus femoris*, the increase in salt content, on a dry matter basis was followed by a slight decrease up to day 127 then the salt content reached a plateau. In the *Semitendinosus*, the level of salt by dry matter increased and reached a plateau from day 127. In the *Biceps femoris*, from day 127, the increase in salt content was noticeably lower

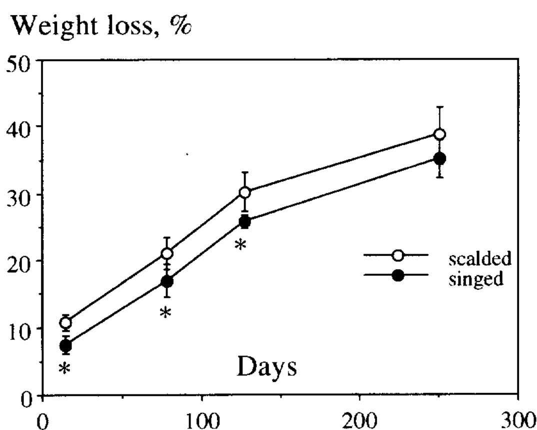

Fig. 2. Time-related changes in weight loss of scalded and singed hams. *Indicates a significant difference among dehairing techniques at a given time.

TABLE 1
Water Content in Four Muscles during Dry-cured Ham Processing

Time, days	*BF*	*RF*	*Muscle* *SM*	*ST*	*lsdm*
0	75.9^{a1}	77.1^{b1}	75.7^{a1}	74.5^{c1}	*0.69*
14	74.9^{a2}	71.2^{b2}	67.4^{c2}	73.9^{d1}	*0.94*
78	70.8^{a3}	66.0^{b3}	65.7^{b3}	67.4^{c2}	*1.26*
127	67.1^{a4}	60.7^{b4}	58.9^{c4}	64.7^{d3}	*0.95*
251	61.6^{a5}	55.8^{b5}	49.1^{c5}	57.5^{b4}	*1.97*
lsdt	*0.91*	*1.38*	*1.24*	*1.37*	

BF: *Biceps femoris*; RF: *Rectus femoris*; SM: *Semimembranosus*; ST: *Semitendinosus*.
[1,2,3,4,5]: means within a column with a same superscript are not significantly different ($p < 0.05$).
[a,b,c,d]: means of a same source within a row with a same superscript are not significantly different ($p < 0.05$).
lsdm: least significant difference among muscles; *lsdt*: least significant difference among sampling times.

TABLE 2
NaCl Content as (i) % Wet Matter (ii) % Dry Matter (iii) in $g\,l^{-1}$ of Water in Four Muscles during Dry-cured Ham Processing

Time, days	*BF*	*RF*	*SM*	*ST*	*lsdm*
			Muscle		
		NaCl (% wet matter)			
0	$0{\cdot}11^{1}$	$0{\cdot}12^{1}$	$0{\cdot}12^{1}$	$0{\cdot}13^{1}$	*0·03*
14	$0{\cdot}68^{a2}$	$4{\cdot}22^{b2}$	$6{\cdot}32^{c2}$	$0{\cdot}78^{a2}$	*0·70*
78	$3{\cdot}65^{a3}$	$7{\cdot}18^{b3}$	$5{\cdot}55^{c3}$	$4{\cdot}37^{d3}$	*0·50*
127	$6{\cdot}22^{a4}$	$7{\cdot}78^{b3}$	$6{\cdot}62^{a2}$	$6{\cdot}16^{a4}$	*0·52*
251	$7{\cdot}72^{a5}$	$8{\cdot}79^{b4}$	$6{\cdot}65^{c2}$	$7{\cdot}59^{a5}$	*0·59*
lsdt	*0·37*	*0·70*	*0·54*	*0·40*	
		NaCl (% dry matter)			
0	$0{\cdot}44^{1}$	$0{\cdot}53^{1}$	$0{\cdot}48^{1}$	$0{\cdot}52^{1}$	*0·11*
14	$2{\cdot}7^{a2}$	$14{\cdot}4^{b2}$	$19{\cdot}3^{c2}$	$3{\cdot}0^{a2}$	*1·9*
78	$12{\cdot}5^{a3}$	$21{\cdot}1^{b3}$	$16{\cdot}2^{c3}$	$13{\cdot}5^{a3}$	*1·4*
127	$18{\cdot}9^{a4}$	$19{\cdot}8^{a3}$	$16{\cdot}1^{b3}$	$17{\cdot}5^{c4}$	*1·3*
251	$20{\cdot}1^{a5}$	$19{\cdot}9^{a3}$	$13{\cdot}1^{b4}$	$17{\cdot}9^{c4}$	*1·2*
lsdt	*1·0*	*1·8*	*1·4*	*1·0*	
		NaCl ($g\,l^{-1}$ of water)			
0	$1{\cdot}4^{a1}$	$1{\cdot}6^{ab1}$	$1{\cdot}6^{ab1}$	$1{\cdot}8^{b1}$	*0·4*
14	9^{a2}	60^{b2}	94^{c2}	11^{a2}	*11*
78	52^{a3}	109^{b3}	85^{c2}	65^{d3}	*8*
127	93^{a4}	128^{b4}	112^{c3}	95^{a4}	*9*
251	126^{a5}	158^{b5}	136^{a4}	133^{a5}	*14*
lsdt	*6*	*13*	*10*	*8*	

BF: *Biceps femoris*; RF: *Rectus femoris*; SM: *Semimembranosus*; ST: *Semitendinosus*.
[1,2,3,4,5]: means within a column with a same superscript are not significantly different ($p < 0{\cdot}05$).
[a,b,c,d]: means of a same source within a row with a same superscript are not significantly different ($p < 0{\cdot}05$).
lsdm: least significant difference among muscles.
lsdt: least significant difference among sampling times.

when expressed on a dry compared to wet basis (+6% vs +24%, respectively). The salt concentrations in muscle water tended to equalize in all muscles, however the salt concentration remained higher in *Rectus femoris* till the end of the processing (Table 2).

The salt content expressed on a wet matter basis was higher in scalded compared to singed hams at day 127 (7·1% vs 6·25%, respectively) and day 251 (8·0% vs 7·1%, $p < 0{\cdot}05$). The same was observed for the concentration of salt in muscle water ($114\,g\,l^{-1}$ vs $100\,g\,l^{-1}$, $p < 0{\cdot}05$ at day 127; $148\,g\,l^{-1}$ vs $129\,g\,l^{-1}$, $p < 0{\cdot}05$, at day 251). However, some of this variation was due to differences in extent of desiccation, since there was no significant difference in the salt content, on a dry matter basis at the end of processing, between both hams (17·6% vs 17·8%).

Nitrogen fractions
The changes in total nitrogen and nitrogen fractions are shown in Table 3. The total nitrogen content of the desalted dry matter (i.e. dry muscle tissue) decreased during the rest period then remained constant in the *Biceps femoris*. In the *Semimembranosus* it continued to decrease to the end of processing. The proportion of water-soluble nitrogen decreased during the salting period, then increased during the resting period in *Biceps femoris* and until day 127 in *Semimembranosus*. Differences between muscles were low. The NPN initially decreased in the *Biceps femoris*, then tended to increase. In the *Semimembranosus*

TABLE 3
Nitrogen Contents in *Biceps Femoris* and *Semimembranosus* Muscles during Dry-cured Ham Processing

	Total nitrogen, g kg⁻¹ of desalted dry matter			*Water-soluble nitrogen, % of total nitrogen*			*Non-protein nitrogen, % of total nitrogen*		
Time, days	*BF*	*SM*	*lsdm*	*BF*	*SM*	*lsdm*	*BF*	*SM*	*lsdm*
0	144^{1}	145^{1}	*6*	$39{\cdot}2^{a1}$	$41{\cdot}5^{b1}$	*1·5*	$17{\cdot}9^{1}$	$18{\cdot}0^{1}$	*3·5*
14	142^{1}	143^{1}	*3*	$32{\cdot}2^{a2}$	$29{\cdot}6^{b2}$	*1·7*	$12{\cdot}6^{a2}$	$18{\cdot}4^{b1}$	*1·5*
78	130^{a2}	138^{b2}	*5*	$35{\cdot}9^{3}$	$34{\cdot}0^{3}$	*2·3*	$16{\cdot}8^{a1}$	$13{\cdot}0^{b2}$	*1·3*
127	133^{a2}	128^{b3}	*4*	$36{\cdot}0^{3}$	$37{\cdot}0^{4}$	*1·7*	$20{\cdot}5^{a4}$	$14{\cdot}6^{b3}$	*2·0*
251	130^{a2}	116^{b4}	*5*	$38{\cdot}0^{13}$	$35{\cdot}9^{4}$	*2·6*	$17{\cdot}6^{a1}$	$15{\cdot}8^{b4}$	*1·2*
lsdt	*5*	*4*		*2·1*	*1·9*		*2·6*	*1·2*	

BF: *Biceps femoris*; SM: *Semimembranosus*.
^{1,2,3,4}: means within a column with a same superscript are not significantly different ($p < 0{\cdot}05$).
^{a,b}: means of a same source within a row with a same superscript are not significantly different ($p < 0{\cdot}05$).
lsdm: least significant difference among muscles.
lsdt: least significant difference among sampling times.

the values decreased during resting then changed little. Dehairing technique did not affect total nitrogen at any processing stage (results not shown). At day 251, singed hams contained more water-soluble nitrogen and NPN than scalded hams (38·4% vs 35·5%, $p < 0{\cdot}05$, and 17·5% vs 15·9%, $p < 0{\cdot}05$, respectively), which indicates more proteolysis.

The content of all free amino acids increased with time, except taurine and glutamine (Table 4). The increase between days 0 and 251 was very variable, but was generally 10–20 times at day 251 (but only about 2 for phosphoserine, 3 for aspartic acid, 1·5 for hydroxyproline, 2 for β-alanine). Taurine changed little during processing (+17% in *Semimembranosus* and −30% in *Biceps femoris*). Glutamine increased to day 78 then decreased below its initial level. The dipeptide carnosine decreased during processing while anserine increased slightly. There was little difference in free amino acid contents between both muscles during processing, generally in fresh hams they tended to be higher in *Semimembranosus*. They were higher in *Biceps femoris* than *Semimembranosus* at day 78, then became higher in *Semimembranosus* (results not detailed). The correlation coefficient between total amino acid contents of *Semimembranosus* and *Biceps femoris* was 0·96. For individual amino acids, most correlation coefficients were higher than 0·7 and generally in the range 0·85–0·97. Exceptions were phosphoserine, 0·67; aspartic acid, 0·61; hydroxyproline, 0·25; β-alanine, 0·51; glutamine, 0·46; taurine, 0·33; it should be noted that in all these latter amino acids the variation during processing was low. For simplicity, the results are not detailed for every amino acid, but Fig. 3 shows typical examples of the relationships between muscles with total amino acids and serine ($r = 0{\cdot}96$). Carnosine and anserine were higher in *Semimembranosus* than in *Biceps femoris*. Dehairing technique only slightly affected the content of taurine, which was higher by 30% in singed hams.

Electrophoresis

The electrophoretic profiles of proteins were comparable in both muscles of the fresh hams. They underwent numerous changes during processing. In electrophoresis of the low ionic strength-soluble fractions, all bands decreased during processing (Fig. 4). The phosphorylase band had almost disappeared at end of resting, while bands corresponding

TABLE 4
Free Amino Acids and Dipeptides (Carnosine and Anserine) in *Biceps Femoris* and *Semimembranosus* Muscles during Dry-cured Ham Processing

	Time, days						*Muscle*		
	0	*14*	*78*	*127*	*251*	*lsdt*	*BF*	*SM*	*lsdm*
n	*16*	*16*	*16*	*16*	*16*		*80*	*8*	
Phosphoserine	26^{a}	22^{b}	27^{a}	35^{c}	41^{d}	*3·6*	27^{a}	34^{b}	*2·9*
Aspartic acid	21^{a}	22^{a}	42^{b}	56^{c}	68^{d}	*9*	37^{a}	46^{b}	*8*
Glutamic acid	24^{a}	35^{a}	109^{b}	206^{c}	269^{d}	*21*	115	138	*33*
Hydroxyproline	1·9^{ac}	1·5^{a}	1·9^{a}	2·5^{bd}	2·3^{cd}	*0·5*	2·0	2·0	*0·3*
Serine	15^{a}	23^{a}	81^{b}	133^{c}	189^{d}	*12*	84	90	*22*
Glycine	32^{a}	37^{a}	90^{b}	138^{c}	205^{d}	12	98	100	*22*
Asparagine	8·8^{a}	14^{a}	55^{b}	81^{c}	91^{d}	*7·2*	48	50	*12*
β-alanine	9·5^{a}	7·3^{a}	8·3^{a}	13^{b}	15^{b}	*2·5*	9^{a}	12^{b}	*1·8*
Glutamine	32^{a}	37^{a}	54^{b}	24^{ac}	16^{c}	*9·2*	33	32	*7·1*
Taurine	98^{a}	68^{b}	88^{a}	90^{a}	89^{a}	*17*	84	90	*11*
Alanine	10^{a}	14^{a}	59^{b}	104^{c}	158^{d}	*10*	66	69	*19*
His + Thr	59^{a}	68^{a}	172^{b}	278^{c}	429^{d}	*27*	187	209	*47*
Arginine	0^{a}	0^{a}	82^{b}	138^{c}	188^{d}	*16*	77	83	26
Proline	9·0^{a}	10^{a}	67^{b}	124^{c}	209^{d}	*12*	79	85	*25*
Methyl-histidine	5·2^{a}	6·3^{a}	23^{b}	37^{c}	42^{d}	*5·0*	22	23	*5·9*
Tyrosine	8·0^{a}	11^{a}	39^{b}	57^{c}	82^{d}	*5·7*	36	43	*9·6*
Valine	13^{a}	19^{a}	87^{b}	148^{c}	244^{d}	*15*	95	105	*9*
Methionine	6·2^{a}	7·7^{a}	32^{b}	47^{c}	79^{d}	*5·3*	32	36	*9·2*
Isoleucine	7·2^{a}	10^{a}	55^{b}	71^{c}	124^{d}	*10*	51	56	*15*
Leucine	12^{a}	22^{a}	102^{b}	145^{c}	240^{d}	*18*	96	108	*29*
Phenylalanine	6·0^{a}	14^{a}	55^{b}	71^{c}	124^{d}	*10*	49	58	15
Tryptophane	1·0^{a}	1·1^{a}	5·7^{b}	8·4^{c}	14^{d}	*1·3*	5·6	6·3	*1·8*
Ornithine	1·4^{a}	1·9^{a}	5·1^{b}	5·0^{b}	14^{c}	*3·3*	4·9	5·9	*2·5*
Lysine	11^{a}	15^{a}	60^{b}	105^{c}	260^{d}	*19*	82	94	*32*
Total FAA	416^{a}	465^{a}	1402^{b}	2124^{c}	3192^{d}	*195*	1417	1571	*357*
N AA/ N, %	0·75^{a}	0·86^{b}	2·87^{c}	4·48^{d}	6·83^{d}	*0·37*	2·87	3·16	*0·74*
N AA/NPN, %	4·6^{a}	6·0^{a}	19·3^{a}	26·5^{b}	39·2^{b}	*2·9*	16·5^{a}	21·2^{b}	*4·5*
Carnosine	894^{a}	732^{b}	724^{b}	656^{b}	548^{c}	*85*	618^{a}	810^{b}	*58*
Anserine	12^{a}	12^{a}	14^{a}	14^{a}	17^{b}	*1·9*	12^{a}	16^{b}	1·2

Amino acids and dipeptides are expressed in μmol g^{-1} N.
N AA: amino acid nitrogen. BF: *Biceps femoris*; SM: *Semimembranosus*.
^{1,2,3,4,5}: means within a column with a same superscript are not significantly different ($p < 0{\cdot}05$).
^{a,b,c,d,e}: means of a same source within a row with a same superscript are not significantly different ($p < 0{\cdot}05$).
lsdm: least significant difference among muscles; *lsdt*: least significant difference among sampling times.

to pyruvate kinase, enolase, creatine phosphokinase, phosphoglycerate mutase and triose-phosphate isomerase remained visible to the end. Important modifications were apparent particularly in the ranges 150–95 kDa and 40–30 kDa. The changes were similar in both muscles, although they were greater between days 0 and 78 (salting-resting period) in *Semimembranosus*. Electrophoresis of the myofibrillar fractions showed changes in all bands except actin (42 kDa) which underwent little change (Fig. 5). The most striking changes were in the ranges 220–95 kDa and 40–20 kDa. A band corresponding to a molecular weight of about 150 kDa increased from the end of the resting period (Fig. 5 and Table 5). At the same time, the intensity of the bands at 220 kDa (myosin heavy

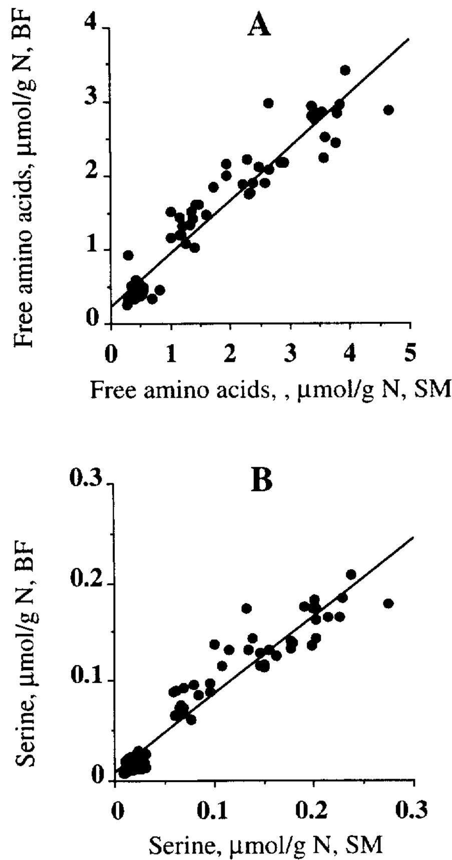

Fig. 3. Relationships between free amino acid contents in *Semimembranosus* and *Biceps femoris* during dry-cured ham processing. A, Total free amino acids; B, serine.

chain, MHC) and 95 kDa, and all major bands in the range 40–20 kDa decreased noticeably. These changes were more marked in the *Semimembranosus* in the earlier processing steps (Table 5). MHC decreased more in scalded than singed hams and the 95 kDa band decreased more in singed hams during processing (results not detailed). The ratio MHC/actin decreased from 1·08 to 0·82 in the former and from 1·06 to 0·67 in the latter, between days 0 and 251. Simultaneously, the ratio 95 kDa/actin decreased from 0·41 to 0·15 in singed hams and from 0·42 to 0·22 in scalded hams. In both cases the differences between dehairing techniques were significant at day 251.

Microscopy

In the fresh hams, numerous ultrastructural changes were observed, such as weakening of Z-lines and transversal breaks of fibres (Fig. 6). However the myofibrillar structure was clearly visible. The changes were similar in both *Biceps femoris* and *Semimembranosus* muscles. At the end of salting, fibre disruptions appeared more frequently than before salting. The myofilaments and the transversal striation were more easily distinguished in

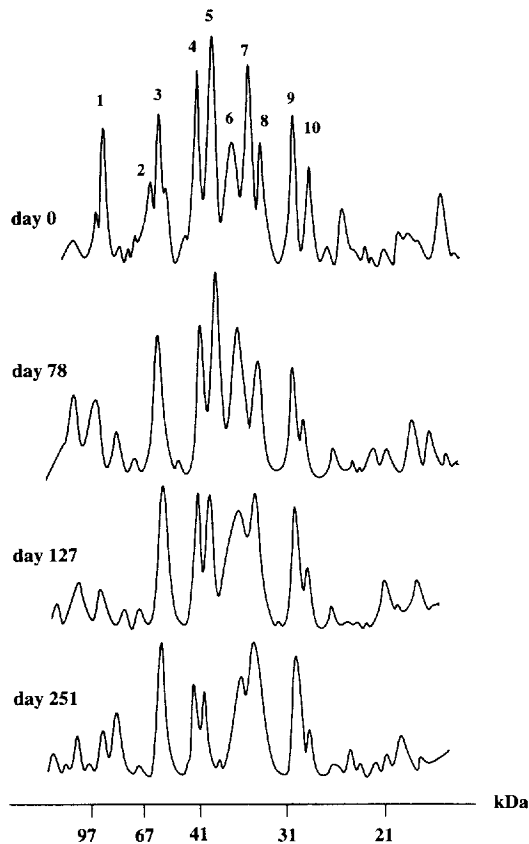

Fig. 4. Densitograms of electrophoretograms of low ionic-strength soluble nitrogen fraction at various steps of processing. Bands identified from McCormick *et al.* (1988) and Savage *et al.* (1990): 1, phosphorylase; 2, phosphoglucomutase; 3, pyruvate kinase; 4. enolase; 5, creatine phosphokinase; 6, aldolase; 7, glyceraldehyde-phosphate dehydrogenase; 8, lactate dehydrogenase; 9, phosphoglycerate mutase; 10, triose-phosphate isomerase.

the *Biceps femoris*. This difference increased during resting. At the end of this period, the M band was still visible in some places in the *Biceps femoris*, but not in *Semimembranosus*. Ultrastructural features were difficult to distinguish in both muscles at the end of processing. The dehairing technique did not affect either the ultrastructure of muscle or the microscopic appearance of fat tissue.

Rheological traits

Hardness was higher in *Biceps femoris* than in *Semimembranosus* at days 0 and 251, while the reverse was observed at day 127. It increased in both muscles during the first half of the processing then returned to values close to the initial in *Semimembranosus* but remained stable in *Biceps femoris* (Table 6). Cohesiveness was increased at day 127 then decreased in *Semimembranosus*. Springiness increased between the beginning and the end of processing in both muscles. Adhesiveness was not significantly different between

muscles at day 0, but it increased much more in *Semimembranosus* than *Biceps femoris* during processing. In the former, it reached a very high value at day 127, then decreased but stayed higher than in *Biceps femoris*. The changes in chewiness with time parallelled

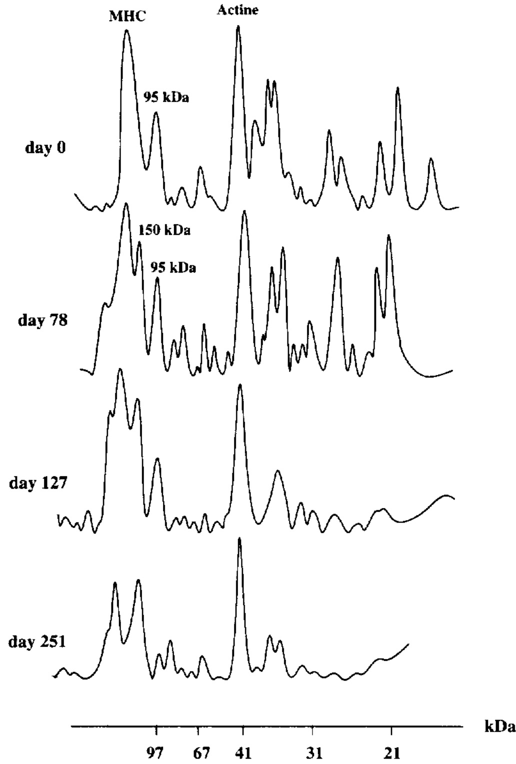

Fig. 5. Densitograms of electrophoretograms of myofibrillar fraction at various steps of processing. MHC and actin bands identified from Iversen (1995).

TABLE 5
Relative Densities of 220 kDa (MHC), 150 kDa and 95 kDa Bands in Myofibrillar Protein Electrophoresis

	MHC/actin			*150 kDa/actin*			*95 kDa/actin*		
Time, days	*BF*	*SM*	*lsdm*	*BF*	*SM*	*lsdm*	*BF*	*SM*	*lsdm*
0	$1{\cdot}07^{12}$	$1{\cdot}06^{1}$	*0·13*	$0{\cdot}49^{a1}$	$0{\cdot}36^{b1}$	*0·09*	$0{\cdot}47^{a1}$	$0{\cdot}36^{b1}$	*0·08*
78	$1{\cdot}26^{a1}$	$0{\cdot}89^{b2}$	*0·25*	$0{\cdot}55^{a1}$	$0{\cdot}45^{b1}$	*0·07*	$0{\cdot}48^{a1}$	$0{\cdot}37^{b1}$	*0·08*
127	$0{\cdot}99^{23}$	$0{\cdot}86^{2}$	*0·17*	$0{\cdot}89^{2}$	$0{\cdot}77^{2}$	*0·20*	$0{\cdot}34^{2}$	$0{\cdot}27^{2}$	*0·10*
251	$0{\cdot}78^{3}$	$0{\cdot}75^{2}$	*0·17*	$1{\cdot}02^{2}$	$0{\cdot}94^{3}$	*0·38*	$0{\cdot}19^{3}$	$0{\cdot}19^{2}$	*0·09*
lsdt	*0·21*	*0·14*		*0·19*	*0·16*		*0·09*	*0·09*	

BF: *Biceps femoris*; SM: *Semimembranosus*.
[1,2,3]: means within a column with a same superscript are not significantly different at the $p < 0{\cdot}05$ level.
[a,b]: means of a same source within a row with a same superscript are not significantly different at the $p < 0{\cdot}05$ level.
lsdm: least significant difference among muscles;
lsdt: least significant difference among sampling times.

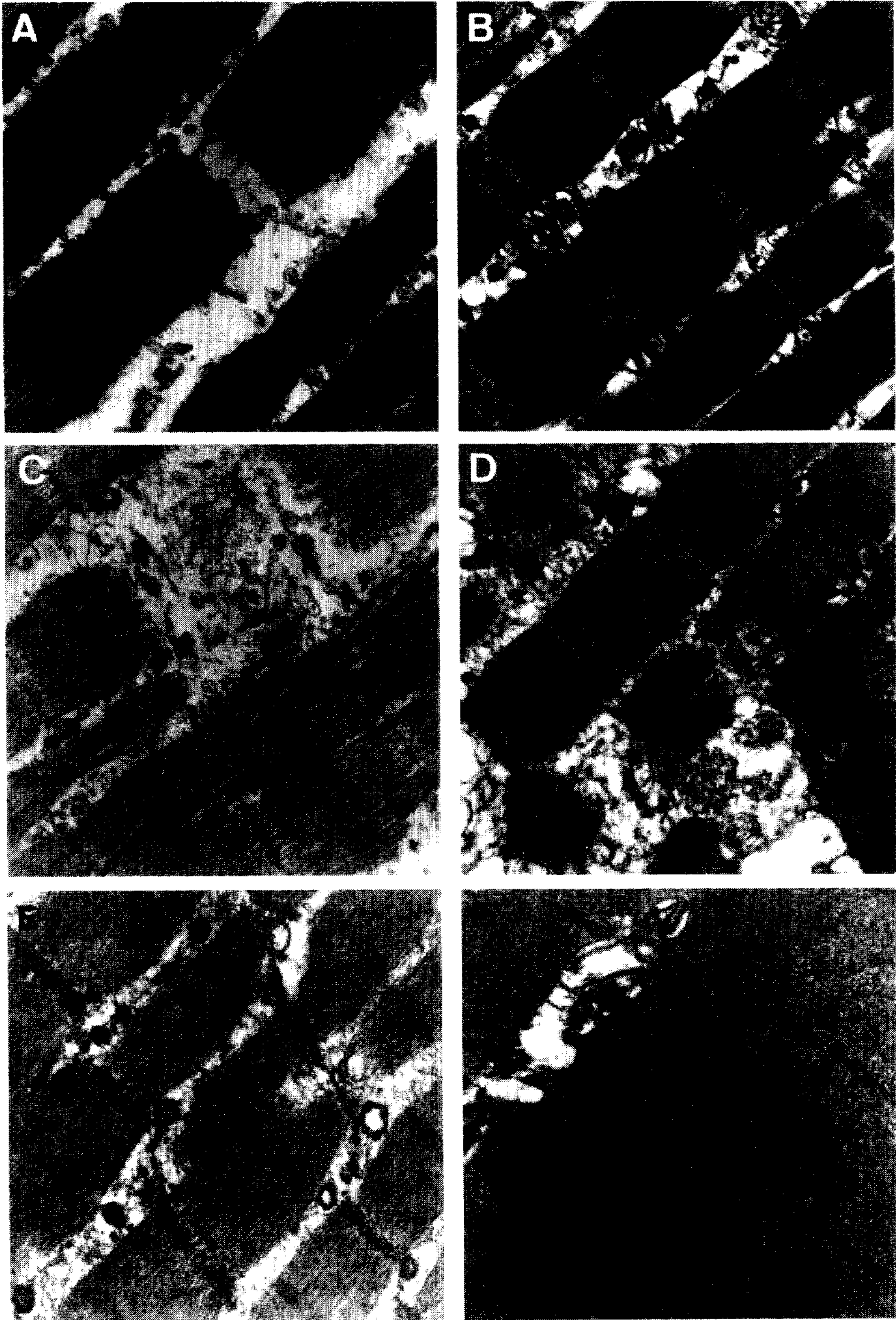

Fig. 6. Electron micrographs of *Biceps femoris* (left pictures) and *Semimembranosus* (right pictures) at days 0 (a, b), 14 (c, d) and 78 (e, f).

TABLE 6
Rheological Traits of 2 Muscles during Dry-cured Ham Processing

	Hardness, kg			*Springiness, cm*			*Adhesiveness, units*		
Time, days	*BF*	*SM*	*lsdm*	*BF*	*SM*	*lsdm*	*BF*	*SM*	*lsdm*
0	1·73^{a1}	0·87^{b1}	*0·31*	13·9^{1}	13·4^{1}	*0·79*	10·9^{1}	7·0^{1}	*4·0*
127	2·38^{a2}	3·59^{b2}	*0·73*	13·2^{a2}	13·9^{b12}	*0·62*	18·0^{a2}	79·6^{b2}	*19·2*
251	2·32^{a2}	1·18^{b1}	*0·29*	15·1^{a3}	14·4^{b2}	*0·54*	21·6^{a2}	33·9^{b3}	*7·5*
lsdt	*0·54*	*0·43*		*0·61*	*0·70*		*6·8*	*15·3*	
	Cohesiveness, units			*Chewiness, units*					
Time, days	*BF*	*SM*	*lsdm*	*BF*	*SM*	*lsdm*			
0	0·45^{1}	0·43^{1}	*0·05*	10·7^{a1}	5·1^{b1}	*2·6*			
127	0·51^{a2}	0·57^{b2}	*0·05*	16·6^{a2}	28·1^{b2}	*6·7*			
251	0·47^{12}	0·46^{1}	*0·04*	16·9^{a2}	7·8^{b1}	*3·0*			
lsdt	*0·05*	*0·03*		*4·9*	*4·1*				

BF *Biceps femoris*; SM: *Semimembranosus*.
^{1,2,3}: means within a column with a same superscript are not significantly different ($p < 0{\cdot}05$).
^{a,b}: means of a same source within a row with a same superscript are not significantly different at the ($p < 0{\cdot}05$).
lsdm: least significant difference among muscles.
lsdt: least significant difference among sampling times.

those observed for hardness. This was because hardness showed the biggest change over sampling time among the values used to calculate chewiness. The scalded hams showed higher hardness ($p < 0{\cdot}05$) and chewiness ($p = 0{\cdot}05$) than the singed ones at day 127.

Sensory traits

The technique of dehairing affected some sensory traits (Table 7). The scalded hams were more salty and pungent. They had more pronounced aromas of dry ham, rancidity and hazelnut, and less aroma of fresh meat. Their texture was dryer and less mellow.

DISCUSSION

As stated in the Introduction, there are numerous reports available on the biochemical changes taking place in dry-cured ham during processing. However comparisons are difficult due to differences in the technologies applied. Overall the results of the chemical analyses were in agreement with those of Buscailhon *et al.* (1994*a*,*b*) who studied extensively the chemical changes in Bayonne hams during processing, except for NPN and free amino acids (this is discussed below).

The changes in salt content relative to the dry matter of the various muscles showed that the transfer of salt within the ham continued to the end of processing. The salt content reached its final level at day 127 in *Rectus femoris* and *Semitendinosus*, and had almost reached it in *Biceps femoris*. However, it continued to decrease in *Semimembranosus*, after the initial rise corresponding to the salting period. Similar observations were recently reported by Arnau *et al.* (1995) in Spanish dry-cured hams studied for 207 days. In the last processing steps, the distribution of the salt in the ham appeared to depend mainly on the water distribution, as the salt concentration in water tended to equalize in the three most important muscles. Thus, during processing, salt initially tends to distribute uniformly in the ham water, then the distribution of salt within the ham stays similar

TABLE 7
Effect of the Carcass Dehairing Technique on the Sensory Quality of Dry-cured Hams

Traits		*Dehairing* *Singeing*	*Scalding*	*P*
Appearance	Red colour	5·8	6·3	ns
	Marbling	5·9	5·7	ns
	Brightness	5·9	5·1	ns
Taste	Salty	5·3	6·1	**
	Acid	3·2	3·5	ns
	Bitter	2·4	2·7	ns
	Pungent	3·1	3·6	*
Aroma	Dry ham	5·0	6·0	**
	Rancid	3·3	3·8	*
	Fresh meat	4·2	3·1	**
	Fresh fat	4·1	3·6	ns
	Butter	3·7	3·3	ns
	Hazelnut	2·9	3·4	**
Texture	Dry	4·2	5·5	*
	Mellow	5·9	4·5	**

Significance of differences: ns, non-significant.
*$p < 0.05$, **$p < 0.01$.

to that of water. A similar conclusion was reached by Arnau *et al.* (1995) and Leon Crespo *et al.* (1991), although the latter used a different experimental design. Thus it can be concluded that the gradients of salt inside the ham are directly determined by the gradients of water, and thus should be closely related to the rate of water extraction from the cut surface.

The levels of water-soluble nitrogen were in the range observed by other authors using water or dilute buffers (e.g. Ambanelli *et al.*, 1968; Flores *et al.*, 1984; Bellati *et al.*, 1985; Toldra *et al.*, 1993; Buscailhon *et al.*, 1994*a*; Hortos Bahi, 1995). During salting, the decrease in soluble nitrogen relates to the denaturation of the proteins due to the rise in salt concentration. The subsequent increase in soluble nitrogen can be attributed to proteolysis. The initial level of NPN was rather high, about 18% in both muscles studied. In *Biceps femoris* sampled the day after slaughter, Buscailhon *et al.* (1994*a*) found NPN values of about 12% using the same technique. The high initial NPN level in the present study was probably due to the fact that the hams were sampled after 3 days of chilling which allowed proteolysis to develop. By contrast, the levels of NPN at the end of seasoning were lower in the present work than reported in *Biceps femoris* of Bayonne hams by Buscailhon *et al.* (1994*a*) as well as by Virgili (1996), i.e. about 22 and 24%, respectively. The changes in NPN during processing in the present study disagree with previous authors who reported an increase during processing (Ambanelli *et al.*, 1968; Flores *et al.*, 1984; Bellati *et al.*, 1985; Astiasaran *et al.*, 1989; Buscailhon *et al.*, 1994*a*; Hortos Bahi, 1995). In general, the content of NPN was found to double during processing, rising from 10–12 to 20–25% of total nitrogen.

Regarding free amino acid contents, the present observations agree with those of numerous authors (Ambanelli *et al.*, 1968; Baldini *et al.*, 1977; Bellati *et al.*, 1985, Cordoba Ramos, 1990; Aristoy and Toldra, 1991; Hortos and Garcia-Regueiro, 1991; Hortos Bahi, 1995) who observed an increase in free amino acids throughout processing. In contrast, Flores *et al.* (1984), Astiasaran *et al.* (1989) and Buscailhon *et al.* (1994*a*) observed a rise in the levels of most free amino acids during the first months followed by a subsequent

decrease. Concerning the differences between muscles, the present results agree with those of Cordoba Ramos (1990) who found that contents of individual amino acids differed little in *Biceps femoris* and *Semimembranosus*. Hortos Bahi (1995) observed that concentrations were higher in the *Semimembranosus* of fresh hams, as in the present study, but higher in the *Biceps femoris* during all further processing steps. This author suggested that proteolysis was more intense in the latter muscle due to higher water and lower salt contents. Clearly this explanation does not explain our results.

The identification of the bands in the electrophoretic profiles were made by comparison with the reports of McCormick *et al.* (1988) and Savage *et al.* (1990) for low ionic strength-soluble fraction, and with Iversen (1995) for the myofibrillar fraction. The changes revealed by electrophoresis of the low ionic strength-soluble fraction showed similarities with those observed by Toldra *et al.* (1992, 1993) and Hortos Bahi (1995). The latter found that phosphorylase kinase, phosphoglyceromutase and triosephosphate isomerase were the most stable proteins of those soluble in water and 1% NaCl solutions, while phosphorylase had disappeared by the end of salting. The changes observed in the ranges 150–95 and 40–30 kDa can be attributed to the degradation of sarcoplasmic proteins and appearance of products from myofibrillar degradation, as suggested by Toldra *et al.* (1993). Concerning the myofibrillar fraction, our findings agree in large part, with those of Cordoba *et al.* (1994) and Hortos Bahi (1995) who carried out electrophoresis of the proteins soluble in 0·1 M phosphate, 1·1 M KI during dry ham processing. These authors observed a decrease in all bands present in the fresh muscle and an increase in a band of about 150 kDa (transitory increase for Hortos Bahi). The band corresponding to actin was retained in the final product. However it is difficult to compare these results in detail due to differences in the techniques used for protein extraction. In particular, our fraction is constituted essentially of myofibrillar protein, because of washing procedure. By contrast, the 0·1 M phosphate, 1·1 M KI-soluble fractions were likely to contain a significant amount of insolubilized sarcoplasmic protein, at least after salting. The faster changes in the myofibrillar electrophoretic profiles of *Semimembranous* during the first steps of processing agree with the fact that ultrastructural changes were seen earlier in this muscle. Sayas-Barbera *et al.* (1990) and Aranda-Catala *et al.* (1991) also found that ultrastructural changes were faster in *Semimembranosus* than in *Biceps femoris*. They suggested that the increase in salt concentration was the cause of the ultrastructural disorganization. The present results agree with this suggestion. It is known that any increase in ionic strength in muscle tissue can contribute to degradation of the myofibrillar structure (Monin and Ouali, 1991). On the whole, these results question the validity of NPN as an index of proteolysis. Indeed, proteolysis appeared more active in the *Biceps femoris* than in the *Semimembranosus* on the basis of NPN content. On the other hand, electrophoretic and ultrastructural data indicated that protein degradation was more active in the *Semimembranosus*. The net value of NPN at any time depends on the balance between the NPN production from proteolysis, NPN transfers by diffusion and NPN losses by amino acid degradation. All these phenomena are affected by salt and moisture levels and pH (Baldini and Raczynski, 1979; Sarraga *et al.*, 1989; Sarraga, 1992; Toldra, 1992; Hortos Bahi, 1995).

The decrease in total nitrogen content of the desalted dry matter in both *Semimembranosus* and *Biceps femoris* deserves discussion. As noted above, the variation in nitrogen level at a given point in the ham may depend partly on diffusion of low molecular weight nitrogen compounds. However, it can be assumed that this diffusion goes from the *Semimembranosus* to the *Biceps femoris*, as is the case for salt, thus increasing the nitrogen content of the latter. That a decrease is observed in both muscles indicates a loss of nitrogen compounds during processing. This loss may occur from the NPN fraction by desamination and/or transformation of amino acids to volatile compounds and might be

important in the present study. Such a loss of nitrogen has been reported previously by Buscailhon (1994*a*) in *Biceps femoris*. Similarly, Coutron (1996) has observed a net loss of dry matter of 4% in the *Biceps femoris* of Corsican hams, which may well result, at least partly, from the same phenomenon. The decrease in nitrogen content of the *Biceps femoris* observed here was greater than reported by Buscailhon *et al.* (1994*a*) (7·5% vs 4%). It was still higher in the *Semimembranosus* (18%), where it continued during all processing while it occurred only during the resting period in the *Biceps femoris*. Possibly diffusion of low molecular weight nitrogen compounds from the former towards the latter muscle contributes to the difference between the muscles. The present results cannot explain the mechanisms which underly the nitrogen loss, but they suggest that proteolysis might be more active than shown by NPN levels, as noted above. From a practical point of view, this loss of nitrogen could be very important. It is noticeable that in the literature on dry-cured ham, there are many qualitative studies, but few attempt to quantify the observed phenomena at the level of the whole ham; the work of Arnau *et al.* (1995) regarding salt is a notable exception. It is of practical interest to verify the extent of this nitrogen loss in the whole ham and to investigate the mechanism. In this respect, the use of labelled molecules to quantify the diffusion phenomena and metabolic chambers to analyse, both quantitatively and qualitatively, exchanges between the ham and its environment could be helpful.

The changes in hardness and chewiness probably depends on both water content and state of proteins. Monin *et al.* (1995*b*) have observed a negative relationship between these traits and water content in six types of dry hams. In post mortem muscle, it is well-known that hardness increases with the initial loss of protein solubility, then decreases with the degradation of the myofibrillar structure (Ouali, 1990). In dry hams, the situation is complicated by the changes in water and salt contents. However, it can be assumed that hardness and chewiness increased during the initial steps of processing due to a decrease in the solubility of proteins and water content, then decreased as proteolysis progressed.

The hams from scalded carcasses absorbed more salt and lost more water than those from singed carcasses. This phenomenon can be attributed to the effect of the dehairing technique on the state of the skin. As noted in the Introduction, hair is pulled out during scalding, which is likely to make microscopic 'holes' in the skin through which salt penetration and water extraction are made easier. The hams from scalded carcasses were saltier than those from singed carcasses. This resulted from their higher salt content, since a good correlation has been found between salt concentration and salty taste (Rousset-Akrim, 1996). The drier and less mellow texture of the scalded hams agreed with their lower water content. Moreover the better mellowness of the singed hams could be related to their higher content of NPN, as Virgili (1996) has observed a positive relation between these traits. The scalded hams got sensory scores indicating a more aged state: higher rancid and dry ham aromas and lower fresh meat aroma.

CONCLUSION

During processing of dry-cured hams, there is a decrease in the muscle nitrogen content. This decrease seems to be related to the intensity of proteolysis as assessed by electrophoretic profiles and ultrastructural changes. It would be useful to confirm and quantify the corresponding nitrogen loss in the whole ham. The technique of carcass dehairing used for producing the hams affected the composition and sensory quality of the product. The singed hams were less salty and more mellow, which is favourable from both nutritional and sensory points of view. On the other hand, scalded hams presented a higher degree of maturity, in terms of aroma, than the singed ones.

Thus the use of singeing in place of scalding may well lengthen the processing time required to obtain a similar aroma quality. This is undesirable on economic grounds.

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