DIFFERENCES IN SARCOPLASMIC PROTEINS FROM HAMS FROM THREE WHITE PIG CROSSES CONTAINING DUROC

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Background

Structural alterations in the sarcoplasmic fraction have been described in meat during *post mortem* ripening (Claeys *et al.,* 1995; Alomirah *et al.*, 1998). Curing brings about degradation of the sarcoplasmic proteins in hams. Córdoba (1990) studied the changes taking place in the electrophoretic bands for the sarcoplasmic proteins at different stages in the Ibérico ham curing process lasting 17½ months and reported that levels of phosphorylase b, enolase, creatine kinase and myoglobin became undetectable. Monin *et al.* (1997) carried out a similar study on Bayonne hams cured for 8 months and found a gradual decrease in the intensity of the electrophoretic bands. Additionally, in Ibérico hams Toldrá *et al.* (1993) recorded the appearance of protein fragments extractable in low ionic strength buffer and Córdoba *et al.* (1994) reported the appearance of bands for the molecular weights of 120, 85, 70 and 67 kDa.

Objectives

The main objective was to compare the changes taking place in the sarcoplasmic proteins from the *Semimembranosus* and *Biceps femoris* muscles from hams from three different crosses extracted in a low ionic strength aqueous buffer 5 days *post mortem* and after curing for 11 months.

Materials and methods

Ham samples: two Duroc (DU) boars and one Duroc x Large White (LW) boar from three breeding enterprises were selected commercially and mated with Duroc x Landrace (LD) sows to yield the following crosses: **A:** 75% DU + 25% LD, **B:** 50% DU + 25% LD + 25% LW, **C:** 75% DU + 25% LD. Slaughter took place at a mean weight of 111 kg and a mean age of 6 months. Hams were processed 5 d *post mortem* using a curing procedure for a period that lasted 11 months. The *Semimembranosus* and *Biceps femoris* muscles from 6 raw hams and 6 cured ham from each of the crosses considered were analized.

Electrophoresis: the proteins soluble in low ionic strength buffer were extracted using the method of Monin *et al.* (1997), dialysed using a 7 000 Da and denatured according to the method of Toldrá *et al.* (1990). The proteins were separated by horizontal SDS-PAGE using a PhastSystem apparatus following the recommendations set out in the instruction manual for the equipment used (Amersham Pharmacia Biotech, 1986a,b). Quantification of the bands was carried out by first scanning the gels using the PhotoPhinish program (TDI, Spain) and then taking densitometric readings using the 1D-Manager program (TDI, Spain). The molecular weights of the protein bands were calculated from the Rf values by interpolation of the calibration curve constructed using markers (Sigma) of known molecular weight spanned a range from 6.5 to 205 kDa.

Statistical analysis: analysis of variance was applied to the data using the SPSS 10.0 program for Windows 2000. The level of significance of the differences was tested using the least significant difference (LSD) test.

Results and discussion

Raw hams

Qualitatively the electrophoretic profiles for both the *Semimembranosus* and the *Biceps femoris* muscles were similar for all three crosses considered (Fig. 1), though there were significant differences in the relative quantities of certain proteins: phosphorylase b and phosphorylase b kinase for both the *Semimembranosus* and the *Biceps femoris* muscles, pyruvate kinase and triosephosphate isomerase in the *Semimembranosus* muscle and phosphoglucomutase, creatine kinase/phosphoglycerate kinase, phosphoglycerate mutase and myoglobin for the *Biceps femoris* muscle.

Cured hams

The electrophoretic profiles for the high molecular mass protein fragments differed by muscle and by sample tested. However, the electrophoretic profiles for the fragments for molecular weights lower than 66 kDa (phosphoglucomutase) were quite similar qualitatively. The bands with molecular masses of 36 (glyceraldehyde-3-phosphate dehydrogenase), 31 and 9 kDa from the *Semimembranosus* muscle exhibited significant differences among the three crosses. In the *Biceps femoris* muscle the 15 kDa band was the only band that displayed any significant differences among the crosses.

Changes undergone by the proteins soluble in low ionic strength buffer extracted from the raw and cured hams

The changes in the sarcoplasmic proteins revealed by the electrophoretic patterns at the end of curing were similar for both of the muscles examined. On the whole, four different findings were observed:

(1) Polypeptides that ceased to be detectable after curing: the muscles from the raw hams displayed bands with molecular weights of 155, 144, 71 25 and 23 kDa as well as those for enolase and which were not recorded in any of the samples from the cured hams. The 16 kDa electrophoretic band, identified as myoglobin, also ceased entirely to be detectable, perhaps because this protein bound to nitric oxide to form nitrosomyoglobin, which is not soluble in aqueous buffers (Córdoba *et al.,* 1994).

(2) Polypeptides whose concentrations underwent changes during curing: decreases in the following proteins were observed: 149 kDa, phosphorylase b/phosporylase b kinase, phosphofructokinase, AMP deaminase, phosphoglucomutase, creatine kinase/fosfoglycerate kinase, aldolase and lactate dehydrogenase. The bands for these proteins even ceased to be detectable in certain of the samples analysed. The

absence of these proteins may have been the outcome of proteolysis and/or insolubilization attributable to the conditions in which the curing process takes place (Klement *et al.,* 1973; Wardlaw *et al.,* 1973; Astiasarán *et al.,* 1990).

Increases in relative intensity were also recorded for phosphoglucose isomerase, glyceraldehyde-3-phosphate dehydrogenase, triosephosphate isomerase, myokinase and the protein with a molecular mass of 12 kDa (in *Biceps femoris* muscle only).

(3) Polypeptides that formed over the course of curing: the new bands recorded in the muscle samples taken from the cured hams but not present in the muscle samples taken from the raw hams may have been produced by proteolysis of the myofibrillar proteins (Toldrá *et al.,* 1993; Córdoba *et al.,* 1994). These bands had molecular weights of 124, 112, 104, 96, 94, 56, 31, 30, 22, 18, 15 and 9 kDa. The 31, 18, 15 and 9 kDa fragments were recorded for all the samples analysed. Fragments with molecular weights of 11 and 10 kDa were recorded for some of the samples from the raw hams but in contrast were present in all the samples from the cured hams.

(4) Polypeptides that did not undergo any variations: pyruvate kinase was the only protein whose concentration remained constant during the study.

Conclusions

The electrophoretic profiles for the two muscles considered from the raw hams were qualitatively similar, but the profiles for the muscles from the cured hams revealed changes in the electrophoretic bands in the range of 66-149 kDa. The relative concentrations of certain proteins differed in both the raw and cured hams from the three crosses. Curing resulted in the loss of electrophoretic bands, chiefly for the higher molecular weights; the appearance of new protein fragments, primarily in the range of 9-22 kDa; and quantitative variations in certain polypeptides.

Pertinent literature

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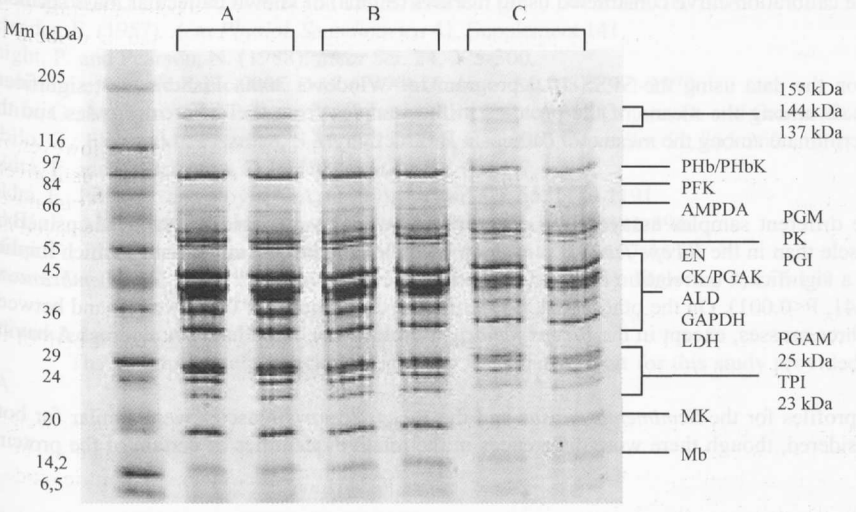

Fig. 1. Electrophoregram for the proteins soluble in low ionic strength buffer (sarcoplasmic proteins) extracted from the *Semimembranosus* muscle in raw hams from crosses A, B and C. PHb: phosphorylase b; PHbK: phosphorylase b kinase; PFK: phosphofructokinase; AMPDA: AMP deaminase; PGM: phosphoglucomutase; PK: pyruvate kinase; PGI: phosphoglucose isomerase; EN: enolase; CK: creatine kinase; PGAK: phosphoglycerate kinase; ALD: aldolase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; LDH: lactate dehydrogenase; PGAM: phosphoglycerate mutase; TPI: triosephosphate isomerase; MK: myokinase; Mb: myoglobin.