DIFFERENCES IN PROTEOLYSIS OF MYOFIBRILLAR PROTEINS IN HAMS FROM THREE WHITE PIG CROSSES CONTAINING DUROC

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Background

During curing proteolytic reactions take place in hams, catalyzed primarily by lysosomal proteases, in particular cathepsins B, L, H, and D. Toldrá *et al.* (1993) reported that after curing for 15 months cathepsins B and L were active and were more closely involved in the breakdown of sarcoplasmic and myofibrillar proteins than were cathepsins H and D.

The changes undergone by the myofibrillar proteins in hams from white pigs during curing have been widely studied using denaturing electrophoresis on polyacrylamide gels (SDS-PAGE). Toldrá *et al.* (1992) observed complete breakdown of myosin heavy chain and detected the presence of a 150 kDa fragment and a large number of peptides in the region between 50-100 kDa in hams cured for 8 months. Tabilo *et al.* (1999) found that myosin heavy chain, α-actinin, and T-troponin ceased to be detectable in hams from Landrace x (Large White x Duroc) crosses cured for 12 months, and they attributed the appearance of two peptide fragments of 150 and 85 kDa in those same hams to the degradation of the myosin heavy chain and sarcoplasmic proteins, respectively. They also recorded an increase in the densities of various polypeptides in the region of 14.4-40 kDa, though according to their findings actin had not undergone any changes at the end of curing. Working with Bayonne hams cured for 8 months, Monin *et al.* (1997) observed changes in all the electrophoretic bands except that of actin, reporting the highest levels of alteration in the regions of 95-220 kDa and 20-40 kDa, where the intensity of the bands for myosin heavy chain, a protein with a molecular mass of 95 kDa, and the major proteins in the region of 20-40 kDa all decreased. The 150 kDa band increased at the end of the resting period (day 78 of curing).

Objectives

The principal objective of this study was to examine the influence of genetic factors on the proteolysis of myofibrillar proteins taking place in hams during curing.

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 six months. Hams were processed 5 days *post mortem* and cured for 11 months. Cathepsin B+L activity in the raw hams was determined in 17 hams from cross A, 23 hams from cross B and 20 hams from cross C, while cathepsin B+L activity in the cured hams was determined in 20 hams from each of the crosses. SDS-PAGE was performed on 6 raw hams and 6 cured hams from each cross chosen at random from the set of sample hams used to analyse cathepsin B+L activity.

Cathepsin B+L activity: The cathepsin B+L extract was obtained according to the method of Etherington *et al.* (1987) and Koohmaraie and Kretchmar (1990). Cathepsin B+L activity was assayed by the fluorimetric method described by Barrett (1980) and Koohmaraie and Kretchmar (1990).

Electrophoresis: The proteins soluble in high ionic strength buffer were extracted using the method of Helander (1957), dialysed using a 7000 Da membrane, 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 employed (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 masses of the protein bands were calculated from the Rf values by interpolation of the calibration curve constructed using markers (Sigma) of known molecular mass spanning a range of from 6.5 to 205 kDa.

Statistical analysis: Analysis of variance was run on the data using the SPSS 10.0 program for Windows 2000. Fisher's least significant difference (LSD) procedure was used to discriminate among the means of the proteins in the samples from the different crosses and the Student Newman Keuls (SNK) test was used to discriminate among the means for cathepsin B+L activity.

Results and discussion

Cathepsin B+L activity: Enzymatic activity in the different samples assayed was observed to be highly variable. Higher cathepsin B+L activity was recorded in the *Semimembranosus* muscle than in the *Biceps femoris* muscle in both the raw and the cured hams, which implies higher proteolysis levels in the former. There was a significant correlation between the enzymatic activity levels in the *Semimembranosus* and *Biceps femoris* muscles in the raw hams ($r=0.641$, $P<0.001$). On the other hand, no significant differences ($P<0.05$) were found between the mean values for cathepsin B+L activity in the three crosses, except in the *Biceps femoris* muscle in the cured hams, with cross A having the highest value and cross C having the lowest.

Electrophoresis: Qualitatively, the electrophoretic profiles for the *Semimembranosus* and the *Biceps femoris* muscles were similar for both the raw and cured hams from all three crosses considered, though there were differences in the relative quantities of certain of the proteins present.

- *Raw hams*: There were significant differences in the proteins with the highest molecular masses in the *Biceps femoris* muscle in the samples from the three crosses: 200 kDa (myosin heavy chain), 102 kDa (α-actinin) and the 59 and 56 kDa proteins were all lower in cross A. Conversely, this same cross exhibited the highest values for the proteins with the lowest molecular masses, 37 (T-troponin), 24 (I-troponin), 18 (myosin light chain II) and 17 kDa. The low values for the high molecular mass proteins and the high values for the low

molecular mass proteins suggest that proteolysis was higher in cross A than in the other two crosses at five days *post mortem*. Furthermore, this result was more pronounced for the *Biceps femoris* muscle.

- *Cured hams*: Significant differences were observed in the *Semimembranosus* muscle for the low molecular mass bands, namely, 35 (tropomyosin), 22, 9, and 8 kDa; in all cases the highest values were recorded for cross B. Significant differences were observed in the *Biceps femoris* muscle for the proteins with masses of 89, 25 (myosin light chain I) and 9 kDa, the mean values for the last two proteins being highest in cross B. The concentration levels for the peptide fragments recorded in both muscles indicated that proteolysis was more pronounced in the hams from cross B over the 11-month curing period, which should make a positive contribution to aroma and flavour development.

- *Changes undergone by the myofibrillar proteins extracted from the raw and cured hams*: The changes in the myofibrillar proteins at the end of the curing period as revealed by the electrophoretic patterns were similar for both the muscles considered. Overall, there were four main categories of findings:

(1) Polypeptides that ceased to be detectable after curing: The molecular masses involved were 200 kDa (myosin heavy chain), 140 kDa (C-protein), 102 kDa (α-actinin), 56 kDa, 18 kDa (myosin light chain II) and 17 kDa. Cleavage of such high molecular mass proteins as myosin may have been caused by proteolysis catalyzed by cathepsins B, D, H, and L (Toldrá and Etherington, 1988; Toldrá *et al.*, 1993), by lower solubility as a result of denaturation brought about by drying (Astiasarán *et al.*, 1990), or by oxidation reactions catalyzed by heavy metals that may be present as contaminants in the salt used in curing, causing bonds to form between molecules, thereby impeding extraction (Knight and Pearson, 1988).

(2) Polypeptides that formed over the course of curing: Bands were present in the cured hams that were not recorded in the raw hams: a 126 kDa band and bands for masses in the regions of 54-89 kDa (54, 63 and 89 kDa) and 8-12 kDa (8, 9, 11 and 12 kDa). Yates *et al.* (1983) suggested that fragments of myosin could fall in the region of 50-100 kDa, where numerous protein fragments were found in the cured hams. The 8 kDa peptide fragment was present in all the *Semimembranosus* muscle samples from the cured hams but only in some *Biceps femoris* muscle samples, which might be indicative of a higher level of proteolysis in the former muscle, which also exhibited higher levels of cathepsin B+L activity.

(3) Polypeptides whose concentrations underwent changes during curing: Slight decreases in the proportions of actin and T-troponin were observed (only in the *Biceps femoris* muscle). The relative intensity of the proteins tropomyosin, myosin light chain I (mainly in the *Semimembranosus* muscle), I-troponin and the protein with a mass of 22 kDa likewise decreased. A band for a protein with a molecular mass of 70 kDa was present in only some of the raw hams but in all the samples from the cured hams. The concentration of myosin light chain III and a polypeptide with a mass of 13 kDa increased slightly in the *Biceps femoris* muscle samples. A 32 kDa band increased, primarily in the *Biceps femoris* muscle samples.

(4) Polypeptides that did not undergo any variation: The relative concentration of C-troponin remained more or less constant.

Conclusions

The content of low molecular mass fragments was higher in the cured hams from cross B (50 % DU + 25 % LD + 25 % LW) than in the cured hams from the other two crosses compared in this study. This provides evidence of higher levels of proteolysis in the hams from cross B during the 11 months of curing. Accordingly, replacing 25 % DU with LW helps intensify the proteolysis taking place during the curing of the hams and thus demonstrates that genetics is one of the factors that influence the level of proteolysis.

Pertinent literature

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