PROTEOLYTIC ENZYME ACTIVITY IS INVOLVED IN MECHANICAL WEAKENING OF MUSCLE FIBRES DURING POST-MORTEM STORAGE

Christensen, M.[1], Larsen L.M.[2], Ertbjerg, P.[1], Purslow P.P.[3]
[1]Institute of Dairy and Food Science, The Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark
[2]Chemistry Department, The Royal Vet. and Agric. University, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark
[3]Department of Biological Sciences, University of Stirling, United Kingdom

Background

Post-mortem degradation of myofibrillar proteins and its relationship to meat tenderness have since long been the subject of considerable research. There is evidence that the cysteine proteases, in particular the calpains are responsible for myofibrillar protein degradation during post-mortem storage (Huff-Lonergan et al., 1996). The effect of proteolysis in relation to meat tenderness has previously been investigated by protein degradation studies or by structural studies using either microscopic techniques or mechanical measurements on whole meat. These techniques do not enable direct measurements of the effect of proteolysis on the mechanical properties of the myofibrillar component of meat, which is directly related to changes in meat tenderness. Alternatively, mechanical tests can be performed directly at the single fibre level after treating the muscle fibres with different proteolytic enzymes or protease inhibitors. Previously, we have shown that addition of exogenous μ-calpain causes mechanical weakening of single muscle fibres (Christensen et al., 2000). In the present work we use inhibitors to investigate the role of endogenous proteases on post-mortem tenderisation. The purpose was to examine the effect of endogenous cysteine proteases (especially the calpains) on the tensile breaking strength and breaking strain of single muscle fibres during post-mortem storage.

Methods

Semitendinosus muscles were obtained from Holstein heifer 24 hours post-mortem. Muscle samples were cut, vacuum packed and frozen at -20°C. Small strips of muscle was dissected from the frozen sample and placed in a solution containing 50 mM MES (pH 5.6), 100 mM KCl, 280 mM Mannitol and 0.2 mM EGTA. Single fibres were isolated under a binocular dissecting microscope at room temperature and rapidly transferred onto aluminium templates so they extended across a gap (2-3 mm) cut in the centre of the plate. The muscle fibres were attached to the aluminium plate by gluing the fibre ends onto the plate with cyanoacrylate adhesive glue. Care was taken not to stretch the fibre during the transfer process. The free length of the fibre was measured using vernier callipers and the diameter was measured using a Leica DMIRB microscope.

Single muscle fibres were immersed in a solution containing 100 mM KCl, 15 mM dithiothreitol (DTT), 250 mM Mannitol and either 50 mM MES (pH 5.6, buffer A) or 50 mM Tris-HCl (pH 7.5, buffer B). Three different calcium concentrations (0.1 mM, 1 mM and 10 mM) were added to buffer A and B, and the single muscle fibres were incubated in these solutions for 8 days at 4°C. As a control, single muscle fibres were incubated in either buffer A or buffer B containing 10 mM EGTA. As a control for storage induced changes, the mechanical properties of single muscle fibres incubated in the above mentioned solutions were compared to the properties of single muscle fibres isolated from 24-hour muscle samples (unaged control). Another experiment was performed in which the effect of the protease inhibitor E-64 on the mechanical properties of single muscle fibres were investigated. Single muscle fibres were incubated in buffer B (+/- 150 μM E-64) containing either 0.1 mM $CaCl_2$ or 1 mM $CaCl_2$. As a control, single muscle fibres were incubated in buffer B containing 10 mM EGTA.

After incubation, the single fibres were transferred to a small mechanical testing device (Lewis & Purslow, 1989). Fibres were stretched at a constant rate of 13.8 μm/s until fracture. Loads and extensions were monitored using a 16-bit A/D converter (National Instruments) running under LabView data acquisition software. Breaking strength and breaking strain of the muscle fibres were then calculated.

Results and Discussion

The calpains are believed to be one of the most important enzyme systems for meat tenderisation. The calpains are calcium-activated enzymes and they are known to display the highest proteolytic activity around physiological pH. In order to investigate if the endogenous calpains mechanically weaken the muscle fibres we performed an experiment in which we incubated single muscle fibres in three different calcium concentrations at two different pH values, one of which is close to the physiological pH (7.5) and one which is the pH present in bovine muscle post-rigor (5.6). After 8 days of incubation in buffer A (pH 5.6) containing $CaCl_2$ the breaking strength and strain of the single muscle fibres were not significantly different from the breaking strength and strain values of unaged control fibres and of fibres incubated in buffer A containing EGTA (Table 1). However, a tendency ($P = 0.07$) towards a weakening of the muscle fibres incubated in buffer A containing 10 mM $CaCl_2$ was observed. Single muscle fibres incubated in buffer B (pH 7.5) were clearly weakened compared to muscle fibres incubated in buffer A. The difference in strength of the fibres at the two pH values could partly be caused by a swelling at the higher pH seen as an increase in cross-sectional area. As shown in Table 1, addition of 1 to 10 mM $CaCl_2$ to buffer B (pH 7.5) dramatically weakened the muscle fibres resulting in fracture of the fibres at the beginning of the testing procedure. Addition of 0.1 mM $CaCl_2$ resulted in significantly weaker fibres compared to the unaged controls. This can be explained by a direct (non-enzymatic) effect of pH, as the mechanical properties of the fibres incubated in 0.1 mM $CaCl_2$ were not different from the fibres incubated in buffer B containing EGTA. Whether the mechanical weakening of the muscle fibres observed at pH 7.5 is due to calcium-induced proteolytic activity or it is a direct result of the calcium ions could not be concluded from these results. In order to answer this question we performed an experiment, in which single muscle fibres were incubated for 8 days at pH 7.5 containing 0.1 mM $CaCl_2$ or 1 mM $CaCl_2$ with or without added protease inhibitor E-64 (Figure 1). Addition of E-64 to solution B containing 0.1 mM $CaCl_2$ did not have any effect on the mechanical properties of the single muscle fibres indicating that 0.1 mM $CaCl_2$ does not result in calpain induced weakening of the fibres (Figure 1). However, addition of E-64 to solution B containing 1 mM $CaCl_2$ completely prevented the mechanical weakening of the fibres. These results clearly indicate that the weakening of the muscle fibres in the presence of 1 mM $CaCl_2$ is caused by proteolytic activity, probably the calpains. Further studies will aim to clarify the role of the different proteolytic enzyme systems.

Pertinent literature

Christensen, M., Larsen, L.M., Purslow, P.P. (2000): The 46th ICoMST. Buenos Aires, Argentina. 460-461.
Huff-Lonergan, E., Mitsuhashi, T., Beekman, D.D., Parrish Jr, F.C., Olson, D.G., Robson, R.M. (1996): Journal of Animal Science 74: 993-1008.
Lewis, G.J., Purslow, P.P. (1989): Meat Science 26: 255-269.

Table 1. The influence of pH and calcium on the breaking strength and strain of single muscle fibres. Values are expressed as means ± SE.

	Breaking strength (kPa)	Breaking strain (%)
pH 5.6 (buffer A)		
0.1 mM $CaCl_2$ (n=9)	366.7 ± 39.8^{a}	52.4 ± 5.2^{ab}
1 mM $CaCl_2$ (n=8)	336.8 ± 25.3^{ab}	53.4 ± 4.9^{ab}
10 mM $CaCl_2$ (n=8)	222.5 ± 31.6^{b}	45.3 ± 7.8^{a}
10 mM EGTA (n=9)	302.5 ± 38.6^{ab}	66.8 ± 5.2^{b}
Unaged control (n=7)	314.7 ± 57.0^{ab}	57.2 ± 8.8^{ab}
pH 7.5 (buffer B)		
0.1 mM $CaCl_2$ (n=5)	101.3 ± 16.0^{c}	29.8 ± 4.2^{c}
1 mM $CaCl_2$ (n=8)	NM	NM
10 mM $CaCl_2$ (n=9)	NM	NM
10 mM EGTA (n=8)	89.4 ± 18.4^{c}	44.1 ± 10.3^{c}
Unaged control (n=7)	187.5 ± 23.6^{d}	42.6 ± 10.3^{c}

NM: non-measurable, the fibres fractured before testing was performed
Within columns, values with the same letter are not significantly different

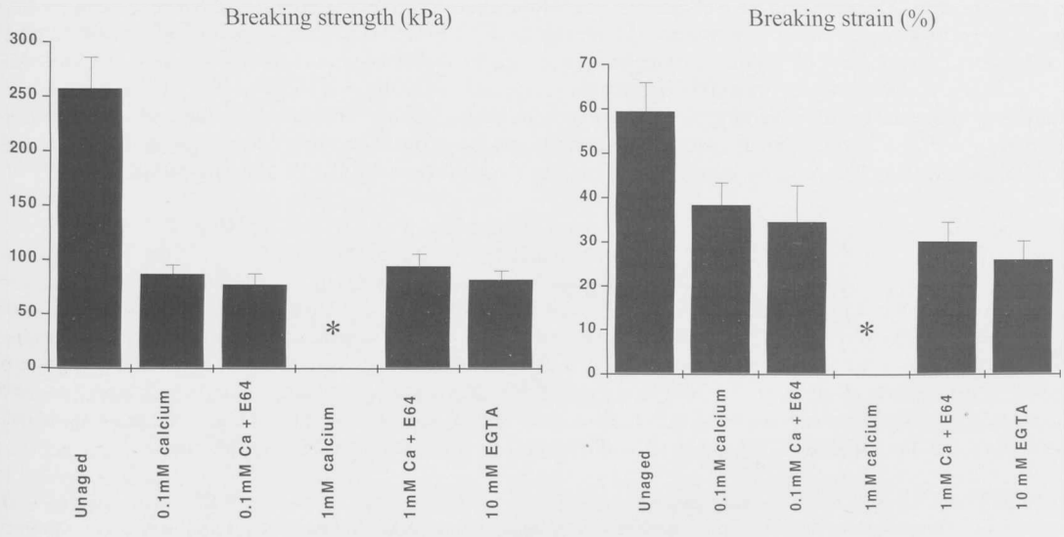

Figure 1. The effect of calcium concentration and application of the protease inhibitor E-64 on the breaking strength (left) and breaking strain (right) of single muscle fibres incubated at pH 7.5. The star designates that incubation of muscle fibres in a solution containing 1 mM of $CaCl_2$ were non-measurable due to extensive weakening. Columns represent means and the error bars are given as standard error ($10 < n < 14$).