62 The effect of incubation temperature on recombinant caspase 3 mediated proteolysis of myofibrillar proteins. C. Kemp* and T. Parr, *University of Nottingham*, *Loughborough, Leicestershire, UK*.

Meat tenderization results from the weakening of myofibrillar structures and has been attributed to proteolytic enzymes. Research into proteases involved in postmortem (PM) proteolysis has predominantly focused on calpains, however it has been proposed that other proteases could contribute (Ouali et al., 2006). Caspases are primarily associated with apoptosis. Once activated they target and cleave a number of substrates including components of the Z-disk and costameres. Studies have shown that caspases are active in skeletal muscle during the PM conditioning period and data indicates that there is a relationship between caspase activity and shear force (Kemp et al., 2006). The aim of this study was to investigate whether recombinant caspase 3 (rC3) was capable of degrading porcine myofibril proteins in vitro at temperatures analogous to that of a cooling carcass.
Myofibrils were prepared from porcine LD muscle according to Goll et al. (1974). rC3 was expressed in E.coli and purified using the AKTA chromatography system (GE Healthcare). rC3 activity was determined using Promega′s Caspase 3/7 activity assay. Myofibrils (5 mg) in a mixed salt solution (MSS: 12mM NaCl, 12.6 mM $MgSO_4 \bullet 7H_20$, 70 mM KH_2PO_4, 3.4 mM NaOH, 64.2 mM KOH, 11.1 mM H_2SO_4, 132 mM lactic acid, 100 mM MES, pH5.8) were incubated with 10 units of rC3 for 24 h at 37, 25, 15 and 4°C. After incubation, myofibrils were centrifuged at 6000xg for 3 min, the supernatant removed and the pellet resuspended in 100 µl MSS. Proteins were separated by 12.5% SDS-PAGE and visualized by Coomassie staining or transferred to nitrocellulose by Western blotting. Blots were probed with anti-caspase 3 ab (1:2000, Merck Biosciences) or anti-desmin ab (1:500, Sigma Aldrich). Protein bands were detected using ECL detection system (GE Healthcare) and intensities quantified (Quantity-One Multi Analyst, BioRad). Bands from Coomassie stained gels were analyzed using MALDI-TOF Mass Spectrotrometer.
Myofibrils incubated with rC3 at higher temperatures showed increased proteolysis in comparison to those incubated at lower temperatures. Degradation products at 18, 20 and 32 kDa were detected in all reactions and identified to result from the proteolysis of myosin light chain, troponin T and actin by MALDI-TOF analysis. Western blot analysis showed that there was more caspase 3 cleavage from its inactive isoform into its active isoform at higher temperatures and also increased proteolysis of desmin (Table 1).
This study has shown that rC3 is capable of causing degradation of myofibrillar proteins. rC3 is active and causes proteolysis of myofibrils through a range of temperatures including 4 °C. Incubation of myofibrils with rC3 resulted in a number of proteins being degraded including troponin T, I, desmin, actin and myosin light chain as detected by Western blotting and Coomassie staining. These degradation patterns correspond with previous research in myofibrils incubated with µ-calpain. These findings therefore strengthen the hypothesis that PM proteolysis and meat tenderization is a multienzymatic process and that caspases could contribute to it.
Goll et al., 1974. Proc Ann Recip Meat Conf 27: 250-267. Kemp et al., 2006. Journal of Animal Science 84: 2841-2846. Ouali et al., 2006. Meat Science 74: 44-58.
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Protein levels of rC3 and desmin in myofibrils at different incubation temperatures

Temp °C	Inactive rC3(32kDa)	Active rC3 (20 kDa)	Desmin
Negative Control	ND	ND	100
4	100	32.5	78.3
15	54.2	77.7	36.0
25	8.0	92.3	39.1
27	0.4	100	32.9

Figures are expressed as percentage of densitometry units obtained from Western blots. ND= Not detectable