Inhibition of cathepsins reduces longitudinal shrinkage and prevents transversal swelling during heating of *semitendinosus* muscle fiber fragments

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I. INTRODUCTION

Meat shrinkage and loss of fluid during cooking can have a negative effect on the eating quality of cooked meat. Single muscle fibers used as models for shrinkage behavior of meat during heating, showed transverse shrinkage during heating over 40-70°C in bovine *semitendinosus* and *psoas major* (1) and longitudinal shrinkage during heating over 70-90°C range in *semitendinosus* (1). The potential role of endogenous meat proteases in the transverse shrinkage of single muscle fibers of *semitendinosus* has previously been suggested (1). Furthermore, cathepsin B and L retain 21 % of their activity in meat heated at 70°C (2). This study aims to identify the role of proteases, with focus on the cathepsins in the dimensional shrinkage of single muscle fibers during heating.

II. MATERIALS AND METHODS

Ten *semitendinosus* muscles were removed from both sides of five bovine carcasses at one-day post slaughter. Muscles from one side were randomly processed either on the day of collection (0 days ageing) or after 14 days ageing in a vacuum bag at 2-4°C. After ageing, 1g samples were cut randomly from four different locations of each muscle and homogenized in 10 ml of cold mannitol buffer (see 1 for detail) with (i) nothing added to buffer (control) or (ii) with 0.02 mM cathepsin protease inhibitor Z-Phe-Ala-diazomethylketone (Bachem) dissolved in 20 % (v/v) DMSO. A drop of each homogenate was transferred to a cavity glass slide (Sigma Aldrich) and observed with a compound microscope Olympus and DP73 camera, at 20x magnification on a heating stage (Linkam PE120). A single field of vision was chosen that had 2 to 3 muscle fiber fragments for each muscle, aging period, and buffer. The temperature was increased at a rate of 10°C/minute with 2 min for equilibration at each temperature. Images were taken at the following temperatures: 22, 40, 45, 50, 55, 60, 65, 70, 75, 80 and 90°C. The length and diameter of the fiber fragments were measured in Image J and volume was derived as $\pi \times (diameter/2)^2 \times length$. Longitudinal, transverse and volume shrinkage at each temperature were calculated as the length, diameter and volume of the fiber fragment at each temperature relative to the same dimensions at 22°C. The effects of the treatments were analyzed using restricted maximum likelihood with a heterogeneous power model to model the repeated measures over temperature. Least significant intervals (LSI) were calculated to compare treatments within each temperature level. Spearman rank correlations were also examined. All analyses were performed using GenStat (VSN International). Due to space limitations, the effect of aging is not discussed in this paper.

III. RESULTS AND DISCUSSION

The changes in the fiber diameter of the control were temperature dependent with transverse shrinkage observed over the range of 40-70°C, while transverse swelling occurred at 75 and 90°C (Figure 1a). When cathepsin inhibitor was added to the buffer, the transverse shrinkage was greater (Figure 1b) and the longitudinal shrinkage (Figure 2a) was reduced at ≥70°C. The addition of cathepsin inhibitor led to increased volume shrinkage overall (Figure 2b). It is highly likely that the transverse swelling is a consequence of the longitudinal shrinkage in some (not all) of the control fibers at ≥70°C. This hypothesis is supported by the high and negative correlation between the transverse and the longitudinal shrinkage at 70, 75, 80 and 90°C, with Spearman`s rank coefficient of correlation of -0.84, -0.81, -0.65 and -0.64, respectively ($n=21$ to 23; $P<0.001$).

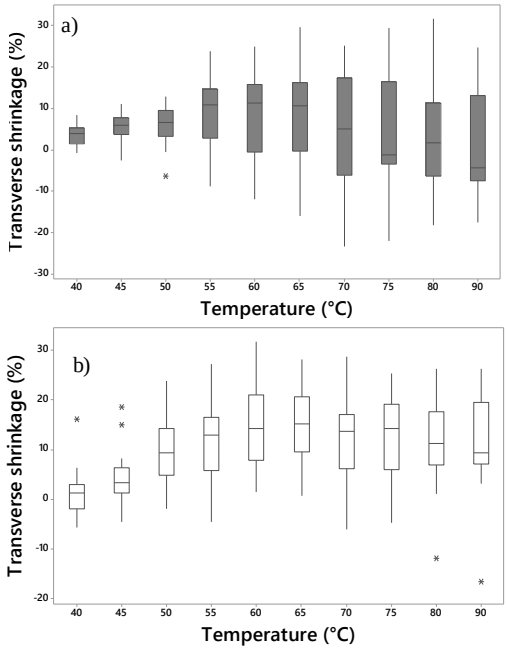

Figure 1. Boxplots of transverse shrinkage of *semitendinosus* fiber fragments as a function of temperature. Boxes represent interquartile ranges, asterix values are outliers. a) control, b) with cathepsin inhibitor

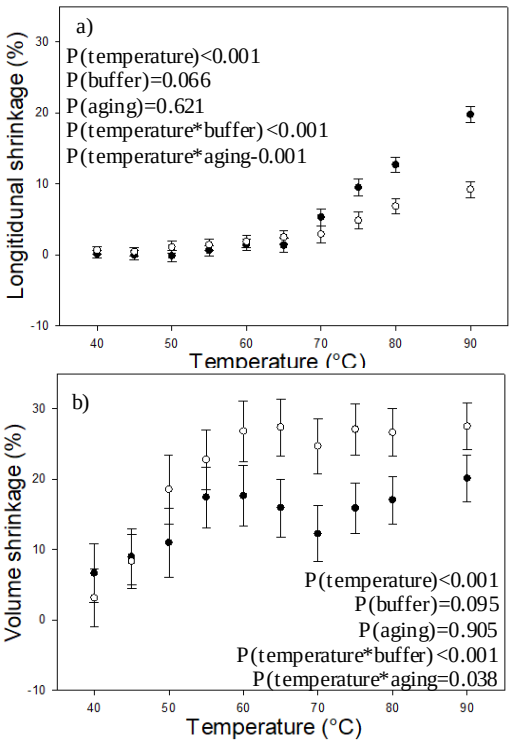

Figure 2. Predicted means and LSIs for a) longitudinal shrinkage, b) volume shrinkage of *semitendinosus* fiber fragments as a function of temperature. Dark circles- control, light circles- with cathepsin inhibitor. SED longitudinal shrinkage =2.208, SED volume shrinkage =3.623, comparisons within each temperature level only

Based on our results, it is likely that cathepsins are active during the heating of *semitendinosus* muscle fibers. Offer *et al*. (3) proposed a theory that shrinkage can be passively driven by the endomysium or actively driven by the myofibrils. At higher heating temperatures (>70°C), we hypothesize that the inhibition of cathepsins reduces the longitudinal shrinkage by preventing the proteolysis of collagen, which is a known target of cathepsins (4). The different extent of transverse shrinkage/ swelling of muscle fiber fragments of *semitendinosus* in the control samples (Figure 1a) could be due to the different denaturation patterns of myosin isoforms (fiber types) in the fiber fragments at heating temperatures below 60°C (5) or different extent of desmin proteolysis potentially occurring during heating. While it is obvious that transverse shrinkage above ≥70°C is dependent on the extent of longitudinal shrinkage, it remains unclear why some fiber fragments remain in their shrunken state and some tend to swell and whether this is related to changes in myosin or desmin. The greater volume shrinkage of fibers heated with cathepsin inhibitor implies that they also lose more water, suggesting a role of cathepsins in water retention during heating.

CONCLUSION

Inhibition of cathepsin activity in muscle fiber fragments of semitendinosus decreased the extent of longitudinal shrinkage and increased the transverse shrinkage, by preventing transverse swelling. The longitudinal and transverse shrinkages were correlated at high temperature which we hypothesize is due to changes in myosin and collagen during heating. Cathepsin activity during heating could have a positive role in preventing shrinking and fluid loss during cooking.

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