EFFECT OF PRE-RIGOR TEMPERATURE AND MUSCLE RESTRAINT ON THE BIOPHYSICAL PROPERTIES OF MEAT TENDERNESS DEVELOPMENT

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Keywords: Sarcomere length, restraint, tenderness, myofibrillar fragmentation index, temperature.

Introduction

Muscle temperature during rigor development is critical in determining meat tenderness, primarily due to the sarcomere shortening produced by low temperatures (cold shortening): cold shortened meat is tougher before ageing and does not tenderise even after prolonged storage when tested by shear force in the cooked state (Davey & Gilbert, 1975). In contrast, shortening induced by high pre-rigor temperatures (heat shortening) causes a limited amount of toughening as measured by shear force (Hertzman *et al.*, 1993). Recently, Simmons *et al* (1996) found that heat shortened meat did not produce an increase in shear force in cooked unaged samples; but the amount of subsequent ageing was limited, an effect attributed to a reduction in the proteolytic activity of the calpains rather than the effect of sarcomere lengths.

Objectives

This study compared the development of tenderness in restrained and unrestrained muscle samples held over a temperature range to establish the relationship between sarcomere length and tenderization. Ageing was measured in the raw state using the myofibrillar fragmentation index (MFI). Tenderness was measured at rigor mortis on cooked samples.

Materials & Methods

The longissimus dorsi (LD) was removed at 20-30 minutes post-mortem from three steers. The animals had been slaughtered using commercial procedures with no electrical stimulation. Each LD was cut into six 150 mm lengths, parallel to the fibre axis, and a pH measurement was taken of each piece, using an Ingold probe. Two muscle pieces from each LD were placed between stainless steel plates and over-wrapped tightly with a cling film which was secured with adhesive tape. The samples were placed in a sealed, weighted plastic bag and immersed in a water bath at either 5 or 35°C. The remaining four pieces were placed in an unrestrained state in water baths at 5, 15, 25 and 35°C.

The pH was measured at regular intervals. When the muscle pieces reached rigor (pH 5.5) they were removed from the water bath. Samples were taken for measurement of the MFI, the sarcomere length measurement and the rigor shear force. The remainder of the muscle was stored at 4°C for MFI measurements at 30 hours, 3 and 7 days. Sarcomere length measurements were determined in quadruplicate by microscopic measurements of single fibres after fixation in 1% gluteraldehyde.

The MFI preparation was carried out using fresh muscle samples in accordance with Watanabe *et al*, (1993). Images of the myofibrillar fragments were produced with phase contrast light microscopy, digitised and stored on VHS video tape, and the MFI was calculated using an image analysis software package (Matrox Inspector). The results were expressed as the percentage of myofibrils that were 1-4 sarcomeres long in relation to the total number of fragments within an image. The analysis was carried out in triplicate for each sample.

Shear force was measured at rigor mortis with a MIRINZ tenderometer after cooking to an internal temperature of 75°C. Ten replicates per sample, each cut into 1 cm x 1 cm and parallel to the fibre axis, were prepared and sheared perpendicular to the fibre axis.

Results

As shown in Figures 1 and 2, LD samples maintained at either 15 or 25°C during the pre-rigor period had similar sarcomere lengths (2.0 and 2.2 μm respectively) and similar rigor shear force values (13.0 and 12.4 kgF). By increasing the rigor temperature to 35°C, the sarcomere length decreased significantly to 1.75 μm ($p<0.01$) but the shear force was unaffected (13.4 kgF). In contrast, holding at 5°C produced a high degree of muscle shortening (1.4 μm) and a concomitant increase in toughness (19.9 kgF; $p<0.001$).

Pre-rigor muscle restraint increased the sarcomere length for both the 35 and 5°C samples to 2.4 μm and 2.5 μm respectively, suggesting a slight degree of stretching. However, while the pre-rigor restraint had no effect on shear force values for 35°C samples (13.2 kgF), it significantly decreased the shear force to 9.8 kgF ($p<0.001$) for the 5°C samples compared to all other temperature treatments.

At rigor, the MFIs were equivalent for all samples, irrespective of treatment (Fig. 3). The MFI of samples held at 15°C pre-rigor and then aged at 4°C increased gradually from the rigor values, reaching 78 at day 7. A different trend was found for samples held at 25 and 35°C pre-rigor. The MFI increased during the first 3 days of ageing but then fell. Unrestrained 5°C samples showed a rapid increase in MFI during ageing compared to the other samples and their MFI was significantly higher at day 7 ($p<0.001$). Pre-rigor restrained samples held at 5°C also had a rise in MFI over time but the effect was less marked than in unrestrained samples. MFI changes during storage were not affected by restraint in samples held at 35°C pre-rigor.

Discussion

Meat tenderness development is largely attributed to calpain-mediated proteolytic events. Superimposed on this is the influence of sarcomere length. The increase in overlap between thick and thin filaments as sarcomeres shorten results in higher levels of the rigid, heat denatured actinomyosin complex on cooking (Marsh & Carse, 1974) and hence meat becomes more resistant to shearing. Toughness in cold shortened meat has also been attributed to a reduction in calpain activity at the lower temperatures (Dransfield, 1994).

Pre-rigor holding at 5°C resulted in an increase in the MFI compared to samples held at the higher temperatures, in the present study. This suggests that the increased intracellular Ca^{2+} resulting from the low temperatures (Jeacocke, 1982) may have stimulated an increase in the proteolytic activity of the calpains. However, increasing intracellular Ca^{2+} also stimulates muscle contraction and hence toughening, and the greater proteolytic activity, implied by the higher MFI, became evident as a reduction in shear force only when the muscle was restrained.

In contrast to the cold shortened toughness, heat shortening (produced by 35°C pre-rigor holding) had little effect on shear force in either the restrained or unrestrained samples. This finding is similar to the observation made by Smulders *et al.*(1990), that tenderness was unaffected by sarcomere length in fast glycolysing muscle but that there was a high correlation between cold shortening and reduced tenderness in slow glycolysing muscles. It is likely that the fast glycolysing muscles will reach rigor earlier and thus at a higher temperature and will, under these circumstances, heat shorten. It is not clear why the heat shortened samples did not toughen, but it might relate to the extensive protein denaturation, particularly myosin, that occurs during the pre-rigor period (Bandman & Zdanis, 1988) or to the temperature-induced increase in proteolysis during the pre-rigor period (Simmons *et al.*, 1996).

References

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Figure 1. Effect of temperature and muscle restraint during rigor development on sarcomere length

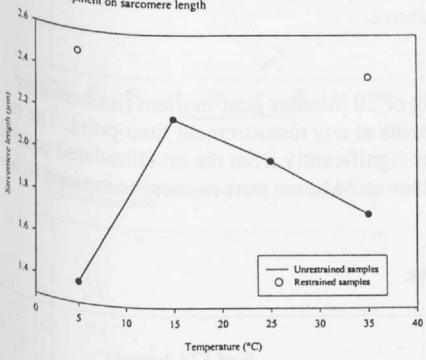

Figure 2. Effect of temperature and muscle restraint during rigor development on shear force

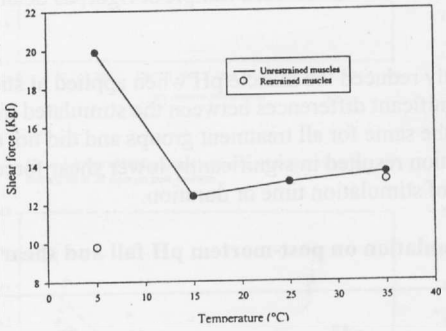

Figure 3. Effect of pre-rigor temperature on the post-mortem myofibrillar fragmentation index