The effect of pre-rigor holding temperature on calpain and calpastatin activity and meat tenderness

N.J. Simmons[1], K. Singh[2],P M. Dobbie[2], C.E. Devine.[1]

[1] Meat Industry Research Institute of New Zealand, P.O. Box 167, Hamilton, New Zealand
[2] AgResearch, Ruakura Agricultural Centre, Private Bag 3123, Hamilton, New Zealand

Keywords: pH, calpains, calpastatin, tenderness, sarcomere length

Introduction

The influence of pre-rigor temperature on sarcomere length has been well documented and it has been proposed that sarcomere shortening occurring at either high or low temperatures causes tougher meat. Cold shortening of between 20 - 40% produces severe toughening and the meat remains tough despite prolonged ageing (Davey and Gilbert, 1973). Similar levels of shortening are produced by high temperatures but the toughening effect tends to be less and more variable (Locker & Daines, 1975, Lee & Ashmore, 1985, Herztman et al, 1993). Intermediate temperatures generally cause less toughening and Tornberg et al (1986) found that a rigor temperature of 15°C was favourable due to the minimal level of sarcomere shortening at this temperature. However, Locker and Daines (1976), cold shortened meat then transferred it to 35 °C until rigor completion and despite a final shortening of 33% the shear force values were identical with those that had gone into rigor at 15 °C thus questioning the effect of sarcomere shortening on meat toughness. Similarly Smulders (1990) demonstrated toughening independent of sarcomere length.

In contrast, Dransfield (1993) has proposed that high temperatures result in an early exhaustion of enzyme activity, so that although proteolysis is initially rapid, little long term ageing occurs. Thus, the implication is that differences in toughness or a failure for toughness to resolve during ageing are due to differences in proteolytic activity rather than sarcomere length.

The aim of this study is to examine the effects of pre-rigor holding temperature on calpain and calpastatin activity, tenderness and sarcomere length in beef. Because holding temperatures affects the rate of pH fall and rigor onset, and thus proteolysis onset and rate, muscle calpain/calpastatin activity will be measured relative to muscle pH rather than time post mortem.

Materials and Methods

The *longissimus* (LD) was removed at 20-30 minutes post mortem from four steers. The muscles were cut into 150 mm lengths, parallel to the fibre axis, and a pH measurement was taken using an Ingold probe. The muscle pieces were placed in a vacuum bag and submerged in a temperature controlled water bath at either 15, 25 or 35°C. When the muscle pH reached 6.5, 6.2, 5.8 and rigor (≤5.5), a 5 g sample was taken from each of the two duplicate pieces of meat for μ-and m-calpain and calpastatin measurement. A further 1 g sample was frozen in liquid nitrogen for subsequent pH measurement by homogenisation in iodoacetate, to confirm the probe reading. Sarcomere lengths were determined by microscopic measurements of single fibres after fixation in 1% gluteraldehyde. The remainder of the meat was transferred to 2 °C for subsequent ageing.

The calpains and calpastatin were extracted and separated on a DEAE Sephacel column using a stepwise NaCl gradient (Wheeler and Koohmaraie, 1991, Sainz et al., 1992). Calpain activities were determined against casein (Hammarsten, Merck, Germany). One unit of calpain activity is defined as the amount of enzyme that catalyses an increase of 1 absorbance unit at 278 nm in 60 minutes at 25 °C. Calpastatin was assayed as the inhibition of m-calpain activity.

Shear force assessments were made on samples at rigor and following 5 days ageing at 2°C. Samples were cooked to an internal temperature of 75°C and peak shear force determined using a MIRINZ tenderometer. Each sample was cut into 10 replicates, each 1cm x 1cm slices parallel to the muscle fibres, and sheared perpendicular to the fibre axis.

Results

The rate of pH decline increased as the pre-rigor holding temperature increased (Fig. 1) from being 0.06 pH unit/hour at 15°C to 0.1 and 0.3 unit/hour at 25 and 35°C respectively. Sarcomeres were significantly shorter ($p< 0.05$) at 35°C (mean length 1.55 μm) than at 25 or 15°C (2.05 and 2.33 μm respectively).

The effect of temperature on μ-calpain activity at specific muscle pH values is shown in Figure 2. At 25°C and 35°C, μ-calpain activity peaked slightly as the pH fell to 6.5, then declined. At 15°C there was no peak and the μ-calpain fell more gradually. By the completion of rigor, a substantial temperature effect was evident: μ-calpain was reduced to 16%, 49% and 74% of initial values for 35, 25 and 15°C respectively.

Temperature effects on m-calpain activity were broadly similar to those for μ-calpain (Fig. 3), although the differences between 15 and 25 °C were less notable. At rigor, the m-calpain activity in muscle held at 35°C was less than half that in muscle held at the lower two temperatures.

Calpastatin activity was unchanged in the pre-rigor period in muscle held at 15°C (Fig. 4) and decreased only slightly at 25°C. In contrast, at 35°C there was an initial marked increase while the pH was > 6, followed by a rapid decline to activity levels below those seen at either 15 or 25°C.

At rigor, meat held at 35°C was significantly (p <0.05) more tender than meat held at 25 or 15°C. However, by 336 hours, meat held at 15°C had aged to a level of tenderness significantly greater (p <0.01) than that of meat held at 35°C.

Discussion

In an effort to consider the temperature effects independent of the rate of pH fall, calpain/calpastatin activity was compared at equivalent pH values in muscle held at different pre-rigor temperatures. It is evident that by the completion of rigor, both μ-and m-calpains were substantially depleted in muscle held at 35°C. This depletion can be attributed to early and rapid proteolytic activity, resulting in the reduced shear force values measured at rigor. However, subsequent ageing post-rigor was limited by the low levels of residual calpain enzyme.

In contrast, muscle held at 15°C showed little change in calpain activity in the pre-rigor period, and had high shear force values at rigor. Proteolysis occurred predominantly in the post-rigor period, and the effects of enzyme activity appear to be more sustained under these conditions, since a higher level of tenderness was reached in meat held at 15°C. Both μ and m-calpain activity was higher at 48 hours post-rigor for meat held at 15°C (data not shown), than meat held at 35°C.

While an effect of reduced sarcomere lengths in producing toughness in meat held at high pre-rigor temperatures cannot be excluded, the present results suggest that toughness in meat held at 35°C pre-rigor is primarily mediated by temperature effects on the calpain/calpastatin system. Furthermore, the lack of ageing appears to be caused primarily by the rapid depletion of calpains, rather than by calpastatin inhibition. These data would support the model detailed by Dransfield (1993), which proposed that high temperatures accelerate autolysis of calpains relative to calpain-mediated proteolysis, leading to a rapid depletion of calpain and failure of subsequent tenderisation.

References

Davey, C.L., Gilbert, K.V. (1973) J.Food Tech. 8, 445
Dransfield, D.E. (1993) Meat Sci, 34, 1
Hertzman, C., Olsson, U., Tornberg, E. (1993) Meat Sci, 1,119
Lee, Y.B., Ashmore, C.R. (1985) J. Anim. Sci, 60, 6, 1588
Locker, R.H., Daines , G.J. (1975) J. Sci. Food and Tech. 26, 1721
Locker, R.H., Daines, G.J. (1976) J. Sci. Food and Agric. 27, 193
Sainz, R.D., Thomson, B.C., Macsood, F.N. (1992) Fed. Am. Soc. Exp. Biol. Proc. 6, A1968
Smulders, F.J.M., Marsh, B.B., Swartz, D.R., Russell, R.L., Hoenecke, M.E. (1990) Meat Sci, 28, 349
Tornberg, E., Larsson, G. (1986) Proc. 32nd Eur. Meat Res. Workers, Ghent, 85
Wheeler, T.L., Koohmaraie, M. (1991) J. Anim. Sci. 69, 1559

Figure 1. Effects of pre-rigor holding temperature on pH decline

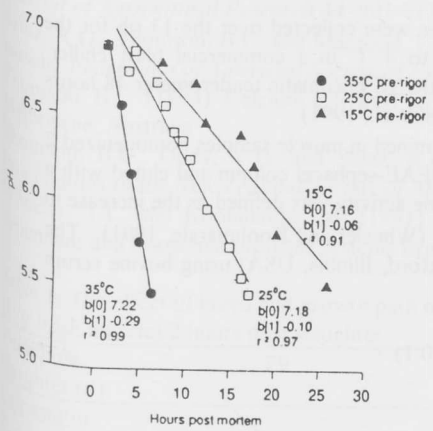

Figure 2. Effects of pre-rigor holding temperature on μ-calpain activity

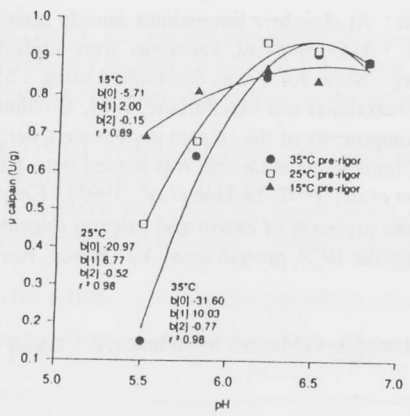

Figure 3. Effects of pre-rigor holding temperature on m-calpain activity

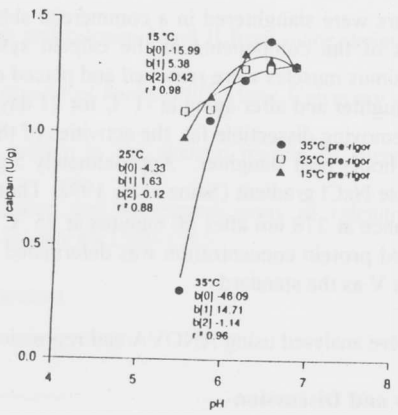

Figure 4. Effects of pre-rigor holding temperature on calpastatin activity

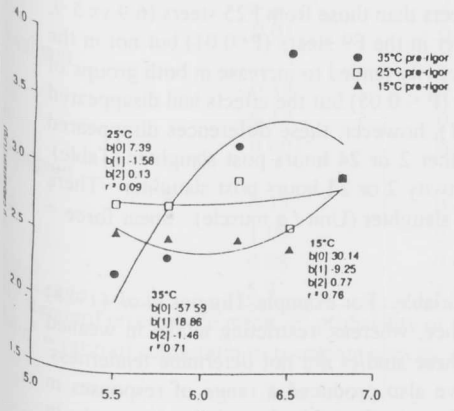

Table 1 Effects of pre-rigor holding temperature on mean post mortem shear force (Kgf) measurements (s.e)

	35 ° C	25 ° C	15 ° C	Significance
At rigor	9.5[a] (0.18)	11.6[b] (0.92)	11.5[b] (0.79)	*
120 hours at 2°C	7.9 (0.35)	6.8 (0.52)	7.0 (0.36)	ns
336 hours at 2°C	7.6[a] (0.30)	6.6[a,b] (0.42)	5.5[b] (0.27)	**

Figures within a row with different superscripts are significantly different
* p<0.05, ** p< 0.01