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THE INFLUENCE OF EARLY POST-MORTEM pH AND TEMPERATURE DECLINE ON TENDERNESS AND AGEING POTENTIAL OF BEEF LOIN MUSCLE

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Background

Under normal chilling conditions, Hwang *et al.* (1998) showed that pH at 45 minutes post mortem (*pm*) was negatively related to shear force, with the magnitude of the correlation increasing with ageing duration. This was consistent with results of Marsh *et al.* (1987) and Pike *et al.* (1993), who showed that early application of electrical stimulation can be detrimental to tenderness. In excised muscle, Simmons *et al.* (1996) showed that the combination of a low pH and high muscle temperature resulted in earlier exhaustion of calpain activity and therefore a decrease in ageing rate. Previous studies have tended to focus on the importance of variation in pH decline, with little quantification of the impact of variation in temperature decline in rapidly glycolysing carcasses on both protease activity and tenderness. In the studies by Pike *et al.* (1993) and Hwang *et al.* (1998) only one chilling regime was applied, or where chilling rates were varied, the temperature differential was small (Smulders *et al.*, 1990), or the results were not reported (Marsh *et al.*, 1987). Also the above *in situ* studies did not examine the impact of pH or temperature decline on protease activity.

Objective

This study investigated the interaction between rate of pH and temperature decline in striploin muscle on shear force and calpain activity. The experimental design aimed to create independent variation in both pH and temperature decline. The duration of electrical stimulation and the chilling regime were varied to achieve this.

Methods

Animals, Experimental Design and Slaughter Procedure: Twenty grass-fed Angus x Hereford steers approximately 22 months old were transported 500 km to the Food Science Australia, Brisbane Laboratory and kept on pasture for a week before slaughter. Groups of four steers were slaughtered over a five-day period. Mean live and carcass weights were 353 and 182 kg, respectively, with an average 12/13th fat thickness of 2.9 mm. The design was a 2x2x4 factorial with two electrical stimulation treatments (either 10 or 40 seconds duration), two chilling regimes (1 or 6 °C for the first 10 hours *pm*) and four ageing times (1, 3, 7 and 14 days). The LVES treatment was applied 3 minutes *pm* to carcasses via a nostril/rectal probe (45 volts, 100 ms on and 12 ms off, 36 pulse per second with 500 mA current). Alternate carcasses were stimulated for either 10 or 40 seconds as a means of generating variation in the rate of pH fall. Variation in chilling rates was achieved by rapidly chilling the right side of each carcass, whilst the left side was cooled slowly. Fast chilling comprised placing sides, within 50 min *pm*, in a 1 °C chiller equipped with a blast fan (air speed, 1.25 m/s, humidity: 86%) and spraying with an ice slurry every 5 to 10 minutes for the first hour in the chiller. Slow chilling comprised keeping sides at about 18 °C for two hours *pm* before being placed in a slow chiller (6 °C, air speed 0.45 m/s, humidity: 83%) for 7-11 hours. Thereafter all sides were moved to the fast chiller for overnight storage.

Measurements: Temperature was logged in the *M. longissimus thoracis et lumborum* (LD) between the 3rd and 4th lumbar vertebrae. Measurements of pH were taken every 15 min for two hours *pm* and then every hour for the next five hours, by collecting 500 mg of muscle from the caudal portion of the loin and immediately placing the sample in liquid nitrogen. pH was then determined in an idoacetate homogenate (Bendall, 1973). For the measurement of calpains and calpastatin activities, approximately 7 g of muscle was collected from the caudal end of the loin at 0.5, 4 and 24 hours *pm*. Immediately after collection, tissue samples were prepared for loading onto 10 mL DEAE-Sephacel columns (Pharmacia Biotech) and the elution performed within 12 days of loading (McDonagh, 1998). The following day striploins were boned from the carcass and portioned into sections, which were vacuum packed and randomly allocated to the four ageing treatments. After 1, 3, 7 or 14 days ageing at 1 °C, samples were frozen at –20 °C. Warner-Bratzler shear force was measured by cooking from the frozen sample using the modified method of Bouton *et al.* (1971).

Statistical Analyses: pH and temperature decline was modeled using the exponential function $pH(t) = pH_\infty + (pH_0 - pH_\infty)e^{-kt}$, where pH(t)=pH at time t, pH_0 =pH at time 0, pH_∞ =pH at time ∞ and k was the rate constant of pH decay. Temperature data for sides in the slow chill treatment were used only up until they were transferred into the fast chiller. Shear force measurements were analysed using a mixed model which contained significant ($P<0.05$) terms for ageing, linear and curvilinear effects for both pH and temperature at 1.5 hours *pm*, loin portion, and the interaction between ageing and pH at 1.5 hours. μ-calpain at 4 hours was analysed using a mixed model which contained linear terms for pH and temperature at 1.5 hours ($P<0.05$), and a random effect for animal. The final model for μ-calpain at 24 hours *pm* contained linear terms for pH and temperature at 1.5 hours and the interaction term between pH and temperature at 1.5 hours ($P<0.05$), and a random effect for animal.

Results and discussions

The experimental design aimed to create independent variation in the rates of pH and temperature fall. This was achieved as there was no correlation between either the decay constants for pH and temperature, or predicted pH and temperature at specific times (*e.g.* at 1.5 hours *pm* the correlation between predicted pH and temperature was -0.02). The decay constant of the exponential function

describes the rate of change in time without reference to the magnitude of the dependent variable. Therefore the predicted pH and temperature values at the time of maximum variation were used as a quantification of the rates of pH and temperature decline. The maximum variance in pH and temperature occurred at 1.5 hours *pm* (5.6 – 6.5 and 19 - 39 °C, respectively).

As there was no interaction between temperature or pH at 1.5 hours *pm,* the response surfaces were plotted separately (see Figures 1 and 2). If glycolysis was not as rapid there are biological reasons why temperature and pH would interact, although this was not evident in this data set. For samples aged 1 day, shear force increased as a curvilinear function of pH at 1.5 hours *pm*. As ageing duration increased a curvilinear function was still apparent, although the effect of ageing on shear force was greater for those samples which had a slow rate of glycolysis. Points of inflexion for the separate ageing curves showed the pH at 1.5 hours to achieve the minimum shear force increased from 5.7 to 5.9 for samples aged from 1 and 14 days. These curves were similar to those described by Pike *et al.* (1993) and Marsh *et al.* (1987) and suggest that rapid glycolysis had a detrimental effect on ageing potential of the meat and that the optimum pH increased as the meat was aged for longer periods. In this experiment, the application of either the 10 and 40 second stimulation treatments meant that glycolysis was very rapid. Other workers have used pH at 3 hours as a predictor of subsequent tenderness and ageing potential (e.g. Marsh *et al.*, 1987). In this study, almost 50% of the sides had entered rigor (i.e. pH 6.0) at 1.5 hours and 75% by 3 hours and so it was important that a measurement of pH be obtained earlier than conventionally undertaken.

Figure 2 shows the changes in shear force as a curvilinear function of temperature at 1.5 hours *pm,* adjusted to pH 6.0. There was little change in shear force at the lower temperatures, although as temperature at 1.5 hours increased, shear force decreased by up to 0.7 kg. In this study, pH at 1.5 hours and temperature at rigor (i.e. at pH 6.0) were highly correlated (r=0.87). Both measures showed a similar pattern, in that a rapid decline in pH, which resulted in high temperatures at rigor gave rise to low shear force at day 1, and reduced ageing potential. Consequently, there was little effect of rigor temperature on tenderness after 14 days of ageing.

Figure 3 showed that the levels of μ-calpain at 4 hours *pm* were linearly related to both pH and temperature at 1.5 hours *pm*. At 4 hours *pm*, both low pH and high temperatures at 1.5 hours *pm* had the effect of lowering levels of μ-calpain. At 24 hours *pm*, there was a significant interaction between pH and temperature at 1.5 hours *pm* on the level of μ-calpain, with muscle which had chilled slowly (i.e. reached only 32 °C at 1.5 hours *pm*) and showed a rapid rate of glycolysis having a lower level of μ-calpain than muscle which had chilled slowly and showed a lower rate of glycolysis (Figure 3). In contrast, if the muscle was chilled rapidly (ie reached 24 °C at 1.5 hours *pm)* there was no relationship between μ-calpain and pH at 1.5 hours *pm*. This was consistent with the lower ageing potential as shown in Figure 1 and the results of Simmons *et al.* (1996).

Conclusion

For the electrically stimulated carcasses, an intermediate pH decline (at 1.5 hours *pm*) resulted in the most tender meat, particularly if it was to be aged. Temperature had little effect on shear force, however higher temperature accelerated a decrease in μ-calpain. In this study an early measurement of pH provided a useful predictor of eating quality and ageing potential.

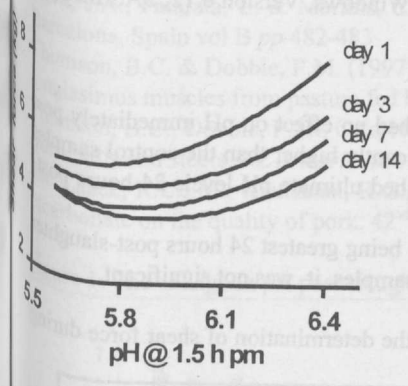

Figure 1. Shear force as a function of pH at 1.5 hours *pm*, adjusted to a temperature of 28 °C at 1.5 hours *pm*

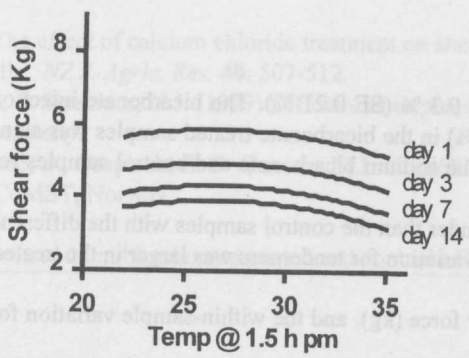

Figure 2. Shear force as a function of temperature at 1.5 *pm*, adjusted to a mean pH 6.0 at 1.5 hours *pm*

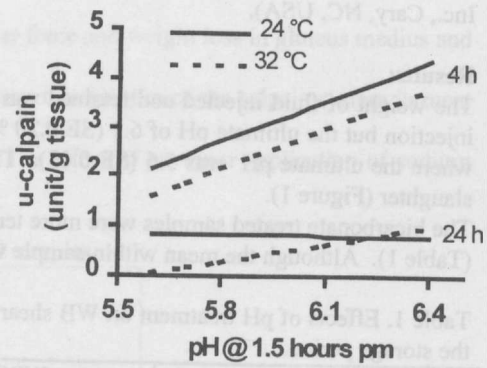

Figure 3. Levels of μ- calpain at 4 and 24 hours *pm* as a function of pH at 1.5 hours *pm*

Pertinent literature

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