MANIPULATION OF THE RATE OF PROTEOLYSIS IN BOVINE M. *LONGISSIMUS DORSI*

P. Sullivan[1,2]*, A. White[1], M. Cairns[2] and A.M. Mullen[1]

[1]Ashtown Food Research Centre (formerly known as The National Food Centre), Teagasc, Ashtown, Dublin 15, Ireland. [2] The National Diagnostic Centre, National University of Ireland, Galway, Ireland. Email: paul.sullivan@teagasc.ie

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Introduction

Tenderness is seen as one of the most important attributes in eating quality of beef (Koohmarie, 1998). Understanding the biochemical basis of meat quality, particularly tenderness, is of great importance to the meat industry. Production of tender meat has led to a number of procedures being put in place to increase the quality of meat, through breeding programmes, slaughtering methods, post-mortem intervention techniques and storage conditions. However variation is still found in beef tenderness (Maher *et al.*, 2004). Several studies have shown the rate of *post-mortem* glycolysis to be an important contributor to this variation (White *et al.*, 2006b; O'Halloran *et al*, 1997; Takahashi *et al.*, 1984), which is also affected by the rate of cooling, which affects rigor onset (White *et al.*, 2006a; Locker and Hagyard, 1963). Interactions between pH and temperature during the onset of rigor directly influence meat tenderness, water-holding capacity and meat colour through their effects on proteolysis, protein denaturation, and myofibrillar shrinkage (White *et al.*, 2006a; Bertram *et al.*, 2004). Close examination of the protein profiles in meat over the ageing period reveals novel indicators of tenderness (Sanchez *et al.*, 2005) which may lead to a greater understanding of the processes contributing to quality.

Meat samples collected through a non-research abattoir can result in samples with variation in sarcomere length are difficult to categorise for tenderness. Manipulation of post-mortem handling of the muscle can allow for divergence in proteolysis, which in turn may result in protein profiles with novel quality indicators. Therefore, the objective of this study was to manipulate the rate of proteolysis, through the application of chilling regimes, to enable closer analysis of products of proteolysis which may be related to quality.

Materials and Methods

A model system was established to produce beef which was divergent for proteolysis through alteration of the rate of glycolysis (White *et al.*, 2004) Hot-boned *M. longissimus dorsi* (LD) muscles (n=5) were cut in half and wrapped by Pi-Vac (PiPatente, Germany) to prevent shortening. Each half was immersed in a water bath set at either 5°C or 20°C for 8 hours *post-mortem* and then placed in a chill set at 2°C for 14 days.

pH and temperature were recorded up to 48hrs *post mortem*. Both sarcomere length (day 2 *post-mortem*) and Warner Bratzler shear force (day 2, 7, 14 and 21 day *post mortem*) were measured according to the methods of Cross *et al.* (1980) and Shackleford *et al.* (1991). Both myofibrillar and TCA extractions were performed on samples taken at 1hr, 3hr, 8hr, 1d, 7d and 14d and these were run on 12% 1-dimensional sodium dodecyl sulphate polyacylamide gel electrophoresis (SDS-PAGE) (Laemmeli *et al.*, 1970).

Results and Discussion

The difference in holding temperature was shown to have a significant effect on the pH of the LD from 3-8hrs *post mortem* ($P < 0.001$) and also at 24hrs and 48hrs ($p<0.05$). LD muscles held at 5°C were found to have a slower rate of pH decline than those muscles held at 20°C (Figure 1).The temperature of storage was found to have no significant effect ($P > 0.05$) on the sarcomere length of the LD and was also shown to have no significant effect ($P >0.05$) on the Warner Bratzler shear force values of the muscle. This may have been due to the muscles being wrapped by Pi-Vac. The mean sarcomere length was 1.81 ± 0.17 for the 5°C treatment and 1.85 ± 0.16 for the 20°C treatment. Hence the impact of pH/temperature alone was not enough to cause a significant difference in the tenderness of these samples.

Initial results from qualitative analysis of the SDS-PAGE gels for both TCA (Figure 2) and myofibrillar extractions show little or no difference between the proteolytic profiles of the two treatments. However, further semi-quantitative analysis is required to verify this. Also use of 2D SDS-PAGE may identify differences which are not evident using 1D SDS-PAGE.

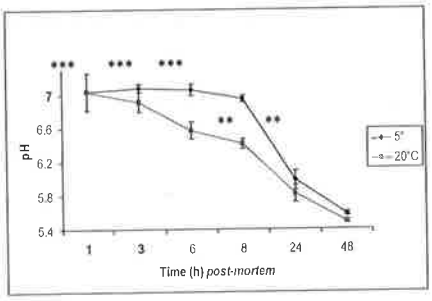

Figure 1: Mean pH value of hot-boned *M. longissimus dorsi* muscle held at 5 ° and 20 °C (bars represent standard error of the mean).

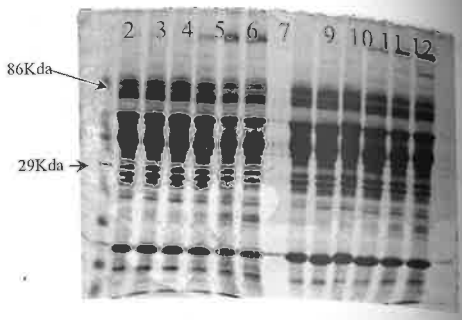

Figure 2: TCA extractions loaded onto 12% 1D SDS-PAGE. Lanes 2-7: 5°C samples taken at 1h, 3h, 8h, 1d, 7d and 14d, respectively. Lanes 9-14: 20°C samples taken at 1h, 3h, 8h, 1d, 7d and 14d, respectively.

Conclusions

Pre-rigor temperature regimes altered the glycolytic pathway of the muscles. Use of the Pi-Vac machine restricted sarcomeres from shortening. This may have contributed to the lack of impact of temperature regimes on Warner Bratzler shear force values. Further work, such as 2-D gel electrophoresis may provide further insight in to the proteolytic profile.

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