# Beef Tenderness Could be pH Compartmentalised

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*Abstract* – **Bull *M. longissimus dorsi* (n = 51) were categorised based on ultimate pH ($pH_u$) into high (pH ≥ 6.2, n = 20) and low (pH ≤ 5.79, n = 31) $pH_u$ groups and aged for 0, 1, 2, 7, 14 and 28 days *post mortem* at -1.5°C. Shear force, enzyme activities were determined for all samples and myofibrillar protein degradation were determined on a subset of samples from each $pH_u$ group for each timepoint. High $pH_u$ meat was acceptably tender at 1 day *post mortem* (shear force < 11 kgF) and low $pH_u$ at 7 days *post mortem*. Rapid titin, nebulin and filamin degradation in high $pH_u$ meat was linked with the early autolysis of μ-calpain at 0 day *post mortem*. In contrast, desmin degradation was concurrent with an increase in cathepsin B levels and tenderisation in low $pH_u$ meat. It is hypothesised that beef tenderness is pH compartmentalised, with tenderness in high and low $pH_u$ meat characterised by the respective rapid degradation of intact high and low molecular weight myofibrillar proteins during ageing.**

*Keywords* – **pH compartmentalisation, ultimate pH, tenderness**

## I. INTRODUCTION

Ultimate pH is a good indicator of meat tenderness, with high (pH ≥ 6.2) and low (pH ≤ 5.79) $pH_u$ meat being consistently tender after ageing. Additionally, high $pH_u$ meat tenderise more rapidly than low $pH_u$ meat [1]. With the inherent pH difference of low and high $pH_u$ groups, and its effect on the plethora of changes taking place in *post mortem* muscle, it is speculated that the mechanisms driving the tenderisation of low and high $pH_u$ meat are potentially different.

It is widely accepted that meat tenderness is largely due to the proteolysis of myofibrillar proteins that are critical in maintaining the highly organised muscle ultra-structure. Calpains and cathepsins are two enzyme systems implicated in *post mortem* proteolysis of muscle/meat proteins [2, 3]. Attempts have been made to reconcile the degradation of several myofibrillar proteins during ageing with meat ultimate tenderness [4]. However, the mechanisms surrounding the difference in the rate of tenderisation observed in low and high $pH_u$ meat is still poorly understood.

This study evaluated the degradation of myofibrillar proteins and proteolytic activity in low and high $pH_u$ meat in order to test the hypothesis that tenderness in beef may be pH compartmentalised due to the differential rate of degradation of myofibrillar proteins in low and high $pH_u$ meat.

## II. MATERIALS AND METHODS

### *A. Animals*

*M. longissimus dorsi* (LD) of bulls (n = 51) slaughtered in a commercial abattoir were used in this study. At 24 hours *post mortem* each LD was equally divided into 6 sub-samples and randomly allocated to 1, 2, 7, 14 and 28 days *post mortem* ageing at -1.5°C. 10 g samples were excised from sub-sample at all timepoints, snap frozen in liquid nitrogen and stored at -80°C for subsequent analysis.

### *B. pH and shear force measurement*

pH of all sub-samples were measured with a Testo® 230 pH meter (Lenzkirch, Germany) directly inserted into the sample. Shear force were measured in all the sub-samples with a MIRINZ tenderometer as described by Pulford *et al.* [5].

### *C. Coomassie blue SDS-PAGE and Western blot analysis*

Separation of larger proteins (>100 kDa) was conducted on total muscle extracts using pre-cast 5% Tris-HCl gels (BioRad). Desmin and μ-calpain were resolved using pre-cast 12% Bis-Tris and 7.5 Tris-HCl gels (BioRad), respectively. Electrophoresis of all gels was conducted in a BioRad Criterion cell system.

Following electrophoresis, 5% Tris-HCl gels were stained with Colloidal Coomassie blue (17% ammonium sulphate, 2% phosphoric acid, 30% methanol, 0.04% Coomassie G-250) for 48 hours. Gel

images were captured with a GS700 calibrated densitometer scanner (BioRad).

Immunoblots for μ-calpain and desmin were conducted as described by Kim *et al.* [6] and Pulford *et al.* [7], respectively, in a subset of low (n = 4) and high (n = 7) samples.

*D. Cathepsin B activity*

Cathepsin B activity was assayed as described by Caballero *et al.* [8] on all samples. Enzyme activity was calculated from a standard curve of purified cathepsin B and expressed as mU cathepsin B mg protein$^{-1}$.

## III. RESULTS AND DISCUSSION

High $pH_u$ samples were more tender than low $pH_u$ samples at all ageing timepoints. With 11 kgF as the upper limit for acceptable tenderness in beef [9], as represented by the dashed line in Fig. 1, high $pH_u$ samples (n = 20) were already acceptably tender 1 day *post mortem*. In comparison, low $pH_u$ samples (n = 31) attained acceptable tenderness levels after 7 days *post mortem*.

μ-Calpain, which is optimal at near neutral pH, undergoes autolysis following activation. Extensive autolysis eventually deactivates the enzyme so that once the large μ-calpain sub-unit had been to 76 kDa, it is no longer active in meat [10]. Autolysis of μ-calpain was detected immediately *post mortem* in high $pH_u$ samples and was initially detected in low $pH_u$ meat at 1 day *post mortem* (Fig. 2). Once activated, enzyme autolysis in the most acidic low $pH_u$ samples was very rapid so that it had completely autolysed after 14 days *post mortem*.

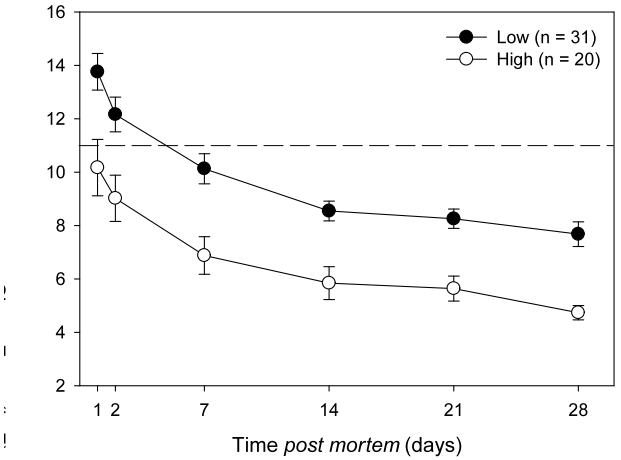


Fig. 1: Shear force of low and high $pH_u$ *M. longissimus dorsi* aged at -1.5 °C for up to 28 days *post mortem*. Error bars are standard errors of the mean.

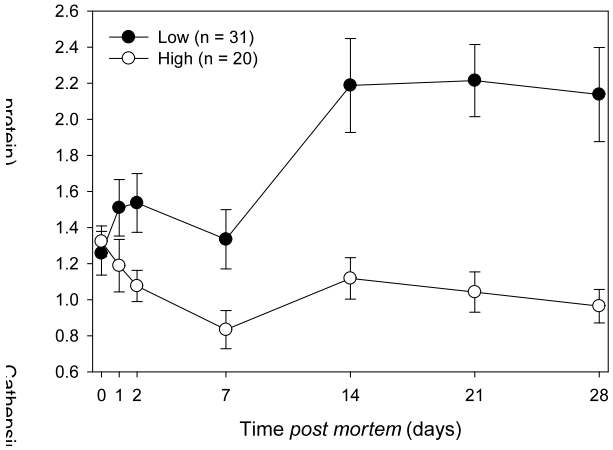


Fig. 2: Cathepsin B activity for all low and high $pH_u$ samples that have been aged at -1.5°C for up to 28 days *post mortem*. Error bars are standard errors of the mean.

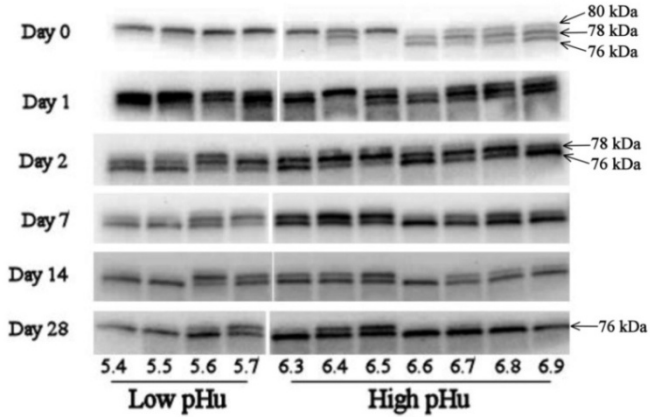


Fig. 3: Representative Western blots showing the autolysis of μ-calpain in low (n = 31) and high (n = 20) $pH_u$ *M. longissimus dorsi* samples that have been aged at -1.5°C for up to 28 days *post mortem*.

Cathepsin B activity is optimal at the pH range of 5.0 – 6.0 and is released from the lysosomes *post mortem*. Cathepsin B levels in low $pH_u$ meat increased with ageing, then plateau after 14 days *post mortem* (Fig. 3). The significant rise of cathepsin B in low $pH_u$ samples after 7 days *post mortem*, indicates the release of this enzyme from the lysosome. Cathepsin B levels in high $pH_u$ remained low throughout ageing. This is believed to be due to the stability of lysosomes at near neutral pH levels [11].

Titin degradation was evident at 0 day *post mortem,* and nebulin and filamin degradation were detected at 1 day *post mortem* in high $pH_u$ samples (Fig. 4). The degradation of these proteins was delayed in low $pH_u$ samples, with titin and filamin

degradation initially detected after 1 and 7 days *post mortem*, respectively.

Desmin degradation was initially detected at 7 days *post mortem* in low and high $pH_u$ samples, and progressively degraded thereafter (Fig. 5). This may be due to the proteolytic activity of residual μ-calpain and elevated levels of cathepsin B in high and low $pH_u$ meat, respectively.

The differential proteolytic activity and degradation rates of myofibrillar protein in low and high $pH_u$ beef during ageing, supports our hypothesis for the $pH_u$ compartmentalisation of tenderness in beef. That is, the basis for the tenderisation of high and low $pH_u$ beef may not be identical.

Our results show the rapid tenderisation of high $pH_u$ samples was concomitant with the early autolysis of μ-calpain and faster degradation of titin, nebulin and filamin. The degradation of these proteins was delayed in low $pH_u$ beef. Higher molecular weight proteins constitute a minority of the total myofibrillar proteins in muscle; however, it is proposed that the faster degradation of these proteins in high $pH_u$ meat has a significant impact in the rapid tenderisation of high $pH_u$ beef.

Titin is located in the I-band and $N_2$ lines of the sarcomere. Researchers have identified the degradation of these regions compromise the integrity of the muscle structure which consequently leads to meat tenderisation [12, 13]. It is therefore proposed that tenderness in high $pH_u$ meat is predominantly due to the weakening of inter-myofibrillar links early *post mortem* due to the proteolysis of large proteins such as titin byμ-calpain.

The tenderisation of low $pH_u$ beef may be due to the proteolysis of smaller myofibrillar proteins. Desmin degradation was concurrent with the shear force decline of low $pH_u$ samples at latter ageing timepoints. Myosin also degraded faster and more extensively in low $pH_u$ samples (data not shown)S. It is important to reiterate that desmin degradation were equivalent during ageing in low and high $pH_u$ samples. However, high $pH_u$ samples were tender long before degradation was detected. As μ-calpain is restricted by the acidic levels in low $pH_u$ meat, it is proposed that desmin proteolysis could be due to cathepsins activity whose increase at 7 days *post mortem* corresponded to subsequent degradation of desmin.

Desmin is a component of costameres, filaments that anchor the myofibrils to the sarcolemma. It is also a constituent of intermediate filaments that link adjacent myofibrils with each other at the Z-disk level. Thus, the weakening of these intra-myofibrillar linkages in muscle due to the degradation of smaller myofibrillar proteins in low $pH_u$ meat significantly

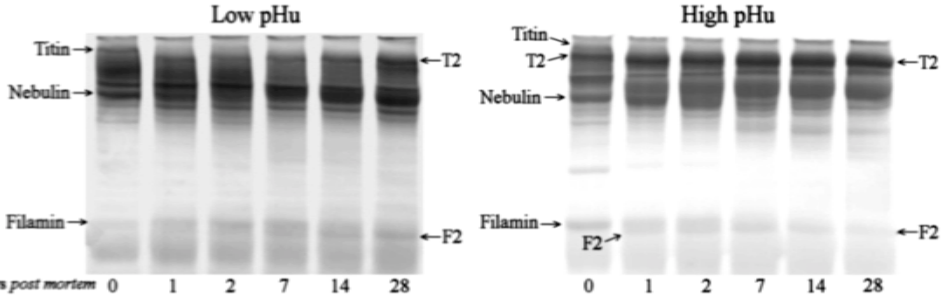


Fig. 4: SDS-PAGE gels of representative low and high $pH_u$ *M. longissimus dorsi* samples that have been aged at -1.5°C for up to 28 days *post mortem*.

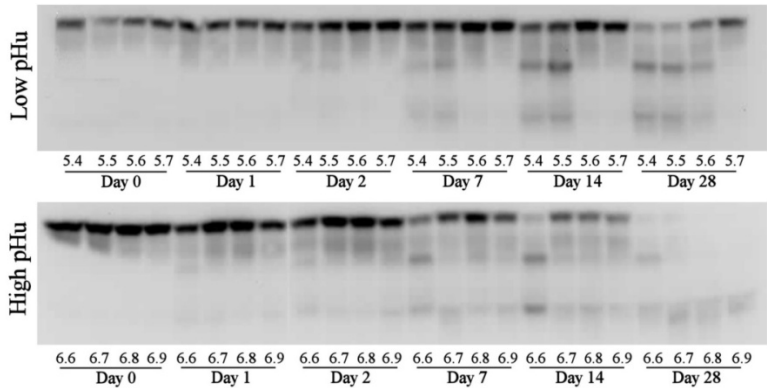


Figure 5: Western blots of representative low, and high $pH_u$ *M. longissimus dorsi* samples aged at -1.5°C.

impacts on the tenderisation of low $pH_u$ meat.

## IV. CONCLUSIONS

The result of this study supports the hypothesis that beef tenderness may be $pH_u$ compartmentalised. The rapid tenderisation of high $pH_u$ meat was concomitant with the faster degradation of high molecular weight proteins and the immediate autolysis of μ-calpain early *post mortem*. In contrast, tenderness in low $pH_u$ beef may be attributed to the proteolytic action of cathepsin B on smaller myofibrillar proteins such as desmin and possibly other proteins associated with inter-myofibrillar linkages. It is proposed that tenderness in the low $pH_u$ beef is due to the fragmentation of intra-myofibrillar linkages in *post rigor* muscle.

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