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Predicting tenderness from proteolytic enzyme activity in the live animal and on-line carcass

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Abstract

Although the role of protease enzymes is generally considered fundamental to post mortem tenderisation, the role of differences in substrate susceptibility to protease activity has not been previously considered within meat science. In the physiology research literature, differences in rates of degradation of cell proteins are recognised to be affected by protein denaturation and post translational modifications such as phosphorylation and glycosylation.

We have developed a methodology for measuring substrate susceptibility in meat homogenates. This methodology is based on measuring the total protease activity in situ using a fluorescent µ-calpain specific substrate (CalS1). To measure protein susceptibility, CalS1 is added to the homogenate at two concentrations: the first uses CalS1 in excess, which determines the total calpain activity in the sample. The second uses a constant but submaximal concentration, which creates a competitive condition for the enzyme: if the affinity for the endogenous substrate is high, the rate of CalS1 degradation is reduced compared to a condition where the affinity for the endogenous substrate is low.

Our investigation of this assay last year identified that, in samples measured at kill or at rigor, the substrate susceptibility assay was highly correlated with the rate of tenderisation in the samples, and these results provide the justification for some further investigation of the underlying mechanisms responsible for differences in susceptibility. In this work, we investigated the effects of different pre-rigor temperatures on substrate susceptibility: high pre-rigor temperatures are known to cause denaturation of both myofibrillar and soluble cytoplasmic proteins, and these effects could be expected to engender changes in myofibrillar susceptibility.

In addition to denaturation, changes in susceptibility could be expected during the ageing period. The likely reason for such changes would be due to degradation of substrate proteins, which might be expected to reduce susceptibility as the total number of binding sites decrease. Alternatively, as the number of breakdown products increase – and many myofibrillar protein breakdown products are soluble - susceptibility might increase as more binding sites become available.

From the results of the current stage, it does not appear that µ-calpain activity alone can explain the increased ultimate toughness of meat incubated at high temperatures during the pre-rigor period. However, the present assay does appear to provide an additional marker for meat that has undergone denaturing conditions that in turn leads to increased toughness.

The overall conclusions of this work were :

- The level of phosphorylation in the myofibrillar and supernatant fraction of muscle tissue varies between animals. This difference is evident both at kill and in post rigor samples.
- Although the experimental numbers were small and the comparisons did not reach statistical significance, the results suggested that the level of phosphorylation in the myofibrillar and supernatant fractions offer potential markers for tenderness
- These results, if confirmed, support the results of in-vitro manipulation of muscle protein phoshorylation, with demonstrated effects on calpain activity.

Contents

1 Background

The rate and extent of proteolysis in meat after slaughter defines the development of tenderness and remains a highly variable attribute of meat. Understanding the sources of variation and manipulating proteolysis to ensure rapid tenderisation would have important commercial implications for the meat industry by, first, reducing the time of chilled storage necessary to ensure acceptable tenderness and, second, avoid the incidence of tough meat that creates to consumer dissatisfaction.

Existing methods to study proteolysis in meat are based on elaborate purification procedures that, ultimately, quantify the amount of enzyme present but offers no indication of how much proteolysis is actually taking place in the meat. This is because enzymes are not always in an activated form, are compartmentalised and/or inhibited by specific endogenous inhibitors. The procedure under development in this objective quantifies the activity a whole tissue homogenate while trying to minimise artifactual activation or inactivation of the enzyme activity, to provide a direct index of the proteolytic activity, rather than the amount of enzyme. Commercially available fluorescent substrates are added to crude homogenates and total activity measured by monitoring changes in fluorescence activity associated with degradation of the substrate. Because the synthetic fluorescent substrates are designed for specificity to specific protease enzymes, the activity of different enzymes in meat samples can be measured independently of each other by appropriate choice of fluorescent substrate.

A recent milestone reported that the CalS1 measured at kill or at rigor showed highly significant correlations with the meat tenderness characteristics. In contrast, the cathepsin B substrate did not show any significant correlation. A second aspect of the calpain fluorescence assay is the opportunity to measure substrate susceptibility to calpain degradation. A third aspect of the calpain assay identified in the last year is the relationship between CalS1 activity and the measured concentration of heat shock proteins (HSP) 20 and 27. A potential role of these chaperone proteins in the tenderisation process was first suggested when they were identified as proteins bound to both calpains and calpastatin in immunoprecipitation experiments. These proteins have also been shown to bind directly with myofibrils. Calpain activity was shown to be highly correlated with HSP 20 concentrations, and the effect of preslaugher stress on HSP20 concentrations is currently being measured. These results point to the possibility that HSP 20 and 27, together with the closely related α-B crystallin, play an important role in mediating variations in tenderness attributes.

2 Project Objectives

The objectives of the research were :

- Optimise the muscle biopsy assays
- Validate the biopsy assays as a scientific procedure

3 Materials & Methods

The following milestones were :

Milestones[1]
1. Develop muscle biopsy procedures to allow routine on-farm measurements of calpain protease activity and HSP expression
2. Measure changes in myofibrillar susceptibility following different processing conditions and following different ageing periods
3. Using serial muscle biopsies, measure variations in calpain enzyme activity, substrate susceptibility and HSP expression
4. Develop procedures for measuring the extent of myofibrillar protein phosphorylation, with particular emphasis on titin
5. Evaluate a possible role for phosphorylation and HSP binding as a source of variation in calpain susceptibility

Specifically the following methods were applied :

3.2 Measure changes in myofibrillar susceptibility following different processing conditions and following different ageing periods

Prime sirloin samples (*m. longissimus dorsi lumborum*) were collected hot from the carcass and immediately transferred to incubation waterbaths. Each muscle was cut in half and incubated at either 15°C and 0°C, or 15°C and 40°C. Ten muscles were collected on each trial and a total of 20 muscles were analysed at 40°C and 0°C (resulting in 40 samples at 15°C). For each sample collection, half the loins were firmly wrapped in polyethylene film to restrict muscle contractions.

An initial 'at kill' susceptibility measurement was made before the samples were allocated to their incubation temperatures. The pH decline for each sample was continuously monitored to determine when rigor mortis was attained. At rigor mortis, a further sample was analysed from each muscle for protease susceptibility, using the procedures for the assay as described earlier. The activity in each sample was measured in triplicate; the measured fluorescence was fitted to an exponential curve (in all cases, the r^2 of the fit exceeded 0.95) and the initial rate calculated from fitted data.

Subsequent to the 'at rigor' measurement, the meat was sliced into 5 portions and vacuum packed. All the samples were then aged at 15ºC for 6 days, and the susceptibility assay undertaken at various intervals to assess changes during the ageing period.

3.5 Evaluate a possible role for phosphorylation and HSP binding as a source of variation in calpain susceptibility

Five striploins were collected during the early pre-rigor period and maintained at 15ºC throughout the pre-rigor and subsequent post-rigor ageing period.
As soon as the samples reached the laboratory, and before the pH had declined below 6.4, a 100mg/mL homogenate was prepared, either with or without the addition of a phosphatase inhibitor cocktail (Phostop, Roche). The homogenate was centrifuged at 4000rpm for 10min to produce the supernatant fraction, the remaining pellet was washed once in homogenisation

[1] Milestones must be achieved to the Steering Committee's reasonable satisfaction.

buffer then resuspended in SDS solubilisation buffer to produce a myofibrillar fraction. Samples were frozen until further use.

The muscle pH's were measured continuously to identify the onset of rigor mortis and a further post-rigor homogenate was prepared for phosporylation measurements in the early post rigor period.

The TPDB assay was carried out as described in the previous milestone. The meat was then tested for tenderness at intervals following rigor mortis to establish at-rigor toughness and ultimate tenderness, and the ageing rate was calculated for each animal by fitting an exponential curve to the tenderness values at each timepoint.

4 Results & Findings

4.1 Develop muscle biopsy procedures to allow routine on-farm measurements of calpain protease activity and HSP expression

We have developed a novel assay for measuring calpain activity in meat and muscle samples which shows promise as a means of predicting rate of tenderisation. Because the assay requires only a few milligrams of sample, a key application is to measure the calpain activity from muscle biopsies taken from animals on farm. This will allow sequential measures of calpain activity, and associated prediction of tenderness characteristics from live animals and allow a new approach to evaluating the effects of environmental effects on a critical meat quality attribute.

Additionally, we have identified that a small molecular weight heat shock protein (HSP) is expressed in muscle tissue in response to environmental stressors, and that these HSPs affect tenderness through their influence on calpain activity. Again, the ability to measure in-vivo expression of HSPs in response to environmental factors will provide a valuable tool to understanding the causes of variability in meat quality.

Fully exploiting the opportunity depends on developing a biopsy procedure that is simple, rapid, requires a minimum of animal restraint and produces a minimum of stress. Our previous approach was based on first introducing a local anaesthetic, then manually removing a biopsy using a rotating needle from the anaesthetised site. The procedure does not meet all the requirements of a system to permit routine and repeated biopsies from the same animal: it requires a significant amount of restraint, is slow and the response to the local anaesthetic can be significant.

In view of the likelihood of on-going research based on muscle biopsies, we have developed a shot biopsy procedure that is intended to allow a sample to be taken without local anaesthetic and only minimal restraint (crowding in a race).

Shot biopsies have already been developed and used successfully for muscle biopsies. These use a captive bolt pistol to propel the biopsy needle but suffer from a number of disadvantages. The gun is noisy and the design for capturing the sample in the biopsy needle results in relative large diameter needles. The large diameter punches a round hole through the skin which can slow the healing process. Also, the use of a cartridge powered captive bolt can also lead to the complexities of requiring a firearm licence.

The objective of this milestone is to develop a shot biopsy needle to overcome these limitations.

The shot biopsy gun.

The propulsion of the shot biopsy is based around a pneumatic cylinder. The valves were selected to transfer large air volumes to produce sufficient speeds. The current speed is estimated at 12 m/sec. The biopsy needle has a 4 mm internal diameter. The tip is bevelled to make an incision through the skin, but the trailing edge of the bevel is made blunt to encourage the needle to follow through the initial incision rather than punch out a plug of skin.

Existing systems use a blade inside the biopsy needle to secure the sample in the needle when the needle begins to retract, but this approach contributes to an increase in needle diameter. To avoid the need for a blade, 3 novel features were introduced. First, the needle was made open-ended, to avoid the build-up of air pressure inside the needle as it is introduced into the muscle. Second, a series of back-facing ridges were cut into the inside surface of the needle to help grip the sample. Last, a cup-type seal, connected through the end of the needle to the housing of the biopsy gun, is held fixed as the needle is propelled into the muscle. This has the effect of generating a reduced air pressure inside the needle to help hold the sample inside the needle when the needle retracts.

Although some further refinements are continuously being made, this range of design features have been found to reliably collect 100-200 mg of muscle tissue taken from beef carcasses within 5 minutes of slaughter.

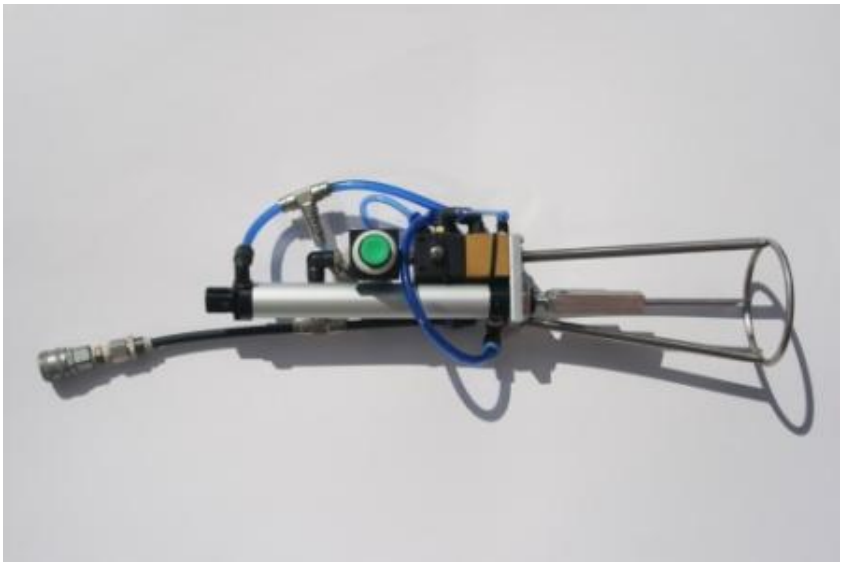


Figure 1: Photograph of the shot-biopsy gun.

In time, a housing will be placed around the unit to ensure it is waterproof and the working parts are protected from damage.

4.2 Measure changes in myofibrillar susceptibility following different processing conditions and following different ageing periods

There was a highly significant effect of incubation temperature on shear force ($p<0.001$) and a significant interaction between incubation temperature and measurement time ($p<0.01$): The measured shear forces demonstrated that the samples incubated at 0ºC were tougher, and the samples incubated at 40ºC more tender than the samples incubated at 15ºC. However, by the end of 6 days of ageing at 15ºC, the high and low temperature treatments were both tougher than the 15ºC samples (Figure 1).

There was also a significant interaction between the effect of pre-rigor muscle restraint and incubation temperature ($p<0.001$): Wrapping the loins in film to restrict muscle expansion, and hence contraction, lowered the initial shear force in the cold shortened samples and appeared to slightly reduce the initial value at 15ºC. Interestingly, the high temperature samples were essentially unaffected by wrapping. By the end of 6 days of ageing at 15ºC, wrapping had a significant effect only the 0ºC treatment.

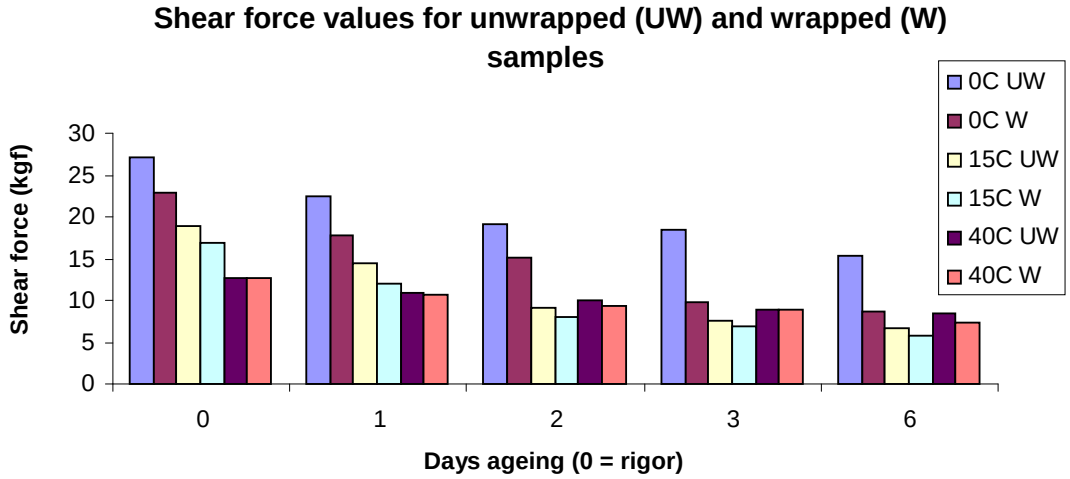
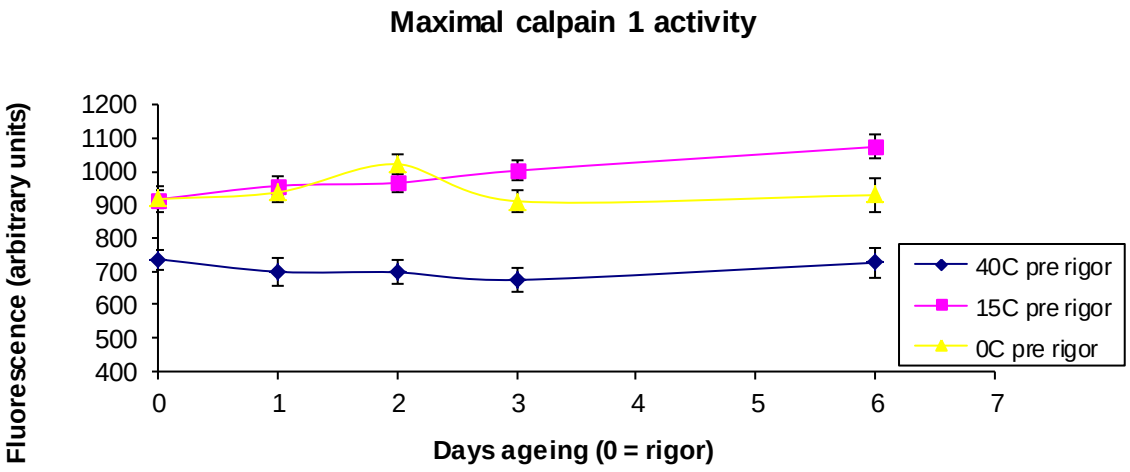
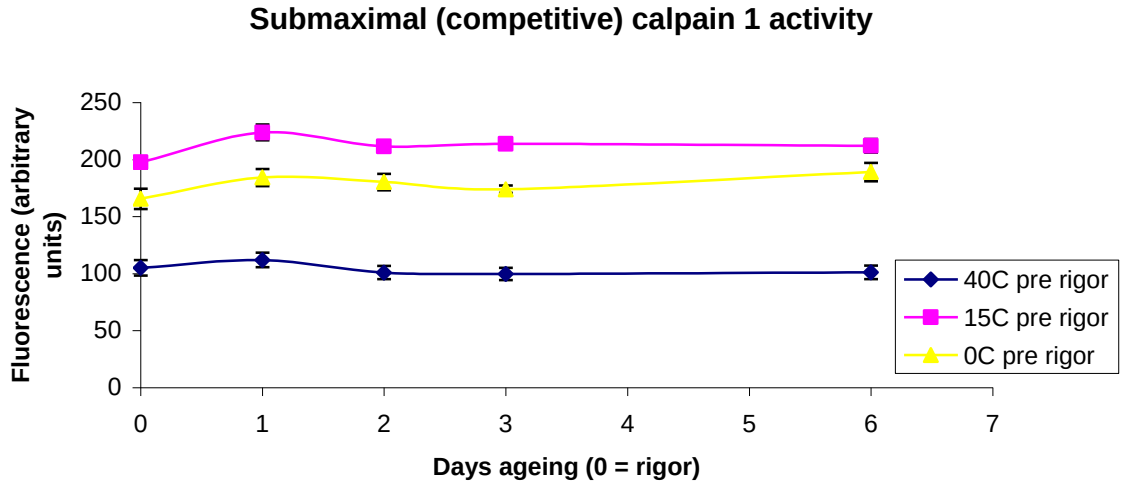
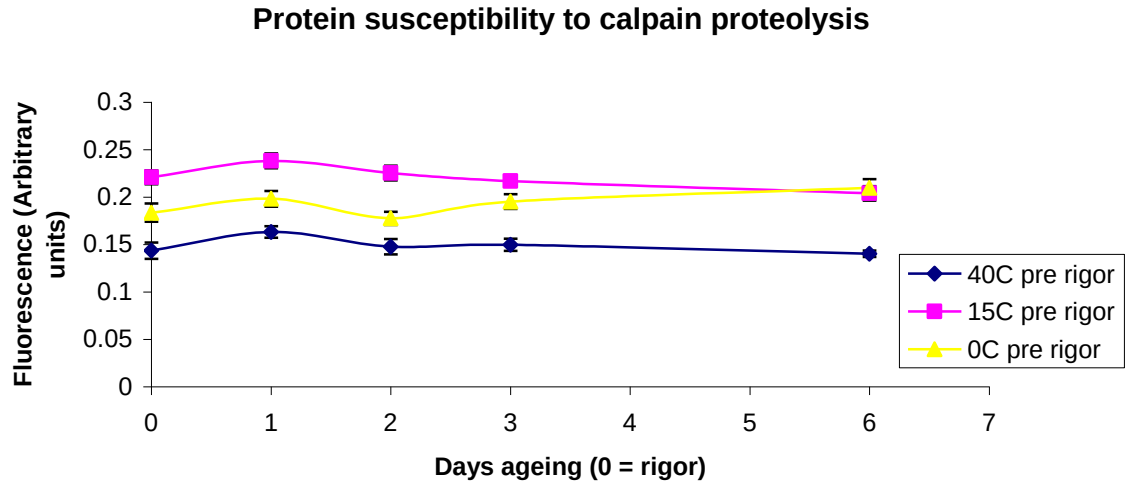


Figure 1. Shearforce values during ageing

Wrapping did not produce any differences in the measured CalS1 activity in any of the treatment groups, and the results for wrapped and unwrapped samples were pooled for each temperature treatment. The effects of pre-rigor temperature and ageing times on maximal calpain activity, competitive activity and the calculated susceptibility are shown in Figures 2 to 4.







Figures 2-4: Effects of pre-rigor temperature treatments on calpain activity at rigor and during ageing at 15ºC.

Incubating hot-boned meat at 40ºC during the pre-rigor period produced a highly significant ($P<0.001$) decrease in maximal activity compared with 15ºC. In contrast, incubation at 0ºC did not produce a difference except after 6 days of ageing.

A similar pattern was evident in the competitive assay, using submaximal CalS1 substrate concentrations. In the case of the 40ºC samples, activity was reduced by approximately 50% throughout the ageing period compared with the 15ºC samples ($P<0.001$). In the 0ºC samples, activity was significantly reduced ($p<0.05$) compared with 15ºC except on the 6th day of ageing.

Susceptibility is calculated as the proportion of the total activity measured at submaximal CalS1 concentrations. Proportionately less activity denotes more competition from endogenous proteins, which is interpreted as higher susceptibility.

Samples incubated at 40ºC pre-rigor had significantly greater calculated susceptibility than samples incubated at 15ºC throughout the ageing period. Therefore, in spite of the reduced total activity in the high temperature samples, the activity measured under competitive conditions was reduced still further. In contrast, maximal activity in the 0ºC samples was comparable to 15ºC, the activity in the competitive condition was reduced and the calculated susceptibility was significantly reduced at all timepoints post mortem except at day 6.

In spite of the effects of temperature on calpain activity and susceptibility, these did not change over the 6-day ageing period.

This study confirmed that exposure of post mortem muscle to high temperatures during the pre-rigor period produces consistent and recognisable effects on post rigor tenderisation: compared with meat held at 15 ºC: muscle maintained at 40ºC has lower initial (at rigor) tenderness but the ultimate tenderness is higher. The reasons for this have not been fully explained, but the absence of any effect of wrapping, to restrict contracture, suggest that sarcomere shortening is not a contributing factor. This is in spite of reports that the extent of contracture at high temperatures (40ºC), is greater than contracture at 0ºC (Locker and Hagyard, 1963). Cold shortening had the expected effect of increasing initial toughness, an effect that could be mitigated by restricting contracture. As expected, the final tenderness is a function of proteolysis and the extent of the initial contracture and hence the shear force of the wrapped samples did decline during the 6-day ageing period reaching an average shear force of 8.6.

An alternative to toughening caused by contracture is an effect on the protease enzymes responsible for tenderisation, and there is evidence that calpain activity is reduced post rigor following incubation at high temperatures (Simmons *et al*, 2006). The present results offer some support to this idea: compared with 15ºC, the maximal calpain activity at rigor in 40ºC samples was reduced to approximately 80% and, on average over the whole ageing period, was approximately 70%. These differences are, however, substantially less than the changes in calpain activity as described in meat science literature using the traditional calpain assay. However, the methodologies are very different: the conventional assay relies on first extracting all the tissue calpain, then activating the enzyme in optimal in vitro conditions to measure the quantity of enzyme present, hence this methodology depends on effective enzyme recovery and cannot take into account differences in activation or inhibition of the enzyme in situ.

The results using a competitive concentration of CalS1 show a still greater decline in the high temperature samples (approximately 50%). This disproportionate reduction is interpreted the substrate providing greater competition for the available enzyme, and this is attributed to a higher affinity for the endogenous proteins. This could be attributed to the denaturation of muscle proteins caused by the high temperatures and could explain the lower shear force levels seen at rigor following incubation at high temperatures.

Cold shortening conditions only had a slight effect on enzyme activity and slightly increased the calculated susceptibility. The toughness induced by cold shortening cannot be attributed to effects on protease activity because degradation of structural protein can be seen to occur at a rate comparable to unshortened meat (Koohmaraie *et al*, 1996). These results confirm that there is limited effect on protease activity and the substrate, if anything, has an increased affinity in the shortened condition.

A surprising finding in this work is the limited change in protease activity during the ageing period, an effect that was independent of pre rigor incubation temperature. The activity of µ-calpains, measured by conventional means, is normally found to reduce quickly in the post mortem period. Indeed, one of the paradoxes of the explanation for post mortem proteolysis is the rapid disappearance of µ-calpains because this enzyme is claimed to be critical for the long-term proteolysis but disappears before the ageing process is complete.

The present results seem to suggest that µ-calpain activity persists relatively unchanged. A number of possible explanations can be offered for this discrepancy. One is that the CalsS1 substrate is not sufficiently specific and is measuring the activity of some other protease. In our initial development of this assay, we undertook to test the specificity of the CalS1 activity by demonstrating calcium dependency, inhibition by calpain-specific protease inhibitors and native gel zymography, and all these tests showed the expected specificity (this contrasts with an earlier substrate, CalS2, which was found by these tests to have significant cross-reactivity with proteasomes and cathepsins in ruminants). However, these tests were not carried out in aged meats, and will be repeated to provide confirmation that an alternative protease activity does not develop during the ageing period.

A second alternative is that the conventional assay does not accurately measure the true µ-calpain activity in aged meat. This could readily be attributed to difficulties with isolating the enzyme in aged meat. A third alternative could be attributed to changes in the degree of activation/inhibition of the calpain activity in the homogenate when measured with the CalS1 assay. By using the whole homogenate, the enzyme activity is measured under conditions where endogenous activators and inhibitors can, potentially, continue to function, and the balance of these may allow increased activity in the face of decreasing enzyme concentrations as the meat ages. It is, however, somewhat surprising that the loss of enzyme concentration is so closely reversed by the increase in activity.

4.3 Using serial muscle biopsies, measure variations in calpain enzyme activity, substrate susceptibility and HSP expression

Over a period of time, biopsy samples were taken from live animals to ascertain the tenderness properties of animals during normal production systems. The results show that consistent trends can be measured successfully using the techniques developed. In the absence of environmental challenges, calpain activity did not vary over time within an animal. However, movement to a processing plant and immediate pre-slaughter handling produced an increase in calpain activity. In contrast, the levels of expressed HSP's tended to fluctuate more than the calpain activity and were highly correlated with the measured tenderness.

4.4 Develop procedures for measuring the extent of myofibrillar protein phosphorylation, with particular emphasis on titin

A number of methodologies have been developed to investigate the possible role of phosphorylation on the rate of protein degradation in post mortem meat. Using the CalS1 assay,

clear effects on the rate of protein degradation can be identified, together with preliminary evidence that the substrate susceptibility is also influenced. Further experiments to understand these relationships further are therefore warranted.

Milestone 4 describes a number of methodologies that can be used to evaluate the level of muscle protein phosphorylation. The results so far strongly suggest that in vitro manipulation of the level of phosphorylation can influence calpain activity and may also affect protein susceptibility.

The objective of this milestone is to evaluate phophorylation in vivo and identify possible effects of this form of post translational modification on the tenderisation process post mortem.

One approach to this milestone was included in the results of milestone 3 (Using serial muscle biopsies, measure variations in calpain enzyme activity, substrate susceptibility and HSP expression). These results were related specifically to the phosphorylation of HSP27, a protein we had previously identified as having a significant role in the activity of calpain enzymes post mortem. The results from the biopsy trials found that the phosphorylated form of HSP27 appears to provide a better tenderness marker, both for the activity of the calpain enzyme and the rate of tenderisation, than the total HSP27 concentration. This finding identifies an important role of the phosphorylation process in the modulation of post mortem tenderisation in meat.

The results described here are based on measurements of the total phosphorylation levels of muscle proteins, based on the total phosphorylation dot blot assay (TPDB).

4.5 Evaluate a possible role for phosphorylation and HSP binding as a source of variation in calpain susceptibility

The objectives were to establish whether the measured phophorylation of myofibrils and supernatant fractions varied between animals, and whether the presence of a phosphatase inhibitor to prevent the dephosphorylation post mortem would help to identify a phophorylation-based influence on post mortem tenderness.

In the absence of the phosphatase inhibitor, differences in the level of phophorylation were measurable both in the pre- and post-rigor periods. The quantified intensity of the dot blot response (where a value of 200 represents no colour and 0 represents pure black) shows the highest value was 75% more than the lowest in the pre-rigor supernatant, and 39% in the myofibrillar fraction. This decreased to 51% in post rigor supernatant but increased to 40% in post rigor myofibrils. On average, the level of phosphorylation was slightly higher in homogenates prepared from post rigor compared with pre rigor samples.

Inhibition of endogenous phosphatase activity increased protein phosphorylation, but the effect was much more marked in the supernatant than the myofibrillar fraction. The effects of the inhibition were comparable in samples prepared either pre or post rigor.

Table 1. Phosphorylation in muscle sirloin - Myofibrills

	Pre rigor	Pre rigo	Post rigor	Post rigort
Animal	control	phos inhib	control	phos inhib
1	137.8	128.6	148.9	134.8
2	148.7	120.3	135.0	107.7
3	164.9	142.1	127.0	125.9
4	135.4	138.7	135.8	134.0
5	174.1	172.6	178.1	174.6
Mean	152.2	140.5	145.0	135.4

Table 2. Phosphorylation in muscle sirloin – Supernatant

	Pre rigor	Pre rigor	Post rigor	Post rigor
Animal	control	phos inhib	control	phos inhib
1	102.8	34.5	61.0	15.4
2	107.3	114.3	86.1	14.9
3	75.9	23.1	57.1	42.2
4	110.6	26.3	79.4	24.8
5	63.5	11.4	80.0	13.0
Mean	92.0	21.9	72.7	22.0

In the control samples, the levels of phosphorylation in the supernatant and myofibrilar samples prepared from pre rigor samples showed a highly significant inverse correlation (r^2 0.9; p=0.01), but this relationship did not persist post rigor.

Comparing the level of phosphorylation in the control and phosphatase-treated samples produced a significant correlation in the post rigor myofibrils only (r^2 0.8; p=0.04). Pre and post rigor myofibrillar samples treated with phosphatase inhibitior were also correlated (r^2 0.86; p=0.02).

Correlations with tenderness attributes.
The correlations between the tenderness attributes – initial and final tenderness, and ageing rate – and the TPDB measurements did not reach statistical significance. With only 5 animals, this is probably not surprising, but some trends were clearly evident. The best relationships were seen between pre- and post-rigor control myofibrillar phosphorylation and initial toughness (Figure 5), and between post rigor control supernatant phosphorylation and ageing rate. This second relationship showed an inverse association (Figure 6).

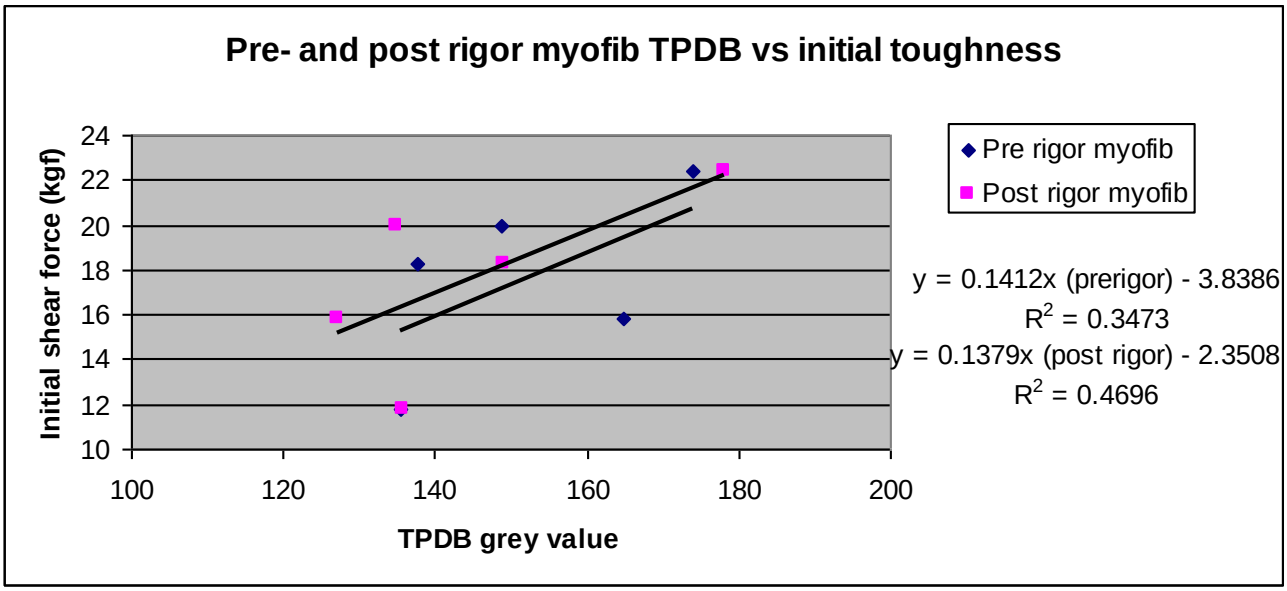


Figure 5: Pre-and post-rigor myofibrillar TPDB vs initial toughness

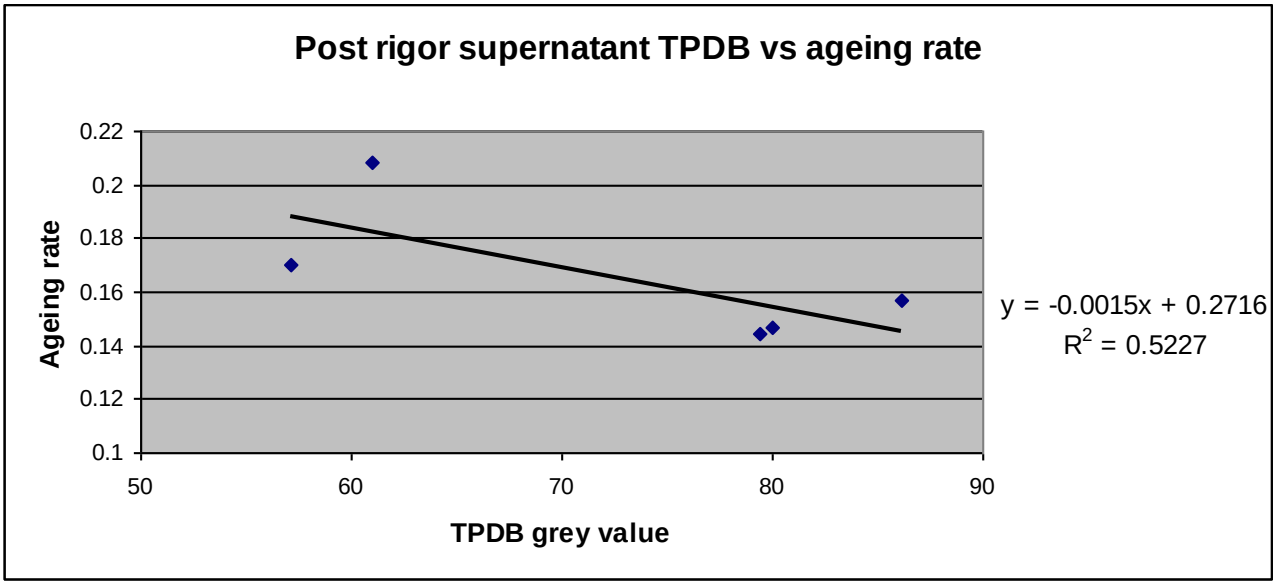


Figure 6: Post rigor supernatant TPDB vs ageing rate

5 Conclusion

CalsS1 activity is significantly reduced by pre-rigor incubation at denaturing temperatures, particularly when measured at low, competitive CalS1 concentrations, but the susceptibility of the substrate proteins increases, presumably because of denaturation. This last result may explain why the initial (at rigor) shear force values are reduced. However, it is surprising to find that the calpain activity is sustained through the 6 days of ageing, and some further work is needed to

understand why this pattern of activity differs from those measured using the conventional calpain assay.

At this stage, it does not appear that μ-calpain activity alone can explain the increased ultimate toughness of meat incubated at high temperatures during the pre-rigor period. However, the present assay does appear to provide an additional marker for meat that has undergone denaturing conditions that in turn leads to increased toughness.

The overall conclusions of this work are:

- The level of phosphorylation in the myofibrillar and supernatant fraction of muscle tissue varies between animals. This difference is evident both at kill and in post rigor samples.
- Although the experimental numbers were small and the comparisons did not reach statistical significance, the results suggested that the level of phosphorylation in the myofibrillar and supernatant fractions offer potential markers for tenderness
- These results, if confirmed, support the results of in-vitro manipulation of muscle protein phoshorylation, with demonstrated effects on calpain activity.

6 Commercial Implications

The ultimate benefit of these methodologies proposed in this application is that they allow environmental factors that can affect tenderness and possibly other meat quality attributes to be assessed directly from small samples of muscle tissue. By allowing repeated measurements of tenderness markers to be made from the same animal, clear indications of environmental effects will be explained and, potentially, the conditions needed to manage animal variability can be identified. If so, then animal husbandry or handling specifications can be developed and, by developing auditing procedures to ensure compliance, a mechanism to increase commercial returns through improved process efficiency can be developed.

7 Recommendations

The ultimate benefit of these methodologies proposed in this application is that they allow environmental factors that can affect tenderness and possibly other meat quality attributes to be assessed directly from small samples of muscle tissue. By allowing repeated measurements of tenderness markers to be made from the same animal, clear indications of environmental effects will be explained and, potentially, the conditions needed to manage animal variability can be identified. If so, then animal husbandry or handling specifications can be developed and, by developing auditing procedures to ensure compliance, a mechanism to increase commercial returns through improved process efficiency can be developed.

The following work is proposed to allow a commercial product to be realised :

- Further refine in vivo tenderness markers, based on HSP phosphorylation and binding to myofibrils to produce indicators of myofibrillar modifications that influence tenderisation rate
  Commercial outcome and date: Improved tenderness predictions from live animal biopsies through development of better tenderness markers - 2008

- Develop animal handling specifications for transport and yarding to improve 24 hour tenderness
  Commercial outcome and date: Industry specifications for live animal management to reduce product variability – starting 2008 and continuing 2010.

- Define the role of HSP in the development of denaturation events on water binding capacity and colour attributes (colour and colour stability).
  Commercial outcome and date: Industry specification for live animal management and post mortem processing to improve processing efficiency and reduce product variability – 2009.

8 References

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