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Process modelling

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Abstract

In the previous research (p.pip.228), a simplified version of a contraction model as described by Reiner & Quintern (1997) was validated. The model was simplified by limiting the data set to stimulation protocols that maximally activated the muscle and restricted the duration of stimulation to significant changes in contraction characteristics attributable to fatigue. This work demonstrated that the model could be effectively fitted to muscle contraction data and provided the basis for providing a more comprehensive description of muscle contraction behaviour.

The main modification needed to upgrade the existing model is to accommodate variable sub-maximal stimulation conditions. Under conditions where two increasing levels of stimulation (fibre recruitment) are applied sequentially, a first cohort of fibres will be subjected to stimulation during the whole stimulation episode, whereas the second cohort, recruited at the higher level of stimulation intensity, will have a distinctly shorter history of stimulation. Therefore, the model needs to track the changes associated with the stimulation in the two cohorts independently. In particular, it is the fatigue related changes in muscle fibre activity that need to be accounted for when sequential changes in recruitment are applied.

Therefore, the first upgrade of the model was to introduce independent tracking of fibre cohorts to accommodate changes in recruitment parameters. However, a second requirement quickly became evident: stimulation of post mortem muscles quickly produce changes in threshold of activation that is not effectively accounted for in the present model. In fact, few muscle contraction models reported in the literature incorporate this phenomenon of membrane fatigue (as opposed to metabolic fatigue, where force generation is reduced but thresholds do no change), possible because the effect is particularly severe in circumstances where blood flow to remove metabolic and ionic products of muscle activation are totally absent, as in post mortem muscle. We previously developed a model for membrane fatigue, which was incorporated into this contraction model.

This study evaluated the potential contribution of these mechanisms. Two experimental approaches were used: first, meat was restrained during the pre-rigor period to prevent contracture; and, second, meat was placed into denaturing conditions after rigor mortis, by which time contraction is prevented and the denaturation of myosin is substantially prevented because of the stabilising effects of the actomyosin bond. The results of the first experimental procedure were reported in a separate milestone report (Pship 150.01D; Milestone 2) as it could conveniently be incorporated into the requirements for that milestone. That study demonstrated that pre rigor wrapping significantly reduced the toughening produced by incubation of the muscle at 0ºC but had no effect on either the initial or final tenderness values of meat incubated at 40ºC. The implications are therefore that sarcomere shortening is not a significant contributor to the impaired meat quality that results from rapid pH fall/high temperatures during the pre-rigor period.

In the current research, high temperature/low pH effects were added to the model.

The model was presented to MSA and key industry representatives in Australia and New Zealand. MSA have identified the MSA database that may be uploaded and included into the model. Finally, feedback on the model will be used to develop industry adoption plans.

Contents

1 Background

The concept of the Central Processing Management System (CPMS) that underpins the MQST program depends on quantifying the interactions of processing variables so that meat quality outcomes can be predicted under a range of processing conditions. The core processing conditions that need to be managed are the rate of change of pH and temperature, and how these interact to affect quality will differ according to which muscle or species of meat animal is being considered. The meat quality attributes that need to be predicted by the model are tenderisation, colour and colour stability and water binding capacity (current process model; Figure 1).

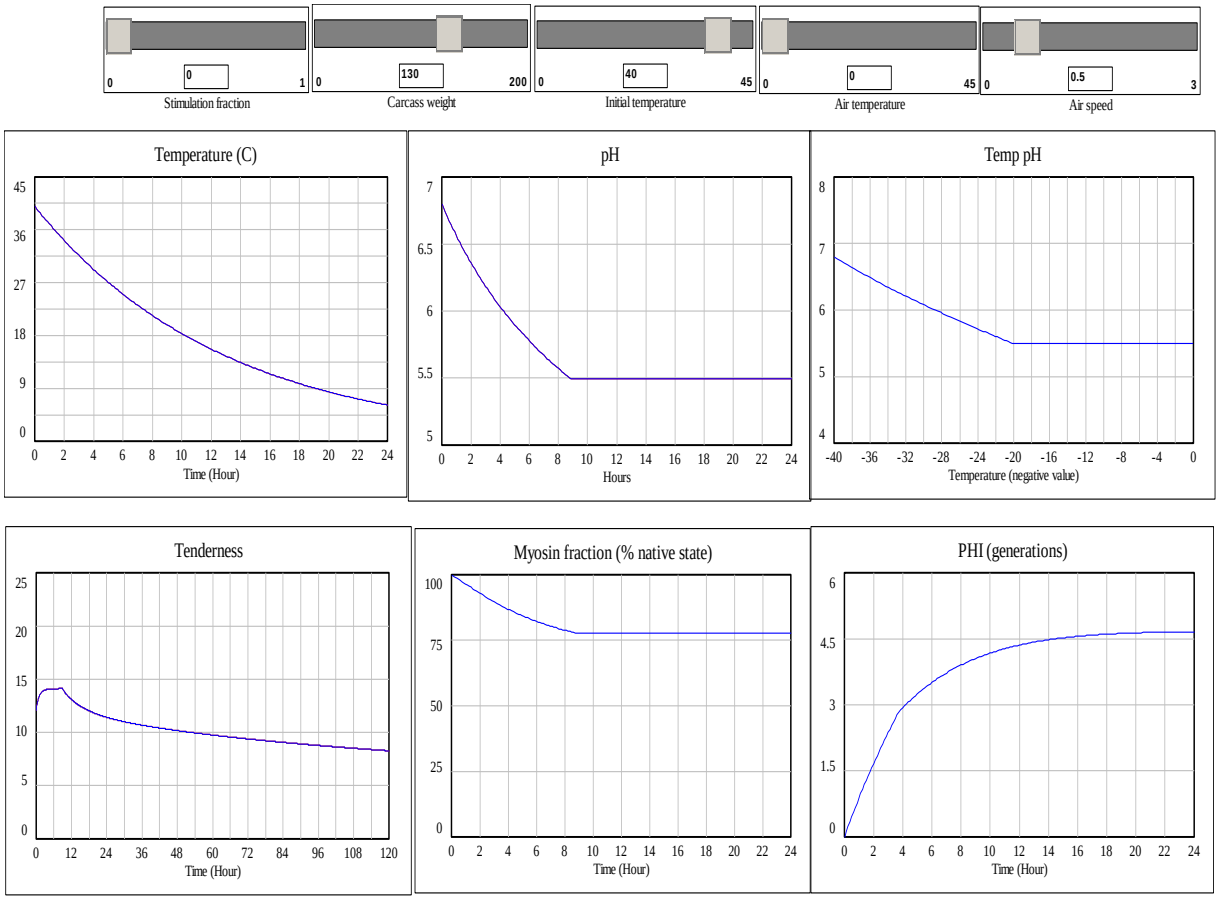


Figure 1 – Critical components of the Process Model.

Two mathematical models have previously been developed at AgResearch: a procedure to calculate the rate of tenderisation under different chilling regimes for electrically stimulated lambs (tenderness model), and a more ambitious pre-rigor model for hot boned meat that calculates the effects of temperature on the rate of pH decline and consequent tenderisation and protein denaturation (Meat Quality Model; MQM). The parameters for the MQM were derived largely from the scientific literature or from experimental data. This model still needs further validation and refinement.

The pre-rigor model was developed for hot boning because hot boning simplifies many of the predictive requirements. Predicting variable temperature gradients within a carcass, or within large muscles in a carcass, could be ignored, as could the need to accommodate differences in the skeletal restraints that either limit the extent of shortening in some muscles, or actually cause stretching. These variables still need to be quantified to produce a pre-rigor model for cold boning.

A second component of the CPMS modelling requirements is to account for the effects of electrical inputs to a carcass. The electrical parameters of these inputs will vary depending on the processing objectives (stunning, immobilisation, rigidity), as will their timing relative to slaughter.

Excessive electrical stimulation or an inherently rapid pH decline in muscle tissue following slaughter can create conditions of high temperature and low pH. These conditions can have significant adverse effects on meat quality: purge is increased, colour stability is reduced and eating quality is reduced. The cause of the loss of eating quality is not fully understood. Although meat that has undergone a rapid pre-rigor pH fall while the temperature remains high is initially more tender than meat produced under control conditions, it fails to reach the same ultimate tenderness, the conclusion is therefore that the full expression of tenderisation is impaired.

Three principal events occur that may contribute to this phenomenon: the contraction of muscle as it enters the rigor state (rigor shortening) is temperature dependent and increases with muscle temperature. Muscle contraction elicited by cold temperatures is a different phenomenon (it is ATP and calcium dependent, whereas rigor shortening is triggered by the loss of ATP and is independent of calcium) but is shown to induce toughening, so a similar mechanism may be responsible for the high temperature effect.

An alternative hypothesis is that the denaturation of muscle proteins, in particular myosin, during the pre rigor period may contribute to structural changes in the muscle that lead to limited tenderisation. A further explanation is that the activity of the calpain enzymes responsible for the tenderisation may be disaffected by these pre-rigor conditions resulting in a failure to proteolyse to an optimal level.

This year high temperature/low pH effects will be added to the model. Although often referred to as heat shortening, it is not yet understood if rigor contractions are truly responsible for the reduced eating quality. Contractures caused by low temperatures increase initial toughness whereas heat shortening results in lower initial toughness than controls. Also, the effects of high temperatures are also evident when temperatures remain high in the post rigor mortis period, when contractures have ended. We have demonstrated that calpain activity is significantly reduced when high temperatures are combined with low pH, and this effect is independent of the onset of rigor mortis. Accordingly, the current model structure considers only the rate of autolysis relative to proteolysis (an approach also used in the original Dransfield calpain model). This concept will be validated using the calpain assay, and the possible need for an additional component to quantify the contribution of contracture will be assessed.

In the current research, the model will be presented to MSA and key industry representatives in Australia and New Zealand. Feedback on the model will be used to develop industry adoption plans. MSA will also be consulted to identify if data from the MSA database may be uploaded and included into the model.

2 Project Objectives

The objectives of the research are :

- Develop a mathematical model of the voltage gradients through carcass during immobilisation and stimulation to allow general principles to be defined.
- Report on the effects of denaturing/shortening conditions either pre- or post-rigor mortis on calpain activity, shear forces and protein denaturation in beef.
- Incorporate shortening/denaturation data into the meat quality model.
- Further assessment of the effects of high temperature denaturation on the ageing rate and final tenderness of meat.
- Update on the development of the commercial dataset developed in collaboration with commercial partners in New Zealand.
- Review and report on what MSA data can be added to Meat Quality Model.

3 Materials & Methods

3.1 Develop a mathematical model of the voltage gradients through carcass during immobilisation and stimulation to allow general principles to be defined.

The use of electrical immobilisation of carcass after a stun is intended to provide additional safety to the operators by controlling animal movement. At the same time, the electrical current can itself be a risk factor and needs to comply with recently developed safety standards. The objective of this milestone is to develop a model for the voltages within an idealised carcass in order to establish qualitatively some of the critical variables that influence the exposure of operators in contact with the carcass to electrical voltages, as well as those that influence the ability of the applied voltage to control carcass movement.

The electrical safety standard requires that the voltage exposure at the point of contact be less than a specified limit. For this modelling exercise, the voltage of a hand contact (Vh) is calculated as a peak pulse voltage, and the appropriate calculation can be made to determine the RMS voltage. In contrast, effective immobilisation of a carcass depends on stimulation the voltage gradient (voltage drop/distance) since it is the voltage difference across a membrane that triggers an action potential. In the model, this is represented as the gradient through the core, Ec.

Some questions regarding the design of the immobilisation system were also addressed. Of particular interest is the common practice of resting the carcass on a conductive platform while it is being stimulated; this platform has the potential to be a parallel pathway that will influence the current pathways during immobilisation. Also, the size of the electrodes and differences in the electrical conductivity of the skin, due primarily to wetness of the hide, are considered.

Carcass model
Baseline model parameters
Dimensions:

Carcass length (m)	2.00	*Hand diametrically opposite to electrode.*
Carcass diamater (m)	0.50	*Hand centre 1/6 of dist. From end of carcass at grounded electrode end.*
Skin thickness (m)	0.03	*Electrode centres 1/3 of dist. from end of carcass.*
Electrode width (m)	0.10	*Single parallel plate*
Electrode length (m)	0.30	
'Hand' width (m)	0.10	
'Hand' length (m)	0.15	
Parallel plate width (m)	0.10	
Dist. Electrode->plate (m)	0.06	

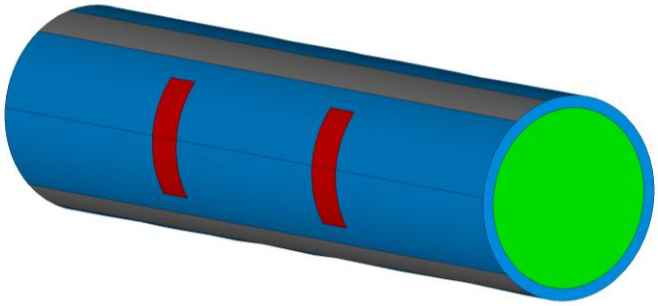
Material properties:

Skin resistivity (Ohm.m)	10.00
Core resistivity (Ohm.m)	1.50
'Person' resistance (Ohm)	500.00

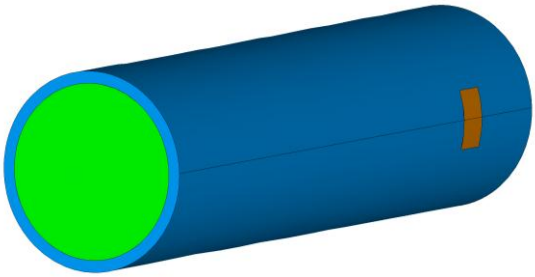
Abbreviations:

Vh	Voltage across the resistor representing a person
Rs	Resistance seen by the source voltage
Ea	Average magnitude of electric field* over the cross section midway between the electrodes
Ec	As above but averaged over the core only
Es	As above but averaged over the skin only
	* Equivalent to magnitude of voltage gradient in 3D

Figure 2 shows the stimulation electrodes (red) and two parallel plates (grey) which represent the conductive platform on which the carcass rests. On the opposite side to the electrodes is the hand location (brown).



(a) Electrodes and parallel plates (2 plate case)



(b) Hand location

Figure 2. Problem Geometry

3.2 Report on the effects of denaturing/shortening conditions either pre- or post rigor mortis on calpain activity, shear forces and protein denaturation in beef.

Both striploins from 5 cows were collected immediately after carcass dressing and incubated at 15°C until rigor mortis. Following rigor mortis, the muscle was subdivided into portions and each was allocated to one of the following treatments:

1. Control: Continued post mortem ageing at 15ºC
2. 2 hour treatment: samples transferred to 40ºC for 2 hours, then returned to 15ºC
3. 6 hour treatment: samples transferred to 40ºC for 6 hours, then returned to 15ºC
4. 10 hour treatment: samples transferred to 40ºC for 10 hours, then returned to 15ºC

After the appropriate high temperature incubation period and equilibration back to 15ºC, an initial sample was collected for analysis. Subsequently, after a further 7 days of ageing at 15ºC to reach a final tenderness, a second sample was analysed to determine the ultimate tenderness characteristics.

3.3 Incorporate shortening/denaturation data into the meat quality model.

The key objective of this milestone is to quantify the effects of denaturing conditions on the subsequent tenderisation process in meat. It is now generally recognised that high temperatures through the pre-rigor period, when low pH conditions develop in the muscle, lead to reduced eating quality, measurable as increased measured shear force. In addition, denaturation reduces water binding capacity, an effect attributed to the denaturation of myosin and increased lattice shrinkage. The lattice shrinkage also contributes to colour changes due to increased reflectance. The objective is to incorporate these data into the meat quality model.

Our work to date has concentrated on denaturing events in the pre-rigor period, and the changes in calpain activity have been quantified using the CalS assay that we have developed over the last year. The pre-rigor period is important to those meat quality attributes that are influenced by contracture as induced by high (rigor shortening) or low (cold shortening) temperatures. Also, myosin is much more susceptible to denaturation when in the unbound, pre-rigor state and denaturation is largely prevented once rigor bonds with actin form.

Understanding the effects of denaturing conditions on calpain activity needs also to consider any differences that may exist between the pre- and post-rigor period. The advantage of the post-rigor period is that any confounding effects of rigor contractures can be separated from the effects on proteolytic enzymes. Therefore, the experimental design reported here is based on varying periods at high, denaturing temperatures immediately after rigor mortis, following a constant pre rigor temperature of 15ºC.

The results from an earlier series of experiments reported as milestone 2 under this objective, clearly demonstrated an increased ultimate shear force in meat maintained at denaturing temperatures in the post rigor period. As little as 2 hours at 40ºC reduced calpain activity, and this change was greater than that seen after maintaining at an equivalent temperature for the whole pre-rigor period. The treatment also increased ultimate shear force, an effect that was more pronounced after 6 hours of high temperature treatment. Of some interest was a suggestion that the 10 hour high temperature treatment reduced the toughening effect compared with the 6 hour high temperature treatment.

The objective of this milestone was to acquire the data needed to describe more fully the effects of several time and temperature environments in the post rigor period on calpain activity and shear force for inclusion into the model. However, obviously, these data cannot be incorporated into the predictive meat quality model until the underlying mechanisms are better understood.

The milestone was therefore modified to examine in more detail the tenderness effects of denaturing conditions.

Both striploins from 5 beef carcasses were collected immediately after dressing and incubated at 15°C until rigor mortis and the pH was monitored until rigor (ultimate pH). At rigor mortis, the muscle was subdivided into portions and each was allocated to one of the following treatments:

- Control: Continued post mortem ageing at 15ºC
- 2 hour treatment: samples transferred to denaturing conditions for 2 hours, then returned to 15ºC.
- 6 hour treatment: samples transferred to denaturing conditions for 6 hours, then returned to 15ºC
- 10 hour treatment: samples transferred to denaturing conditions for 10 hours, then returned to 15ºC
- 24 hour treatment: samples transferred to denaturing conditions for 10 hours, then returned to 15ºC

Two denaturing conditions of 35º and 40ºC were used.

After the appropriate high temperature incubation period and equilibration back to 15ºC, an initial sample was collected for analysis. Subsequently, after a further 5 days of ageing at 15ºC to reach a final tenderness, a second sample was analysed to determine the ultimate tenderness characteristics.

The analyses were:

- shear force measurements immediately after denaturing treatments and again after aging for 5 days at 15ºC until ultimate tenderness was attained
- Calpain activity and substrate susceptibility
- Water binding capacity, measured using either the centrifuge or the filter paper press tests
- Total reductive capacity using the MTT tetrazolium assay.

3.4 Further assessment of the effects of high temperature denaturation on the ageing rate and final tenderness of meat

The main objective of this milestone was to determine whether or not an interaction exists between denaturation during the pre-rigor period, when muscle shortening is possible and myosin in particular is susceptible to denaturation, compared with denaturation of meat during the post-rigor period. In particular, the effects on the phenomenon of toughening only during transient exposure to high temperatures was of interest.

Denaturing conditions in meat, produced by the combination of low pH and high temperatures, are now recognised as causing a toughening effect in meat. This phenomenon is likely to be mediated by effects of denaturing conditions on the activity of calpain enzymes, since both the conventional measurements of calpain activity (Simmons et al 1996) and our more recently developed CalS1 assay have both demonstrated reduced calpain activity following denaturation.

However, our recent milestone (Milestone 3) identified a more complex response to denaturing temperatures. Under experimental conditions where post rigor meat was maintained for differing time periods of up to 24 hours at 40ºC, the ultimate tenderness increased in response to exposure to denaturing conditions for up to 12 hours, but then decreased again, to reach normal levels, when denaturing conditions were maintained for longer periods of up to 24 hours. The implication seems to be that sustained exposure to denaturing temperatures allows the tenderisation process to proceed normally and reach a normal ultimate tenderness, but a

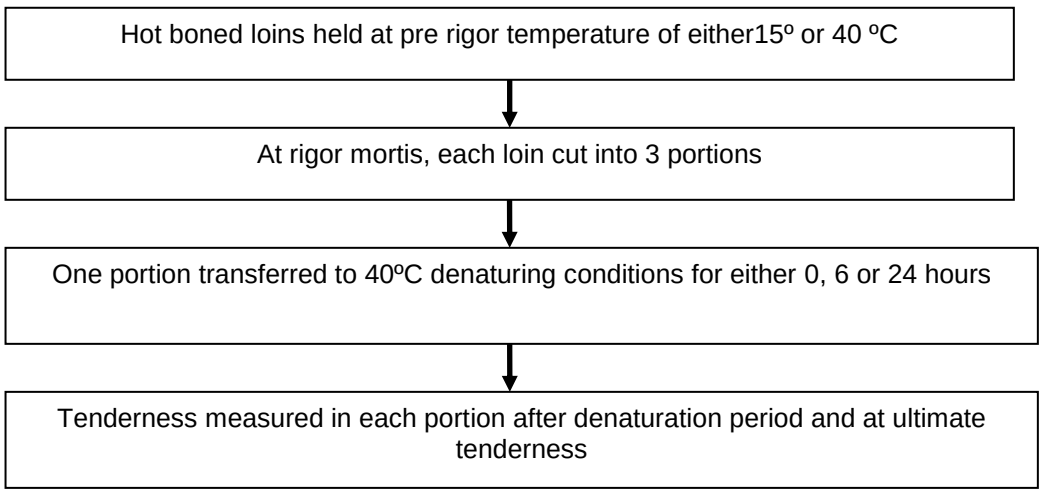
temperature transition to a lower temperature (in this case, from 40º to 15ºC) when tenderisation is only partially completed generates the inhibition of the tenderisation process.

It is difficult to explain these effects based on a simple denaturing effect of high temperatures on calpain enzymes. If the behaviour is mediated by calpain activity, then it probably relates to a complex temperature dependency between level of inhibition by calpastatin, rates of inactivation of calpains through autolysis and the resultant rate and extent of proteolysis. Alternatively, an interaction between substrate degradation through proteolysis and direct denaturation of the substrates, particularly the myofibrillar proteins, may also be a contributing factor.

At this stage, some better understanding of the behaviour of the system is needed to help formulate an appropriate hypothesis and provide some indication of potential commercial uses of these findings. So far, the work has only considered the effects of denaturation on post rigor meat, in order to minimise the complication of pre-rigor contractures and the high susceptibility of myosin to denaturation during the pre-rigor period. This experiment therefore extended the experimental conditions to include the post-rigor period, in order to assess if the phenomenon of interest is still evident when the denaturation conditions are extended to include different exposure times in the post-rigor period.

Ten striploins from 5 cattle were removed pre-rigor as soon as the carcass was dressed. One side was immediately cooled in a 15ºC water slurry while the other side was maintained at 40ºC. The pH decline was monitored in samples at both temperatures until rigor mortis (ultimate pH) was attained.

At rigor, each sample was cut into 3 portions, which represented 3 time periods of exposure to denaturing conditions: these were 0, 6 and 24 hours. Each period of exposure to high temperatures began at rigor mortis and, at the end of the period, the samples were returned to 15ºC. After equilibrating to 15ºC, half of each sample was cooked and its tenderness determined; the remaining half was maintained at 15ºC until final tenderness was attained (120 hours), then cooked and shear force measured (see Schematic 1).



Schematic 1 – Process used to measure tenderness after denaturation period for incorporating into the Process Model.

4 Results

4.1 Develop a mathematical model of the voltage gradients through carcass during immobilisation and stimulation to allow general principles to be defined.

Some key diagrammatic results are shown below.

Figure 3 shows a contour plot of the voltage through a cross section of the carcass at the site of the live electrode. This shows that the high resistivity of the skin relative to the core means that most of the voltage drop is through the skin. Since the live and return electrodes are exactly symmetrical, the majority of the voltage is, necessarily, at a midway voltage value – approximately 45 volts.

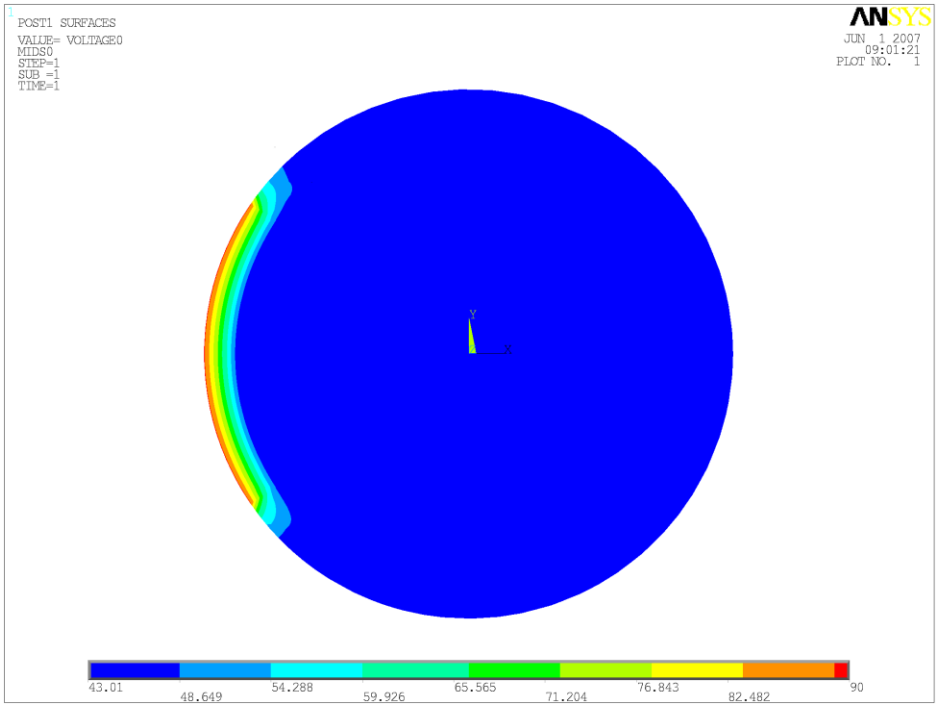


Figure 3 - Contour plot of the voltage through a cross section of the carcass at the site of the live electrode.

This will apply throughout the carcass, including at the surface of the skin. This means that, irrespective of the resistance of the skin, the contact voltage will be approximately ½ of the peak voltage as long as the contacts of the live and return electrodes are similar. If the resistance of the return electrode is lower (larger electrode area or better contract), the carcass voltage will be lower than the midway value; but if the contact if the live electrode is the better, then the carcass voltage will be greater than the midway value.

Figure 4 confirms the points made earlier. This shows the distribution of voltage across the surface of the carcass, and this represents the exposure voltage if contact is made. Clearly, the position of contact will have very little on exposure voltage unless the position is immediately adjacent to the live electrode.

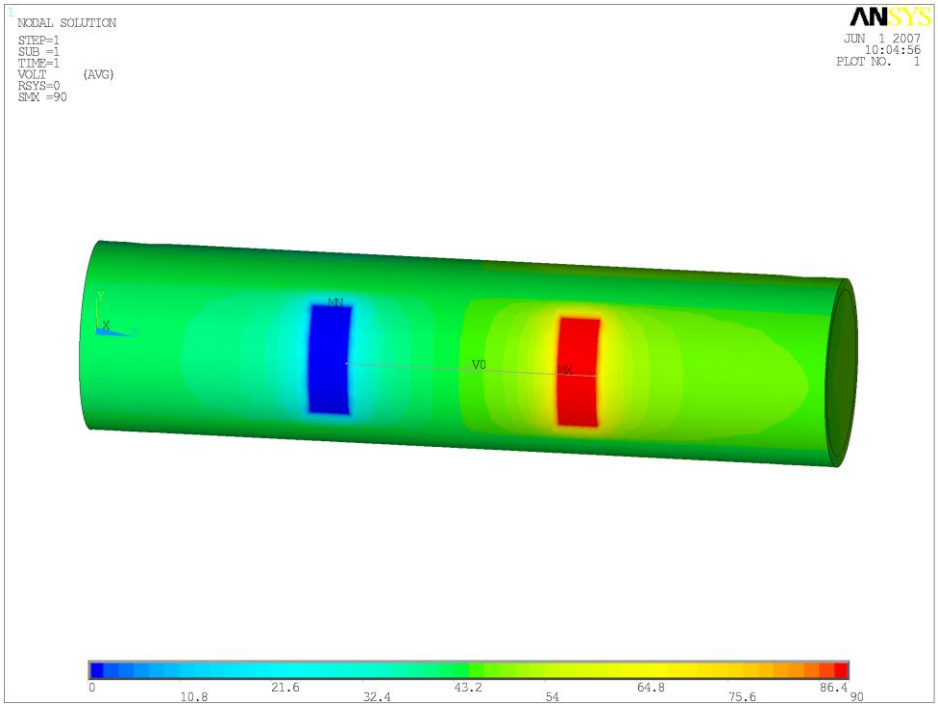


Figure 4: Carcass surface voltages.

Figure 5 shows the voltage gradient through the carcass at a cross section midway between the electrodes. The voltage gradient is a vector quantity, so this graph shows the sum of the values in the three dimensions, but the majority of the gradient at this point in the carcass is parallel to the length of the carcass.

The graph shows that the largest gradient is found on the side of the stimulation electrodes and is less pronounced on the opposite side of the carcass. The difference across the carcass for this Figure, based on a 10 Ω.m skin resistivity (quite low, assumed to represent a wet hide) is reasonably significant.

Figure 6 shows the same calculation if the resistivity of the skin is 75 Ω.m and shows not only a much lower gradient value but also a more even distribution of gradient (note that the scale of the colour contours has changed). Both figures show the presence, and effect, of a single support plate.

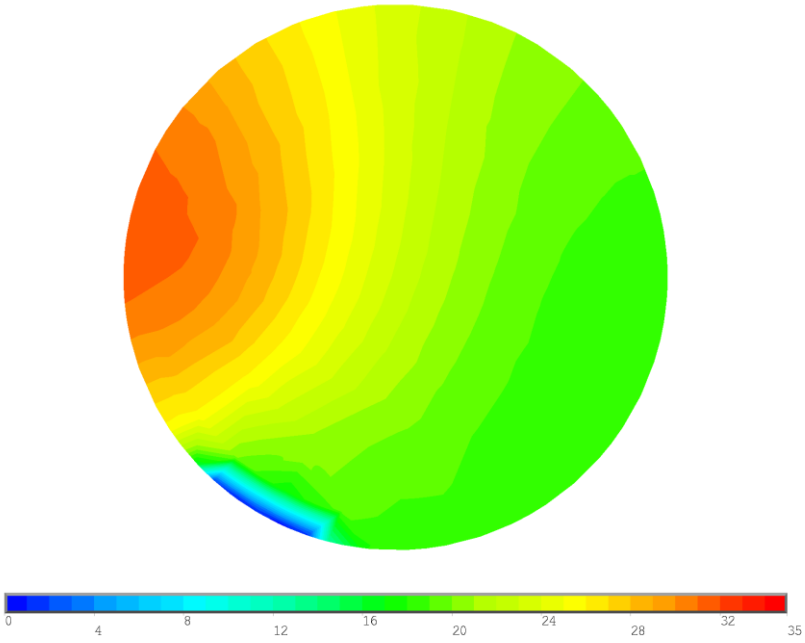


Figure 5: Voltage gradient in cross section using 10 Ω.m skin resistivity.

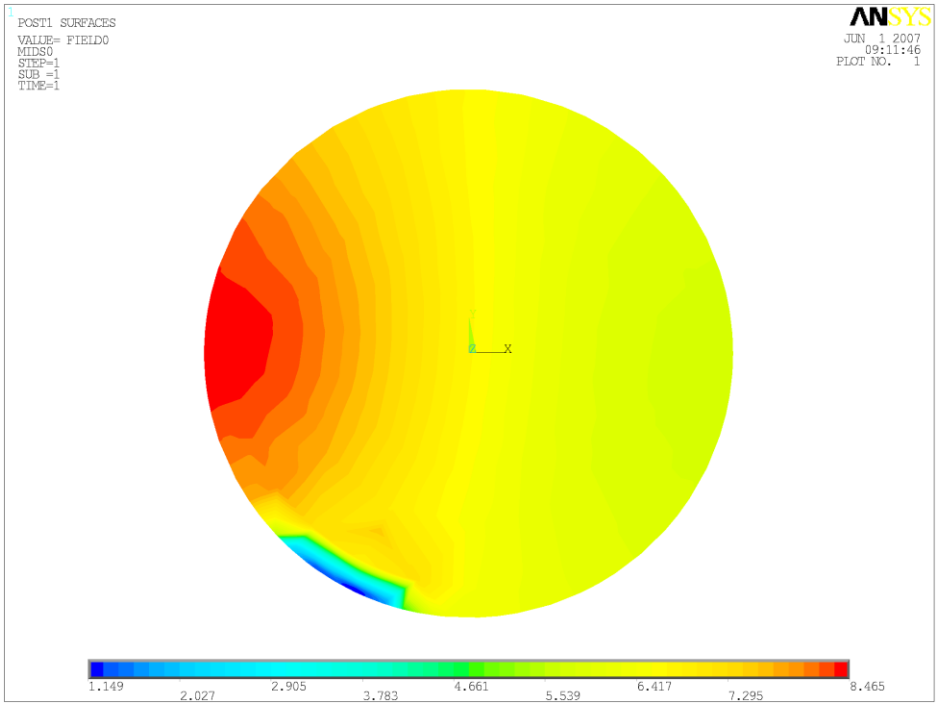


Figure 6: Voltage gradient in cross section using 75 Ω.m skin resistivity.

The results of the simulations are shown in the tables below. The two critical columns are Vh – voltage exposure during contact with the carcass – and Ec – the gradient through the core and, hence, effectiveness at immobilisation.

Table 1: Effect of increasing the number of parallel plates – plates electrically isolated from earth.

Number of plates	Vh	Rs	Ea	Ec	Es
1	37.8	21.8	22.6	22.8	21.8
2	39.9	20.7	16.7	17.1	15.3
3	40.7	20.5	14.5	15.0	12.8
4	41.3	20.3	12.7	13.3	10.7

This results shows that the exposure voltage (Vh) is unaffected by the surface area of contact with a conductive support platform. However, the mean voltage gradient through the cross section at the midpoint between the electrodes (Ec) shows a decrease of nearly 50% as the surface area of contact with the plates is increased 4 fold.

Table 2: Effect of altering the distance between the parallel plate and the electrodes.

Distance (mm)	Vh	Rs	Ea	Ec	Es
30	38.0	21.3	22.1	22.3	21.8
60	37.8	21.8	22.6	22.8	21.8
90	37.8	21.9	22.9	23.1	22.1
120	37.7	22.0	23.2	23.5	22.4
150	37.8	22.1	23.4	23.6	22.7

Where the support plates are relative to the stimulating electrodes had no discernable effects on either safety or immobilisation.

Table 3: Effect of increasing the number of parallel plates (plates earthed).

Number of plates	Vh	Rs	Ea	Ec	Es
1	4.3	11.9	31.4	24.9	47.5
2	1.5	10.8	25.8	18.4	44.4
3	0.5	10.5	24.3	16.6	43.3
4	0.4	10.4	22.8	15.0	41.8

This simulation considers the effect of the surface area of contact to support plates that are earthed (the output of the stimulator is also earthed). Because this effectively decreases the resistance to the return by increasing the area of contact of the return electrode, the safety is substantially increased. There is some loss in the immobilisation, but this is a comparatively small effect.

Table 4: Effect of skin resistivity.

Skin resistivity (Ω.m)	Vh	Rs	Ea	Ec	Es
10	37.8	21.8	22.6	22.8	21.8
23	37.5	41.0	15.0	15.2	14.5
36	36.8	59.4	11.4	11.5	11.0
49	35.9	77.5	9.2	9.3	9.0
62	34.9	95.2	7.8	7.9	7.6
75	33.9	112.7	6.7	6.8	6.5

Skin resistivity had little effect on Vh – as discussed earlier, the carcass voltage assumes a ½ maximal voltage if the resistances through the two electrodes are symmetrical. However, the immobilisation effect decreases dramatically as the resistivity increases.

It should be recognised that, while the voltage at the point of contact remains the same irrespective of the skin resistivity, the current flow through a person making the contact decreases in proportion to the increased resistivity. It does seem somewhat anomalous that the safety is considered equivalent when the current flow is clearly very different, but this is a consequence of basing the safety standard on voltage alone, rather than, for example, the energy (e.g. volts x amps) at the point of contact.

Table 5: Effect of electrode length (no parallel plates) for a range of skin resistivities.

	Vh		
Electrode length/skin resistivity	10 Ω.m	40 Ω.m	70 Ω.m
20 mm	38.7	34.2	29.8
115 mm	36.4	36.1	33.3
210 mm	34.2	36.0	34.0
305 mm	31.9	35.4	33.9
400 mm	29.9	34.7	33.7

	Ec		
Electrode length/skin resistivity	10 Ω.m	40 Ω.m	70 Ω.m
20 mm	9.0	2.9	1.8
115 mm	19.9	6.8	4.2
210 mm	28.3	10.3	6.4
305 mm	36.0	13.7	8.6
400 mm	42.7	16.9	10.6

Increasing electrode length effectively reduces the contact resistance with the carcass. This is therefore the opposite of increasing the skin resistivity. Although Vh is relatively unchanged by these different parameters, Ec is very significantly affected: large surface area of contact contributes to the voltage gradient through the core and ensures effective immobilisation.

New components of the process model incorporating cold-shortening & contraction shown in the Appendix (Figure A1).

4.2 Report on the effects of denaturing/shortening conditions either pre- or post rigor mortis on calpain activity, shear forces and protein denaturation in beef.

4.2.1 Shear force

The initial shear forces were measured at rigor for the control samples and immediately after the appropriate period of high temperature incubation in the treatment groups. This protocol allowed for a significant amount of post-rigor ageing in the treatment groups and, depending upon the duration of incubation at the high temperature, a significant amount of ageing would have been anticipated. As expected, the initial shear force decreased in proportion to the duration of the high temperature incubation and the effects on the ultimate shear forces were marked: The ultimate shear force was clearly increased ($p<0.01$) compared to controls even in those samples that had been incubated for 2 hours at 40ºC post rigor (Figure 7).

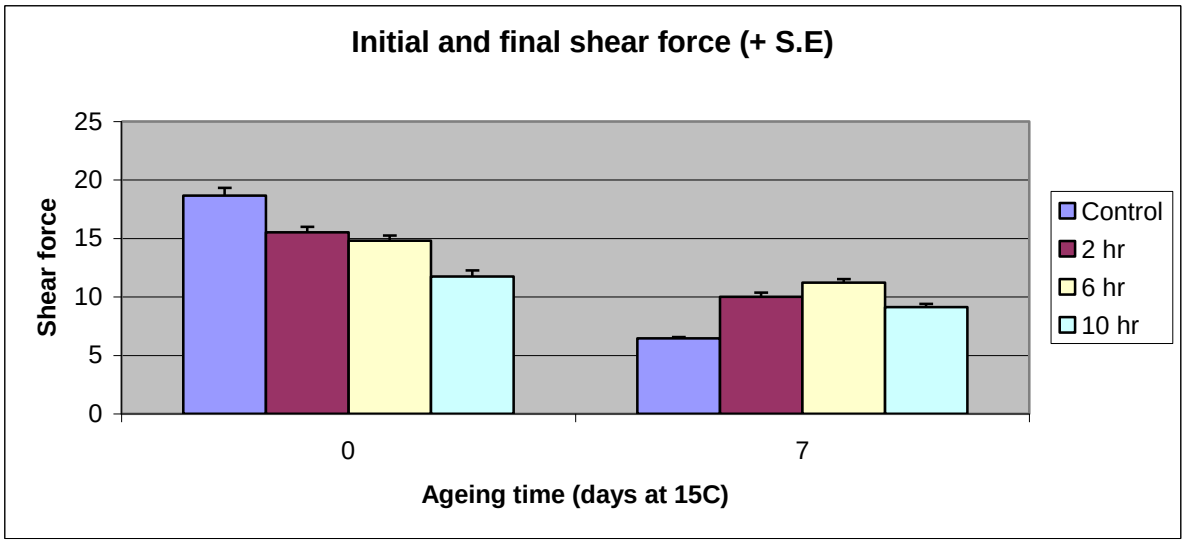


Figure 7 : Initial and final shear force

While the 6 hour incubation period increased the ultimate shear forces still further, there is some evidence that the longest period of high temperature incubation, 10 hours, began to reverse this effect. The average shear forces in the 10 hour treatment group were significantly less ($p<0.05$) than the 6 hour treatment group. However, the total change in tenderness during the ageing period for samples in this treatment group was least (Figure 8).

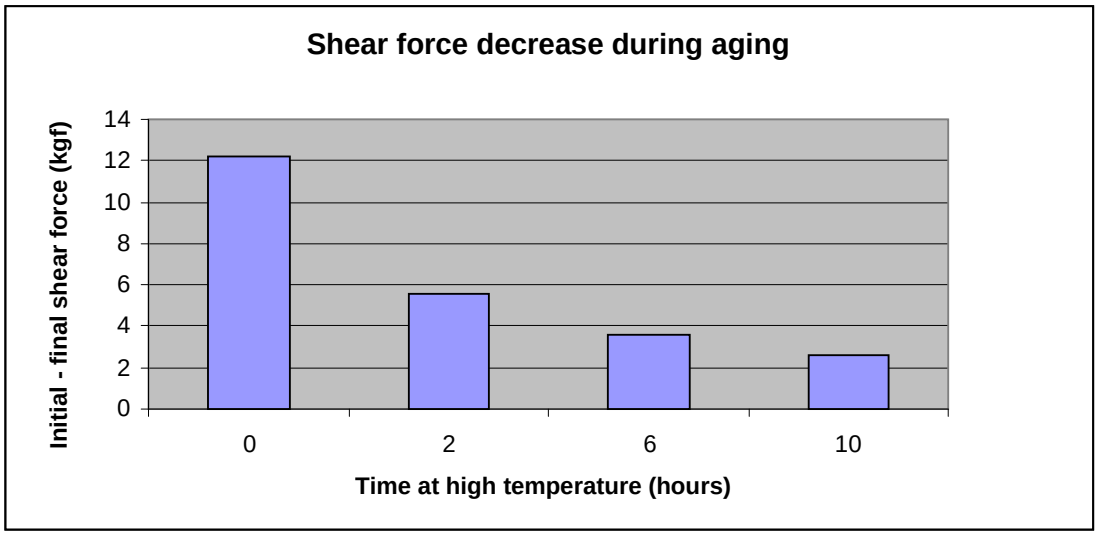


Figure 8 : Shear Force decrease during ageing.

4.2.2 Water binding capacity

The effects of high temperature post-rigor incubation on water binding capacity (WBC) was also measured, using both the filter paper press test or centrifuge test. Both methods show that the WBC was reduced following high temperature incubation, an effect that was significant using both measurement systems, even after 2 hours of incubation ($P<0.05$).

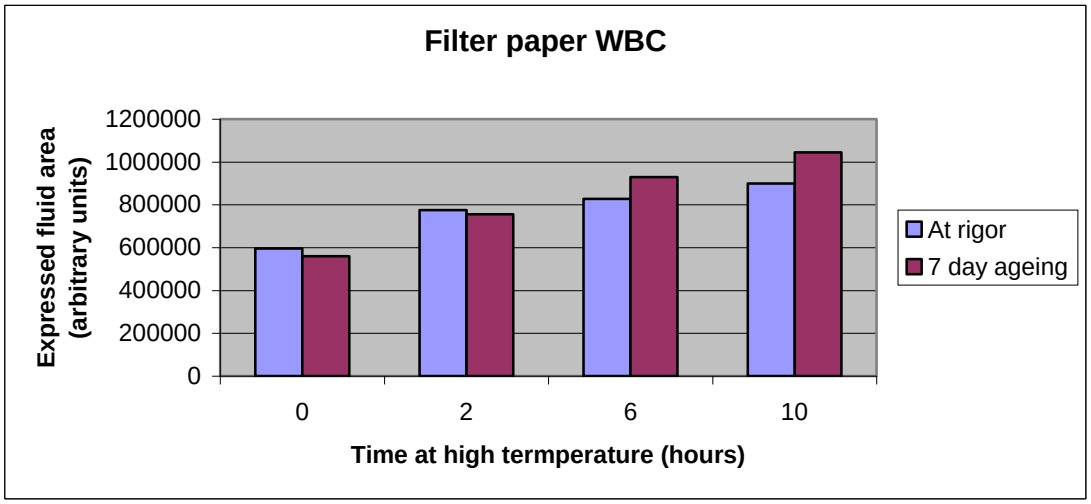


Figure 9 : Water binding capacity as expressed fluid area.

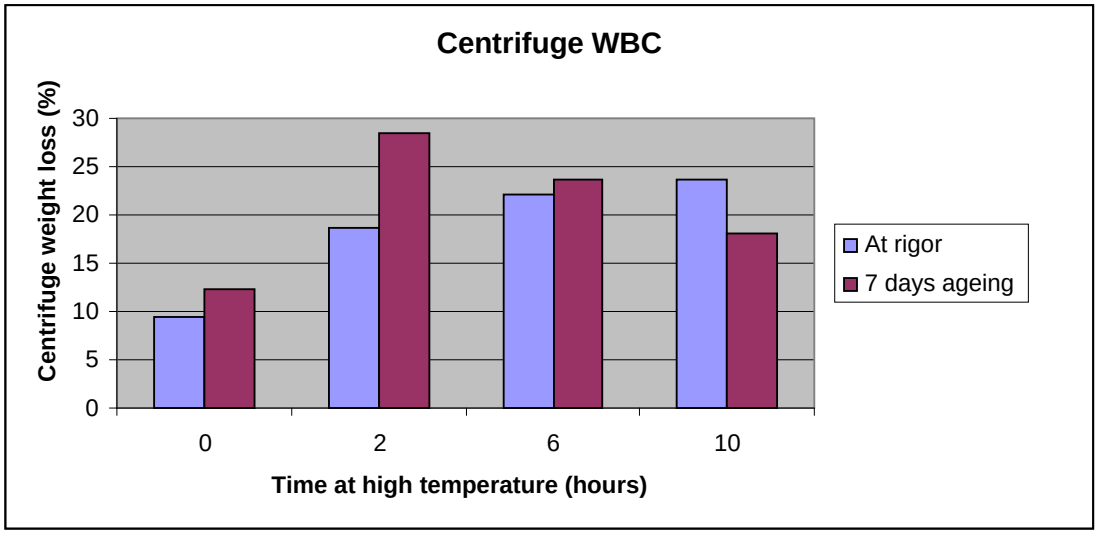


Figure 10 : Water binding capacity as expressed centrifuge weight.

4.2.3 Calpain activity

The calpain activity was significantly affected by post rigor high temperature incubation periods (Table 6).

Table 6: Effect of post rigor high temperature incubation periods on calpain activity.

Hours at high temperature	0	2	6	10
Maximal calpain activity	821 (65)	216 (21)	405 (96)	417 (87)
Competitive calpain activity	176 (7.7)	26.5 (5.1)	46.3 (14.4)	49.1 (7.8)
Substrate susceptibility	0.22 (0.027)	0.12 (0.013)	0.11 (0.013)	0.12 (0.007)

Figures given are means (standard errors)

Maximal activity was significantly reduced following all periods of post-rigor high temperature incubation, but, surprisingly, the greatest effect was seen following the 2 hour incubation treatment. On average, the maximal activity was reduced to 40% of the control group, and this effect was most marked for the low CalS1 concentration (23% of the 40% reduction in activity). This disproportionately low activity at the competitive concentration is interpreted to mean more competition from endogenous proteins, so the calculated susceptibility is found to be twice that of the control group.

The effects of post-rigor incubation at a high temperature on the final tenderness of meat was found to be equivalent to the effects of pre-rigor exposure to 40°C. This finding tends to confirm that the reported reduction in sarcomere length following high pre-rigor temperature incubation is unlikely to be a significant contributing factor to the reduction in tenderness following high temperature treatments.

Also, since the binding of myosin to actin at rigor mortis reduces the susceptibility of the myosin head to denaturation, it could be concluded that myosin denaturation is unlikely to be a cause of the reduced tenderisation. However, the WBC was also significantly reduced and might implicate some degree of myosin denaturation, since myosin denaturation results in myofibrillar lattice shrinkage and, hence, loss in WBC. We have previously found that post rigor exposure to high temperatures does not alter myofibrillar density, a sensitive measure of myofibrillar protein denaturation, so the loss of WBC might be attributed instead to an increase in soluble sarcoplasmic protein denaturation.

What is clearly demonstrated in this study is the importance of temperature in the post-rigor muscle. As little as 2 hours exposure to 40ºC produced effects on shear force comparable to exposure to this same temperature during the whole pre-rigor period. Furthermore, the calpain activity appears to be even more sensitive to post rigor temperatures: whereas pre-rigor exposure reduced maximal activity by about 20% and the competitive concentration by about 50%, the post –rigor exposure essentially double the magnitude of this effect. Effects on myofibrillar susceptibility were comparable, but remained substantial even after a 2 hour exposure to high temperature.

One interesting feature evident in these results is the possibility that sustained exposure to high temperatures (10 hours) may have less effect on ultimate tenderness than shorter exposures. This suggestion is supported by earlier studies designed to determine the temperature-dependence of ageing rates. In these studies, samples were maintained at 15ºC during the pre-rigor period but then transferred to high temperatures for the whole ageing period; these studies, in both beef and lamb, did not identify a particularly obvious failure to tenderise. Some further exploration of this phenomenon may help to identify the mechanism underlying the failure to tenderise at high temperatures.

4.3 Incorporate shortening/denaturation data into the meat quality model.

4.3.1 Shear force.

The previous results based on denaturation at 40ºC for up to 10 hours suggested that the ultimate tenderness was less affected by the 10 hour treatment than the 2 and 6 hour treatments. To confirm this, an additional treatment of 24 hours at denaturing temperatures was added, as well as an additional temperature treatment, 35ºC.

As expected, the samples tenderised significantly during the high temperature conditioning period (Figure 11), reaching 8.3 and 7.2 kgf, at 35ºC and 40ºC respectively. The ultimate tenderness of the control samples, aged for 5 days at 15ºC, averaged 5.8 kgf (Figure 12); which means that the 24 hours at the denaturing temperatures reached 83 and 91% of the total tenderisation of the control samples.

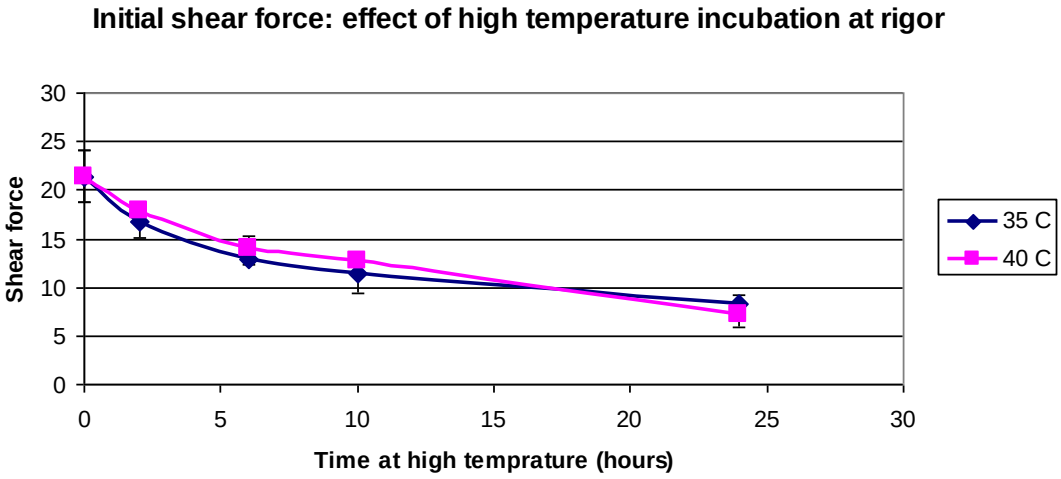


Figure 11: Effect of high temperature incubation at rigor: Initial shear force (kgf).

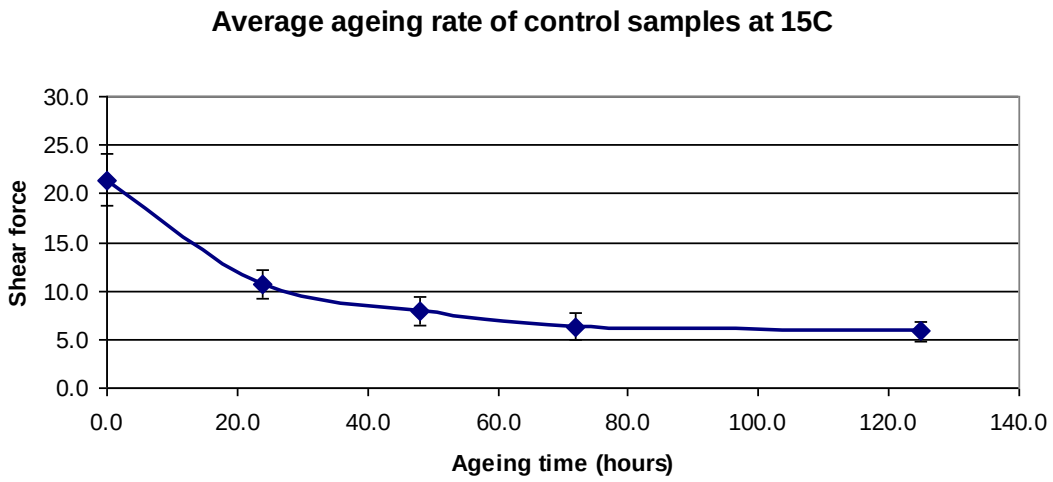


Figure 12: Average ageing rates of control samples at 15'C.

The ultimate shear forces were measured after the denaturing treatment and a subsequent period of 5 days of ageing at 15ºC. The results are shown in Figure 13.

As seen previously, the final shear force values were increased by a period of conditioning at denaturing temperatures and this effect was more severe at 40ºC compared with 35ºC. After 6 hours at high temperatures, the ultimate shear was nearly doubled to 10 kgf, which represents an unacceptable eating quality.

However, some improvement was seen in the 10 hour treatment at 35ºC. Exposure to both denaturing temperatures for 24 hours did not produce a significant increase in the ultimate tenderness compared to controls

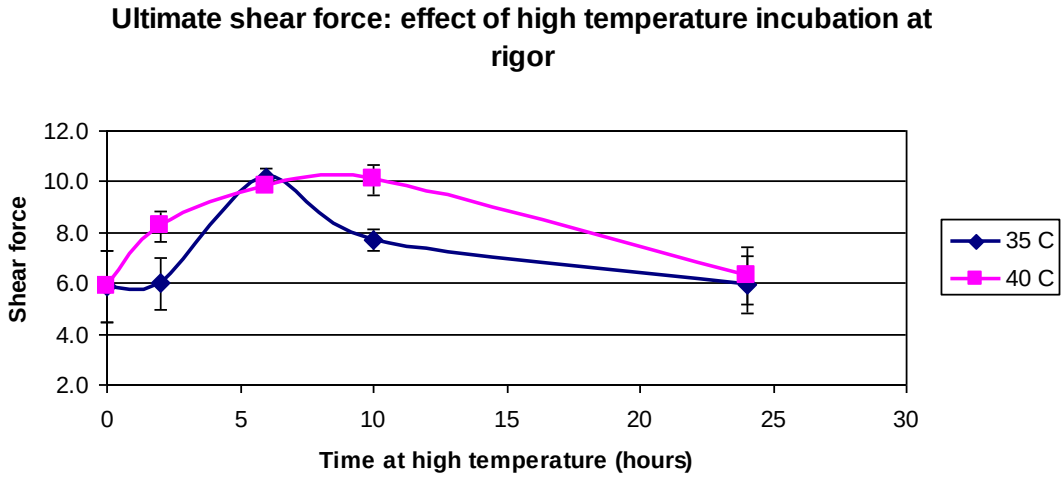


Figure 13: Effect of high temperature incubation at rigor: Ultimate shear force (kgf).

4.3.2 Combined pre-and post-rigor denaturing temperatures.

To further explore this phenomenon of reduced toughening with the more prolonged exposure to denaturing conditions, an additional 5 sirloins samples were maintained at 40ºC throughout the pre-rigor period and for a further subsequent 24 hours. Thereafter, these samples were aged at 15ºC for 5 days and compared with samples that were maintained at 40ºC during the pre-rigor period only or at 15ºC throughout the pre- and post-rigor period.

The results are in keeping with the earlier studies: High temperatures during the pre-rigor period only significantly increased the ultimate tenderness from 4.8 kgf in the control 15ºC samples to 8.3 kgf ($p<0.01$). However, the high temperatures during the pre-rigor period combined with an additional 24 hours post-rigor produced a final tenderness of 5.5 kgf, which was not significantly different from the controls.

4.3.3 Calpain activity.

The pattern of calpain activity showed a similar pattern to previous results. In the control sample, activity did change much or show any consistent pattern during ageing.

Even brief exposure to high temperatures produced a very significant decrease in CalS1 activity (Table 7), to an extent comparable to previous results. The susceptibility calculation also showed a significant increase in substrate susceptibility. However, the duration of exposure did not significantly affect either the activity or the susceptibility and, while there was a tendency for a large effect at 40ºC compared with 35ºC, this difference was not significant.

Table 7: Effect of denaturing temperatures on initial calpain activity and susceptibility.

	5 mM CalS1	0.2 mM CalS1	Susceptibility
15ºC	922	148	0.164
35ºC	634*	69*	0.109*
40ºC	585*	50*	0.087*

An effect of duration of exposure to denaturing condition was evident in the calpain activity and susceptibility measured after 5 days of ageing at 15ºC. These are shown in Figures 14-16.

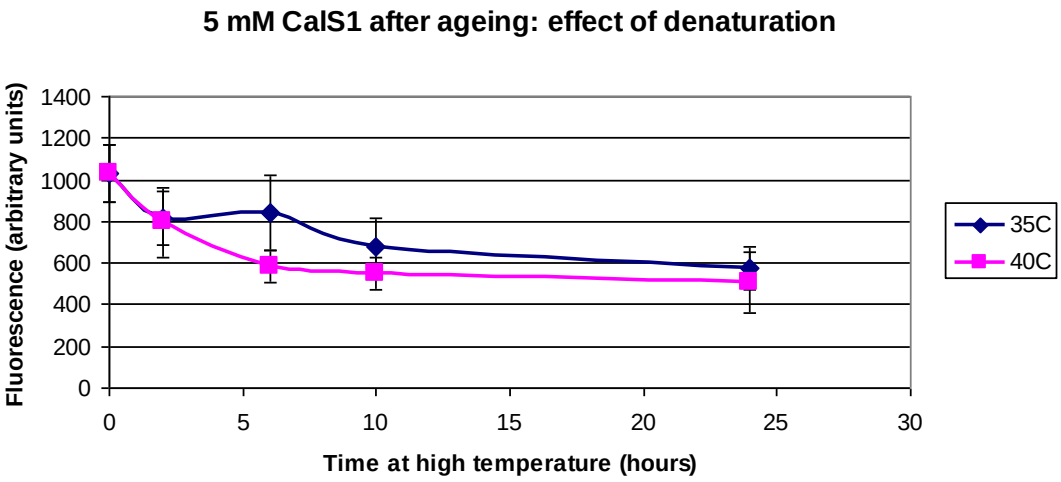


Figure 14: Effect of calpain activity exposed to denaturation

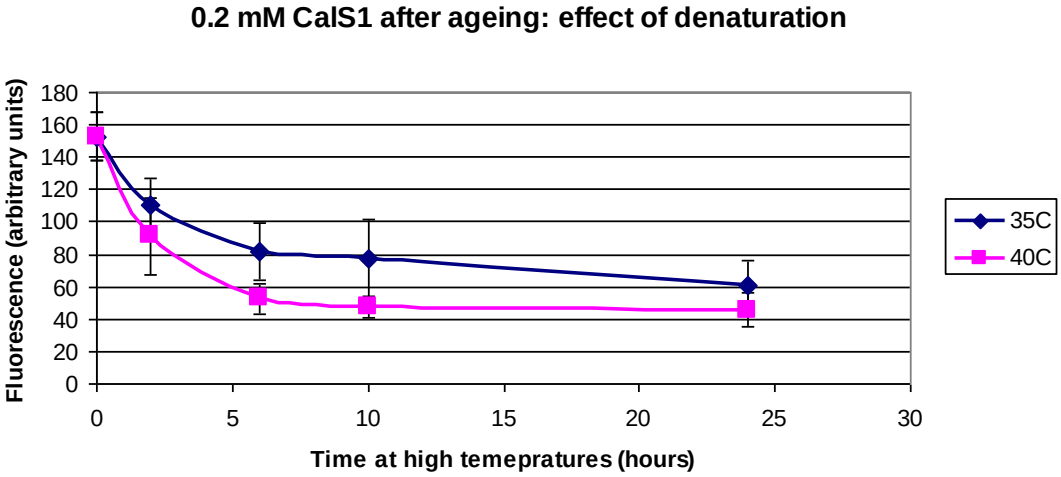


Figure 15: Effect of calpain activity exposed to denaturation

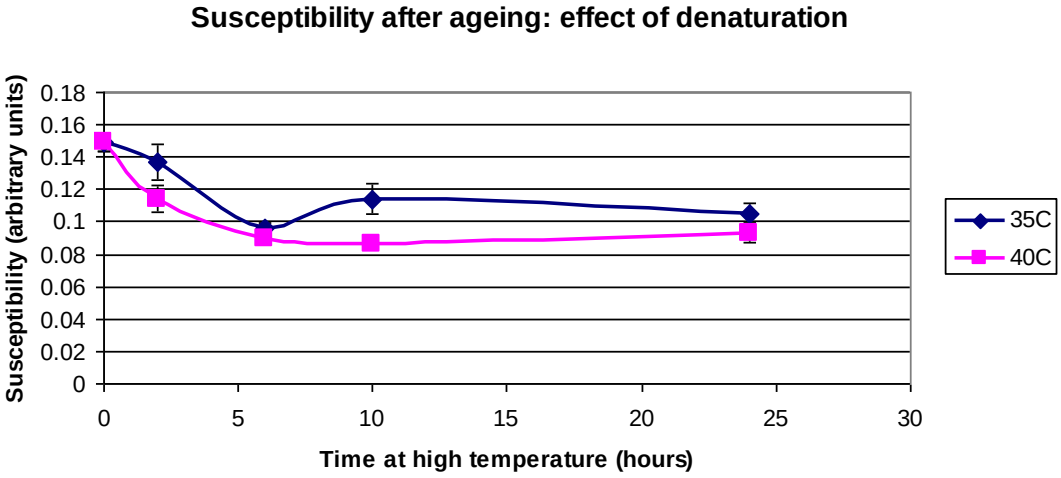


Figure 16: Effect of calpain activity exposed to denaturation

4.3.4 Water binding capacity.

A comparison was made of two separate methods for measuring water binding capacity of meat - the filter press method and the centrifuge method. Both show the characteristic of decreasing WBC during the ageing period, although a clear difference was more evident during the first 24 hours post rigor (Figures 17 & 18).

In general, both methods show a slight decrease in the initial WBC in proportion to the duration of high temperature denaturation. However, these effects were not large, particularly when comparing with the effects of denaturing conditions in the pre-rigor period, and are unlikely to contribute much to the tenderness effects (Figures 19 & 20).

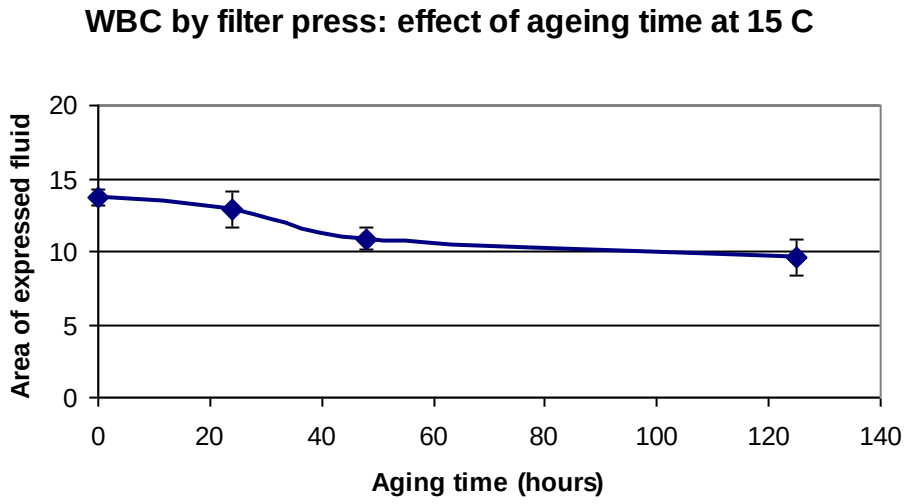


Figure 17: Effect of ageing time at 15'C on water binding capacity (by filter press).

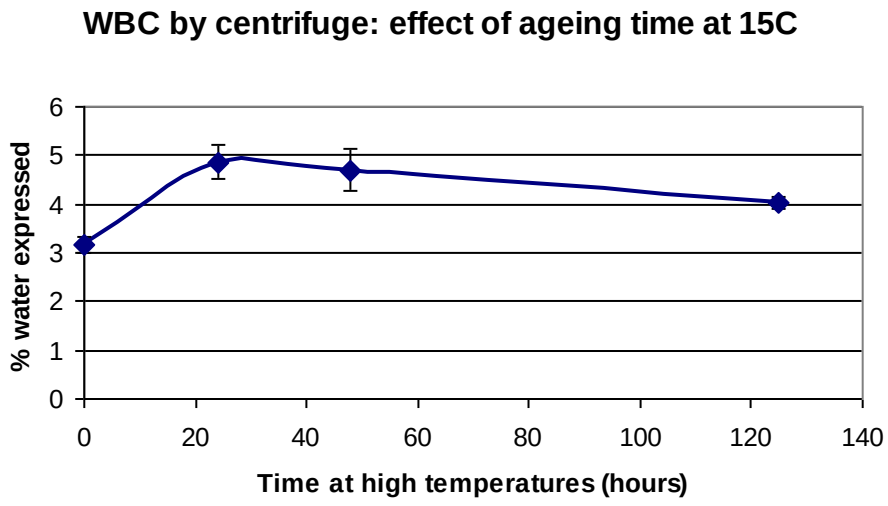


Figure 18: Effect of ageing time at 15'C on water binding capacity (by centrifuge).

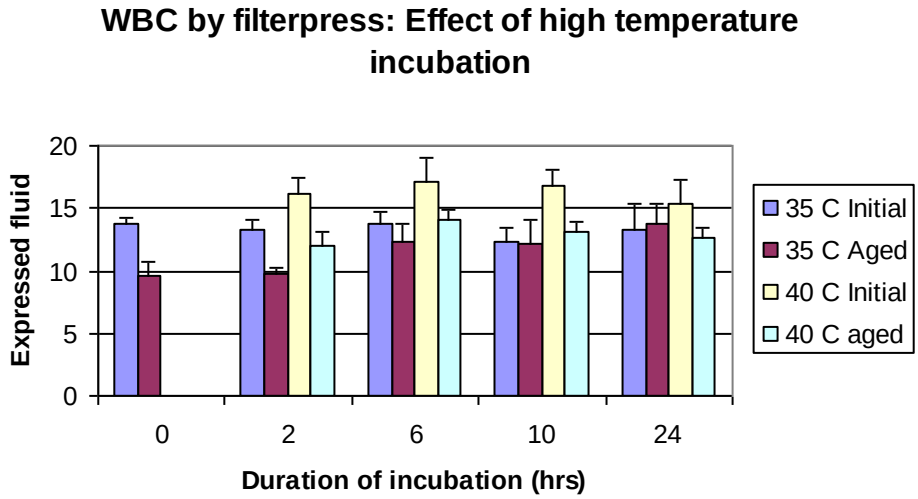


Figure 19: Effect of high temperature on water binding capacity (by filter press).

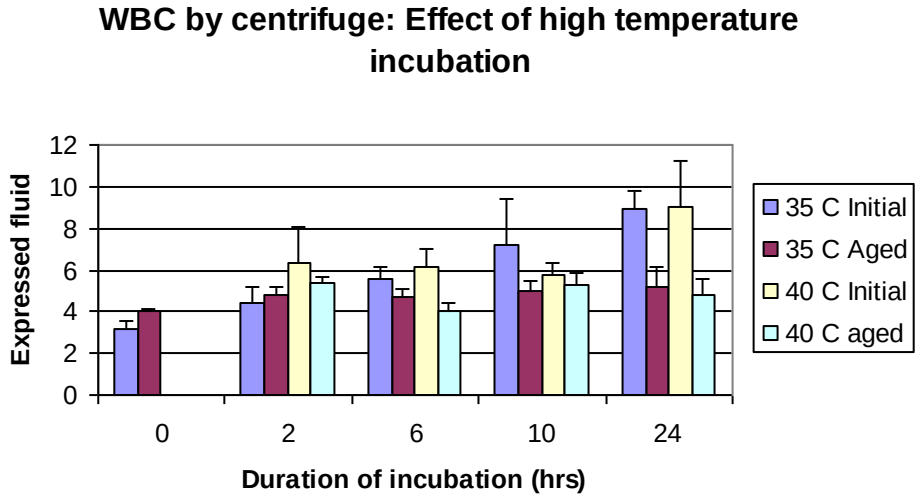


Figure 20: Effect of high temperature on water binding capacity (by centrifuge).

4.3.5 Total reducing capacity.

Total reducing activity measured using the MTT assay measures primarily mitochondrial reducing capacity associated with oxygen consumption and reductase enzymes, included metmyoblobin activity. The activity of these systems has been previously shown to be reduced by denaturing conditions and by post mortem ageing. The purpose of including this assay in these experiments was to assess an alternative enzyme system to compare with the changes to the calpain system.

As previously demonstrated, the MTT activity decreases with time post mortem in a temperature dependent manner (Figure 21). Consequently, 6 hours of exposure to high temperatures reduced MTT activity to a level comparable to 125 hours ageing at 15ºC (Figure 22).

Consistent with previous results, the response of the MTT activity to the high temperature denaturing treatments showed the normal time/temperature sensitivities.

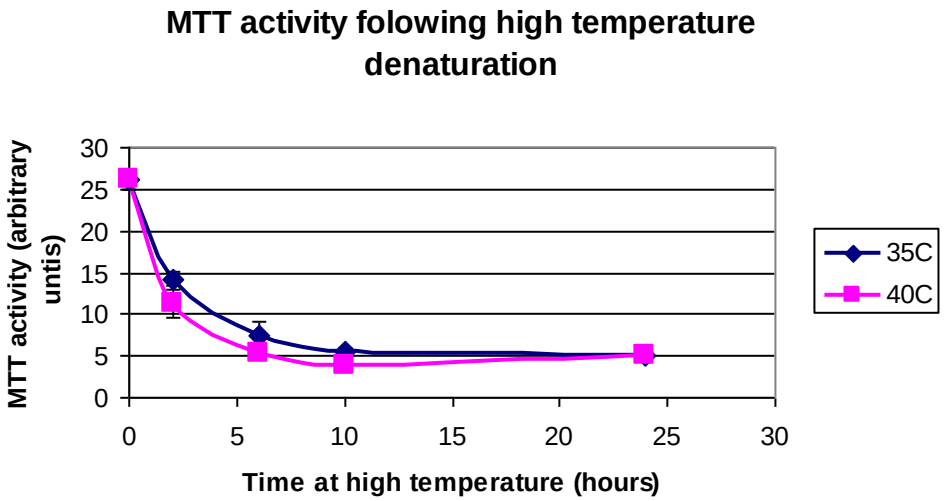


Figure 21: MTT activity following high temperature denaturation.

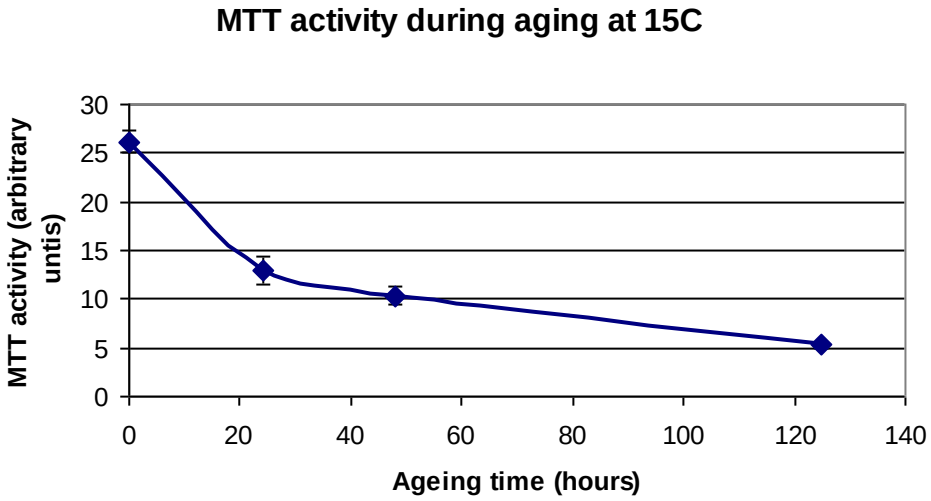


Figure 22: MTT activity during ageing at 15'C.

The key observation from these experiments is that sustained exposure (24 hours) to denaturing temperatures reversed the toughening effects on ultimate tenderness found after shorter exposures (6 hours). This result applied whether the samples were exposed to denaturing temperatures in the pre-rigor or exclusively post-rigor period.

We undertook the MTT assay to confirm that the samples had indeed undergone denaturation throughout the treatments and ensure that nothing anomalous was happening during the experiments. These measurements showed the expected increase in denaturation (loss of mitochondrial enzyme activity) in proportion to both the denaturing temperature and duration of exposure to the high temperatures.

Changes in WBC were also used to identify changes in the protein conformations that might help to understand the tenderness changes. Although some decrease in WBC was evident from exposure to denaturing conditions, these effects were not large and were largely absent after the ageing period.

The implication is that sustained exposure to the high temperature during the post-rigor period when proteolytic activity is high does not affect the tenderisation process. In contrast, a temperature transition, in this case from the denaturing temperatures to the 15ºC ageing temperature, while the tenderisation process was still largely incomplete produces the characteristic denaturation-related increase in ultimate tenderness.

The toughening associated with denaturation has been attributed to loss of proteolytic activity, possible because autolysis of the calpains may be faster than the rate of proteolysis, so that the total amount of proteolysis is reduced. This hypothesis is not supported by the results of the measured calpain activity: while these show a substantial reduction in activity, these effects occur within the first 2 hours and the subsequent calpain activity remains relatively unchanged.

An effect of time of exposure to denaturing temperatures was evident when calpain activity was measured after 5 days of ageing at 15ºC. However, the activity is less after 24 hours high temperature treatment and so does not contribute to explaining the reversal of the toughening effect.

The alternative explanation for the phenomenon is a substrate mediated effect. One mechanism may relate directly to denaturation of substrate proteins, and a consequence of this is a decrease in water binding capacity. We found that there is a decrease in WBC following the denaturation treatments, although these effects are relatively small compared with the effects of denaturing temperatures during the pre-rigor period. However, the loss of WBC becomes greater with time of exposure to denaturing temperatures and, once again, these measurements do not offer an explanation for the reversal of the toughening effect.

The calculated susceptibilities in aged samples show a gradual increase in susceptibility associated with increased high temperature exposure. At the same time, the total proteolytic activity is reduced. It is therefore not obvious how these measures help to explain the effect in question.

New components of the process model incorporating cold-shortening & contraction shown in the Appendix (Figure A2).

4.4 Further assessment of the effects of high temperature denaturation on the ageing rate and final tenderness of meat

Overall, the shear force values in this experiment were higher than normal, particularly for the ultimate tenderness values.

Considering first the samples maintained at 15ºC during the pre-rigor period, then transferred to denaturing temperatures for varying times, the final tenderness shows the same pattern described in an earlier milestone: 24 hours in denaturing conditions resulted in the same ultimate tenderness as avoiding denaturing conditions altogether, but 6 hours in denaturing conditions significantly increased final tenderness by 3 kgf ($p<0.01$). However, most of the ageing of the 24 hour treated samples actually took place during the period of high temperature treatment, as the shear force immediately after the treatment was essentially equivalent to the ultimate shear force.

Table 8: Effect of pre-rigor temperature on initial and final tenderness (measure by shear force)

Prerigor Temp	Time at 40°C	Initial tenderness	Final tenderness

15	0	17.3	7.1
15	6	12.6	10.2
15	24	7.7	7.0
40	0	14.9	8.5
40	6	14.1	9.6
40	24	7.7	6.3

The samples maintained at 40ºC throughout the pre-rigor period again showed the expected pattern of shear force changes: compared to the 15ºC samples, the shear force was lower at rigor mortis (14.9 vs 17.3) but, even in the absence of any post rigor high temperature treatment, the ultimate shear force was significantly increased from 7.1 to 8.6 kgf ($p<0.05$). However, a further 6 hours of high temperature treatment after rigor mortis produced a very similar final tenderness to that seen after the 15ºC pre-rigor equivalent (9.6 vs 10.2).

The critical observation is the effect of 24 hours at 40ºC after a pre-rigor exposure to the 40ºC temperature. The final tenderness was 6.3 kgf, compared with 7 kgf in the 15ºC controls. This clearly demonstrates that the same behaviour is evident in the pre-rigor period, irrespective of the pre-rigor temperatures. In spite of potential differences in muscle contracture and myosin denaturation, the meat showed an equivalent ability to reach ultimate tenderness if the high temperatures are maintained throughout.

5 Industry implications

The Meat quality model has a number of potential applications. In its simplest form, it can provide a decision support tool for the meat industry to help identify improved processing conditions. Beyond this, it can integrate with the CPMS framework to integrate on-line measurements and processing controls with meat quality outcomes. Last, the modelling framework provides a useful environment to identify research requirements and to test meat science principles. At this stage in the development of the model, a structure for most of the key meat quality considerations has been constructed.

6 Conclusion

This research had two primary objectives: the first was to continue the development of the pre-rigor model to accommodate the requirements of cold boning; the second was to develop the electrical stimulation component to meet the requirements of the CPMS. Wherever possible, data from other projects within the MQST program was incorporated to develop the models and used to validate aspects of the model as they were developed.

The findings of the research were :

- At this stage, a number of possible permutations of the immobilisation procedures have been evaluated to establish some general principles. Perhaps the most interesting results comes from the contribution of the support plates which, if uninsulated, provide a parallel pathway (not earthed) or additional electrode surface area (earthed). In the case of the former arrangement, the safety is not in any way improved but the immobilisation (Ec) is reduced; whereas, the in the second case, the safety is substantially improved with only a much smaller effect on immobilisation. Now that this simple model has been established, it can be used to assess additional scenarios as they develop. In addition, should the

requirement develop, it can be enhanced to better anatomical accuracy and provide more quantitative measures of either the carcass responses or the safety implications of different systems.

- Exposure to 40ºC temperature for as little as 2 hours immediately post rigor produced a dramatic reduction in ultimate tenderness, decreased WBC and decreased µ-calpain activity. The pre-rigor period is generally considered the critical determinant of meat quality, and it is claimed that this is due to the high internal muscle temperatures resulting in sarcomere shortening and toughness. These results show that the temperature in the immediate post rigor period may have a still greater effect, but a more detailed, quantitative measure of the post rigor temperature effects will be needed before this can be confirmed. If this proves to be the case, then the carcass temperature curve post rigor may need to be considered more carefully than has been the case so far.

- Sustained exposure to denaturing temperatures allowed a normal level of tenderness to develop, whereas a temperature transition during the tenderisation transition, from the denaturation temperature to an ageing temperature, cause an elevated ultimate tenderness. The mechanism for this effect is not clear at this stage. However, this observation could have significant commercial implications: the possibility that the effects of denaturing conditions can be modulated by the rate of change of temperature offer a way of managing rapid processing conditions to minimise adverse effects on tenderness.

- These results clearly show that pre-rigor exposure to denaturing conditions are not additive to changes that occur to tenderness in response to denaturation in the post rigor period. In particular, the ability of meat to reach normal tenderness if the high temperature is maintained throughout the ageing period is clearly evident. The implication of this work is that there are mechanisms affecting ultimate tenderness in meat which relate to the changes in temperatures during the aging period, rather than the absolute temperature. With further exploration of the time/temperature profile underlying this mechanism, the opportunity to produce accelerated tenderness while minimising the adverse effects of denaturing conditions/heat shortening on eating quality could be optimised.

7 Recommendations

Although often referred to as heat shortening, it is not yet understood if rigor contractions are truly responsible for the reduced eating quality. Contractures caused by low temperatures increase initial toughness whereas heat shortening results in lower initial toughness than controls. Also, the effects of high temperatures are also evident when temperatures remain high in the post rigor mortis period, when contractures have ended. We have demonstrated that calpain activity is significantly reduced when high temperatures are combined with low pH, and this effect is independent of the onset of rigor mortis. Accordingly, the current model structure considers only the rate of autolysis relative to proteolysis (an approach also used in the original Dransfield calpain model).

This concept will be validated using the calpain assay, and the possible need for an additional component to quantify the contribution of contracture will be assessed.

Appendix 1 – New components of the Process Model.

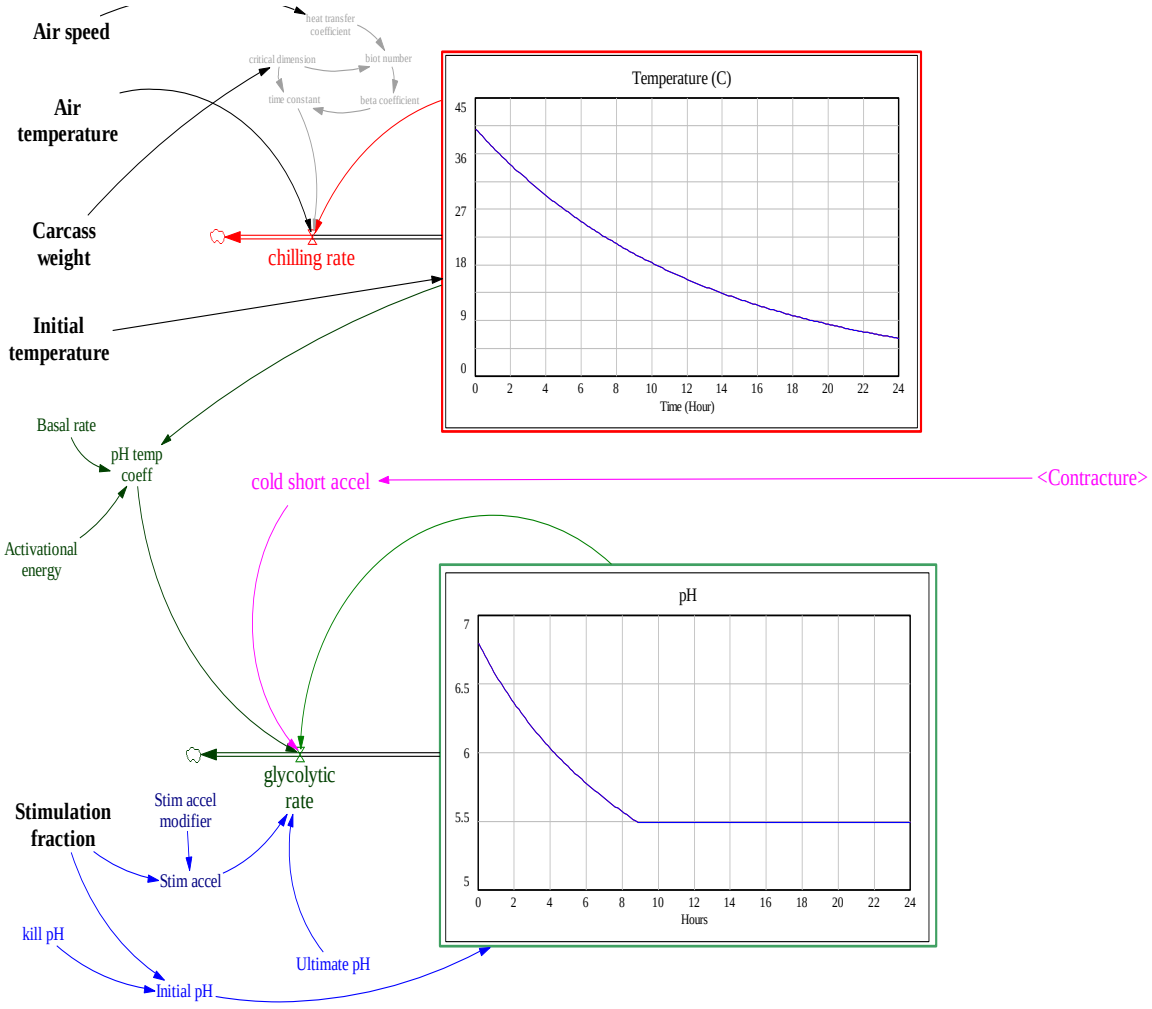


Figure A1 – New components of the Process Model incorporating cold-shortening & contraction.

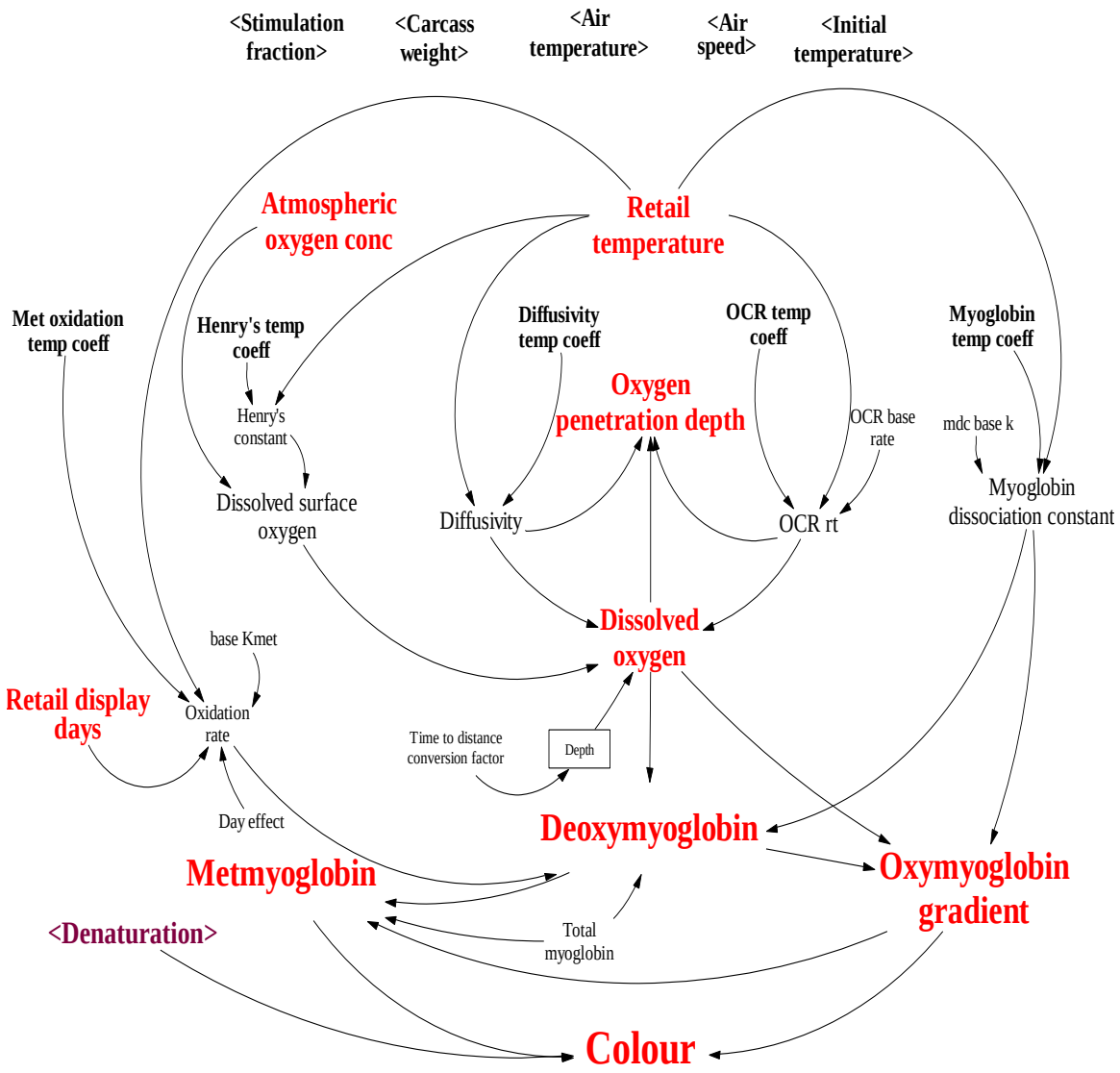


Figure A2 – New components of the Process Model incorporating meat denaturation & shortening.

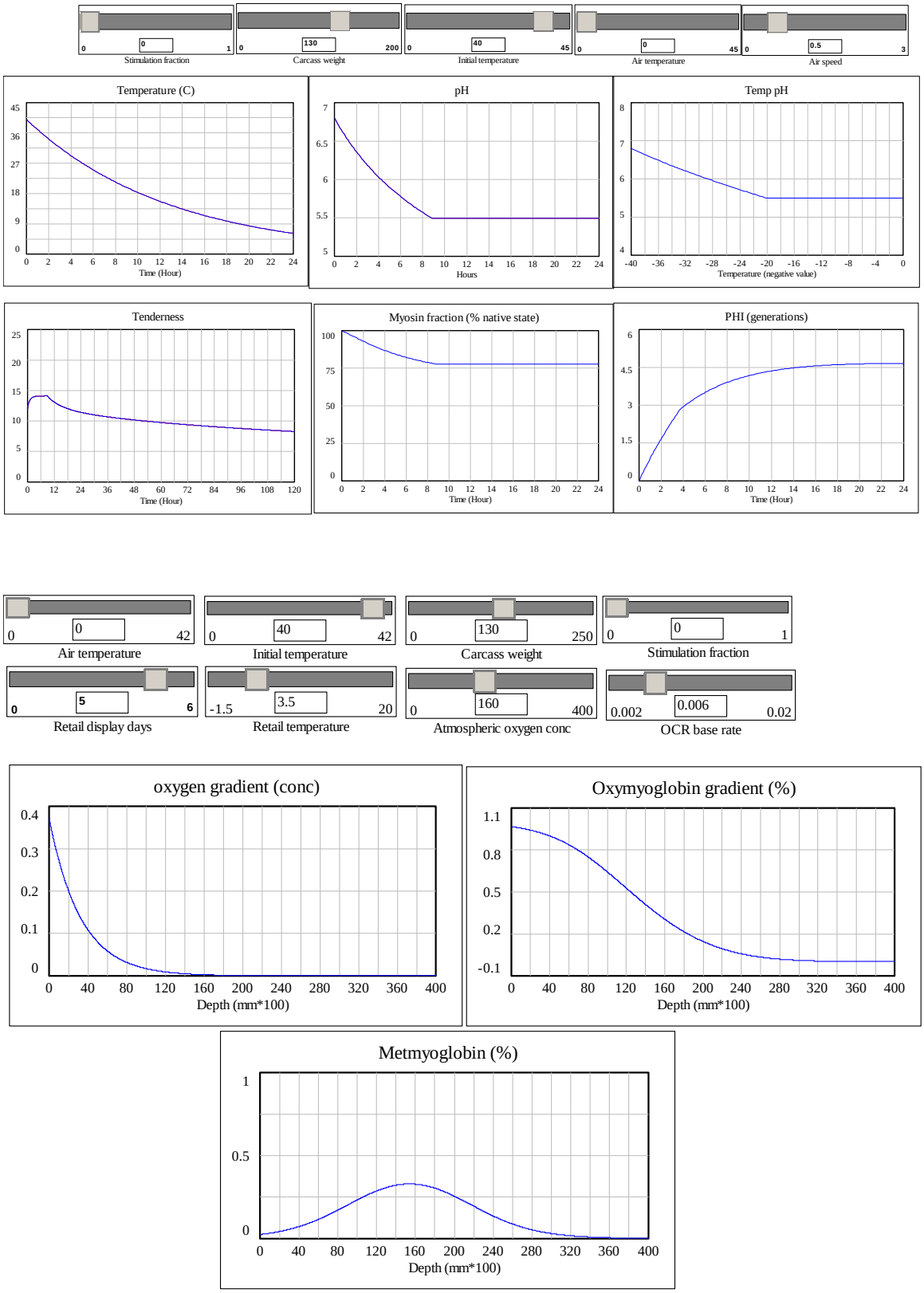


Figure A3 – Screen shot of fields available on Process Model incorporating meat denaturation & shortening.