final report

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VFC and HPP Tenderisation Mechanisms

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Executive summary

The Australian meat industry operates in a demanding and sophisticated market place. The consumer is becoming more knowledgeable, discerning and health-conscious and enjoys access to a greater range of food choices. One factor important to the consumer is consistent eating quality, and it is vital for the Australian red meat industry to continue to invest in this area to maintain a profitable and sustainable business. The adoption of new technologies is one pathway to achieve this which will enable the industry to remain at the cutting edge and remain competitive against alternative muscle food options (e.g. chicken, seafood).

Very fast chilling (VFC) is known to impart tenderness in hot-boned meat by reducing muscle temperature post-slaughter to less than 0°C within 2 hours post-mortem. Previous Meat and Livestock Australia (MLA) research focused on VFC has been based around two areas: (i) the efficiency of particular refrigeration technologies in attaining the appropriate temperature profile to achieve the tenderisation window, and (ii) the combined application of VFC and salt to achieve other qualitative improvements in minced products. More recently, accelerated tenderisation was reported when lamb loins were chilled to -3°C within 1 hour of slaughter (Jacob et al. 2012). The time and temperature at the end of the VFC period were concluded to be important for consistent tenderness. Little is known of the mechanisms behind this process and how it achieves positive changes in quality, particularly texture.

The potential of high pressure processing (HPP) as a technology to improve meat tenderness and increase cook yields in low-value meat cuts has previously been investigated by MLA and CSIRO has reported (A.MTP.0013, Sikes et al. 2010) on the effectiveness of applying high pressure combined with mild heat to improve tenderness and cook yields on post-rigor beef meat cuts of varying connective tissue content (neck muscle, topside, eye round, striploin). More recent project work (A.MPT.0032, A.MPT.0041) found that applying high pressure at low temperature to high connective tissue cuts (brisket) resulted in significant tenderisation. However, when these conditions were applied to lower connective tissue content cuts, such as topside and tenderloin, there was a reduced effect and no effect, respectively, on tenderisation. Similar to VFC, the mechanism behind this tenderisation, including variation between muscles, is not well understood.

This Final Report summarises the important issues relating to the use of low-temperature HPP (50 - 600 MPa at 0 - 8°C for 5 min) and VFC (-3°C within 1 hour post-slaughter) on post-rigor and pre-rigor meat, respectively, and outlines the impacts of these technologies on quality and the potential for improving the value of meat cuts by tenderisation.

Jacob, R., Rosenvold, K., North, M., Kemp, R., Warner, R. and Geesink, G. (2012) Rapid tenderisation of lamb M. longissimus with very fast chilling depends on rapidly achieving sub-zero temperatures. Meat Science 92:16-23.

Sikes, A.L., Tornberg, E. and Tume, R. (2010) A proposed mechanism of tenderising post-rigor beef using high pressure-heat treatment. Meat Science 84:390-399.

In this project, VFC treatment was successful for improving tenderness in both beef striploin and brisket muscle as shown by lower and similar shear force values, respectively, compared to the control treatment (conventional chilling). The method of VFC was important in producing an improvement in texture, as shown by the waterbath immersion chilling resulting in equivalent texture (brisket) or more tender (striploin) meat than the controls, whereas chilling using dry ice resulted in tougher meat in both muscles compared to the control treatment. These two methods differed in their rate of chilling. Therefore, the rate of chilling appears to be critical in achieving tenderisation using VFC. The effect of VFC was dependent on the muscle type, with a larger effect shown on the striploin muscle (low connective tissue content) compared to the brisket muscle (high connective tissue content) in terms of texture (lower shear force values in the striploin), pH decline (slower pH decline in the striploin) and water-holding capacity (lower purge and cook losses in the striploin). The results reported suggest that this tenderisation occurred independently of muscle sarcomere shortening (cold-shortening), and was also independent of denaturation or degradation of muscle proteins (proteolysis). The microscopy images indicate possible changes in muscle structure due to ice crystal formation during VFC. However, pH results indicate that the rate of pH decline and ultimate pH are significant modulators of tenderisation using VFC, and should be investigated further.

Freezing of muscle was shown to have no influence on the effect of high pressure treatment on the texture of beef muscle. No difference in texture was found between fresh and frozen beef cuts (brisket and outside flat) when pressure (50 - 600 MPa for 5 min) was applied at low temperature (4°C). Freezing muscle (brisket, topside) after high pressure treatment at both low (4°C, 400 MPa for 5 min) and high temperatures (60°C, 200 MPa for 20 min) did not affect the texture of the cooked product as measured by Warner-Bratzler shear force. Therefore, inconsistencies in texture results reported in previous projects could be due to structural, pH or fibre type differences between muscles, but not from the effects of freezing.

Low temperature-HPP conditions (50 - 600 MPa at 4°C for 5 min duration) were defined for an improvement in texture for brisket and outside flat muscles, and the amount of pressure required is muscle-dependent; a lower pressure necessary for outside flat (50 - 200 MPa) compared to brisket (200 - 300 MPa). The textural changes of the pressure-treated meat appear to be the result of the effect of treatment on the myofibrillar component of muscle, although muscle differences were apparent in respect to the effect of HPP on connective tissue. Thermal stability of extracted connective tissue was shown to be dependent on the sequence of extraction and pressure treatment. Even though a reduction in shear force value was measured in both muscles when optimum HPP conditions were applied, this improvement in tenderness would not be sufficient for consumer acceptability of the product. Pressurised muscles also had reduced water-holding capacity and increased cooking losses compared to untreated muscle. Therefore, there appears to be limited opportunity to add value to high connective tissue secondary cuts using low-temperature (4°C) HPP.

A combination of VFC and HPP applied to pre-rigor beef brisket muscle was successful in producing tender meat at 24 hours post-slaughter. VFC of pre-rigor brisket muscle using dry ice was followed by high pressure treatment, using a range of pressure and temperature combinations. The VFC only treatment produced tougher meat than the untreated controls. This was also found later in the project when two methods of VFC were compared, and

confirmed that VFC using dry ice was the least preferred method for rapid chilling. However, when high pressure was applied after VFC (dry ice chilling), a reduction in shear force values was observed when pressure was applied at 200 MPa at 30°C or 60°C and 600 MPa at 60°C. When pressure was applied at 600 MPa at 30°C, the meat was as tough as the control and VFC only brisket samples. The processes applied in combination resulted in brisket muscle that had a high ultimate pH which suggests that post-mortem glycolysis had been inhibited. This would be expected to influence water-holding capacity and therefore, the final product yield, but the cooking losses were similar to the untreated control. There appears to be potential in applying a combination of VFC and HPP to achieve tenderisation although no comparison of this treatment was made with HPP only of pre-rigor muscle. Therefore, the improvement in texture found under these conditions could have been due to HPP alone and that there was no additive effect due to VFC.

The research work reported in this final report outlines processes capable of adding value and possible new pathways for predicted eating quality beyond the current Meat Standards Australia (MSA) protocols through advanced processing, and therefore the opportunity to increase the profitability of the red meat industry. These value-added products meet consumer demands of consistent eating quality and convenience.

Contents

Project team

Project leader:	Robyn Warner / Anita Sikes
Collaborator:	Robin Jacob (DAFWA)
Project staff:	Janet Stark, Joanne Hughes, Neil McPhail, Sandra Crameri, Vicki Eggleston, Aarti Tobin

1 Background

Very fast chilling (VFC) and high pressure processing (HPP) are novel processing techniques that have been studied independently of one another. VFC is defined as reducing the temperature in muscle to sub-zero within 5 hours of stunning (Joseph, 1996) and interest in this technique initially focussed on reducing evaporative losses in beef (Bowater, 1997). HPP is commercially applied to meat at pressures usually 600 MPa or higher for several minutes, with the aim of reducing microbial growth and improving shelf life. VFC by definition is a pre-rigor process whilst HPP has until now been mainly done post-rigor. Both are applicable to sheep meat and beef although different advantages apply to the various species.

Initial interests in VFC and HPP have related to a range of different processing outcomes but subsequent investigations have established potential effects on meat tenderness for both techniques. For VFC, this represents a paradox in the sense that tender meat is achievable with temperature conditions pre-rigor expected to cause cold shortening and toughening. For HPP, the tenderising effects of HPP on post-rigor meat have been reported but the tenderisation varies substantially between studies, particularly where the conditions of application of HPP vary with time, temperature and pressure, as well as scale. Furthermore, recent results from a previously funded MLA project (A.MPT.0032) have suggested that tenderisation occurs when applying HPP at 400 MPa at ~4°C for 5 min, which has not previously been reported. The mechanisms for tenderisation associated with VFC and HPP at low temperature are therefore unknown and may fall outside of the range of the three different mechanisms normally associated with tenderness or toughening, being: sarcomere length; post-mortem proteolysis; and collagen cross-linking. As well as prevention of sarcomere shortening, another mechanism appears to be involved in the observed increase in tenderisation with VFC that is not explained by proteolysis.

The aim of this project was to elucidate biochemical and biophysical changes associated with tenderisation for VFC and HPP.

Bowater, F. (1997) Economies of meat chilling and freezing. In “Proceedings of the Institute of Refrigeration”, London. Pp. 1-11.
Joseph, R. (1996) Very fast chilling of beef and tenderness - a report from an EU concerted action. Meat Science 43, 217-227.

2 Project objectives

To design a process that will facilitate tenderisation within the first 24 hours post slaughter for both low and high value beef cuts.

The outcomes include:

1. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to VFC
2. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to HPP

The sequence of experiments in this project is outlined in the table below. Experiments relating to one processing technology were not conducted consecutively. However, for the purpose of this final report, experiments relating to each processing technology are presented together.

Milestone	***Activity***	***Experiment(s)***
1	Project commenced	
2	Literature review of methods	
3A	Processing effects of low temperature HPP	Effect of freezing after post-rigor HPP Defining HPP conditions at low temperature for 2 beef muscles
3B	Processing effects of low temperature HPP	Characterisation of beef muscle treated with low temperature HPP
4	Development of VFC system	Modelling of VFC system
5A	Refinement of the VFC system	Further modelling to achieve the target chilling rate
5B	Processing effects of VFC (Delayed VFC and VFC using waterbath)	Effects of VFC on the biophysical changes in pre-rigor LD beef muscle
6 and 7	Effects of a combination of VFC and HPP	VFC (dry ice) followed by HPP on the texture of beef muscle
8	VFC method comparison (dry ice vs waterbath)	Effects of two VFC methods on two pre-rigor beef muscles

The HPP experiments for Milestone 3 (A and B) were conducted on post-rigor beef muscle at low temperature.

This work was followed by the development and modelling of the VFC system using immersion of muscle samples in a waterbath (Milestones 4 and 5A) which subsequently led to the analysis of the effects of VFC on pre-rigor beef striploin (Milestone 5B).

The next scheduled experiment investigated the approach to applying a combination of VFC and HPP to achieve tender meat. Due to the logistics of conducting the VFC process at Teys using the immersion waterbath method (identified in Milestone 5B) and transporting the chilled samples to the Coopers Plains laboratory for HPP, a different method of VFC (dry ice) was trialled to hasten the chilling process (Milestones 6 and 7).

From the outcomes of Milestones 5B, and taking the alternative VFC method used for Milestones 6 and 7 into consideration, further VFC work involved a comparison of VFC methods (dry ice versus waterbath immersion) on two pre-rigor beef muscles.

3 HPP at low temperature of post-rigor beef muscle

3.1 Effect of freezing after HPP treatment on the texture of cooked meat

Inconsistent results of texture between muscles treated with HPP were previously reported in MLA-funded project work (A.MPT.0032, A.MPT.0041). One difference between these trials was whether HPP-treated muscles had been frozen prior to cooking. In the early stages of this project, an experiment was designed to investigate if freezing after HPP treatment impacted on the texture of the cooked product. Results presented showed that there was no effect of freezing beef muscles (brisket, *M. pectoralis profundus* or topside, *M. semitendinosus*) after HPP treatment at either low (4°C) or high temperature (60°C), on the texture of the cooked product. Therefore, inconsistencies between experiments in texture could be due to structural, pH or fibre type differences between muscles, but not sue to freezing.

Key Results - refer to Appendix - Milestone Report 3A

3.2 HPP conditions and choice of muscles

Discussions with MLA on the results of research conducted in a previous MLA-funded HPP project (A.MPT.0041) determined that the research focus in this project would be HPP (0.1 - 600 MPa) applied to post-rigor beef muscle at low temperature (-18 to 10°C) rather than at high temperature. As beef brisket (*M.* pectoralis profundus) had been used in previous trials with HPP at low temperature with positive results, it was decided to continue to use this muscle. It had been agreed that the HPP work would be conducted on 2 muscles, and as the potential industry benefits of this HPP process is adding value to low-value cuts, the outside flat muscle, *M. biceps femoris* was chosen, as it is low-value, easily accessible and large enough for many subsamples.

3.3 HPP at low temperature of frozen meat

Initially, to narrow down the temperature range (-18 to 10°C) of the meat for HPP, the effect of pressure treatment (0.1 - 600 MPa at 4°C for 5 min) on fresh (4°C) or frozen (-18°C) brisket and outside flat muscle was investigated and the texture after cooking was measured by the Warner-Bratzler shear force method. Results showed that there was no difference in the texture of brisket and outside flat muscle when pressure was applied to either fresh or frozen muscle.

Key Results - refer to Appendix - Milestone Report 3A

3.4 HPP at low temperature - defining optimum conditions

Subsequent to the trials conducted on frozen muscle, pressure (0.1 - 600 MPa) was applied to brisket and outside flat muscle at temperatures between 0 and 8°C and the texture of cooked muscle was determined. For both muscles, the main effect impacting texture was the pressure applied, irrespective of the temperature of processing. Higher pressures (500 and 600 MPa) applied to brisket and outside flat muscles resulted in toughening of meat after cooking. The optimum pressure to achieve an improvement in tenderness (approximately, a 10 Newton (N) reduction) was muscle-dependent: 200 to 300 MPa for brisket and lower pressure, 50 - 200 MPa for outside flat muscle.

Key Results - refer to Appendix - Milestone Report 3A

3.5 Characterisation of beef muscle treated with HPP at low temperature

Using the optimum HPP conditions identified to achieve tenderisation in each muscle, pressure was applied to brisket (300 MP) and outside flat (50 MPa) muscles at 4°C for 5 min and samples were analysed to determine the effect on biophysical characteristics. In addition to measurements on whole muscle, muscle protein fractions (sarcoplasmic, myofibrillar, connective tissue) were extracted from raw muscle and pressure-treated as individual fractions, as well as from muscle that had been pressure-treated.

The peak force values for both the brisket and the outside flat muscles were reduced to ~60 N when high pressure was applied at the optimum pressure conditions for each muscle. The peak force value was significantly lower than the untreated muscle samples; however, the improvement in tenderness would not be substantial enough for consumer acceptability of the product in terms of Warner-Bratzler shear force (~40 N). There was no effect of pressure on the sarcomere lengths and the textural changes of the pressurised meat appeared to derive from the myofibrillar component of the muscle.

Pressurised muscles had reduced water-holding capacity and increased cooking losses compared to untreated muscles, indicating denaturation of proteins. This denaturation was confirmed by thermal stability studies, which showed that pressure treatment partially denatured myosin and totally denatured actin. A change in the properties of muscle proteins, such as myosin and actin, will subsequently affect the overall structure, impacting on the functionality of the muscle. The connective tissue appeared to be largely unaffected by pressure treatment, as assessed by thermal stability studies, although differences in the effect of HPP on connective tissue were apparent between the two muscles, as seen from the force deformation curves.

Examination of muscle fibre lengths showed that pressure treatment at low temperature resulted in shorter muscle lengths, with the cut ends having a very distinct, ‘sharp’ appearance compared to the untreated muscle. This fracturing pattern is similar to that seen when combined pressure and heat was applied to beef muscle (A.MPT.0013).

Thermal stability measurements of the extracted connective tissue from the brisket muscle indicate the effect of high pressure is dependent on when the connective tissue is extracted from the muscle. An effect of pressure treatment was only evident when the connective tissue was extracted from pressure-treated muscle.

Key Results - refer to Appendix - Milestone Report 3B

Conclusions:

Freezing of beef muscle (brisket, topside) after pressure treatment at low (4°C, 400 MPa for 5 min) and high temperature (60°C, 200 MPa for 20 min) had no effect on the texture of the cooked product as measured by Warner-Bratzler shear force. Similarly, there was no difference in texture between fresh and frozen beef muscle (brisket, outside flat) when pressure treatment (50 - 600 MPa for 5 min) was applied at low temperature (4°C).

Optimum HPP conditions (pressure, temperature,) were defined for improvement in texture for brisket and outside flat muscles, and the amount of pressure required was

muscle-dependent. Even though a reduction in shear force value was measured in both muscles when optimum HPP conditions were applied, this improvement in tenderness would not be sufficient for consumer acceptability of the product. Pressurised muscles also had reduced water-holding capacity and increased cooking losses compared to untreated muscle. Therefore, the process tested (low-temperature HPP) does not appear to provide an improved quality meat product from low-value cuts and thus does not provide benefits for the meat industry.

The textural changes of the pressure-treated meat appear to be the result of the effect of treatment on the myofibrillar component of muscle, although muscle differences were apparent in respect to the effect of HPP on connective tissue. Thermal stability of extracted connective tissue was shown to be dependent on the sequence of extraction and pressure treatment.

4 VFC for accelerated tenderisation of beef muscle

Previous project work funded by MLA (P.PSH.0326) reported that accelerated tenderisation occurred when lamb loins were chilled to -3°C within 1.5 hour of slaughter. The time and temperature at the end of the chilling period were concluded to be important for consistent tenderness and it was suggested that lamb loins of 40 mm thickness needed to be cooled to less than 0°C at 1.5 hours post-mortem (Jacob et al., 2012). Therefore, the target for chilling beef muscles in this work was -3°C within 1 hour of obtaining muscle.

4.1 Development of VFC system

Two Julabo water baths were purchased, commissioned and calibrated. Initially, computational fluid dynamics (CFD) was used to determine critical process parameters of the VFC process to achieve a target temperature of -3°C in the muscle core after approximately 1 h. From the different models, it was found that in a 40 % propylene glycol / water mixture, the target temperature could be achieved in 1 to 1.5 hours when the fluid temperature was in the order of -14 to -20°C and the flow rate between 0.5 and 3 m/s.

Key Results - refer to Appendix - Milestone Report 3A and Milestone Report 4

The CFD modelling results were validated on beef muscle portions (75 x 90 x 180 mm, w x h x l) and found that a target temperature of -3°C within 1 hour for beef muscle of approximately 80 mm thickness was possible. Initial lab trials using the information from this model used pre-rigor beef neck muscle (*M. sternomandibularis*) and thick skirt (the lumbar portion of the diaphragm), due to the accessibility and availability from a cold-boning process, rather than being the ideal choice from an industrial perspective. However, inconsistent sample size and shape of these muscles led to difficulties in achieving the chilling rate of -3°C in an hour.

Therefore, refinement of the VFC system originally developed was required to achieve the targeted chilling rate. A software program developed by MIRINZ, Food Product Modeller, was used to develop a cooling model that allowed simulation of the cooling process to enable prediction of cooling times for muscle samples of different sizes, initial temperatures and cooling medium temperatures to those tested. Samples dimensions, the location of the temperature recorders in the chilled muscle, and the fluid temperature were entered into the software program, with this model predicting the cooling rate at the thermocouple location with reasonable accuracy. The actual data and predicted cooling rates from the model were a similar fit and showed clearly that chilling beef striploin muscle (55 - 66 mm thick) to -3°C within 1 hour from an initial temperature of approximately 30°C was difficult to achieve. The Food Product Modeller software was then used to predict that a maximum sample thickness of 45 mm would allow a core temperature of -3°C to be achieved within 1 hour when the initial meat temperature was 30°C and the glycol bath temperature set to -20°C.

Key Results - refer to Appendix - Milestone Report 5A

Jacob, R. et al. (2012) Rapid tenderisation of lamb M. longissimus with very fast chilling depends on rapidly achieving sub-zero temperatures. Meat Science 92:16-23.

4.2 VFC effects on metabolism, structure and biophysical changes

Using the results from the VFC lab trials described above, a trial was conducted at Teys Australia, Beenleigh to rapidly chill beef striploin muscle using the VFC water bath system. To gain some insight into the events that lead to tenderisation due to VFC, an additional treatment was designed into this trial to separate the prevention of cold shortening from other possible unknown mechanism(s). This was achieved by including a treatment to delay VFC through holding the pre-rigor muscle at 15°C for 2 hours (delayed VFC).

The VFC treatment was successful in accelerating tenderisation, with shear values being lower than the control and delayed VFC samples after 2 days of ageing. This confirmed findings from previous studies that the timing of VFC is critical and needs to occur within a few hours of slaughter. There was no difference in sarcomere lengths between treatments which provided an opportunity to investigate the unknown mechanism, independent of sarcomere length.

The VFC treatments resulted in a slower pH decline and a higher ultimate pH at 24 hours than the conventionally chilled treatment. This high pH existed after 2 and 5 days ageing but after 14 days ageing, there was no difference in pH between treatments. The slower pH decline and high pH at early ageing times would be expected to impact on the ability of the muscle to retain water and this was reflected in the lower purge and cooking losses for the VFC treatments compared to the control treatment.

Results suggest that the tenderisation is independent of denaturation of muscle proteins as measured by thermal analysis (differential scanning calorimetry, DSC). There was also no difference in protein solubility and no evidence of degradation of major muscle proteins (e.g. myosin, actin) or sarcoplasmic proteins with either fast chilling treatment compared to the control sample.

Visualisation of the muscle samples using microscopic methods (light microscopy and transmission electron microscopy, TEM) showed that VFC treatments have some effect on muscle structure, with fracturing and fragmentation of muscle fibres evident in fast chilled muscle. There appeared to be more fracturing in the VFC samples compared to the delayed VFC samples.

The VFC treatments had an effect of the surface colour of the striploin muscle. The control samples were lighter and redder in colour than both the VFC treatments. The difference in redness suggests an increased oxygen consumption rate in the VFC treated samples.

Key Results - refer to Appendix - Milestone Report 5B

4.3 Comparison of the effects of two VFC methods on low and high connective tissue beef muscles

Discussions between MLA and the project team defined the direction of the experimental work for the last stage of this project. It was agreed to further investigate the mechanism of tenderisation of beef muscle under VFC conditions. It was decided to focus the investigation on the possibility of either the prevention of cold-shortening or the modification of metabolism being the mechanism.

VFC was achieved using the two methods previously developed - dry ice chilling and waterbath immersion chilling. Chilling conditions for each method were determined based on the criteria that the centre of the muscle reached the middle of the freezing plateau. That is, the aim was to reach a target temperature (approximately -1°C) in the centre of the muscle rather than a chilling rate. Time of chilling for striploin portions was 35 min using the waterbath immersion method and 50 min using dry ice. As the brisket samples were smaller, chilling took less time than the striploin samples; 25 min for immersion chilling and 30 min in dry ice. Therefore, the two chilling methods in effect provided two different chilling rates, and as the muscle portions were different sizes (thicknesses) for each muscle, the rates were also different between muscles.

Chilling of pre-rigor striploin (low connective tissue content) and brisket (high connective tissue content) muscles were successfully conducted at Teys Australia, Beenleigh, using two chilling methods. The waterbath immersion VFC treatment was successful for both striploin and brisket muscles, as the shear force values were similar (brisket) or lower (striploin) than the (untreated) controls. However, VFC of both muscles using dry ice produced tougher meat than control samples. This suggests that the rate of chilling is critical for improved texture of very fast chilled muscle.

Cold-shortening did not occur with either VFC method as there was no difference in sarcomere lengths between treatments for both muscles. The rate of pH decline was muscle-dependent, with a slower decline in striploin muscle and a faster decline in brisket muscle compared to control samples.

Key Results - refer to Appendix - Milestone Report 8

Conclusions:

Overall, it was shown that there was success in reproducing a tenderising VFC effect in hot-boned beef striploin and confirmed that the timing of VFC is critical. Tenderisation occurred independently of cold-shortening, denaturation or degradation of muscle proteins. VFC was accompanied by a slower pH decline, a higher ultimate pH and lower purge and cooking losses compared to conventionally chilled striploin muscle. However, some fragmentation and fracturing of the muscle fibres was evident when visualised by microscopic methods.

The investigation of two different chilling methods, dry ice chilling and waterbath immersion chilling, showed that the rate of chilling is critical to achieve meat with similar or improved texture compared to control muscle. VFC by immersion in 45% propylene glycol resulted in muscle with an improved (striploin) or similar (brisket) texture to control samples. VFC using dry ice resulted in tougher meat than the control samples for both muscles. Muscle differences were apparent in the effect of VFC on texture, cooking loss and metabolism (as measured by pH decline). In the absence of cold-shortening, the rate of pH decline appears to be a significant modulator of tenderisation using VFC.

5 Combined VFC and HPP on the quality of beef brisket

As outlined in Section 2, the trial combining VFC and HPP was conducted before the second VFC only trial comparing the effects of two VFC methods (Section 4.3). Separate investigations were conducted on the effects of VFC and HPP on the texture of meat. It was agreed to evaluate the potential of a 'novel' approach of combining the two processing technologies and determine the impact that pressure and temperature changes have at the sarcomere and cellular level of pre-rigor red meat. Therefore, pre-rigor beef muscle was subjected to VFC followed by HPP to determine the potential for achieving tender meat within 24 hours post-slaughter.

In an initial trial, it was determined that dry ice (snow) could be effectively used for chilling pre-rigor meat samples. Samples achieved sub-zero temperatures within 18 minutes, indicating a simple and cost-effective method for very fast chilling of meats.

A subsequent trial investigated the effect of applying a combination of VFC and HPP on pre-rigor brisket muscle (high connective tissue content, low-value cut). Under the conditions used, VFC without HPP resulted in very tough meat, similar to the control samples. However, when high pressure (200 MPa/30°C, 200 MPa/60°C or 600 MPa/60°C) was applied to very fast chilled muscle, a reduction in peak shear force was measured. Subjecting brisket samples to VFC and HPP at 600 MPa/30°C resulted in tough meat, similar to the VFC only and control samples.

The combination of VFC and HPP resulted in meat with a high ultimate pH which suggests that post-mortem glycolysis was inhibited. This would be expected to positively impact the water-holding capacity, however, the cook losses of the treated samples were similar to the controls.

Key Results - refer to Appendix - Milestone Report 6 and Milestone Report 7

Conclusions:

A combination of VFC and HPP applied to beef brisket muscle was successful in producing tender meat at 24 hours post-slaughter.

6 Recommendations

6.1 High pressure processing

As low-temperature HPP (at 4°C) appears to have limited commercial potential for producing an improved eating quality product, the following recommendations consider the pressure-heat application for tenderisation of low-value meat cuts:

- Evaluate the need for optimisation of conditions for quality improvement (texture, water-holding capacity) for different muscles. This would be necessary if a cost-benefit analysis was required.
- Investigate the safety (microbiological), stability (chemical) and shelf-life (microbiological, rancidity, packaging) of processed meat products.
- Assess consumer acceptance of high pressure-treated product by conducting consumer sensory analysis.
- Investigate and find solutions to commercial scale-up problems:
    - larger quantities of meat product in a batch process,
    - kinetics of heating during HPP,
    - development of an insulated sample carrier.
- Investigate the structure-function-stability relationships of connective tissue with combined HPP-heat application.
- Investigate the pressure-induced changes in microstructure and water distribution, which impacts yield, in raw and cooked beef.

6.2 Very fast chilling

- Further investigation of the effect of different chilling rates on individual muscles on metabolism and texture of very fast chilled muscle.
- Investigate the importance of the rate of pH decline on resultant texture of very fast chilled muscle from the analysis of metabolomic data.
- Investigate how muscle differences (e.g. different connective tissue content, fibre type) influence the effects of VFC (texture, pH, water-holding capacity).

6.3 Combination of VFC and HPP

- Re-assess tenderisation of pre-rigor muscle using a combination of VFC and HPP, using the waterbath immersion method for chilling rather than the dry ice method. Also, the inclusion of a HPP only treatment on pre-rigor muscle into the experimental design would be beneficial in determining the effect of the two technologies used in combination.

7 APPENDIX

Milestone Report 2: Method development and evaluation

MILESTONE REPORT

MLA project code:	**A.MQT.0053**
MLA project title:	**VFC and HPP tenderisation mechanisms**
Project leader:	**Robyn Warner / Anita Sikes**
MLA project manager/coordinator:	**Phil Franks**
Milestone number:	**2**
Date:	**31 January 2012**

Milestone

Report on methods to be used to assess processing effects (VFC, HPP) on muscle. Review of literature and a description of methods developed and evaluated for texture, structure, metabolism, osmotic effects, protein denaturation and degradation. Report on the confirmed HPP conditions for tenderness at low and high temperature.

Abstract

High pressure processing (HPP) and very fast chilling (VFC) are novel processing techniques that have gained recent industry interest. These initial interests have related to a range of different processing outcomes but subsequent investigations have established potential benefits for meat tenderness for both technologies. The use of these technologies on pre- and post-rigor meat has the potential to reduce processing costs for the industry (e.g. reduced chilling costs) and improve the eating quality of a consistent quality product which will assist the meat industry in improving profitability and sustainability. Many of the methods to be used for assessing processing effects (HPP, VFC) on muscle have been described in this progress report. Well-established methods have been evaluated and where necessary, development or modification of methods is underway and on-track. Preliminary results of some techniques have been reported.

Project objectives

To design a process that will facilitate tenderisation within the first 24 hours post-slaughter for both low- and high-value beef cuts.

Outcomes:
1. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to VFC
2. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to HPP

Success in achieving milestone

This milestone was successfully achieved. Progress on development and evaluation of methods that will be used to assess processing effects (VFC, HPP) on muscle are given below. Details of the different physico-chemical attributes that will be measured are also described. Several methods for analysis of meat are well established, and are referenced appropriately below.

Development and evaluation of methods that will be used to assess processing effects (VFC, HPP) on muscle

Texture measurement

Meat is a combination of myofibre and connective tissue structures which when chewed, gives us an indication of the tenderness (or toughness) of the meat. Tenderness can be defined by a consumer as the ease with which the meat is masticated (chewed). Mastication involves the ability of the meat to be sheared, compressed and ground during consumption. The problem with this is the texture of meat cannot be analysed by a single mechanical parameter because of its multidimensional sensory characteristics. Mastication involves load, strain and strain rate, all of which are not covered by one objective assessment.

In the fields of meat research, quality control and processing, the most widely used (used by 78% of researchers; Lepetit & Culioli, 1990) mechanical method is the Warner-Bratzler test, developed by Warner (1928). This test remains the main reference with an 80% satisfaction rate (Lepetit & Culioli, 1990). It is used in conjunction with sensory and consumer panels to determine acceptability grading for meat. Other methods used to describe mechanical properties of meat are compression tests (Stanley *et al.,* 1971) and tensile tests (Bouton & Harris, 1972c), penetrometry and multi-blade shearing (Kramer, 1951) and bite tests (Volodkevich, 1938) (36%, 27%, 25%, 16% and 10% of researchers use these methods respectively; Lepetit & Culioli, 1990). Most tests for meat tenderness have been well established, and only slight modifications have been made to these in regards to sample size, blade dimensions and the rate the test is performed. Each method has its advantages and disadvantages, with no single method providing a complete tenderness profile.

The mechanical methods that will be developed and evaluated for this project, help describe the texture of meat. The results of the tests used in combination, give us a better understanding of the mechanics of meat tenderness.

Warner-Bratzler (WB) shear force

Warner-Bratzler shear measurements involve a combination of tensile, compressive and shear stresses which provide greater interpretational difficulties than other measurements. The orientation of the strains applied in relation to the myofibres influences the shear force values. When the shearing plane is perpendicular to the muscle fibres, this configuration in cooked meat is closely related to the myofibrillar component of the meat.

The method uses a straight edged shear blade with a rectangular shaped sample so that the length of the blade in contact with the sample is independent of shear force values. This provides a deformation curve which presents an initial yield (IY) and a peak force (PF). The initial yield can be an indication of myofibre resistance; in some instances this may not be apparent on the deformation curve. The peak force can express either myofibrillar or connective tissue resistance depending on how the meat is cooked and on the contracted state of the muscle as measured by the sarcomere length. The difference between PF and IY (PF-IY) can indicate the resistance of the connective tissue.

The tenderness or toughness of meat samples was determined by using a modification of the Warner-Bratzler shear device (Bratzler, 1932) and a Lloyd Instruments LRX materials testing machine fitted with a 500N load cell (Lloyd Instruments Ltd., Hampshire, UK). Samples used in the Warner-Bratzler device had a rectangular cross-section 15mm x 6.7mm (1cm² cross-sectional area), and were cut with the fibre orientation parallel to the long axis, and at right angles to the knife blade of the device. The force required to shear through the clamped samples with a triangulated 0.64mm thick blade pulled upward at a speed of 100mm/min was measured. Data was collected using the Nexygen 4.5 software (Lloyd Instruments Ltd., Hampshire, UK); the parameters measured from the shear force deformation curves were peak force (PF), initial yield (IY), and peak force minus initial yield (PF-IY). Six determinations were made on each sample and the mean recorded. All analysis was performed at room temperature.

Results from a previous MLA project (A.MTP.0013) showed a reduction in Warner-Bratzler shear force values (20-40%) of pressure-heat treated samples, which give a good indication of improved tenderness.

Texture profile analysis (TPA)

When a sample is stressed in one direction, it deforms not only in the direction of the applied strain, but also in two other directions at right angles to the applied strain, called free strain directions,. This makes analysing the mechanical behaviour of muscle complex. Depending on the strain direction occurring in the sample, different mechanical properties are involved. Texture profile analysis (TPA) was developed by (Szczesniak and Torgeson, 1963), and modified by (Szczesniak, 1968) to a multi-point system where many parameters could be identified and quantified. A high correlation between chewiness as measured by compression and the sensory attribute of tenderness was demonstrated by (Bouton *et al.,* 1975).

Samples were analysed using a Lloyd Instruments LRX materials testing machine fitted with a 500N load cell (Lloyd Instruments Ltd., Hampshire, UK). A 6.3mm diameter flat-ended plunger was driven vertically 80% of the way into a 10mm ± 1mm

thick sample of meat. The samples were cut and presented so that the fibres were perpendicular to the direction of the plunger penetration. The plunger was driven twice into the same location at 50mm/min, and the mean of six determinations recorded. Data was collected using the Nexygen 4.5 software (Lloyd Instruments Ltd., Hampshire, UK); the parameters measured from the work and force deformation curves were hardness, cohesiveness, springiness, gumminess, chewiness, fracture force and adhesiveness. All analysis was performed at room temperature.

Results from a previous MLA project (A.MTP.0013) showed that Texture profile analysis also supported an improvement of hardness, gumminess and chewiness, when comparing the control to the treated samples.

Tensile tests

Tensile tests are better suited for structural investigations (Purslow, 1985) rather than for sensory evaluation of tenderness. Tensile tests give information concerning the changes in the relative strengths of the fibres and of the connective tissue holding the fibres together. Stress applied along the muscle fibres in a tensile test, are 10-fold higher than those measured across the fibres, which indicate a contribution of myofibres to the strain.

Samples were analysed using a Lloyd Instruments LRX materials testing machine fitted with a 500N load cell (Lloyd Instruments Ltd., Hampshire, UK), and two pneumatic operated jaws set 25mm apart. The samples were cut initially the same as the Warner-Bratzler rectangular shape 1.5mm x 0.67mm (1cm^2 cross-sectional area), with the fibre orientation parallel to the long axis. To prevent sample breakage at the jaws, the samples were then cut into dumbbell shapes by cutting around a template; the cross-sectional area in the centre of the sample was 0.44 (±10%) cm^2. The jaws moved apart at 100mm/min until a break occurred. Data was collected using the Nexygen 4.5 software (Lloyd Instruments Ltd., Hampshire, UK); the parameters measured from the force deformation curves were peak force (PF), total energy to fracture, breaking strain and extension of peak force. Large amounts of connective tissue in the samples tested caused high variability, so a minimum of eight determinations were tested with the mean recorded. All analysis was performed at room temperature.

When measuring the raw samples, there was a problem with slippage of the samples from the clamps during the application of the stress along the fibres. Various methods were tested to prevent the slippage; increasing the air pressure to the jaws and using an abrasive medium between the jaws and the sample, all of which did not give a good success rate. A better method will hopefully be developed after consultation with a visiting scientist (Peter Purslow) in February, on technical problems encountered.

Adhesion tests

In tensile tests across the myofibres the primary event seen is the opening up of cavities between fibre bundles involving the perimysial-endomysial junction (Purslow, 1985). Secondly, the cavities join up and finally the perimysial strands rupture. The breaking strength is determined by the amount and resistance of the perimysium. So this is a good measure of the connective tissue strength between the fibre bundles. Samples were analysed using a Lloyd Instruments LRX materials testing machine fitted with a 500N load cell (Lloyd Instruments Ltd., Hampshire, UK), and two

pneumatic operated jaws set 20mm apart. The samples were cut the same as the Warner-Bratzler rectangular shape 1.5mm x 0.67mm (1cm² cross-sectional area), with the fibre orientation perpendicular to the long axis. Sample breakage at the jaws occurred infrequently so the rectangular shape was used for the measurements. The jaws moved apart at 100mm/min until a break occurred. Data was collected using the Nexygen 4.5 software (Lloyd Instruments Ltd., Hampshire, UK); the parameters measured from the force deformation curves were peak force (PF), total energy to fracture, breaking strain and extension of peak force. Large amounts of connective tissue in the samples tested caused high variability, so a minimum of eight determinations were tested with the mean recorded. All analysis was performed at room temperature.

Results from previous MLA project (A.MPT.0013) showed that there was an insignificant effect on adhesion values between the treatments which suggests that connective tissue had not been noticeably affected.

Hydrothermal Isometric Tension (HIT)

Isometric tension tests are used to analyse the heat behaviour of collagen and the myofibril component of meat. They measure the tension developed during continuous heating, in a neutral isotonic medium, up to boiling temperature. The maximum force per unit section area and the temperature at which this force occurs have been linked to the heat stable molecular cross-links of the collagen and to its thermal solubility (Kopp & Bonnet, 1987). Isometric tension measures the tension generated in the material already denatured.

Maximum hydrothermal isometric tension (HITm) was measured as described by Beilken and Harris (1987). The length and weight of strips of muscle approximately 30 mm long and 2-6 mm wide was recorded and the average cross-sectional area determined assuming a density of 1.04 g/cm³ for raw meat. The muscle fibres were parallel to the longest length of the sample. Each strip was immersed in distilled water and held tight between two clamps on Lloyd Instruments TA Plus materials Testing Machine fitted with a 500 N load cell (Lloyd Instruments ltd., Hampshire UK). The distance between the clamps was 10mm. Slight tension (0.05N) was applied to the sample and a circulating water bath was used to raise the temperature at a rate of approximately 2˚C/min until the force began to decline after reaching a maximum (around 90˚C). Samples were analysed in triplicate and the mean result recorded.

Preliminary HIT analysis of untreated and pressure treated (200 MPa, 60°C, 20 min) brisket muscle has been previously reported (A.MPT.0041), and this measurement will continue to be investigated for processing treatments and muscles used in this project.

Changes in Microstructure of Muscle Induced by High Pressure Processing

High pressure processing (HPP) is a technique that uses high pressures, generally in the range of 100 to 900 mega Pascals (MPa) or 1,000 to 9,000 bars, in order to preserve or enhance properties of a particular food product, such as meat. The application of this technology to improve characteristics of muscle foods and meat products is of considerable interest. Muscles from animals from ovine, porcine and bovine species have been studied in relation to the improvements in tenderness,

water-holding capacity (WHC) and colour of the muscle. These attributes need to be understood at the structural level, so many investigations have used microscopy as a tool. Various forms of microscopy can be used in order to visualise changes occurring within the muscle fibres, the myofibrils and the sarcomere. In addition, the connective tissue and extracellular components can also be viewed.

This section will focus on the effects of HPP on muscle foods in relation to the contractile proteins and connective tissue present in the meat. Four popular types of microscopy will be reviewed; light microscopy (LM), confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Light Microscopy (LM)

The light microscope allows for the generation of images that are approximately 5 to 1000 times magnified. This means the resolution is fairly poor in comparison to some of the other microscopy methods discussed here. But LM allows the microscopist to generate a good overview of the muscle structure. The changes in connective tissue (epimysium, perimysium and endomysium) and also in the muscle fibres can be visualised easily. Fibres can be measured both transversely to gain insight into the fibre diameter and also longitudinally to measure levels of contraction. Also, structures such as the blood vessels can be seen. This technique is often used alongside immunohistochemistry techniques in order to visualise specific structural proteins and enzymes of particular interest.

In terms of preparation, samples are normally sectioned very thinly in order to allow for the penetration of light. For this reason, muscle or meat samples are normally sectioned (approximately 5-10µm thick) and mounted on a glass slide. The phase contrast microscope is one application of the light microscope and can easily be used for visualisation of an unstained sample. This technique was used to visualise beef myofibrillar shortening with application of HPP (Sikes *et al.*, 2010). The HPP treatment (200 MPa, 20 minutes) with heating (60°C) decreased the length of myofibrils significantly in comparison to heat controls and was thought to be partially responsible for the reduction in tenderness values.

The most common application of the light microscope is using bright field microscopy. There can be difficulty in viewing unstained sections, so they are normally stained. In order to preserve the structure, samples are normally fixed prior to staining. Some of the first visualisation of HPP treated beef *semitendinosus* muscle occurred using formalin (a common fixative) prior to wax embedding and subsequent staining with haematoxylin and eosin (Bouton *et al.*, 1977). High pressure processing induced a disruption in the myofibrillar structure of the pre-rigor HPP treated muscle, in comparison to the control.

Beef rump muscle was treated between 100 to 600 MPa (10 minutes, room temperature), and at around 300 MPa, sarcomere length increased whereas at 500 MPa the sarcomere length decreased (Liu, 2010). This could be a result in differences in the muscles and treatment temperatures or the method of embedding (cryosections compared to paraffin wax). At low pressures, protein solubility increased and could be a result of slippage of the contractile proteins and lengthening of the sarcomere. At higher pressures, proteins became denatured and had a lower solubility; this resulted in gaping between fibres, and overstretching and fracturing of the myofibrils, thus shortening the sarcomere.

Recent light microscopy images of brisket and topside muscles (Appendix 1) show that the appearance of the muscle fibres is very regular. The muscle fibres are arranged neatly in parallel bundles with thin gaps between them (containing connective tissue). In the heat controls (Appendix 1, B and F), there is more gaping between the fibres possibly due to cooking loss. In addition, the edges of the muscle fibres are no longer smooth and consistent, but are more ragged and frayed. The connective tissue also appears more aggregated, with some clumping, especially in longitudinal sections.

With pressure applied at low temperature (Appendix 1, C and G), there is an appearance of crimping of the muscle fibres along the length (especially with the topside). These fibres appear bent in a zigzag-like pattern, almost like they have been compressed and subsequently released. In some areas where connective tissue is most abundant, there are signs of aggregation and clumping.

In both muscles, P-H treatment induced aggregation in the connective tissue (Appendix 1, D and H), especially in areas of high density. There appears to be large voids present, which could have originally been occupied by either connective tissue or adipocytes. In brisket, muscle fibres appear organised but there is more gaping between fibres. Whereas in topside, the muscle fibres show a wavy, zigzag-like pattern along the length. Muscle fibres are also cracked and show clear signs of breakage. There also appears to be some breakage in the centre of some of the muscle fibres. Again, there is an increase in gaping between muscle fibres.
In short, heat treatment results in shrinkage of the muscle fibres and solubilisation of connective tissue. With pressure treatment, there is also some shrinkage, but not to the same extent as heat alone. Pressure also seems to induce cracking and some sort of contraction of the muscle fibres, that makes them appear wavy or crinkled, especially in the topside.

The applications of LM are fairly limited due to the minimal level of resolution. Staining is often required to improve the visibility of the structures within the meat system. Regardless, this technique provides microscopists with a commendable overview of the structural changes occurring within the sample. HPP treatment induces gaping between myofibres and a reduction in cell area. At high temperatures, deposits of sarcoplasmic proteins can be visualised. However, if a higher level of resolution is required, confocal microscopy is a favourable option.

Confocal Laser Scanning Microscopy (CLSM)

The CLSM, is the most recently developed microscopic technique for viewing. It was developed in the 1950's and the application of the lasers came later in the 1980's. One of the major advantages of CLSM is that sample preparation is fairly minimal and thin sections are not required for viewing. Quite often, samples as large as a few millimetres thick are used and this reduces the time spent on sample preparation. Normally samples are stained with a fluorescent dye to maximise visualisation. Other advantages of this technique are that it is relatively cheap (in comparison to electron microscopy) and samples can be viewed in three dimensions, due to the use of three focal planes (x, y and z).

Minimal work has been performed with CLSM in relation to HPP effects on muscle structure. However, this technique seems to have more useful applications in the visualisation of meat products (Chattong *et* al., 2007; Bertram *et al*., 2006) which will not be discussed in detail in this report. For muscle structural characterisation, the majority of publications seem to be using electron microscopy.

Scanning Electron Microscopy (SEM)

SEM uses electrons to scan the surface of the sample and in turn can generate images of a much higher resolution (up to 100,000 times magnification), which allows viewing down to 3 to 4 nm. For preparation, samples can either be fixed or frozen and then normally dehydrated. The exposed surface is then coated in a conducting material, such as gold or carbon and is viewed using an electron beam.

The first SEM images of HPP-treated meat related to meat emulsions and will be discussed briefly. Colmenero *et al*. (1997) published images of low and high fat pork emulsions before and after HPP treatment at 300 MPa for 20 minutes, and visualised differences in the filamentous structure of the product that varied according to fat content. Later, HPP protein denaturation of blue whiting batters was investigated and ultrastructure was viewed using SEM (Fernandez-Martin *et al*., 1998). The mechanical and textural differences observed revealed a dense compact matrix at low temperatures and low pressure (7°C, 200 MPa for 10 minutes), whereas with higher temperatures and pressures (37°C, 375 MPa for 20 minutes) the structure was more porous. This decrease in density was reflected in lower values recorded in the cohesiveness and penetration tests.

This increase in porosity has also been observed in trout muscle at lower temperatures (Basaran-Akgul *et al*., 2010). HPP-treated (414 and 517 MPa, 20°C for 5 minutes) muscle tissue was less compact and more porous in comparison to the untreated control. This increase in porosity was consistent with increasing pressure. The effect of using two different sample dehydration techniques; lyophilisation with liquid nitrogen and critical point drying (CPD), using carbon dioxide was also investigated. The CPD method showed greater muscle protein aggregation, which could have been caused by remaining sarcoplasmic proteins from the washing stage of preparation.

A lyophilisation technique was also used in the preparation of restructured pork meat (Hong *et al*., 2008). Meat was treated at 4°C, at two different pressures (100 and 200 MPa) and two different pressurisation times (10 and 30 minutes), with the addition of a cold set binder. In the control, there were no network structures in the junctions within the meat. When pressure was applied at 100 MPa, a continuous network was observed, but the structure was not as intense as at 200 MPa, where there seemed to be a significant network formation. This network formation was thought to be responsible for an improvement in water-holding capacity and an increased binding strength in the restructured pork meat.

In relation to muscle and connective tissue, a disruption in the honeycomb structure of the endomysium with HPP-treated beef shoulder was observed (Ueno *et al*., 1999). The post-rigor beef was pressurised at 100, 200, 300 or 400 MPa for 5 minutes at 4°C and the connective tissue was prepared for SEM visualisation by using the cell maceration method (Ohtani *et al*., 1988). This extraction method allows for the removal of the cellular elements (by immersion of fixed tissues in sodium hydroxide) and exposure of collagen fibrils in their natural locations. The endomysium was visualised as a honeycomb structure that had a wavy appearance with no pressure (Ueno *et al*., 1999). As soon as pressure was applied, the wavy appearance disappeared; tearing and roughening at 300 MPa and disruption in areas at 400 MPa. These changes did not seem to be related to the effects seen with ageing.

Similarly, bovine shoulder muscle samples were prepared using the cell-maceration method for viewing connective tissue of HPP-treatments at 150 and 500 MPa for 5 minutes at approximately 8°C (Ichinoseki *et al*., 2006). They found gaping between the perimysium and endomysium and this gaping became larger with increasing pressures. At low pressures (150 MPa), a loosening effect in the collagen fibres within the perimysium was observed, but after treatment at 500 MPa they were disrupted and had formed separate fibres. They related these changes to a reduction in collagen stability and in turn, the increase in tenderness values as measured by shear force.

Use of SEM has also indicated the disruption of the endomysial sheath of pre-rigor beef semitendinosus with HPP treatment at approximately 100MPa for 2 minutes, 35°C (Elgasim and Kennick, 1980; Kennick, 1981). With HPP treatment, there was an alteration of amino acid composition, which resulted in a weakening or degradation effect on collagen and destruction of the sarcolemma. This was correlated to an improvement in tenderness and was thought to be most effective on tougher cuts of meat.

From the literature, SEM is an important technique when visualising structural changes occurring within the connective tissue. It is also useful for observing modifications in meat products, both in terms of porosity and network formation. These characteristics can be attributed to changes in gelling properties or textural attributes of the muscle or meat product. Care must be taken in the sample preparation technique which could alter the native configuration of the proteins and consequently, lead to artefacts on the image generated.

Transmission Electron Microscopy (TEM)

By far, the majority of images published in relation to HPP have been generated using TEM, particularly in the early stages of research. In terms of mechanism, the TEM is similar to the SEM in that it also uses electrons to generate an image. In TEM, an electron beam is directed towards an ultra thin section of the sample and the image is created by detecting the number of electrons passing through the sample, where contrast is obtained by differences in electron absorption. The TEM supersedes SEM in terms of magnification (300,000 times) and therefore also improves resolution (0.2 to 1 nm). Disadvantages of using this method are that sample preparation is extensive and involves a series of fixation, dehydration, embedding, sectioning and staining steps. Therefore, this can be an expensive process and a laborious one in terms of sample preparation. Nonetheless, this technique seems to be superior and more popular method, especially for the visualisation of muscle tissue.

Some of the earliest work performed on HPP-treated skeletal muscle, in relation to TEM visualisation, was performed by Morton *et al*. (1973). The effects of HPP treatment on the mitochondria of skeletal muscle were visualised. This paper provides an insight as to the superior resolution of the TEM to visualise intracellular structures in detail. Three pre-rigor muscles (*semitendinosus*, *semimembranosus* and *adductor*) of sheep were pressure-treated at 15,000 psi (approximately 100 MPa) for either 5 or 60 minutes at 10°C. At the longer exposure time, intracristal structures and matrix granules were seen in the mitochondria, however these changes were not seen when HPP was for only 5 minutes. The authors correlated this difference to a decline in pH after pressurisation and subsequently accelerated levels of glycolysis.

Both pre- and post-rigor sheep *semimembranosus* have been investigated in relation to the effects of HPP on the structure of the muscle (Macfarlane and Morton, 1978). For pre-rigor muscle, they agreed with the findings of Bouton *et al*. (1977) which indicated the extensive structural damage had resulted in a contraction band formation. A contraction band with TEM after treatment of muscle at 100 MPa, 25°C for 1 minute has also been viewed (Macfarlane and Morton, 1978). The bulging appearance is thought to be a result of extreme shortening by many sarcomeres. The sarcolemma had been distorted outwards, which was also seen later using SEM (Kennick *et al*., 1980). The loss of the M-line protein and a distortion in the A-band as a result of opposing movement by adjacent fibres had already been reported (Macfarlane and Morton, 1978). A summary of the modifications of pre-rigor muscle due to HPP is given in Appendix 2.

In post-rigor muscle, the M-line protein appeared absent in both the longitudinal and transverse sections (Macfarlane and Morton, 1978). The bridges joining the myosin filaments in the M-line were reported as either absent or disrupted. Also, aggregation of a granular material around the I-band especially near the Z-line had been reported. A summary of modifications on post-rigor muscle is given in Appendix 3.

These authors continued their work on post-rigor beef *semimembranosus* and *longissimus dorsi* in relation to textural attributes (Macfarlane *et al*., 1981). They visualised the muscle with and without HPP treatment (150 MPa at 0°C for 3 hours) and also the changes seen after cooking (80°C for 1 hour). Similarly, with pressure treatment, a loss in the integrity of the I-band and M-line regions was found. These two changes in ultrastructure were not seen to influence shear values in stretched muscle, so were thought to be less important in relation to toughness. However, in cold-shortened muscle, the authors theorised that the compression of myosin into the Z-line removed the I-band as a zone of weakness and therefore resulted in increased shear values. The results seen were also related to differential scanning calorimetry observations, where pressure was found to be responsible for the denaturation of actin.

Also in the early eighties, a group from New Zealand (Locker and Wild, 1984) studied the effects of pressure on the tenderisation of meat and visualised the effect of low pressures (60 MPa) on pressure-heat (P-H) treated ox *sternomandibularis* muscles. In addition, these authors employed a “yieldmeter” to stretch the muscles by 50% during fixation. With treatment at 60°C, they discussed the appearance of the filaments around the M-line. In the 60°C heat control, the M-line, A- and I- filaments were intact, the gap-filaments, spanning the M- to Z- line, were described as slack, but were also intact. Upon HPP treatment at the same temperature, these gap-filaments appeared broken around the Z-line. The Z-lines were noticeably out of register, the A-filaments appeared destroyed and the M-line appeared absent or faint.

The authors also investigated the effects of pre-treatment cold-shortening and post-treatment cooking at 80°C under the same P-H conditions. In the cold-shortened muscles, the results seen with heat and P-H at 60°C were very similar. Both treatments seemed devoid of A-filaments and images had the appearance of a coagulum of material spanning the I-bands, which is consistent with the granular material as described previously (Macfarlane *et al*., 1981). In the P-H sample, the coagulum was confined to a narrower central zone near the H-zone. In the second instance, where the muscle had not been cold-shortened, the post-treatment cook was at 80°C for 40 minutes. The 60°C cooked control showed practically identical features to heat alone. However, the 60°C P-H samples showed some differences in that the sarcomeres had extended more uniformly and were still continuous. The authors concluded with a discussion on meat structure in relation to tenderness.

They summarised that there was a dissociation of A-filaments and the gap-filaments were seriously weakened but the I-filaments remained morphologically intact even after P-H (60°C). From these results, the mechanism of tenderness was still uncertain, but both unshortened and cold-shortened meat could be tenderised by the use of HPP and are consistent with others (Bouton *et al.*, 1977).

Investigations on stretched and cold-shortened sheep *semimembranosus* when treated with heat (60°C) and P-H (150 MPa, 60 minutes and 60°C) have been completed (Macfarlane *et al.*, 1986). With P-H, there was a removal of material from the M-line as well as from the pseudo-H-zone, which was thought to be due to the disruption of the myosin rod portions and other structures that may be present. Clumping in the I-band was observed, with voids appearing and overall disruption around the region. This contrasted with the work of Locker *et al*. (1984) who found the region intact, but as discussed by the authors, this could be due to the lower pressures that they used.

Interestingly, at lower temperatures (20°C), no remarkable change was observed in the ultrastructure of beef shoulder muscle up to 150 MPa, with 5 minutes exposure time. This is consistent with (Jung *et al.*, 2000) who found similar at 130 MPa (260 seconds, 10°C) with cow *Biceps femoris*. Although Macfarlane *et al*. (1981) found defects in the structure at 0°C and at similar pressure (150 MPa). However these authors had a substantially longer exposure time of 3 hours. In addition, at 20°C there was a loss of the M-line integrity at 200 MPa, but no loss of density and uniformity of the I-filament until 300 MPa exposure (Suzuki *et al.,* 1990). These authors also saw the deposit of dense material (tufts) on the A-filament. Similar losses of structure in the M-line and I-band at 325 MPa (10°C) have been reported (Jung *et al.,* 2000).

In comparison to longitudinal images, the transverse section also provides an insight as to structural changes occurring (Jung *et al.*, 2000). At 325 MPa, changes occurred in the intermyofibrillar space with the visualisation of many organelle fragments. At 520 MPa, myofibrils appeared as indistinguishable shapes and myofilaments were disrupted. The area of the myofibrils was reported to be larger with all pressure treatments. The visual appearances are thought to be related to changes observed in the protein solubility and in turn the electrophoretic patterns and differences in tenderness.

Japanese scientists have also been investigating the mechanism of HPP tenderisation in chicken *pectoralis profundus* muscle (Suzuki *et al.*, 2001). The HPP induced conversion of α-connectin to β- connectin by use of immunoelectron microscopic localization has been observed. The muscle was fixed to a glass rod and pressure-treated (100-300 MPa) at 0-2°C for 5 minutes. The connectin epitope appeared as an electron dense line at either side of the H-zone. Upon pressurisation, the epitope became dispersed and migrated with the change in sarcomere length. Changes were accelerated with increasing pressure and were significantly different to those seen with post-mortem storage. The modifications seen were thought to be one of the causes of the HPP tenderisation effect.

Further work on the pressure-induced mechanism of proteolytic tenderisation was continued with rabbit *Longissimus thoracis* (Kubo *et al.*, 2002). Pressure-induced modifications occurred to the lysosomes and the location of the proteolytic enzyme, cathepsin D. After treatment at 100 MPa, a loss in the round nature of the lysosomes was observed using an immunoelectron microscopic localization method. Between 150 to 400 MPa, cathepsin D was liberated from the lysosome and subsequently

became bound onto the myofibrils. These effects seen with pressure were drastic compared to those seen with conditioning for up to 14 days.

Control TEM images of brisket muscle (Appendix 4, A and D) illustrate regular banding patterns within myofibrils with intact and clear Z-lines and definite M-line present, surrounded by a definite H-zone. Both the I-bands and A-bands (electron dense region) appear clear and can easily be distinguished. Membranes of mitochondria are intact and cristae are clearly visible.

With the application of pressure at low temperature (Appendix 4, B and E), the Z-line is distinguishable, however, around the area, the I-band contains aggregated and clumped material. The M-line is no longer visible, whilst the H-zone is still present but has shrunk. The A-band also appears more intact but there is some aggregation. There are empty voids present between some myofibrils, which could be a result of bursting of mitochondria. The mitochondria that are still present in the structure look elongated and ready to burst.

The most pronounced alterations in structure appear to occur with P-H treatment (Appendix 4, C and F). The I-band as a whole seems destroyed. There are some proteins linking Z-line to A-band but these structures have a high level of aggregation and clumping and look denatured. Within the I-band, there are coagula with very electron dense material present (about 100-200nm diameter) which could be protein aggregates. The A-bands are also lacking clarity and appear as one aggregated mass, rather than being smooth and having distinct fibres. The H-zone looks completely melted and there is no appearance of the M-line. There is no sign of mitochondria present within the structure.

In summary, pressure treatment seems to induce some sort of aggregation and clumping of the proteins, especially within the I-band region. This seems to result in the formation of coagulum within the same area. The H-zone and M-line regions appear distorted and have lost their integrity. Mitochondria organelles appear to have ruptured and dissipated. The changes occurring under P-H conditions are similar to that of P-L, but seem more pronounced. This is particularly evident in the I-band region, where there is minimal protein intact after P-H treatment.

Overall, TEM is an extremely useful microscopy method for viewing ultrastructural changes in muscle that have been induced by HPP treatments. In pre-rigor meat, one of the most specific differences seen with HPP treatment is the contraction of the sarcomere, followed by the disruption of myofibrillar structure. With post-rigor meat, the difference seen tends to be dependent on the level of pressure treatment used and also the temperature and time for which it is applied. In general, at lower pressures, the M-line, I-band and Z-line lose their integrity and there is a loss of material. As pressure increases, there seems to be the further disorganisation of the sarcomere structure and also the appearance of granular deposits. As temperature increases, these modifications seem to occur at lower pressures.

Integration of Methods

Some authors have used combinations of the microscopy methods described above in order to investigate meat systems. In relation to pre-rigor bovine muscles, the combination of LM, SEM and TEM have been shown to be very informative about revealing the detail about HPP induced modifications (Elgasim, 1982). Longissimus muscle treated at approximately 100 MPa (37°C, 2 minutes) displayed many structural changes. In terms of the sarcomere, it was found to be shorter due to

contraction bands with a disappearance of the H-zone, M-line and Z-line. There was more gaping between muscle fibres and myofibrils appeared more loosely packed and fragmented. The sarcoplasmic reticulum, sarcolemma and mitochondria were all affected by pressure treatment, being distorted, swollen or burst in some way. Using SEM, the appearance of a globular material was evident and was thought to be breakdown products of collagen or sarcoplasmic proteins. These changes were only studied at one set of conditions, so other combinations of pressure and temperature treatments should be considered when analysing pre-rigor results.

In terms of post-rigor bovine shoulder muscle, a combination of SEM and TEM was used to observe HPP-induced modifications (Rusman *et al.,* 2007). SEM was used to visualise the changes in transverse sections of muscle fibres and also in the endomysium/ perimysium, using the cell maceration method (Ohtani *et al.,* 1988). The TEM was used for viewing longitudinal sections and measuring the lengths of the sarcomeres. They used temperatures of either 30 or 60°C for 5 minutes at HPP conditions of 100, 150, 200, 300 and 400 MPa. SEM results for HPP treatment at 30°C illustrated an increase in gaping up to 200 MPa and then a decrease in the width of the gaps (300 and 400 MPa), but at 60°C this decrease was not seen (Rusman *et al.*, 2007). The honeycomb structure of the intramuscular connective tissue was also visualised at temperatures of 30 and 60°C (Rusman *et al.,* 2007). In untreated samples, they visualised wavy collagen fibres and subsequent tearing with pressure. These authors reported the tearing occurred at low pressures; over 150 MPa at 30°C and over 100 MPa at 60°C. This is different to that seen by others (Ueno *et al.*, 1999)who found tearing in beef shoulder muscle at 300 MPa, but these authors used a lower temperature (4°C) for the same treatment time (5 minutes).

In relation to TEM longitudinal sections, a loss of M-line integrity and contraction of the sarcomere was observed at 100MPa and collapse of I-filament and Z-lines at higher pressures. These results are in agreement with others (Macfarlane *et al.*, 1981; Ashie and Simpson, 1998). Macfarlane *et al*. (1981) saw disruption in the I-band and a loss of material from the M-line after HPP treatment at 150 MPa, 3 hours, 0°C whereas Ashie and Simpson (1998) reported the I-band as being indistinguishable at 100 MPa in fish muscle. This author also reported the disintegration of the Z-line at 200 MPa.

The use of the different microscopic methods allows the scientist to develop an improved overview of the whole muscle or meat product structure. It can provide information on both the transverse and longitudinal properties of the muscle fibres and can also be used for the influence of the connective tissue component. This can allow for the integration of knowledge from different microscopic techniques in order to generate a more accurate profile of events that occur with treatments such as HPP.

Tensile testing in combination with microscopy

As well as using combinations of microscopic methods for studying the ultrastructure of muscle, it is also possible to use rheological methods in combination with microscopy. For example, the CLSM has an attachment that enables tensile stresses to be placed on samples whilst simultaneously viewing the sample.

When searching the literature, only a few papers have reported simultaneous structure analysis during tensile stretching of meat samples. Pieces of tissue, single muscle fibres and connective tissue have been studied and structural information such as sarcomere length, fracture appearance and crimps have been obtained. The

samples have been either pork or beef. There doesn't seem to be any recent publications in this area and no analysis of HPP-treated samples was found.

Pieces of bovine semitendinosus muscle, both raw and heated (90°C, 45 minutes) were stressed either parallel or perpendicular to the muscle fibre axis by Carroll *et al.* (1978). Samples were studied by light microscopy (video recording) and scanning electron microscopy (static stress). Stress parallel to the fibre axis resulted in the initial rupture of the muscle fibre-endomysium sheath. Perpendicular stress caused initial rupture at the endomysium-perimysium junction with the muscle fibres remaining undisturbed.

Perimysial connective tissue from raw (frozen and thawed) and cooked (50-90°C, 1 h) bovine *M. semitendinosus* was tensile stretched (6.5 mm/min) in saline solution by Lewis and Purslow (1989). The initial 'toe' region of a J-shaped load-extension curve was progressively lost with increasing cooking temperature, the curve becoming more linear. Breaking strength increased from raw to cooked at 50°C, thereafter decreasing at higher cooking temperatures. Only light microscopy images of non-stretched samples are presented in the paper but the tensile device is made to be fitted under a microscope. During cooking, crimps of the collagen fibres straighten.

Mutungi *et al*. (1995) looked at tensile tests (3.9 µm/s) on single muscle fibres from raw and cooked (80°C, 1 h) porcine *longissimus thoracis* in conjunction with light microscopy analysis (camera + video) of sample deformation and fracture properties. The muscle fibres initially resisted being stretched until a yield point, which occurred when strains of between 2 and 5% in raw fibres, and 10 and 20% in cooked fibres were reached. After that, the fibres required less force to be extended, thereby producing an r-shaped stress-strain curve. Surface cracking was followed by unequal sarcomere stretching, with those sarcomeres in the cracked regions extending faster than those in areas still covered by the surface membrane which remained relatively unchanged. The results showed that the deformation was not uniform along individual fibres, especially in the raw case and the endomysium had an important contribution to this non-uniform deformation. They concluded that a possible link exists between gap filaments and meat toughness.

Willems and Purslow (1996) examined the effect of postrigor sarcomere length on mechanical (tensile test, 4.2 µm/s) and structural (light microscopy) characteristics of raw and cooked (80°C, 1 h) single muscle fibres from porcine *longissimus lumborum*. The samples developed rigor at 2°C (cold-shortened), at 22°C or at 22°C after being stretched. Scanning electron microscopy was applied to analyse the effect of longitudinally splitting of cold-shortened heat-denatured muscle fibres. The results showed that raw, stretched muscles displayed a prerigor increase and a postrigor decrease in sarcomere length. The sarcomere length was positively correlated to maximum stress. Cooking led to a decreased sarcomere length for all samples. The heat-denatured, cold-shortened muscle fibres showed a tremendous increase in stress at large strains indicating that the structural mechanism to deform and fracture structural elements of heat-denatured, cold-shortened muscle fibres is different than control and stretched ones. The crack propagation was different between the samples where the cold-shortened muscle fibres exhibited a progressive fracture of separate myofibrils and the other samples had an almost immediate propagation straight across the muscle fibre. This reduced cooperativity of fracture between myofibrils in cold-shortened muscle and suggested that the balance between the axial strength along the myofilaments and the transverse strength of the lateral structures such as transverse cytoskeletal elements (intermediate filaments) is altered. The transverse filaments might even be detached in cold-shortened muscle fibres.

Following this study, they extended the research to encompass groups of muscle fibres (Willems and Purslow, 1997). The conclusions were that muscle fibres extend and break in a different way when linked to neighbouring muscle fibres by the endomysium in a small group of fibres from raw meat. This load-sharing through the endomysium serves to even out local weakness and variances in extensibility so as to allow groups of fibres to extend far more uniform than single fibres. Cooking does reduce the influence of these linkages. These findings suggest that connections between myofibrils and extracellular connective tissue via cytoskeletal filaments and costamere structures must be taken into account in any model of meat toughness.

Dransfield *et al*. (1995) studied fractures in stretched beef (*M. Pectoralis profundus*) after dismounting from Instron testing (2.5 mm/min). Samples were stored at 15°C for 1.5, 27 or 73 hours after stunning and then cooked in water under 0.11 N/cm^2 stress (1.5°C/min to 70°C + 10 minutes at 70°C) and longitudinal sections were viewed by light microscopy. The ultimate tensile strength and extensibility decreased exponentially during storage. Fresh meat had a brittle fracture and sequential fibre fracture. In aged meat, discontinuous fractures were prominent within each fibre. The changes with time aligned to a gradual reduction in cohesion within the meat and indicated that the weakening of the focal adhesions is primarily responsible for post-mortem tenderisation.

Tensile testing in combination with CLSM will be developed for analysis of processed meat in this project.

Protein denaturation

Differential scanning calorimetry (DSC) is a popular tool for investigating the thermodynamic properties of foods and food components and to date, the most common method for monitoring denaturation of proteins in muscle systems. The principle of DSC is to measure the difference in heat flow between the sample and a reference which are heated or cooled at the same rate. Changes in the sample that are associated with absorption or evolution of heat cause a change in the differential heat flow which is recorded as a peak. The area under the peak is directly proportional to the enthalpic change and its direction indicates whether the thermal event is endothermic or exothermic. The assignment of transitions in beef muscle to the denaturation of myosin, sarcoplasmic proteins, collagen and actin is based on the calorimetric studies of Wright et al. (1977) on purified muscle proteins, and the temperatures at which these transitions occur are dependent on the heating rate.

DSC has been used in a previous MLA-funded project (A.MPT.0013) and has shown changes in muscle protein components with high pressure processing when combined with heat (Sikes et al., 2010). When pressure (200 MPa) was applied with heat (60°C) to beef neck muscle, myosin and actin were totally denatured. Minimal effect of this pressure treatment on the peak corresponding to collagen was observed.

The measurements were performed using a micro-differential scanning calorimeter (Micro DSC III, Setaram Inc., Lyon, France) equipped with a refrigerated circulating bath (Julabo). The instrument was calibrated for temperature and enthalpy using naphthalene as standard. The beef muscle samples (~100 mg) were sealed in a hastelloy sample vessel. Duplicate samples were heated from 25°C to 90°C at a scanning rate of 1°C/min, with an equivalent weight of water (or buffer) as reference.

The transition temperature (T_m) was recorded and the transition enthalpy (ΔH) was calculated from the peak area using the Setaram software and expressed in J/g.

The thermograms produced from recent studies using DSC to investigate denaturation of muscle proteins of high pressure processed topside and brisket muscle, at low and high temperatures, are given in Appendix 5.

DSC will continue to be used as a tool for investigating denaturation of muscle proteins of processed meat (HPP and VFC), analysing both whole muscle samples and isolated muscle protein components.

The glass transition of foods has gained recent focus in food science because food properties related to molecular mobility, such as texture, microbial growth, chemical reactions and shelf life, are affected by the glass transition temperature (Tg) (Slade and Levine, 1991). The glass transition concept was developed on the basis of polymer science. Only in recent years has it been applied to food science, after the recognition that most foods are non-equilibrium or metastable systems and glass formation is a plausible mechanism through which such non-equilibrium systems can be stabilised. Therefore, this concept has been used to predict product quality, safety and storage stability. The consequence of this in terms of food products is that a small change in temperature in the vicinity of the glass transition temperature will result in pronounced changes in the sensory properties of texture (Simatos *et al*., 1995). It is proposed that changes in phase and glass transition temperatures due to processing of muscle, particularly VFC, will be important in understanding the mechanisms of tenderisation.

The glass transition is the temperature where the food goes from a hard, glass-like state to a rubber-like state. DSC defines the glass transition as a change in the heat capacity as the sample goes from the glass state to the rubber state. This is a second order endothermic transition (requires heat to go through the transition), so in the DSC thermogram, the transition appears as a step transition and not a peak. Above the glass transition temperature, the system is in the rubbery state (capable of flow in real time) and below Tg, the system is in the glassy state.

Information in the literature on muscle Tg is focussed on measuring the phase transitions of dehydrated (i.e. freeze dried) muscle tissue with varying water contents and its subsequent impact on the stability of the meat product. Very few references describe the methods and values of Tg for 'fresh' muscle tissue.

Brake and Fennema (1999) highlighted the fact that no standardised procedures for measuring Tg using DSC have been developed, with various cooling rates, heating rates, holding times and temperatures, annealing conditions (or lack of) and sample sizes reported. These differences can affect the maximum freeze concentration and therefore the measurement of Tg values. Brake and Fennema (1999) developed a procedure for the determination of Tg which involves annealing and has subsequently been used by other researchers (Delgado and Sun, 2002, Sablani *et al*., 2007). Annealing is an important step in the process, and involves the sample being frozen followed by warming of the sample to above the Tg and held for long enough to allow either more or less ice to form. The annealed sample is then cooled to below the Tg before the DSC scan.

Reported Tg values of beef vary considerably, and with the exception of two reports, are curiously low: -60°C (Rasmussen, 1969), -40°C (Simatos *et al*., 1975), >-5°C (Levine and Slade, 1989), -12°C (Brake and Fennema, 1999) and -13°C (Akköse and Aktaş, 2008). Sunooj *et al*. (2009) investigated various factors (annealing conditions,

moisture content, heating rate) that influence the determination of Tg in chicken and mutton samples. Tg values of -17°C and -13°C for chicken and mutton respectively, were reported from these experiments. The Tg value for chicken was in agreement with that reported by Delgado and Sun (2002). Sunooj *et al*. (2009) concluded that correct selection of annealing conditions (temperature and time) is critical in the determination of Tg. DSC determination of glass transition was optimised for sea bass muscle and this procedure was used for Tg determination on high-pressure treated (200 – 600 MPa, 10°C, 10 min) sea bass muscle (Tironi *et al*., 2009).

As there are no standardised procedures for measuring Tg using DSC (as stated in the literature), approximately 20 mg samples of minced topside and brisket were sealed in 40 µL aluminium pans and heated from -20°C to 100°C at a heating rate of 5°C/min using a Perkin Elmer Diamond DSC. The 5°C/min heating rate was chosen as it was commonly used by others (Brake and Fennema, 1999; Delgado and Sun, 2002). Both topside and brisket samples showed small peaks at approximately 0°C, 60°C (possibly collagen) and a larger peak at approximately 78°C (possibly actin). Both samples also exhibited a Tg at approximately 50°C.

In order to emulate the conditions used in VFC, where beef is chilled to subzero within 1 hour of stunning, samples of topside and brisket were cooled from 40°C to -20°C at 1°C/min. The topside sample exhibited a Tg at approximately 36°C and brisket at approximately 21°C. These results were difficult to interpret.

However, as meat exhibits subzero Tg temperatures (e.g. -13°C for mutton, and -17 ºC for chicken (Sunooj *et al*., 2009), it was not possible to visualise these Tg's using the Perkin Elmer equipment which is coupled to an intracooler with a minimum temperature of -20°C. Lower temperatures (-60°C or less) are required in order to determine the Tg of meat, as annealing at temperatures such as -20°C are necessary for visualisation of the Tg (Brake and Fennema, 1999; Sunooj *et al*., 2009). The acquisition of an intracooler that is capable of reaching these lower temperatures is in progress and this will also allow protocols which include annealing to be investigated for determining the Tg of processed (VFC, HPP) meat.

Protein degradation

There have been many studies reported in the literature showing that proteolysis of myofibrillar, intermediate filament and cytoskeletal proteins has a direct impact on tenderisation that occurs during normal post-mortem ageing of beef (for example Koohmaraie, 1996; Taylor *et al*., 1995). Many changes occur in the structure of the myofibril during post-mortem ageing (Parrish *et al*., 1973) and changes in some proteins are important indicators of tenderness (Ho *et al,* 1994; Huff-Lonergan *et al.,* 1996*).* Many of these changes have been identified by well-established methods using one-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. More recently, Anderson *et al.* (2012) have investigated the use of two-dimensional difference in gel electrophoresis (2D DIGE) of muscle which allows a more detailed study of post-mortem changes that alter the structure of the myofibril to identify potential indicators of tenderness (Hollung *et al*., 2007).

Many myofibrillar and cytoskeletal proteins have been implicated in the post-mortem changes observed to myofibrillar structures and proteins. Of these, nebulin, titin, troponin, desmin, vinculin and dystrophin have been suggested to play important

roles (Koohmaraie, 1992; Ouali, 1992; Roncales *et al*., 1995; Greaser and Fritz, 1995).

The degradation of nominated myofibrillar and cytoskeletal proteins in muscle samples treated by HPP or VFC will be analysed using these established methods.

Metabolism

Muscle metabolism in skeletal muscle is a phenomenal process and in general, glycogen is the preferred substrate for production of ATP (the energy unit of the cell), either through oxidative phosphorylation (aerobic metabolism) or through anaerobic glycolysis (anaerobically). Early post-mortem muscle obviously uses the anaerobic pathway, as oxygen supplies to the cells are rapidly depleted. Lactate (lactic acid) is the end product of anaerobic glycolysis which results in pH decline. Therefore, the determination of muscle glycogen and lactate is important in understanding the metabolism of pre-rigor muscle and will be measured quantitatively using commercially available kits.

Osmotic effects

The osmotic pressure of post-rigor beef has been established as being about 380 – 540 mOs which is equivalent to a solution containing 275 – 295 mM NaCl (Winger and Pope, 1981; Ouali, 1990; Ouali *et al*., 1991). This is nearly double that of pre-rigor muscle (300 mOs). The low molecular weight components were suggested to account for the osmotic pressure increase in post-rigor muscle (winger and Pope, 1981). The intracellular osmotic pressure (ionic strength) is closely related with pH during the development of rigor (Ouali, 1990) but it has received comparatively little focus in meat research studies. Ouali *et al*. (1991) suggested that the pH drop altered the proteins to which ions are bound, hence increasing the osmotic pressure. Conclusions from studies investigating high ionic strength on the solubilisation of structural proteins (Wu and Smith, 1985, 1987), changes in myofibrillar ATPase activity with ageing (Ouali, 1992) and osmotic pressure of muscles with varying contraction speeds (Geesink *et al*., 1992; Ouali *et al*., 1991, 1992) suggested that elevated osmotic pressure, along with enzymatic proteolysis, has an impact on myofibrillar proteins that could be associated with tenderisation mechanisms.

Heffron and Hegarty (1974) reported a decrease in fibre diameter in pre-rigor muscle with time, and at the same time, an increase in extracellular space (ECS). These authors suggested that this could be related to a rapid increase in osmotic pressure due to the transfer of ions towards the ECS through the sarcoplasmic membrane. Osmotic pressure of muscles has been measured using different methods based on the determination of either vapour pressure, cryoscopic parameters or muscle weight changes after soaking in various solutions (reviewed by Maffly and Leaf, 1959; Winger and Pope, 1981). Osmotic pressure has been assessed in pre-rigor and post-rigor muscles by osmotic determination of muscle juice osmolality using an automatic osmometer (Ouali, 1990; Ouali *et al*., 1991) and it was found that as muscle pH decreased, osmotic pressure increased exponentially. This conventional osmometer, which measures the freezing point depression of salt solutions against pure water, cannot be used for solid samples. Bonnet *et al*. (1992) stated that osmotic pressure is a difficult characteristic to measure and that no convenient and efficient method was available and subsequently developed a DSC method based on

the determination of the melting onset point of the frozen water in the muscle. This method was compared to a freezing point osmometric method and a soaking method (Winger and Pope, 1981). It was concluded that the DSC method appeared more practical and reliable than the other methods.

It is undecided at this point in time as to which method will be used for determining the osmotic pressure in pre-rigor muscle (VFC) but this will be investigated in future work.

Small deformation rheology

Although biochemical and structural studies provide information on changes in meat with processing, less is understood how these changes affect texture. Dynamic mechanical analysis (DMA) is an approach that studies changes in isolated mechanical properties under small deformations, including both elastic and viscous responses. In polymer science, DMA has been used to relate mechanical properties to molecular level properties (Menard 1999). DMA also provides temperature programming, so that processes such as cooking can be mimicked and transitions occurring at specific temperatures can be monitored (similar to DSC).

Kerr et al. (2000) measured the dynamic mechanical properties of chicken breast muscle marinated with different phosphate types, before, during and after cooking, and related these measurements to changes in muscle proteins and ultrastructure. Marination caused a reduction in the storage modulus (G′) and an increase in Tan delta (Tan δ) of raw chicken meat. These changes in the dynamic moduli were explained by the solubilisation of muscle proteins by salt and phosphate and subsequent breakdown of sarcomere structure which resulted in a less elastic and more viscous system.

Preliminary investigations of high pressure treated meat using DMA (TA Q800) indicated differences between control, heat control (60°C, 20 minutes) and pressure-heat treated (200 MPa, 60°C, 20 minutes) beef neck muscle (Appendix 6). However, there were large variations in replicates within each sample; the potential source of this variation could be due to difficulty with sample preparation (cutting of sample, loading of sample into clamp assembly) or moisture loss from the sample during heating. There are two possible solutions for overcoming the moisture loss during heating of the sample: a) purchase the submersion sample/heating accessory supplied by the manufacturer of the DMA where samples can be tested submerged in a liquid; b) modify the program on the Lloyd texture analyser that is used for the HIT analysis to enable an oscillating force to be applied to the sample while heating submerged in water or a buffer. These possible solutions will be further investigated.

DMA can also be used to determine the glass transition (Tg) as well as to identify transitions corresponding to other molecular mobilities. DMA can measure the viscoelastic properties of the materials as a function of frequency of oscillatory deformation. In the glassy state, the material will exhibit solid-like characteristics and have a measurable shear modulus. In the rubbery state, the shear modulus will be much lower. The glass transition can be detected as the temperature where the storage modulus starts to fall rapidly with increasing temperature from the very high value in the glassy state to a lower value in the rubbery state. A peak in the ratio of the loss to the storage modulus (Tan δ) can also be used to indicate the glass transition temperature.

Preliminary investigations tested samples of topside (raw) and brisket (raw) using the duel cantilever method of the DMA instrument (TA Q800). Minced meat was placed in the powder holder and scanned from -50°C to 50°C at 3°C/min at 2 Hz and deflection of the samples was 15µm. The observed Tg's for topside and brisket are given in Table 1. The method appears to give a reasonable transition of the minced samples of topside and brisket, with clear traces for storage modulus and loss modulus (Appendix 7). Although the method seems promising to give transition data for meat samples, there is a question as to its validity. As the samples are frozen prior to heating, is the method looking at the properties of water in the meat rather than the muscle tissue?

Table 1: Observed glass transition temperatures (Tg) for raw topside and brisket minced muscle, as measured using DMA.

Sample	***Tg (°C) Tangent***	***Tg (°C) Loss modulus max.***
Topside	-11.4	-1.55
	-12.2	-1.56
Brisket	-16.7	-3.35
	-14.9	0.00
	-18.2	-6.03

The samples (topside and brisket) were subsequently tested by cooling from 25°C to -50°C and the modulus monitored. Minced muscle was held at 25°C for 3 min then cooled to -50°C at 3°C/min. The sample was deflected by 15µm at 2 Hz. A very different curve was obtained to that of the heating method (Appendix 8). The modulus of the minced muscle remained low until the temperature reached -20°C. At this temperature, the storage modulus increased progressively, with the loss modulus and Tan δ reaching a maximum.

Overall progress of the project

Many of the methods to be used for assessing processing effects (HPP, VFC) on muscle have been described. Well-established methods have been evaluated and where necessary, development or modification of methods is underway and on-track. Preliminary results of some techniques have been reported.

Recommendations

Discussions between MLA and CFNS representatives at a recent project meeting held at CFNS-Werribee (January 30, 2012) concluded that future HPP experiments will be performed at low temperature (less than 10°C). An experimental plan will be developed and distributed to all parties for discussion and confirmation of processing conditions.

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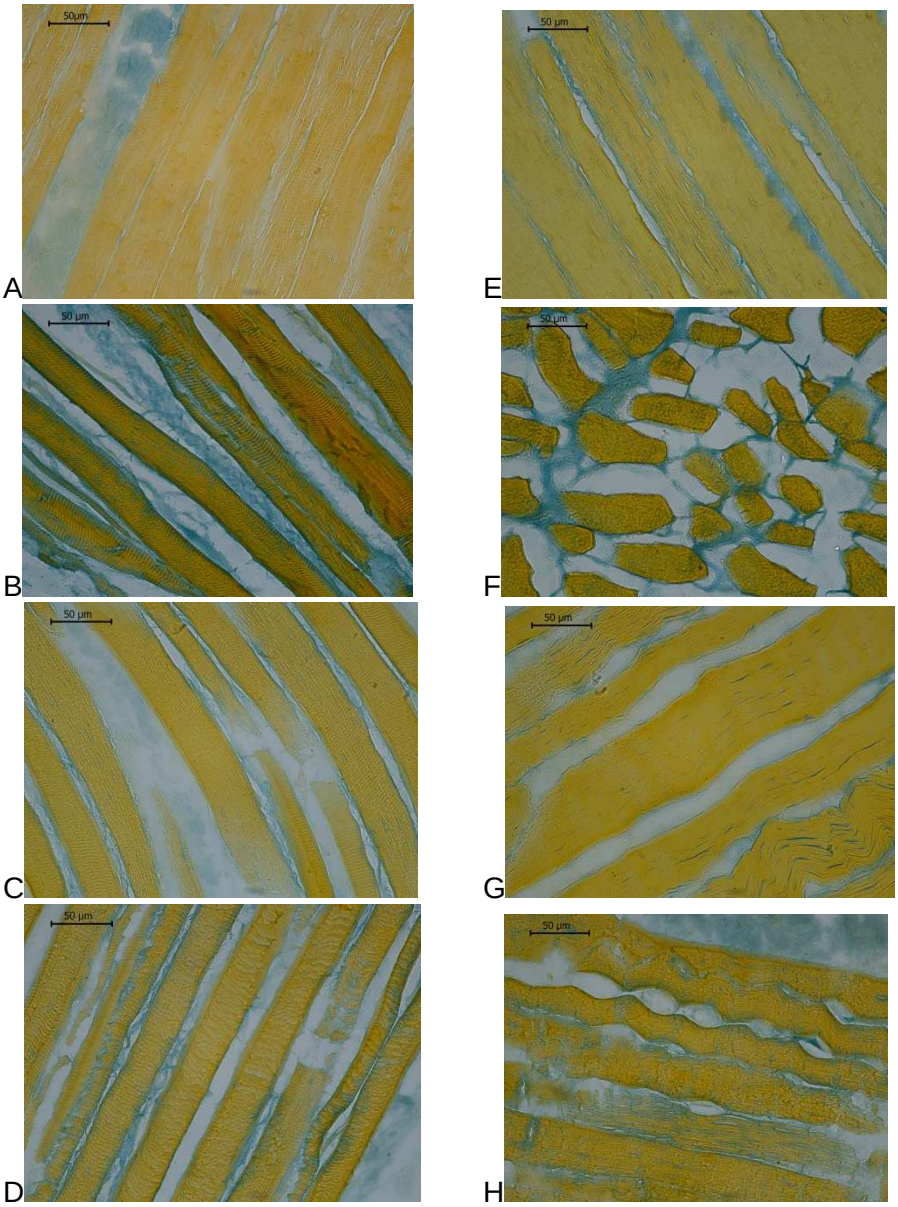
Appendix 1: Recent light Microscopy from CSIRO

Light microscopy images of brisket (A-D) and topside (E-H) muscles, cryosectioned (10µm) and stained using orange G (muscle fibres) and aniline blue (collagen). Treatments were control -A and E; heat control (60°C, 20 minutes) -B and F; pressure low (400 MPa, 4°C, 5 minutes) - C and G; pressure high (200 MPa, 60°C, 20 minutes) - D and H).



Appendix 2: Summary of HPP Modifications to pre-rigor muscle, as viewed microscopically.

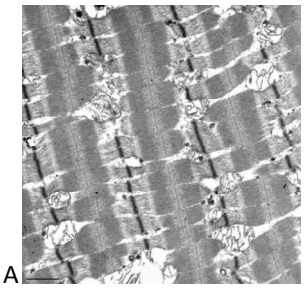
Author	*Year published*	*Animal and muscle*	*Microscopic method*	*Conditions of HPP treatment*	*Modifications seen in HPP-treated sample*
Bouton *et al*.	1977	Beef, semitendinosus	LM	Approximately 100 MPa, 4 minutes, 35°C	Disruption in myofibrillar structure
Macfarlane and Morton	1978	Sheep, semimembranosus	TEM (longitudinal)	100 MPa, 1 minute, 25°C	contraction band formation, extreme shortening of sarcomeres, distortion of sarcolemma outwards, distortion of A-band, loss of M-line protein
Kennick *et al*.	1980	Beef/sheep, supraspinatus	SEM (longitudinal)	Approximately 100 MPa, 2 minutes, 35°C.	Sarcolemma disrupted and convoluted, interaction between contractile filaments and connective tissue, fibres frayed.
Elgasim	1982	Beef longissimus	SEM & TEM (both longitudinal & transverse)	Approximately 100 MPa, 2 minutes, 35°C.	Appearance of contraction bands on sarcomere, disappearance of the H-zone, M-line and Z line. gaping between muscle fibres, myofibrils fragmented. Sarcoplasmic reticulum & sarcolemma distorted, mitochondria swollen or burst.

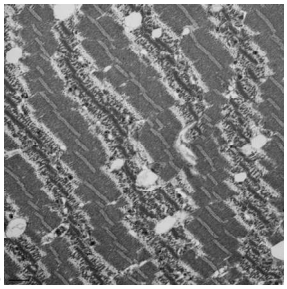
Appendix 3: Summary of HPP Modifications to post-rigor muscle, as viewed microscopically.

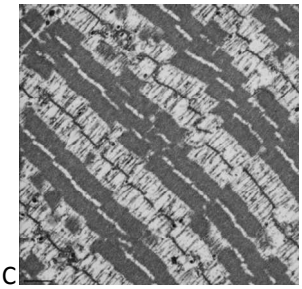
Author	*Year published*	*Animal and muscle*	*Microscopic method*	*Conditions of HPP treatment*	*Modifications seen in HPP-treated sample*
Macfarlane and Morton	1978	Sheep semimembranosus	TEM (longitudinal)	100 MPa, 60 minutes, 25°C	Loss of M-line protein, aggregation of granular material around I-band (near Z-line).
Macfarlane *et al*.	1980	Beef semimembranosus and longissimus dorsi	TEM (longitudinal)	150 MPa, 3 hours, 0°C	Loss in integrity of I-band and M-line regions.
Locker and Wild	1984	Beef sternomandibularis	TEM (longitudinal)	60 MPa, 20 minutes, 55°C (50% stretched)	G-filament broken around Z-line, Z-lines out of register, A-filaments destroyed, M-line absent or faint.
				60 MPa, 1 hour, 60°C (cold shortened)	Similar to heat control, coagulum of material near H-zone. I-filaments morphologically intact.
				60 MPa, 1 hour, 60°C (cooked 80°C, 40 minutes)	Sarcomeres extended more uniformly.
Macfarlane *et al*.	1986	Sheep semimembranosus	TEM (longitudinal)	150 MPa, 1 hour, 60°C	Removal of material from M-line and pseudo-H-zone, clumping in I-band and voids appearing.
Suzuki *et al*.	1990	Beef shoulder	TEM (longitudinal)	150 MPa, 5 minutes, 20°C	No remarkable change.
				200 MPa, 5 minutes, 20°C	Loss of M-line integrity.
				300 MPa, 5 minutes, 20°C	Loss of density and uniformity of I-filament and deposit of dense material (tufts) on the A-filament.
Jung *et al*.	2000	Beef (cow) biceps femoris	TEM (longitudinal)	130 MPa, 260 seconds (4.3 minutes) 10°C	No remarkable change.
				325 MPa, 260 seconds (4.3 minutes) 10°C	Loss of structure in M-line and I-band.
			TEM (transverse)	325 MPa, 260 seconds (4.3 minutes) 10°C	Visualisation of many organelle fragments
				520 MPa, 260 seconds (4.3 minutes) 10°C	Myofibrils appeared as indistinguishable shapes, myofilaments disrupted, area of myofibrils larger
Ashie and Simpson	1998	Bluefish dorsal region	TEM (longitudinal)	100 MPa, 30 minutes, ambient temperature	I-band indistinguishable from A-band
				200 MPa, 30 minutes, ambient temperature	Sarcomeres not in register, Z-disk disintegration, degradation of connective tissue network (SEM observation)
				300 MPa, 30 minutes, ambient temperature	Further disintegration and disorganisation of sarcomere
Rusman *et al*.	2007	Beef shoulder muscle	TEM (longitudinal)	100 MPa, 5 minutes, 30°C	Contraction of sarcomere
				200 MPa, 5 minutes, 30°C	Deformation of Z-line
				300 MPa, 5 minutes, 30°C	Loss of M-line, thickening of Z-line, collapse of I filament
				100 MPa, 5 minutes, 60°C	Contraction of sarcomere, slight loss of M-line structure
				200 MPa, 5 minutes, 60°C	Collapse of I-filament and Z-line in several places
				300 MPa, 5 minutes, 60°C	Decreased length of sarcomere.

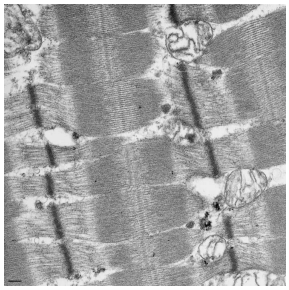
Appendix 4: Transmission electron microscopy images of brisket from recent trials conducted at CSIRO.

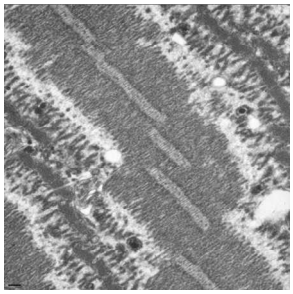
Treatments were control - A and D; pressure low (400 MPa, 4°C, 5 minutes) - B and E; pressure high (200 MPa, 60°C, 20 minutes) - C and F. Scale bars represent 1µm (A – C) and 200nm (D – F).

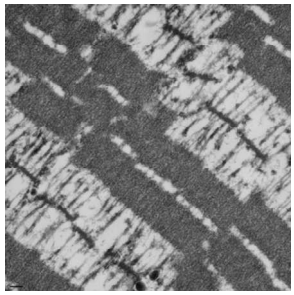
A 

B 

C 

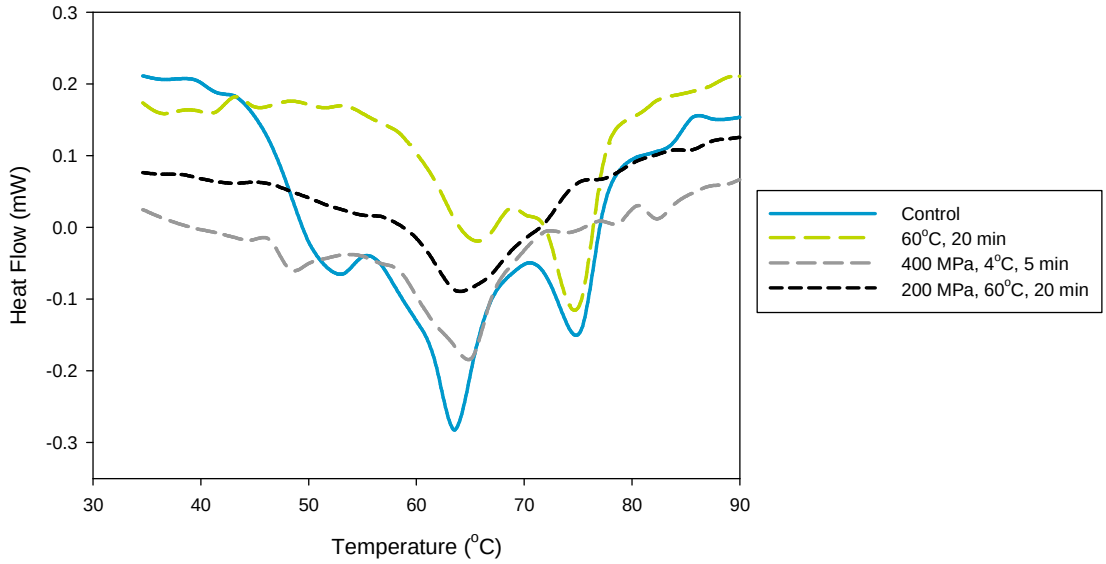
D 

E 

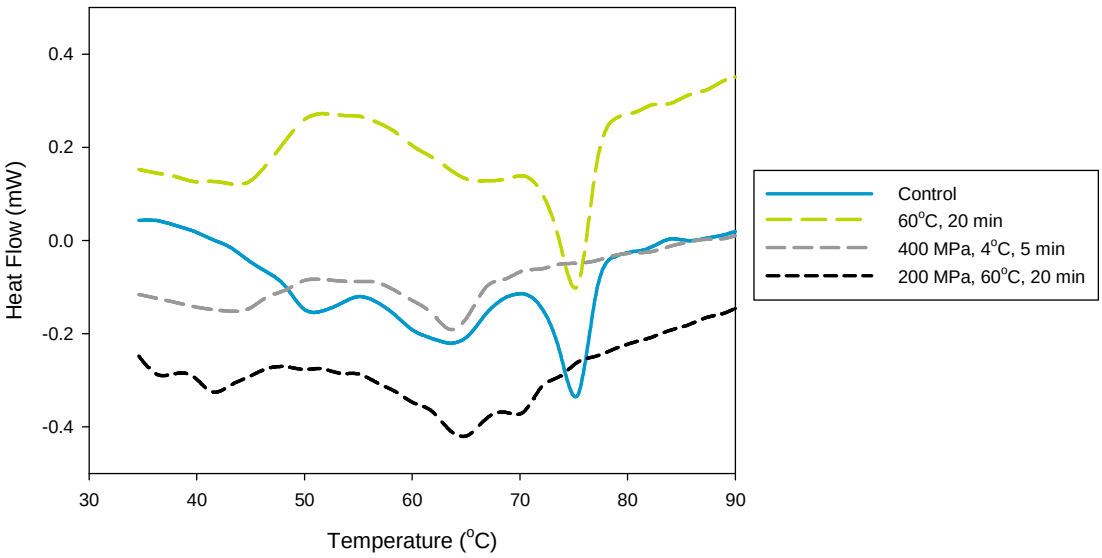
F 

Appendix 5: DSC thermograms of (A) brisket and (B) topside muscle, Control (no treatment).

A.

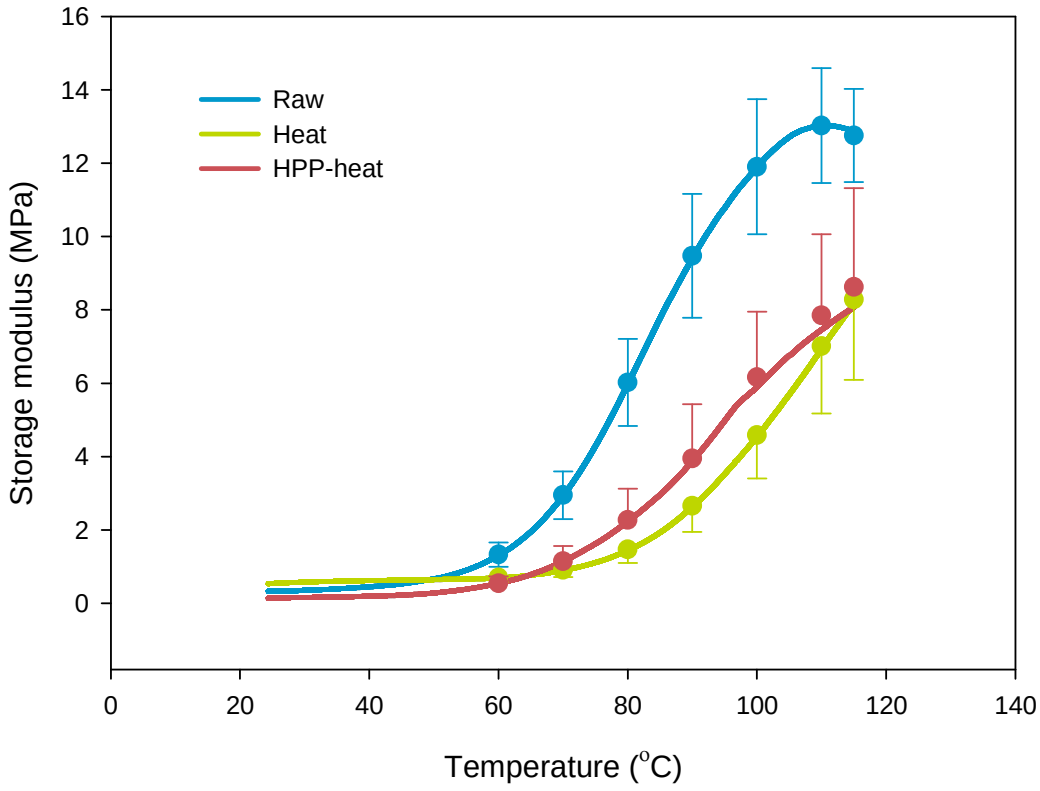


B.

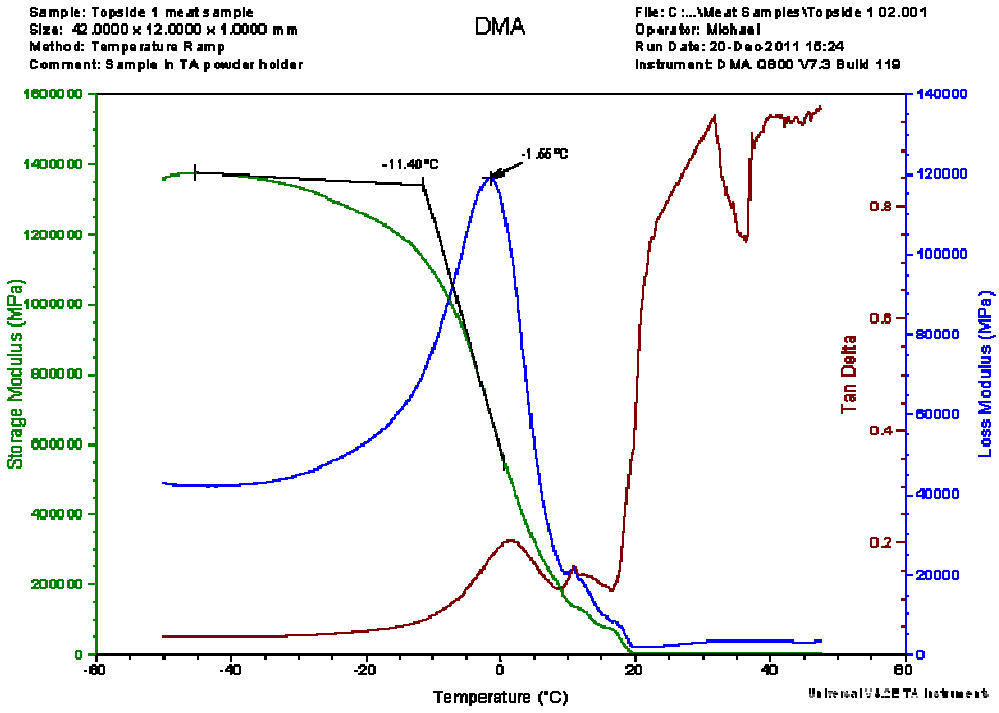


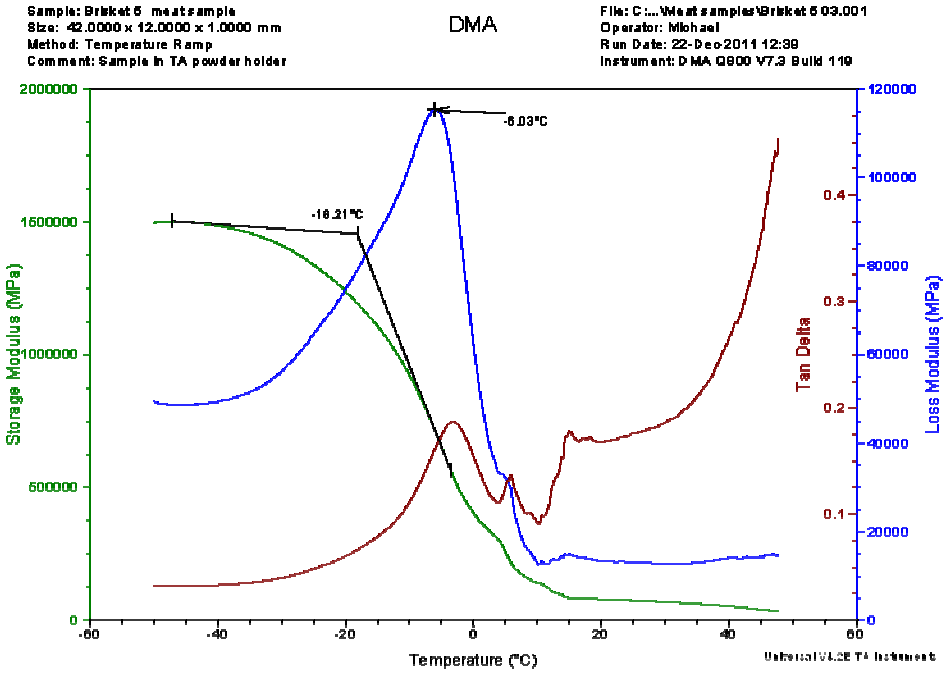
Appendix 6: Dynamic mechanical analysis (DMA)

Preliminary results of differences in raw (untreated), heated (60°C, 20 min) and HPP-heat-treated (200 MPa, 60°C, 20 min) beef neck muscle.

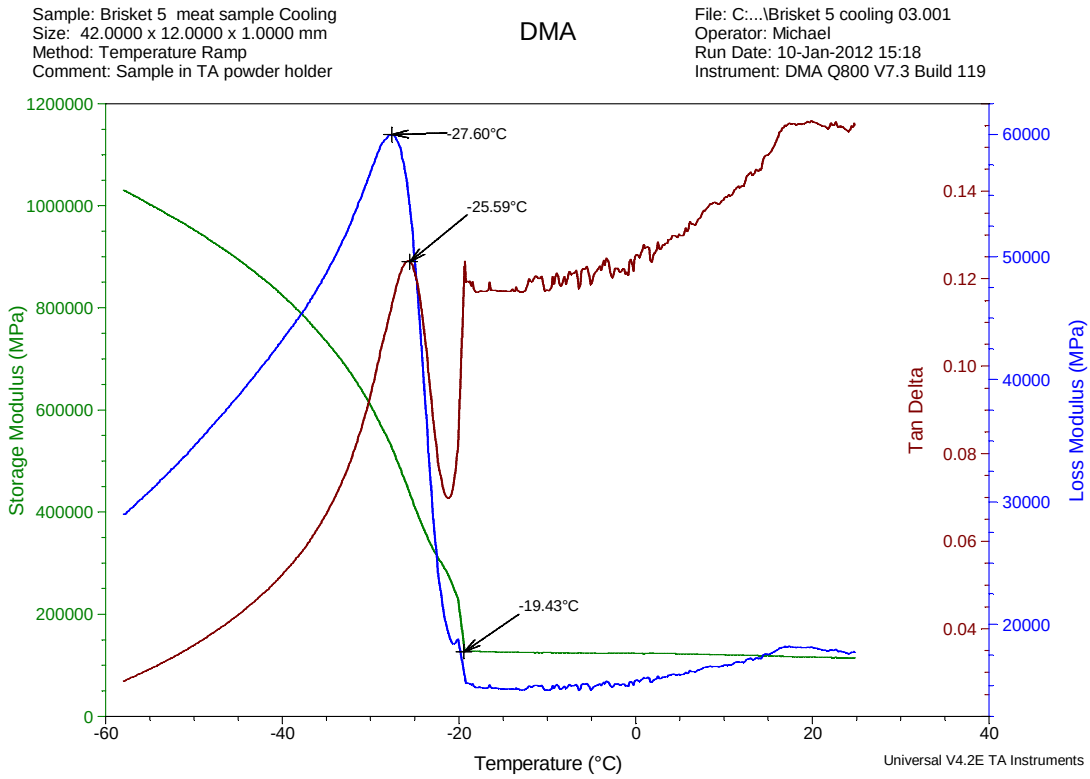


Appendix 7: Examples of the examination of topside (top) and brisket (bottom) muscle for the determination of the glass transition temperature (Tg) using the DMA heating method.





Appendix 8: An example of the examination of brisket muscle by DMA for the determination of glass transition temperature (Tg) using the cooling method.



Milestone Report 3A Optimisation of HPP conditions at low temperature for tenderisation

milestone report

MLA project code:	**A.MQT.0053**
MLA project title:	**VFC and HPP tenderisation mechanisms**
Project leader:	**Robyn Warner / Anita Sikes**
MLA project manager/coordinator:	**Phil Franks / Michael Lee**
Milestone number:	**3A**
Date:	**29 June 2012**

Milestone

A progress report submitted on the processing effects on the tenderness of selected beef muscles.

- Outline the optimum processing conditions (pressure, temperature) for tenderisation of brisket and biceps femoris muscles
- Report on the commissioning and calibration of Julabo waterbath for VFC system

Abstract

Inconsistent results of texture between muscles treated with high pressure processing (HPP) have been previously reported. One factor noted as being different between these trials was that HPP-treated muscles had been frozen prior to cooking. An experiment was designed to investigate if freezing after HPP treatment impacted on the texture of the cooked product. Results presented show that there was no effect of freezing muscles after HPP treatment (at low and high temperature) on the texture of brisket or topside.

The second aim of this work was to define the optimum processing conditions for selected muscles. Brisket and outside flat muscles were subjected to pressures between 0.1 and 600 MPa at temperatures between -18°C and 8°C for 5 minutes and the texture after cooking was measured by the Warner-Bratzler shear force method. For logistical purposes, initial trials involved high pressure treatment (0.1 – 600 MPa) at 4°C of frozen muscles (-18°C). It was found that there was no difference in the texture of brisket and outside flat muscle when pressure was applied to either fresh or frozen muscle.

Further experimental work investigated the effect of applying a range of pressures (0.1 – 600 MPa) at temperatures between 0°C and 8°C on the texture of brisket and outside flat muscles. For both muscles, the main effect impacting texture was from the pressure applied, irrespective of the temperature of processing. The application of higher pressures (500 and 600 MPa) to brisket and outside flat muscles resulted in toughening of meat after cooking, as measured by peak force. The results from this work suggest that the optimum pressure for tenderisation of brisket muscle at low temperature is within the range of 200 to 300 MPa, and 50 – 200 MPa for outside flat muscle.

Project objectives

To design a process that will facilitate tenderisation within the first 24 hours post-slaughter for both low- and high-value beef cuts.

Outcomes:
1. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to VFC
2. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to HPP

Success in achieving milestone

In a recent project report (A.MPT.0041 Final Report), inconsistencies were observed between muscles, depending on whether HPP was applied at high (60°C) or low (4°C) temperature, as well as differences in results from the objective texture measurements. It was suggested that structural, pH and fibre type differences between the muscle types and the effect of freezing on cooked texture could be factors to consider. As inconsistencies in texture results compared to previous work have occurred when pressure-treated muscle samples had been frozen prior to cooking (for logistical reasons), the first part of this milestone report (Section 5) outlines the effects of freezing after pressure treatment (low and high temperature) on the texture of *Pectoralis profundus* (brisket) and *M. semimembranosus* (topside) muscle.

Discussions with MLA (January 30, 2012) resulted in suggested pressure-temperature ranges of 50 – 600 MPa and -18 – 10°C; the temperature noted being the initial temperature of the meat. Initially, to narrow down the temperature range to investigate, the intention was to investigate the effect of pressure-treating frozen (-18°C) brisket, at pressures between 50 and 600 MPa for 5 minutes duration. It was also suggested that two muscles be investigated under these conditions; a delay in experiments was experienced in waiting for a decision/confirmation from MLA on a second muscle to investigate, in addition to brisket. A decision was made to use the *Biceps femoris* (outside flat) as it is low-value, easily accessible and large enough to cut many samples from. Trials were conducted investigating the effect of pressure-treating frozen outside flat on cooked texture. Therefore, Section 6 outlines results of pressure treatment of frozen brisket and outside flat on texture. Subsequently, pressures (50 – 600 MPa) applied at temperatures between 0°C and 8°C (temperature of compression fluid) were investigated on the texture of brisket and outside flat (Section 7).

The last section (Section 8) describes the commissioning and calibration of the waterbaths for the VFC system.

Effect of freezing after HPP on the texture of brisket and topside

The aim of this experiment was to investigate the effect of freezing on the texture of high pressure-treated beef (at low and high temperature) as a means of explaining previous inconsistencies observed in texture of pressure-treated samples.

Paired, post-rigor topside muscles (cap off/inside red) were collected from six animals from Teys Pty Ltd (Beenleigh). Portions (2.5.x 2.5 x 10 cm, approximately 80g) were randomly allocated to treatments:

Treatment	*Storage*
Control - low (CL)	Fresh
Heat control (HC) (60°C, 20 min)	Fresh
HPP at low temp (400 MPa, 4°C, 5 min) (PL)	Fresh
HPP at high temp (200 MPa, 60°C, 20 min) (PH)	Fresh
Control – high (CH)	Fresh
Control - low (CL)	Frozen
Heat control (HC)	Frozen
HPP at low temp (400 MPa, 4°C, 5 min) (PL)	Frozen
HPP at high temp (200 MPa, 60°C, 20 min) (PH)	Frozen
Control – high (CH)	Frozen

Weights and pH were recorded pre- and post-treatment. After cooling for 10 minutes in an ice slurry after pressure treatment, fresh samples were cooked using the Silex protocol (on the same day as processing) and stored at 5°C overnight prior to texture analysis. The remainder of the samples were vacuum-packed and stored at -18°C for 11 days. The samples were thawed at 4°C overnight and weights recorded. Samples were cooked and texture measurements performed the next day.

Analysis of variance (ANOVA) was used to determine the effects of pressure treatment and storage on texture as measured by the Warner-Bratzler method. All statistical analyses were performed using GenStat (GenStat Committee, 2008).

No significant difference in peak force (PF) was observed between fresh and frozen storage after pressure treatment for any of the treatments (Appendix 1, Figure 2). Subsequent analysis which excluded storage conditions indicated that pressure treatment (200 MPa) for 20 minutes at high temperature (60°C) significantly reduced (P<0.001) the peak force of topside muscle compared to both the control and the heat control (Appendix 1, Figure 3) whereas pressure treatment (400 MPa) for 5 minutes at low temperature (4°C) significantly increased (P<0.001) peak force compared to the untreated control sample (Appendix 1, Figure 3). The reduction in the PF value for pressure treatment at high temperature appears to be the result of the degradation of the myofibrillar component (IY) under these pressure and temperature conditions, with no effect of the connective tissue component (PF-IY) (Appendix 1, Figure 3). This confirms results from previous project work for topside muscle.

A similar experiment was designed using brisket muscle. Due to technical issues, samples for pressure treatment at low (4°C) and high (60°C) temperature were performed at different times (i.e. samples were from different animals) and are therefore analysed separately.

No significant difference in texture, as measured by Warner-Bratzler peak force, was found between fresh (unfrozen) and frozen brisket muscles which had been pressure-treated (400 MPa) for 5 minutes at low temperature (4°C) (Appendix 2, Figure 4). When the storage condition was removed from the analysis, there was no significant effect of pressure at low temperature on the peak force (PF) or the other texture parameters as measured by the WB measurement (Appendix 2, Figure 5).

Pressure treatment at high temperature (200 MPa, 60°C, 20 min) gave some conflicting results when fresh versus frozen samples were analysed. Control brisket samples (untreated) that had been stored frozen at -18°C for 11 days were significantly more tender (P=0.005) than fresh control brisket muscle whereas fresh brisket treated with pressure combined with heat was significantly more tender (P=0.005) than brisket that had been stored frozen after pressure treatment (Appendix 3, Figure 6). This was evidenced by lower peak force (PF) values. There was no effect of frozen storage on the texture of the heat control samples.

Effect of pressure–treating frozen brisket and outside flat on texture

The aim of this experiment was to investigate the effect on texture of high pressure–treated frozen beef (brisket and outside flat), by measuring Warner-Bratzler (WB) shear force, with the intention of narrowing the suggested temperature range (-18 – 10°C).

Paired, post-rigor brisket muscles (point end, deckle off) were collected from five animals from Teys Pty Ltd (Beenleigh). Portions (2 x 2 x 11 cm, approximately 60g) were cut from the left and right sides of muscles from each animal and randomly allocated to treatments:

- Control-fresh – untreated
- Control-frozen – untreated (stored at -18°C overnight for processing the next day)
- HPP-fresh – seven pressure treatments (50, 100, 200, 300, 400, 500, 600 MPa) applied at 4°C for 5 minutes to fresh (unfrozen) brisket samples on the same day as collection. The temperature during pressure treatment (4°C) relates to the temperature of the compression fluid.
- HPP-frozen – seven pressure treatments (50, 100, 200, 300, 400, 500, 600 MPa) applied at 4°C for 5 minutes to frozen (-18°C) brisket samples on the day after collection.

Due to the storage required for frozen samples, the fresh samples were all measured on the same day (the day of collection) while frozen samples were measured on the following day. For each day, i.e. fresh or frozen measurements, samples from within an animal were measured for WB texture in a batch with the order randomly allocated.

Measurements for pH, colour and weights were recorded pre- and post-treatment. Colour measurements on pre-treated samples were taken after blooming for 20 minutes at 4°C. After pressure treatment, samples were cooled for 10 minutes in an ice slurry and stored at 5°C until cooking. After cooling, pressure-treated frozen samples were stored at 5°C for 30 minutes for equilibration prior to physical measurements. Samples were cooked using the waterbath protocol (80°C/30 min; cooled 10 min in ice slurry) and weights recorded to determine cook loss. Samples were stored at 4°C overnight prior to texture analysis.

For the brisket experiment, analysis of variance was used to determine the effect of storage (fresh vs frozen) and pressure treatments on WB texture. Pressure treatments were nested within storage condition with the blocking used being animal nested within storage condition. Treatment, cook or total loss were also tested separately as a co-variate and were only retained if significant. All statistical analyses were performed using GenStat (GenStat Committee 2008).

Within the high pressure treatments of fresh brisket, the peak force value for the brisket pressure-treated at 400 MPa was significantly lower (P=0.05) than the untreated brisket and pressure treatment at 600 MPa resulted in a significantly higher (P=0.05) peak force than the fresh brisket treated at 400 MPa (Appendix 4, Table 1). When pressure was applied at 600 MPa to frozen brisket muscle, the peak force value was significantly increased (P=0.05) compared to untreated frozen brisket or brisket that was pressure-treated at 100 and 300 MPa (Appendix 4, Table 1). There was no significant enhancement of texture (i.e. reduction in peak force value) of frozen brisket with any of the pressures applied.

It was concluded from analyses of the effect of storage (i.e. fresh vs frozen) at a particular pressure, that there is no effect of storage condition on texture (peak force) of brisket at any given pressure (Appendix 4, Table 1).

The appearance of the fresh and frozen brisket after pressure treatment is shown in Appendix 5, Figure 7. As reported in previous project reports, an increase in pressure causes a discolouration of muscle, giving it a cooked appearance. Visually, there doesn't appear to be a difference in the colour between fresh and frozen brisket at a specific pressure. The internal colour of the muscle after pressure treatment of frozen brisket appears to be different from that of the surface colour, with the cut surface being darker and redder in colour, particularly with the application of higher pressures (Appendix 5, Figure 8).

The details and conditions were the same for the experiment using the outside flat muscles. Analysis of variance (ANOVA) was used to determine the effect of storage (fresh vs frozen) and pressure treatments on WB texture (Appendix 6, Table 2).

Pressure applied at low intensities (50 – 400 MPa) had no significant effect on the texture (peak force) of fresh (unfrozen) outside flat muscle compared to untreated controls. However, pressure applied at 500 and 600 MPa to fresh outside flat muscle at 4°C for 5 minutes had significantly higher (P<0.001) peak force values, indicating a tougher product. This increase in toughness appears to be due to the myofibrillar component, with a significant increase (P<0.001) in the IY value for muscles treated at these pressures (Appendix 6, Table 2).

When pressure was applied to frozen outside flat muscle, all pressures except 50 MPa resulted in a significant increase (P<0.001) in toughness as indicated by higher peak force values. The myofibrillar component of the muscle was again implicated in this increased toughness with significantly higher (P<0.001) IY values at these pressures. With the application of 300 MPa of pressure to frozen muscle, there was a significant reduction in the PF-IY value compared to the untreated control which indicates a modification to the connective tissue component of the muscle.

Effect of various pressure treatments (50-600 MPa) at low temperature (0°C-8°C) on the texture of brisket and outside flat

As the results of Section 6 indicate that there is no benefit of pressure-treating frozen muscle (brisket or outside flat) compared to fresh muscle on texture, the aim of this experimental work was to define the optimum pressure and temperature conditions for tenderisation, within a narrower range of temperature. The same muscles and range of pressure conditions were used as described in Section 6 (50 – 600 MPa) and the temperature range investigated was 0 – 8°C, noting that this was the temperature of the compression fluid. For logistical reasons, smaller numbers of replicates were processed and therefore the experiment repeated to increase statistical rigour.

Paired post-rigor muscles (brisket and outside flat) were collected on separate occasions from two animals from Teys Pty Ltd (Beenleigh). Therefore, there were four replicates for each muscle. Portions (25 x 25 x 100 mm) were cut from the left and right sides of muscles from each animal and randomly allocated to treatments, using a factorial design with seven pressures and three temperatures:

- Control – untreated
- HPP – pressure treatment (50, 100, 200, 300, 400, 500, 600 MPa) at temperatures (0, 4, 8°C) for 5 minutes duration on the day after collection. The temperature during pressure treatment (0 – 8°C) relates to the temperature of the compression fluid

Samples from within a replicate were randomly allocated to high pressure treatment at a given temperature and texture measurement by Warner-Bratzler shear force.

Measurements for pH, colour and weights were recorded pre- and post-treatment. Colour measurements on pre-treated samples were taken after blooming for 20 minutes at 5°C. After pressure treatment, samples were cooled for 10 minutes in an ice slurry and stored at 5°C until cooking. Samples were cooked using the waterbath protocol (80°C/30 min; cooled 10 min in ice slurry) and weights recorded to determine cook loss. Samples were stored at 5°C overnight prior to texture analysis.

Analysis of variance was used to determine the effect of pressure and temperature and any interactions on WB texture, as measured by the peak force value. The blocking used was experiment number (day of processing) and animal.

For both the brisket and the outside flat muscle, there was no significant effect ($P>0.05$) of processing temperature (0 – 8°C) on the texture of the pressure-treated (0.1 – 600 MPa) meat, as measured by Warner-Bratzler peak force (Appendix 7, Figures 9 and 10). Similarly for both muscles, the interaction of the range of pressures applied and the processing temperature was not significant ($P>0.05$) (Appendix 7, Figures 9 and 10). The main effect for each muscle was pressure treatment.

Analysis of pressure-treated brisket (Appendix 8, Table 3), showed that the application of 500 and 600 MPa of pressure resulted in a significant increase ($P<0.001$) in peak force compared to the untreated control. The application of pressures below 500 MPa did not significantly affect ($P>0.05$) the texture of brisket compared to the control. The texture of brisket muscle was similar when pressures of 50, 100 and 400 MPa was applied, and this peak force value was similar to the

untreated control. When comparisons were made across the treatments, the texture of brisket treated at 200 and 300 MPa were similar to each other but different to all other treatments. Although samples treated at 200 and 300 MPa were not significantly different to the untreated control, the peak force values were lower and the design of the experiment did not allow enough replicates of the control sample. From these analyses, it can be suggested that this pressure range (200 – 300 MPa) has the potential of reducing the peak force of brisket (i.e. tenderisation) across a processing temperature of 0 – 8°C.

Similar to brisket muscle, analysis of pressure-treated outside flat muscle showed that pressure applied at 500 and 600 MPa significantly increased (P<0.001) the peak force value, indicating a toughening the muscle compared to the untreated sample (Appendix 8, Table 4). Pressures applied at less than 500 MPa did not significantly affect texture compared to the control. Comparisons across treatments showed that the texture of outside flat samples were similar when pressure was applied at 50, 100, 200 and 300 MPa and that this was different (P<0.001) to the texture of samples treated at the higher pressures (400 – 600 MPa). These results suggest that for tenderisation of outside flat muscle using high pressure processing at low temperature that pressure in the range of 50 – 200 MPa is required.

System for VFC

Two Julabo F38-ME waterbaths (Figure 1) were purchased – one located at CFNS-Werribee and the other at CFNS-Coopers Plains. The waterbath located at Werribee was installed and tested to verify that its performance conforms to the manufacturer's specifications. The unit is designed to control the temperature of specific fluids in the bath tank. Testing was carried out using a bath fluid of water/propylene glycol mixture (3:5 by vol) to achieve the desired temperature of -3°C. The bath tank was filled with 40 litres of the fluid which is within the recommended filling level (the maximum filling volume of the waterbath is 45 litres). The temperature of the fluid at 6 different locations across the bath tank was continuously measured using calibrated T-type thermocouples interfaced a PC-based data acquisition system.

The results of the test showed a uniform temperature distribution across the bath tank with a temperature difference of less than 0.18°C between locations. It took about 2.75 hours to cool the fluid from its initial temperature of 20°C down to the desired level of -3°C and the temperature stability at this level was within $\pm$0.32°C. It appears that the cooling process was a bit slow due to the low capacity (i.e. 920-320W) of the cooling unit. In general, the unit was found to perform in accordance to its specifications. It is assumed that the second unit located at CFNS-Coopers Plains will operate under the same specifications.

Figure 1: Julabo waterbath purchased for the very fast chilling process. A waterbath is located at both Coopers Plains and Werribee.

Overall progress of the project

The project experienced some acknowledged delays in exploring the HPP conditions at low temperature and also in deciding on the second muscle to be investigated. This resulted in an agreed revised schedule, with Milestone 3 being split into two components, Milestone 3A (this report) and Milestone 3B.

Recommendations

As the optimum pressure conditions have been investigated for the tenderisation (as measured by Warner-Bratzler shear force) of two muscles (brisket and outside flat) at low temperature, and outlined in this progress report, the next stage of the project will able to be planned in detail. This involves characterisation of the effects of the optimum HPP conditions described here, using previously developed methods that were outlined in Milestone 2 report.

Due to the delay in planning the next stage of the work, a revised submission date of August 27 for Milestone 3B is proposed.

Appendix 1: Effect of freezing after high pressure treatment at low (4°C) and high (60°C) temperature on the texture parameters of topside

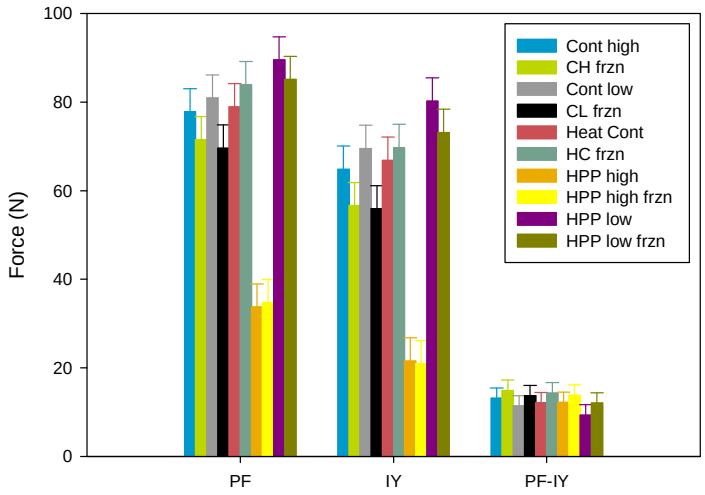


Figure 2: Warner-Bratzler (WB) texture parameters (PF, peak force, IY initial yield and PF-IY, peak force minus initial yield) of pressure-treated topside muscle. Mean ± SED, n=6.

Cont high = control muscle for pressure at high temperature (60°C), no pressure treatment, no freezing (fresh)
CH frzn = frozen control for pressure at high temperature, no pressure treatment, stored at -18°C for 11 days
Cont low = control muscle for pressure at low temperature (4°C), no pressure treatment, no freezing (fresh)
CL frzn = frozen control for pressure at low temperature, no pressure treatment, stored at -18°C for 11 days
Heat Cont = heat control; heated at 60°C for 20 min
HC frzn = frozen heat control, stored at -18°C for 11 days
HPP high = pressure treatment (200 MPa) at high temperature (60°C) for 20 min, no freezing (fresh)
HPP high frzn = pressure treatment (200 MPa) at high temperature (60°C) for 20 min followed by storage at -18°C for 11 days
HPP low = pressure treatment (400 MPa) at low temperature (4°C) for 5 min, no freezing (fresh)
HPP low frzn = pressure treatment (400 MPa) at low temperature (4°C) for 5 min followed by storage at -18°C for 11 days

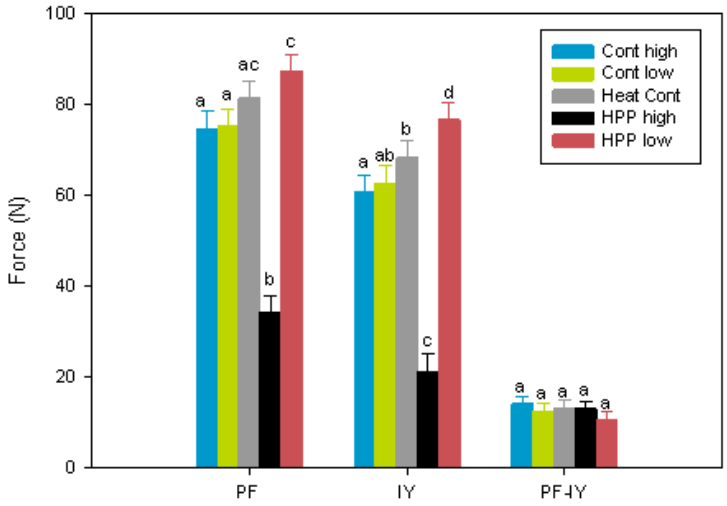


Figure 3: Effect of pressure at high (60°C) and low (4°C) temperature on Warner-Bratzler (WB) parameters (PF, peak force, IY, initial yield and PF-IY, peak force minus initial yield) of topside muscle, irrespective of storage (fresh vs frozen). Different letters within the same WB parameter indicates significance, $P < 0.001$.

Cont high = control muscle for pressure at high temperature (60°C), no pressure treatment, no freezing (fresh)
Cont low = control muscle for pressure at low temperature (4°C), no pressure treatment, no freezing (fresh)
Heat Cont = heat control; heated at 60°C for 20 min
HPP high = pressure treatment (200 MPa) at high temperature (60°C) for 20 min, no freezing (fresh)
HPP low = pressure treatment (400 MPa) at low temperature (4°C) for 5 min, no freezing (fresh)

Appendix 2: Effect of freezing after high pressure treatment at low (4°C) temperature on the texture parameters of brisket

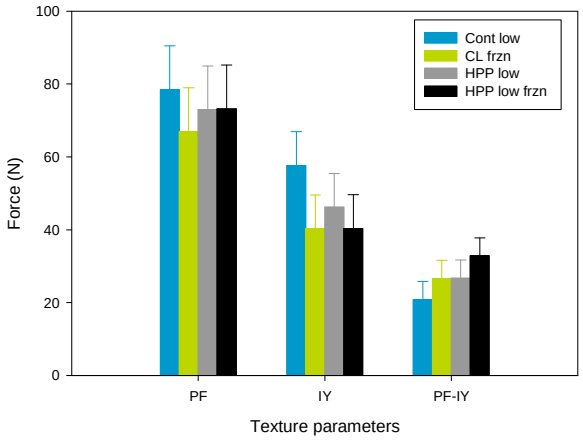


Figure 4: Warner-Bratzler (WB) texture parameters (PF, peak force, IY, initial yield and PF-IY, peak force minus initial yield) of brisket muscle pressure treated (400 MPa) for 5 minutes at low temperature (4°C). Mean ± SED, n=6.
CL = control muscle for pressure at low temperature (4°C), no pressure treatment, no freezing (fresh)
CL frzn = frozen control for pressure at low temperature, no pressure treatment, stored at -18°C for 11 days
HPP low = pressure treatment (400 MPa) at low temperature (4°C) for 5 min, no freezing (fresh)
HPP low frzn = pressure treatment (400 MPa) at low temperature (4°C) for 5 min followed by storage at -18°C for 11 days

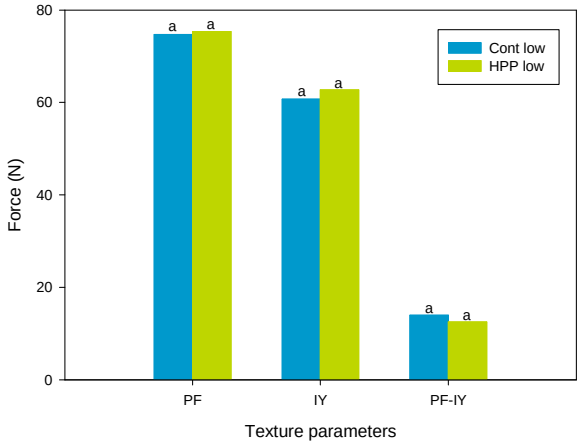


Figure 5: Warner-Bratzler (WB) texture parameters (PF, peak force, IY, initial yield and PF-IY, peak force minus initial yield) of brisket muscle pressure treated (400 MPa) for 5 minutes at low temperature (4°C), irrespective of storage. Mean ± SED, n=6.
Cont low = control muscle for pressure at low temperature (4°C), no pressure treatment, no freezing (fresh)
HPP low = pressure treatment (400 MPa) at low temperature (4°C) for 5 min, no freezing (fresh)

Appendix 3: Effect of freezing after high pressure treatment at high (60°C) temperature on the texture parameters of brisket

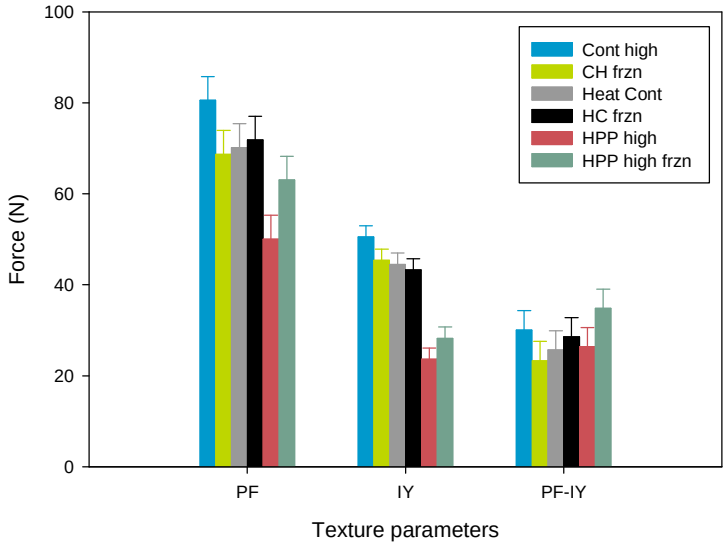


Figure 6: Warner-Bratzler (WB) texture parameters (PF, peak force, IY, initial yield and PF-IY, peak force minus initial yield) of brisket muscle pressure treated (200 MPa) for 20 minutes at high temperature (60°C). Mean ± SED, n=6.

Cont high = control muscle for pressure at high temperature (60°C), no pressure treatment, no freezing (fresh)
CH frzn = frozen control for pressure at high temperature, no pressure treatment, stored at -18°C for 11 days
Heat Cont = heat control; heated at 60°C for 20 min
HC frzn = frozen heat control, stored at -18°C for 11 days
HPP high = pressure treatment (200 MPa) at high temperature (60°C) for 20 min, no freezing (fresh)
HPP high frzn = pressure treatment (200 MPa) at high temperature (60°C) for 20 min followed by storage at -18°C for 11 days

Appendix 4: Effect of pressure treating fresh or frozen brisket at low (4°C) temperature on the texture parameters of brisket

Table 1: Mean Warner-Bratzler shear force parameters (natural log transformed), peak force (PF), initial yield, (IY) and peak force minus initial yield (PF-IY) of brisket muscle (fresh or frozen) after treatment (Control, Cont vs high pressure treatment at various pressures, 50, 100, 200, 300, 400, 500, 600 MPa, at 4°C for 5 minutes), n=5. Means for PF and PF-IY are adjusted for the co-variate treatment loss.

Values in parentheses are back-transformed means, with l.s.d. referring to the transformed data.

Storage	Fresh								Frozen								l.s.d. (*P*=0.05)	l.s.d. (*P*=0.05)
Treatment	Cont	50	100	200	300	400	500	600	Cont	50	100	200	300	400	500	600	For comparisons within a storage condition	For all other comparisons
PF (N)	4.44	4.30	4.38	4.31	4.38	4.24	4.34	4.42	4.30	4.39	4.31	4.35	4.28	4.38	4.40	4.48	0.166	0.207
	(84.61)	(73.92)	(79.52)	(74.51)	(79.52)	(69.55)	(76.71)	(82.68)	(74.00)	(80.56)	(74.66)	(77.71)	(71.95)	(79.44)	(81.78)	(88.15)		
IY (N)	3.95	3.84	3.94	3.71	3.55	3.70	3.94	4.16	3.88	3.83	3.83	3.86	3.89	4.10	4.23	4.36	0.146	0.212
	(52.09)	(46.39)	(51.32)	(40.89)	(34.71)	(40.33)	(51.21)	(63.75)	(48.62)	(45.92)	(46.15)	(47.61)	(48.96)	(60.46)	(68.65)	(78.02)		
PF-IY (N)	3.34	3.18	3.20	3.41	3.72	3.26	3.11	2.90	3.27	3.62	3.42	3.45	3.14	3.02	2.97	2.80	0.460	0.479
	(28.08)	(23.95)	(24.51)	(30.36)	(41.10)	(26.10)	(22.49)	(18.10)	(26.34)	(37.15)	(30.51)	(31.34)	(23.17)	(20.49)	(19.41)	(16.44)		

Appendix 5: Effect on colour (appearance) of fresh (unfrozen) and frozen brisket after pressure treatment (0.1 – 600 MPa) at 4°C for 5 minutes.

Figure 7: Appearance of fresh (unfrozen) and frozen brisket muscle after pressure treatment (Control 0.1 MPa, 50, 100, 200, 300, 400, 500 and 600 MPa) at 4°C for 5 minutes.

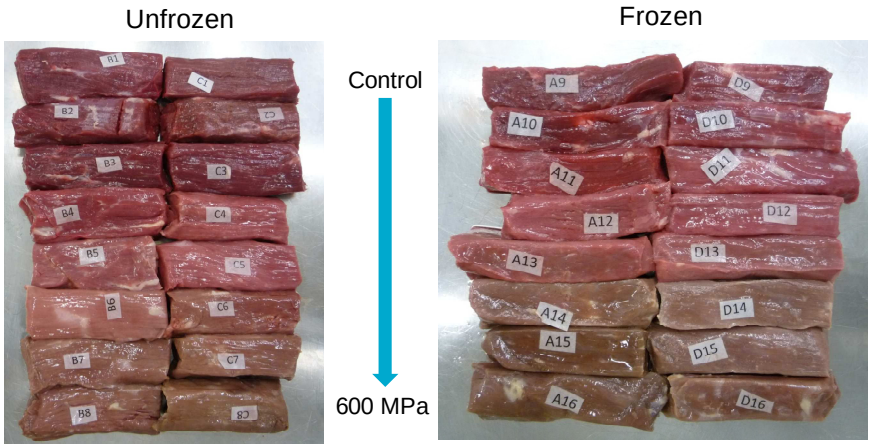


Figure 8: Appearance of frozen brisket muscle after pressure treatment (0.1 – 600 MPa) at 4°C for 5 minutes. Portions of muscle on the left side of the picture show the colour of the cut surface.

Appendix 6: Effect of pressure treating fresh or frozen outside flat at low (4°C) temperature on the texture parameters of brisket

Table 2: Mean Warner-Bratzler shear force parameters, peak force (PF), initial yield, (IY) and peak force minus initial yield (PF-IY) of outside flat muscle (fresh or frozen) after treatment (Control, Cont vs high pressure treatment at various pressures, 50, 100, 200, 300, 400, 500, 600 MPa, at 4°C for 5 minutes), n=5.

Storage	Fresh								Frozen								l.s.d. (*P*=0.05)
Treatment	Cont	50	100	200	300	400	500	600	Cont	50	100	200	300	400	500	600	
PF (N)	78.8	84.1	81.0	74.3	82.7	89.4	106.8	109.7	61.4	59.3	80.7	85.1	88.9	84.9	97.2	82.8	17.80
IY (N)	65.1	68.1	64.7	60.0	68.3	71.3	93.4	72.9	44.9	46.3	70.0	71.5	81.0	72.7	82.8	95.3	19.35
PF-IY (N)	13.7	16.1	16.3	14.4	14.5	18.1	13.4	9.9	16.5	13.0	10.7	13.6	7.9	12.2	14.4	14.4	7.37

Appendix 7: Effect of various pressures applied at low temperature on the texture of brisket and outside flat muscle

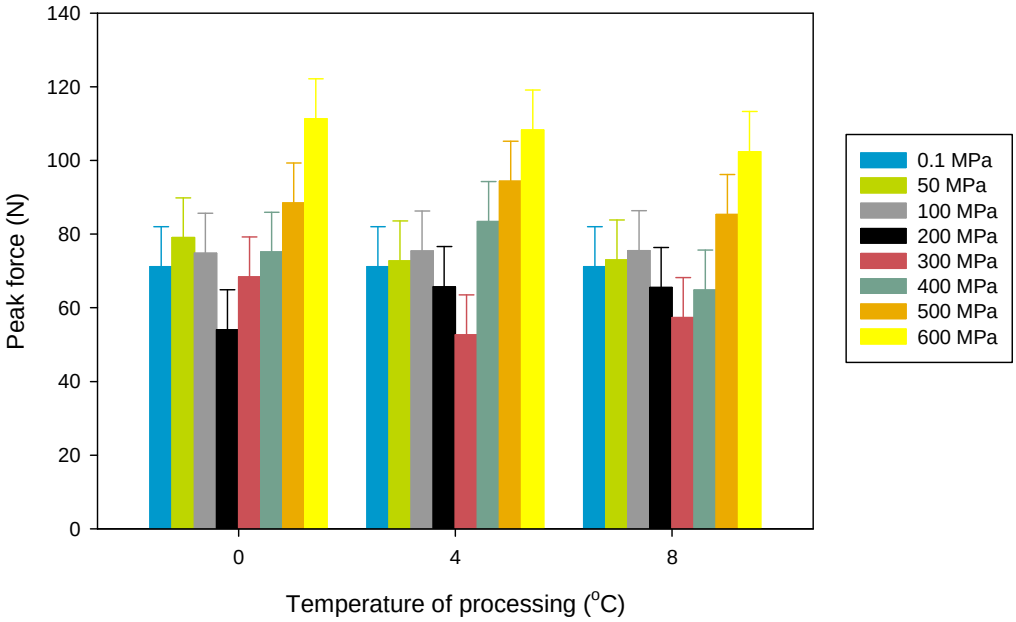


Figure 9: The effect of pressure (0.1 – 600 MPa) applied at various temperatures (0 – 8°C) on the texture, as measured by Warner-Bratzler peak force, of brisket muscle. Mean ± SED, n=4.

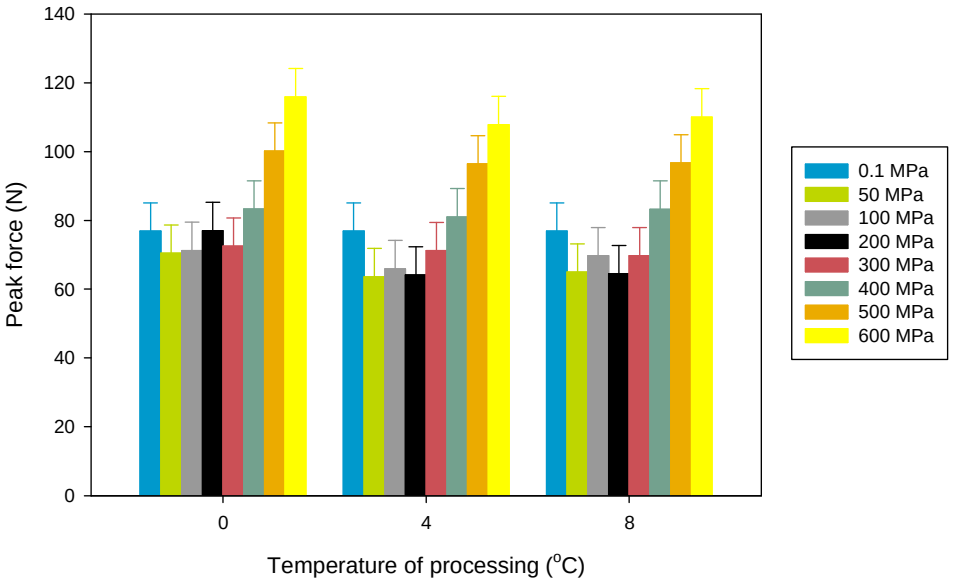


Figure 10: The effect of pressure (0.1 – 600 MPa) applied at various temperatures (0 – 8°C) on the texture, as measured by Warner-Bratzler peak force, of outside flat muscle. Mean ± SED, n=4.

Appendix 8: Statistical analysis of the main effect (pressure treatment) of pressure-treating brisket and outside flat muscle

Table 3: Mean Warner-Bratzler peak force values of brisket muscle after treatment, irrespective of temperature of treatment (Control, 0.1 MPa vs high pressure treatment at 50, 100, 200, 300, 400, 500, 600 MPa), n=4.

Treatment (MPa)								l.s.d. (*P*=0.05) For comparisons with the control	l.s.d. (*P*=0.05) For all other comparisons
0.1	50	100	200	300	400	500	600		
71.17	74.92	75.26	61.78	59.46	74.45	89.39	107.34	17.722	12.531

Table 4: Mean Warner-Bratzler peak force values of outside flat muscle after treatment, irrespective of temperature of treatment (Control, 0.1 MPa vs high pressure treatment at 50, 100, 200, 300, 400, 500, 600 MPa), n=4.

Treatment (MPa)								l.s.d. (*P*=0.05) For comparisons with the control	l.s.d. (*P*=0.05) For all other comparisons
0.1	50	100	200	300	400	500	600		
76.86	66.33	68.95	68.53	71.13	82.51	97.74	111.23	13.452	9.512

Milestone 3B: Characterisation of Low Temperature HPP beef muscle

milestone report

MLA project code:	**A.MQT.0053**
MLA project title:	**VFC and HPP tenderisation mechanisms**
Project leader:	**Robyn Warner / Anita Sikes**
MLA project manager/coordinator:	**Phil Franks / Michael Lee**
Milestone number:	**3B**
Date:	**26 October 2012 (updated)**

Milestone

A progress report submitted on the processing effects on the tenderness of selected beef muscles.

- Characterise the effects of agreed (optimum) HPP conditions applied to post-rigor beef, using developed methods, for textural, structural and biophysical changes

Abstract

High pressure applied at low temperature to brisket and outside flat muscles has no effect on the sarcomere length of either muscle compared to the untreated samples. The optimal processing conditions previously identified for each muscle (Milestone 3A) resulted in a reduction in the peak force values. However, the improvement in tenderness would not be substantial enough for consumer acceptability for texture of the product. The textural changes of the pressurised meat appear to be the result of the effect of treatment on the myofibrillar component of the muscle. Thermal stability studies of brisket muscle using differential scanning calorimetry indicates that pressure applied at 300 MPa at 4°C partially denatures myosin and totally denatures actin. The connective tissue fraction appears to be largely unaffected by pressure treatment, although muscle differences are apparent in respect to the effect of HPP on connective tissue. Pressurised muscles had reduced water-holding capacity and increased cooking losses compared to untreated muscle. High pressure processing under low temperature conditions does not appear to provide a beneficial improved quality meat product for the meat industry.

Project objectives

To design a process that will facilitate tenderisation within the first 24 hours post-slaughter for both low- and high-value beef cuts.

Outcomes:

1. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to VFC
2. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to HPP

Success in achieving milestone

The research outcomes and completion of Milestone 3A were essential for directing the planning and experimental details required for research work to complete Milestone 3B. As the completion of Milestone 3A was delayed, this has obviously impinged on the completion of Milestone 3B. Also, the analyses of samples from two muscles involved comprehensive and time-consuming laboratory work, therefore some delay in reporting of these analyses was experienced.

Materials and methods

Experimental design

The experimental plan used for the project work required to complete Milestone 3B is outlined below (Figure 1). Two muscles (brisket, *M. pectoralis profundus*, H.A.M. 2353 and outside flat, *M. biceps femoris*, H.A.M. 2050) from 4 animals were processed using this experimental design, with the HPP conditions being 4°C (compression fluid) for 5 minutes and the pressure varied according to the optimal conditions for tenderisation for each muscle as outlined in Milestone 3A. This was 300 MPa for brisket and 50 MPa for outside flat. Biophysical measurements on control and pressure-treated raw, whole muscle included sarcomere length (SL), water-holding capacity (WHC), myofibrillar fragmentation index (MFI) and protein denaturation using differential scanning calorimetry (DSC). Texture analysis using the Warner-Bratzler (WB) shear force method was done on whole muscle samples after cooking. Extracted fractions (sarcoplasmic, myofibrillar and connective tissue) were prepared either before high pressure treatment (i.e. fractions extracted from untreated muscle and the individual fractions subjected to pressure) or after the muscle was pressure-treated. The experimental procedure used for sample preparation and analysis was the same for brisket and outside flat muscles.

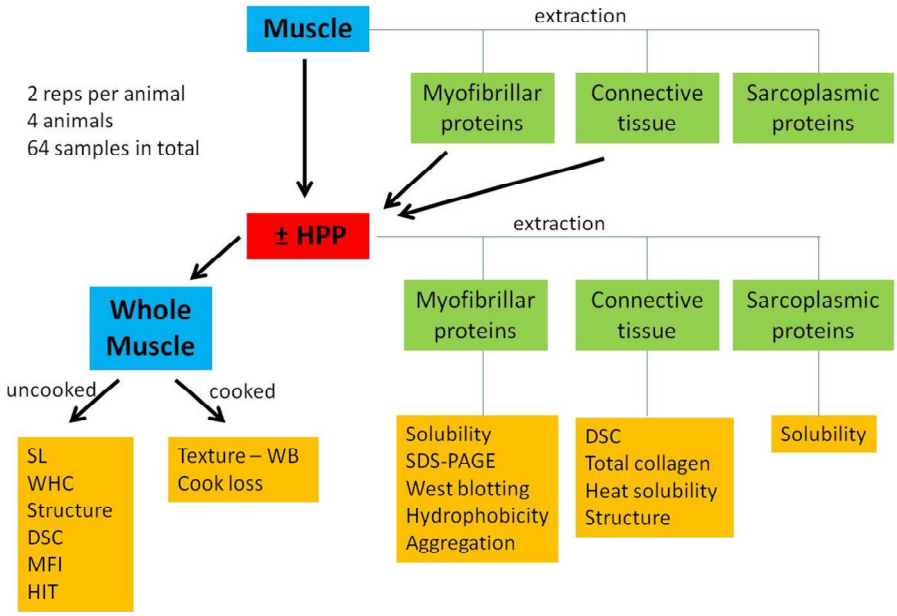


Figure 1: Experimental plan for Milestone 3B. SL – sarcomere length; WHC – water-holding capacity; DSC – differential scanning calorimetry; MFI – myofibrillar fragmentation index; WB – Warner-Bratzler shear force

Samples were randomly allocated to 8 treatments (outlined below), with 2 replicates from the left and right sides of muscles from each animal, as outlined below and in Figure 1. Therefore, there were 16 samples from each animal, a total of 64 samples for each muscle.

The treatments were:

- Control raw whole muscle
- HPP raw whole muscle
- Control whole muscle cooked
- HPP whole muscle cooked
- Control extracted fractions (S, sarcoplasmic; M, myofibrillar; C, connective tissue) (extracted before treatment)
- HPP extracted fractions (M, C, S) (extracted before treatment)
- Control extracted fractions (M, C, S) (extracted after treatment)
- HPP extracted fractions (M, C, S) (extracted after treatment)

Sample preparation:

Muscles were trimmed of subcutaneous fat and connective tissue (epimysium) and cut into portions approximately 25 x 25 x 100 mm. Colour measurements were taken on cut ends (Minolta CR-400, light source D65) following blooming at 5°C for 20 minutes. The pH of muscle portions was also recorded. Samples were weighed and samples identified for extraction were processed immediately; samples for HPP treatment were vacuum packed and stored overnight at 5°C until processing the following day.

Extraction

Extraction of muscle protein fractions was carried out according to Busch et al., (1972). Briefly, 20 g of minced muscle was homogenised in 200 mL extraction buffer (50 mM Tris-HCl pH 7.0, 100 mM KCl, 5 mM EDTA) in a Waring blender for 15 s at low speed, followed by 15 s at high speed. All extraction steps took place at 5°C. The homogenate was centrifuged (1000 g/10 min/4°C) and the supernatant was retained (sarcoplasmic fraction) and the pellet resuspended in 100 mL extraction buffer. After 3 washes, the homogenate was filtered through nylon mesh to collect the connective tissue fraction. After a further 2 washes, the myofibrillar fraction was collected.

High pressure treatment

Pressure was applied to whole muscle and extracted protein fractions of brisket samples (300 MPa) and outside flat samples (50 MPa) at 4°C for 5 min on the day after collection. Muscle samples were vacuum-packed; extracts (sarcoplasmic, myofibrillar and connective tissue) were heat-sealed (double-bagged) to reduce the incidence of leakage into the HPP vessel.

Cooking

Whole muscle samples were cooked at 70°C in a waterbath for 30 min and cooled in an ice slurry for 10 min, with weights recorded to determine cook loss. Samples

were stored at 5°C overnight prior to texture analysis by the Warner-Bratzler (WB) shear force measurement.

Measurements

Whole muscle

Sub-samples from the left and right sides from each animal were collected for total collagen content. Sub-samples of the raw muscle were taken after treatment (control or HPP) for:

- Sarcomere length
- Drip loss (hanging method)
- Differential scanning calorimetry (DSC)
- Myofibrillar fragmentation index (MFI)
- Remaining muscle was frozen

Sarcoplasmic fraction (S)

The volume of the sarcoplasmic fraction of each sample was recorded and samples subsequently frozen at -20°C until further analysis.

Myofibrillar extract (MF)

After extraction, an aliquot (1 mL) of the MF extract was frozen for the determination of the protein concentration. After treatment (control or HPP), myofibrillar extracts were stored at -20°C in a solution of 50% glycerol suspended in extraction buffer with 1 mM dithiothreotol (DTT), until required for analysis of:

- Solubility
- Protein composition (SDS-PAGE, Western blotting)
- MFI
- Hydrophobicity
- Aggregation

Connective tissue extract (CT)

A portion of CT (0.5 g) was taken after extraction for total collagen determination.
After treatment (control or HPP), a sub-sample was frozen in extraction buffer for DSC analysis and the remaining sample was freeze-dried for total collagen and heat solubility measurements.

Characterisation of brisket

Whole muscle

The total amount of connective tissue in the muscle was determined by measuring the hydroxyproline content in lyophilised muscle according to the International Standard method (ISO 3496:1994). The average total collagen content of the brisket muscle from the four animals used in this study was 2.52% (± 0.12 SE). The sarcomere length of the muscle before and after high pressure treatment was very similar (2.53 µm ± 0.173 and 2.52 µm ± 0.090, respectively).

Pressure applied at 300 MPa to brisket muscle resulted in a significant (*P*=0.010) decrease in the peak force value (PF), indicating more tender muscle due to HPP (Figure 2). As seen in previous work, pressure resulted in a significant (*P*<0.001) reduction in the IY peak which is affected predominantly by treatments influencing the myofibrillar component of muscle. The peak force minus initial yield (PF-IY) value which reflects the contribution of connective tissue to the overall texture of muscle, was significantly (*P*=0.003) increased in brisket muscle by high pressure treatment.

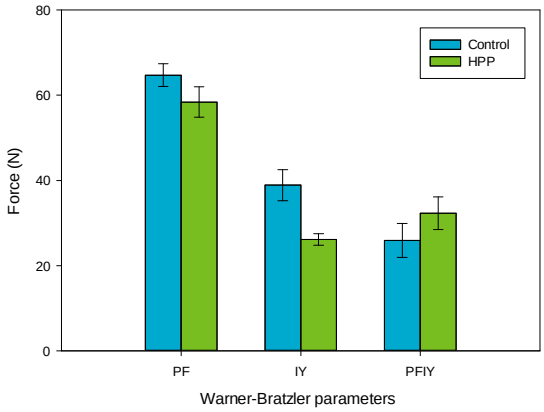


Figure 2: The effect of high pressure processing (300 MPa, 4°C, 5 min) on the texture of brisket muscle as measured by the Warner-Bratzler shear force method. PF – peak force; IY – initial yield; PFIY – peak force minus initial yield. Mean ± SE, n=4.

Yields (losses) were measured at several steps along the processing chain. The application of pressure resulted in increased moisture losses compared to the untreated control sample (Table 1). Drip loss which is an indication of the water-holding capacity (WHC) of the muscle was also higher in the pressure-treated brisket muscle (Table 1). This was measured using the hanging method, as shown in Figure 3.

Table 1: Moisture losses at different stages of processing of brisket muscle, control (untreated) or pressure treated (HPP; 300 MPa, 4°C, 5 min). Mean ± SE

Treatment	Treatment loss (%) [a]	Drip loss (%) [b]	Cook loss (%) [c]	Total loss (%) [d]
Control	0.9 ± 0.12 [x]	1.1 ± 0.08 [x]	14.8 ± 0.41 [x]	15.9 ± 0.48 [x]
HPP	1.4 ± 0.11 [y]	1.7 ± 0.13 [y]	23.1 ± 0.69 [y]	24.4 ± 0.76 [y]
P-value	0.004	<0.001	<0.001	<0.001

a loss during treatment, i.e., control (untreated) or HPP
b drip loss as calculated by the hanging method
c loss after cooking
d overall loss due to treatment and cooking
Columns with different letters indicate significance

Figure 3: The hanging method for measuring drip loss of whole muscle.

When beef muscle is heated in a differential scanning calorimeter (DSC), three major endotherms are usually observed. These events are attributed to the thermal denaturation of muscle proteins. The first transition has been attributed to myosin. The second transition was assigned to collagen and the sarcoplasmic proteins, although both isolated actomyosin and myosin and its subunits undergo transitions in this same temperature range. The third transition has been consistently assigned to actin. These three transitions are clearly indentified in the control sample in Figure 4 (Peaks 1, 2 and 3, respectively).

The application of pressure had no effect on the transition temperature of the myosin endotherm (Table 2, Peak 1) but there was a reduction in the enthalpy. In contrast, pressure resulted in an increase in the enthalpy of Peak 2, which is associated with the sarcoplasmic and connective tissue fractions. Pressure applied at 300MPa to brisket muscle totally denatured actin (Peak 3) (Table 2 and Figure 4). The total enthalpy change was significantly lower for the pressure-treated samples, probably due to the denaturation of actin during high pressure processing.

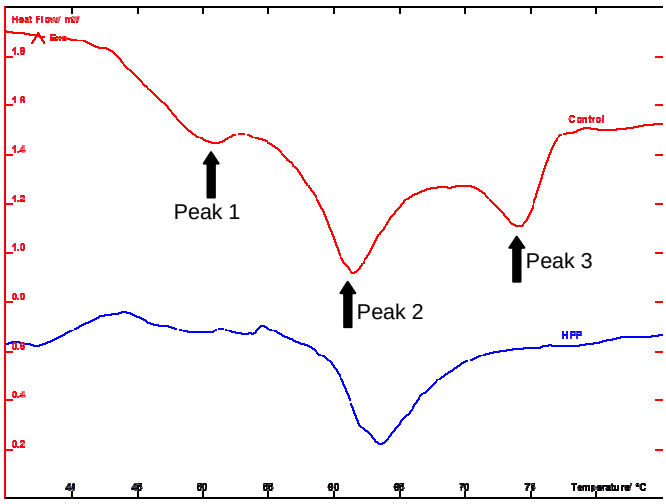


Figure 4: An example of DSC thermograms of control (untreated) and HPP (high pressure processing, 300 MPa, 4°C, 5 min) brisket muscle.

It was apparent in some of the samples after pressure treatment that a new low melting transition was seen around 35°C (Figure 5). A rescan was carried out on selected control and treated samples to check if there was any reversible modifications of the proteins (Figure 6). On rescanning, there was little evidence of new structures seen in HPP samples being retained or reformed on cooling (Figure 6B).

Table 2: DSC analysis of control (untreated) and pressure treated (300 MPa, 4°C, 5 min) brisket muscle. T_m, denaturation temperature; ΔH, enthalpy. Mean ± SE, n=8.

Transition	DSC Parameter	Treatment	
		Control	HPP
Peak 1[a]	T_m (°C)	52.1 ± 0.27	51.0 ± 0.62
	ΔH (J/g)	0.82 ± 0.047	0.31 ± 0.032
Peak 2 [b]	T_m (°C)	62.1 ± 0.42	63.2 ± 0.14
	ΔH (J/g)	0.79 ± 0.031	0.92 ± 0.069
Peak 3 [c]	T_m (°C)	74.5 ± 0.14	indistinct
	ΔH (J/g)	0.54 ± 0.017	indistinct
Total	ΔH (J/g)	4.95 ± 0.161	2.30 ± 0.228

[a] myosin
[b] sarcoplasmic proteins and connective tissue
[c] actin

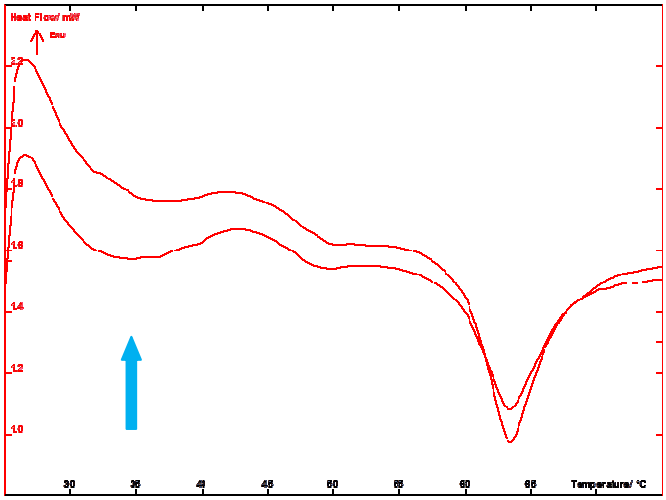


Figure 5: DSC thermograms of duplicate scans from the same high pressure-treated brisket muscle sample. The arrow indicates a potential new peak formed around 35°C.

(A) **(B)**

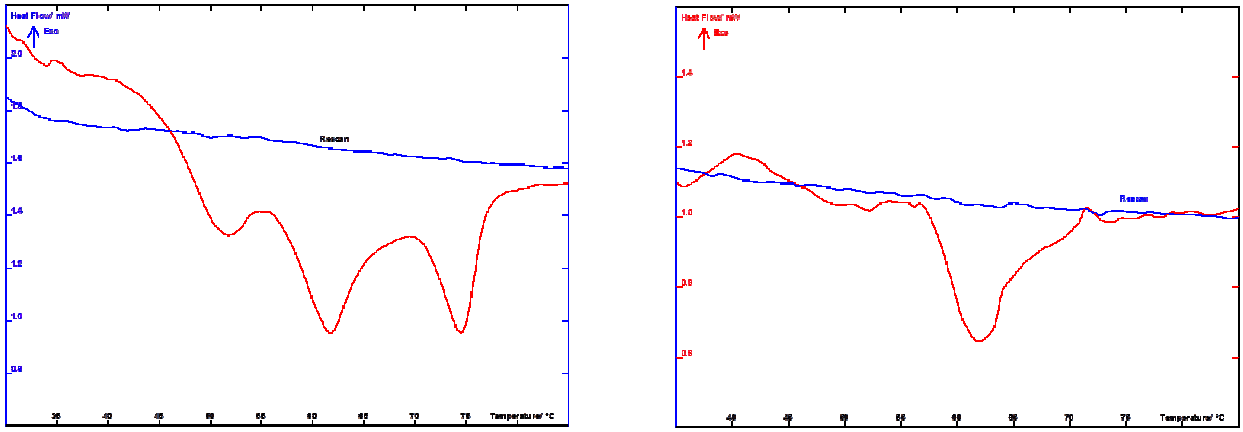


Figure 6: DSC thermograms of control brisket muscle (A) and high pressure-treated brisket muscle (B), showing corresponding rescans of the samples.

In order to visually examine any differences in muscle with high pressure treatment, homogenates of whole brisket muscle were viewed by light microscopy. Homogenates of untreated muscle clearly showed the extraction of many myofibres (Figure 7). The 'before' and 'after' extraction treatments for the control samples were essentially the same, so there was no difference (P>0.05) in the mean fibre length for these samples; 220 µm and 188 µm, respectively (Figure 8Figure). Similarly, the mean fibre length for the 'before' treatment for the HPP samples was 235 µm (Figure 8). This would be expected as the muscle homogenates were prepared before HPP treatment, so in essence, no different to the control treatment. However, homogenates prepared from the pressure-treated muscle were very different to the untreated, control homogenates. It can be seen that the fibre lengths have been degraded into smaller fragments (mean 121 µm, P=0.002) (Figure 8) by high pressure treatment and that the cut ends are very distinct (sharp) compared to the control samples. This phenomena was also reported for muscle homogenates of beef muscle after combined pressure-heat treatment (Sikes et al., 2010).

There was no significant difference (P>0.05) in muscle fibre width between any of the treatments (Figure 8).

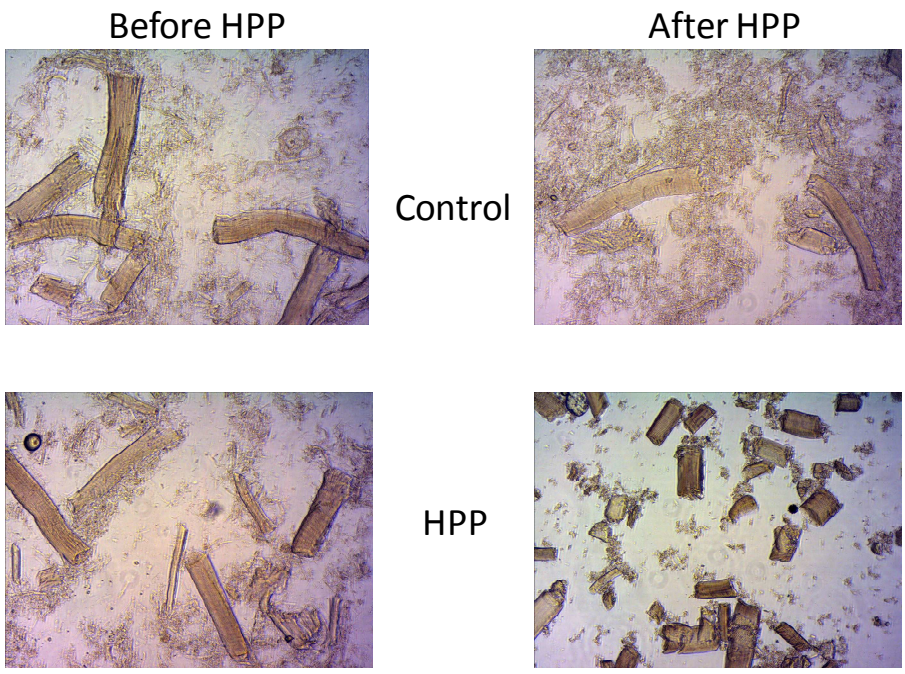


Figure 7: Whole muscle homogenates viewed by light microscopy. Left, homogenates prepared before HPP. Right, homogenates prepared after HPP. Top, Control (untreated) samples. Bottom, HPP (300 MPa, 4°C, 5 min).

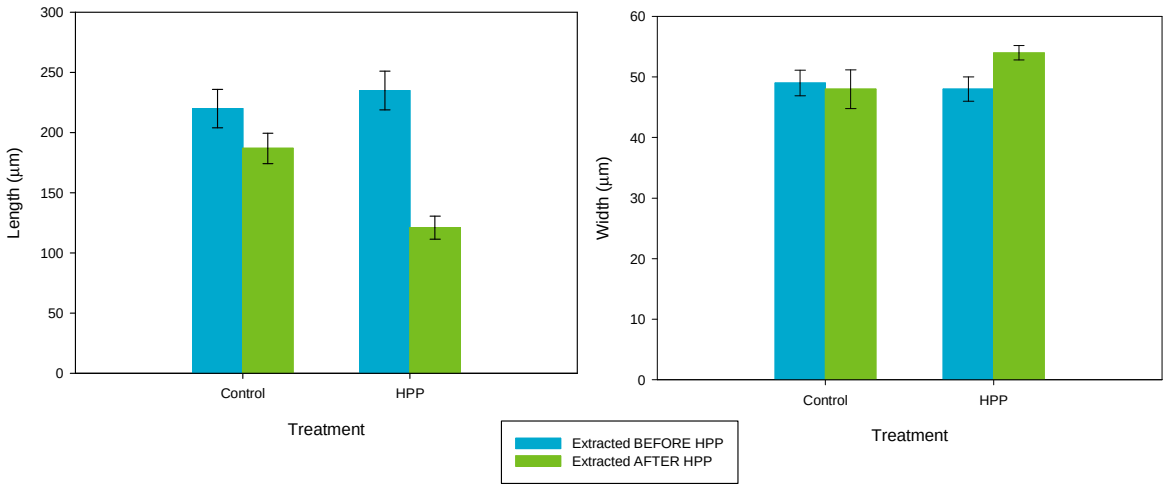


Figure 8: The effect of treatment (Control, untreated; HPP, 300 MPa, 4°C, 5 min) and time of extraction (homogenates prepared before or after HPP treatment) on muscle fibre lengths (left) and widths (right) of brisket muscle, viewed and measured using light microscopy. Mean ± SE, n=4.

Extracted fractions

On initial inspection of the extracted fractions after pressure treatment, it was difficult to observe any obvious differences (Figure 9). However, it appeared that the connective tissue had solubilised to a slight degree and this was also evidenced by the viscous nature of the solution when removed from the packaging. Precipitation

and/or aggregation of proteins in the sarcoplasmic fraction occurred after standing for some time.

Figure 9: Extracted protein fractions from brisket muscle, Sarc, sarcoplasmic; MF, myofibrillar; Coll, connective tissue fractions. Left, before pressure treatment. Right, after pressure treatment.

The thermal stability of the extracted connective tissue fraction after the muscle was pressure-treated was measured using DSC. The results suggest that there was no difference in the onset (T_o) or denaturation temperatures (T_m) compared to the connective tissue from the untreated muscle (Table 3, Figure 10). However, there was a significant (*P*=0.004) reduction in the enthalpy of the pressure-treated sample, indicating less energy was required to denature the connective tissue.

Table 3: Mean DSC parameters of intramuscular connective tissue extracted from brisket muscle before and after pressure treatment at 300 MPa for 5 min at 4°C. T_o, onset temperature; T_m, temperature of denaturation; ΔH, enthalpy of denaturation of the collagen peak, n=6.

Time of Extraction	Treatment	T_o (°C)	T_m (°C)	ΔH (J/g)
Before	Control	58.02	62.46	5.978
	HPP	57.88	62.58	5.010
	P value	0.628	0.473	0.084
	s.e.d.	0.273	0.162	0.4798
After	Control	58.62	62.83	5.999
	HPP	58.18	62.54	4.623
	P value	0.041	0.034	0.009
	s.e.d.	0.173	0.107	0.3881

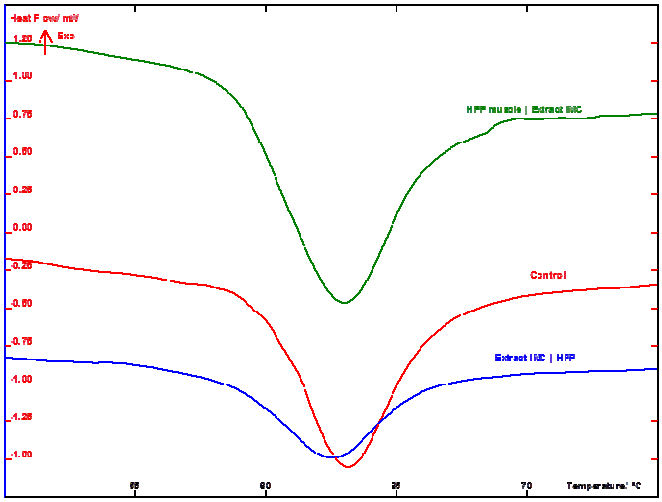


Figure 10: An example of DSC thermograms of untreated (control) and pressure-treated connective tissue (HPP) from brisket muscle. The HPP connective tissue was extracted from the muscle and pressure-treated at 300 MPa for 5 minutes at 4°C.

Characterisation of outside flat

Limited analysis of samples from the outside flat muscle has been completed to date. This data and interpretation of results will be included in the final report.

As for the brisket muscle, the sarcomere length of the untreated outside flat muscle was similar to that of the pressure-treated muscle (1.75 µm ± 0.014 and 1.71 µm ± 0.025, respectively).

The peak force value for outside flat was significantly (*P*<0.001) reduced when pressure was applied under these conditions (Figure 9). Both the IY and PF-IY values were significantly (*P*<0.001 and *P*=0.045, respectively) reduced in the outside flat muscle by high pressure treatment (Figure 9). This was in contrast to the brisket muscle where the application of pressure increased the PF-IY value.

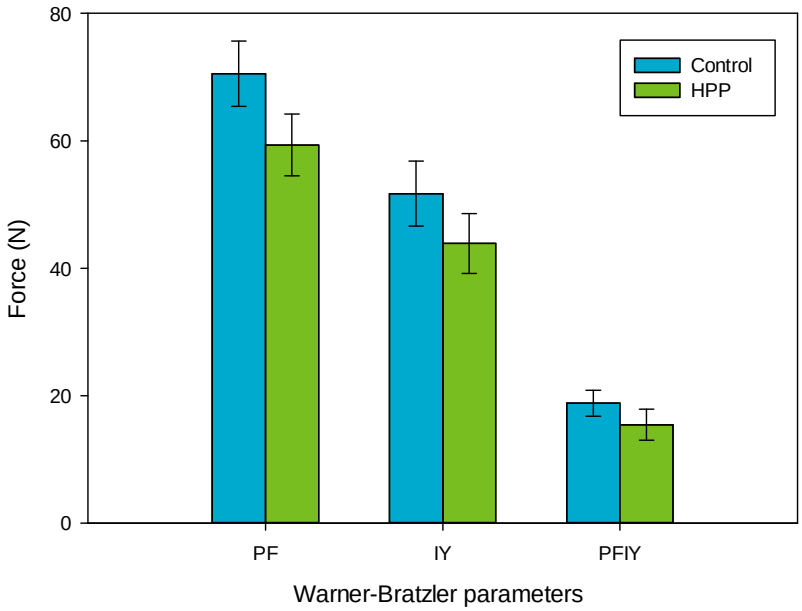


Figure 9: The effect of high pressure processing (50 MPa, 4°C, 5 min) on the texture of outside flat muscle as measured by the Warner-Bratzler shear force method. PF – peak force; IY – initial yield; PFIY – peak force minus initial yield. Mean ± SE, n=4.

There was no effect (*P*>0.05) of pressure on the treatment loss of outside flat muscle but the drip loss, cooking loss and overall processing loss was higher in the pressure-treated samples compared to the control samples (Table 4).

Table 4: Moisture losses at different stages of processing of outside flat muscle, control (untreated) or pressure treated (50 MPa, 4°C, 5 min). Mean ± SE, n=8.

Treatment	Treatment loss (%) [a]	Drip loss (%) [b]	Cook loss (%) [c]	Total loss (%) [d]
Control	1.44 ± 0.135 [x]	1.36 ± 0.110 [x]	17.38 ± 0.948 [x]	18.72 ± 1.029 [x]
HPP	1.42 ± 0.100 [x]	1.86 ± 0.115 [y]	19.84 ± 0.840 [y]	21.10 ± 0.878 [y]
P-value	0.894	<0.001	0.003	0.007

a loss during treatment, i.e., control (untreated) or HPP
b drip loss as calculated by the hanging method
c loss after cooking
d overall loss due to treatment and cooking
Columns with different letters indicate significance

Overall progress of the project

Despite the difficulty in completing this milestone report, the overall progress of this project is on target. Several key milestone dates have been revised due to competing project commitments and also to provide a more realistic time frame for completion of the work. The project team is appreciative of this revision.

Conclusions

- Optimum HPP conditions have been defined for an effect on texture for 2 beef muscles and the amount of pressure required for this effect varies between the muscles.
- A reduction in Warner-Bratzler shear force was measured in both of the muscles when optimum pressure conditions were applied at 4°C for 5 min.
- However, this improvement in tenderness would not be substantial enough for consumer acceptability of the product.
- The textural changes of the pressurised meat appear to be the result of the effect of treatment on the myofibrillar component of the muscle.
- Muscle differences are apparent in respect to the effect of HPP on connective tissue.
- Pressurised muscles had reduced water-holding capacity and increased cooking losses compared to untreated muscle.
- Thermal stability of extracted connective tissue is dependent on the sequence of extraction and pressure treatment.

Recommendations

To continue the analysis of brisket and outside flat samples from these experiments for the characterisation of the effects of HPP to post-rigor beef. This information will provide a solid platform of knowledge and understanding to assist in developing a theory for the mechanisms of tenderisation of post-rigor beef under these processing conditions.

Milestone 4: Development of system for VFC

milestone report

MLA project code:	**A.MQT.0053**
MLA project title:	**VFC and HPP tenderisation mechanisms**
Project leader:	**Robyn Warner / Anita Sikes, Kai Knoerzer**
MLA project manager/coordinator:	**Phil Franks / Michael Lee**
Milestone number:	**4**
Date:	**31 July 2012**

Milestone
Milestone report on the system developed for VFC, outlining the boundaries determined for the very fast chilling parameters (time, temperature, pH of muscle).

Abstract
Computational Fluid Dynamics (CFD) was used to determine critical process parameters of the VFC process to achieve a target temperature of -3°C in the muscle core after approximately one hour.

Different models were set up, varying in numerical complexity, and a parametric study was completed for varying fluid temperatures and flow velocities.

It was found that in a 40% propylene glycol / water mixture, the target temperature could be achieved in 1 to 1.5 hours when the fluid temperature was in the order of -14 to -20°C and the flow rate between 0.5 and 3 m/s. A functional relationship between flow velocity and time required to reach target temperature in the muscle core was found for the different medium temperatures.

The model predictions seem reasonable; however, a full validation has not been done, but forms part of the next stage of the process. Once validated, this model can be used to quickly determine the critical process parameters for any given product size.

Project objectives
To design a process that will facilitate tenderisation within the first 24 hours post-slaughter for both low- and high-value beef cuts.

Outcomes:
1. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to VFC
2. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to HPP

Success in achieving milestone
Modelling of the VFC process for meat involved the following tasks:

- Model development and coding in COMSOL Multi-physics
  - 2D formulation
  - Heat transfer only
- Determination of model parameters
  - Literature search for the thermo-physical properties of the meat and fluid
- Preliminary testing and validation using literature data (if available)
- Model simulation to identify critical/key process parameters (e.g. fluid temperature, thickness of the sample, etc) to achieve the desired cooling rate

Model Setup
COMSOL Multiphysics™ (Ver 3.5a, COMSOL AB, Stockholm, Sweden), a commercially available software package based on the finite element method and incorporating modules for various Multiphysics phenomena, was used to develop the computational model of the VFC process.

The meat was approximated in 2D considering only half for symmetry, given that 2 dimensions are sufficient for predicting transient temperature evolution in a meat sample with fluid inflow directed on one of the shorter sides (see Figure 1). The depth, which is greater than the height and width, of the product will in this case not contribute to the time required to achieve the target temperature.

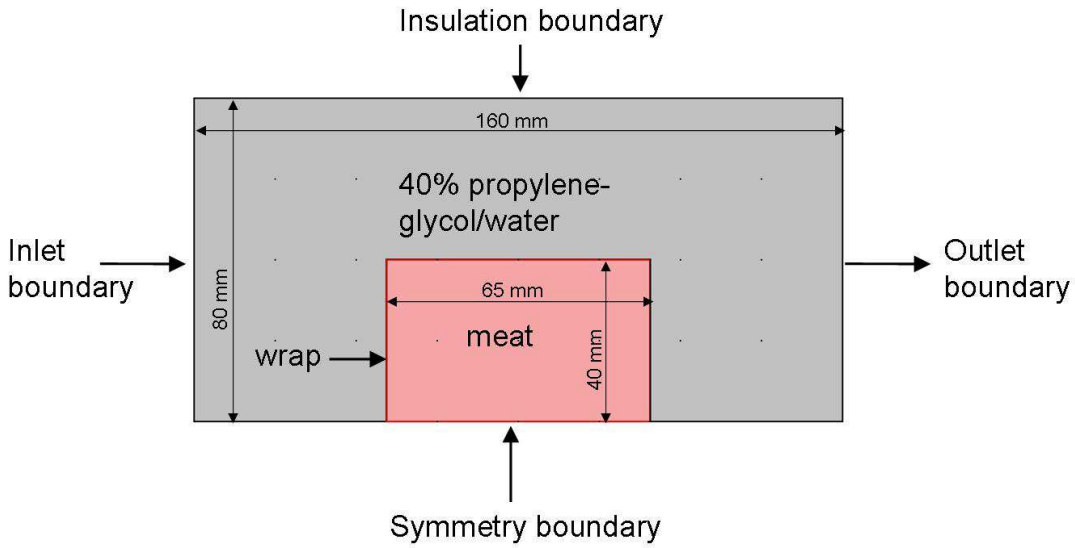


Figure 1: Domain of the modelled scenario, including dimensions.

The numerical approximation of the system included the cooling medium domain, a meat sample with a rectangular cross-section and a plastic wrap around the meat. The cooling medium used was a 40% propylene glycol / water mixture with a freezing point of -22°C. The temperature- and concentration-dependent thermophysical properties for the cooling medium were taken from M. Conde Engineering (2011), the properties for the meat were taken as constants from published data (Fontana et al, 1999).

Three different models were set up with varying complexity:

1. Heat transfer only; i.e., a heat transfer coefficient was calculated from the dimensionless Reynolds, Nusselt and Prandtl numbers, depending on fluid velocity, fluid properties and sample dimensions.
   This model was not expected to provide accurate results as differences in fluid velocity streaming over the sample leading to changes in the heat transfer coefficient were not taken into account; furthermore, the characteristic length required for the calculation of the heat transfer coefficient was taken as constant irrespective of the location on the sample boundary.
   Nonetheless, this model was considered a good starting point for roughly determining the ranges of temperatures and flow velocities required.
2. Convective heat transfer: this model included the prediction of flow around the sample and, therefore, adjusting the heat transfer coefficient according to the local velocities on the boundary was possible. However, also here only one characteristic length could be applied; the model was expected to be more accurate than the heat transfer model only; still, accurate predictions were not expected.
3. Turbulent heat transfer: In this numerical scenario, the heat transfer coefficient was replaced by a so-called turbulent thermal wall function. This numerical approach recognises the laminar sublayer on the meat sample but at the same time also the increased turbulent viscosity and turbulent thermal conductivity, generated through the turbulent eddies in the fluid domain. In other work (Knoerzer et al., 2012), this approach has proven to give most accurate results in turbulent heat transfer problems.
   For this scenario a range of medium temperatures (-14, -18, -20°C) and fluid inlet velocities (0.25 to 3 m/s) were investigated in a parametric study.

Results

The turbulent thermal wall function model showed that for different medium velocities a steep decline in required cooling time occurs initially, approaching a plateau (see Figure 2), i.e., a further increase in velocity will not lead to further decrease of time to reach target temperature in the muscle core. This decline followed nicely a power law function.

Cooling time for different medium temperatures can be estimated with the following equations:

$$t_{-14°C}(s) = 363.8 \cdot v_{inlet}^{-0.9772} + 4117$$

$$t_{-18°C}(s) = 394.7 \cdot v_{inlet}^{-1.144} + 3548$$

$$t_{-20°C}(s) = 454.3 \cdot v_{inlet}^{-1.113} + 3364$$

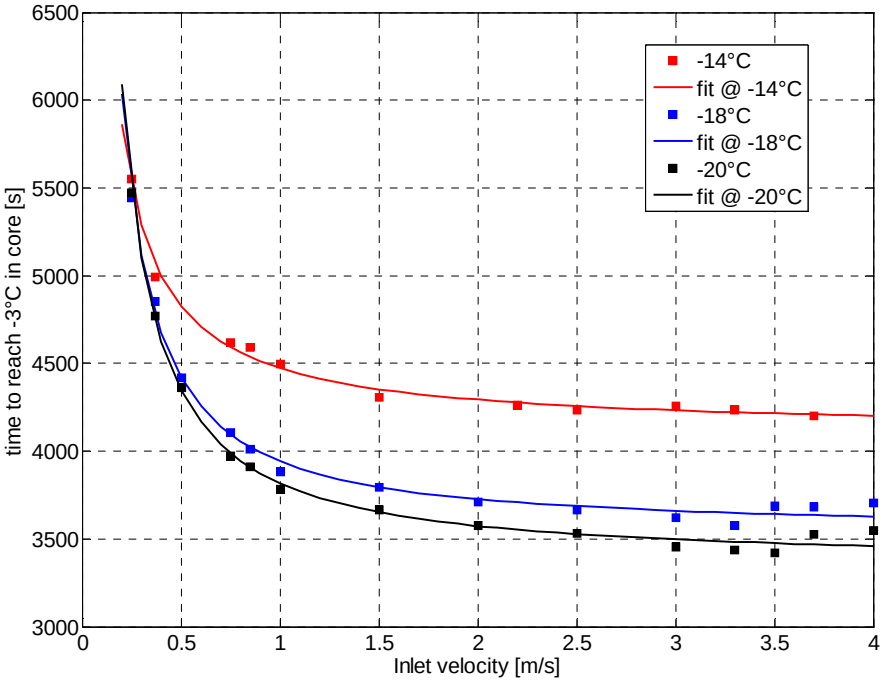


Figure 2: Time required to reach -3°C at different fluid temperatures as a function of fluid inlet velocity.

Conclusions and outlook

The results of the modelling study shows that for a maximum muscle size of 80 mm, cooling medium temperatures should be lower or equal to -14°C to achieve a core temperature of -3°C within 1 to 1.5 hours at feasible fluid flow velocities in the order of 1 to 3 m/s.

Reducing the sample size will allow for shorter cooling times required or fluid temperatures above -14°C.

The model has not been validated yet due to the lack of extensive data available in the published literature. The validation and potential model finetuning (e.g., including phase change phenomena, the thermophysical properties of meat as a function of temperature or mesh refinement) will form part of the next stage of the project once experimental data has been collected. After validation, the model can be set up as a platform with the capability to quickly change process parameters and muscle size and shape for fast determination of cooling time. Also, the platform can be used in reverse fashion where the outcomes of the process are defined, i.e., required cooling time for a given muscle, and the model then predicts combinations of process conditions, i.e., medium temperature and flow velocity, which will be suitable to achieve the required outcome.

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M.Conde Engineering, 2011. Thermophysical Properties of Brines. M.Conde Engineering.

Knoerzer, Baumann, Buckow, 2012. An iterative modelling approach for improving the performance of a pulsed electric field (PEF) treatment chamber, Computers and Chemical Engineering 37, 48-63.

Milestone 5A: Modification of VFC system

milestone report

MLA project code:	**A.MQT.0053**
MLA project title:	**VFC and HPP tenderisation mechanisms**
Project leader:	**Robyn Warner / Anita Sikes**
Prepared by:	**Anita Sikes / Neil McPhail**
MLA project manager/coordinator:	**Phil Franks / Michael Lee**
Milestone number:	**5A**
Date:	**11 January 2013**

Milestone

An interim milestone report submitted outlining the approach to develop the very fast chilling system for beef muscle, including the refinements and modifications developed to overcome the challenges encountered to achieve the desired chilling rate.

Abstract

Previous research funded by MLA identified that very fast chilling (VFC) has the potential for accelerating tenderisation in lamb loins. From this work, a chilling rate of -3°C within an hour was defined for exploring the effects of VFC on beef muscle. A system for conducting pilot plant trials for VFC of beef muscle was developed. Several trials have been conducted to overcome challenges faced (sample size and shape, temperature monitoring, presence of fat layer) and subsequent remodelling to achieve an acceptable chilling rate (temperature and time). Experimental planning for an on-site plant trial is currently underway to obtain samples for the characterisation of the effects of VFC on pre-rigor beef muscle.

Project objectives

To design a process that will facilitate tenderisation within the first 24 hours post-slaughter for both low- and high-value beef cuts.

Outcomes:

1. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to VFC
2. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to HPP

Success in achieving milestone

Previous research funded by MLA and conducted collaboratively between the Department of Agriculture and Food Western Australia (DAFWA) and Ag-Research Ltd, MIRINZ centre (P.PSH.0326) indicated that accelerated tenderisation occurred when lamb loins were chilled to -5°C within 1 hour of slaughter. Jacob et al. (2012) concluded that both time and temperature at the end of the chilling period contributed

to variations in tenderness (as measured by shear force) and that to achieve low shear force values, lamb loins (40 mm thick) needed to be cooled to less than 0°C at 1.5 hours post mortem. Therefore, the target for chilling beef muscles in this work was set at -3°C within 1 hour of receipt of the muscle.

Section 5 outlines the approach to develop the VFC system (Scoping study) and Section 6 details refinements and modifications to the developed system to achieve the target cooling rate (Fine tuning).

As described below, this chilling target proved to be challenging for beef muscles of thicknesses greater than 45 mm. Therefore this interim report outlines the problems encountered and solutions trialled to achieve the target cooling rate. Discussions with Teys Australia the week of December 10, 2012 confirmed trials to be conducted on-site at Beenleigh starting the week of January 28, 2013. Samples collected in these plant trials will be used for the characterisation work (metabolism, structure, biophysical effects) for Milestone 5B.

Scoping study

Validation of the model

From the modelling study detailed in Milestone 4, beef muscle portions (60-75 mm wide, 70-90 mm high, 160-180 mm long) were equilibrated to either 33°C or 28°C. Muscle samples were placed in a water bath containing 40% propylene glycol, set at -20°C and cooled until a temperature of -3°C was reached. From Figure 1, it can be seen that from a starting temperature of 28°C, it took 1 hour to reach -3°C and 1.5 hours from 33°C. Therefore, it seemed from the COMSOL modelling that a target of -3°C within 1 hour was possible for beef muscle of approximately 80 mm thickness.

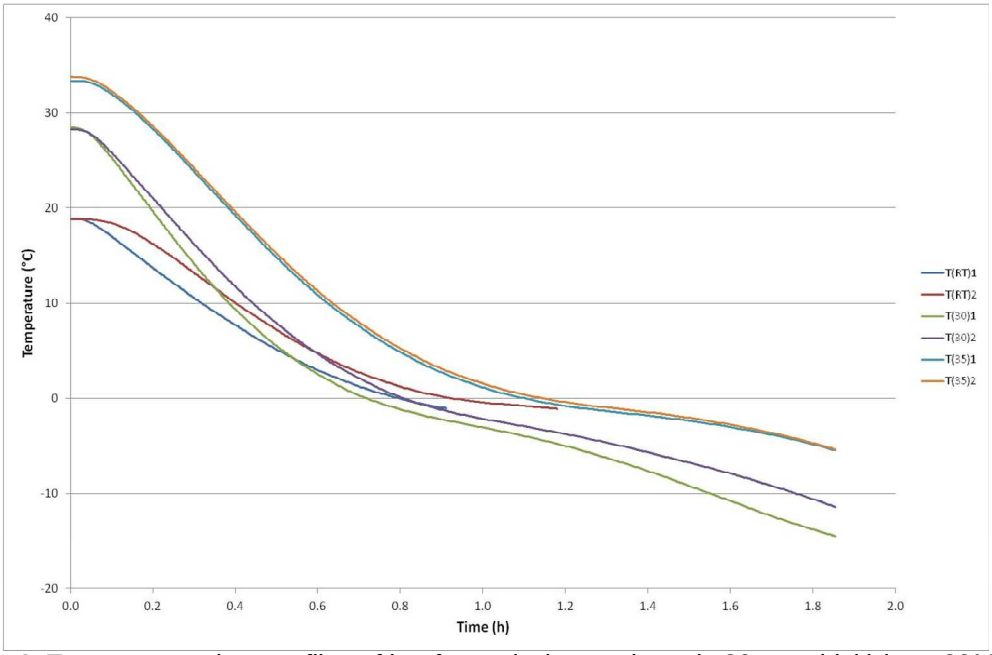

Figure 1: Temperature-time profiles of beef muscle (approximately 80 mm thick) in a -20°C waterbath (40% propylene glycol) with flow velocities of 3 m/s.

Choice of muscle

The VFC process by its nature is for use on pre-rigor muscle. Therefore, the choice of muscle for the scoping study was dependent on accessibility (from a cold-boning process) and proximity to the laboratory, rather than the best choice from an industrial perspective. After discussions with MLA (Michael Lee) and JBS Brooklyn (Rod Mitchell), it was concluded that 2 muscles available for use in the scoping study were the tongue root fillet, also known as the neck muscle (*M. sternomandibularis*), and the thick skirt (the lumbar portion of the diaphragm) (Figure 2). Initial trials at CAFHS Werribee used both of these muscles but subsequently the tongue root fillet was rejected due to its small size.

Development of VFC system

The modelling, validation of the model and choice of muscle had been confirmed at this stage of the scoping study. It was necessary to further develop the system for the VFC process. This included devising a method for suspending the meat samples

in the water bath. Examples of the initial development and refinement of the process is shown in Figure 3.

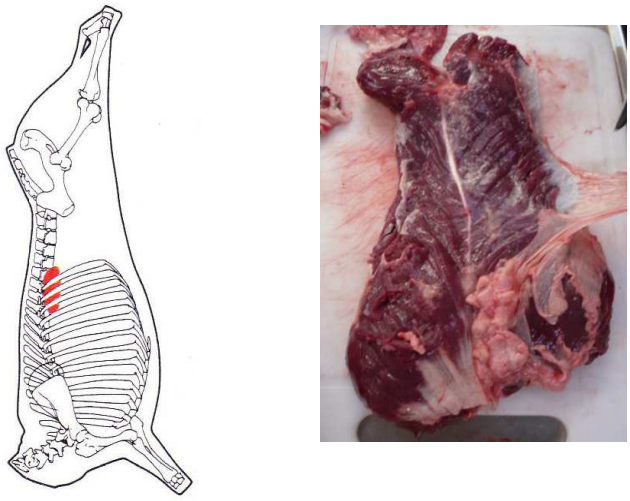

Figure 2: The thick skirt muscle showing the position in the carcase (left) and as received off the slaughter floor (right).

Figure 3: Development and refinement of the system for VFC

Temperature measurement

Initially, the only means of monitoring the temperature decline during chilling was by using Thermocron I-Button data loggers. For initial chilling runs, slits were made in the muscle and the I-Buttons placed as centrally as possible within the muscle. For subsequent thick skirt samples that were 'rolled' (see below), the I-Buttons were placed centrally in the muscle prior to 'rolling'.

Geometry of the muscle

The dimensions of the thick skirt varied from run to run (thickness 12–20 mm), depending on the types of animals that were being slaughtered. In an attempt to achieve a consistent size and shape, the thick skirt muscles were 'rolled', as shown in Figure 4.

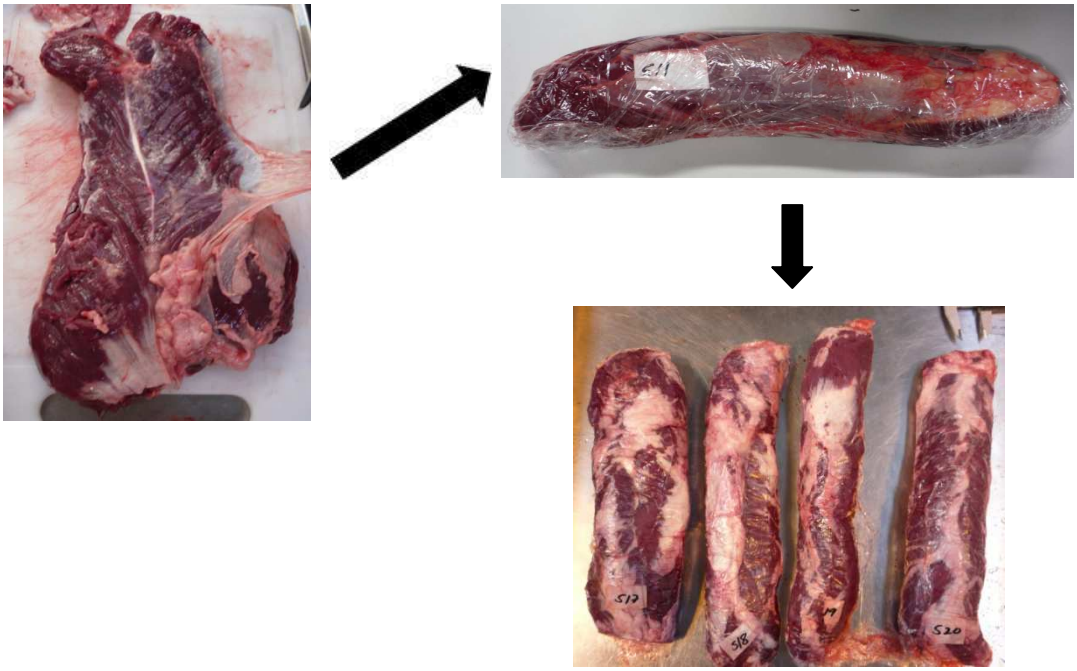

Figure 4: Thick skirt muscle 'rolled' to achieve a consistent thickness for the VFC process

Results

The system for the VFC process was developed in this scoping study. Initial trials on the neck muscle and thick skirt experienced difficulties in consistent sample size and shape to achieve the target chilling rate of -3°C in an hour. The I-Buttons were essentially frozen inside the muscle after each chilling run. Therefore, the temperature profiles obtained during these runs were difficult to interpret as there was no pertinent method for knowing where they were located within the muscle. A subsequent method developed for identifying the location of the data loggers within the muscle (detailed in Section 6) shows that it was not always possible to position the data loggers in the centre of the 'rolled' thick skirt (Figure 5).

Figure 5: Examples of the placement of I-Button data loggers in 'rolled' thick skirt muscles.

Fine tuning of VFC process

As the VFC system had been developed in the scoping study (Section 5), further modifications were necessary to reach the chilling rate required.

Temperature measurement

The temperature of the cooling medium and the meat samples was measured during very fast chilling (VFC) using two methods:

1. Thermocron I-Button temperature loggers (supplied in Australia by On Solution Pty Ltd, Baulkham Hills, NSW), and
2. Thermocouples made from 0.2 mm Type T (copper-constantan) wire connected to a Grant Squirrel Series 1250 data logger.

I-Button measurement

Three I-Button loggers were programmed to record at 15 second intervals for each trial. One logger was placed in the water bath basket to record the temperature of the cooling medium and the other two inserted into the meat samples. In the case of the LD samples, a slit was made with a knife in the end of each sample at half the height and to a depth of 50 – 60 mm into which the I-Button was inserted.

Thermocouple measurement

A small slit was made in the side of each of two vacuum bags through which the thermocouple wire was inserted so that the measuring junction reached past halfway across the bag. The slit in the bag was sealed to the thermocouple wire using Sikaflex®-227 polyurethane flexible sealant and allowed to cure overnight (Sika Australia, Wetherill Park, NSW).

For each trial, the meat cut to be cooled was placed into the vacuum bag and the thermocouple measuring junction inserted into the end of the LD muscle at approximately half the height of the cut to a depth of 50 – 60 mm. The vacuum bags were then evacuated and sealed in a vacuum-packaging machine with the vacuum level set to low. The two samples were then fixed to the water bath basket so that they were suspended vertically with liquid movement around all surfaces. The third thermocouple was attached to the basket to record the cooling medium temperature.

The thermocouples were plugged in to the Squirrel temperature logger which was set to record at 30-second intervals. Logging was commenced as the basket was lowered into the bath and continued until the slowest cooling sample reached -3°C.

Modelling

After removal from the bath, the frozen samples were placed into a chiller at -1 or +2°C for several hours or overnight. The still firm (partly frozen) samples were then sliced across the long axis until the I-Button loggers and the thermocouple junctions were located. The cross-sectional dimensions of the samples at these points were measured and the location of the loggers and thermocouples recorded (Figure 6).


Figure 6: Examples of identifying the location of thermocouple junctions (left) and I-Button loggers in VFC-processed LD muscle (fat layer on).

Food Product Modeller (FPM) v 2.0 (MIRINZ, Ag-Research, NZ) was used to develop a cooling model to allow simulation of the cooling process to enable prediction of cooling times for samples of different sizes, initial temperatures and cooling medium temperatures to those tested. A suitable generic shape was selected in FPM and the sample dimensions, thermocouple location and fluid temperature entered. The heat transfer coefficient value was adjusted until the model predicted the cooling rate at the thermocouple location with reasonable accuracy.

Results

Initial trials were carried out with pieces of beef striploin which were approximately one quarter the length of a whole striploin. These had been stored frozen and were thawed and then warmed to 30°C in a water bath to simulate hot-boned product. Some samples were also equilibrated to 15°C to simulate conditioning prior to very fast cooling.

Initially striploin samples were only cooled for one hour in attempts to achieve the cooling parameters of -3°C in one hour but a centre temperature of only 2 to 5.7°C was achieved in that time with a glycol bath temperature of -15 to -20°C. The subcutaneous fat layer on one side of the striploin significantly affected the cooling rate and the lower thermal conductivity of the fat resulted in the thermal centre of the sample being offset from the geometric centre. Subsequent trials were undertaken with samples that were trimmed of the external fat layer and other muscles so that the sample comprised the *m. longissimus dorsi* (LD) alone. The bath temperature was set to -20°C.

When the external fat was trimmed and the position of the samples in the glycol bath alternated each 15 minutes to compensate for variations in fluid velocity, the thermal centre of the samples was at the geometric centre. A typical cooling curve for the centre of the LD muscle recorded from the thermocouple readings is shown in Figure 7.

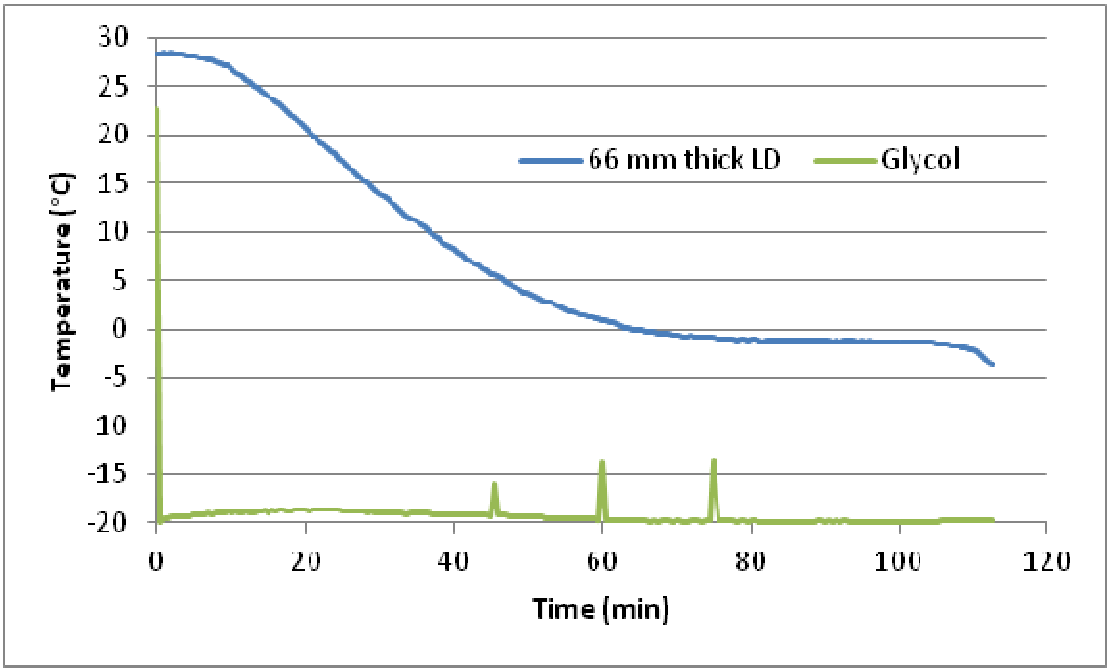

Figure 7: Cooling rate at the centre of a trimmed LD muscle (66 mm thick) in a glycol bath set to a temperature of -20°C.

This shows clearly that for a normal-sized beef LD muscle the aim of cooling to -3°C within one hour from an initial temperature of approximately 30°C is very difficult to achieve. When the samples were conditioned to 15°C, a centre temperature of -3°C within one hour also could not be achieved as shown in Figure 8.

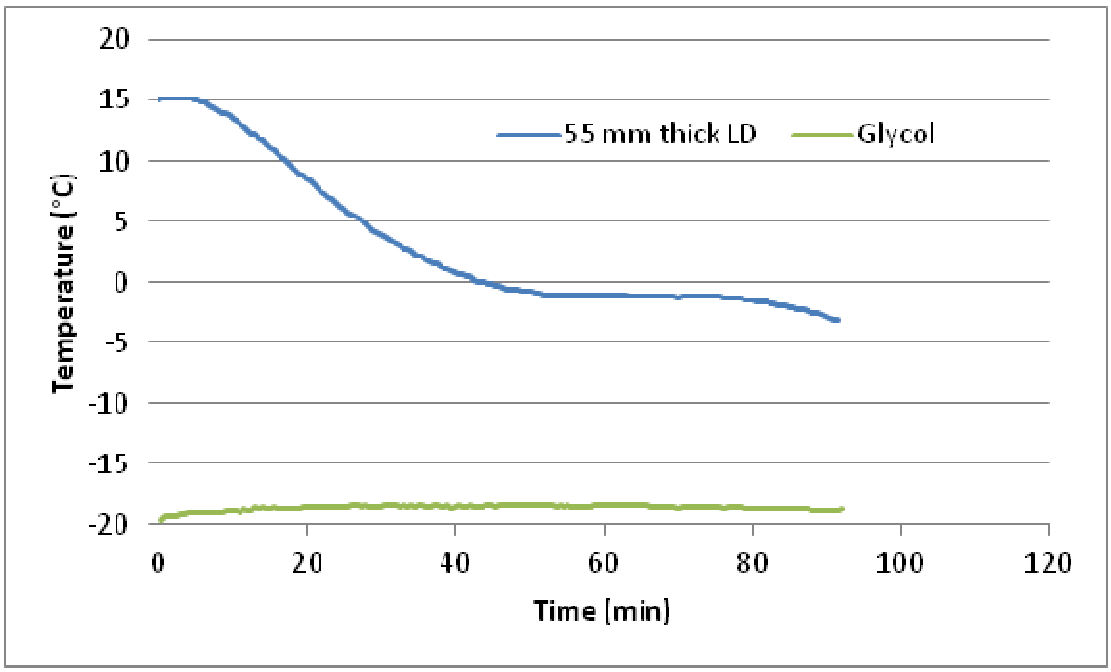

Figure 8: Cooling rate at the centre of an LD muscle (55 mm thick), conditioned to 15°C, in a glycol bath set to -20°C.

When the LD sample cross-sectional dimensions, glycol temperature, and initial sample temperature were entered into the FPM model, quite a good prediction of the sample centre cooling rate was achieved (Figure 9).

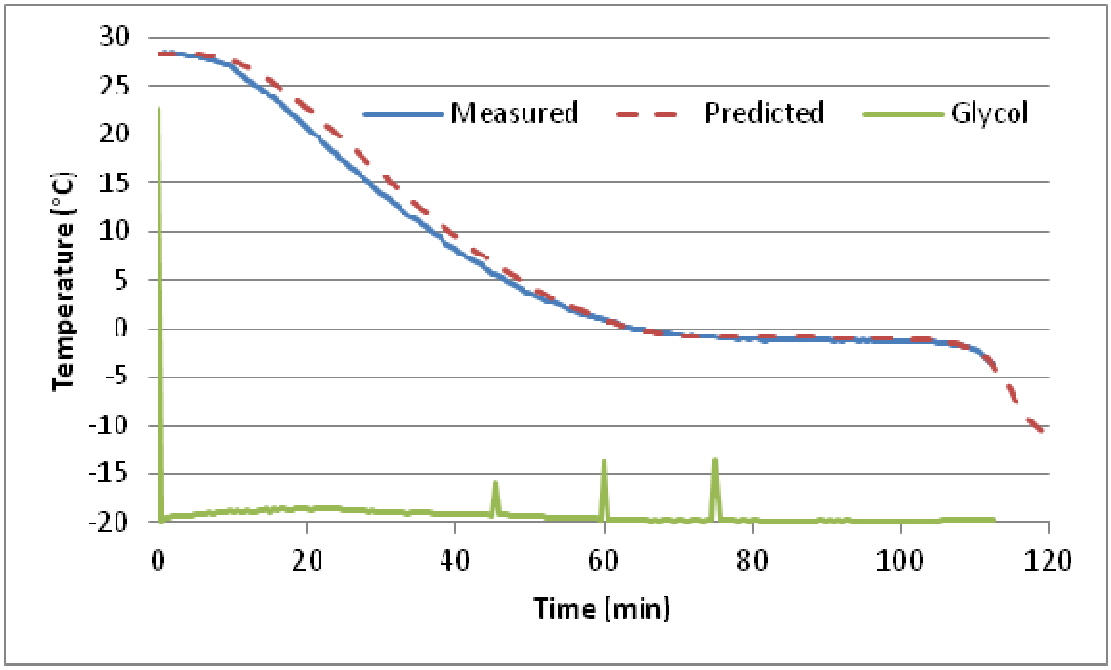

Figure 9: Actual and predicted cooling rates at the centre of 66 mm thick beef LD muscle in glycol bath at -20°C

When the same parameters were applied to samples at an initial temperature of 15°C, a similar good fit between predicted and actual centre temperature was obtained. FPM was then used to predict the maximum sample thickness that would allow a centre temperature of -3°C to be achieved within 1 hour. When the initial meat temperature was 30°C, the maximum thickness of the sample was estimated to be 45 mm using a glycol bath temperature of -20°C.

Overall progress of the project

The VFC system has been developed and the process for positioning samples in the waterbath has been established. Challenges experienced around sample size and shape, real-time temperature monitoring and presence of the fat layer in respect to achieving the target chilling rate have been overcome.

Due to these challenges, a planned on-site plant trial has been delayed until the end of January 2013, when the plant reopens after the Christmas break. Therefore, biophysical and structural characterisation of the effects of VFC on pre-rigor beef muscle will not be reported until April 2013 (Milestone 5B).

Recommendations

Schedule a project meeting to discuss:

- Progress to date
- Challenges faced with reaching the chilling rate defined for this project work
- Redefining the chilling rate for beef muscle and exploring the boundaries of temperature and time
- Confirm on-site plant trial

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Milestone 5B: Processing effects of VFC on biophysical changes in pre-rigor muscle

milestone report

MLA project code:	**A.MQT.0053**
MLA project title:	**VFC and HPP tenderisation mechanisms**
Project leader:	**Robyn Warner / Anita Sikes**
Prepared by:	**Anita Sikes / Robin Jacob**
MLA project manager/coordinator:	**Phil Franks / Michael Lee**
Milestone number:	**5B**
Date:	**30 March 2014**

Milestone

A milestone report submitted on the processing effects of very fast chilling (VFC) applied to pre-rigor beef muscle on muscle structure, metabolism and biophysical changes. Determine the importance of biophysical changes in VFC muscle relative to structure, protein modifications and initial metabolic measurements and evaluate if further investigation of metabolism and proteases is warranted for assessment in Milestone 7.

Abstract

An experiment was conducted on-site at Teys Australia, Beenleigh to obtain samples for the characterisation of the effects of VFC on pre-rigor beef muscle. The VFC treatment was successful in accelerating tenderisation, as shown by the shear values being lower than the control and delayed VFC after only 2 days of ageing. As there was no difference in sarcomere lengths between treatments, this provides an opportunity to investigate a novel tenderisation mechanism which is independent of sarcomere length, as intended in the design of this experiment. This also confirms findings from previous studies that the timing of VFC is critical and needs to occur within a few hours of slaughter. Results from other analyses in this work suggest that the tenderisation is also independent of proteolysis and denaturation and degradation of muscle proteins. Future work in exploring the mechanism of tenderisation by very fast chilling treatments should focus on the importance of the prevention of muscle shortening due to crust freezing as well as the modification of metabolism during the VFC process and the effect this has on glycolysis and hence texture. Overall, this experiment was successful in reproducing a tenderising VFC effect in hot-boned beef loin.

Project objectives

To design a process that will facilitate tenderisation within the first 24 hours post-slaughter for both low- and high-value beef cuts.

Outcomes:

1. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to VFC

2. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to HPP

The aims of the work reported in this interim milestone report were:
(a) to repeat the VFC process and rapid tenderisation reported by Jacob et al. (2008 a, b)
(b) to investigate the effect of VFC on texture and metabolism during ageing
(c) from these results, gain some insight into the mechanism(s) of tenderisation due to VFC

Success in achieving milestone

A very fast chilling (VFC) experiment was conducted at Teys Australia, Beenleigh over a 3 week period from January 31, 2013. Due to local flooding in the Brisbane area at the end of January, the planned start date of VFC trials at Teys Australia was delayed by a week but subsequently the experiment was completed successfully. Several hypotheses of the mechanism for tenderisation due to very fast chilling of beef striploin muscles have been tested.

Aims/Background

Research to date suggests that two separate processes are responsible for accelerated tenderisation due to VFC. Firstly sarcomere length is maintained (i.e., does not cold-shorten) despite early pre-rigor temperature being very low. Secondly, an unknown mechanism reduces shear force further to prevention of cold shortening, the nature of which is not clear (novel tenderisation mechanism = NTM). The aim of this experiment was to separate prevention of cold shortening from NTM to enable further investigation of NTM. This was done by comparing a VFC treatment applied within 1 hour post-slaughter with a VFC treatment which was delayed by a 15°C conditioning period for 2 hours. With conventional cooling, sarcomeres shorten when the temperature of the muscle falls below 15°C in the pre-rigor period, and the pH is still above 6. Delaying the VFC with a 15°C conditioning period should therefore prevent cold shortening and allow comparison of VFC independent of sarcomere shortening.

Experimental design

The experiment was a 3x3 factorial design consisting of three chilling treatments Control, Delayed VFC and VFC (Table 1), and three post-slaughter ageing periods; 2 days, 5 days and 14 days. Due to limitations with water bath capacity, there were 12 slaughter dates with all treatments applied on each slaughter date and one animal per treatment per replicate. The intention was to collect 3 muscles from 3 different animals for each replicate (slaughter date) from within the same mob. On two occasions, this was not the case (Table 2).

Table 1: Explanation of treatments used for very fast chilling experiments.

Treatment	***Abbreviation***	***Conditions***
Control	C	Conventionally chilled through normal processing
Delayed VFC	F	Hot-boned muscle placed in 15°C water bath until centre reaches 15°C or until the previous VFC completed; then placed in -20°C water bath until internal temperature of <1°C
Very fast chilling	V	Hot-boned muscle placed in -20°C glycol water bath until internal temperature reaches <1°C

Materials and methods

Animals

A total of 36 striploins were collected from 36 beef carcasses over a 3 week period at Teys Australia, Beenleigh. The animals were graded as cows (6-8 tooth), with carcass weights ranging from 204 to 336 kg (mean = 262 kg). All striploins were collected from the left side of each carcase. Pre-rigor (i.e. hot-boned) *M. longissimus dorsi* (LD, striploin) muscles were collected from one side (left) from 24 animals, while a further 12 LD muscles from separate animals were tagged after slaughter and collected the following day in the boning room (controls). Carcase information was obtained for each muscle sample. Each LD muscle was allocated to a treatment (control, delayed VFC or VFC) and cut into thirds, approximately 120 x 120 x 60 mm, with the rump end of the striploin always to the right when cutting. Each third was randomly allocated to an ageing period.

Table 2: Animals from each mob number in each replicate (slaughter date).

Mob	Slaughter date											
	31-Jan	4-Feb	5-Feb	6-Feb	7-Feb	11-Feb	12-Feb	13-Feb	14-Feb	18-Feb	19-Feb	21-Feb
426	3											
125		3										
210			3									
308				1								
307				2								
410					3							
106						3						
212							1					
213							1					
216							1					
311								3				
417									3			
103										3		
225											3	
413												3
Total	3	3	3	3	3	3	3	3	3	3	3	3

Sample preparation

The LD's identified for the control treatment were tagged with a CSIRO carcase tag and the pH and temperature of the LD measured at approximately 1 h post-slaughter. The hot-boned, intact LD's were also measured immediately for pH and temperature. The time from slaughter to carcase tagging and hot-boning ranged from 41 to 76 minutes, with an average of 47 minutes. The fat layer and cap were removed from the hot-boned LD's, the intact muscle cut into thirds and each piece randomly allocated to an ageing period (2, 5 or 14 days). The weight and dimensions (length, width, thickness) of each sample was recorded, and the pH and temperature again recorded before sealing. The average weight of the samples was 1.19 kg (0.796 to 1.72 kg). A thermocouple was inserted into the centre of the sample and the sample vacuum-packed with the thermocouples sealed inside the bag to enable real-time temperature monitoring.

Conventionally chilled, control samples

Samples from carcasses identified as control samples were conventionally chilled as per standard plant protocol (0°C for 12-15 hours with spray chilling) and the striploin boned out from the carcase at approximately 24 hours post-slaughter.

Very fast chilling

The water bath was filled with approximately 40 L of 45% (v/v) propylene glycol in water. The three samples (from the one LD) were chilled until the freezing plateau (approximately -1 to -1.5°C) was reached (see Appendix 1) and then left for approximately 5 min before removal from the bath. The freezing plateau was identified by real-time temperature monitoring. The three samples were removed from the water bath based on the sample with the slowest temperature decline. After the VFC process, a subsample was taken and frozen in dry ice and stored at -80°C for pH measurement by homogenisation. Samples were re-vacuum packed and stored at approximately -1.5°C for the designated ageing periods.

Conditioning then very fast chilling

The second water bath was filled with water and the temperature of the water was maintained at 15°C. Samples were held in the water bath until the core of the sample was close to 15°C (± 1°C) or until the concurrent VFC treatment had completed the cycle. Prior to VFC, the vacuum pack was opened, the pH and temperature recorded, a subsample taken for pH measurement by homogenisation, and the sample resealed before the VFC process. After the VFC process was completed as described above, a subsample was taken, frozen in dry ice and stored at -80°C for pH measurement by homogenisation. Samples were re-vacuum packed and stored at approximately -1.5°C for the designated ageing periods.

Temperature profiles

The temperature of the cooling medium and the meat samples was measured during VFC and delayed VFC using thermocouples made from 0.2 mm Type T (copper-constantan) wire connected to a Grant Squirrel Series 1250 data logger.

A small slit was made in the side of the vacuum bag through which the thermocouple wire was inserted. The slit in the bag was sealed to the thermocouple wire using Sikaflex®-227 polyurethane flexible sealant and allowed to cure overnight (Sika Australia, Wetherill Park, NSW).

For each replicate, the sample to be cooled was placed into the vacuum bag and the thermocouple inserted into the end of the LD muscle at approximately the centre of the exposed LD (length, width and depth). The vacuum bags were then evacuated

and sealed in a vacuum-packaging machine with the vacuum level set to low. The three samples were then fixed to the water bath basket so that they were suspended vertically with liquid movement around all surfaces. A fourth thermocouple was attached to the basket to record the cooling medium temperature.

The thermocouples were plugged in to the Squirrel temperature logger which was set to record at 30-second intervals. Logging of the temperature commenced as the basket was lowered into the bath and continued until the slowest cooling sample reached -3°C.

Ageing

Samples were aged at -1.5°C for the designated times (2, 5 and 14 days). At each time point, the muscle and bag were weighed. Samples were cut in half perpendicular to the backbone and steaks were cut for colour. Two steaks were placed cranial side facing up onto black snopak foam trays (205 x 130 x 12 mm). Further subsamples were taken for Warner-Bratzler shear force, sarcomere length and myofibrillar particle size analysis. The remainder was minced and subsamples taken for subsequent analyses for protein denaturation and glycogen and lactate analysis. All subsamples were frozen until required.

Colour measurement

After opening of the packs at each time point, the muscle samples were bloomed (5°C for 60 min, in light conditions) and triplicate colour measurements (L*, a* and b* values) were conducted at room temperature (approximately 25°C) using a Hunterlab Miniscan EZ (illuminant A, observer angle 10°, aperture size 5 cm). Steaks remained on the black trays during measurement. Where required, standardisation of the Hunterlab was conducted through clingwrap, similar to sample wrapping.

pH measurement

Muscle pH was measured on whole muscle samples using a digital pH meter (TPS WP80) fitted with a combination electrode (Ionode IJ44 glass body with a spear tip) with temperature compensation.

Approximately 0.5 g of frozen (dry ice) subsample was homogenised for 15 s in 5 mL of 5 mM iodoacetate in 0.15 M KCl, pH 7 buffer using an Ultra Turrax. The pH of the homogenate was measured using a digital pH meter as described above.

Sarcomere length

Sarcomere lengths were measured using a helium-neon gas laser diffraction technique on unfixed portions taken from frozen (-20˚C) samples. The laser has a wavelength of 635nm, and was used as the light source to obtain diffraction patterns from muscle fibre samples held between glass microscope slides. Sarcomere length was determined from the diffraction pattern displayed on a frosted screen (Bouton *et al.* 1973). Sarcomere length (µm) was calculated from the average distance (mm) of the inner and outer diffraction bands from the centre of the screen.

Purge

The purge for each sample was calculated on a weight loss basis. The intact packs were weighed prior to opening and the weight of the empty bag and thermocouple (where present) was subtracted from the initial weight. The purge was expressed as a percentage of the initial weight of the sample.

Myofibrillar particle size

Myofibrillar particle size was measured according to the method of Karumendu et al. (2009) by homogenising approximately 1 g of muscle (sliced along the fibre direction) in 15 mL of ice-cold phosphate buffer (25 mM potassium phosphate, 0.1 M KCl, 1 mM EDTA, pH 7.0), using an Ultra Turrax® with a 10 mm diameter shaft at 16,000 rpm. The samples were homogenised twice for 30 s with a 30 s break on ice. The homogenate was filtered through nylon mesh (1 mm^2) and left on ice until tested for particle size using a laser diffraction particle size analyser (Malvern Mastersizer 2000). The instrument measures the size distribution of particles suspended in a liquid (water) by using the principle of light scattering. The data produced include the volume mean diameter (D[4,3]), which arithmetic average particle diameter (volume based, µm).

Warner-Bratzler shear force and cooking loss

At each storage time point, a subsample was frozen at -20°C for textural analysis. Subsamples were thawed overnight at 5°C, cooked in a water bath at 70°C for 60 min, chilled and stored at 5°C. Samples were weighed prior to cooking and were reweighed after cooking and blotting dry. Cooking loss is expressed as the initial weight minus the final weight, and expressed as a percentage of the initial weight. The cooked samples were cut for textural analysis using Warner-Bratzler (WB) shear force. Details of sample thickness, shape and fibre orientation are given in Bouton et al. (1971) and Bouton and Harris (1972). All textural measurements were made on a Lloyd Instruments LRX Materials testing machine fitted with a 500 N load cell (Lloyd Instruments Ltd., Hampshire UK). Six subsamples having a rectangular cross-section of 15 mm wide by 6.7 mm deep (1 cm^2 cross-sectional area) were cut from all samples, with the fibre orientation parallel to the long axis, and at right angles to the shearing surface. The force required to shear through the clamped subsample with a 0.64 mm thick blade pulled upward at a speed of 100 mm/min at right angles to fibre direction was measured as shear force (N). The mean of the peak force for the six subsamples was recorded.

Free glucose and glycosyl unit content

The protein fraction of frozen muscle samples (1 g) was removed by homogenisation (1:10 w/v) in 30 mM HCl using an Ultra-turrax (22,000 rpm for 3 x 10 s bursts). The homogenate was left on ice for 20-30 min. An aliquot (200 µL) of the settled supernatants was added to a 1 mL solution containing amyloglucosidase (100 mg/100 mL, Roche 102857) diluted 1:100 in acetate buffer (40 mM, pH 4.8) and incubated with shaking at 37°C for 90 min. The mixture was centrifuged at 10,000 g for 5 min. The glucose concentration (µmol/g muscle), including glucose from glycogen plus any free glucose and glucose-6-phosphate, was determined in duplicate using a glucose assay kit (Boehringer Mannheim 716251) and glucose as a standard. The absorbance of both samples and standards was measured at 340nm.

Lactic acid content

D-lactic acid content was measured using lactic acid/UV test kits (r-Biopharm, 11 112 821 035), with modification. Muscle (1.25 g) was homogenised in 5 ml chilled 1 M perchloric acid for 30 s (Ultra-turrax 20,000 rpm) on ice. Milli-Q water was added (2 ml) and muscle proteins were removed by centrifugation at 3,000 rpm, 4°C for 10 min. The pH of the supernatant was adjusted to between pH 10-11 using a saturated potassium hydroxide solution. Contents were transferred quantitatively into a 20 ml volumetric flask and the mixture was shaken. Samples were held at 5°C for 20 min to allow for potassium perchlorate precipitation and subsequently filtered through Whatman #1 filter paper. The clear solution was used for the analysis as described in the lactic acid test kit procedure, and values were expressed as µmol D-lactic acid/g muscle.

Protein content
The protein content of the muscle samples was determined by the Kjeldahl method.

Protein denaturation
The measurements of the thermal stability of whole muscle samples (2 d and 14 d storage) were performed using a micro-differential scanning calorimeter (Micro DSC III, Setaram Inc., Lyon, France) equipped with a refrigerated circulating bath (Julabo, Germany). The instrument was calibrated for temperature and enthalpy using naphthalene as a standard. Each sample (~100 mg) was sealed in a hastelloy sample vessel. Duplicate samples were heated from 25 to 90°C at a scanning rate of 1°C/min, with an equivalent weight of water as a reference. The transition temperature (T_m) was recorded and the transition enthalpy (ΔH) for individual peaks was calculated from the peak area using the Setaram software (SetSoft) and expressed as J/g. The total enthalpy was also calculated using a temperature range of 35-80°C.

Protein solubility
Muscle samples were removed from -80°C storage and tempered for approximately 30 min. Duplicate 2 g samples were used to extract the sarcoplasmic and myofibrillar proteins according to the method of Warner et al. (1997). The protein concentration (mg/g) of myofibrillar and sarcoplasmic protein fractions was determined using the Biuret method with bovine serum albumin as a standard (Gornall et al., 1949).

Protein degradation
Protein extracts were diluted to 2 mg/ml using the appropriate extraction buffer and subsequently diluted to 1 mg/ml with SDS-reducing reagent (Laemmli, 1970). Gel preparations were stored at -80°C until analysis. After thawing, gel preparations were boiled for 5 min to dissociate the proteins and centrifuged at 14,500 rpm for 2 min. Gel preparations and a Precision Plus unstained standard (Bio-Rad 161-0363, 10-250 kDa) were loaded at 20µl onto a Criterion™ TGX stain-free gel, 4-20% (Bio-Rad 567-8094) and run for 55 min at 200 V. Gels were imaged using the Gel Doc EZ system (Bio-Rad 170-8270) and associated software (Image Lab Software).

Collagen content
The total amount of connective tissue in each muscle sample was determined by measuring the hydroxyproline content in lyophilised muscle according to the International Standard method (ISO 3496, 1994).

Structural modifications

Light microscopy (LM)
Subsamples (approximately 1 cm^3) were taken from the centre of each control and treated muscle sample, cryosectioned (10 µm) and stained using Bouin's fixative, Orange G (muscle fibres) and aniline blue (collagen).

Transmission electron microscopy (TEM)
Subsamples were fixed in glutaraldehyde and osmium tetroxide followed by embedding in an araldite/Epon resin mixture.

Statistical analyses
Statistical analyses for the shear force data were done using Genstat 15th edition (http://www.vsni.co.uk/software/genstat). A linear mixed model using REML was

fitted to the shear force data. The fixed effects were treatment*ageing time and the random effects kill date/mob number/animal. Five samples were removed from the shear force data due to high residual values (1 from control, 1 from VFC and 3 from delayed VFC). These all had a pH_u > 5.8. Analyses of all other data are presented as means ± standard error (SE).

Results and Discussion

Protein and collagen contents

Subsamples of each muscle portion were taken at the 2 d storage period for analysis of protein and collagen contents. It was expected that these contents would not change with storage (ageing), hence no samples were collected at the 5 d and 14 d time points.

The mean (mean ± SE) total protein contents (%) for the control, delayed VFC and VFC treatment groups were 22.43 ± 0.305, 22.88 ± 0.371 and 22.58 ± 0.423, respectively.

There was no significant difference (P>0.05) in total collagen content (%) between the 3 treatment groups, with a mean (mean ± SE) of 1.41 ± 0.055 in the control group, 1.57 ± 0.150 in the delayed VFC samples and 1.69 ± 0.112 in the VFC treated samples.

Therefore, delayed VFC or VFC only had no effect on the total protein and total collagen content of the striploin muscle.

Temperature profiles

The temperature loggers experienced some problems, possibly due to moisture from condensation or electrical interference. This was reflected by unstable readings. Therefore it was very difficult to identify the freezing plateau. An example of the cooling rates of LD muscle under the two different chilling regimes is given in Appendix 1.

pH decline and storage

The delayed VFC and VFC treatments resulted in a slower pH decline than the conventionally chilled treatment (Figure 1). The pH at 24 hours post-slaughter was higher in both the very fast chilling treatments (pH 5.69 ± 0.051 for delayed VFC, pH 5.64 ± 0.020 for VFC) compared to the control (pH of 5.49 ± 0.016). This high pH was also evident after 2 and 5 days ageing (Figure 2) but there was no effect of treatment on pH after 14 days ageing. The slower pH decline and high pH at early ageing times of the VFC treated muscle would be expected to impact on the ability of the muscle to retain water and therefore influence purge and cooking losses, as well as affect muscle colour.

Sarcomere length

There was no effect of either chilling treatments (delayed VFC or VFC) on sarcomere length at 2 days storage (Figure 3). At 5 days ageing, sarcomere length appears to be shorter in the delayed VFC muscle samples (1.88 ± 0.066 µm) than in the control (2.11 ± 0.081 µm) or VFC samples (2.01 ± 0.031 µm). However, at 14 days ageing, the sarcomere length of the delayed VFC muscle is longer (2.00 ± 0.023 µm) than

the control (1.87 ± 0.040 µm) or VFC muscle (1.92 ± 0.039 µm). All of the sarcomere lengths were above 1.6 µm, which is the length at which the peak force would be expected to increase. These values also suggest that there was no cold-shortening of muscle during chilling treatments.

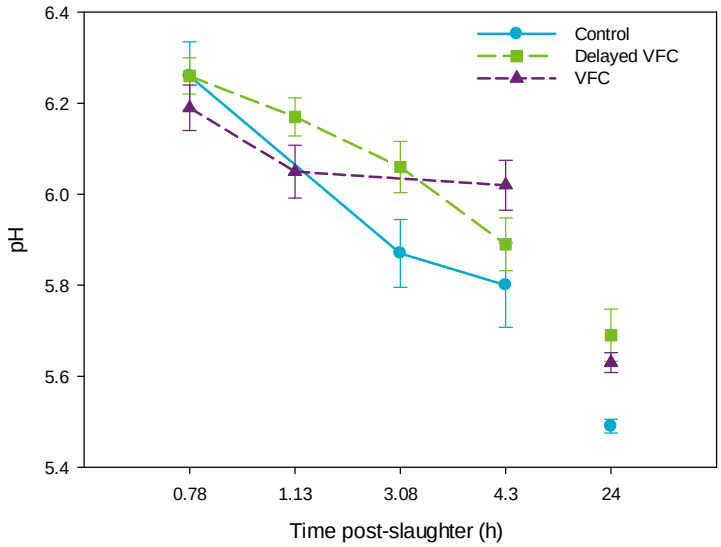

Figure 1: Decline of pH with time post-slaughter of LD muscle. Control (conventionally chilled); delayed VFC (hot-boned muscle held at 15°C prior to very fast chilling, VFC); and VFC (hot-boned muscle chilled in a glycol water bath set at -20°C, very fast chilling). Mean ± SE, n=12.

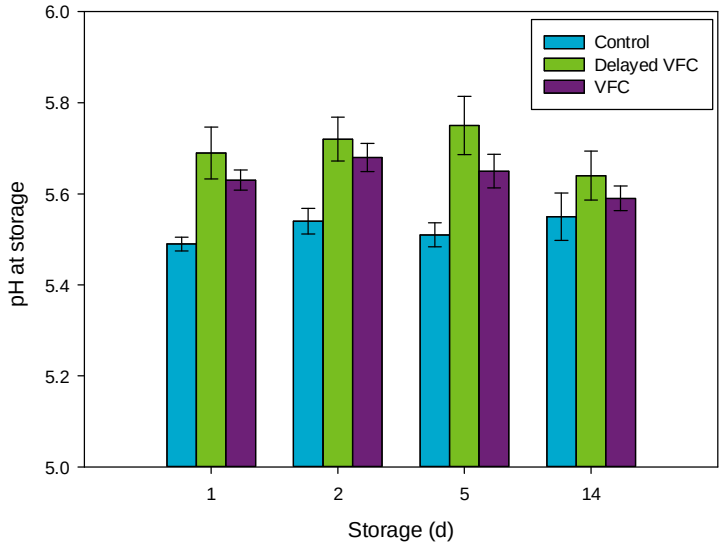

Figure 2: The effect of chilling treatments (control – conventionally chilled; delayed VFC – hot-boned muscle held at 15°C prior to very fast chilling; VFC – very fast chilling, hot-boned muscle chilled in a glycol water bath at -20°C) on pH at different ageing times (days). Mean ± SE, n=12.

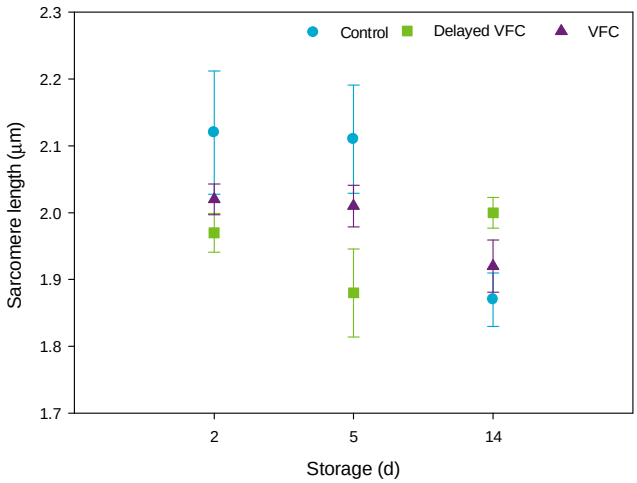

Figure 3: Sarcomere length changes after different chilling regimes of LD muscle. Control – conventionally chilled; delayed VFC – hot-boned muscle held at 15°C prior to very fast chilling; VFC – very fast chilling, hot-boned muscle chilled in a glycol water bath at -20°C. Mean ± SE, n=12.

Colour changes

The surface colour of the LD after blooming appears to have been influenced by both treatment and ageing period in the case of lightness (Figure 4), but treatment only for redness (Figure 5). The control meat was lighter and redder in colour than the both VFC treatments, and meat aged for 14 days was lighter than meat aged for 2 days. The difference in redness between treatments suggests that meat from both VFC treatments had a higher oxygen consumption rate due to mitochondrial activity than the control treatment. This could result in the meat from these treatments being less stable in colour during retail display. The lack of any effect of ageing period on redness suggests that oxygen consumption rate may have changed little during this time for all cooling treatments. The change in lightness with ageing period is consistent with findings from previous studies (Swatland 2004).

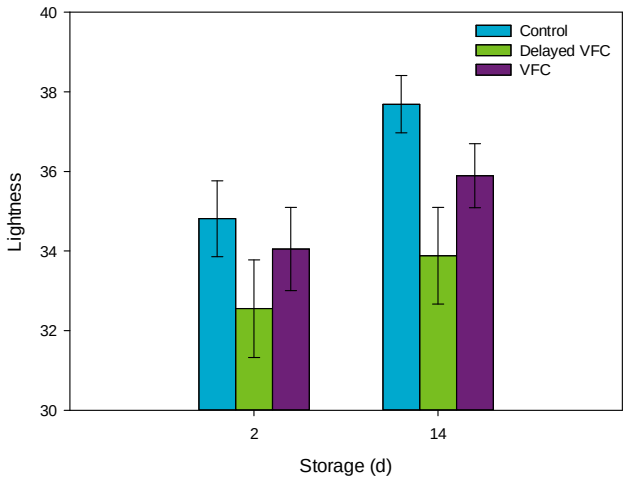

Figure 4: Bloom colour (lightness) of LD muscle at 2 and 14 days ageing subjected to different chilling treatments. Control, conventional chilling; Fifteen, muscle held at 15°C prior to very fast chilling (delayed VFC); and VFC, very fast chilling treatment. Mean ± SE, n=8.

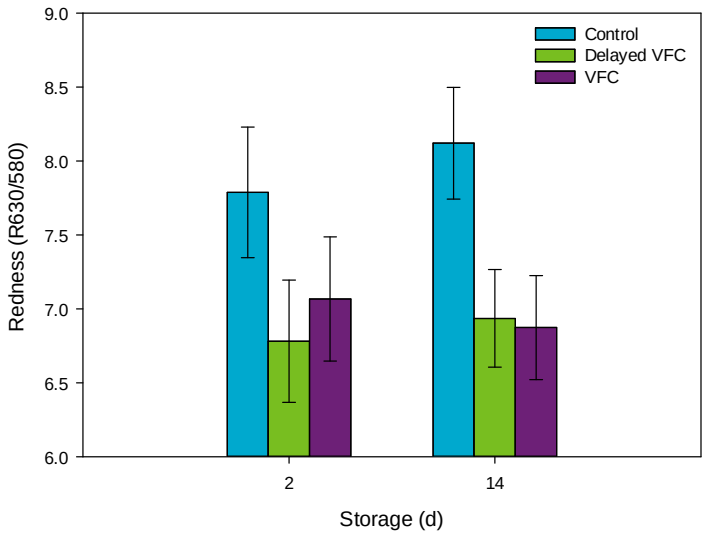

Figure 5: Bloom colour (redness) of LD muscle at 2 and 14 days ageing subjected to different chilling treatments. Control, conventional chilling; Fifteen, muscle held at 15°C prior to very fast chilling (delayed VFC); and VFC, very fast chilling treatment. Mean ± SE, n=8.

Purge

Very fast chilling treatments had a significant effect on the moisture lost from the vacuum-packed muscles during ageing (Figure 6). Purge from control samples was 0.2 ± 0.03%, 0.5 ± 0.08% and 1.1 ± 0.16% at 2, 5 and 14 days ageing, respectively. Purge from both VFC treatments was approximately 0.03% for all ageing periods. This difference is possibly due to the slower pH decline and higher ultimate pH of the very fast chilled samples compared to the control.

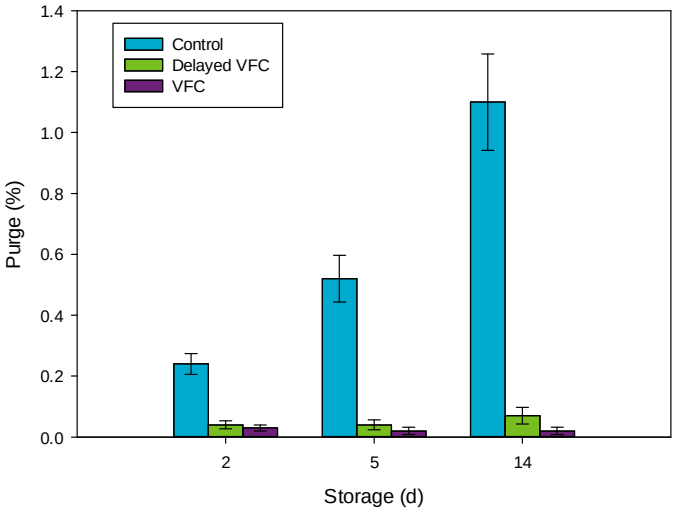

Figure 6: The effect of chilling treatment and ageing time on the purge of LD muscle. Control, conventionally chilled; delayed VFC, hot-boned muscle held at 15°C prior to very fast chilling; VFC, very fast chilling, hot-boned muscle chilled in a glycol water bath at -20°C. Mean ± SE, n=12.

Myofibrillar particle size

There was no effect of chilling treatment on the particle size of myofibrils after 2 days of ageing (Figure 7). After 5 and 14 days ageing, the myofibrillar particle size of the muscles subjected to VFC was smaller than that of the control and the delayed VFC treatment. This method is an indication of the extent of muscle myofibrillar protein degradation under post-mortem conditions and suggests that the VFC treatment exhibited more proteolysis after 5 days and 14 days of ageing compared to the delayed VFC and conventionally chilled muscle.

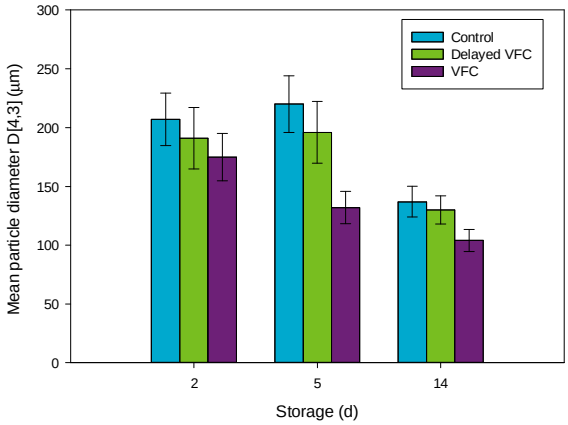

Figure 7: The effect of chilling treatment and ageing time of the myofibrillar particle size (diameter in µm) of LD muscle. Control, conventionally chilled; delayed VFC, hot-boned muscle held at 15°C prior to very fast chilling; VFC, very fast chilling, hot-boned muscle chilled in a glycol water bath at -20°C. Mean ± SE, n=12.

Warner-Bratzler shear force

Muscles subjected to VFC treatment were more tender at 2 days of ageing, having a lower shear force value than control and delayed VFC muscle. There was no difference in treatments at 5 or 14 days ageing. This suggests accelerated tenderisation at 2 days storage.

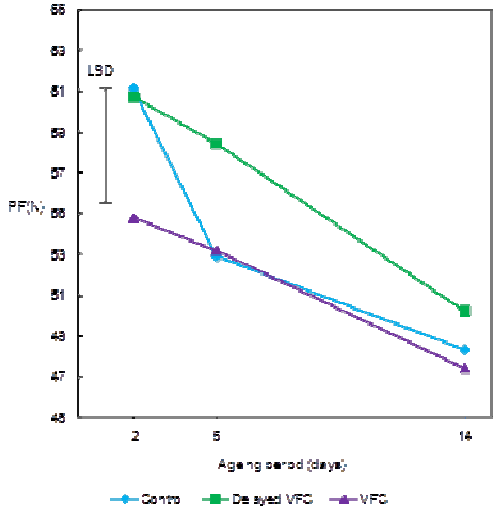

Figure 8: Effect of chilling treatments (control – conventionally chilled; delayed VFC – hot-boned muscle held at 15°C prior to very fast chilling; VFC – very fast chilling, hot-boned muscle chilled in a glycol water bath at -20°C) and ageing time (storage) on texture (peak force) of LD muscle. Values are predicted means with the LSD bar representing the least significant difference (P<0.05) for comparing means for control and VFC treatments on day 2.

Cook loss

The loss due to cooking in a water bath at 70°C for 1 h was lower in the VFC and delayed VFC treatments than in the conventionally chilled treatment after 2 and 5 days ageing (Figure 9). There was no effect of chilling treatment on cooking loss after 14 days ageing. This reduction in cooking loss in VFC treated samples, like the low purge, is probably related to the higher pH at each storage point.

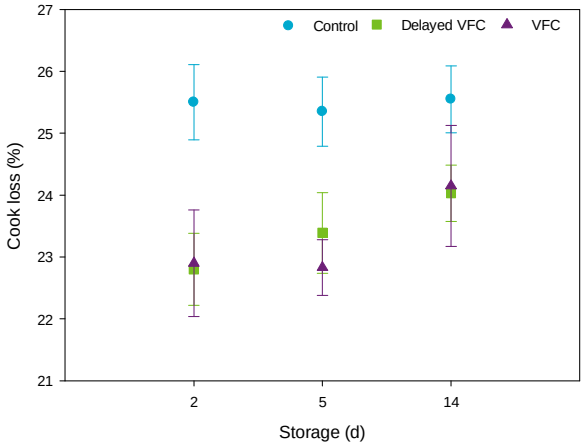

Figure 9: Cook loss (%) from LD muscle subjected to three chilling treatments at 2, 5 and 14 days ageing. Control, conventionally chilled; delayed VFC, hot-boned muscle held at 15°C prior to very fast chilling; VFC, very fast chilling, hot-boned muscle chilled in a glycol water bath at -20°C. Mean ± SE, n=12.

Glucose and Lactate content

Irrespective of ageing, there was a significant effect (P=0.004) of treatment on the glucose content of the beef LD (Figure 10). The predicted means for the control, delayed VFC and VFC treatments were 15.7, 10.9 and 13.6 µmol/g respectively. Conversely, there was no effect of treatment at any storage point on the lactate concentration but there was a significant (P<0.001) increase after 14 days storage (Figure 11).

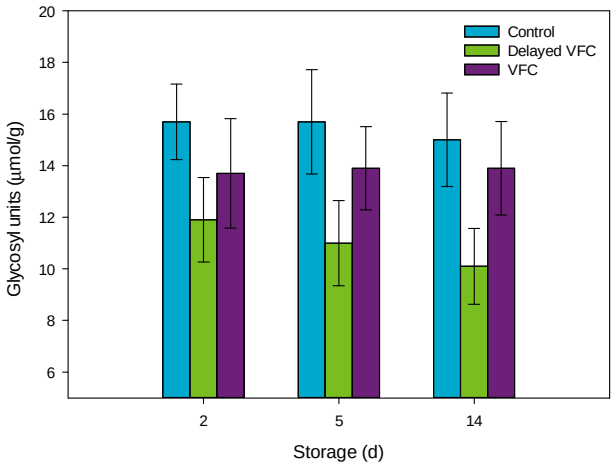

Figure 10: Glucose (glycosyl units) content (µmol/g), including glucose from glycogen plus free glucose and glucose-6-phosphate, of beef LD muscle subjected to chilling treatments and stored for 2, 5 and 14 days. Control, conventionally chilled; delayed VFC, hot-boned muscle held at 15°C prior to very fast chilling; VFC, very fast chilling, hot-boned muscle chilled in a glycol water bath at -20°C. Mean ± SE, n=12.

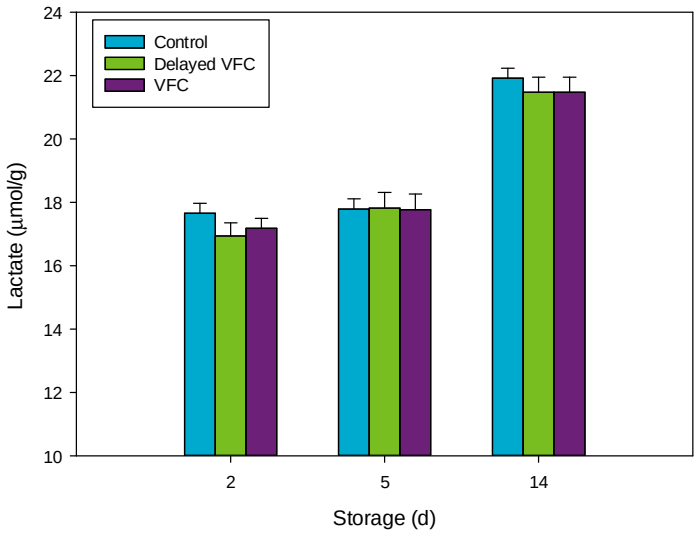

Figure 11: Lactate content (µmol/g) of beef LD muscle subjected to chilling treatments and stored for 2, 5 and 14 days. Control, conventionally chilled; delayed VFC, hot-boned muscle held at 15°C prior to very fast chilling; VFC, very fast chilling, hot-boned muscle chilled in a glycol water bath at -20°C. Mean ± SE, n=12.

Protein denaturation

The three major endothermic transitions of beef muscle are attributed to myosin (~52°C), collagen (~63°C) and actin (~74°C). The sarcoplasmic proteins also contribute to the second major endothermic transition. Very fast chilling treatments (delayed VFC and VFC) did not have any effect on the major muscle proteins (Table 3), as measured by DSC.

Table 3: Thermal characteristics (T_m, transition temperature; ΔH, enthalpy) of beef striploin muscle after chilling treatments; Control, conventionally chilled; delayed VFC, hot-boned muscle held at 15°C prior to very fast chilling; VFC, very fast chilling, hot-boned muscle chilled in a glycol water bath at -20°C. Mean ± SE, n=12.

Treatment	Storage (days)	Peak 1 (myosin)		Peak 2 (sarcoplasmic, collagen)		Peak 3 (actin)		Total enthalpy
		T_m (°C)	ΔH (J/g)	T_m (°C)	ΔH (J/g)	T_m (°C)	ΔH (J/g)	ΔH (J/g)
C	2	51.86±0.157	0.646±0.0782	62.84±0.221	0.484±0.0164	74.60±0.140	0.610±0.0180	4.235±0.2600
	14	52.10±0.157	0.582±0.0544	63.23±0.188	0.452±0.0314	74.85±0.061	0.635±0.0214	4.031±0.3066
Delayed VFC	2	51.41±0.506	0.568±0.0658	63.44±0.242	0.578±0.0208	74.41±0.131	0.596±0.0204	4.234±0.2255
	14	52.14±0.404	0.757±0.0577	63.16±0.210	0.453±0.0248	74.59±0.125	0.621±0.0215	4.364±0.1444
VFC	2	51.42±0.281	0.514±0.0822	63.40±0.278	0.591±0.0229	74.32±0.182	0.598±0.0244	4.077±0.2839
	14	51.69±0.255	0.533±0.0768	63.10±0.192	0.481±0.0252	74.41±0.163	0.589±0.0166	3.785±0.3030

Protein solubility

Neither chilling treatment (delayed VFC and VFC) nor ageing had an effect on the concentrations of the myofibrillar and sarcoplasmic proteins from beef LD muscle (Figure 12).

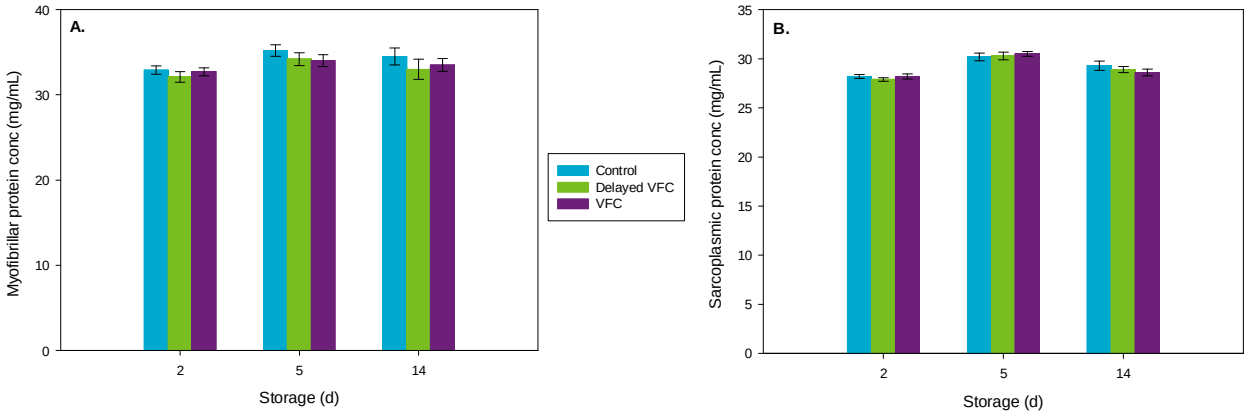

Figure 12: Myofibrillar and sarcoplasmic protein concentration (mg/mL) of LD muscle extracts after chilling treatments and storage at -1°C for 2, 5 and 14 days. Control, conventionally chilled; delayed VFC, hot-boned muscle held at 15°C prior to very fast chilling; VFC, very fast chilling, hot-boned muscle chilled in a glycol water bath at -20°C. Mean ± SE, n=12.

Protein degradation

An example of the separation of the myofibrillar proteins from each of the various treatments of the muscle can be seen in Figure 13. This shows extracts from muscle samples from 2 animals (numbers 9 and 10). Figure 14 shows the volume intensity (arbitrary units) of the major myofibrillar proteins obtained from the myofibrillar fractions from the three differently treated samples. As seen in Figure 13, there is no visual evidence of myosin, α-actinin, desmin or actin degradation with either very fast chilling treatments compared to the control sample.

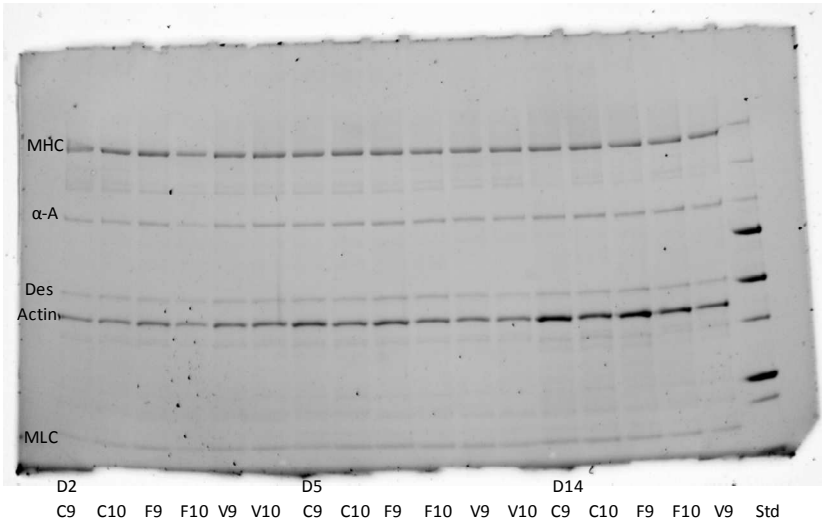

Figure 13: SDS-PAGE (4-20% gel) of myofibrillar proteins from Control (C9, C10), delayed VFC (F9, F10) and VFC (V9, V10) beef LD muscle with storage (D2, 2 days storage; D5, 5 days storage; D14, 14 days storage). MHC, myosin heavy chain; α-A, α-actinin; Des, desmin; MLC, myosin light chain.

However, from the histogram (Figure 14), the relative intensities of the actin bands for the 5 day and 14 day storage periods from the VFC samples are lower than the control and delayed VFC samples, suggesting that the solubility of actin had decreased in the SDS sample buffer used to solubilise these proteins for SDS-PAGE.

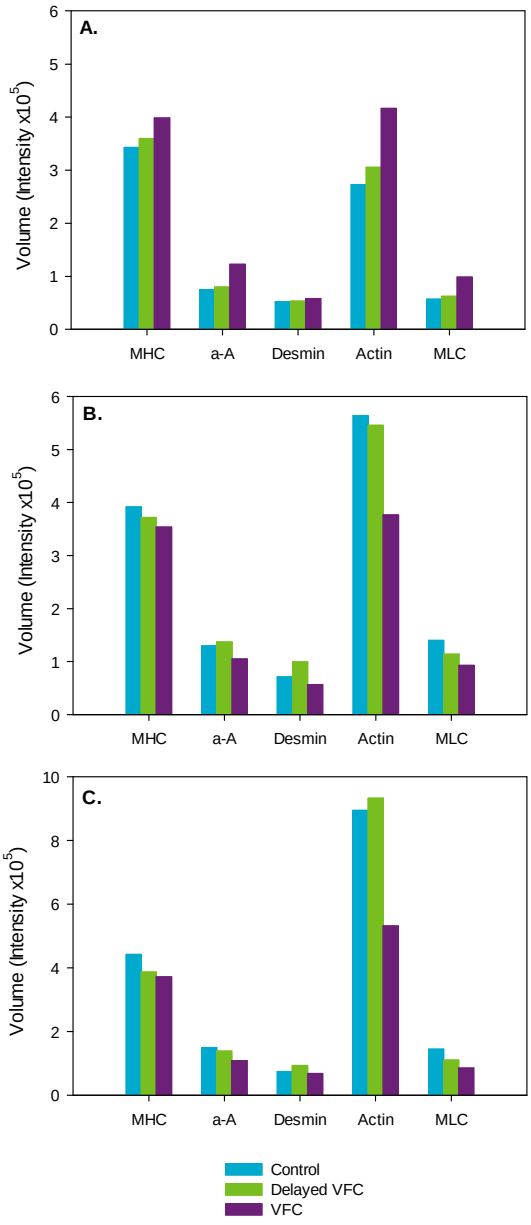

Figure 14: The volume intensity of the different bands (MHC, myosin heavy chain; a-A, α-actinin; Desmin; Actin; MLC, myosin light chains) run on SDS gel electrophoresis (4-20% gel) for the myofibrillar fraction (see Figure 13). A. 2 days storage; B. 5 days storage; C. 14 days storage

The type and distribution of proteins present in the sarcoplasmic fraction of muscle from each treatment can be seen in Figure 15. The control and chilled samples (delayed VFC and VFC) contained similar amounts of the most common sarcoplasmic proteins.

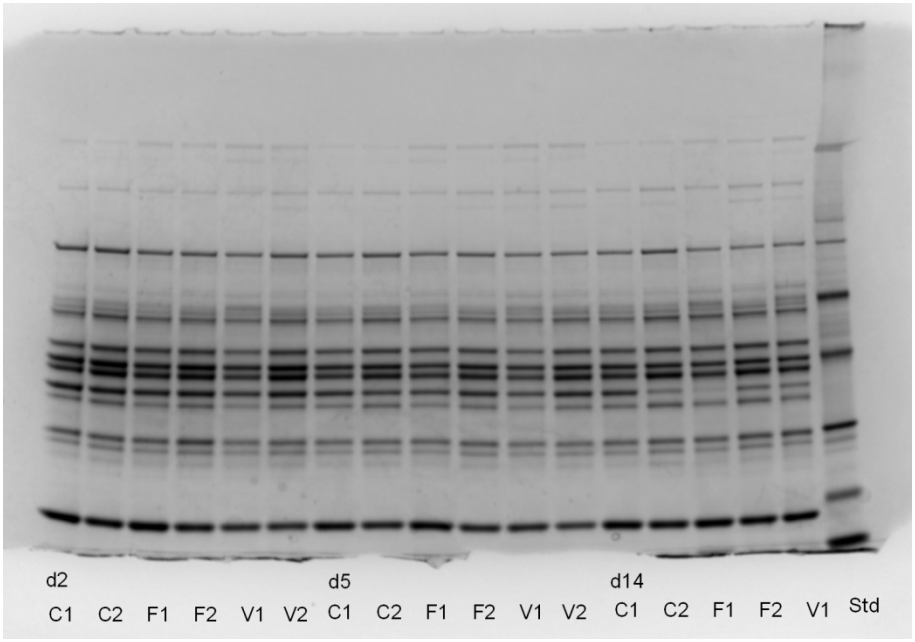

Figure 15: SDS-PAGE (4-20% gel) of sarcoplasmic proteins from Control (C9, C10), delayed VFC (F9, F10) and VFC (V9, V10) beef LD muscle with storage (D2, 2 days storage; D5, 5 days storage; D14, 14 days storage).

Structural modifications

From the light micrographs (Figure 15), it can be seen that a greater number of gaps between fibres is evident in the control samples after 14 days storage and the connective tissue appears to be more aggregated. In the images of the delayed VFC samples, fragmentation of fibres is evident which is more obvious after 14 days storage compared to 2 days storage and could be due to the formation of ice crystals. Freezing damage is more obvious in the VFC samples and there appears to be no difference in storage at 2 or 14 days.

The TEM images of the VFC samples after 2 days storage shows some misalignment of the sarcomeres and possibly greater spaces between the fibres compared to the control and delayed VFC samples (Figure 16). This is more evident in the samples aged for 14 days.

Visualisation of the muscle samples using microscopic methods suggests that very fast chilling has some effect on muscle structure and should be pursued further.

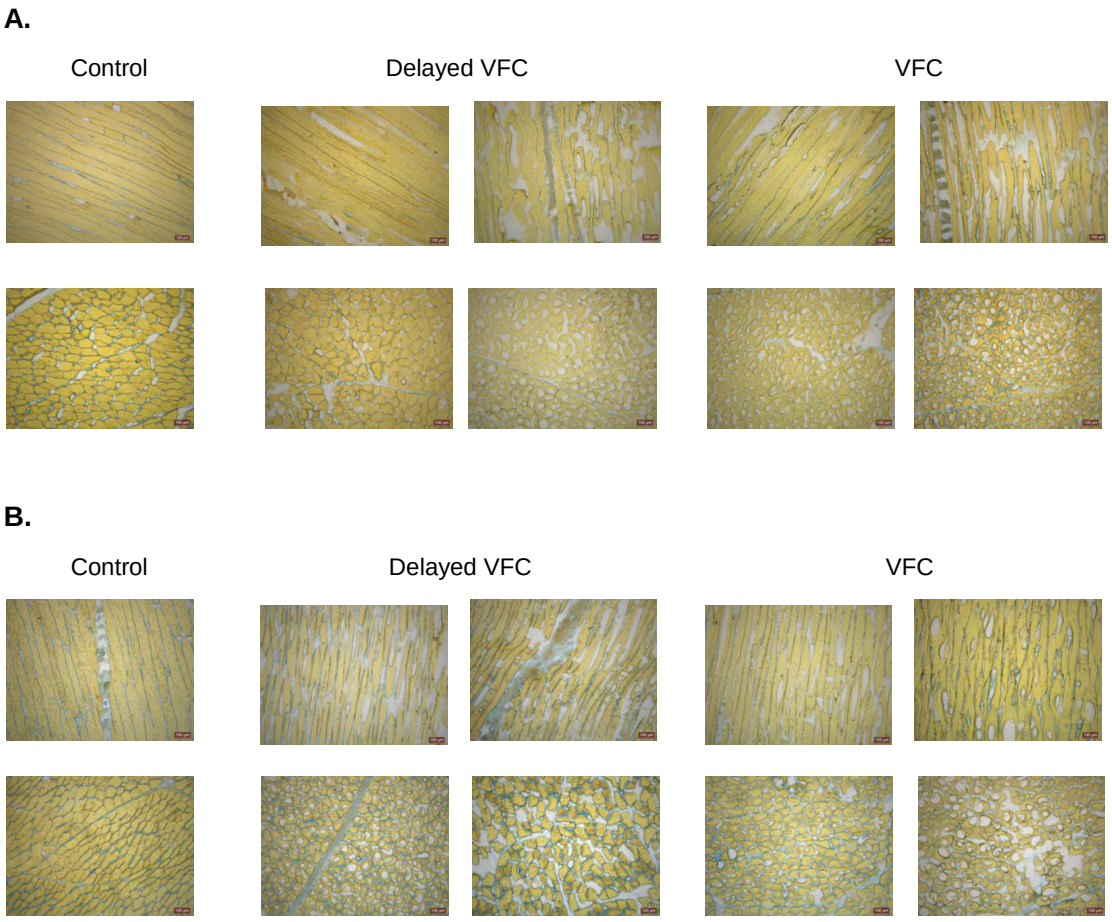

Figure 15: Light microscopy images of beef striploin samples after treatment - Control, conventionally chilled; delayed VFC, hot-boned muscle held at 15°C prior to very fast chilling; VFC, very fast chilling, hot-boned muscle chilled in a glycol water bath at -20°C. Muscle samples were cryosectioned (10 µm) and stained using Bouin's fixative, Orange G and aniline blue. A. after 2 d storage; B. after 14 d storage. Scale bar = 100 µm.

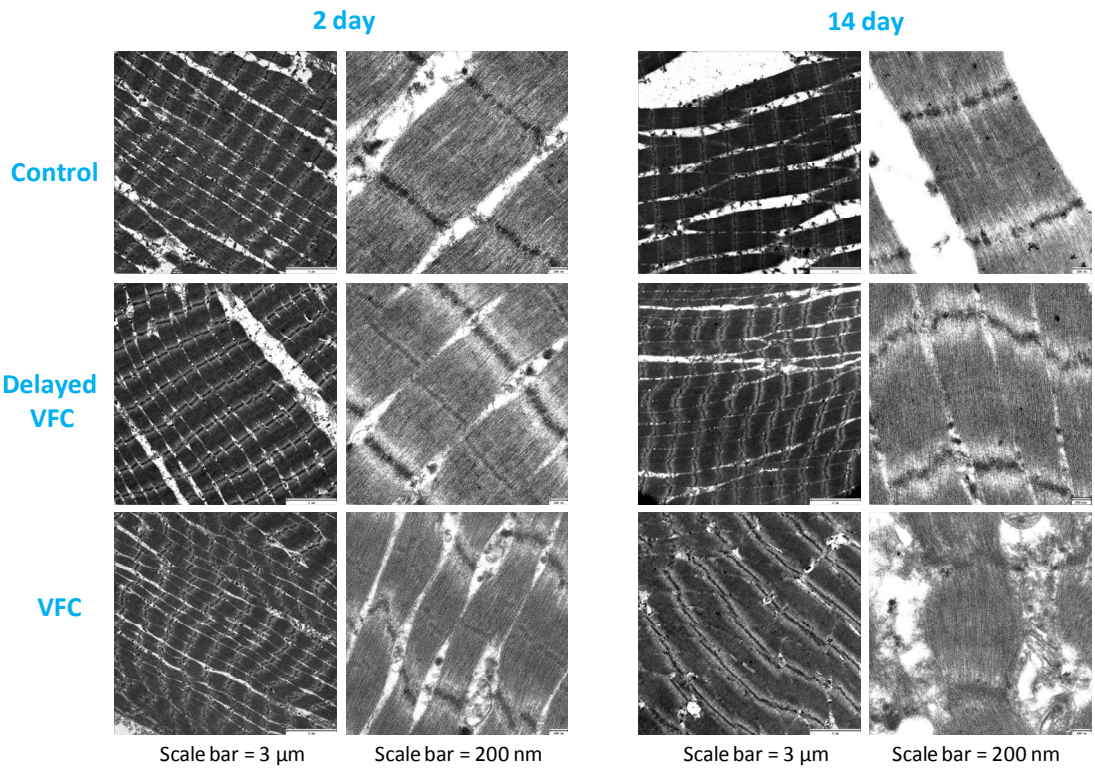

Figure 16: Transmission electron microscopy (TEM) images of beef striploin samples after treatment (Control, conventionally chilled; delayed VFC, hot-boned muscle held at 15°C prior to very fast chilling; VFC, very fast chilling, hot-boned muscle chilled in a glycol water bath at -20°C) and storage at -1°C for 2 days and 14 days.

Conclusions

VFC treatment was successful because the shear values were lower than both the control and delayed VFC after only 2 days of ageing. This suggests accelerated tenderisation occurred due to this treatment and confirms previous studies that the timing of VFC is critical (Jacob, Rosenvold *et al.* 2012). As there was no difference in sarcomere lengths between treatments, this provides an opportunity to investigate NTM independently of sarcomere length with this experiment. There was no effect of treatment on sarcomere length and sarcomeres were longer than the value at which shear force would be expected to be increased (1.6µm), in all treatments. The lack of effect of VFC treatments on the denaturation and degradation of muscle proteins shown in this work using DSC and SDS-PAGE, respectively, also suggests that tenderisation is independent of proteolysis. However, the results of the metabolism analyses (i.e. pH measurements) indicate that the rate of pH decline as well as the ultimate pH are significant modulators of tenderisation using VFC. The light microscopy and TEM images also indicate possible changes in muscle structure due to very fast chilling.

Overall progress of the project

A VFC experiment was successfully carried out at a local processing plant. The differences obtained for shear force between the delayed VFC and VFC treatments provides a good contrast for further comparison of tenderisation mechanisms.

Recommendations

Results from this experiment suggest that tenderisation by VFC of beef LD muscle was independent of muscle shortening and proteolysis. Further work in understanding the mechanism of tenderisation using VFC should focus on 2 aspects:

- Is the prevention of shortening due to crust/zone freezing important?
- Does the modification of the metabolism of VFC meat influence the rate of glycolysis?

Future work using LM or TEM for visualisation of muscle structure changes could involve the development of a technique for measuring the gaps/spaces between the muscle fibres.

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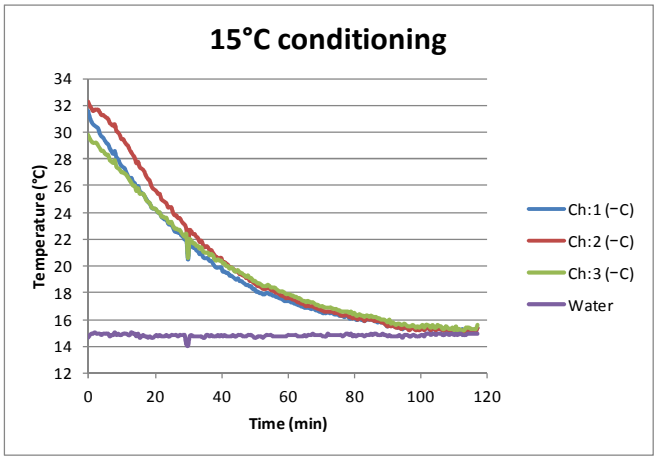
Appendix 1 Temperature-time profiles of beef striploin muscle in a -20°C water bath (45% propylene glycol).

A. Cooling rate of LD muscles held in a 15°C water bath containing water (conditioning).

B. Cooling rate of LD muscles conditioned at 15°C followed by chilling in a glycol water bath set at -20°C (conditioning then VFC).

C. Cooling rate of hot-boned LD muscles in a glycol water bath set at -20°C (VFC).

A.



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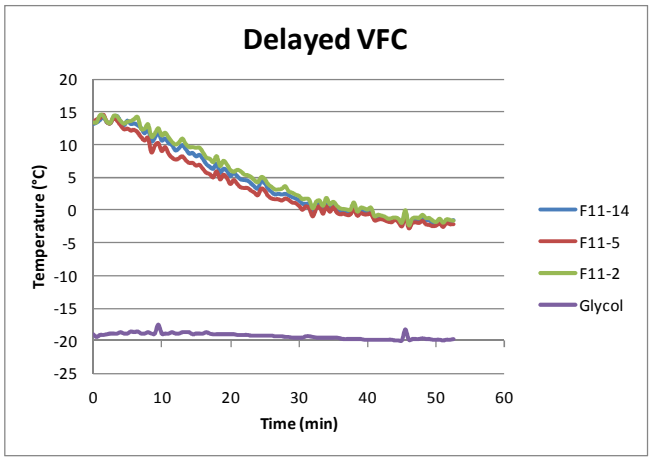

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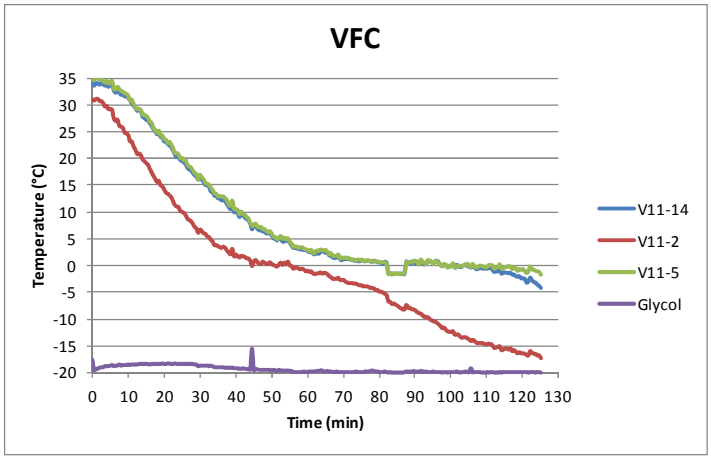


Milestone 6: Defining conditions for applying a combination of VFC and HPP to pre-rigor muscle

milestone report

MLA project code:	**A.MQT.0053**
MLA project title:	**VFC and HPP tenderisation mechanisms**
Project leader:	**Robyn Warner / Anita Sikes**
Prepared by:	**Anita Sikes**
MLA project manager/coordinator:	**Phil Franks / Michael Lee**
Milestone number:	**6**
Date:	**31 December 2013**

Milestone

A progress report submitted outlining the approach to applying a combination of very fast chilling (VFC) and high pressure processing (HPP) to achieve tender meat within 24 hours post slaughter:

- Define the optimum chilling rate (temperature, time) to be achieved prior to HPP treatment
- Outline the optimum HPP conditions (pressure, temperature, time) necessary to tenderise previously very fast chilled muscle

Abstract

Thirty-three *pectoralis profundus* (brisket) muscles, hot-boned from beef carcasses at 30 min post-slaughter, were used to investigate the effect of VFC and HPP applied to pre-rigor beef muscle on texture, cook loss and metabolism and to define the optimum HPP conditions. We have analysed the shear force and sarcomere length data and the remaining data will be included in a subsequent report. This experiment showed that;

- Dry ice can be effectively used to subject meat samples to very fast chilling. Samples achieved sub-zero temperatures within 18 min of being placed in the dry ice. Processing plants generally have a supply of dry ice. Thus this is a simple and cost-effective method for very fast chilling of meat cuts and warrants further investigation.
- Under the conditions used in this experiment, very fast chilling of pre-rigor brisket, without HPP, resulted in very tough meat, similar to control samples.
- Subjecting pre-rigor brisket to a combination of very fast chilling and high pressure processing under conditions of 200 MPa/30°C, 200 MPa/60°C or 600 MPa/60°C resulted in acceptably tender meat (assessed by peak shear force) at 24 hrs post-slaughter. Subjecting brisket samples to VFC and HPP at 600 MPa/30°C resulted in tough meat, similar to VFC and control samples.
- The next experiment should investigate the effect of a defined set of HPP pressure-temperature conditions, applied to pre-rigor meat which has been very fast chilled, either at a larger scale (similar to commercial, depends on availability and accessibility of hot-boned beef meat) or applied to more muscles.

Project objectives

To design a process that will facilitate tenderisation within the first 24 hours post-slaughter for both low- and high-value beef cuts.

Outcomes:

1. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to VFC
2. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to HPP

The aims of the work reported in this interim milestone report were:

a) to repeat the VFC process and rapid tenderisation reported by Jacob et al. (2008 a, b)
b) to investigate the effect of VFC on texture and metabolism during ageing
c) from these results, gain some insight into the mechanism(s) of tenderisation due to VFC

Success in achieving milestone

1. A progress report submitted outlining the approach to applying a combination of VFC and HPP to achieve tender meat within 24 hours post slaughter. We have been successful in producing tender meat at 24 hrs post-slaughter, using a combination of HPP and VFC.

- Define the optimum chilling rate (temperature, time) to be achieved prior to HPP treatment. The optimum chilling rate has been defined and success was achieved in using dry ice to achieve VFC.
- Outline the optimum HPP conditions (pressure, temperature, time) necessary to tenderise previously very fast chilled muscle. Optimum pressure and temperature conditions during high pressure processing were defined from the experiment conducted. These conditions are a pressure of 200 MPa, combined with 30°C or 60°C, or 600 MPa, with 60 °C.

Aims/Background

To investigate the effect of VFC and HPP applied to pre-rigor beef muscle on texture, cook loss and metabolism and to define the optimum HPP conditions.

Experimental design

Due to the complexity of the logistics of the proposed experiment, extensive consultation and discussion was used to determine the final treatments applied and which facilities to use for hot-boning and HPP. At an all day meeting between all project staff (Robin Jacob, Robyn Warner, Anita Sikes, Neil McPhail, Janet Stark, Joanne Hughes), all previous data on VFC and HPP was reviewed and all possible combinations of treatments for VFC and HPP were discussed as well as all possible facilities in eastern Australia. The design presented below was the outcome of these intensive discussions.

In order to undertake HPP and VFC on pre-rigor beef meat, the experiment required (1) access to a beef processing plant amenable to hot-boning, (2) the same processing plant to be close (within 1 hr drive) to the HPP facilities at either Coopers Plains or at Werribee. Teys-Beenleigh was the only beef processing plant considered to be amenable to hot-boning as well as being close to a HPP vessel (at Coopers Plains). Greenham-Tongala and Midfields-Warrnambool undertake hot-boning of

beef carcasses but are 2-3 hrs drive from Werribee. JBS-Brooklyn is close to Werribee but is generally not as cooperative with R&D experiments.

Pre-rigor brisket muscles were collected from the right sides of 32 beef carcases over a period of 3 weeks (Table 1). There were a total of 6 treatments:

- Control – pre-rigor samples wrapped and stored at 15°C for 24 hrs,
- VFC – Very fast chilling (VFC) comprising holding pre-rigor strips of muscle in dry ice for 18 min followed by storage at 5°C for 24 hrs,
- VFC_HPP200 at 30°C – comprising holding pre-rigor strips of muscle in dry ice for 18 min (VFC) followed by storage at 5°C, followed by high pressure processing (HPP) at 200 MPa at 30°C (compression fluid), followed by storage at 1°C for 24 hrs,
- VFC_HPP200 at 60°C – as for VFC_HPP200 at 30°C except the HPP was conducted at 60°C,
- VFC_HPP600 at 30°C - as for VFC_HPP200 at 30°C except the HPP was conducted at 600 MPa,
- VFC_HPP600 at 60°C - as for VFC_HPP200 at 30°C except the HPP was conducted at 600 MPa and 60°C.

For logistical reasons, only one temperature of high pressure treatment could be done on any day (16 animals per temperature of HPP). Therefore, the processing temperatures were alternated on each day (Table 1). Each brisket, on any collection day, was cut into 7 portions and allocated to 3 treatments (control, 2 pressure treatments at one temperature), with 2 replicates for each treatment within a muscle. The seventh sample was an additional control sample, used to monitor the pH during the 24 hour post-slaughter period. The briskets were collected on the slaughter-floor, within 30 min. of slaughter. The samples were subjected to very fast chilling VFC within 60 min. of slaughter. The high pressure processing was applied to samples within 2-3 hrs post-slaughter.

Figure 1: The brisket being hot-boned from a beef carcass

Figure 2: The hot-boned brisket is rapidly taken to the boning room and cut up into slices, for the different treatments

Figure 3: Showing the layout of equipment in the boning room

Figure 4: Control samples in an esky

Figure 5: Samples being subjected to very fast chilling, using dry ice

Figure 6: Samples being subjected to high pressure processing, after being transported back to the CSIRO laboratory at Coopers Plains

Table 1: Temperature (°C) of high pressure treatment of brisket muscle collected from animals on different days.

Animal	Slaughter date									
	13-Nov	14-Nov	18-Nov	19-Nov	20-Nov	21-Nov	25-Nov	26-Nov	27-Nov	28-Nov
1	30°C									
2	30°C									
3		60°C								
4		60°C								
5		60°C								
6			30°C							
7			30°C							
8			30°C							
9				60°C						
10				60°C						
11				60°C						
12				60°C						
13					60°C					
14					60°C					
15					60°C					
16						30°C				
17						30°C				
18						30°C				
19						30°C				
20							60°C			
21							60°C			
23								30°C		
24								30°C		
25								30°C		
26								30°C		
27									60°C	
28									60°C	
29									60°C	
30									60°C	
31										30°C
32										30°C
33										30°C

Materials and methods

Very fast chilling on dry ice

Preliminary trials were conducted to determine the effectiveness of dry ice for VFC. Brisket (point end deckle off) was purchased from a local butcher. The brisket muscle was cut into portions, approximately 20 x 20 x 100 mm. Meat to be chilled was placed in vacuum bags with a thermocouple wire sealed to the bag using a polyurethane flexible sealant (Sikaflex®-227; Sika Australia, Wetherill Park, Australia) and the thermocouple (0.2 mm Type T, copper-constantan wire) was inserted into the end of the brisket muscle. The vacuum bags were evacuated and sealed in a vacuum-packaging machine. The thermocouples were plugged in to the Grant Squirrel Series 1250 temperature logger (Grant Instruments Ltd., Cambridge, UK) which was set to record at 30-second intervals. Logging was commenced and the samples were warmed in a circulating water bath (Julabo F38-ME; Julabo Labortechnik GmbH, Seelbach, Germany) set at 35°C to a core temperature of 30°C (to simulate hot-boned muscle). Once a core temperature of 30°C was reached, the samples were placed in dry ice (3 mm pellets; Air Liquide, Wacol, Australia) and chilled until the freezing plateau was reached (approximately -1.5°C).

The temperature-time profiles of a total of 12 samples are given in Appendix 1. These profiles show that the freezing plateau is reached by 18 min and therefore, this time was chosen for the in-plant VFC of muscle portions of this size.

Animals

A total of 33 briskets (*M. pectoralis profundus*; HAM 2353, point end deckle off) were collected from 33 beef carcases over a 3 week period at Teys Australia, Beenleigh. The animals were collected from a variety of animal types (see Appendix 2), with carcase weights ranging from 217 to 368 kg (mean = 297 kg). Carcase information was obtained for each sample. Pre-rigor (i.e. hot-boned) briskets were collected from the right side of each carcase. One of the samples collected (animal number 22 on 25/11/2013) had a high pH (6.6) after 24 hours post-slaughter (control sample) and therefore was discarded from the data set. Therefore, data from a total of 32 briskets from 32 animals was used in the data analysis.

Sample preparation

The pH and temperature of the intact brisket was measured immediately after hot-boning and the time was also recorded.

Briskets were trimmed of overlying fat and 7 portions, approximately 20 x 20 x 120 mm were cut from each muscle and allocated to treatments.

Control samples

Samples identified as control samples were individually wrapped in cling wrap and packaging tape immediately, transported to the Coopers Plains laboratory in an esky containing bubble wrap and a freezer brick and subsequently stored at 15°C until the pH was 5.8 or less.

Very fast chilling

A thick layer of dry ice “snow” (obtained from Teys) was placed on the bottom of a plastic tub and the samples for VFC were placed on top of this layer and fully covered with more snow. After 18 min, the samples were removed and placed in an esky containing bubble wrap. The samples were transported to the Coopers Plain laboratory and either stored at 5°C prior to HPP or treated with high pressure immediately.

High pressure processing

Pressure treatments were performed using a 0.3 L capacity 850 Mini FoodLab high pressure vessel (Stansted Fluid Power Ltd., Stansted, UK) connected to a circulating water heater (PolyScience 9702, Niles, USA) set so that the temperature of the compression fluid (30% propylene glycol in water, v/v) could be adjusted to 30 and 60°C. Pressure was applied at 200 and 600 MPa at 30 and 60°C, with a hold time of 10 min. The inherent ramp rate was 20 MPa/s and a decompression procedure over a period of 45 s was used. Following release of pressure, samples were held in ice water for 10 min. After cooling, samples were repackaged in vacuum bags and stored at 5°C overnight prior to texture assessment.

pH measurement

Subsamples were taken for pH measurement at designated points along the VFC_HPP process and immediately frozen in CO_2 snow (at the abattoir) or -80°C freezer (at the laboratory) for subsequent analysis:

- Intact, hot-boned muscle
- Individual samples prior to sealing before VFC
- Individual samples before HPP (for the control sample, subsample removed from the additional control portion)
- Individual samples after HPP (for the control sample, subsample removed from the additional control portion)
- Individual samples prior to cooking (> 24 h post-slaughter)

Approximately 1 g of frozen subsample was homogenised for 15 s in cold 10 mL of 5 mM iodoacetate in 0.15 M KCl, pH 7 buffer using an Ultra-Turrax® (T25; IKA Labortechnik, Staufen, Germany), as described by Bendall (1973). The pH of the homogenate was measured using a digital pH meter (TPS WP-80, Springwood, Australia) and intermediate junction pH electrode (Ionode IJ44, Tennyson, Australia) with temperature compensation.

Warner-Bratzler shear force and cooking loss

After 24 hrs of storage of all samples, individual brisket samples were cooked in a water bath at 75°C to an internal temperature of 72°C. After cooling in an ice slurry for 15 min, samples were stored at 5°C for at least one hour prior to cutting for texture analysis. Samples, previously weighed prior to cooking, were removed from the bags and carefully dried with a paper towel and reweighed. Cooking loss was expressed as a percentage of the initial weight. The cooked samples were prepared for measurement of Warner-Bratzler (WB) shear force. Details of sample thickness, shape and fibre orientation are given in Bouton et al. (1971) and Bouton and Harris (1972). All textural measurements were made on a Lloyd Instruments LRX Materials testing machine fitted with a 500 N load cell (Lloyd Instruments Ltd., Hampshire, UK). Six subsamples having a rectangular cross-section of 15 mm wide by 6.7 mm deep (1 cm^2 cross-sectional area) were cut from all samples, with the fibre orientation parallel to the long axis, and at right angles to the shearing surface. The force required to shear through the clamped subsample with a 0.64 mm thick blade pulled upward at a speed of 100 mm/min at right angles to fibre direction was measured as shear force (N). The mean of the peak force for the six subsamples was recorded.

Colour measurement

Before cooking, triplicate surface colour measurements (L*, a* and b* values) were conducted at room temperature (approximately 25°C) with a chromameter (Konica Minolta Inc., Osaka, Japan; illuminant D65, aperture 10 mm).

Sarcomere length

Sarcomere lengths were measured using a helium-neon gas laser diffraction technique on unfixed portions taken from frozen (-20°C) samples. The laser has a wavelength of 635 nm, and was used as the light source to obtain diffraction patterns from muscle fibre samples held between glass microscope slides. Sarcomere length was determined from the diffraction pattern displayed on a frosted screen (Bouton *et al.* 1973). Sarcomere length (µm) was calculated from the average distance (mm) of the inner and outer diffraction bands from the centre of the screen.

Statistical analyses

Data was subjected to REML (Restricted Estimated Maximum Likelihood) with treatment, temperature an interaction as fixed effects and random effects included in the statistical model were Day/Animal/Replicate.

Results and Discussion

Temperature profiles

The temperature profiles are shown in Appendix 3. Generally, the brisket samples reached 0°C within 10-20 min of being placed in the dry ice.

pH decline

These data are not yet available.

Warner-Bratzler shear force

The control, and very fast chill (VFC) treatments produced very tough brisket meat (Figure 1) with a peak shear force of 70-85 N (cutoff for consumer acceptability ~40 N) which was tougher then three of the VFC/HPP treatment combinations. Brisket strips which were subjected to VFC, then high pressure processing (HPP) applied at 200 MPa, resulted in tender meat (~35-45 N) whether the HPP was applied at 30°C or 60°C, relative to control and VFC. When the HPP was applied at 600 MPa, only the 60°C resulted in tender meat (<40 N), the 30°C treatment resulted in very tough meat >80N).

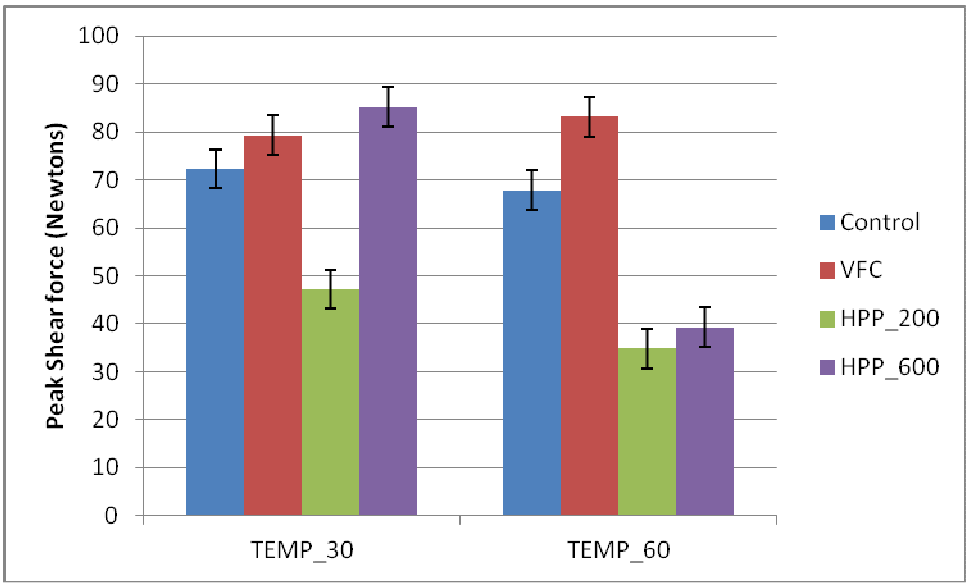


Figure 7: The effect of temperature during processing (Temp_30, HPP conducted at 30°C; Temp_60, HPP conducted at 60°C) and treatment (Control, samples were wrapped pre-rigor and stored for 24 hrs; VFC, Samples were wrapped pre-rigor, subjected to very fast chilling on dry ice, then stored for 24 hrs; HPP_200, samples were wrapped pre-rigor, subjected to VFC, then to HPP at 200 MPa, then stored for 24 hrs; HPP_600, samples were wrapped pre-rigor, subjected to VFC, then to HPP at 600 MPa, then stored for 24 hrs) on the peak shear force of the brisket (*Pectoralis profundus*). Treatment P<0.001, Temperature (<0.001), Treatment x temperature P<0.001. Each bar is a predicted mean and the LSD (least significant difference) for the interaction is shown as a vertical line on top of the bar.

Cook loss

These data are not yet available.

Sarcomere length

The VFC and VFC-HPP treatments resulted in similar sarcomere lengths of about 1.7-1.85 µm. The control treatment resulted in a longer sarcomere length of over 1.9 µm (P<0.001, Figure 8). Thus it is evident that the tenderisation due to HPP was not due to a change in sarcomere length. The toughness of the VFC-only treatment was either similar to the control (30°C), or tougher (60°C), which may have been explained by the shorter sarcomere length than the control.

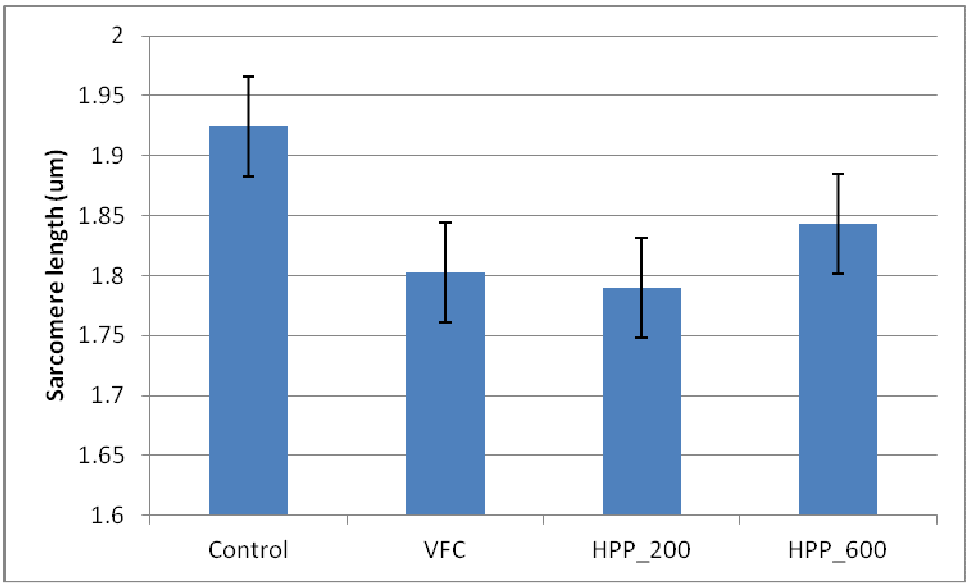


Figure 8: The effect of treatment (Control, samples were wrapped pre-rigor and stored for 24 hrs; VFC, Samples were wrapped pre-rigor, subjected to very fast chilling on dry ice, then stored for 24 hrs; HPP_200, samples were wrapped pre-rigor, subjected to VFC, then to high pressure processing at 200 MPa, then stored for 24 hrs; HPP_600, samples were wrapped pre-rigor, subjected to VFC, then to high pressure processing at 600 MPa, then stored for 24 hrs) on sarcomere length of the brisket (*Pectoralis profundus*). Treatment P<0.001. Each bar is a predicted mean and the LSD (least significant difference) is shown as a vertical line on top of the bar.

Colour changes

These data are not yet available.

Overall progress of the project

The milestones and objectives were previously modified and the project is now on-track to be completed, with results which have implications for the commercial application of VFC and HPP.

Summary and recommendations

This experiment showed;

- Dry ice can be effectively used to subject meat samples to very fast chilling. Samples achieved sub-zero temperatures within 18 min of being placed in the dry ice. Processing plants generally have a supply of dry ice. Thus this is a simple and cost-effective method for very fast chilling of meat cuts and warrants further investigation.
- Under the conditions used in this experiment, very fast chilling of pre-rigor meat, without HPP, resulted in very tough meat.

- Subjecting pre-rigor brisket to combination of very fast chilling and high pressure processing under conditions of 200 MPa/30^{o}C, 200 MPa/60^{o}C or 600 MPa/60^{o}C resulted in acceptable tender meat (assessed by peak shear force).
- The next experiment should investigate the effect of a defined set of HPP pressure-temperature conditions, applied to pre-rigor meat which has been very fast chilled, under larger scale (similar to commercial) conditions.

Bibliography

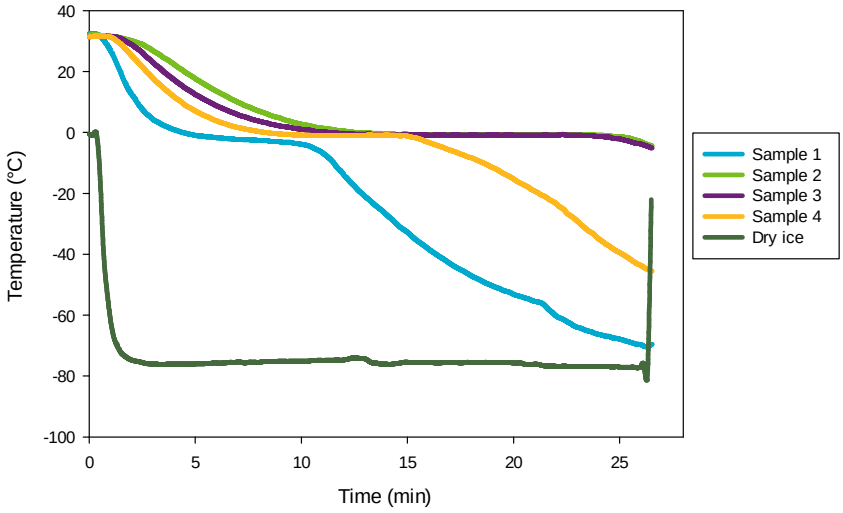
Bouton,, P.E., Harris, P.V. and Shorthose, W.R. (1971) Effect of ultimate pH upon the water-holding capacity and tenderness of mutton. *Journal of Food Science* 36:435-439.

Bouton, P.E. and Harris, P.V. (1972) A comparison of some objective methods used to assess meat tenderness. *Journal of Food Science* 37: 140-144.

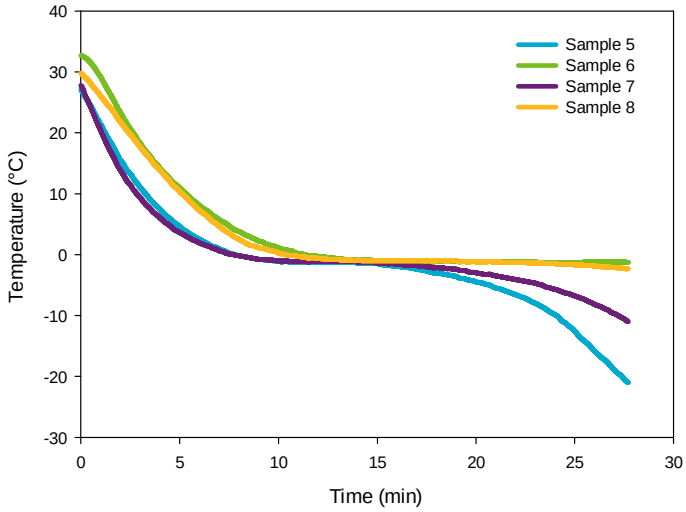
Bouton, P.E., Fisher, Anne L., Harris, P.V. and Baxter, R.I. (1973) A comparison of the effects of some post-slaughter treatments on the tenderness of beef. *Journal of Food Technology* 8(1): 39-49.

Appendix 1 Preliminary trials to determine the effectiveness of dry ice for VFC. Temperature-time profiles of beef brisket muscle in dry ice.

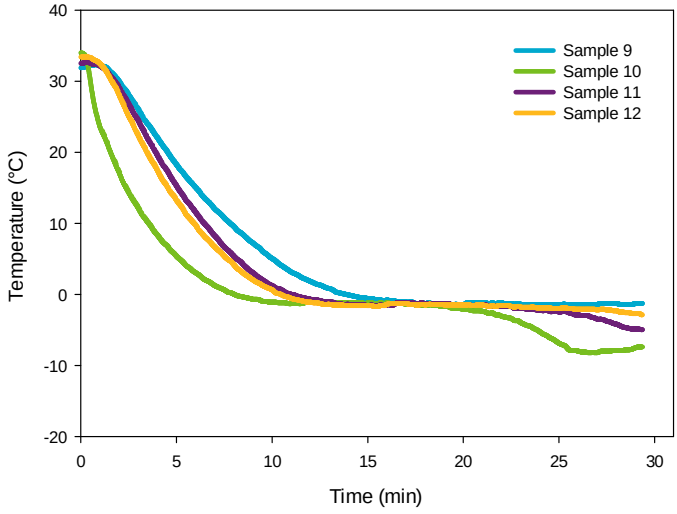
A. Run 1, Nov 8, 2013



B. Run 2, Nov 8, 2013



C. Run 3, Nov 11, 2013

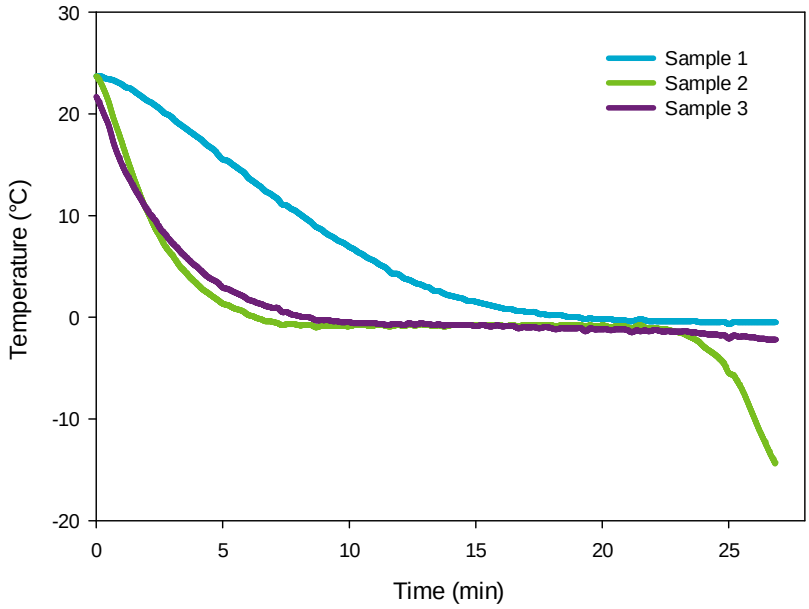


Appendix 2 Number of samples collected in each animal category over the 3 week period (n=33). The highlighted row indicates the sample that had a high pH after 24 hours and therefore the data from this animal was discarded (n=32).

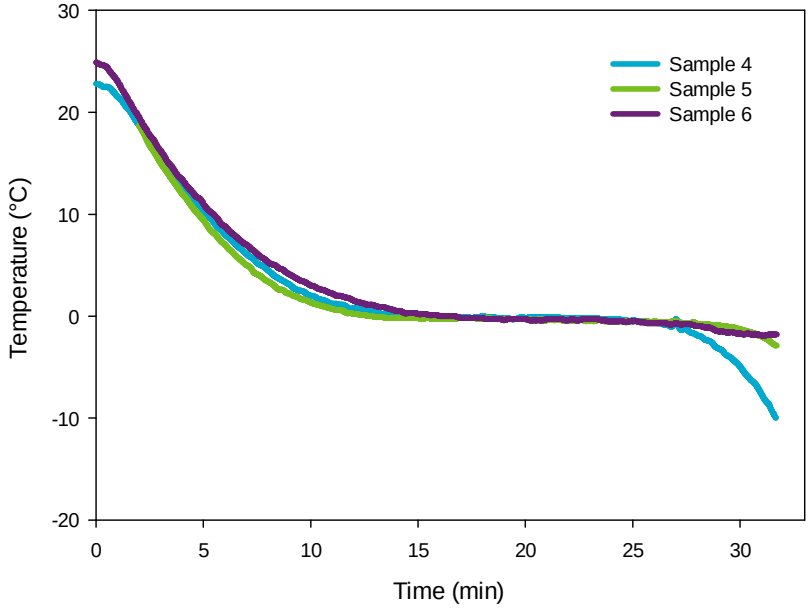
Animal category	*No. of animals*
EU YP	2
Grainfed 300-320 kg	1
MSA EU YP <285kg	2
MSA EU YP >320 kg	3
MSA EU YP 285-320kg	4
MSA GFYG 70 Day Grain	1
MSA YG 100 Day Grain	5
MSA YG Grass <285kg	5
MSA YG Grass >320kg	2
MSA YG Grass 285-320kg	2
MSA YP 100 Day Grain	1
MSA YP Grass >320kg	2
Organic YP	1
YG Grass >320kg	1
YG Grass 285-320kg	1

Appendix 3 Temperature-time profiles of beef brisket muscle in dry ice during VFC runs conducted at Teys Australia, Beenleigh

A. Samples processed on Nov 13, 2013



B. Samples processed on Nov 14, 2013



Milestone 7: Effects of a combination of VFC and HPP on the texture, cook loss and metabolism of pre-rigor beef muscle

milestone report

MLA project code:	**A.MQT.0053**
MLA project title:	**VFC and HPP tenderisation mechanisms**
Project leader:	**Robyn Warner / Anita Sikes**
Prepared by:	**Anita Sikes / Robyn Warner / Robin Jacob**
MLA project manager/coordinator:	**Phil Franks / Michael Lee**
Milestone number:	**7**
Date:	**28 March 2014**

Milestone

From the outcomes of Milestone 6 (optimum VFC and HPP conditions), submit a report outlining the initial results of the effects of applying a combination of VFC and HPP on the texture, cook loss and metabolism of pre-rigor beef muscle.

Abstract

Thirty-two *M*. *pectoralis profundus* (brisket) muscles, removed from beef carcasses about 30 min post-slaughter, were used to investigate the effect of VFC and HPP on texture, cook loss and metabolism. This experiment showed that under the conditions used, very fast chilling of pre-rigor brisket, without HPP, resulted in very tough meat, similar to control samples. However, subjecting pre-rigor brisket to a combination of very fast chilling and high pressure processing under conditions of 200 MPa/30°C, 200 MPa/60°C or 600 MPa/60°C resulted in acceptably tender meat (assessed by peak shear force) at 24 h post-slaughter. Subjecting brisket samples to VFC and HPP at 600 MPa/30°C resulted in tough meat, similar to VFC and control samples. The application of HPP after VFC treatment also resulted in meat with a high ultimate pH which suggests that the combination of VFC and HPP inhibited post-mortem glycolysis. This would be expected to impact the water-holding capacity (increased moisture loss due to denaturation of proteins), however the cook losses of VFC_HPP samples was similar to the controls. The next experiment should investigate the effect of a defined set of HPP pressure-temperature conditions, applied to pre-rigor meat which has been very fast chilled, to confirm these tenderisation results and to further investigate the mechanism of tenderisation.

Project objectives

To design a process that will facilitate tenderisation within the first 24 hours post-slaughter for both low- and high-value beef cuts.

Outcomes:

3. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to VFC
4. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to HPP

Success in achieving milestone

A milestone report submitted outlining the initial results of the effects of applying a combination of VFC and HPP on the texture, cook loss and metabolism of pre-rigor muscle. We have been successful in producing tender meat at 24 h post-slaughter, using a combination of VFC and HPP.

Aims/Background

To investigate the effect of VFC and HPP applied to pre-rigor beef muscle on texture, cook loss and metabolism.

Materials and methods

Animals

A total of 33 briskets (*M. pectoralis profundus*; HAM 2353, point end deckle off) were collected from 33 beef carcases over a 3 week period at Teys Australia, Beenleigh, QLD. The animals were collected from a variety of animal types (see Appendix 1), with carcase weights ranging from 217 to 368 kg (mean = 297 kg). Carcase information was obtained for each sample. Pre-rigor (i.e. hot-boned) briskets were collected from the right side of each carcase. One of the samples collected (animal number 22 on 25/11/2013) had a high pH (6.6) after 24 hours post-slaughter (control sample) and therefore was discarded from the data set. Therefore, data from a total of 32 briskets from 32 animals was used in the data analysis.

Experimental design

There were a total of 6 treatments:

Treatment name	*Description*
Control	Wrapped, stored at 15°C until pH ≤5.8
VFC	Placed in foam box containing dry ice for 18 min
VHPP200T30	VFC then HPP @ 200 MPa, 30°C for 10 min
VHPP200T60	VFC then HPP @ 200 MPa, 60°C for 10 min
VHPP600T30	VFC then HPP @ 600 MPa, 30°C for 10 min
VHPP600T60	VFC then HPP @ 600 MPa, 60°C for 10 min

Once the various treatments were completed, all samples were stored at 5°C until 24 h after slaughter.

The experiment was conducted over a period of 3 weeks, commencing 13/11/2013 and finishing 28/11/2013. This was done due to time constraints imposed by logistical considerations associated with commencing the VFC and control treatments at the plant and the HPP treatment at the laboratory.

After removal from the carcase, each brisket was cut into 7 portions, each of which was allocated to one of 3 treatments (control, 2 pressure treatments at one temperature), with 2 replicates for each treatment within a muscle. The spare sample (seventh) was allocated to the control treatment and used to monitor the pH during the 24 h post-slaughter period (Table 1). VFC only was not originally designed into the experiment as a treatment, but 9 samples were collected during the course of the experiment to see the effect of VFC only. The briskets were collected on the slaughter floor, within 30 min of slaughter. VFC commenced within

60 min of slaughter and high pressure processing within 2-3 h post-slaughter for the samples allocated to these treatments. For the HPP treatments, only one temperature setting could be used on any one day, due to the time required for the temperature of the chamber to equilibrate. Therefore, the HPP temperatures (30°C and 60°C) were swapped each alternate day (Table 2).

Table 1: The number of samples allocated to each treatment (V=VFC, VHP200=VFC and HPP at 200 MPa, VHP600=VFC and HPP at 600 MPa, VHT30=VFC and HPP at 30°C, VHT60=VFC and HPP at 60°C.

	Control	*VHT30*	*VHT60*	*Total*
Control	64			64
V	9			9
VHP200		32	32	64
VHP600		32	32	64
	73	64	64	201

Table 2: The numbers of animals sampled and the HPP temperature assigned for each date.

Date	*HPP temperature*		*Total*
	30°C	*60°C*	
13/11/2013	2		2
14/11/2013		3	3
18/11/2013	3		3
19/11/2013		4	4
20/11/2013		3	3
21/11/2013	4		4
25/11/2013		3	3
26/11/2013	4		4
27/11/2013		4	4
28/11/2013	3		3
Total	16	17	33

VFC using dry ice

A thick layer of dry ice “snow” (obtained from Teys) was placed on the bottom of a plastic tub and the samples for VFC were placed on top of this layer and covered with more snow. After 18 min, the samples were removed and placed in an esky containing bubble wrap for transport to the Coopers Plain laboratory. As described in the Milestone 6 Report, the core temperature of brisket muscle portions, approximately 20 x 20 x 120 mm in size, reached a plateau 18 min after being placed in dry ice, signifying the commencement of freezing. This time was therefore chosen for the in-plant VFC treatment.

Sample preparation
The pH and temperature of the intact brisket was measured immediately after hot-boning and the time was also recorded.

Briskets were trimmed of overlying fat and 7 portions, approximately 20 x 20 x 120 mm were cut from each muscle and allocated to treatments.

Control samples
Samples identified as control samples were individually wrapped in cling wrap and packaging tape immediately, transported to the Coopers Plains laboratory in an esky containing bubble wrap and a freezer brick and subsequently stored at 15°C until the pH was 5.8 or less.

High pressure processing (HPP)
Pressure treatments were performed using a 0.3 L capacity 850 Mini FoodLab high pressure vessel (Stansted Fluid Power Ltd., Stansted, UK) connected to a circulating water heater (PolyScience 9702, Niles, USA) set so that the temperature of the compression fluid (30% propylene glycol in water, v/v) could be adjusted to either 30 or 60°C. Pressure was applied at 200 and 600 MPa at 30 and 60°C, with a hold time of 10 min. The inherent ramp rate was 20 MPa/s and a decompression procedure over a period of 45 s was used. Following release of pressure, samples were held in ice water for 10 min. After cooling, samples were repackaged in vacuum bags and stored at 5°C overnight prior to texture assessment.

pH measurement
Subsamples were taken for pH measurement at designated points along the VFC_HPP process and immediately frozen in CO_2 snow (at the abattoir) or -80°C freezer (at the laboratory) for subsequent analysis:

- Intact, hot-boned muscle
- Individual samples prior to sealing before VFC
- Individual samples before HPP (for the control sample, subsample removed from the additional control portion)
- Individual samples after HPP (for the control sample, subsample removed from the additional control portion)
- Individual samples prior to cooking (> 24 h post-slaughter)

Approximately 1 g of frozen subsample was homogenised for 15 s in chilled 10 mL of 5 mM iodoacetate in 0.15 M KCl, pH 7 buffer using an Ultra-Turrax® (T25; IKA Labortechnik, Staufen, Germany), as described by Bendall (1973). The pH of the homogenate was measured using a digital pH meter (TPS WP-80, Springwood, Australia) and intermediate junction pH electrode (Ionode IJ44, Tennyson, Australia) with temperature compensation.

Warner-Bratzler shear force and cooking loss
After 24 h of storage of all samples, individual brisket samples were cooked in a water bath at 75°C to an internal temperature of 72°C. After cooling in an ice slurry for 15 min, samples were stored at 5°C for at least one hour prior to cutting for texture analysis. Samples, previously weighed prior to cooking, were removed from the bags and carefully dried with a paper towel and reweighed. Cooking loss was expressed as a percentage of the initial weight. The cooked samples were prepared for measurement of Warner-Bratzler (WB) shear force. Details of sample thickness, shape and fibre orientation are given in Bouton et al. (1971) and Bouton and Harris (1972). All textural measurements were made on a Lloyd Instruments LRX Materials Testing Machine fitted with a 500 N load cell (Lloyd Instruments Ltd., Hampshire,

UK). Six subsamples having a rectangular cross-section of 15 mm wide by 6.7 mm deep (1 cm^2 cross-sectional area) were cut from all samples, with the fibre orientation parallel to the long axis, and at right angles to the shearing surface. The force required to shear through the clamped subsample with a 0.64 mm thick blade pulled upward at a speed of 100 mm/min at right angles to fibre direction was measured in Newtons (N) as peak force (PF). The mean of the peak force for the six subsamples was recorded. This method provides a deformation curve which also presents an initial yield (IY) value. The difference between PF and IY (PF-IY) can indicate the resistance of the connective tissue component of the muscle.

Colour measurement

Before cooking, triplicate surface colour measurements (L*, a* and b* values) were conducted at room temperature (approximately 25°C) with a chromameter (Konica Minolta Inc., Osaka, Japan; illuminant D65, aperture 10 mm).

Sarcomere length

Sarcomere lengths were measured using a helium-neon gas laser diffraction technique on unfixed portions taken from frozen (-20°C) samples. The laser has a wavelength of 635 nm, and was used as the light source to obtain diffraction patterns from muscle fibre samples held between glass microscope slides. Sarcomere length was determined from the diffraction pattern displayed on a frosted screen (Bouton *et al.* 1973). Sarcomere length (µm) was calculated from the average distance (mm) of the inner and outer diffraction bands from the centre of the screen.

Statistical analyses

A confidence level of 5% was used to compare significant differences between means ($P<0.05$) using students *t*-test unequal variance (Microsoft Excel, 2007).

Results and Discussion

Metabolism - pH

The pH of the control sample at 2.3 h post-slaughter was higher (P=0.003) than the samples very fast chilled in dry ice prior to high pressure treatment (Figure 1). However, after pressure treatment (2.73 h post-slaughter), the pH increased (P<0.001) to 6.46, 6.50 and 6.58, 6.64 for the 200 MPa and 600 MPa high pressure treatments (at 30 and 60°C), respectively, whereas the pH of the control sample was 6.25. This increased pH of the VFC_HPP samples remained constant until 27 h post-slaughter (Figure 1). This suggests that the combination of VFC using dry ice and high pressure treatment inhibited post-mortem glycolysis. These results are in contradiction to the work done by Macfarlane in the 1970s (Macfarlane, 1973, 1985) who treated pre-rigor sheep *M. semimembranosus* muscles with high pressure treatments (100-150 MPa, 2 min-3 h, 0-30°C) and found that there was a decrease in pH, indicating that post-mortem glycolysis had been greatly accelerated.

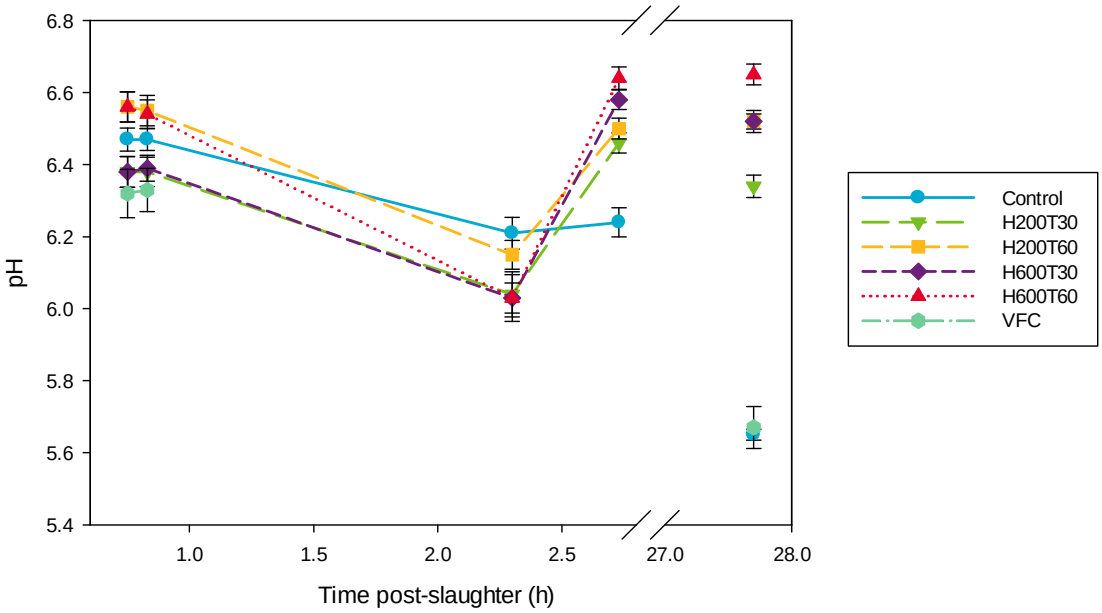


Figure 1: Decline of pH with time post-slaughter of brisket muscle. Control (wrapped pre-rigor, stored at 15°C for 24 h), H200T30 (pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 30°C), H200T60 (pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 60°C), H600T30 (pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 30°C), H600T60 (pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 60°C) and VFC (pre-rigor samples subjected to VFC and stored at 1°C for 24 h). Means ± SE

The control and the VFC only treatments resulted in an ultimate pH (at 27 hours post-slaughter) of 5.6-5.7 (Figure 1). The high pH of the VFC_HPP treated muscle at 27 h post-slaughter would be expected to impact on the water holding capacity of the muscle and therefore influence cooking losses, as well as affect muscle colour.

It should be noted that any comparisons between the control and VFC_HPP treatments with the VFC only treatment must be treated with caution as there were only 9 samples (from 5 animals) used to collect the data for the VFC only treatment.

Texture measurement

The meat from the control and VFC treatments had peak shear forces of 71-82 N (Figure 2) which is greater than the benchmark for consumer acceptability of ~40 N. Brisket strips which were subjected to VFC, then high pressure processing (HPP) applied at 200 MPa, resulted in meat that would be acceptable to consumers (~35-48 N) whether the HPP was applied at 30°C or 60°C. When the HPP was applied at 600 MPa, only the 60°C resulted in meat that would be acceptable to consumers (<40 N), with the 30°C treatment resulting in meat with a shear force of 85 N.

The results for the initial yield values (Figure 3) follow the same pattern as the peak shear force results. The initial yield of the control and VFC treated samples (~63-73 N) was significantly higher ($P<0.001$) than the VFC treatment followed by high pressure treatment at 200 MPa applied at 30°C or 60°C (18-33 N) and the 600 MPa treatment applied at 60°C (~24 N). However, the initial yield of the samples exposed to VFC followed by pressure at 600 MPa and 30°C resulted in similar values to the control and VFC treatments (~74 N). Conversely, the control and VFC treatments

resulted in lower (P<0.001) PF-IY values (8-10 N) than the treatments using a combination of VFC and HPP (11-17 N) (Figure 4). This indicates that the tenderisation effect found with the VFC_HPP treatments is due to modification to the myofibrillar protein components (IY values) of the muscle rather than the connective tissue component (PF-IY values).

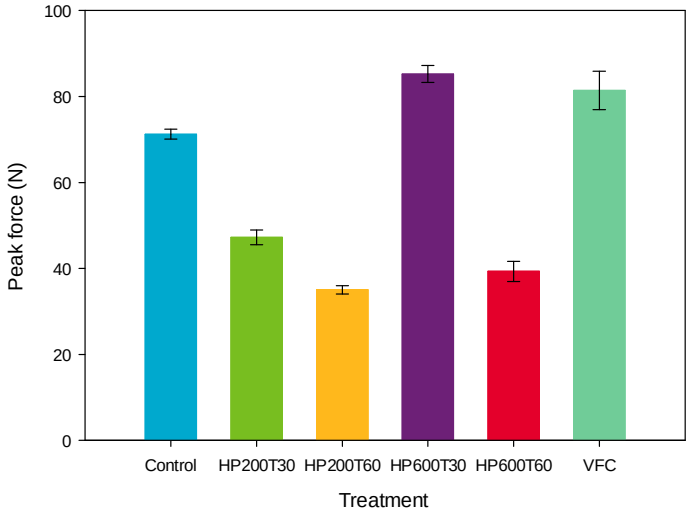


Figure 2: The effect of treatment (Control, wrapped pre-rigor, stored at 15°C for 24 h; HP200T30, pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 30°C; HP200T60, pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 60°C; HP600T30, pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 30°C; HP600T60, pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 60°C; VFC, pre-rigor samples subjected to VFC and stored at 1°C for 24 h) on the peak shear force of the brisket (*M. pectoralis profundus*). Means ± SE.

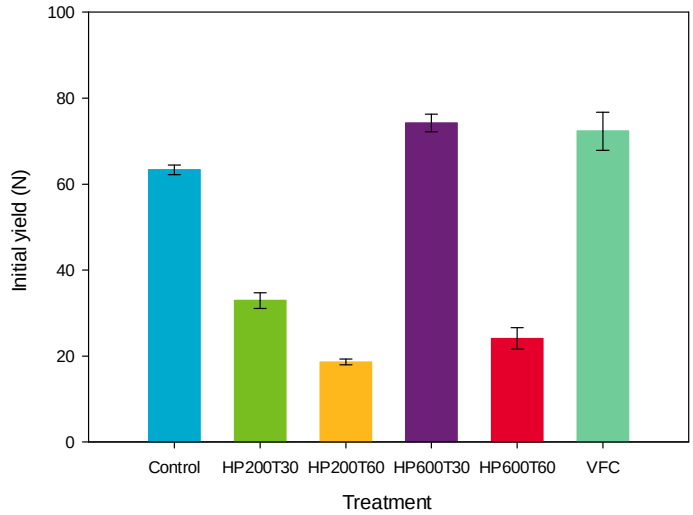


Figure 3: The effect of treatment (Control, wrapped pre-rigor, stored at 15°C for 24 h; HP200T30, pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 30°C; HP200T60, pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 60°C; HP600T30, pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 30°C; HP600T60, pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 60°C; VFC, pre-rigor samples subjected to VFC and stored at 1°C for 24 h) on the initial yield of the brisket (*M. pectoralis profundus*). Means ± SE.

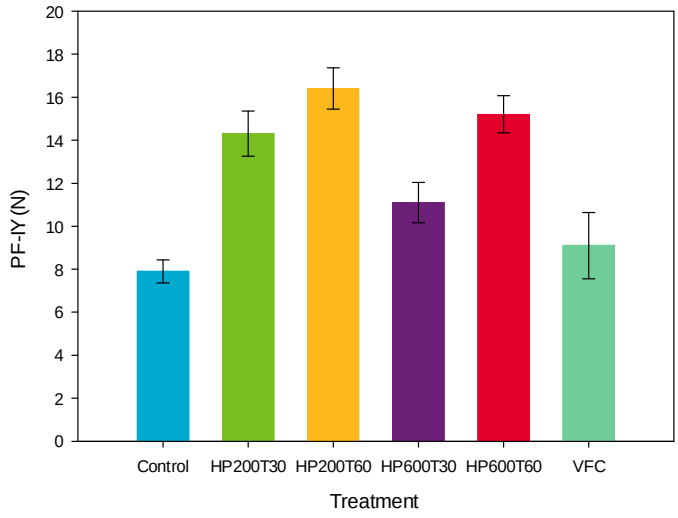


Figure 4: The effect of treatment (Control, wrapped pre-rigor, stored at 15°C for 24 h; HP200T30, pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 30°C; HP200T60, pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 60°C; HP600T30, pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 30°C; HP600T60, pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 60°C; VFC, pre-rigor samples subjected to VFC and stored at 1°C for 24 h) on the peak force minus initial yield (PF-IY) of the brisket (*M. pectoralis profundus*). Means ± SE.

Cook loss

The VFC treatment followed by pressure treatment applied at 600 MPa, at 30°C or 60°C, produced the highest (P<0.001) cook losses (12-16%) (Figure 5). The cook loss for the control, VFC and 200 MPa treatments were similar (~8-10%).

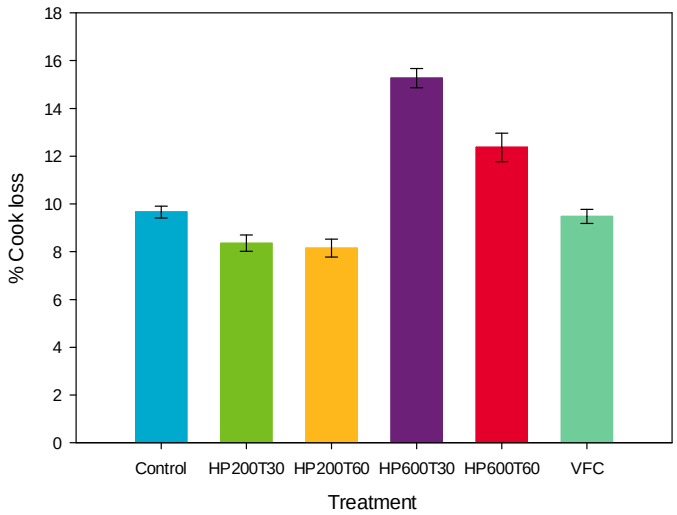


Figure 5: The effect of processing treatments (Control, wrapped pre-rigor, stored at 15°C for 24 h; HP200T30, pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 30°C; HP200T60, pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 60°C; HP600T30, pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 30°C; HP600T60, pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 60°C; VFC, pre-rigor samples subjected to VFC and stored at 1°C for 24 h) on the cook loss (% of initial weight) of beef brisket at 27 hours post-slaughter. Means ± SE.

Sarcomere length

The VFC and VFC_HPP treatments (other than 600 MPa at 60°C) resulted in sarcomere lengths that were not different (P>0.05) of about 1.77-1.81 µm. The control treatment and VFC followed by HPP at 600 MPa and 60°C resulted in significantly (P=0.010, Figure 6) longer sarcomere lengths of over 1.9 µm. However, sarcomere length determination of the samples treated at the higher pressure and temperature (i.e. 600 MPa and 60°C) was more difficult and hence less accurate. Thus it is evident that the tenderisation due to HPP was not due to a change in sarcomere length. The toughness of the VFC only treatment was either similar to the control (30°C), or tougher (60°C), which may have been explained by the shorter sarcomere length than the control.

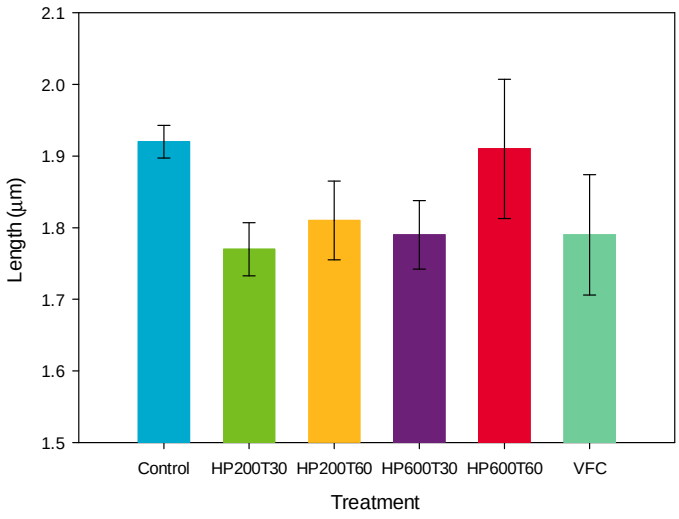


Figure 6: The effect of treatment (Control, wrapped pre-rigor, stored at 15°C for 24 h; HP200T30, pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 30°C; HP200T60, pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 60°C; HP600T30, pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 30°C; HP600T60, pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 60°C; VFC, pre-rigor samples subjected to VFC and stored at 1°C for 24 h) on sarcomere length of the brisket (*M. pectoralis profundus*). Means ± SE.

Colour changes

Measurements of colour taken before cooking showed that the control and VFC-only brisket portions were darker (smaller L* value) than the briskets treated with VFC followed by HPP (Table 3). Overall, the VFC_HPP samples had a 'cooked' appearance after treatment and before cooking compared to the control and VFC-only samples.

Table 3: Effect of treatment (Control, wrapped pre-rigor, stored at 15°C for 24 h; HP200T30, pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 30°C; HP200T60, pre-rigor samples subjected to VFC followed by HPP at 200 MPa, 60°C; HP600T30, pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 30°C; HP600T60, pre-rigor samples subjected to VFC followed by HPP at 600 MPa, 60°C; VFC, pre-rigor samples subjected to VFC and stored at 1°C for 24 h) on colour parameters (L*, lightness; a*, redness; b*, yellowness) of brisket muscle. Means ± SE.

Treatment	L*		a*		b*	
	Mean	***SE***	***Mean***	***SE***	***Mean***	***SE***
Control	35.7	0.38	20.9	0.31	0.94	0.309
H200T30	38.0	0.89	19.3	0.30	0.36	0.544
H200T60	38.2	0.75	18.8	0.38	-0.18	0.491
H600T30	42.6	0.58	22.1	0.42	2.96	0.451
H600T60	53.6	0.43	18.1	0.40	6.63	0.258
VFC	31.6	0.84	16.6	0.65	-4.90	0.690

Overall progress of the project

The milestones and objectives were previously modified and the project is now on-track to be completed, with results which have implications for the commercial application of VFC and HPP.

Summary and recommendations

This experiment showed:

- Under the conditions used in this experiment, very fast chilling of pre-rigor meat, without HPP, resulted in meat with a high shear force and was likely unacceptable to consumers (minimum number of samples/animals analysed).
- Subjecting pre-rigor brisket to a combination of very fast chilling and high pressure processing under conditions of 200 MPa/30°C, 200 MPa/60°C or 600 MPa/60°C resulted in acceptable tender meat (assessed by peak shear force).
- Texture data suggests that tenderisation using a combination of VFC followed by HPP is due to modification(s) of the myofibrillar proteins rather than the connective tissue proteins.
- The pH at 2.3 h post-slaughter (before HPP treatment) was significantly lower in VFC samples than the control samples.
- After pressure treatment (2.73 h post-slaughter), the pH of the VFC_HPP samples was significantly higher than the control samples.
- Ultimate pH (27.7 h post-slaughter) of VFC_HPP samples remained constant (pH 6.4-6.6) after HPP treatment and was significantly higher than the ultimate pH of control and VFC-only samples (pH 5.6-5.7). This suggests that the combination of VFC and HPP inhibited post-mortem glycolysis.
- The next experiment should investigate the effect of a defined set of HPP pressure-temperature conditions, applied to pre-rigor meat which has been very fast chilled, to confirm the tenderisation results of this experiment and to further investigate the mechanism of this tenderisation process.

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Appendix 1 Number of samples collected in each animal category over the 3 week period (n=33). The highlighted row indicates the sample that had a high pH after 24 hours and therefore the data from this animal was discarded (n=32).

Animal category	*No. of animals*
EU YP	2
Grainfed 300-320 kg	1
MSA EU YP <285kg	2
MSA EU YP >320 kg	3
MSA EU YP 285-320kg	4
MSA GFYG 70 Day Grain	1
MSA YG 100 Day Grain	5
MSA YG Grass <285kg	5
MSA YG Grass >320kg	2
MSA YG Grass 285-320kg	2
MSA YP 100 Day Grain	1
MSA YP Grass >320kg	2
Organic YP	1
YG Grass >320kg	1
YG Grass 285-320kg	1

Appendix 2: Photographs depicting the steps during the VFC process using dry ice and subsequent HPP.

Figure 1: Brisket muscle being hot-boned from the carcase

Figure 2: The hot-boned brisket is taken to the boning room and cut up into slices, and allocated to the different treatments

Figure 3: Samples being subjected to very fast chilling using dry ice

Figure 4: Control samples, wrapped in glad wrap and packaging tape, placed in an esky, for transport to the CSIRO-Coopers Plains laboratory

Figure 5: Samples being subjected to high pressure processing, after being transported to the CSIRO-Coopers Plains laboratory

Figure 6: The general layout of equipment in the boning room at Teys, Beenleigh for the VFC process

Milestone 8: Effects of two VFC methods on two pre-rigor beef muscles, specifically on the prevention of muscle shortening and the modification of metabolism

milestone report

MLA project code:	**A.MQT.0053**
MLA project title:	**VFC and HPP tenderisation mechanisms**
Project leader:	**Anita Sikes**
Prepared by:	**Anita Sikes**
MLA project manager/coordinator:	**Phil Franks / Michael Lee**
Milestone number:	**8**
Date:	**30 May 2014**

Milestone

A milestone report submitted outlining the effects of two VFC methods (immersion, dry ice) on texture of two pre-rigor beef muscles, focussing on the importance of:

- The prevention of muscle shortening due to crust freezing;
- The modification of metabolism (rate of pH decline and ultimate pH)

Abstract

A very fast chilling (VFC) experiment was successfully conducted at Teys Australia, Beenleigh, QLD. Sixteen striploin and brisket muscles, hot-boned from beef carcasses (average HSCW 305 kg) at 40 min post-slaughter, were used to investigate the effect of two different very fast chilling methods (dry ice, waterbath immersion) on texture, metabolism, sarcomere length and cook loss. The waterbath immersion VFC treatment was successful for both striploin and brisket muscles as the shear force values were similar (brisket) or lower (striploin) than the (untreated) controls. However, VFC of striploin and brisket muscle using dry ice produced tougher meat than control samples, suggesting that the rate of chilling is critical for improved texture of very fast chilled muscle. There was no difference in sarcomere lengths between treatments for both muscles. This indicates that cold-shortening did not occur with VFC. The rate of pH decline differed depending on the muscle, with a slower decline in striploin muscle and a faster decline in brisket muscle compared to control samples. Therefore, future work should focus on the rate of chilling and the rate of pH decline and the resultant effect on texture of very fast chilled muscle.

Project objectives

To design a process that will facilitate tenderisation within the first 24 hours post-slaughter for both low- and high-value beef cuts.

Outcomes:

5. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to VFC
6. Definition of the chemical and/or physical mechanisms associated with accelerated tenderisation due to high pressure processing (HPP)

Success in achieving milestone

A VFC experiment was conducted at Teys Australia, Beenleigh, QLD, over a 2 week period from May 5, 2014. Samples from 16 animals were collected and processed in-plant and samples transported to Coopers Plains, QLD. The last sample collection day was May 15, 2014. Analyses for texture, sarcomere length and pH measurement have been completed and preliminary statistical analysis of this data is presented in this report. Samples from one animal have been sent to CSIRO-AAHL in Geelong, VIC, for transmission electron microscopy analysis. A VFC trial was successfully completed with kind cooperation from Teys Australia staff with sample collection and processing completed in-plant on May 15, 2014. Analyses are completed and preliminary statistical analysis of results are presented.

Aims/Background

Discussions from a project meeting (Go/No Go point) held on 18 March, 2014 between MLA (Phil Franks, Raj Margapuram) and CSIRO (Robyn Warner, Anita Sikes, Neil McPhail, Katrina Spencer) defined the direction of the experimental work for Milestone 8. A change of scope (from that originally outlined in the contract) was discussed and agreed to based on the results of the previous milestones. Further investigation of the mechanism of tenderisation (or lack of toughening) of beef muscle using VFC is warranted, focussing on the prevention of cold-shortening and the modification of metabolism. A comparison of the texture between the surface and the core of the muscle samples will also be carried out. Samples for metabolomic analysis will be taken and stored frozen and it was agreed that this analysis would not be completed within the timeframe remaining in this project.

Experimental design

Pre-rigor brisket *(M. pectoralis profundus*) and striploin (*M. longissimus dorsi*) muscles were collected from the left sides of the same animal from a total of 16 beef carcases over a period of 2 weeks. Each muscle from one animal was cut into 3 portions and allocated to treatments (Table 1). The muscles were collected on the slaughter-floor within 40 min of slaughter and subjected to VFC within 60 min of slaughter.

Table 1: Explanation of treatments used for in-plant very fast chilling.

Control (C)	Pre-rigor muscle wrapped in glad wrap and masking tape, held at 15°C until pH is 5.8 or less (approx. 24 h)
Dry ice VFC (D)	Samples placed in Warwick tray containing dry ice: 30 min for brisket; 50 min for striploin Storage at 5°C for 24 h
Waterbath VFC (W)	Samples placed in waterbath containing 45% propylene glycol set to -20°C: 25 min for brisket; 35 min for striploin Storage at 5°C for 24 h

Materials and methods

Pre-trial chilling conditions

Temperature profiles from work conducted prior to the in-plant trials on brisket and striploin muscle samples (specific dimensions) to identify the appropriate chilling times for both brisket and striploin muscles in dry ice and immersion chilling are given in Appendix 1. Based on these results, it was decided to chill brisket muscles for 25 min by immersion and 30 min in dry ice; striploin muscles will be held in the waterbath for 35 min and 50 min in dry ice. These times were chosen as they indicate the time to reach the middle of the freezing plateau.

Animals

A total of 16 briskets (*M. pectoralis profundus*; HAM 2353, point end deckle off) and 16 striploins (*M. longissimus dorsi*, HAM 2140) were collected from the left side of 16 beef carcases over a 2 week period at Teys Australia, Beenleigh, QLD. The animals were collected from a variety of animal types and categories (see Appendix 2), with carcase weights ranging from 192 to 402 kg (mean = 305 kg). Carcase information was obtained for each sample.

Sample preparation

Briskets were trimmed of overlying fat and the deckle removed. The striploin had the fat layer and cap removed. Three portions were cut from each muscle (Figure 1): approximate dimensions for the brisket were 25 x 80 x 100 mm and 40 x 90 x 100 mm for the striploin. The portions were randomly allocated to treatments, weights recorded and the samples for very fast chilling treatments were vacuum packed.

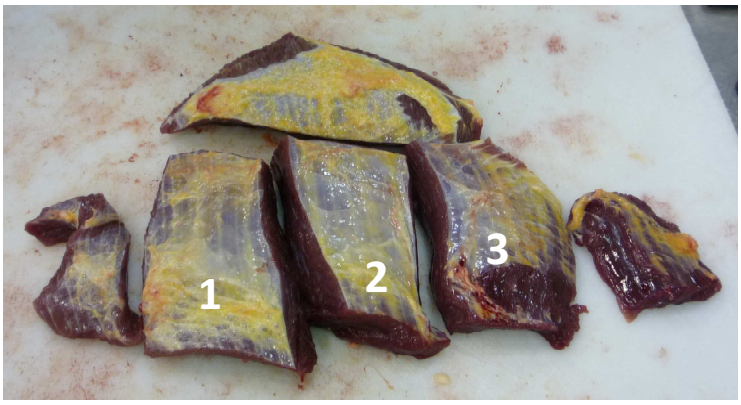

Figure 1: Preparation and cutting of brisket muscle, indicating the 3 portions allocated to treatments.

Control samples

Samples identified as control samples were individually wrapped in cling wrap and packaging tape immediately, stored in an esky at room temperature or above and transported to the Coopers Plains laboratory in an esky and subsequently stored at 15°C until the pH was 5.8 or less.

Very fast chilling on dry ice

Muscle samples were placed on a layer of dry ice (supplied by Teys, “snow”) in a Warwick tray and covered with sufficient dry ice to completely cover the muscles and form a thick layer. The briskets were held on the dry ice for 30 min and the striploins for 50 min. After the designated times, the bags were opened, subsamples taken, the bags resealed and samples stored at -1.5°C until transported to Coopers Plains (4-5 h from collection of the first sample) and subsequently stored at 5°C for approximately 24 h.

Very fast chilling in waterbath (immersion)

Muscle samples were immersed in a waterbath (F38-ME, Julabo Labortechnik GmbH, Seelbach, Germany) containing 45% (v/v) propylene glycol set to -20°C. Brisket samples were immersed for 25 min and striploin samples for 35 min. After VFC, the bags were opened so subsamples could be removed and the bags resealed and stored at -1.5°C until transported to Coopers Plains and subsequently stored at 5°C for approximately 24 h.

pH measurement and subsampling for metabolomic analysis

Subsamples (approximately 1 g) were taken for pH measurement and metabolomic analysis at designated time points, immediately frozen in liquid nitrogen (in-plant and at the laboratory) and stored at -80°C for subsequent analysis:

- Before VFC (T1)
- After VFC (T2)
- 3 h after hot-boning (T3)
- 6 h after hot-boning (T4)
- ≥ 24 h (i.e. before cooking for texture analysis) (T5)

For pH measurement, approximately 1 g of frozen subsample was homogenised for 15 s in chilled 10 mL of 5 mM iodoacetate in 0.15 M KCl, pH 7 buffer using an Ultra-Turrax® (T25; IKA Labortechnik, Staufen, Germany), as described by Bendall (1973). The pH of the homogenate was measured using a digital pH meter (TPS WP-80, Springwood, Australia) and intermediate junction pH electrode (Ionode IJ44, Tennyson, Australia) with temperature compensation.

Samples for metabolomic analysis are stored at -80°C until analysis at a later date (as agreed).

The location of subsamples taken at 24 h is shown in Figure 2. Please note the placement of a blue pin (indicated by an arrow) in the front/left corner of each sample to ensure orientation of each sample is the same. The remaining sample (bordered by the dotted lines in Figure 2) was used for texture analysis.

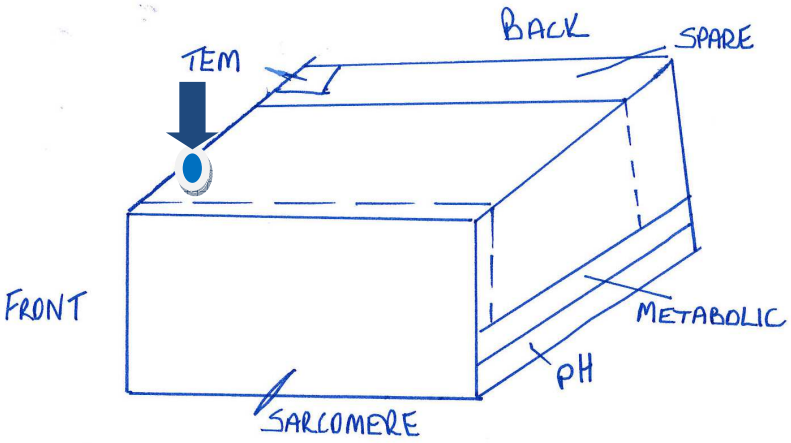

Figure 2: Location of subsamples taken for each analysis at 24 h. The orientation of the sample on the cutting board was the same at all times (see blue pin placement).

Sarcomere length

Sarcomere lengths were measured using a helium-neon gas laser diffraction technique on unfixed portions (2-3 mm thick) taken from frozen (-20°C), raw samples. Cooked strips (numbers 3 and 4) from the striploin samples were also frozen (-20°C) after texture analysis. The laser has a wavelength of 635 nm, and was used as the light source to obtain diffraction patterns from muscle fibre samples held between glass microscope slides. Sarcomere length was determined from the diffraction pattern displayed on a frosted screen (Bouton *et al.*, 1973). Sarcomere length (µm) was calculated from the average distance (mm) of the inner and outer diffraction bands from the centre of the screen.

TEM analysis

A subsample (approximately 10 x 15 x 15 mm) was taken from all treated samples from one animal, as shown in Figure 2. These samples were longitudinal sections, with the fibre direction on the smaller surface of the meat (Figure 3). The samples were fixed in 2.5% glutaraldehyde in Tyrodes buffer (pH 5.5)

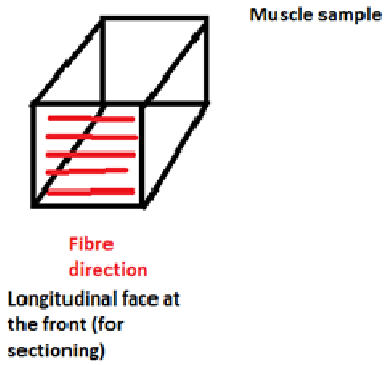

Figure 3: Sectioning of muscle sample for TEM analysis.

Warner-Bratzler shear force and cooking loss

After approximately 24 h of storage of all samples, samples were cooked in a water bath at 74°C to an internal temperature of 72°C: 40 min for brisket and 70 min for striploins (Appendix 3). After cooling in an ice slurry for 30 min, samples were stored at 5°C for at least one hour prior to cutting for texture analysis. Samples, previously weighed prior to cooking, were removed from the bags and carefully dried with a paper towel and reweighed. Cooking loss was expressed as a percentage of the initial weight. The cooked samples were prepared for measurement of Warner-Bratzler (WB) shear force, as shown in Figure 4 for brisket and Figure 5 for striploin. This sample preparation was done so that a comparison of the texture on the surface and the centre of the muscle was possible.

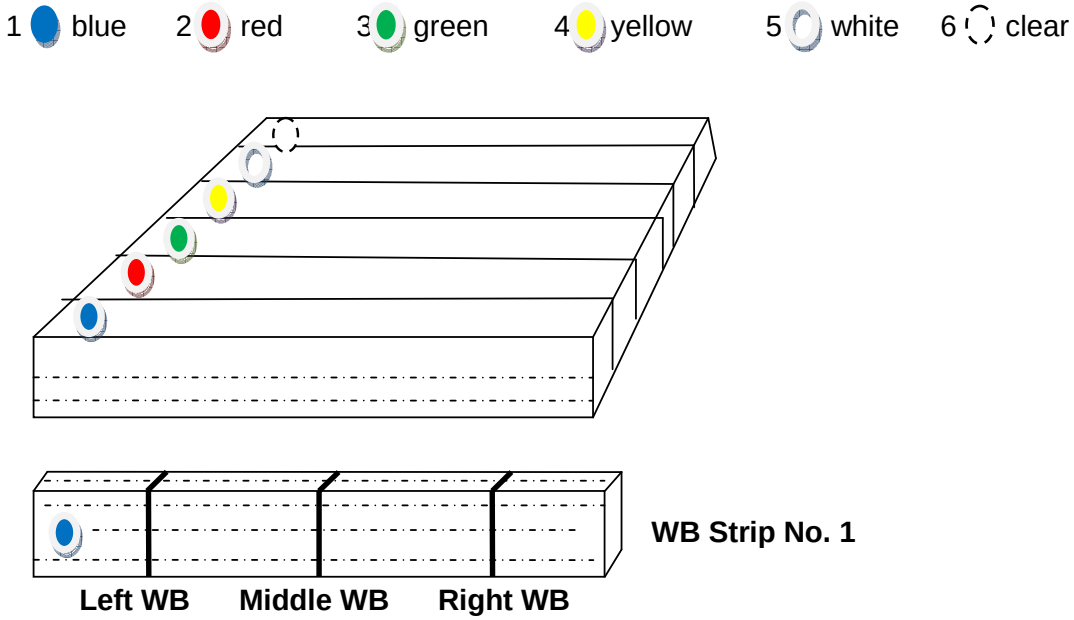

Figure 4: Sample preparation and cutting of strips for texture analysis of brisket muscle.

For both muscles, the front edge of the sample was trimmed and 6 strips were marked along the grain with the 6.7 mm cutter. Once cut, the 6 strips were kept in order and another mark was made with the 15 mm cutter, along the fibre direction of each strip. The orientation of the 6 strips was maintained and each strip was numbered with coloured pins, placing the pin in the left side of the strip (Appendix 4). The strips were measured for texture, cutting the strips in 3 places - left, middle and right - ensuring the strip number and position was recorded. Therefore, a total of 18 shear force measurements were made for each sample.

Details of sample thickness, shape and fibre orientation are given in Bouton et al. (1971) and Bouton and Harris (1972). All textural measurements were made on a Lloyd Instruments LRX Materials Testing Machine fitted with a 500 N load cell (Lloyd Instruments Ltd., Hampshire, UK). Six subsamples having a rectangular cross-section of 15 mm wide by 6.7 mm deep (1 cm^2 cross-sectional area) were cut from all samples, with the fibre orientation parallel to the long axis, and at right angles to the shearing surface. The force required to shear through the clamped subsample with a 0.64 mm thick blade pulled upward at a speed of 100 mm/min at right angles to fibre direction was measured in Newtons (N) as peak force (PF). A PF value of

approximately 40 N is considered to be the cut-off for consumer acceptability for tenderness. The mean of the peak force for the six subsamples was recorded. This method provides a deformation curve which also presents an initial yield (IY) value. The difference between PF and IY (PF-IY) can indicate the resistance of the connective tissue component of the muscle.

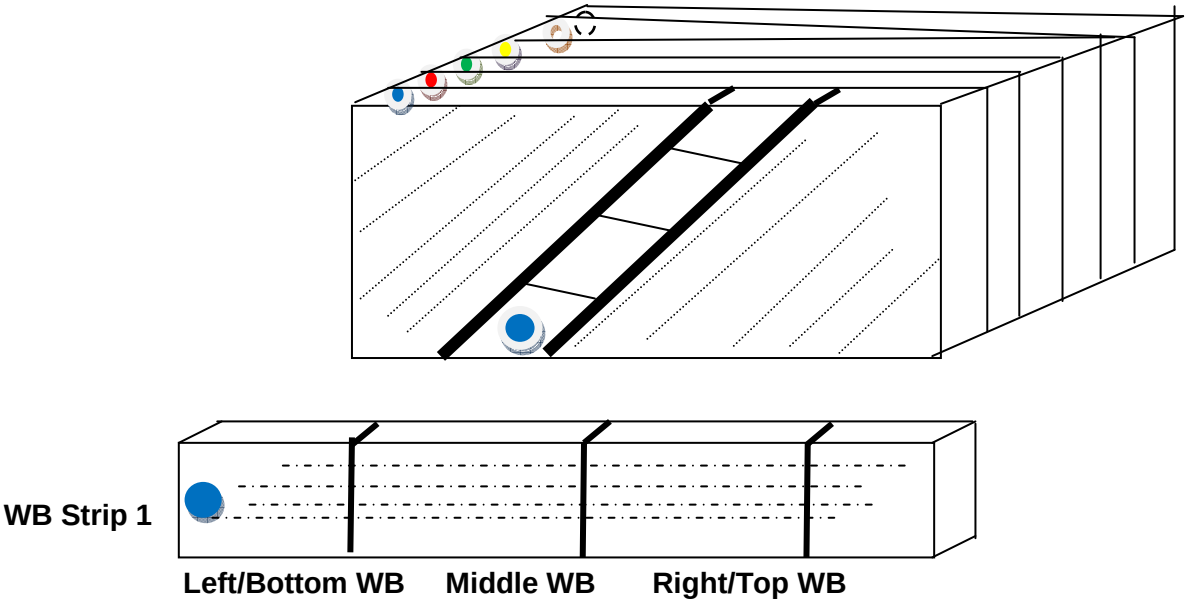

Figure 5: Sample preparation and cutting of strips for texture analysis of striploin muscle.

Statistical analyses

For preliminary analysis, data was subjected to ANOVA using Genstat 15th edition (http://www.vsni.co.uk/software/genstat). Data for each muscle was analysed separately as a comparison between muscles was not possible as the muscles were exposed to different chilling rates which was dependent on the muscle size. The main effect for each muscle (treatment) was analysed, with a blocking structure of Day/Mob/Animal.

Results and Discussion

Metabolism - pH

The dry ice and waterbath VFC treatments applied to the striploin muscle resulted in a slower pH decline than the control treatment (Figure 6). However, the pH at 24 hours post-hot boning was similar for all treatments ($P>0.05$). Interestingly, after VFC using each method (at 1 h), there was a very slight increase in pH compared to the control sample; significant ($P<0.05$) for the waterbath chilled sample but not significant ($P>0.05$) for the dry ice chilled sample. This was not seen in the previous VFC trial using the immersion method on striploin muscle (Milestone Report 5B) but was similar to the effect reported by Jacob et al. (2008, 2012) on lamb striploins. These authors did not provide any suggestions for this increase in pH but it appears that this does not have an impact on the resultant tenderness of the VFC muscles.

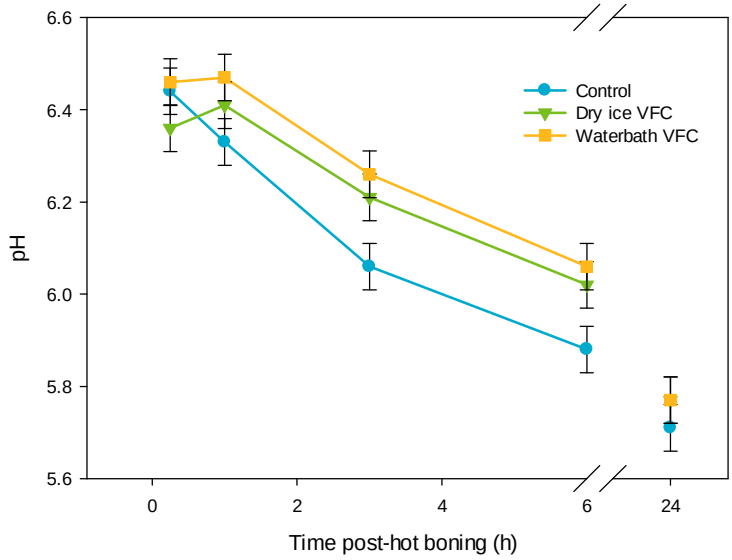

Figure 6: Decline of pH with time post-hot boning of striploin muscle. Control (wrapped pre-rigor, stored at 15°C for 24 h), dry ice VFC (pre-rigor samples subjected to VFC in dry ice for 50 min), waterbath VFC (pre-rigor samples subjected to VFC by immersion for 35 min in 45% propylene glycol set to -20°C). Actual means ± s.e.d., n=16.

In contrast to the pH decline in striploin, the pH decline in very fast chilled brisket muscle was similar to the control sample at 1 h, after which time the VFC samples had a faster decline than the control sample (Figure 7). The pH at 24 hours was similar for all samples. This shows that the pH / temperature decline using VFC varies between the muscles used in this experiment. This could be due to physiological and/or biochemical muscle differences or the different sizes of the muscle portions affecting the chilling rates.

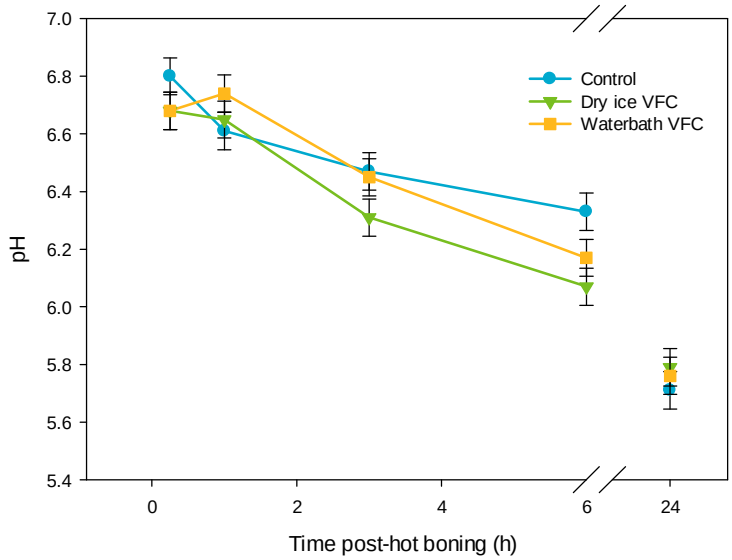

Figure 7: Decline of pH with time post-hot boning of brisket muscle. Control (wrapped pre-rigor, stored at 15°C for 24 h), dry ice VFC (pre-rigor samples subjected to VFC in dry ice for 30 min), waterbath VFC (pre-rigor samples subjected to VFC by immersion for 25 min in 45% propylene glycol set at -20°C). Actual means ± s.e.d., n=16.

Texture measurement

The sample preparation and cutting of samples for the analysis of texture for the striploin samples was explained in Figure 5. Analysing the data of the middle measurement of strips 3 and 4 provides an indication of the texture at the "true" centre of each striploin sample. This is shown in Figure 8 as the "centre" measurement. As it was expected that there would be a temperature gradient across the samples during chilling, this was important to measure to see if there was a corresponding "gradient" of texture across the sample.

Very fast chilling using dry ice produced very tough striploin meat (Figure 8A and 8D), with a peak shear force of 80-98 N and total work done of 419-512 Nmm. This was tougher (P<0.001) than both the control and waterbath chilled samples. The waterbath chilled samples were more tender (P<0.001) than the control samples, with a peak force value of ~69 N compared to ~78 N for the control striploin samples.

The changes in the peak force values were reflected in the initial yield values, with the pattern and effects being similar. The initial yield value (~87 N) for the dry ice chilling of striploin samples was significantly higher (P<0.001) than both the values for the control (~74 N) and waterbath chilled samples (~66 N).

The connective tissue component of the striploin muscle samples was not affected by either VFC treatments, as there was no effect on the PF-IY values (Figure 8C).

The only significant effect due to the position of the texture measurement along the muscle strip was found in the striploin samples chilled using dry ice, with the "right" measurement being significantly lower for the peak force value (P=0.039), initial yield (P=0.035) and work (P=0.033) than the other positions measured (Figure 8 A, B and D).

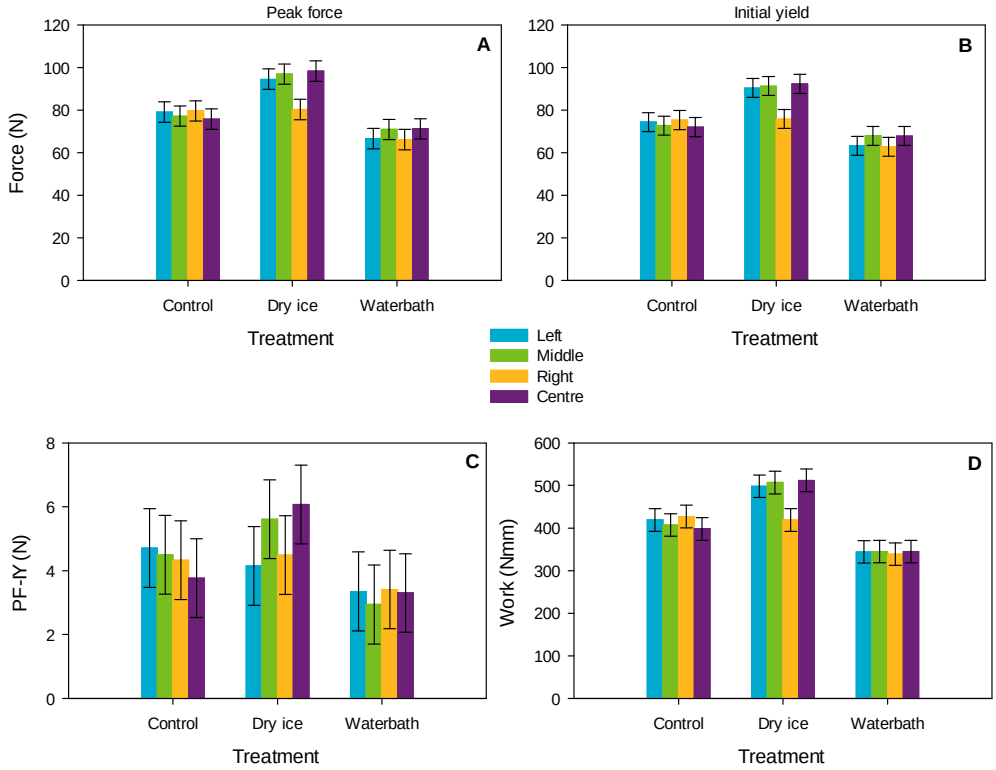

Figure 8: The effect of treatment (Control, wrapped pre-rigor, stored at 15°C for 24 h; Dry ice, pre-rigor samples subjected to VFC in dry ice for 50 min; Waterbath , pre-rigor samples subjected to VFC by immersion for 35 min in 45% propylene glycol set to -20°C) and position (left, middle, right, centre) of the strip within the sample on Warner-Bratzler shear force parameters (A. peak force; B. initial yield; C. PF-IY, peak force minus initial yield; D. Work) of beef striploin. Actual means ± s.e.d. for the interaction, n=16.

The control and waterbath chilled brisket samples had similar peak force (~70 N) and work (~420 Nmm) values, whereas the dry ice chilled samples were significantly (*P*<0.001) tougher (PF ~85 N, Work ~500 Nmm) than either of these treatments (Figure 9A and 9D). All of these brisket samples would be regarded as tough as the cut-off for consumer acceptability is ~ 40 N. Surprisingly, the control brisket samples (~70 N) were more tender than the control striploin samples (~80 N).

The results for the initial yield values (Figure 9B) follow the same pattern for the treatments as the peak shear force and work values. The initial yield values of the dry ice chilled samples (~78 N) were significantly higher (*P*<0.001) than the control (~60 N) and immersion chilled (~63 N) brisket samples.

There was no effect of either chilling treatment on the PF-IY value compared to the control. These results indicate, as previous work has also shown, that any effect on the texture of very fast chilled beef muscle is due to modification of the myofibrillar protein component (IY value) rather than the connective tissue component (PF-IY value).

In the brisket muscle, there was no effect of the position (left, middle, right) of the texture measurement within in each strip cut from the brisket samples for any of the treatments or any of the Warner-Bratzler texture parameters (Figure 9).

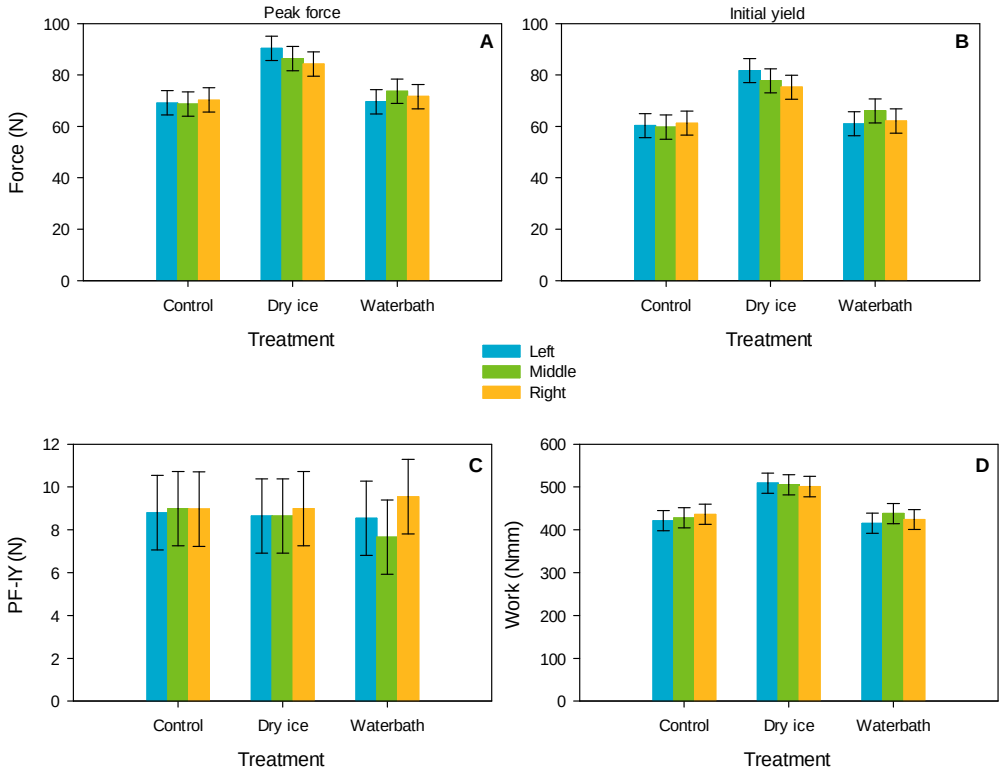

Figure 9: The effect of treatment (Control, wrapped pre-rigor, stored at 15°C for 24 h; Dry ice, pre-rigor samples subjected to VFC in dry ice for 30 min; Waterbath, pre-rigor samples subjected to VFC by immersion for 25 min in 45% propylene glycol set to -20°C) and position (left, middle, right) of the strip within the sample on Warner-Bratzler shear force parameters (A. peak force; B. initial yield; C. PF-IY, peak force minus initial yield; D. Work) of brisket. Actual means ± s.e.d. for the interaction, n=16.

Cook loss

The cook losses for the striploin samples were similar (~19-22%, *P*>0.05), regardless of treatment, and were higher than the loss from the brisket samples (~10-13%). The cook losses for the chilled, brisket samples were significantly higher (*P*<0.001) than the control brisket sample.

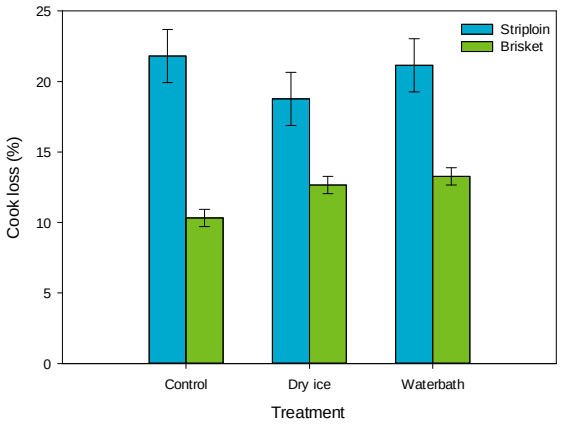

Figure 10: The effect of processing treatments (Control, dry ice VFC, waterbath VFC) on the cook loss (% of initial weight) of beef striploin and brisket at approximately 24 hours post-slaughter. Actual means ± s.e.d., n=16.

Sarcomere length

The sarcomere length of all striploin samples ranged from 1.82 - 1.91 µm. The lengths for brisket samples were longer, 1.90 - 1.96 µm. There was no effect (*P*>0.05) of either chilling treatment (dry ice or waterbath immersion) on sarcomere length for either muscle (Figure 11). All of the sarcomere lengths were above 1.6 µm, which is the length at which the peak force would be expected to increase. These values suggest that there was no cold-shortening of striploin or brisket muscle during either chilling treatment.

Sarcomere length determination for cooked striploin samples proved very difficult and accurate readings were not possible. Therefore, this analysis was not pursued for cooked samples.

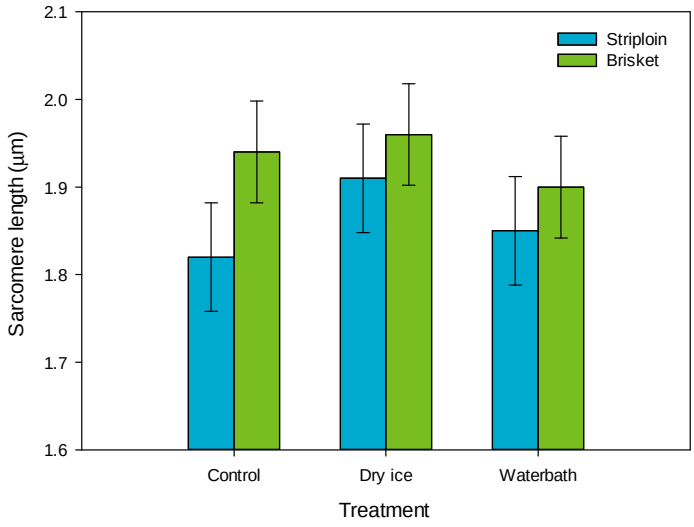

Figure 11: The effect of treatment (Control, dry ice VFC, waterbath VFC) on sarcomere length of striploin and brisket muscles. Each bar is the actual mean and the error bar is the s.e.d., n=16.

Overall progress of the project

A VFC experiment was successfully designed and completed at a local processing plant. The results of this experiment complete the objectives and milestone report for Milestone 8. The submission of Milestone 9 (Final Report) will finalise the end of this project.

Summary and recommendations

This experiment showed:

- Immersion of beef striploin muscle in a waterbath with 45% (v/v) propylene glycol chilled to -20°C resulted in more tender meat than non-chilled (control) samples.
- Very fast chilling (VFC) of beef striploin muscle using dry ice produced tougher meat than control samples.
- Waterbath chilling (immersion) of beef brisket muscle resulted in meat with a similar texture to control brisket muscle.
- Dry ice VFC brisket muscle was tougher than control or waterbath chilled muscle.
- The rate of VFC is critical to achieve meat with similar or improved texture.
- The texture on the surface and the centre of each muscle was similar for each chilling method, indicating the temperature gradient under these conditions had no effect of the resultant texture. Therefore, future sampling can be taken from either part of the muscle sample and the texture result be indicative of the whole sample.
- Texture data suggests that modified texture is due to modification(s) of the myofibrillar proteins rather than the connective tissue proteins.
- Cooking loss in very fast chilled brisket muscle was higher (up to 29%) than control brisket muscle; however, there was no effect of chilling by either method on the cooking loss of striploin muscle.
- Sarcomere lengths in both muscles were maintained with VFC treatments, indicating cold-shortening did not occur.
- The decline of pH was slower in the chilled striploin muscles compared to the control but the ultimate pH (after 24 h) was similar in all samples.
- The chilled brisket muscles had a faster pH decline, up to 6 h, compared to the control brisket but all samples had a similar ultimate pH.
- These results suggest that in the absence of cold-shortening, the rate of pH decline is important to the resultant texture of very fast chilled muscle.

Recommendations for further work:

- Further investigation of the effect of different chilling rates on individual muscles on metabolism and texture of very fast chilled muscle.
- Metabolomic analysis of stored samples will provide information on the importance of the rate of pH decline on resultant texture of very fast chilled muscle.
- Investigate how muscle differences (e.g. different connective tissue content, fibre type) influence effects of VFC.

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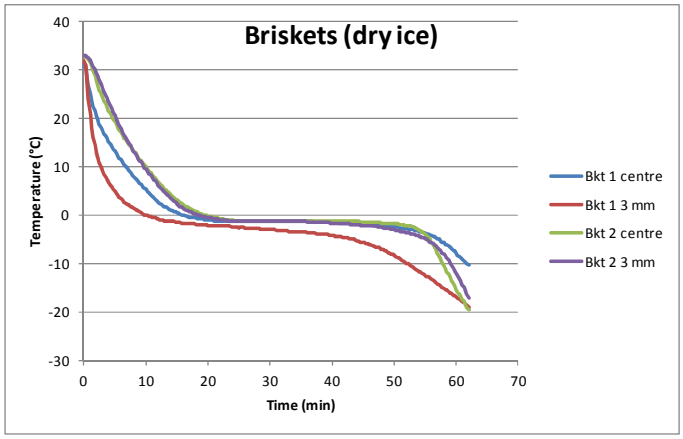
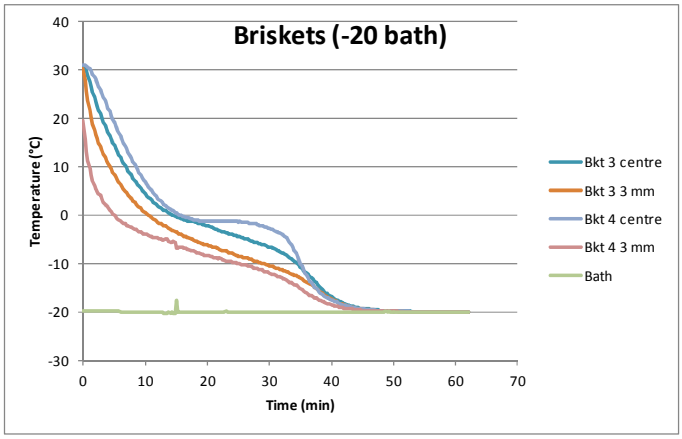
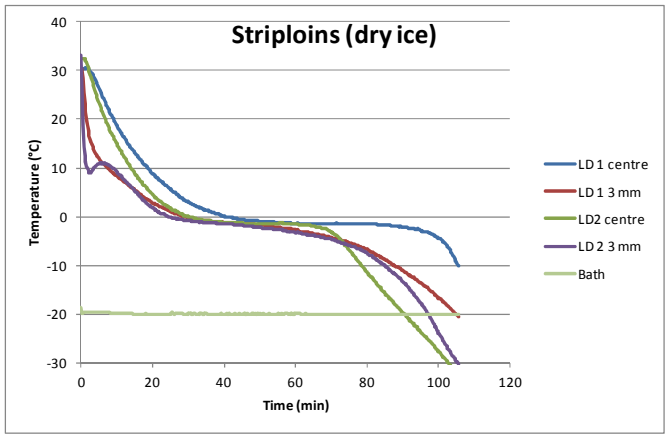
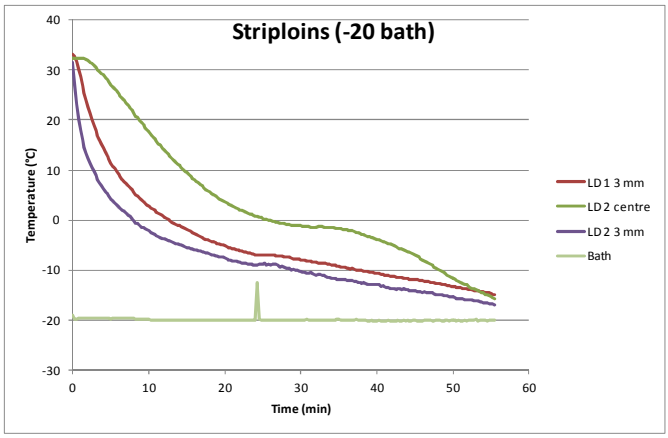
Jacob, R., Rosenvold, K., North, M., Kemp, R., Warner, R. and Geesink, G. (2012) Rapid tenderisation of lamb *M. longissimus* with very fast chilling depends on rapidly achieving sub-zero temperatures. *Meat Science* 92:16-23.

Appendix 1 Temperature profiles from pre-trial work to identify chilling times for each muscle in the two chilling mediums.





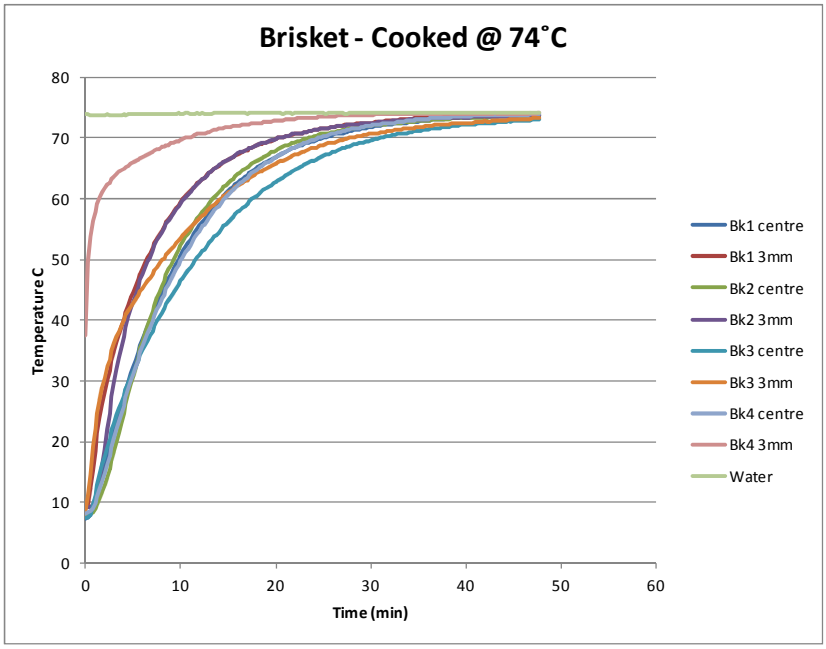


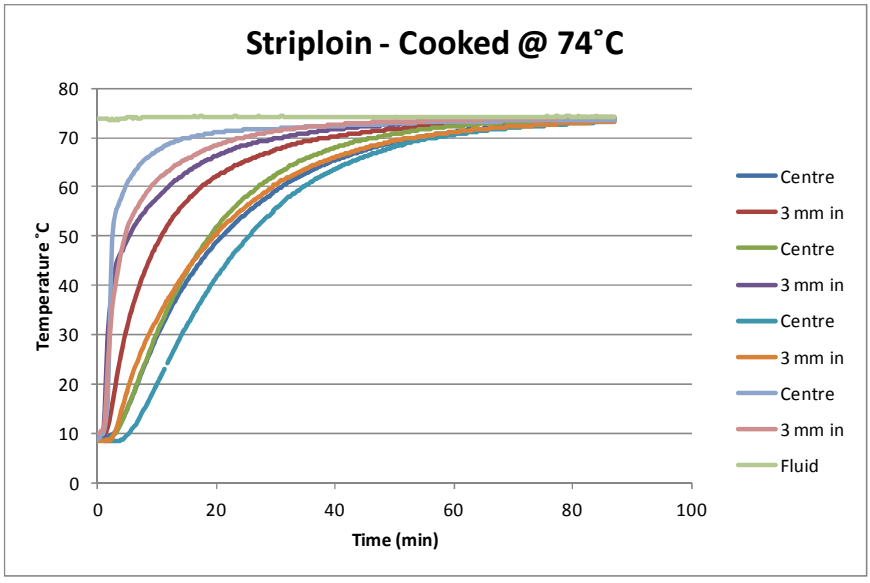


Appendix 2 The animal types, categories and number of samples collected in each animal category over the 2 week period (n=16).

Animal type	*Category*	*No. of animals*
MSA EU YP 100 Day Grain fed	YPS	2
MSA EU Angus YP >320 kg	YPS	1
HGP Free Cow (Grass)	C	3
MSA EU Grass Ox 280 kg	PRS	1
MSA EU YP 285-320 kg	YS	2
MSA EU YP 240-285 kg	Y	1
MSA EU YG 100 Day Grain fed	YGS	1
MSA EU YG 100 Day Grain fed-HQ	YPS	1
MSA YP 100 Day Grain fed	YPS	1
MSA EU YP 285-320 kg	YGS	1
EU Grass Ox 180-280 kg	YP	1
Budget Cow Grass	C	1

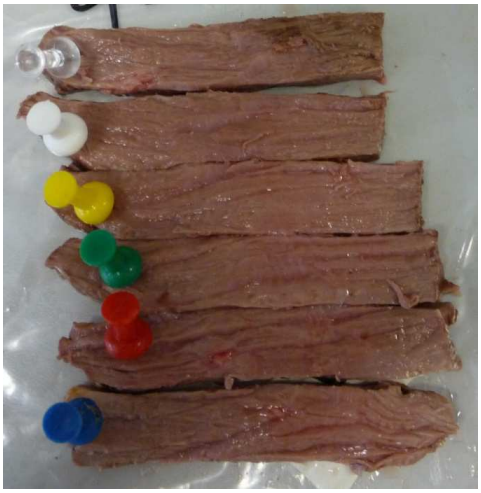
Appendix 3 Temperature profiles to determine the time of cooking for brisket and striploin muscle samples.



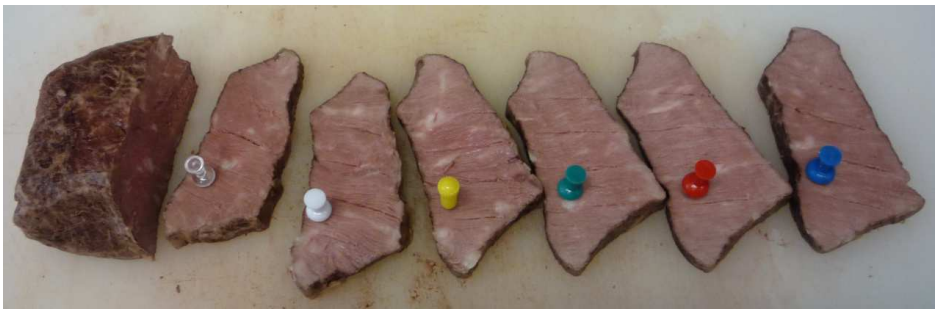


Appendix 4 Cutting of muscle strips for texture analysis, indicating placement of coloured pins. A. brisket B. and C. striploin

A.



B.



C.