final report

Project Code: A.MPT.0048

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Date published: October, 2014

PUBLISHED BY
Meat and Livestock Australia Limited
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Sensitivity of High Pressure Processed (HPP) product eating quality

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

Abstract

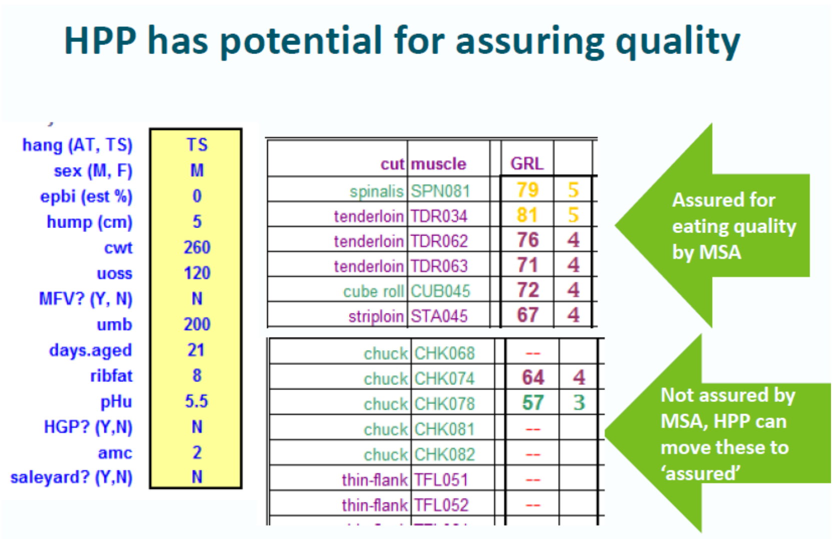
MLA introduced to the Australian red meat industry High Pressure Processing (HPP) in November 2009 at a workshop hosted by CSIRO to showcase the technology and value adding red meat opportunities – namely to increase shelf life and addition of another level of food safety and potential for less reliance on preservatives degradation of vitamins and flavour reduced to a minimum. As with any new technology platform, the learning curve has continued by exploring the HPP mechanisms at various time, temperature, pressure settings and muscle profiling and biophysical assessments (texture, cook loss, colour).

The overall objective of this project was to compare the impact of HPP at low and high temperature to other interventions and cook methods – in particular, evaluation of tenderisation (texture), colour and yield for control and aged samples for several beef cuts.

The research found optimum HPP conditions for improvement in texture (using both assessments by a chef and Warner-Bratzler shear force) and moisture loss yield of beef topside, brisket and chuck pieces were 600 MPa at 90°C for 15 min.

It was found that there was no effect of pressure (425 MPa, 5 min) applied at 70°C on the texture of either brisket or chuck muscle. However, when pressure (600 MPa, 15 min) was applied at 90°C, there was a definite muscle difference: (a) chuck meat pieces were tenderised; (b) tenderisation occurred in brisket muscle only after ageing and with no further cooking prior to texture assessment. The lowest moisture losses occurred when muscle samples were not further cooked after high pressure treatment.

These results indicate the high temperature HPP may provide a pathway for meat tenderisation and improved yield to add value to beef secondary cuts – this could include potential Meat Standards Australia (MSA) pathway. Further HPP as a technology platform may enable provisions for enterprises to “cook” raw meat packaged and HPP treated similar to Sous Vide technology but in 15 minutes compared to 6 hours to achieve similar eating quality but with improved yields.



Contents

The project was commissioned over a number of stages to define the degree of sensitivity and viability of high pressure processing (HPP) for use in red meat by comparing the impact of HPP at low and high temperature to other interventions and cook methods. With extended shelf life well reported as a benefit from HPP, this project focused on the biophysical assessment of beef cuts (texture, cook loss, colour) following various HPP treatments identified as promising by "chef" assessments.. Tenderisation and low cook loss (higher juiciness) from HPP of low value (neck, brisket, blade) and high value cuts (topside, silverside and striploin) may represent an attractive opportunity for the Australian Red Meat industry to value add.

1. Preliminary Findings

- Limited trials of HPP (600 MPa) at high temperature (70°C) indicated dramatic improvement in the texture of brisket and chuck and acceptable improvement for blade
- Confirmation of improvement in texture of brisket with HPP at low temperature but not significant enough to be commercially relevant. No improvement in texture of chuck or blade with low temperature HPP treatment.
- Boundaries defined at high temperature (425 MPa, 5 min, 70°C) for improved texture and yield of topside and chuck, indicating commercial benefits

1.1 Impact of HPP at low temperature across a broad matrix of different interventions

The initial trial design was to repeat the tenderisation found with the application of high pressure to brisket at low temperature (400 MPa, 4°C, 5 min) (A.MPT.0032, A.MPT.0041) and include two other muscles for comparison.

Brisket (*M. pectoralis profundus*), chuck piece (*M. serratus ventralis*) and blade (*M. latissimus dorsi*) were subjected to various pressures (300, 400, 600 MPa) at 4°C for 5 minutes. The samples treated at 600 MPa were also processed for 10 minutes. A range of interventions and cooking methods was also trialled on each muscle, as outlined below:

Intervention	Cooking method
• Control	• Fry
• HPP	• Roast
• Needle injection	• Thin slice (stir fry)
• Cook in bag / HPP	• Sous vide

Treated samples were cooked (as indicated) immediately after processing and informally assessed for eating quality.

1.1.1 Results

Tenderisation of brisket using 400 MPa at 4°C for 5 minutes was achieved as in previous projects (A.MPT.0032, A.MPT.0041). However, these conditions were not conducive to tenderisation in the other muscles. Therefore, lower and higher pressures were applied and also longer pressure holding times were used. This resulted in either no further effect on texture (i.e. similar to the control) or a reduction in tenderness (i.e. tougher meat).

Several different cooking styles and cutting techniques were then incorporated into the trial design to investigate any textural changes. With the exception of the slow cook, no improvement was evident from the chef's informal sensory assessment. The slow cook results were considered unreliable as a result was predictable based on the amount of time in the cooking medium and no improvement was able to be attributed to the HPP / cook or the cook / HPP process.

In an effort to produce a tenderisation effect, discussion and review of previous research (A.MPT.0013) resulted in the application of pressure (600 MPa) at a higher temperature (vessel temperature set at 70°C) for holding times of 5, 10 and 16.65 minutes. The maximum holding time of the 35 L HPP unit is 999 s (i.e. 16.65 min or 16 min 39 s).

A holding time of 5 minutes at a pressure of 600 MPa at 70°C resulted in a definite improvement in tenderness for brisket and chuck but not for blade, as assessed by the chef. An increased holding time of 10 minutes under these conditions produced a larger improvement in texture for brisket and chuck but not for blade. Using the maximum hold time for the 35 L HPP unit (16.65 minutes), an excellent outcome was achieved for brisket and chuck, with an acceptable texture for blade. Also encouraging from these results was the minimal yield loss of the products treated with HPP. Conventional and sous vide cooking of high collagen content meat cuts like brisket and chuck can typically lose up to 45% of original green weight during cooking; the HPP treated brisket lost only 19%. Commercially, this could equate to $1.30 per kg of recoverable meat improvement.

The chef's assessment of the range of interventions applied to brisket, chuck and blade is tabulated in Appendix 1.

1.2 Further investigation of HPP at high temperature

From the above preliminary results of high pressure (600 MPa) at higher temperature (70°C), a further trial was designed to confirm the chef's assessment and to explore the pressure and time boundaries for the tenderisation of beef muscle. The direction of the steak cut (i.e. transverse or longitudinal) and a smaller sample size (i.e. cubes) were also examined for the impact on the texture of high pressure-treated beef.

Topside cap off (*M. semimembranosus*) and chuck pieces (*M. serratus ventralis*) were purchased from Woolworths and cut into portions (25 x 25 x 60 mm for topside;

25 x 25 x 40 mm for chuck) and randomly allocated to treatments. Four pressure treatments (0.1, 200, 400, 600 MPa) were applied for two holding times (5, 16.65 min) with the vessel temperature set at 70°C to the topside and chuck muscle portions. Although three replicates for the topside were run per treatment, only one sample was cooked for sensory assessment. Due to the limited amount of chuck available, one replicate was run per treatment. Two controls were run in parallel for comparison – control (no treatment prior to cooking) and control-heat (no pressure applied but samples heated at 70°C for either 5 or 16.65 min). After treatment, samples were cooked immediately (frying to an internal temperature of 72°C), with no cooling prior to cooking. No more than 10 min elapsed between removal of samples from the HPP unit and commencement of cooking. Weights were recorded after treatment and prior to cooking to determine yield.

1.2.1 Results

A range of pressures (200, 400, 600 MPa) applied at 70°C to topside and chuck resulted in improved texture and yield at 400 MPa, and no difference was observed between 5 or 16.65 minutes at this pressure. When the boundaries around 400 MPa were further explored (300, 350, 425, 450, 475, 500 MPa), the optimum pressure at 70°C for 5 minutes for texture and yield improvement of topside and chuck was 425 MPa. The assessment of these products is tabulated in Appendix 2. It must be noted that this assessment was on one portion of muscle only per treatment.

Two samples of topside were cut longitudinally to the fibre direction prior to HPP treatment at 425 MPa for 5 min at 70°C. When samples were cut across the fibres after cooking, the meat was very tender and noticeable more juicy. Diced portions of both topside and chuck gelled/clumped together during HPP treatment (425 MPa for 5 min at 70°C). This gelling was probably because the HPP conditions used in this work (pressure/temperature) solubilised the muscle proteins (e.g. myosin, actin), causing binding of the individual meat pieces to each other. The gelling of meat pieces was negated when marinade/sauce was added to the diced portions prior to HPP treatment. This is also evident in sous vide cooking where sauce is used to avoid clumping of commercial cooked diced beef. It is assumed in sous vide cooking that the viscosity of the sauce provides aeration which helps to create a buffer between the meat pieces. The reason for the anti-clumping during the HPP process is unknown.

2. Key Research Findings

2.1 Experimental Design:

- Muscles for processing:
  - Brisket, point end deckle off (*M. pectoralis profundus*, HAM 2353*)*
  - Chuck rib meat pieces (*M. serratus ventralis*, HAM 2640)
- Treatments:
  - Control and HPP (two sets of conditions)
  - Fresh and aged
- HPP conditions:
  - HPP90 – 600 MPa at 90°C for 15 min (conditions used for concept product development for Coles meeting, February 8, 2013)
  - HPP70 – 425 MPa at 70°C for 5 min (optimum conditions found in Stage 1)

Day 1	Day 2
Control, fresh	Control, fresh
Control, aged	Control, aged
HPP90, fresh	HPP70, fresh
HPP90, aged	HPP70, aged

Control = no treatment

Fresh = no ageing

Aged = stored at 5°C for 7 days, vacuum packed

HPP90 = 600 MPa for 15 min at 90°C

HPP70 = 425 MPa for 5 min at 70°C

- A total of 96 samples from each muscle was processed; a total of 192 samples
- Meat was sourced by CSIRO from JBS Brooklyn
- Trials completed at CSIRO Werribee

In Stage 1, optimum HPP conditions for improvement in texture and yield of topside and chuck pieces were found to be 425 MPa at 70°C for 5 minutes. Stage 2 of this project was to confirm the chef's assessment (found in Stage 1) and conduct biophysical assessment of the treated muscle. This was undertaken by assessing replicates in two muscles (for statistical analysis) for objective texture and colour measurements, and yield.

Twelve replicates from each muscle were allocated to either control (untreated) or two high pressure treatments (HPP90 = 600 MPa, 90°C, 15 min or HPP70 = 425 MPa, 70°C, 5 min), and two ageing times (fresh or aged, 7 days at 5°C). Measurements for moisture loss and colour were taken before and after treatment. After the appropriate storage periods, samples were analysed objectively for texture using the Warner-Bratzler shear force method.

2.2 Assessments related to Preliminary findings

The chef's assessment of tenderness of chuck meat pieces treated with 425 MPa at 70°C for 5 min found in Stage 1 was not confirmed in this trial using objective texture measurements. There was no effect of pressure (425 MPa, 5 min) applied at 70°C on the texture of either brisket or chuck muscle. However, when pressure (600 MPa, 15 min) was applied at 90°C, there was a definite muscle difference: (a) chuck meat pieces were tenderised; (b) tenderisation occurred in brisket muscle only after ageing and with no further cooking prior to texture assessment. The lowest moisture losses occurred when muscle samples were not further cooked after high pressure treatment.

Generally,

- Different effects on texture between the two muscles with pressure treatment at 90°C
- Greater tenderisation of chuck meat pieces than brisket with pressure treatment at 90°C
- More tenderisation of chuck meat pieces with pressure applied at 90°C (600 MPa, 15 min) than at 70°C (425 MPa, 5 min)
- As expected, there was an impact on colour (increase in total colour difference) on both muscles with both HPP treatments

Specifically,

With 600 MPa, 15 min, 90°C HPP treatment:

- Tenderisation was evident in aged, but not unaged, brisket muscle when the meat is not further cooked prior to objective texture measurement
- Tenderisation of fresh (unaged), but not aged, chuck muscle was evident
- Lowest moisture loss occurred when brisket and chuck muscle were not further cooked after high pressure treatment
- When cooked after HPP, cooking losses were lower in the pressure-treated brisket muscle compared to the cooked, control muscle

With 425 MPa, 5 min, 70°C HPP treatment:

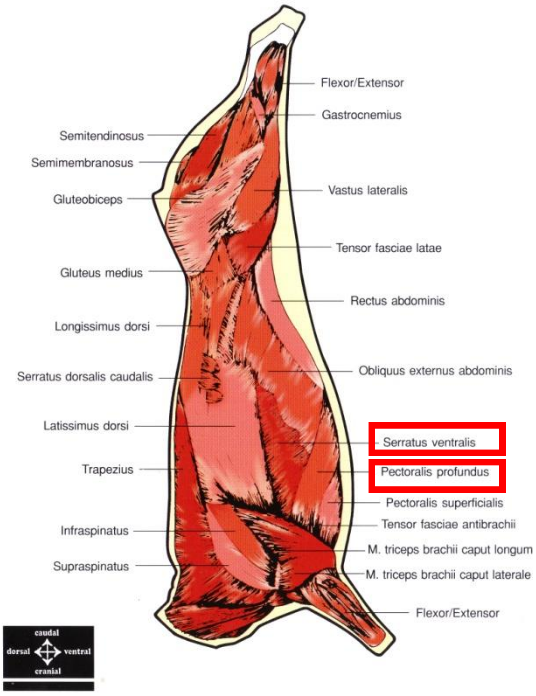
- There was no effect on texture (tenderness), as measured by Warner-Bratzler shear, on either brisket or chuck muscle
- Cooking for longer times (30 min) at 70°C reduced the peak force value of control (untreated) and pressure-treated brisket muscle
- Cooking at 70°C for 30 min after HPP reduced the moisture loss compared to the control

2.3 Material and Methods

Following on from the work and results presented in Stage 1 of this project, the initial part of this trial design was to confirm the tenderisation found with the application of high pressure at high temperature (425 MPa, 70°C, 5 min) to chuck and topside muscle (A.MPT.0048 Milestone 2 report). The second part of this trial investigated the effect of previous HPP conditions (600 MPa at 90°C for 15 min) used for the development of concept products (Coles meeting February 8, 2013) on the texture of beef muscles.

Twelve briskets (*M. pectoralis profundus*, point-end, deckle off, HAM 2353) and thirty-six chuck rib meat pieces (*M. serratus ventralis*, HAM 2640) (Figure 1) were collected from JBS Brooklyn.

A.



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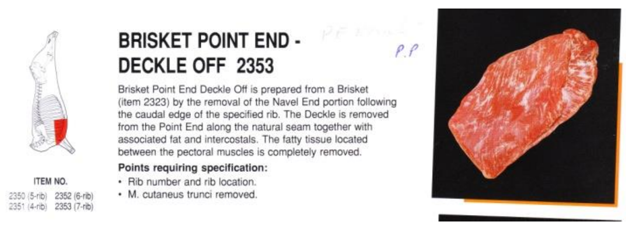

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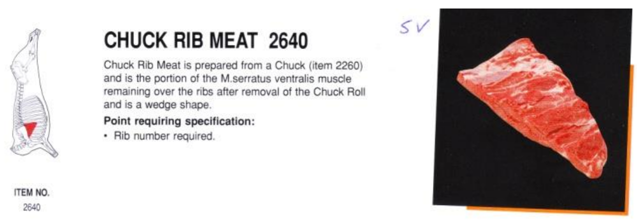

Figure 1: A. Carcase side indicating location of brisket (*M. pectoralis profundus*) and chuck rib meat (*M. serratus ventralis*); Ausmeat Handbook information on B, brisket, and C, chuck rib meat.

The muscles were not trimmed of excess fat or connective tissue prior to preparation. Each brisket muscle was trimmed square and cut into 8 portions of varying size; a total of 96 samples. Twenty-four of the chuck rib meat pieces were cut into quarters of varying size; a total of 96 samples. The average pH of the brisket muscles was 5.58 ± 0.023 (se) and 5.72 ± 0.021 (se) for the chuck meat pieces. All samples were randomly allocated to treatments, with the original experimental design having 12 replicates for each treatment (Table 1). However, a mix-up of samples on Day 1 of processing resulted in 14 brisket samples identified for HPP at 70°C on Day 2 being processed on Day 1, i.e. HPP at 90°C. Therefore, samples for Day 2 processing were reallocated to treatments, resulting in 8 replicates of the control treatments (fresh and aged) and 9 replicates of the HPP treatments (fresh and aged), instead of the planned 12 replicates in each treatment. This number of replicates was only for the brisket muscle treated at 70°C, the chuck meat pieces had 12 replicates per treatment.

Table 1: Four treatments conducted on each day of processing. Twelve replicates of chuck meat pieces allocated to each treatment on each day; twelve replicates of brisket allocated to 90°C treatment (Day 1) and eight replicates for control and nine replicates for high pressure treatment allocated to 70°C (Day 2).

Processing Day 1	Processing Day 2
Control, fresh	Control, fresh
Control, aged	Control, aged
HPP90, fresh	HPP70, fresh
HPP90, aged	HPP70, aged

Control = no treatment

Fresh = no ageing

Aged = stored at 5°C for 7 days

HPP90 = 600 MPa for 15 min at 90°C

HPP70 = 425 MPa for 5 min at 70°C

All samples were prepared on Day 1, irrespective of which day processing occurred. Prior to vacuum-packaging, all samples were weighed and assessed for colour (Minolta CR-300, D65 light source).

High pressure processing combined with temperature involves setting the temperature of two separate components, (1) the vessel temperature and (2) the fill tank water. For processing on Day 1, the HPP vessel was set at 90°C, with the fill tank water temperature set at 75°C. On Day 2, both the vessel and fill tank temperature were set at 70°C. After high pressure treatment, samples were cooled in an ice slurry for a minimum of 10 min.

After treatment on each day, all fresh (unaged) samples (control and pressure-treated) were unpacked, weighed, colour measurements recorded and samples vacuum-packed and frozen at -20°C. Samples identified for ageing were kept in the original packaging and stored at 5°C for 7 days. After this storage period, samples were weighed, assessed for colour and repacked prior to freezing at -20°C. All samples were transported frozen to Coopers Plains for texture assessment.

Weight loss following treatment (treatment loss) was expressed as a percentage of the original weight. Averaged colour values (L*, lightness; a*, redness; b*, yellowness) were used to calculate the total colour difference (ΔE), with the untreated control sample used as a reference, according to the equation, $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$.

Prior to texture assessment, all samples were thawed overnight at 2.5°C ± 1°C. The brisket samples were large in size, so all brisket samples were cut into two pieces. The samples were cooked at two cooking times to determine if further cooking had an effect on the texture of the samples. For the brisket Control samples allocated to the 600 MPa, 15 min, 90°C treatment, one piece was cooked at 90°C for 15 min (heat control), which was the same conditions as the HPP sample but without the pressure, and the other piece was cooked at 70°C for 30 min. For the brisket Control samples allocated to the 425 MPa, 5 min, 70°C treatment, one piece was cooked at 70°C for 5 min (heat control), while the other piece was cooked at 70°C for 30 min (standard cooking temperature/time for texture measurement of these size samples). For the brisket HPP samples for both treatments, one piece had no further cooking, while the other piece was cooked at 70°C for 30 min. All samples had cooking losses recorded. This is summarised in Figure 2.

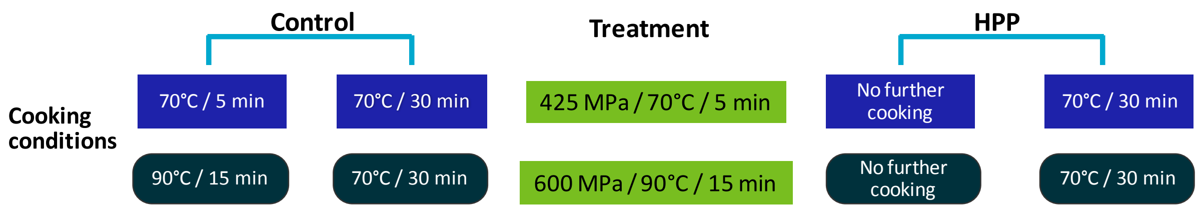

Figure 2: Sample allocation to cooking time and temperature for brisket samples.

The chuck samples were not large enough to cut into two pieces to repeat what was conducted on the brisket. Therefore, the ideal cook conditions were derived from the brisket results. The chuck Control samples allocated to the 600 MPa for 15 min at 90°C treatment were cooked at 90°C for 15 min (heat control) and the chuck Control samples for the 425 MPa for 5 min at 70°C treatment were cooked at 70°C for 30 min. These samples were cooked for 30 min because 5 min only heated the surface of the meat with the core remaining raw, while the core of the HPP samples were all cooked due to the increased heat due to the combination of pressure and temperature. The chuck HPP samples for all treatments had no further cooking. All samples had cooking losses recorded. The sample allocation for cooking of chuck samples is summarised in Figure 3.

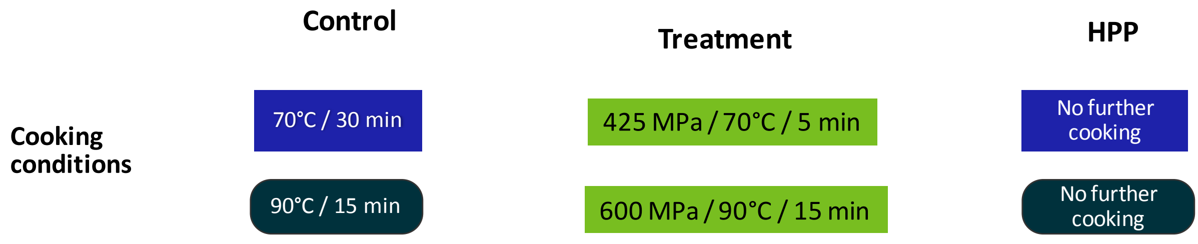

Figure 3: Sample allocation to cooking time and temperature for chuck samples.

All cooked samples were cooled and held in a 2˚C chiller for a minimum of 2 hours prior to being analysed for texture on the same day as the HPP samples which received no further cooking. This ensured there was no aging effect between the two groups.

All samples were analysed for texture according to the method of Bouton et al. (1972), using a Lloyd Instruments LS2.5 materials testing machine fitted with a 500N load cell (Lloyd Instruments., Hampshire, UK). Six Warner-Bratzler shear measurements were performed on each sample and the mean of the subsamples recorded. The Warner-Bratzler shear measurement for texture provides a deformation curve which presents an initial yield (IY) and a peak force (PF). The initial yield is an indication of myofibre resistance and the peak force expresses the total resistance of the muscle. The difference between PF and IY (PF-IY) indicates the contribution of the connective tissue to the force.

Statistical analysis of Warner-Bratzler shear measurements, treatment and cook losses and colour difference was carried out using Genstat 15th edition. A linear mixed model using REML was fitted to the data. The fixed effects were storage, treatment and storage*treatment and the random effects were storage/cook temperature and storage/cook temperature/cook time.

2.4 Results

2.4.1 HPP setting: 600 MPa at 90°C for 15 min

2.4.1.1 Brisket

After high pressure treatment at 90°C, brisket samples were slimy/gelatinous in appearance and to touch, possibly indicating solubilisation of connective tissue.

There was no effect on the peak force value for brisket samples treated with high pressure treated (600 MPa, 15 min) at 90°C, followed by cooking at 15 min. However, there was a significant effect on the IY value (P=0.004) and the PF-IY value (P=0.003) (Figure 4). It must be noted that for this treatment, the control samples were cooked at 90°C for 15 min, whereas the HPP-treated samples were not further cooked after the high pressure treatment. Under this same cooking regime, there was no significant effect of storage on texture but there was a significant interaction between treatment and storage on the PF value (P=0.024) and the PF-IY value (P=0.001) (Figure 4).

When the brisket samples were cooked at 70°C for 30 min (both control and HPP-treated), there was no significant effect of either treatment, storage or the interaction of storage and treatment on any of the Warner-Bratzler measurements (PF, IY or PF-IY) (Figure 4).

Therefore this trial has shown that when pressure is applied at 600 MPa for 15 min at 90°C to brisket muscle, tenderisation is only evident in aged muscle when the meat is not further cooked prior to objective texture measurement by the Warner-Bratzler shear method.

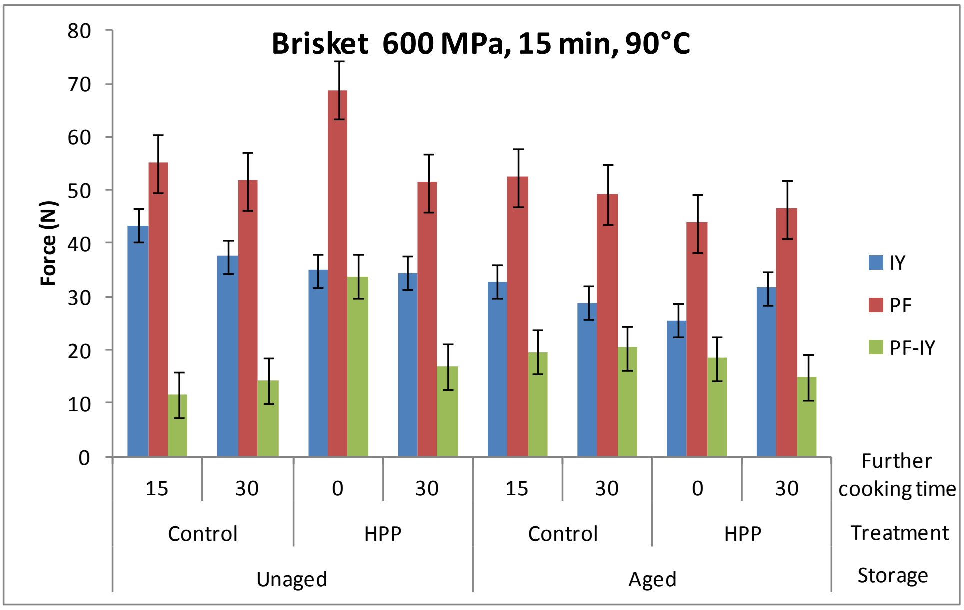

Figure 4: Warner-Bratzler shear force measurements (IY - initial yield, PF - peak force, PF-IY, peak force minus initial yield) of unaged and aged (storage), untreated (control) and pressure treated (HPP, 600 MPa, 15 min, 90°C) brisket samples. Control samples were cooked at 90°C for 15 min or 70°C for 30 min; HPP samples were analysed either by cooking at 70°C for 30 min or without further cooking (0 min). Predicted mean ± s.e.d.

As expected, there was a significant effect ($P<0.001$) of high pressure treatment at 90°C on the treatment loss of brisket muscle compared to the untreated control but this was less than 0.2% (Table 2). Obviously, the avoidance of further cooking after high pressure treatment of muscle has a huge impact on the total moisture lost (Table 2, HPP 15, $P<0.001$). When the pressure-treated brisket muscle was cooked after treatment (70°C for 30 min), the cooking losses were lower ($P<0.001$) than the cooked control samples (Table 2). The control brisket muscle had a lower cooking loss when cooked at 70°C for 30 min compared to cooking at 90°C for 15 min, for both the non aged and aged samples (Table 2). Overall, there was a significant effect of storage on treatment loss (P<0.001) but these losses were very low (0.1 - 0.16%) (Table 2).

Table 2: Effect of treatment (Control - no treatment, HPP - 600 MPa for 15 min at 90°C), storage (unaged, aged) and cooking time and temperature (15 - 90°C for 15 min, 30 - 70°C for 30 min) on treatment and cook loss of brisket muscle.

	Unaged				Aged					P-value						
	Control		HPP		Control		HPP									
	15	30	0	30	15	30	0	30	s.e.d.	Storage St	Cook Time CT	Treatment Tr	St x CT	St x Tr	CT x Tr	St x CT x Tr
% cook loss	27.51	25.35	0.10	21.94	27.18	22.98	0.16	18.50	1.452	0.039	<0.001	<0.001	0.06	0.814	<0.001	0.618
% treatment loss	0.00	0.00	0.10	0.10	0.02	0.02	0.16	0.16	0.012	<0.001	-	<0.001	-	<0.001	-	-

2.4.1.2 Chuck

After high pressure treatment at 90°C, small fat globules had adhered to the outer surface of the chuck meat pieces.

There was a significant reduction of the peak force (PF, *P*=0.014) and the initial yield (IY, *P*=0.009) of chuck meat pieces due to high pressure treatment (600 MPa, 15 min, 90°C) in both unaged and aged samples (Figure 5). However, there was no effect on the connective tissue under these conditions (PF-IY). There was no significant effect of storage (unaged vs aged) or the interaction of storage and treatment on chuck meat pieces.

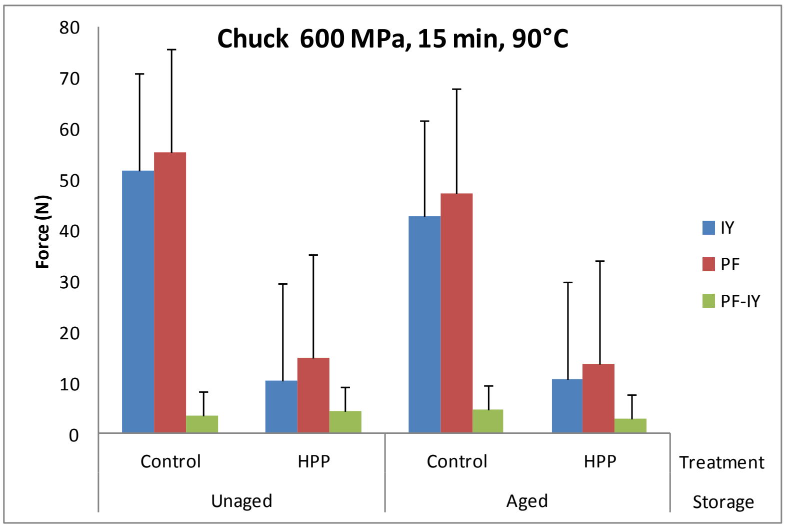

Figure 5: Mean Warner-Bratzler shear force measurements (IY - initial yield, PF - peak force, PF-IY, peak force minus initial yield) of unaged and aged (storage), untreated (control) and pressure treated (HPP, 600 MPa, 15 min, 90°C) chuck samples. Control samples were cooked at 90°C for 15 min; HPP samples were analysed for texture without further cooking. Mean ± s.e.d.

High pressure applied at 600 MPa for 15 min at 90°C resulted in a significant (*P*<0.001) increase in the amount of fluid lost from chuck muscle (treatment loss) compared to the untreated control, although this was only 0.3% (Table 3). There was a significant increase (*P*<0.001) in moisture loss due to cooking in the untreated control samples compared to the pressure-treated chuck muscle, and it must be remembered that the control samples were cooked at 90°C for 15 min whereas the HPP-treated samples were not further cooked after the high pressure treatment (600 MPa, 15 min, 90°C) (Table 3). There was no significant effect of storage and treatment on either treatment loss or cook loss.

Table 3: Moisture losses in chuck muscle due to processing (treatment and cooking) and storage (unaged and aged). The treatment loss was recorded after treatment (Control, no treatment and HPP, 600 MPa for 15 min at 90°C). Cook loss for controls was measured after cooking at 90°C for 15 min; HPP samples received no further cooking.

	Unaged		Aged			P-value		
	Control	HPP	Control	HPP	s.e.d.	Storage St	Treatment Tr	St x Tr
% cook loss	21.60	0.32	22.30	0.31	2.818	0.862	<0.001	0.860
% treatment loss	0.00	0.32	0.02	0.31	0.039	0.835	<0.001	0.743

As expected with high pressure processing of red meat, there was a significant effect (*P*<0.001) on the total colour difference (ΔE) of both brisket and chuck muscles treated at 600 MPa for 15 min at 90°C compared to the untreated control (Table 4). There was also a significant (*P*<0.001) interaction of storage and treatment on the total colour difference of the brisket muscle.

Table 4: Total colour difference (ΔE) for brisket and chuck muscles after treatment (control, HPP - 600 MPa, 15 min, 90°C) and storage (unaged, aged).

Treatment	Unaged		Aged		s.e.d.	P-value		
	Control	HPP	Control	HPP		Storage St	Treatment Tr	St x Tr
Brisket	2.34	20.64	7.93	23.65	0.677	<0.001	<0.001	<0.001
Chuck	3.51	16.46	6.32	19.35	2.748	0.149	<0.001	0.984

2.4.2 HPP setting: 425 MPa at 70°C for 5 min

2.4.2.1 Brisket

Visually, there appeared to be more drip loss in brisket muscles compared to chuck meat pieces after high pressure treatment at 70°C. The brisket samples treated at 70°C under high pressure (425 MPa, 5 min) didn't appear as 'slimy' as those treated at 90°C (600 MPa, 15 min).

There was no effect of high pressure treatment (425 MPa, 5 min, 70°C) on the texture (PF) of brisket muscle as measured by Warner-Bratzler shear measurement, regardless of the cooking protocol used (Figure 6). When the pressure-treated samples were cooked after processing (70°C for 30 min), there was a significant reduction (*P*=0.006) in the IY value compared to the cooked control sample. Cooking at 70°C for 30 min significantly reduced (*P*<0.001) the peak force (PF) value of all samples compared to cooking at 70° for 5 min (Figure 6, note the unaged HPP PF values).

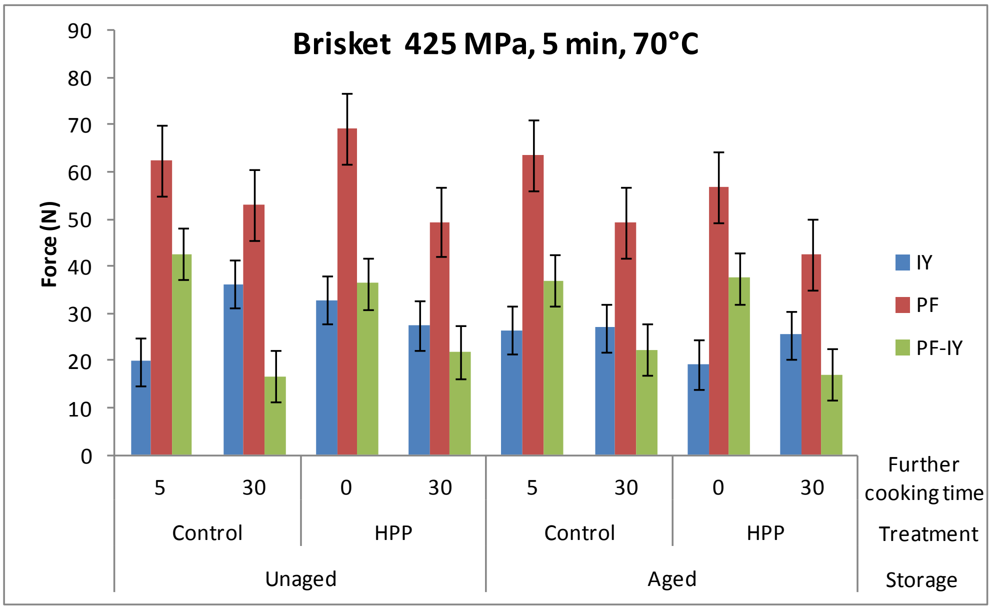

Figure 6: Warner-Bratzler shear force measurements (IY - initial yield, PF - peak force, PF-IY, peak force minus initial yield) of unaged and aged (storage), untreated (control) and pressure treated (HPP, 425 MPa, 5 min, 70°C) brisket samples. Control samples were cooked at 70°C for 5 and 30 min; HPP samples were analysed either by cooking at 70°C for 30 min or without further cooking (0 min). Predicted mean ± s.e.d.

Cooking at 70°C for 5 min resulted in the untreated (control) brisket samples losing about 6% moisture whereas cooking at 70°C for 30 min increased the cook loss to about 24% (Table 5). Brisket muscle treated with 425 MPa pressure at 70°C for 5 min lost less than 0.1% moisture. Cooking at 70°C for 30 min after pressure treatment significantly reduced (*P*=0.016) the cooking loss compared to the cooked control sample (Table 5).

Table 5: Effect of treatment (Control - no treatment, HPP - 425 MPa for 5 min at 70°C), storage (unaged, aged) and cooking time and temperature (5 - 70°C for 5 min, 30 - 70°C for 30 min) on treatment and cook loss of brisket muscle.

	Unaged				Aged					P-value						
	Control		HPP		Control		HPP									
	5	30	0	30	5	30	0	30	s.e.d.	Storage St	Cook Time CT	Treatment Tr	St x CT	St x Tr	CT x Tr	St x CT x Tr
% cook loss	5.36	23.95	0.06	22.45	6.02	24.35	0.07	21.64	8.705	0.927	<0.001	<0.001	0.515	0.279	<0.001	0.739
% treatment loss	0.00	0.00	0.06	0.06	0.02	0.02	0.07	0.07	0.006	<0.001	-	<0.001	-	0.229	-	-

2.4.2.2 Chuck

High pressure (425 MPa, 5 min) at 70°C did not significantly affect the peak force value (PF) of chuck muscle, indicating no effect on tenderness (Figure 7). This result does not confirm the chef's assessment of chuck meat pieces found in the trial conducted in Stage 1. Possible reasons for the contradictory results could include: (a) difference in raw material source, e.g. different animal type and age, different period of ageing of muscle; (b) a higher loading of muscle samples into the HPP unit in this trial which could result in a different heat transfer to that of samples trialled in Stage 1; and (c) the objective texture measurement does not reflect textural changes as assessed by sensory evaluation.

The initial yield (IY, reflecting myofibrillar resistance) was significantly reduced (*P*=0.007) and the PF-IY value (connective tissue) was significantly increased (*P*=0.038) with pressure treatment (Figure 7). There was no effect of storage or the interaction of storage and treatment on any of the Warner-Bratzler shear measurements.

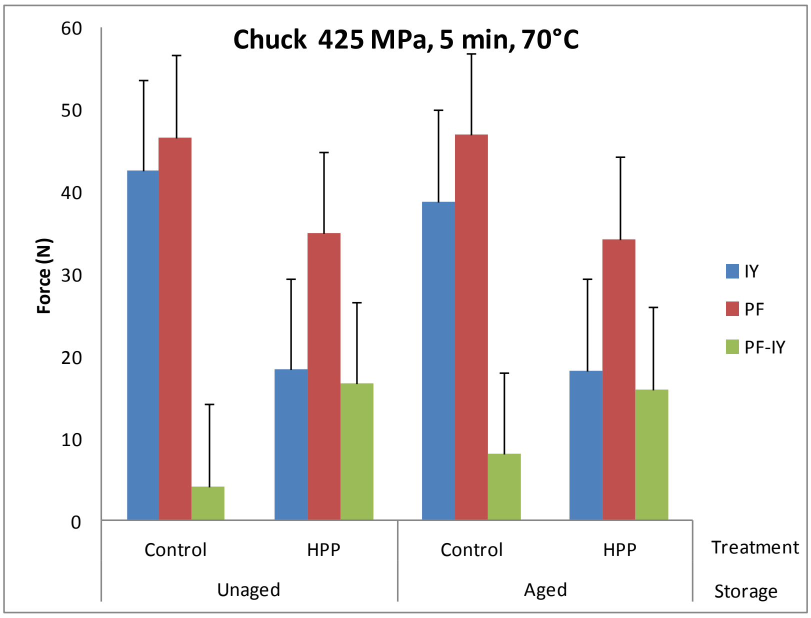

Figure 7: Warner-Bratzler shear force measurements (IY - initial yield, PF - peak force, PF-IY, peak force minus initial yield) of unaged and aged (storage), untreated (control) and pressure treated (HPP, 425 MPa, 5 min, 70°C) chuck samples. Control samples were cooked at 70°C for 30 min; HPP samples were analysed for texture without further cooking. Predicted mean ± s.e.d.

Moisture loss from chuck muscle due to pressure treatment (425 MPa, 5 min) at 70°C was less than 1% compared to the untreated control but this was significant (*P*<0.001) (Table 6). When untreated control chuck samples were cooked at 70°C for 30 min, there was an increase (*P*<0.001) in cook loss compared to the pressure-treated chuck samples that were not further cooked (Table 6). There was no effect of storage or the interaction of storage and treatment on moisture losses (treatment or cook loss).

Table 6: Moisture losses in chuck muscle due to processing (treatment and cooking) and storage (unaged and aged). Treatment loss recorded after treatment (Control, no treatment and HPP, 425 MPa for 5 min at 70°C). Cook loss for controls were measured after cooking at 70°C for 30 min; HPP samples received no further cooking.

	Unaged		Aged			P-value		
	Control	HPP	Control	HPP	s.e.d.	Storage St	Treatment Tr	St x Tr
% cook loss	19.88	0.06	21.15	0.09	3.115	0.768	<0.001	0.779
% treatment loss	0.00	0.06	0.03	0.09	0.023	0.070	<0.001	0.945

Similar to high pressure treatment (600 MPa, 15 min) at 90°C, lower pressure (425 MPa) applied for a shorter time (5 min) at a lower temperature (70°C) significantly increased (*P*<0.001) the total colour difference of brisket and chuck muscle (Table 7).

Table 7: Total colour difference (ΔE) for brisket and chuck muscles after treatment (control, HPP - 425 MPa, 5 min, 70°C) and storage (unaged, aged).

Treatment	Unaged		Aged		s.e.d.	P-value		
	Control	HPP	Control	HPP		Storage St	Treatment Tr	St x Tr
Brisket	4.39	20.29	8.78	20.52	1.126	<0.001	<0.001	<0.001
Chuck	4.03	16.38	4.99	16.42	3.23	0.827	<0.001	0.841

3. Conclusions and recommendations

- As the results of this trial do not confirm the chef's assessment of texture for the chuck meat pieces treated with pressure (425 MPa, 5 min) at 70°C in Stage 1, it is suggested that:
    - The scale up of the process (i.e. higher loading of samples) be investigated
    - The objective measurement for texture of high pressure treated muscle be re-evaluated
- Further investigation to define the optimum conditions for tenderisation in the range of 600 MPa, 15 min, 90°C and to explore muscle differences
- Quantification of the reproducibility of HPP tenderising effect for a given HPP protocol and a given cut of meat – across multiple carcases and MSA scores (or at least animal breed, age, days post rigor)
- Next steps – commercialisation plan needs to consider:
    - how to "maintain" consistent elevated high temperature and pressure settings as current five units in Australia are currently "cold" pasteurisation in the pack HPP operations. Relevant IP, freedom to operate terms and wider red meat industry dissemination of HPP opportunities to create and capture value need to be developed.

- Meat Standards Australia (MSA) Pathways committee update – consideration for further research for HPP as potential MSA Pathway for predicted eating quality

Appendix 1: Preliminary findings from Chef's assessment of the impact of a range of interventions on the eating quality of brisket, chuck and blade

Brisket (*M. pectoralis profundus)*

4 degrees C 600mpa 5 minutes	Steak	Roast	thin slice	Comment
Control	Fail	Fail	Fail	
HPP	Pass	Fail	Fail	HPP product noticeably better. Not as good as control slow cook
Inject	Fail	Fail	Fail	
HPP/Inject	Fail	Fail	Fail	
Inject/HPP	Fail	Fail	Fail	
HPP/Cook	Fail	Fail	Fail	
HPP/Inject/Cook	Fail	Fail	Fail	
slow cook	Excellent			The slow cooked product performed very w ell. No conceivable difference comparing cook to HPP/Cook

4 degrees C 400mpa 5 minutes	Steak	Roast	thin slice	Comment
Control	Fail	Fail	Fail	
HPP	Fail	Fail	Fail	
Inject	Fail	Fail	Fail	
HPP/Inject	Fail	Fail	Fail	
Inject/HPP	Fail	Fail	Fail	
HPP/Cook	Fail	Fail	Fail	
HPP/Inject/Cook	Fail	Fail	Fail	

4 degrees C 300mpa 5 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Fail			
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				

4 degrees C 600mpa 10 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Fail			
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				

Brisket (*M. pectoralis profundus)*

4 degrees C 600mpa 10 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Fail			
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				

70 degrees C 600mpa 5 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Improved			
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				
Cook	excellent			

70 degrees C 600mpa 10 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Good			
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				
Cook	Excellent			

70 degrees C 600mpa 16.5 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Very good			Great brow ning on re cook, low er yield loss very apparent from control cooked piece 19 %. Acceptable tenderness
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				
Cook	Excellent			45 % yield loss

Chuck Piece *M. serratus ventralis*

4 degrees C 600mpa 10 minutes	Steak	Roast	thin slice	Comment
Control	Fail			Light cherry red colour occurred on repeat
HPP	Fail			
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				

70 degrees C 600mpa 5 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Improvement			
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				
Cook	excellent			

70 degrees C 600mpa 10 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	very good			melted connective tissue, can see aplication for rib finger style product matched with sauce as a low cost ready meal alternative
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				
Cook	Excellent			

70 degrees C 600mpa 16.5 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Excellent			Great browning on re cook, lower yield loss 14%. Excellent eating quality
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				
Cook	Excellent			

Blade *M.latissimus dorsi*

4 degrees C 600mpa 5 minutes	Steak	Roast	thin slice	Comment
Control	Fail	Fail	Fail	
HPP	Fail	Fail	Fail	
Inject	Fail	Fail	Fail	
HPP/Inject	Fail	Fail	Fail	
Inject/HPP	Fail	Fail	Fail	
HPP/Cook	Fail	Fail	Fail	
HPP/Inject/Cook	Fail	Fail	Fail	

4 degrees C 400mpa 5 minutes	Steak	Roast	thin slice	Comment
Control	Fail	Fail	Fail	
HPP	Pass	Fail	Fail	Product starting to become acceptable
Inject	Fail	Fail	Fail	
HPP/Inject	Fail	Fail	Fail	
Inject/HPP	Fail	Fail	Fail	
HPP/Cook	Fail	Fail	Fail	
HPP/Inject/Cook	Fail	Fail	Fail	

4 degrees C 300mpa 5 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	improvement			Start of improvement to texture
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				

4 degrees C 600mpa 10 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Fail			
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				

Blade *M.latissimus dorsi*

4 degrees C 600mpa 10 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Fail			
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				

70 degrees C 600mpa 5 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Fail			
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				
Slow Cook	excellent			

70 degrees C 600mpa 10 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Fail			
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				
Slow Cook	Excellent			

70 degrees C 600mpa 16.5 minutes	Steak	Roast	thin slice	Comment
Control	Fail			
HPP	Better			
Inject	Fail			
HPP/Inject				
Inject/HPP				
HPP/Cook				
HPP/Inject/Cook				
Slow Cook	Excellent			

Appendix 2 : Preliminary Chef's assessment of the eating quality of topside & chuck after high pressure treatment at high temperature (70°C)

Muscle	Treatment	Pressure (MPa)	Time (min)	Total wt (g)	Drip (g)	% loss	Sensory assessment
Topside	Control-heat	0.1	5	70.58	8.06	11.4	
	HPP	200	5	62.75	7.4	11.8	Improvement; no 'Who cares' factor
Chuck	Control-heat	0.1	5	41.26	2.21	5.4	
	HPP	200	5	31.8	1.97	6.2	Improvement; no 'Who cares' factor; better than improvement in topside
Topside	Control-heat	0.1	16.5	41.87	13.28	31.7	
	HPP	200	16.5	46.75	9.1	19.5	Improvement; no 'Who cares' factor
Chuck	HPP	200	16.5	32.87	1.38	4.2	Initial improvement, residual bolus
At 200 MPa, topside better at 16.5 min; chuck at 5 min - no perceived benefit							
Topside	Control-heat	0.1	5	45.48	4.51	9.9	
	HPP	400	5	47.89	4.35	9.1	'Who cares' factor
Chuck	Control-heat	0.1	5	42.9	2.73	6.4	
	HPP	400	5	43.92	0.35	0.8	'Who cares' factor
Topside	Control	0.1	16.5	52.08	1	1.9	Tough
	Control-heat	0.1	16.5	53.31	16.61	31.2	Tough, dry
	HPP	400	16.5	55.7	8.07	14.5	Large improvement
Chuck	Control	0.1	16.5	35.7	0	0.0	
	Control-heat	0.1	16.5	42.86	8.2	19.1	
	HPP	400	16.5	45.97	3.18	6.9	Improvement
At 400 MPa, 5 min duration the best for both topside and chuck - definite commercial benefit - texture and yield							

Muscle	Treatment	Pressure (MPa)	Time (min)	Total wt (g)	Drip (g)	% loss	Sensory assessment
Topside	Control-heat	0.1	5	63.32	6.14	9.7	
	HPP	600	5	44.1	4.85	11.0	Not as good as 400 MPa
Chuck	Control-heat	0.1	5	36.93	2.14	5.8	
	HPP	600	5	46.42	1.74	3.7	
Topside	Control	0.1	16.5	55.15	0	0.0	
	Control-heat	0.1	16.5	69.2	18.76	27.1	
	HPP	600	16.5	66.02	12.86	19.5	As tough/tougher than control; better than 5 min HPP
Chuck	Control	0.1	16.5	41.5	0	0.0	
	Control-heat	0.1	16.5	35.39	9.7	27.4	
	HPP	600	16.5	43.84	2.94	6.7	Improvement, not as good as 400 MPa
At 600 MPa, topside HPP tougher than control; improvement in chuck but not as good as 400 MPa							
Optimal conditions identified as 400 MPa for 5 minutes							

?

Defined conditions around 400 MPa and 5 min; new control 400 MPa, 5 min samples							
Muscle	**Treatment**	**Pressure (MPa)**	**Time (min)**	**Total wt (g)**	**Drip (g)**	**% loss**	**Sensory assessment**
Topside	HPP	300	5	55.23	3.94	7.1	Not as good as 400 MPa
Chuck	HPP	300	5	33.65	0.75	2.2	Not as good as 400 MPa
Topside	HPP	500	5	60.18	2.35	3.9	Not as good as 400 MPa (or 300 MPa)
Chuck	HPP	500	5	46.93	4.5	9.6	400 MPa better
Topside	HPP	400	2				Tough

Expand boundaries around 400 MPa further, using 400 MPa for 5 min as controls							
Topside	HPP	350	5	53.1	4.32	8.1	450 ≥ 400 ≥ 350
	HPP	400	5	40	5.47	13.7	
	HPP	450	5	48.66	6.79	14.0	
Chuck	HPP	350	5	37.44	2.13	5.7	400 ≥ 350 ≥ 450
	HPP	400	5	35.61	1.5	4.2	
	HPP	450	5	39.92	4.29	10.7	
Topside	HPP	400	5	44.07	6.04	13.7	Optimum 400 - 425 MPa
	HPP	425	5	39.95	4.34	10.9	
	HPP	475	5	34.9	3.24	9.3	
Chuck	HPP	400	5	38.19	3.54	9.3	Optimum 400 - 425 MPa
	HPP	425	5	31.06	3.74	12.0	
	HPP	475	5	36.26	1.29	3.6	
		* NB 400 MPa samples reheated in hot water					**Optimum pressure = 425 MPa**

Investigate duration at 425 MPa

Muscle	Treatment	Pressure (MPa)	Time (min)	Total wt (g)	Drip (g)	% loss	Sensory assessment
Topside	HPP	425	3	56.98	7.6	13.3	X
	HPP	425	4	34.26	2.1	6.1	OK
	HPP	425	5	-	-	-	Best
	HPP	425	6	54.3	5.94	10.9	OK
Chuck	HPP	425	3	48.11	1.28	2.7	
	HPP	425	4	35.09	1	2.8	
	HPP	425	5	34.78	1.03	3.0	
	HPP	425	6	47.5	1.05	2.2	
							Optimum time 4 - 5 minutes