final report

Project code:	A.MPT.0032
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Date submitted:	January 2011
Date published:	June 2011

PUBLISHED BY
Meat & Livestock Australia Limited
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NORTH SYDNEY NSW 2059

High pressure processing concept product development trials

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.

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Abstract

Previous project work conducted with funding from MLA has shown that tender meat can be achieved from muscles which are normally low value, through the application of high pressure processing (HPP), combined with heat. This work was conducted using the small scale equipment at CFNS-Coopers Plains, using small pieces of meat, and texture assessed by objective measurements (Warner-Bratzler, WB).
The trial work conducted in this project was designed to reproduce the effects found using the small scale equipment at Cannon Hill-Coopers Plains, on the large scale (35L) equipment at Werribee. In addition, these trials were co-ordinated to provide rapid progress and results to assist in answering a number of previously raised questions.

Project objectives

- Determine if pressure alone can achieve tenderisation in low and high collagen cuts (tri-tip, topside, tenderloin, brisket)
- Determine if tenderisation can be achieved using low pressures and high temperatures using CSIRO's Werribee HPP facility
- Determine the impact on tenderness, if any, of pre-freezing samples (-20°C)
- Determine the impact of brine and HPP on texture of HPP-treated product

Methodology

Trial 1 (Nov 3-5)

In order to ensure that the muscles undergoing trial work were of a known origin and specification, muscles were collected from Teys Bros, Beenleigh on November 1, 2010. The muscles were selected on the basis that they represented a cross-section of meat cuts and textures and were commercially available. The muscles chosen were the brisket point end - deckle off (*M. pectoralis profundus*, brisket), the topside cap off (*M. semimembranosus*, topside) and chuck rib meat (*M. serratus ventralis*, chuck) (Figure 1).

Figure 1: Selected muscle cuts used for HPP processing, brisket (left), topside cap (middle), and chuck (right).

Paired muscles (from left and right sides of the same carcase) from 3 animals were collected post-rigor; 18 muscles in total. On arrival at CFNS at Coopers Plains, it was discovered that we received the topside cap (*M. gracilis*, cap) rather than the topside cap off (topside), and that "paired" muscles from 2 of the animals originated from different carcasses (Appendix 1).

Animal information, post-mortem pH values and sarcomere lengths for the muscles are given in Appendix 1.

Pairs of tongue roots or neck muscle (*M. sternomandibularis*, neck) from 11 carcasses (n=22 total) were also collected pre-rigor from Teys Bros, transported to CFNS, wrapped in Clingwrap® and packaging tape and stored at 15°C for approximately 20 hours, to prevent cold-shortening.

Animal information and post-mortem pH values for the neck muscles used for processing from 6 of the animals are given in Appendix 2.

The individual muscle sections were dissected out on November 1 and 2. The original intention was to cut steak-size portions, approximately 150-200g and 20-25 mm thick. Due to the size of the muscles, it was only possible to cut muscle strips of 50-130 g and 2.5 cm thick, as shown in Figure 2. Weights of the muscle strips were recorded.

Figure 2: Muscle portions cut from the brisket muscle for Trial 1 experiments.

Post-mortem pH was recorded for all muscle samples and sarcomere lengths for the 3 commercial muscles (brisket, chuck, topside cap) were measured according to the method of Bouton et al. (1973).

Muscle samples were air-transported on ice to CFNS Werribee, where combinations of pressure, temperature and time were applied to individual muscle strips in the 35L HPP unit (Figure 3). Details of the conditions of each HPP run will be explained in the Results and Discussion Section. For all HPP cycles, that is, for each set of processing conditions, a control sample was allocated. This control was untreated and fried for comparison to treated samples for sensory assessment.

Figure 3: Method of attachment of the meat sample to the insulated sample basket for HPP treatment in the 35L unit.

Prior to pressure treatment, all samples were weighed and an initial weight recorded. Following pressure treatment at high temperatures (above ambient), treated samples were cooled at 2-3°C for at least 10 minutes. In some instances, colour measurements were recorded and this was done using a Minolta chromameter on the cut surface of longitudinally sliced samples. After treatment, weights were recorded and a treatment loss (TL) calculated based on the initial weight of the sample before treatment.

Samples for informal sensory assessment were fried to an internal temperature of 62°C, ensuring that the minimum surface temperature was greater than 75°C. Samples were immediately consumed for sensory scoring. Weights after cooking were recorded for calculation of cooking loss (CL). A final yield or total loss during processing was calculated. This yield calculation incorporated losses after treatment and after cooking, relative to the initial weight. In some HPP runs, samples were also cooked at 80°C for 1 hour in a water-bath, to mimic the cooking procedure used for the Warner-Bratzler texture analysis.

Samples collected for assessment of meat texture using the WB shear force measurement were frozen and transported to CFNS Coopers Plains. Samples were thawed at 4°C for approximately 8 hours and heated at 80°C for 30 minutes. Cooked samples were cut into sub-samples for textural analysis. Details of sample thickness, shape and fibre orientation for samples used for shear force and compression measurements are as described by 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 500N load cell (Lloyd Instruments Ltd., Hampshire UK). Six sub-samples having a rectangular cross section of 14.7 mm wide by 6.7 mm deep were cut from each sample, with fibre orientation parallel to the long axis, and at right angles to the shearing surface. The force required to shear through the clamped sub-sample with a 0.64mm thick blade pulled upward at a speed of 100 mm/min at right angles to fibre direction was measured as shear force. This allowed the determination of peak force (PF), initial yield (IY) and peak force minus initial yield (PF-IY). PF is a measure of the contribution of the myofibrillar and connective tissue toughness, IY is a measure of the myofibrillar toughness, and PF-IY is a measure of the connective tissue (Beilken et al., 1986).The mean for the sub-samples was recorded.
Thermo-eggs (thermocrons) were placed in each HPP run to record the temperature profile during each cycle, and examples of these are given in Appendix 3.

Trial 2 (Nov 15-17)

In addition to the topside, brisket and chuck used in the first set of trials, flank steak (*M. tensor fasciae latae)*, tenderloin (*M. psoas major*) and tri tips (*M. rectus abdominis)* were collected. Muscles for the second set of trials were sourced from:

a. Munster Quality Meats, Port Macquarie (by MLA) – muscles from a stirk veal (very light yearling)
    i. Topside cap off
    ii. Butt tenderloin
    iii. Flank steak
    iv. Tri tip

    N.B. Only the topside and tenderloin muscles were processed
b. Swifts Brooklyn abattoir (by CFNS) – muscles from 6-8 tooth ox (250-350 kg)
    i. Topside cap off
    ii. Brisket
    iii. Tenderloin
    iv. Flank steak

The tenderloin, having a low connective tissue content, was used as a “running control” for the process conditions.

Muscles were cut into strips and allocated to various pressure-time-temperature profiles as described below in the Results section.

A control sample, no treatment, was allocated for each set of processing conditions. Where pressure was combined with temperature, a “heat control” sample provided a further comparison to the pressure-heat-treated samples. This heat control sample was subjected to the same conditions of pre-heating that the treated samples were exposed to.

Initial weight of the samples was recorded prior to application of any treatments. Following pressure treatment at high temperatures (above ambient), treated samples were cooled at 2-3°C for at least 10 minutes. In some instances, colour measurements were recorded and this was done using a Minolta chromameter on the cut surface of longitudinally sliced samples. After treatment, weights were recorded, and a treatment loss (TL) calculated.

Samples for informal sensory assessment were fried to an internal temperature of 62°C, ensuring that the minimum surface temperature was greater than 75°C. Samples were immediately consumed for sensory scoring. Weights after cooking were recorded for calculation of cooking loss. A final yield or total loss during processing was calculated.

Samples collected for assessment of meat texture using the WB shear force measurement were cooked in a water-bath (80°C, 30 min) on the same day as pressure treatment and analysed for texture at CFNS Werribee the following day. These textural measurements were made on an Instron machine (model 5564) fitted with a 500N load cell. Details of the WB analysis were performed as described for Trial 1.

Two thermo-eggs (thermocrons) were used to map the temperature profiles of each HPP cycle in this set of trials. Unfortunately, one of the thermocrons was damaged and only limited data was recovered. The profiles for the HPP cycles using pressure and high temperature on 2 separate days are shown in Appendix 4 and the profile using 450 MPa, for 5 minutes at 4°C is given in Appendix 5, with an explanation of compression cooling that occurs during these processing conditions.

Temperature Profiling in 3L Unit

In order to investigate if it is possible to replicate the processing conditions found with the small unit at Coopers Plains at large scale, separate temperature profiling of meat samples was conducted using the 3L HPP unit at Werribee.

Meat samples, approximately 3 x 3 x 10 cm, were tightly wrapped in Cling Wrap® (Figure 4) and pre-conditioned at approximately 4°C. The vessel (and glycol) temperature was set at 60°C. The temperature was measured at 2 positions within the meat sample, as well as the temperature of the compression fluid (glycol), at pressures of 200 and 400 MPa for a hold time of 20 minutes. The compression rate was 20 MPa/s and decompression took place over a period of 45.

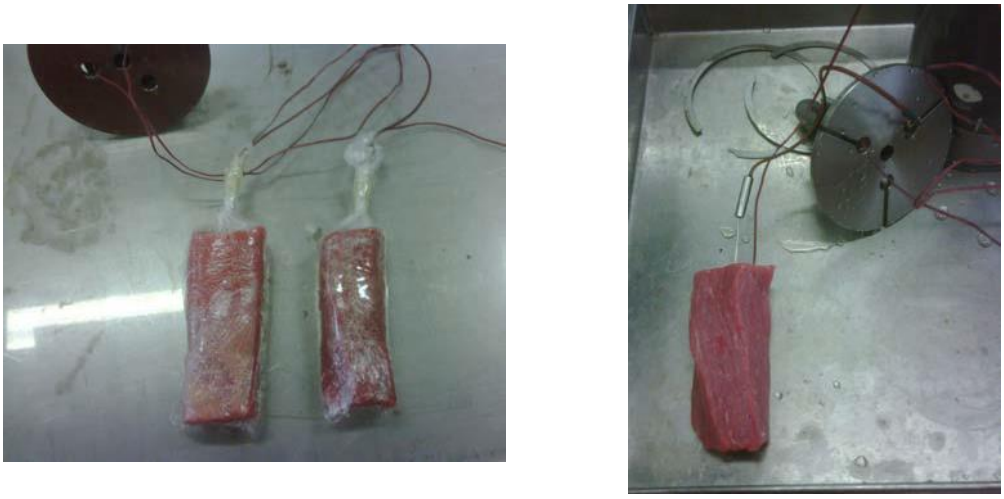


Figure 4: Sample preparation and positioning of thermocouples for temperature profiling in the 3L HPP unit.

Results and discussion

Trial 1 (Nov 3-5)

Pressure combined with heat, all muscles

All muscles, brisket, chuck, cap and neck were pre-heated at 50°C for 35 minutes. Pressure at increasing levels (200, 400, 600 MPa) was applied to the brisket, in sequential runs, to achieve internal target temperatures in the meat of 57.7, 65.4 and 72.6°C respectively. Pressure holding time was 5 or 15 minutes. Pressures of 200 and 600 MPa for 15 minutes were applied to the chuck, cap and neck portions. Pressure treatment (200 MPa) for 15 minutes was also performed on a brisket sample, without pre-heating, to attempt to replicate the conditions used in previous work on the small HPP unit (0.3L) in Brisbane. It is important to note that for this cycle, the fill water temperature was 60°C, with the vessel wall temperature set at 60-65°C and the samples were placed in the non-insulated basket.

It is also important to note that 3 different cooking procedures were used in these trials:

i. Frying to an internal temperature of 62°C for sensory analysis (Werribee)
ii. Water-bath 80°C for 1 hour to try and compare WB method to sensory (Werribee)
iii. Water-bath 80°C for 30 minutes for reduced sample size for texture analysis using the WB method (Coopers Plains)

The raw data is tabulated in Appendix 6. A summary of the data (means calculated where more than 1 sample was processed) is given in Table 1.

The control brisket was rated by the informal sensory as very, very tough (Table 1). Pressure at 200 MPa improved the sensory rating, with a longer holding time (15 min) under HPP having a greater impact than the shorter time of 5 minutes. This trend was similar for the application of 400 MPa. A higher pressure of 600 MPa for 5 minutes had a comparable effect on sensory scores to that of 200 and 400 MPa at 15 minutes. However, pressure applied at 600 MPa for 15 minutes had a negative effect on sensory scores of texture and was assessed as being slighter tougher but more tender than the control.
Interestingly, the brisket sample with no pre-heating (blue row, Table 1) prior to pressure treatment of 200 MPa for 15 minutes, had a sensory score comparable to the pre-heated sample exposed to similar pressure and time conditions.

The WB objective measurements of texture of the brisket indicated that regardless of the pressure holding time, the 200 MPa pressure-treatment reduced the peak force (PF) value of brisket to 62N (control 89N) (Table 1). The majority of the reduction in PF for the HPP treated brisket appeared to be a reduction in IY, with some reduction also in PF-IY. As for the sensory scores, the PF values were reduced for the 400 MPa HPP treatment relative to the control and were a little higher than the 200 MPa HPP treated samples. The application of 600 MPa appeared to have less effect on objective measurements with only a small reduction in PF, IY and PF-IY relative to control. The application of a range of pressures (200, 400 and 600 MPa) to achieve internal target temperatures (58, 65 and 73°C, respectively) and the effects on the texture of the meat, as measured by WB, are also presented in Figure 5. It is clearly seen that with a targeted internal temperature in the meat of 58°C (i.e. 200 MPa of applied pressure), the peak force value is reduced in all meat cuts, particularly the brisket and the topside cap.

It was noted that for the brisket, frying produced a seared outer "skin" that appeared to be influential on the sensory perception of toughness. A different cooking method (e.g. steam combi oven) may produce a different sensory texture result.

Pressure treatment (200 or 600 MPa) had no effect on the sensory scores of chuck portions, The objective measurements showed that chuck samples subjected to pressure at 200 MPa for 15 minutes decreased the PF to 46N (from 61N, control) (Table 1). The application of 600 MPa had a minimal effect on the texture of the chuck.
For the topside cap, no differences in sensory scores after treatment at 200 MPa for 15 minutes were seen, compared to the untreated control sample. Objective measurements showed a similar trend to that of the other muscles (brisket and chuck), showing a decrease in PF with 200 MPa treatment, with all of the difference being accounted for by differences in IY, rather than in PF-IY. There was less of an impact with 600 MPa treatment. The application of 200 and 600 MPa pressure to the neck muscle for 15 minutes showed a reduction in PF of (53N) compared to the control (65N), which was accounted for by a reduction in PF-IY, rather than in IY.

Enzyme treatment

Brisket obtained from a local Werribee butcher was sliced into portions and injected (15% weight of muscle) with a 1% brine solution containing actinidin (kiwifruit protease). Muscle samples were incubated for 60 minutes or 16 hours at 4°C, pre-heated at 50°C for 7 minutes and pressure treated at 200 MPa for 15 minutes. This combination of pre-heating and pressure was set to achieve an internal target temperature of 35°C in the meat.

The baseline tenderness was assessed by the sensory panellists as being ok (a rating of 4 on the texture scale) (Appendix 6), with incubation for 1 hour having no impact on texture but an improvement in texture was noted with a 16 hour incubation. The WB measurement gave variable results, with the peak force ranging from 35 to 51N. As there was no control sample collected for WB measurement, it is difficult to draw any conclusions on the effect of the enzyme and pressure treatment on texture.

Table 1: A summary of the effects of pressure (200 – 600 MPa) combined with temperature (target internal meat temperature, Temp.), time and cook method on the yield (TL = weight loss due to treatment, CL = weight loss due to cooking, Yield = weight loss due to treatment and cooking), texture (Sensory = Sensory scores, PF = Peak force, IY = Initial yield) and surface colour (L*, a*, b*) of brisket, chuck, topside cap and neck muscle. This set of conditions (Trial 1) was similar to those used in the small HPP unit (0.3L). Where more than 1 sample was allocated per treatment, means have been calculated.

Muscle	***Pressure (MPa)***	***Temp (°C)***	***Time (min)***	***Cook Method***	***TL (%)***	***CL (%)***	***Yield (%)***	***PF (N)***	***IY (N)***	***PF-IY (N)***	***Sensory*** [a]	***L****	***a****	***b****
Brisket	Control			Fry			14.74				1			
	200	58	5	Fry			24.34				2	47.88	21.67	18.42
	200	58	15	Fry			27.04				3	52.51	19.58	7.41
	200	No preheat	15	Fry			15.49				3			
	Control			80°C/30min	6.08	40.83	44.43	89.23	39.48	49.75				
	200	58	5	80°C/30min	15.99	33.03	43.75	62.56	24.70	37.86				
	200	58	15	80°C/30min	16.83	35.18	46.09	62.34	19.49	42.84				
	400	65	5	Fry			21.72				2.5	55.92	10.99	10.42
	400	65	15	Fry			25.12				3	53.92	12.78	9.86
	400	65	5	80°C/30min	19.94	33.83	47.03	68.76	29.65	39.11				
	400	65	15	80°C/30min	20.23	34.66	47.88	64.55	26.11	38.45				
	Control			80°C/60min			44.07				2.5			
	400	65	15	80°C/60min			26.82							
	600	73	5	Fry			34.74				3	55.77	11.46	11.42
	600	73	15	Fry			40.13				2	55.79	12.63	11.16

Muscle	*Pressure (MPa)*	*Temp (°C)*	*Time (min)*	*Cook Method*	*TL (%)*	*CL (%)*	*Yield (%)*	*PF (N)*	*IY (N)*	*PF-IY (N)*	*Sensory* [a]	*L**	*a**	*b**
	600	73	5	80°C/30min	16.74	35.11	45.97	77.67	32.46	45.22				
	600	73	15	80°C/30min	23.70	34.78	50.28	88.00	35.10	52.90				
Chuck	Control			Fry			6.81				3			
	200	58	15	Fry			14.81				3.5	45.72	22.47	6.18
	600	73	15	Fry			19.12				3	53.21	15.57	10.99
	Control			80°C/30min	4.80	37.08	40.10	61.21	44.16	17.07				
	200	58	15	80°C/30min	20.30	29.16	43.54	45.59	25.60	20.00				
	600	73	15	80°C/30min	19.34	32.89	45.88	56.26	31.00	25.26				
Cap	Control			Fry			6.46				3.5			
	200	58	15	Fry			8.81				3	53.42	21.13	9.5
	600	73	15	Fry			9.07					53.50	13.03	11.70
	Control			80°C/30min	4.65	38.74	41.59	50.63	42.64	7.99				
	200	58	15	80°C/30min	15.84	29.17	40.40	27.87	18.71	9.15				
	600	73	15	80°C/30min	21.74	31.39	46.32	40.71	26.09	14.63				
Neck	Control			80°C/30min	3.04	28.67	30.84	64.66	41.03	23.64				
	200	58	15	80°C/30min	7.99	25.53	31.48	53.10	39.74	13.36				
	600	73	15	80°C/30min	7.18	30.29	35.22	52.70	38.47	14.23				

[a] Sensory score based on tenderness assessment. 1 – very, very tough; 2 - very tough; 3 - tough; 4 - ok; 5 - good

Samples analysed in Brisbane for texture (Warner-Bratzler shear force, WB)

Treatment done in non-insulated basket, with no pre-heating, to achieve similar conditions to small unit trials

Samples cooked in water-bath at 80°C for 1 h

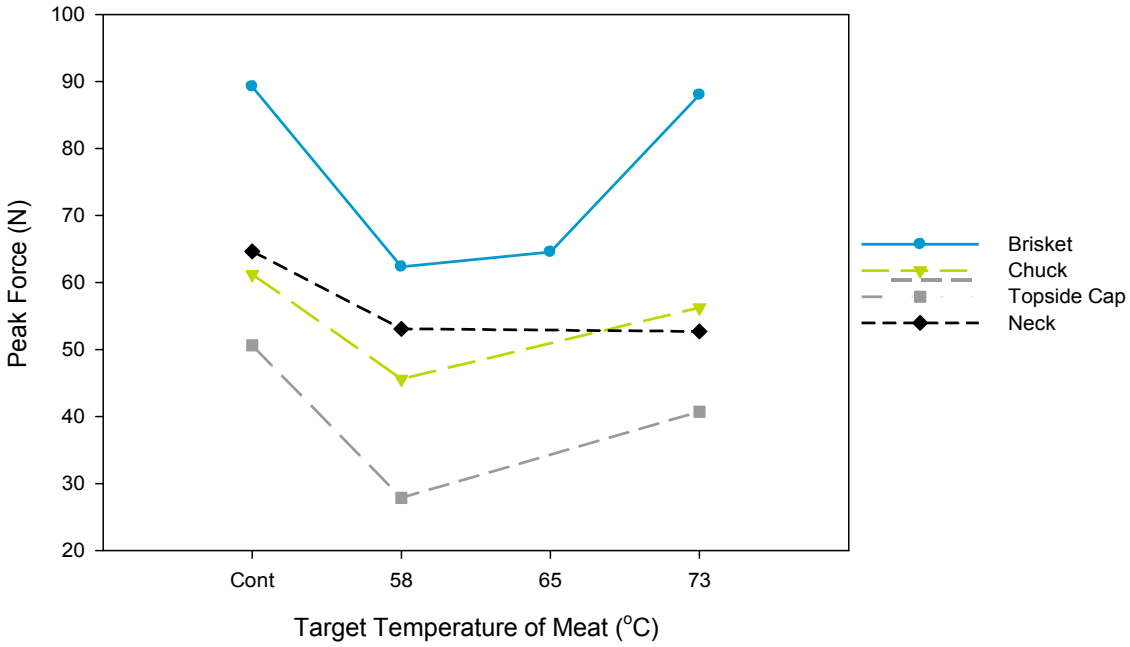


Figure 5: The effect of high pressure (200, 400, 400 MPa) and target temperature (58°C, 65°C or 73°C) applied for 15 minutes on the texture (as measured by peak force) on brisket, chuck, topside cap and neck muscle. The pressures required to reach the target temperatures of the meat were: 200 MPa to reach 58°C; 400 MPa for 65°C and 600 MPa for 73°C.

Summary of Trial 1

For the three muscles brisket, topside cap and chuck, HPP treatment resulted in a reduction in objectively measured texture. The 200 MPa HPP treatment consistently resulted in a greater reduction than either 400 or 600 MPA. The 100 MPa HPP treatment had little effect on the objective measurements of texture or on the sensory scores. As these results were from single samples, it would be advisable to repeat the treatments on a larger number of samples, in order to verify the effects seen in these trials.

Trial 2 (Nov 15-17)

Low pressure, high temperature

Topside and tenderloin portions were subjected to 100 MPa for 5 minutes at either 70°C or 80°C (target temperature). To achieve 70°C, the samples were pre-heated at 65°C for 30 minutes; for 80°C, 75°C for 30 minutes. Corresponding heat control samples were heated under the pre-heating conditions and not pressure-treated. Warner-Bratzler measurements were performed on both the treated samples after frying as well as after the conventional cooking method for WB (water-bath 80°C for 30 minutes).

Low pressure (100 MPa, 5 min) combined with temperatures of 70°C or 80°C had a marginal negative effect on toughness of the topside as assessed by the informal sensory panel (Table 2) and PF (Table 2 and Figure 6A). For the tenderloin treated with 100 MPa for 5 min at 70°C, the tenderness was improved as indicated by both sensory scores (Table 2) , and PF values (Table 2 and Figure 6B) . The decrease in PF value was derived from a reduction in IY, indicating that the myofibrillar component was responsible, rather than connective tissue. However, pressure treatment at 80°C of topside increased the toughness compared to the control.

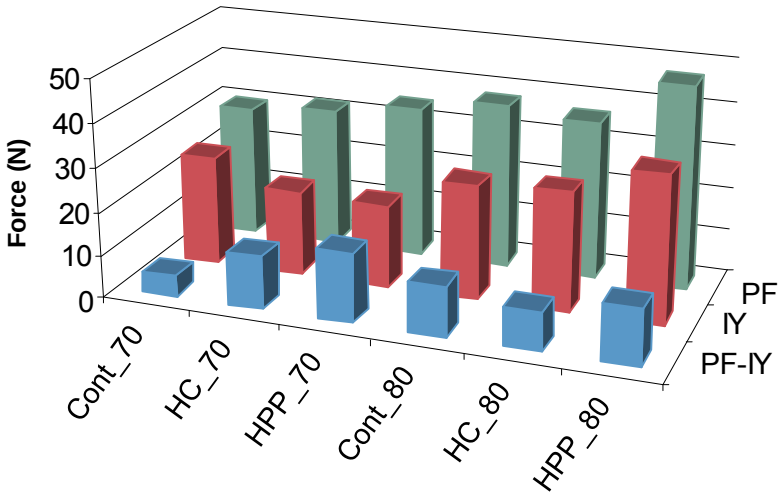
The objective measurements of texture of the brisket samples treated under the same pressure-temperature-time conditions as above showed that at 70°C, both the heat control and HPP-treated samples had slightly reduced PF values, and at 80°C the control and heat control samples had similar PF values, whereas tenderisation occurred with pressure treatment (Table 2, Figure 6C).

Table 2: The effects of low pressure (100 MPa) and high temperature (70 and 80°C), applied for 5 minutes, on the yield (TL = weight loss due to treatment, CL = weight loss due to cooking, Yield = weight loss due to treatment and cooking), and texture (Sensory scores, PF = Peak force, IY = Initial yield) of topside, tenderloin and brisket. Samples were pre-heated for 30 minutes prior to pressure treatment.

Sample	*Pre-heat*	*Pressure (MPa)*	*Target Temp (°C)*	*Time (min)*	*TL (%)*	*CL (%)*	*Yield (%)*	*PF (N)*	*IY (N)*	*PF-IY (N)*	*Sensory* [a]
Topside											
SMC[a]70	Control				0.00	16.61	16.61	30.09	25.25	4.84	3.5
SMHC[b]70	65		70		15.21	16.38	29.10	31.96	19.56	12.41	3.5
SM10070	65	100	70	5	24.57	10.43	32.43	34.86	18.93	15.93	3
SMC80	Control				0.00	-	-	37.92	26.51	11.41	4
SMHC80	75		80		24.62	-	-	36.50	27.58	8.92	4
SM10080	75	100	80	5	35.98	-	-	47.09	34.18	12.91	3.5
Tenderloin											
PMC70	Control				1.55	22.53	23.73	24.35	18.29	3.59	4
PMHC70	65		70		10.57	11.45	20.81	24.62	19.63	4.99	4.5
PM10070	65	100	70	5	57.11	9.22	61.07	12.92	11.58	1.33	5
PMC80	Control				0.00	23.80	23.80	22.24	11.76	10.48	
PMHC80	75		80		32.35	10.86	39.70	26.84	22.25	4.59	
PM10080	75	100	80	5	30.60	8.94	36.80	25.48	22.85	2.63	
Brisket											
PP70CX[c]	Control						29.41	65.70	36.65	29.05	
PP70HX	65		70				34.38	50.75	34.71	16.04	
PP70X	65	100	70	5			36.08	56.46	28.59	27.87	
PP80CX	Control						24.53	64.28	35.51	28.77	
PP80HX	75		80				34.11	65.45	29.46	35.99	
PP80X	75	100	80	5			37.28	51.48	41.53	9.94	

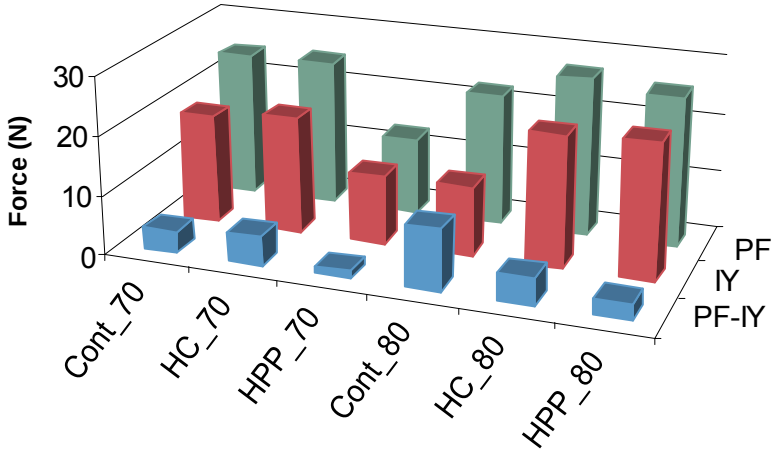
[a] C Control sample – no treatment
[b] HC Heat control – exposed to heating regimes but no pressure treatment
[c] X Samples analysed in Werribee for texture (Warner-Bratzler shear force, WB)

(A) Topside



PF	peak force
IY	initial yield
PF-IY	peak force minus initial yield

(B) Tenderloin



(C) Brisket

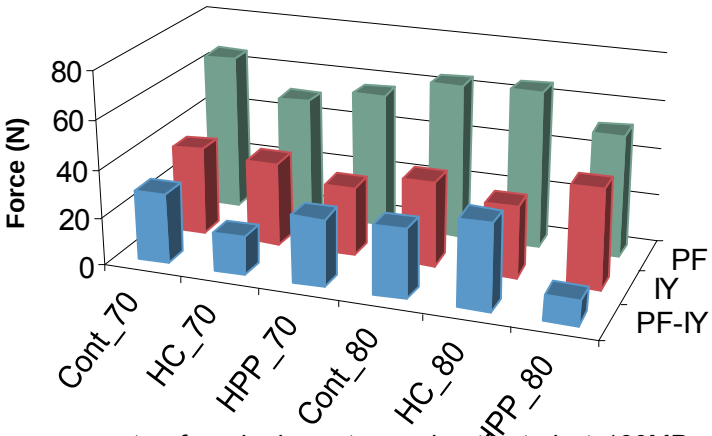


Figure 6: Texture measurements of cooked meat samples treated at 100MPa for 5 minutes at 70°C (70) and 80°C (80). (A) topside, (B) tenderloin and (C) brisket. Cont = control sample, no treatment; HC = heat control, subjected to similar heating conditions with no pressure treatment; HPP = pressure treated

Pressure and frozen meat

A topside sample was frozen overnight at -18°C and subjected to 400 MPa for 5 minutes. The vessel wall temperature was set at 65°C, the fill temperature was approximately 4°C and was established by adding an ice slurry. The post-chamber temperature was 36.5°C. A corresponding "chilled" topside (at 4°C) was subjected to similar conditions.

There was an improvement in sensory score in both the frozen and chilled samples treated with pressure (compared to the control), with a larger effect on the chilled topside (Table 3). A difference in the flavour of the frozen HPP-treated sample was noted but it was not necessarily better.

Similarly, with the tenderloin sample, an improvement in the chilled sample under these conditions was observed.

No samples were collected for texture analysis using the WB method.

Table 3: Effects of high pressure treatment at 400 MPa for 5 minutes on the yield (TL= weight loss due to treatment, CL= weight loss due to cooking, Yield =weight loss due to treatment and cooking) and sensory assessment of chilled (4°C) and frozen (-18°C) topside and tenderloin.

Sample	***Pressure (MPa)***	***Target Temp (°C)***	***Time (min)***	***TL (%)***	***CL (%)***	***Yield (%)***	***Sensory*** [a]
Topside							
SMC	Control			0.00	22.53	22.53	3
SM Froz [b]	400	4	5	1.43	22.04	23.16	4
SM Chill [c]	400	4	5	2.17	20.90	22.62	4.5
Tenderloin							
PMC	Control			-31.32	43.75	26.13	4.5
PM Chill	400	4	5	2.23	24.22	25.91	5

[a] Sensory score based on tenderness assessment. 1 – very, very tough; 2 – very tough; 3 – tough; 4 – ok; 5 - good
[b] Sample frozen overnight at -18°C
[c] Meat chilled at 4°C prior to treatment

Pressure only

Table 4 gives the sensory assessment and yields for topside and tenderloin samples that were high pressure treated at 300 MPa for 5 minutes, with ambient water fill temperature (21°C). No samples were collected for WB measurement. The treated topside was given a slightly higher sensory score (tougher) than the control and there was no apparent difference in the tenderloin with pressure treatment.

Table 4: Sensory scores and yields (TL = weight loss due to treatment, CL = weight loss due to cooking, Yield = weight loss due to treatment and cooking) for topside and tenderloin samples treated at 300 MPa for 5 minutes at ambient temperature.

Sample	***Pressure (MPa)***	***Target Temp (°C)***	***Time (min)***	***TL (%)***	***CL (%)***	***Yield***	***Sensory*** [a]
Topside							
SMC	Control			0.00	23.25	23.25	3
SM300	300	Ambient	5	2.49	20.07	22.06	2.5
Tenderloin							
PMC	Control			0.00	22.02	22.02	4
PM300	300	Ambient	5	1.60	20.15	21.43	4

[a] Sensory score based on tenderness assessment. 1 – very, very tough; 2 – very tough; 3 – tough; 4 – ok; 5 - good

Pressure at low temperature

Following the observed tenderising effect of pressure at low temperature (400 MPa, 4°C, 5 min; Table 3), a range of pressures (100, 250, 600 MPa) was applied to topside and tenderloin samples to investigate if there was an optimum pressure value for tenderisation at this temperature (Table 5).

There was no effect of high pressure treatment at either 100 or 250 MPa on the sensory scores of topside or tenderloin samples. At 600 MPa, in both muscles, high pressure treatment resulted in lower sensory scores and a drier product, and the loss of juiciness was reflected in the yield results (Table 5).

It was decided to confirm the tenderisation with 400 MPa, using topside, tenderloin and brisket, with collection of samples for WB measurement. This combination of pressure-time-temperature (400 MPa, 5 min, 4°C) again resulted in a distinct increase in sensory scores (tenderisation) in brisket, with a slight impact on sensory scores of the topside and tenderloin (Table 5).

Further cycles were conducted to narrow down the pressure range, with the application of 350, 400 and 450 MPa (Table 5). The application of 350 MPa produced variable results; the topside sample was distinctly higher in sensory scores (more tender) whereas the brisket and tenderloin showed minimal differences compared to the control. This variation probably occurred due to a spike in pressure (up to 370 MPa) and a temperature gradient evident within the sample chamber (4-40°C). Pressure treatment at 450 MPa had no effect on topside or brisket muscles but reduced sensory scores for the tenderloin portion (Table 5).

The WB data for the samples collected from the 350, 400 and 450 MPa cycles showed that compared to the control samples, all muscles had higher PF (tougher) at 350 MPa, there was a reduction in PF in the brisket but minimal effect on the topside and tenderloin at 400 MPa, and there was no effect on brisket or topside at 450 MPa but the tenderloin had slightly higher PF.

Using the results in Table 5, the relationship between sensory scores and peak force for brisket, topside and tenderloin at pressures of 250 – 500 MPa at low temperature (4°C) was investigated (Figure 7). A negative linear relationship was observed (R^2 = 0.578).

Table 5: The effects of pressure (100 – 600 MPa) at low temperature (~ 4°C, ice slurry) for 5 min. on the yield (TL = weight loss due to treatment, CL = weight loss due to cooking, Yield = weight loss due to treatment and cooking) and texture (Sensory = sensory scores, PF = Peak force, IY = Initial yield) of topside, tenderloin and brisket.

Sample	***Pressure (MPa)***	***Target Temp (°C)***	***Time (min)***	***TL (%)***	***CL (%)***	***Yield (%)***	***PF (N)***	***IY (N)***	***PF-IY (N)***	***Sensory*** [a]
SMC	Control			0.00	19.84	19.84				3
SM600	600	4	5	2.14	22.05	23.72				2.5
PMC	Control			0.00	22.78	22.78				4
PM600	600	4	5	4.36	23.82	27.14				3
SMC	Control			0.00	17.79	17.79				3
SM100	100	4	5	0.34	14.50	14.79				3
PMC	Control			0.00	16.43	16.43				4
PM100	100	4	5	1.71	17.88	19.28				4
SMC	Control			0.00	25.67	25.67				3
SM250	250	4	5	0.68	22.66	23.18				3
PMC	Control			0.00	18.25	18.25				4
PM250	250	4	5	1.52	22.72	23.89				4
SMC	Control			0.00	22.90	22.90				3
SM400	400	4	5	1.49	27.46	28.54				3.5
PMC	Control			0.00	18.25	18.25				4
PM400	400	4	5	2.66	25.24	27.22				4.5
PPC	Control			0.00	19.39	19.39				1
PP400	400	4	5	1.57	24.30	25.49				3
SMC	Control			0.00	34.19	34.19				3
SM350	350	4	5	20.56	17.02	34.08				4
PMC	Control			0.00	16.32	16.32				4
PM350	350	4	5	1.65	16.25	17.63				4
PPC	Control			0.00	12.89	12.89				2
PP350	350	4	5	3.08	21.69	24.10				2

Sample	*Pressure (MPa)*	*Target Temp (°C)*	*Time (min)*	*TL (%)*	*CL (%)*	*Yield (%)*	*PF (N)*	*IY (N)*	*PF-IY (N)*	*Sensory* [a]
PP350A	350	4	5	1.27	15.10	16.18				
SMC	Control			0.00	17.84	17.84				3
SM450	450	4	5	0.78	20.51	21.14				3
PMC	Control			0.00	23.18	23.18				4
PM450	450	4	5	2.93	30.32	32.36				3
PPC	Control			0.00	18.31	18.31				2
PP450	450	4	5	1.01	21.14	21.93				2
SMCX	Control					32.95	34.69	27.89	6.80	
SM350X	350	4	5			47.85	48.94	46.98	1.96	
SM400X	400	4	5			37.01	29.83	17.46	12.37	
SM450X	450	4	5			32.19	34.15	20.20	13.95	
PMCX	Control					29.01	31.96	24.72	7.24	
PM350X	350	4	5			48.48	43.00	39.36	3.64	
PM400X	400	4	5			33.60	28.45	24.95	3.50	
PM450X	450	4	5			41.78	40.40	34.95	5.45	
PPCX	Control					30.40	69.88	42.58	27.30	
PP350X	350	4	5			42.30	88.84	33.52	55.32	
PP400X	400	4	5			36.44	56.60	34.61	21.99	
PP450X	450	4	5			36.02	69.22	35.67	33.55	

X Samples analysed in Werribee for texture (WB) (Cooked 80°C for 30 minutes in a water-bath)

[a] Sensory score based on tenderness assessment. 1 – very, very tough; 2 - very tough; 3 - tough; 4 - ok; 5 – good

SM topside; PM tenderloin; PP brisket

C contro

Relationship between sensory scores and peak force

The relationships between sensory scores and the peak force (PF), initial yield (IY) and PF-IY for the data in Table 5 were analysed. The prediction of the sensory score from either PF or PF-IY was reasonably good, with about 60% of the variation accounted for by a linear model. Figure 7 shows a plot and equation for PF and identifies the muscles used. The prediction of sensory score from IY was not as good, with less than 1% of the variation explained. It is probable that the prediction of sensory score could be improved by the inclusion of a greater range of samples. Also, with the use of a replicated consumer panel, an investigation of which components of the sensory experience are not being captured by the objective measurements would be recommended. An investigation of alternative methods for objective measurement of texture is also recommended.

The relevant equations are;

(1) Sensory = 5.010-(0.0401*PF), R^2=0.578, SEE =0.694,
(2) Sensory = 3.857-(0.0481*(PF-IY)), R^2=0.570, SEE =0.527,
(3) Sensory = 4.12-(0.0326*IY), R^2=0.0828, SEE =1.023.

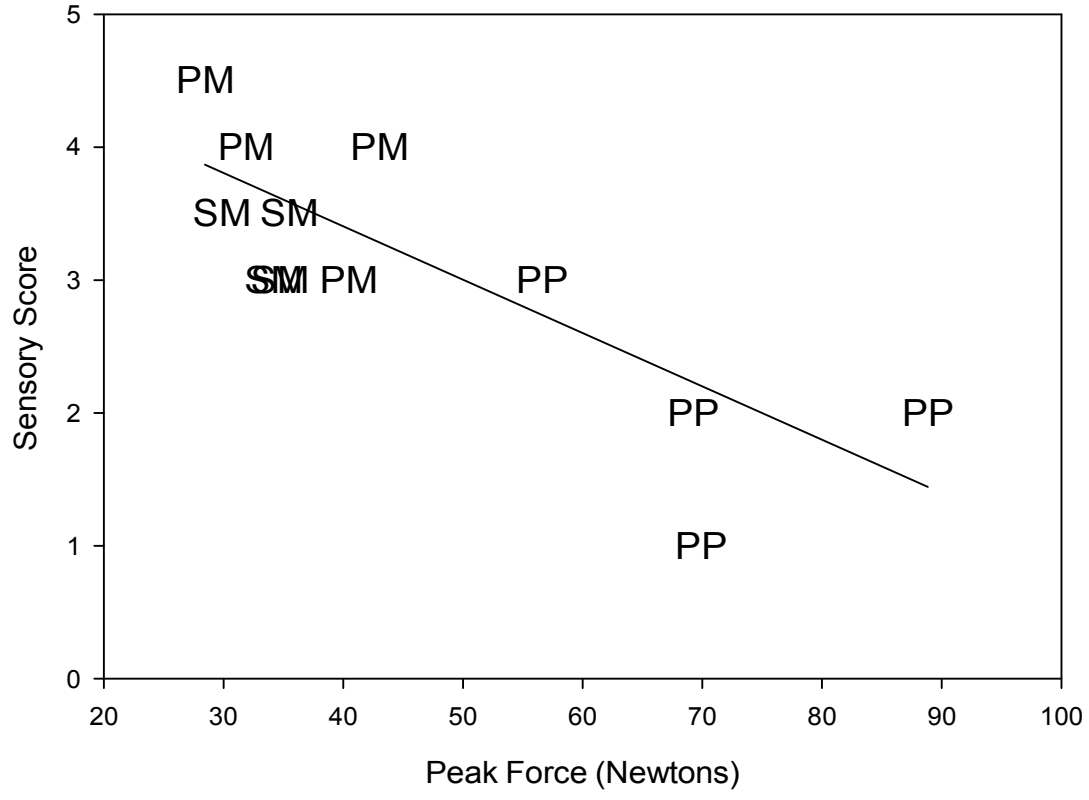


Figure 7: Relationship between sensory scores and peak force in the brisket (PP), topside (SM) and tenderloin (PM) for the samples subjected to either no pressure treatment or 250-500 MPa at a low temperature (4°C, see Table 5).

Summary of Trial 2

High pressure treatment at 100 MPa, using a variety of target temperatures and pre-heating conditions and pre-freezing prior to high pressure treatment had minimal effect on the texture of meat. Topside and brisket samples held at 4°C and subjected to high pressure treatment at 400 MPa, resulted in more tender meat, assessed through both sensory and objective measurements. The same samples held at 4°C and subjected to 250-600 MPa showed no improvement in tenderness. A reasonable correlation was achieved between sensory and objective measures of texture, which can be improved with more investigation.

Temperature Profiling in the 3L Unit

In order to simulate the temperature the meat 'sees' in the 0.3L HPP unit at Coopers Plains, the temperature profiles of the meat samples at 200 and 400 MPa at 60°C for 20 minutes were measured and are shown in Figures 8 and 9, respectively. The temperature was measured at 2 positions: T1, in the centre of the sample, and T2, slightly offset between the surface and the centre of the meat sample. The temperature of the glycol mixture was also recorded (Tcontrol). This was positioned below the non-insulated sample carrier, just underneath the samples.

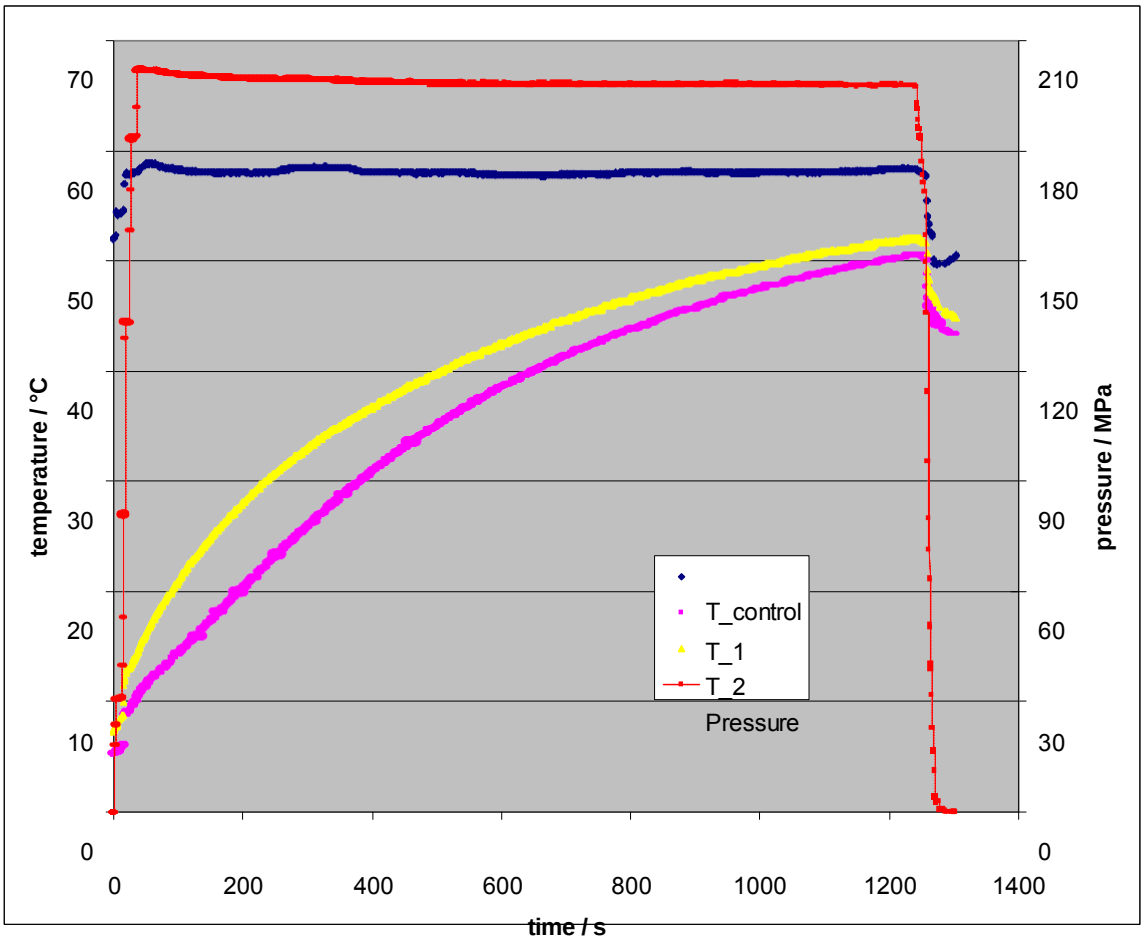


Figure 8: Temperature profile of a meat sample (3 x 3 x 10 cm) processed in the 3L HPP unit at Werribee at 200MPa and 60°C, held for 20 minutes. T_control = temperature of the compression fluid (glycol); T_1 = temperature in the centre of the meat sample; T_2 = temperature in the sample close to the surface of the meat, Pressure = pressure applied

At a pressure of 200 MPa, a small spike in temperature in the compression fluid was seen due to adiabatic heating when pressure was applied (Figure 8, Tcontrol). This had minimal impact on the glycol temperature, with the temperature of the compression fluid remaining constant over the 20 minute hold time. However, the temperature was slightly less than the 60°C set temperature (approximately 58°C). At 400 MPa, an increase to 65°C in the glycol mixture was seen with adiabatic heating, followed by a decrease in temperature to 60°C at around 5-6 minutes. This remained constant, with a final temperature of 59°C by the end of the 20 minute hold time.

As would be expected, the temperature in the centre of the meat sample (T1) lagged behind the temperature closer to the meat surface (T2). This was seen at both 200 and 400 MPa of applied pressure (Figures 8 and 9).

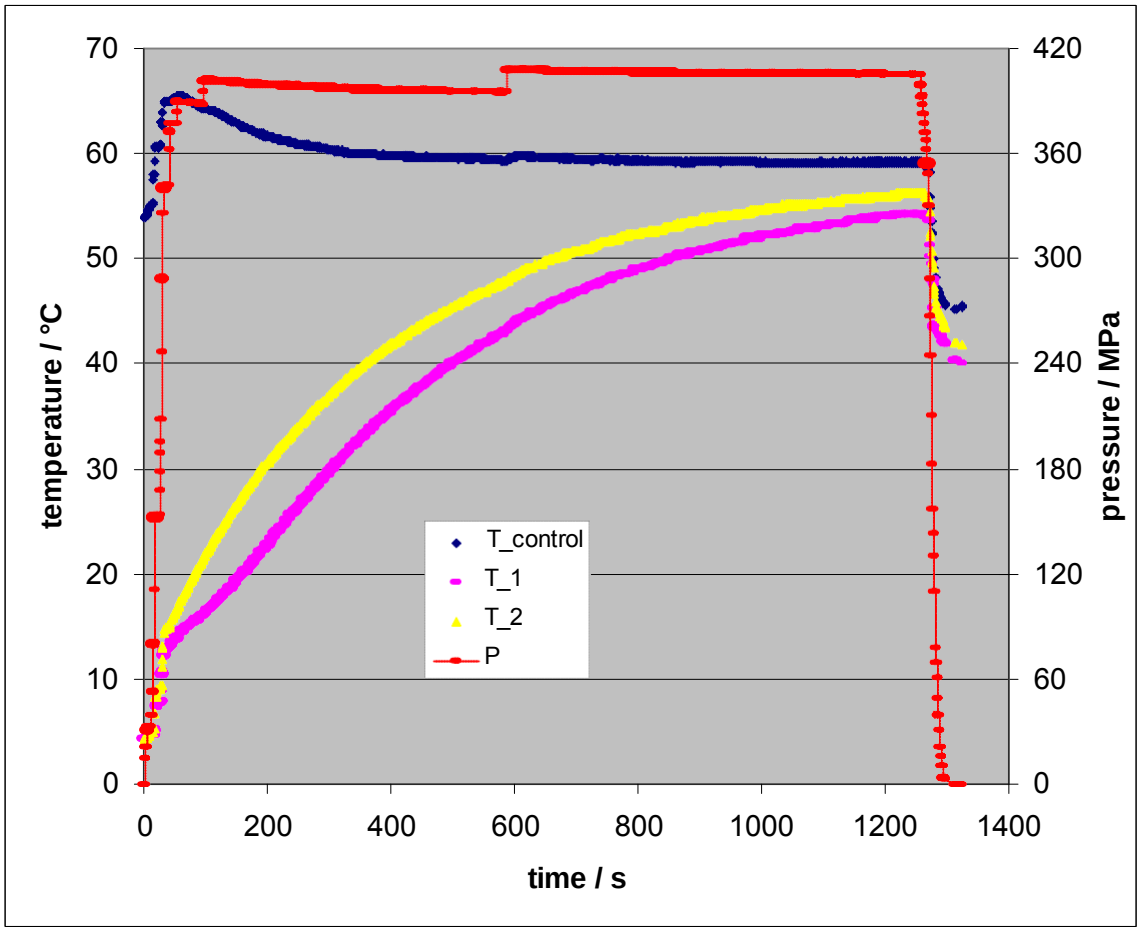


Figure 9: Temperature profile of a meat sample (3 x 3 x 10 cm), pressure treated at 400MPa at 60°C for 20 minutes, in a 3L HPP unit. T_control = temperature of the compression fluid (glycol); T_1 = temperature in the centre of the meat sample; T_2 = temperature in the sample close to the surface of the meat Pressure = Pressure applied.

With pressure applied (200 or 400 MPa), the temperature profiles of the meat samples appeared to be biphasic, regardless of the position of the temperature probe (Figures 8 and 9). A linear increase in temperature to 10 minutes was evident, followed by a gradual increase from 10 minutes to 20 minutes. This was more apparent with 400 MPa of pressure.
With 200 MPa, the maximum temperature reached occurred at the end of the 20 minute run and was 50°C at the centre of the meat sample and 52°C closer to the surface. These temperatures were 4°C higher with the application of 400 MPa – 54°C for the central position and 56°C closer to the surface (Figure 9).

Success in achieving milestone

In these trials, it was not possible to replicate the tenderising effect using the pressure-heat conditions with the small unit (200 MPa, 60°C, 20 min) at CSIRO-Cannon Hill on large scale equipment at CSIRO-Werribee. Pressure alone, without pre-heating, and at ambient temperature, had a negative effect on the texture of topside and tenderloin. A range of pressure-time-temperature conditions were investigated to obtain a tenderising effect on a variety of muscles containing varying amounts of connective tissue.

High pressure applied to frozen samples did not improve tenderness. However pressure (400 MPa) applied at low temperatures (approximately 4°C) appeared to give a tenderising effect on brisket as assessed by the sensory panel and objective measurements. This result requires replication and validation in order to develop a value proposition for High Pressure Processing.

High pressure treatment (200 MPa, 60°C) of brisket with an actinidin solution resulted in the actinidin activity being reduced.

Recommendations

Areas that need to be further investigated and questions that have arisen from these set of trials include the need to:

- Confirm tenderisation conditions (400 MPa, 5 min, 4°C) found for brisket in the large scale equipment, with replicates, with a heated vessel wall.
- Investigate the effect of 400 MPa for 5 min at 4°C without a heated vessel wall, on brisket and other muscles.
- Using the outcomes of the temperature modelling in the 3L unit, investigate the possibility of replicating the processing conditions found on the small unit at Coopers Plains at large scale.
- Establish a correlation between objective measurements of texture and consumer sensory preference for texture, using the industry standard for consumer panel (MSA). This should also include an evaluation of the cooking methods used for assessment by subjective and objective means, which components of the sensory experience are not being captured by the objective measurements and investigation of alternative methods for objective measurement of texture.

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Appendices

Appendix 1

Animal information for muscle samples (topside cap, chuck, brisket) collected for Trial 1.

Animal No.	*Category*	*Body*	*Dent*	*Fat*	*Sex*	*Wt*
1	PR-Jap Ox	692 L	6	11	M	163.2
		692 R	6	11	M	141.6
2	S-Jap Ox	690 L	8	6	M	163.2
		693 R	8	10	M	150.6
3	YP Supergrass	688 L	4	8	M	170.6
	S-Jap Ox	690 R	8	6	M	168.4

Sarcomere and pH data for the two sides from muscles from three animals (above) (Trial 1)

Animal No.	*Muscle*	*Side*	*pH*	*Sarcomere (µm)*
1	Topside cap	A	-	2.00
		B	5.91	2.00
	Chuck	A	5.48	2.42
		B	5.73	3.62
	Brisket	A	5.57	3.52
		B	5.55	2.87
2	Topside cap	A	6.02	2.09
		B	5.98	1.79
	Chuck	A	5.48	3.67
		B	5.73	3.17
	Brisket	A	5.55	2.91
		B	5.47	2.85
3	Topside cap	A	5.94	2.05
		B	5.76	1.90
	Chuck	A	5.84	3.67
		B	5.69	3.70
	Brisket	A	5.54	3.33
		B	5.55	2.85

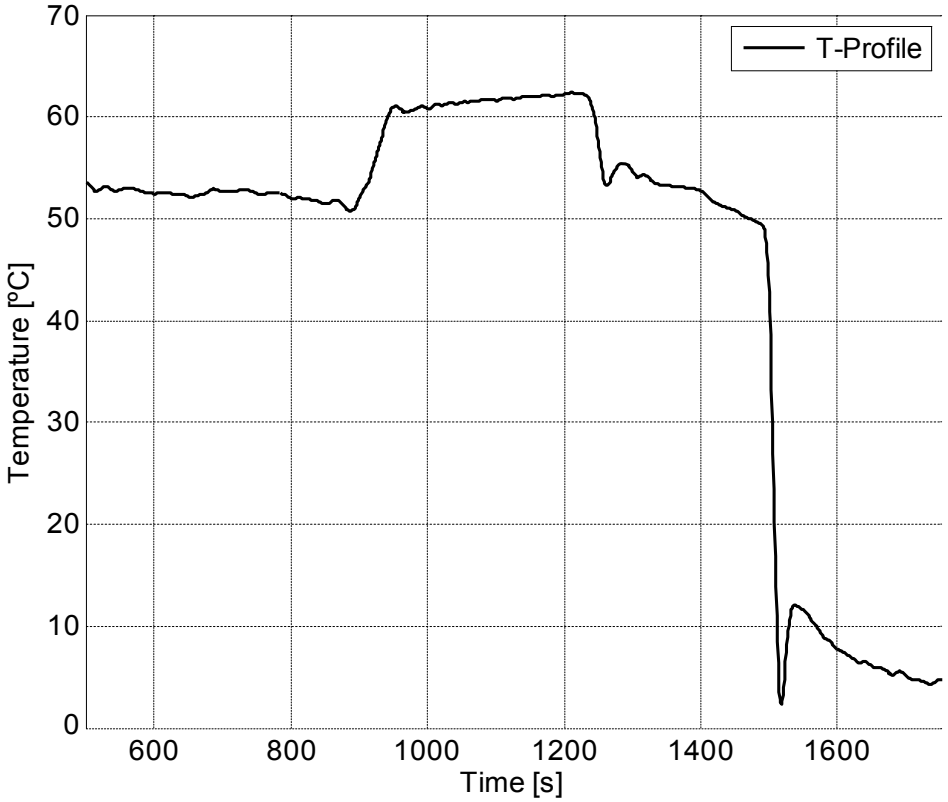
Appendix 2

Animal information for beef neck muscles (*M. sternomandibularis*) collected for Trial 1.

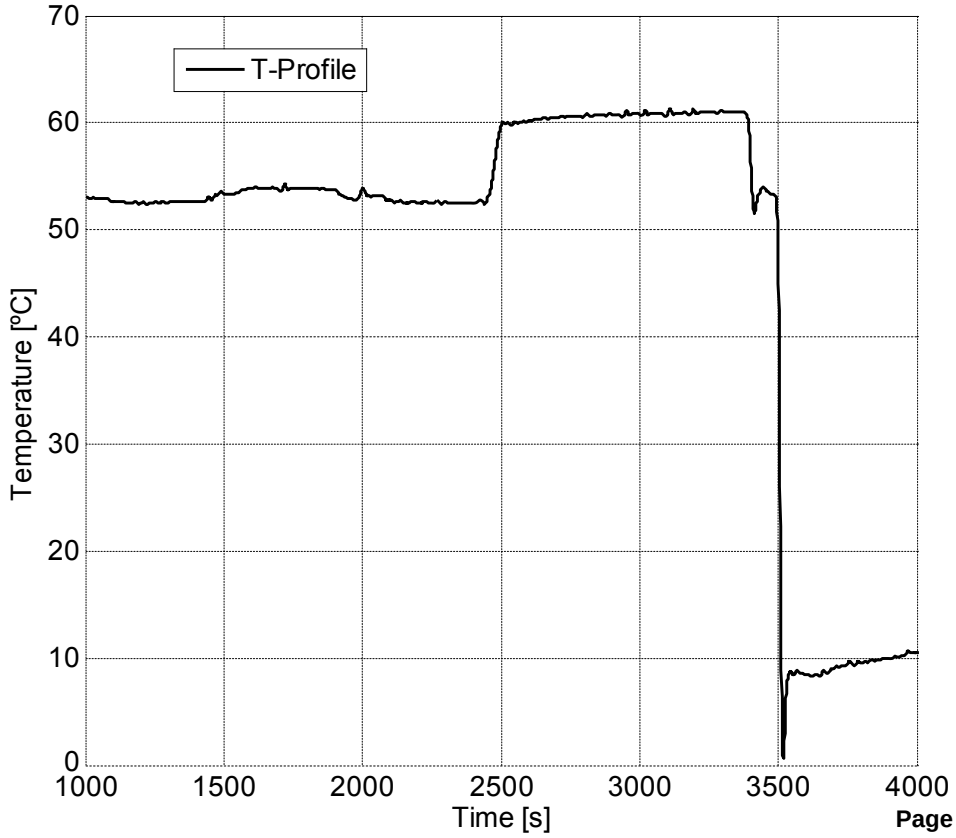
Sample ID	***Category***	***Dent***	***Fat***	***Wt***	***pH***
4	YG Grass	2	10	258.4	5.65
6	YP Supergrass	3	12	310.0	5.61
7	YG Grass	2	20	331.6	5.70
8	S-Jap Ox	8	10	391.8	5.70
10	YG Grass	2	10	304.6	5.72
11	YG Grass	2	25	327.8	5.64

Appendix 3

Temperature profile during the 200 MPa, 5 minute HPP cycle, with preheating at 50°C (Trial 1).

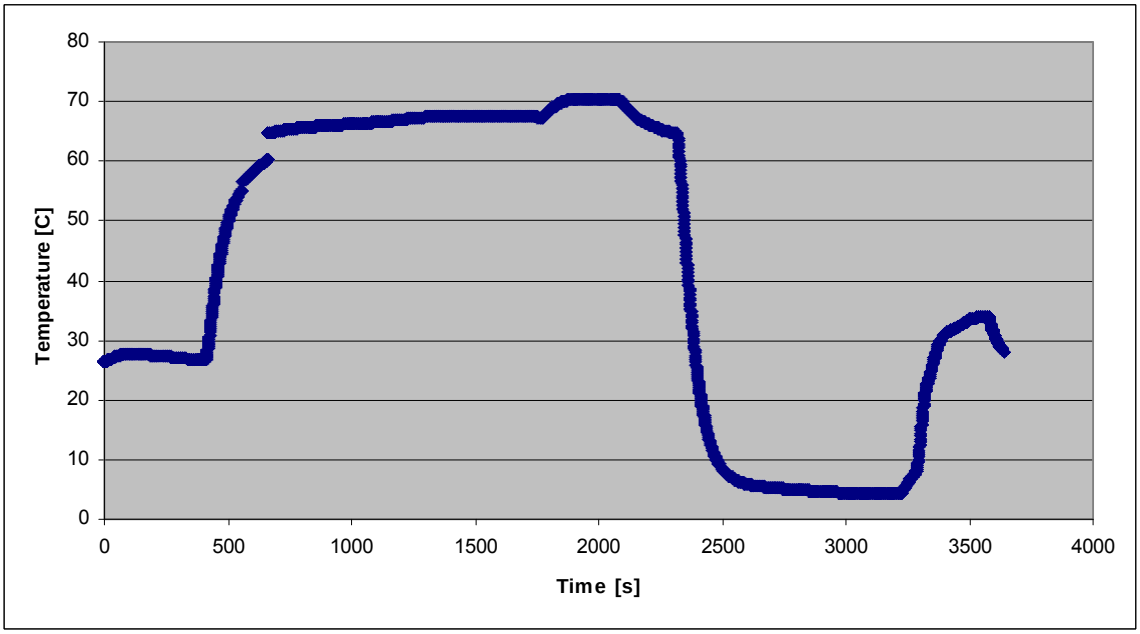


Temperature profile during the 200 MPa, 15 minute HPP cycle, with preheating at 50°C (Trial 1).

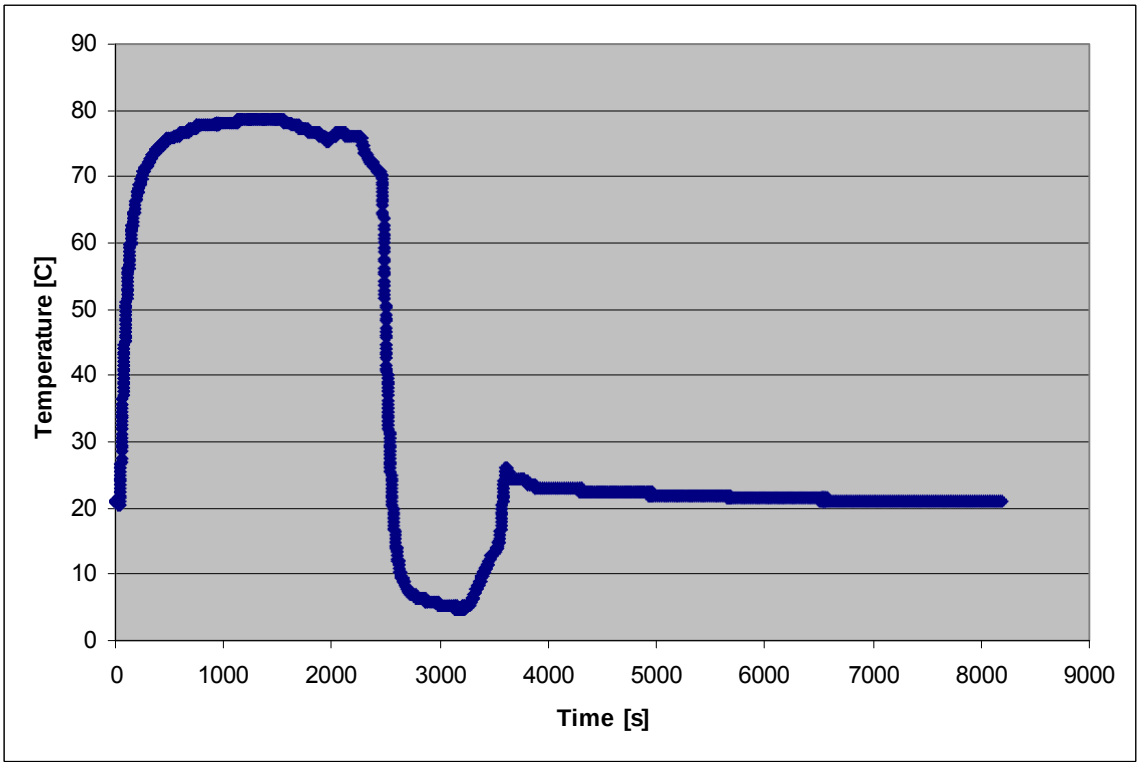


Appendix 4

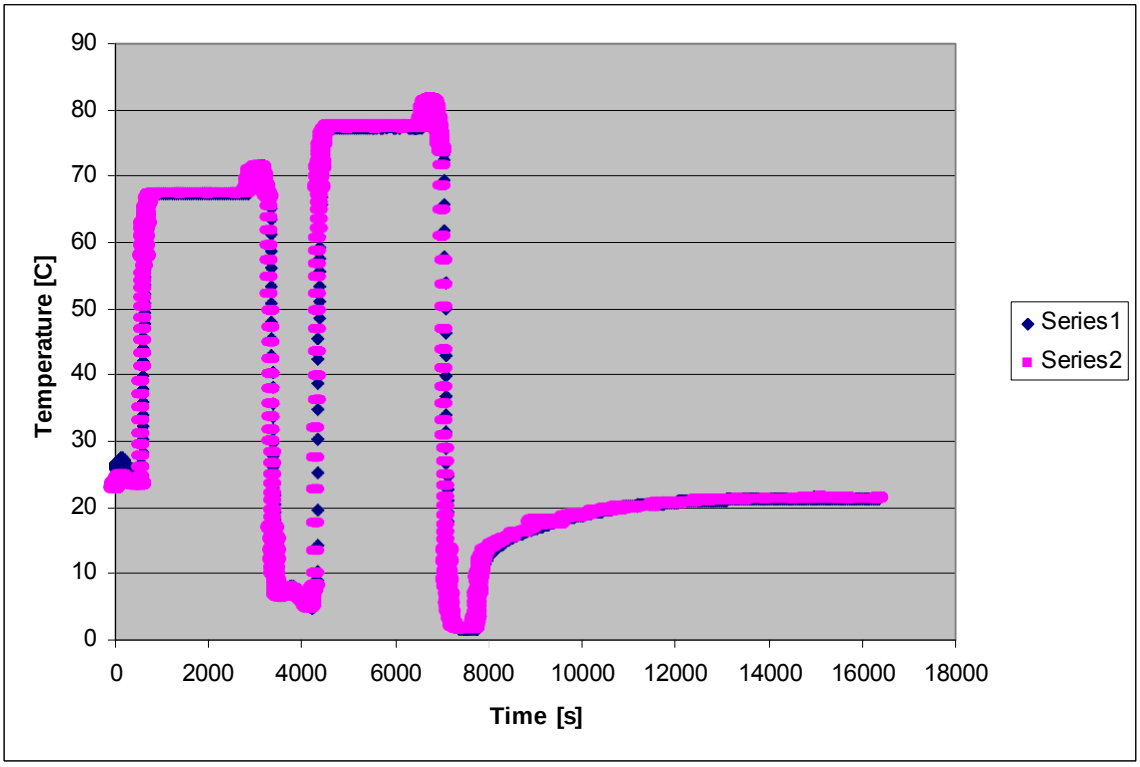
Temperature profile for the HPP cycle, 100 MPa / 5 min / 70°C (Trial 2, Nov 15)



Temperature profile for the HPP cycle, 100 MPa / 5 min / 80°C (Trial 2, Nov 15)

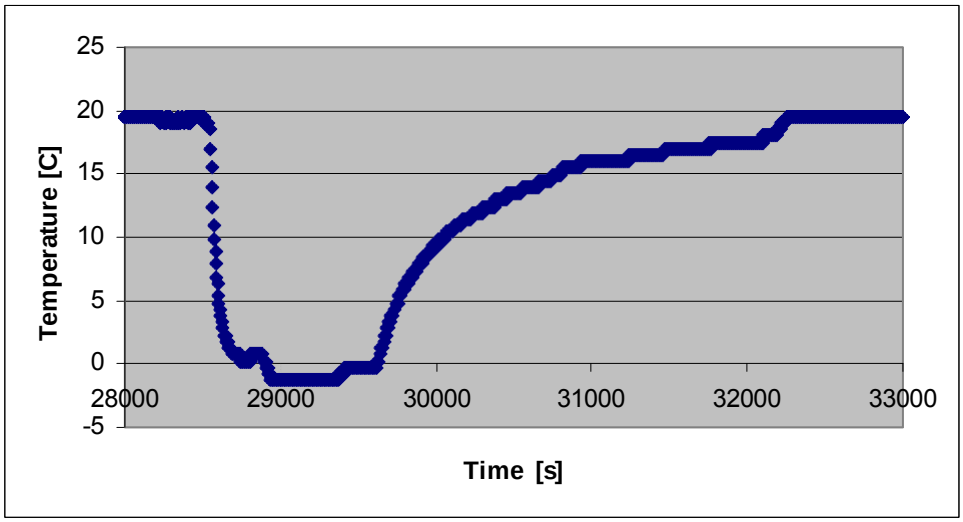


Temperature profiles for HPP cycles, 100 MPa / 5 min / 70 and 80°C (Trial 2, Nov 17)
Two thermo-eggs were recorded for temperature (Series 1 and 2)



Appendix 5

Temperature profile for pressure at low temperature (450 MPa, 5 min, 4°C) (Trial 2, Nov 16)



At temperatures below 4°C, the temperature where water has its highest density at ambient pressures, the thermal expansion coefficient (α) shows negative values. According to equation 1, also the compression heating coefficient (k_C) is negative at these temperatures.

$$k_C = \frac{\alpha}{\rho \cdot C_P} \qquad (1)$$

With r is the density of water (in kg/m^3) and C_P the specific heat capacity (in J/(kg*K)).

Equation 2 describes the change in temperature with changes in pressure:

$$\frac{dT}{dP} = k_C \cdot T \qquad (2)$$

With T is the temperature (in K) and P the pressure (in Pa)

As can be seen from equation 2, negative values for k_C lead to a decrease in temperature with increasing pressure, also referred to as compression cooling. This effect was observed during the last run on 16 November, where the samples and the Thermo-Egg were placed in ice water. The temperature prior to compression was clearly below 4°C; hence, compression cooling was expected to occur. However, temperatures below 0°C can not be explained by the compression cooling alone. Following equation 2, temperatures should only decrease for pressures below 30 MPa. The further decrease to temperatures below 0°C is likely caused by the energy needed for temporary melting of the ice under pressure and possibly changes in ice crystal structure, which is an endothermic reaction.

Appendix 6

Raw data collected from Trial 1, pressure-heat-time combinations applied to brisket (PP), chuck (SV), topside cap (GR) and neck muscle (TR).

Sample	*Pre-heat*	*Pressure (MPa)*	*Target Temp (°C)*	*Time (min)*	*TL (%)*	*CL (%)*	*Yield (%)*	*PF (N)*	*IY (N)*	*PF-IY (N)*
3PPA1	50	200	58	5			23.90			
3PPA2	50	200	58	5			24.36			
3PPA3	50	200	58	5			24.89			
3PPA4	50	200	58	5			24.20			
3PPA5	50	200	58	5	15.47	33.28	43.60	61.86	24.56	37.30
3PPA6	50	200	58	5	15.10	33.95	43.92	58.50	26.05	32.45
3PPA7	50	200	58	5	17.41	31.87	43.73	67.33	23.50	43.83
3PPA8										
3PPA9	50	200	58	15			21.74			
3PPB1	50	200	58	15			28.98			
3PPB2	50	200	58	15			24.93			
3PPB3	50	200	58	15			32.52			
3PPB4										
3PPB5	50	200	58	15	16.51	35.13	45.84	66.77	20.42	46.35
3PPB6	50	200	58	15	18.03	35.04	46.75	54.74	18.26	36.48
3PPB7	50	200	58	15	15.96	35.38	45.69	65.50	19.80	45.70
3PPB8	Control				6.08	40.83	44.43	89.23	39.48	49.75
1PPB2	None	200	60 (Fill)	15			15.49			
2PPB1	50	400	65	5			19.06			
2PPB2	50	400	65	5			23.01			
2PPB6	50	400	65	5			23.10			
2PPB7	Control						14.74			

Sample	*Pre-heat*	*Pressure (MPa)*	*Target Temp (°C)*	*Time (min)*	*TL (%)*	*CL (%)*	*Yield (%)*	*PF (N)*	*IY (N)*	*PF-IY (N)*
2PPB3	50	400	65	5	21.43	33.44	47.70	71.81	36.68	35.12
2PPB4	50	400	65	5	19.67	34.61	47.47	71.65	26.66	45.00
2PPB5	50	400	65	5	18.72	33.45	45.91	62.82	25.61	37.22
2PPA1	50	400	65	15			26.82			
2PPA5	Control						42.59			
2PPB8	50	400	65	15			24.45			
2PPB9	50	400	65	15			25.79			
2PPA2	50	400	65	15	20.91	35.50	48.99	78.23	32.06	46.17
2PPA3	50	400	65	15	19.84	34.54	47.53	64.65	22.88	41.78
2PPA4	50	400	65	15	19.95	33.93	47.11	50.78	23.39	27.39
2PPA6	50	600	73	5			34.64			
1PPA1	50	600	73	5			46.06			
1PPA2	50	600	73	5			34.83			
1PPA3	Control						41.72			
2PPA7	50	600	73	5	17.81	35.58	47.05	78.48	33.39	45.10
2PPA8	50	600	73	5	16.06	36.74	46.89	79.04	34.17	44.87
2PPA9	50	600	73	5	16.35	33.02	43.97	75.49	29.81	45.68
1PPA4	50	600	73	15			43.07			
1PPA5	50	600	73	15			47.80			
1PPA6	50	600	73	15			29.52			
1PPB1	Control						47.91			
1PPA7	50	600	73	15	20.81	37.20	50.27	103.25	32.02	71.22
1PPA8	50	600	73	15	24.15	33.96	49.91	85.03	32.58	52.46
1PPA9	50	600	73	15	26.15	33.19	50.67	75.72	40.69	35.03

Sample	*Pre-heat*	*Pressure (MPa)*	*Target Temp (°C)*	*Time (min)*	*TL (%)*	*CL (%)*	*Yield (%)*	*PF (N)*	*IY (N)*	*PF-IY (N)*
SV1B2	50	200	58	15			12.60			
SV2A2	50	200	58	15			17.02			
SV2B4	Control						5.63			
SV3A2	50	200	58	15	19.95	28.92	43.10	44.94	25.83	19.11
SV3B2	50	200	58	15	20.65	29.39	43.97	46.24	25.36	20.89
SV1A4	Control	200	58	15	5.23	36.72	40.03	57.55	44.37	13.18
GR3A2	50	200	58	15			3.70			
GR3B1	50	200	58	15			13.91			
GR2B3	Control						6.87			
GR1B1	50	200	58	15	17.54	27.74	40.41	29.16	20.46	8.69
GR2B2	50	200	58	15	14.13	30.59	40.39	26.57	16.96	9.61
GR1A1	Control				4.09	39.60	42.07	47.50	40.19	7.31
TR10B	50	200	58	15	6.90	25.74	30.87	57.19	36.67	20.52
TR6B	50	200	58	15	9.00	25.35	32.07	57.05	39.78	17.27
TR7A	50	200	58	15	8.01	24.85	30.87	54.08	41.97	12.11
TR7B	50	200	58	15	8.04	26.17	32.11	44.07	40.53	3.54
TR10A	Control				2.53	26.62	28.48	58.84	45.96	12.87
TR4A	Control				3.40	27.41	29.88	67.07	36.57	30.50
TR4B	Control				3.70	31.12	33.67	65.68	37.02	28.67
TR6A	Control				2.53	29.53	31.31	67.06	44.55	22.51
SV3A1	50	600	73	15			19.44			
SV3B3	50	600	73	15			18.89			
SV1A1	Control						8.00			

Sample	*Pre-heat*	*Pressure (MPa)*	*Target Temp (°C)*	*Time (min)*	*TL (%)*	*CL (%)*	*Yield (%)*	*PF (N)*	*IY (N)*	*PF-IY (N)*
SV1A2	Control				4.37	37.43	40.17	64.88	43.94	20.95
SV2A1	50	600	73	15	20.10	31.61	45.36	63.41	39.44	23.97
SV2B2	50	600	73	15	18.57	34.17	46.40	49.10	22.56	26.54
GR1A3	Control						6.04			
GR2A3	50	600	73	15			13.78			
GR3A3	50	600	73	15			4.36			
GR2A1	50	600	73	15	18.32	31.80	44.29	44.71	27.34	17.38
GR3B3	50	600	73	15	25.15	30.98	48.34	36.70	24.83	11.87
GR1A4	Control				5.20	37.88	41.11	53.75	45.09	8.67
TR11A	50	600	73	15	10.31	32.63	39.58	53.47	42.00	11.47
TR11B	50	600	73	15	8.88	33.68	39.56	46.76	39.67	7.09
TR8A	50	600	73	15	5.01	26.90	30.56	52.06	38.45	13.61
TR8B	50	600	73	15	4.51	27.94	31.19	58.51	33.75	24.76

Yield = Losses due to treatment, TL (i.e. HPP or heating) AND cooking, CL (i.e. frying or cooking at 80°C)
Samples analysed in Brisbane/Werribee for texture (Warner-Bratzler shear force, WB)
Treatment done in non-insulated basket, with no pre-heating, to achieve similar conditions to small unit trials
Samples cooked in waterbath at 80°C for 1 h

Appendix 6

Raw data collected from Trial 1 on brisket bought from local butcher, incubated with a brine/actinidin mixture, pre-heated at 50°C for 7 min and treated with pressure at 200 MPa for 15 min.

Sample	*Enz Hold (h)*	*Pre-heat temp (°C for 7 min)*	*Pressure (MPa, 15 min)*	*Total Loss (%)*	*PF (N)*	*IY (N)*	*PF-IY (N)*	*Sensory*	*L*	*a*	*b*
CFD03	1	50	200	26.48				4	56.64	17.26	8.98
CFD04	1	Control	Control	23.23				4			
CFD06	16	50	200	25.14				5	55.08	15.88	7.29
CFD07	16	50	200	20.86							
CFD08	16	Control	Control	21.02							
CFD10	16	50	200	34.73	48.69	19.41	29.28				
CFD11	16	50	200	33.96	50.88	18.69	32.18				
CFD12	16	50	200	33.88	34.73	19.02	15.71				

Samples cooked at 80°C for 30 minutes for WB measurement