SHELF LIFE EXTENSION OF FRESH MEAT USING HIGH PRESSURE PROCESSING – FINAL REPORT

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1.0 EXECUTIVE SUMMARY

The aim of this project was to investigate the limits of high pressure processing (HPP) that can be applied to fresh meat for maintaining 'acceptable' colour with no detrimental effects on other eating quality parameters, and to determine the impact of this pressure treatment on shelf life. The project consisted of four stages: (1) defining the pressure limits for colour stability and indicative shelf life; (2) verifying the optimum HPP conditions at pilot scale; (3) assessment of the shelf life stability of pressure-treated meat at two storage temperatures; and (4) investigation of the impact of HPP on shelf life and meat quality of sliced, steak portions.

Initially, the limits of pressure to achieve colour stability were investigated on smaller portions (~30x30x120 mm and ~75 g) of beef striploin. High pressure (0.1–600 MPa) was applied at ambient temperature for 2 and 5 min. The pressure threshold for producing an 'acceptable' meat colour was between 200–300 MPa. This range of pressure was identified from colorimetric measurements, which showed that pressures above 300 MPa resulted in striploin muscle that was significantly lighter/paler and less red than the untreated control samples. Pressures applied between 200–300 MPa also resulted in minimal impact on drip loss and texture of cooked samples. Three pressures (200, 250 and 300 MPa) which resulted in acceptable colour stability were chosen to gather preliminary shelf life data. Data from inoculated striploin samples showed that pressures of 250 and 300 MPa resulted in a significant reduction of total viable counts (TVC) at 2 d storage compared to the control and 200 MPa samples. Increased overall growth was noted for 250 and 300 MPa after 40 d although there was no difference in the final TVC.

The pressure threshold identified on small portions of beef striploin was verified at a larger scale. Beef striploin portions (~100x120x150 mm, ~1100 g) were allocated to one of 3 pressures (0.1, 200 and 250 MPa) and 6 time points (0, 4, 8, 12, 16 and 20 weeks) and stored vacuum packed at -1°C. Visual assessment (meat colour, fat colour and overall acceptability) was conducted at each time point. HPP treatment at 250 MPa generated a lighter product, which consumers scored unfavorably and suggests that 200 MPa would be a more suitable threshold pressure for achieving a 'close to fresh' meat product. Microbiology counts remained low (< 7 $\log_{10}$ cfu/cm^2) across the storage period, up to 20 weeks. There was no difference in microbiology between treatments, but at week 4, HPP appeared to delay growth and indicates HPP treatment could be useful for striploins stored for around this timeframe and warrants further investigation. Although an increase in drip loss with pressure treatment was observed, pressure-treated striploin samples were more tender than control samples, showing that HPP did not negatively impact other meat quality attributes.

The shelf life of beef striploins over a 20 week storage period was further explored at two storage temperatures (-0.5 and 4°C). Additionally, processed samples were evaluated for eating quality by eight trained sensory panelists, based on MSA protocols. Beef striploin portions (~150x120x150 mm, ~1600 g) were allocated to a total of 24 treatment combinations: two pressures (0.1 and 200 MPa), 2 storage temperatures (-0.5 and 4°C) and six storage times (0, 4, 8, 12, 16, 20 weeks). No difference between controls and pressure-treated samples was observed in visual appearance by consumers. However, colorimetric data indicated that applied pressure resulted in increased lightness and yellowness values. Trained sensory panelists assessed pressure-treated samples as more tender, with a higher flavour and overall liking compared to the control samples. Treatment of striploin portions

with 200 MPa of pressure for 5 min did not adversely affect the microbial count, and storage at -0.5°C resulted in a significantly lower microbial count over 20 weeks storage than storage at 4 °C.

The extension of shelf life of a retail product such as sliced steak portions would benefit processors through increased production and processing efficiencies. Vacuum-packed beef striploin primals (n=20) were stored at -0.5°C for 14 d. On opening of packs, eight steaks were sliced from each primal, individually vacuum-packed and allocated to one of two pressure treatments (0.1 and 200 MPa, 5 min, ambient) and four storage time points (0, 7, 14 and 21 d at -0.5°C) (n=10). At each storage time point, steaks were removed from vacuum packs, over-wrapped on black foam trays, and put on retail display for up to 7 d. During the retail display, colour measurements and photographs were taken daily, and visual evaluation was conducted at 0, 4 and 7 d. High pressure applied at 200 MPa had no detrimental effect on microbial counts compared to untreated steaks and suggests that under the processing conditions used in this study, steaks may be stored for up to 7 d post-HPP treatment before retail display. Also, visual assessment scores by consumers showed no differences between untreated and pressure-treated steaks, and there was minimal impact on other quality attributes, such as texture and cook loss. However, objective colour measurements indicate that high pressure increased lightness, redness, yellowness and chroma values. In addition, longer storage (7, 14 and 21 d) of steaks at -0.5°C prior to retail display resulted in faster deterioration of colour attributes; 2–3 d retail display compared to 5 d retail display for steaks not stored after high pressure treatment.

Although HPP is an established processing technology in the food industry for the shelf life extension of many products, including ready-to-eat meat products, the results from this project indicate that the pressure optimum/threshold (200 MPa) required to maintain a 'close to fresh' meat product does not significantly impact on the microbial populations to provide a beneficial extension of shelf life of fresh meat primals or sliced steak portions, over and above traditionally aged meat. However, there was minimal impact on other meat quality attributes such as moisture loss and tenderness, using these HPP conditions.

The increase in lightness of muscle that occurred at pressures of 250–300 MPa, combined with no change in redness or yellowness, indicates that non-pigment related changes contributed to the colour stability of muscle. The potential therefore exists to use HPP as an intervention to improve the colour of dark-cutting meat.

Consumer assessment of samples showed that consumers could not visually differentiate between control and pressure-treated samples at any storage point, and a trained sensory panel assessed pressure-treated samples as having an improved eating quality – more tender, better flavour and a higher overall liking – compared to untreated control samples. So, although these HPP conditions had no effect on shelf life or objective texture, the eating quality was improved. This data supports consumer perceptions and likely acceptance of HPP as a food processing technology and the potential success of a pressure-treated meat product in the market place.

2.0 INTRODUCTION

High pressure processing (HPP) is now an established processing technology in the food industry for the shelf life extension of many products, including fruit juices, sauces, guacamole and dips, meal kits, seafood in their shells, and ready-to-eat meat products. HPP has been applied successfully to ready-to-eat products such as sliced ham and salami for the extension of shelf life and the destruction of pathogenic organisms without the use of further heat or preservatives. HPP for cold pasteurisation applications involves pressures of up to 600 MPa at ambient temperatures for a few minutes (Lau and Turek, 2007). However, this pressure treatment at low temperatures induces paleness and lightness in fresh meat products, which is often perceived by industry as a 'cooked-like' (browner) appearance, with the magnitude dependent on the pressure applied. This obviously limits the usefulness of HPP for the extension of shelf life of fresh meat products.

At the retail level, vacuum packaged primals and sliced steak portions are typically assigned a shelf life of 60 and 14 d at 4–5°C, respectively (discussion with retail supplier). Therefore, if a process was developed which could extend shelf life whilst maintaining colour and other eating quality parameters, this would allow companies deeper market access and penetration within the constraints of existing distribution chains. For example, the extension of shelf life of sliced steaks to 21 d could enable a processor to produce products at a central facility in Brisbane and transport to other states. The extension of the shelf life would add to the bottom line of the business through increased production and processing efficiencies, reduction in waste, and improved sustainability whilst meeting increasing consumer demands for such products.

Industry representatives have communicated interest and the value of exploring HPP (as a processing technology) to design 'close to fresh' meat products with extended shelf life and good textural, colour and sensorial attributes. HPP has become a commercial reality for the meat industry within the past twenty years and shared use of HPP equipment makes the technology accessible for small and medium enterprises.

The aim of this project was to investigate the potential of HPP as a processing technology for the extension of shelf life of fresh meat products, whilst ensuring minimal impact on appearance (colour), texture, yield and eating quality.

3.0 PROJECT OBJECTIVES

3.1 Overarching objectives of the project

- Define the limits of applied pressure to achieve a fresh meat product with 'acceptable' colour and appearance, without impacting objective texture and yield.
- Evaluate effects of 'optimum' pressures on microbiological and chemical stability during chilled storage.
- Investigate the effect of HPP for shelf life extension on different product format, e.g. primals vs steak portions.
- Assess consumer acceptance of high pressure processed fresh meat products.

3.2 Specific objectives

The specific objectives for each stage of the project are as follows:

1. Define the pressure limits for colour stability and indicative shelf life:
   (i) Define the pressure limits for colour stability of beef striploins.
   (ii) Assess the impact of high pressure on other meat quality attributes (pH, moisture loss, texture).
   (iii) Collect preliminary shelf life data.

2. Verify the optimum HPP conditions at pilot scale:
   (i) Verify the HPP conditions identified in Stage 1 for colour stability on larger portions of striploin muscle.
   (ii) Assess the impact of high pressure on shelf life (20 week storage).
   (iii) Assess the impact of high pressure on other meat quality attributes (pH, moisture loss, texture) with storage.

3. Shelf life stability of pressure-treated meat at two storage temperatures:
   (i) Conduct a large-scale trial using optimum HPP conditions identified in Stage 2 for colour stability on striploin muscle.
   (ii) Assess the impact of high pressure on shelf life (20 week storage) at two temperatures (-0.5°C and 4°C).
   (iii) Assess the impact of high pressure on other meat quality attributes (pH, moisture loss, texture, lipid stability) with storage.
   (iv) Develop and validate a consumer sensory test specific for pressure-treated whole muscle samples using a trained panel and evaluate the organoleptic properties of cooked meat samples.

4. Impact of HPP on shelf life and meat quality of sliced, steak portions:
   (i) Conduct a large-scale trial using optimum HPP conditions identified in Stages 2 and 3, for colour stability of beef striploin steaks, during chilled storage and retail display.
   (ii) Assess the impact of high pressure on the shelf life of vacuum-packed steak portions (3 week storage at -0.5°C, and retail display).
   (iii) Assess the impact of high pressure on other meat quality attributes (moisture loss, texture) with storage.

4.0 METHODOLOGY

In consultation with the AMPC and meat industry representatives, the striploin (*longissimus lumborum*), as a 'valuable' retail muscle, was selected as the muscle of choice for processing in this project. The experimental methods used for each stage are outlined more fully in Section 4.5.

4.1 Defining pressure limits for colour stability and preliminary shelf life data (Stage 1)

In the initial stage, the limits of pressure that can be applied to fresh striploin (pairs from 6 animals) for maintaining an 'acceptable' colour and appearance, without impacting objective texture and yield, was investigated, using small portions of muscle (30x30x120 mm, ~75 g portions, 26 portions per animal). Pressures up to 600 MPa were applied at ambient temperature (20–25°C) for 2 and 5 min. Colour stability was assessed using a Minolta chromameter, and pH, cook loss and objective texture measurements were recorded.

As one of the objectives of this stage was to collect preliminary shelf life data, the experimental design was separated into two distinct parts: the results from trials defining the pressure limits for colour stability informed the selection of pressures to be applied for microbiological data collection – 200, 250 and 300 MPa.

4.2 Verification of optimum HPP conditions for colour stability at pilot scale and initial shelf life studies (Stage 2)

Pressures identified in the first stage were used to verify the colour stability at a larger scale. Beef striploins (n=18) were cut into three portions (100x120x150 mm, ~1.1 kg) and allocated to one of three pressures (0.1, 200 and 250 MPa, 2 min, ambient temperature) and six time points (0, 4, 8, 12, 16 and 20 weeks) and stored vacuum packed at -1°C. Microbiological evaluation was conducted over the 20 week storage period to assess the impact of identified pressures on shelf life of beef striploin. Other meat quality attributes such as pH, yield (moisture loss) and texture (objective measurement) were also assessed.

4.3 Shelf life stability and sensory assessment of HPP fresh meat stored at two temperatures (Stage 3)

This stage of the project involved a large-scale trial and aimed to systematically evaluate the impact of three main effects: pressure treatment (0.1 MPa and 200 MPa); storage temperature (-0.5°C and 4°C); and storage period (0, 4, 8, 12, 16, 20 weeks), on individual meat attributes. The aim was to keep the sample size (dimensions) as large as practical which resulted in 4 striploin portions (150x120x150 mm, ~1.6 kg) per animal (paired striploins). These constraints made the application of the same animals across all six time points impossible. As a result, we chose to randomise the same animals across four time points so that: 1) the effects of different animals used for different pressure and temperature treatments would be minimised; and 2) the same animal samples across time points provided the crucial linking information which allowed the comparison of the results across different time points with high confidence. In total, 24 animals were used in the experiment. For each given combination (i.e. treatment/temperature/time, 24 combinations), there were four animals. A total of 96 samples were obtained for the analysis. The experimental design is shown in Table 1.

Table 1: Detailed experimental design highlighting the sample allocation (animal number) to treatment combinations.

Pressure		0.1 MPa		200 MPa	
Temperature		-0.5°C	4°C	-0.5°C	4°C
Storage week	0	1–4	5–8	9–12	13–16
	4	5–8	9–12	1–4	17–20
	8	9–12	1–4	13–16	5–8
	12	13–16	21–24	5–8	17–20
	16	17–20	21–24	21–24	1–4
	20	21–24	13–16	17–20	9–12

4.4 Impact of HPP on sliced, steak portions (Stage 4)

The first three experimental stages of this project focused on developing a process for the extension of shelf life of primals or smaller primal portions whilst maintaining a 'fresh-like' appearance. At a retail level, a process for extending the shelf life of sliced steaks would greatly benefit the supply chain, as often these products are prepared at a central processing plant for distribution, locally and interstate. An extension of shelf life could allow for transportation of the primals to the retail outlets and still have an acceptable shelf life.

Vacuum-packed striploin primals (pairs from 10 animals) were aged for 14 d at -0.5°C. Eight steaks (25 mm thick) were cut from each primal and randomly allocated to one of two pressure treatments (0.1 and 200 MPa) at ambient temperature for 5 min and four storage time points (0, 7, 14 and 21 d) at -0.5°C. Vacuum-packed steaks were subjected to HPP and stored at the respective storage periods. At each storage time point, analysis of meat quality attributes and microbiological assessment was conducted, as well as retail display, which is outlined in Section 4.5.10.

4.5 Experimental methods

4.5.1 Collection of muscles and sample preparation

Beef striploins (*longissimus lumborum*) were collected from a local abattoir (Queensland) at 24 h post-mortem. Muscles were randomly selected from male and female carcasses (0–4 tooth), ranging in weight from 224 to 334 kg HSCW (Hot Standard Carcass Weight). Grading data was collected for all animals. Vacuum packed primals were transported to the laboratory in insulated containers. Depending on the stage, muscles were cut into several portions and randomly allocated to treatments, vacuum packed, and stored at 5°C until processing.

4.5.2 High pressure treatment

High pressure was applied to the striploin portions within 24–48 h post-mortem. Pressure treatments were performed using either a 0.3 L capacity 850 Mini FoodLab high pressure vessel (Stansted Fluid Power Ltd., Stansted, UK) or a 35 L unit (Flow Pressure System QUINTUS®, Food Press Type 35L-600 sterilisation machine, Avure Technologies, Kent, WA, USA). Selected pressures for each experimental stage were applied at ambient temperature (20–25°C) for 2 or 5 min. After HPP, samples were immediately cooled in an ice slurry for 5 min and stored at 5°C until required for measurements.

4.5.3 Colour measurements

Minolta chromameter

A Minolta chromameter CR-400 (Minolta Co., Ltd., Japan) was used for colorimetric measurements on small muscle portions (Stage 1), using illuminant D65 and an 8 mm diameter aperture. Measurements were taken before and after HPP. The instrument was calibrated using a standard white calibration tile and triplicate colour measurements were taken on the cut end of each sample after blooming for at least 60 min at 5°C. The surface colour (L*, a*, b*) was recorded for each sample.

Hunterlab colorimeter

For the shelf life and steak portion trials (Stages 2, 3 and 4), triplicate colour measurements (L*, a*, b*) and % reflectance at each wavelength from 400–700 nm were measured on the cut surface of either the sample portions or the inside cut surface of 20–25 mm steaks. Samples were bloomed for 60 ± 10 min at 10°C prior to measurement using a Hunterlab Miniscan EZ (light source A, observer angle 10°, aperture size 5 cm). The instrument was calibrated at the same temperature as measurement, using white and black calibration tiles (Novasys Group Pty Ltd, Ferntree Gully, VIC, Australia). Colour parameters for hue = [arctangent (b*/a*)] and chroma = $(a^{*2} + b^{*2})^{½}$ were calculated. In addition, based on AMSA (2012) and Krzywicki (1979), the relative proportions of each myoglobin form (MMb – metmyoglobin; DMb – deoxymyoglobin; OMb – oxymyoglobin) were calculated as follows:

Reflectance (R) was converted to reflex attenuance (*A*) using equation 1:

- Equation 1: $A = \log 1/R$

where R was the reflectance at a specific wavelength expressed as a decimal fraction.

- Equation 2: % MMb = (1.395 – ((*A*572 – *A*700)/(*A*525 – *A*700))) * 100
- Equation 3: % DMb = (2.375 * (1-((*A*473 – *A*700)/(*A*525 – *A*700)))) *100
- Equation 4: % OMb = 100 – (%MMb + %DMb)

4.5.4 Microbiological testing

The preliminary indicative microbiological data in Stage 2 was collected by preparing a typical beef bacterial inoculum and incubating on individual beef samples. These methods are outlined below.

Preparation of bacteria for beef sample inoculation

A bacterial cocktail was prepared by repeatedly sampling the lean and adipose surfaces of beef striploins and cube rolls that had been aged for 20 weeks at -0.5±1°C. Samples were diluted 1:10

(cm^2/vol) in sterile 0.85% saline and subsequently stomached for 30 s. The resulting suspensions were filtered using a Bagfilter P bag (Interscience) and combined. The bacterial cocktail was then diluted in sterile 0.85% saline to achieve approximately 6.00 $\log_{10}$ cfu/mL in a final volume of 1 L.

Beef sample inoculation

The beef samples were individually painted with the inoculum to achieve approximately 3.00 to 3.50 $\log_{10}$ cfu/cm^2 on the surface. Samples were individually vacuum packed before treatment at 200, 250 and 300 MPa pressure. At day 0, before HPP treatment, six control samples were tested. At day 2 and day 40, five samples from each HPP treatment group and six samples from non-treated controls were tested for total viable count (TVC). Cores were taken from two different surfaces (2x5 cm^2) and stomached for 1 min in 50 ml of 0.85 % saline. The sample was then plated on Petrifilm total viable count plates and incubated at 25 °C for 4 d before being counted and recorded.

Microbial contamination on the external surface of striploin portions and individual steak samples from Stages 2, 3 and 4 was determined as follows. Four × 10 cm^2 surface slices comprised of two from each side of the steak samples; for striploin portions, this consisted of two subcutaneous fat and two lean portions of meat. Surface slices from the same sample were combined with 100 mL of 0.85% saline in sterile bags and stomached for 1 min. An aliquot of each sample was decimally diluted in 0.85% saline and plated onto Petrifilm Aerobic Count (AC) plates (3M Microbiology, Minnesota, USA) for determining total viable counts (TVC) and *E. coli*/Coliform (EC) Count plates (3M) for determining *E. coli*/Coliform counts. Parallel dilutions were also prepared in de Man, Rogosa, Sharpe (MRS) broth (Thermo Fisher, Australia) and plated onto AC plates for enumerating lactic acid bacteria (LAB). AC plates were incubated at 25°C ± 1°C for 96 ± 3 h; EC plates were incubated at 37°C ± 1°C for 48 ± 2 h and LAB plates were incubated anaerobically at 25°C ± 1°C for 120 ± 3 h. Samples with counts of zero (no colony forming units) were arbitrarily assigned a value of half the limit of detection (i.e. 0.1 $\log_{10}$ cfu/cm^2 for TVC and EC and 1.10 $\log_{10}$ cfu/cm^2 for LAB).

4.5.5 Cook loss and texture measurements

Cook loss was determined by weighing the samples before and after cooking (75°C for 30 min to an internal temperature of 72°C). Cook loss was expressed as the initial weight minus the final weight and presented as a percentage of the initial weight.

Texture measurements were carried out using a Lloyd LS 2.5 with a 500 N load cell (Lloyd Instruments, West Sussex, United Kingdom) and a modified Warner-Bratzler shear device (Bouton and Harris, 1972, Bouton et al., 1971). The samples were cut into rectangular shapes with dimensions of 15 mm width, 6.7 mm height, giving a cross-sectional area of 1 cm^2, and at least 25 mm long to enable secure clamping of the sample into the holder. For the steak portions, sample dimensions were 10x10 mm, giving a cross-sectional area of 1 cm^2. A triangular shaped blade with a thickness of 0.64 mm was attached to an overhead clamp and was pulled up through the muscle fibres, perpendicular to the fibre direction, at a speed of 100 mm/min. The maximum peak force (PF) and initial yield (IY) were objectively determined using Nexygen Plus V3.0 software (Lloyd Instruments, West Sussex, United Kingdom). The difference between these measurements (PF-IY) was also reported. At least six measurements were made on each sample and the mean recorded.

4.5.6 pH measurement

The pH was measured on control and pressure-treated samples before and after treatment, and at each storage point, using a TPS WP-80 pH meter with a polypropylene spear-type gel electrode (IJ 44) and temperature probe (TPS Pty Ltd, Brisbane, QLD, Australia). Calibration was conducted using pH 4 and pH 7 buffers equilibrated at a similar temperature to the sample.

4.5.7 Consumer visual evaluation

Visual assessment of the meat was conducted at each storage time point. A panel of 5–10 consumers assessed steaks cuts from larger sample portions (Stages 2 and 3) and on retail display ready steaks at 0, 4 or 7 d in the cabinet (Stage 4). Consumers rated appearance of meat on a scale of 1 (extremely dislike) to 10 (extremely like) for meat colour, fat colour and overall acceptability, and also on the purchase intent of these products. The form used for visual assessment is shown in Appendix 1.

4.5.8 Sensory assessment

Steaks (n=4) of approximately 25 mm thickness were cut from frozen striploin portions, individually vacuum packed and stored frozen (-20 °C) until sensory testing (Stage 4). For food safety reasons, samples with high (>7.00 $\log_{10}$ cfu/cm^2) microbiological counts were excluded from the sensory assessment: this included all samples stored at 4°C, except for the week 0 samples; and all week 20 samples stored at -0.5°C. For other storage times at -0.5°C, the number of replicates in each treatment group ranged between two to four samples. Therefore, a total of 44 samples were subjected to sensory assessment. On the day before testing, samples were thawed overnight on trays at 4°C. The eating quality of the steaks was determined by eight trained sensory panelists (Queensland Department of Agriculture & Fisheries), based on MSA protocols. The panelists consisted of five females (40–60 years old) and three males (31–61+ years old).

Four steaks were grilled (220°C, 2 min) in a clamshell grill (Silex GTT20, Germany) with the lid closed. Steaks were cut in half and the two pieces were placed on two separate plates, labelled with a 3-digit code, and served to panelists according to a randomized serving order. Panelists were instructed to consume a minimum of one to two mouthfuls of each sample, from a similar part of the steak. Panelists assessed tenderness (*not tender* to *very tender*), juiciness (*not juicy* to *very juicy*), flavour liking (*dislike extremely* to *like extremely*) and overall liking (*dislike extremely* to *like extremely*) using a 100 mm line scale on a printed paper ballot. The quality of each product was also rated, from *unsatisfactory*, *good everyday*, *better than everyday* or *premium quality*. The sensory questionnaire is given in Appendix 2. Eleven samples were assessed by each panelist each day over four separate days. Carbonated water and plain crackers were provided as palette cleansers between samples.

4.5.9 Lipid stability

Lipid stability was determined on shelf life samples at each storage point (Stage 3) by the thiobarbituric acid reactive substances (TBARs) assay as per Witte et al. (1970), with modifications. Samples of diced striploin were ground in an Oskar food processor and 2.00 ± 0.01 g were weighed into scintillation vials and capped. Samples were cooked in a 75°C water bath for 20 min and allowed to cool at 5°C for 30 min before extraction. Samples were homogenized on ice in 6 mL of chilled TCA solution (7.5% trichloroacetic acid, 0.1% propyl gallate and 0.1% EDTA) using an Ultra Turrax for 30 s at 13 000 rpm. The homogenate was filtered and rinsed with an additional 2 mL of TCA solution. Aliquots (2.5 mL) of

the filtrate were transferred, in duplicate, to test tubes, diluted with 2.5 mL distilled water and reacted for 16 h with 5 mL of 0.02M thiobarbituric acid solution at room temperature, in a dark cupboard. The absorbance of the resulting solutions was read at 532 nm on a visible light spectrophotometer (Novaspec II) and the TBARs value as mg/kg malondialdeyde (MDA) equivalents was determined against a standard curve prepared form 1,1,3,3-tetraethoxypropane. Samples collected at each storage point were stored at -80°C before analysis.

4.5.10 Retail display of steaks

At each storage time point, ten steaks from each treatment were used for simulated retail display (Stage 4). Steaks were removed from vacuum packs and bloomed at 5°C for 60 min prior to colour measurement. Steaks were transferred onto black foam trays (205x130x12 mm), over-wrapped with Cling Wrap® and photographed using an Olympus digital SLR camera (E330), under 4 light struts angled at +/- 45° to the sample surface (1652–1820 lux).

Samples from each treatment were placed on the top, middle and bottom shelves of 2 retail display cabinets, perpendicular to the light source. The light source in the cabinets (A and B) were illuminated 24 h a day and provided by 3 light tubes (Osram Natural L 36W/76) which were on the top and sides of the interior of the cabinet. These provided the highest intensity of light on the top shelves (A:1081 lux, B: 298–835 lux), medium level of light on the middle shelf (A: 350–721 lux, B: 64–403 lux) and lowest intensity on the bottom shelves (A: 290–474 lux, B: 212–682 lux). Steaks on retail display were rotated daily between cabinets, and top, middle and bottom shelves.

Steaks were displayed for 7 d (from storage weeks 1, 2 and 3) and 11 d (from storage week 0 only) and photographs were taken daily, using the camera described above. Colour measurements were also made daily, with the over-wrap intact on the meat, using the Hunterlab colorimeter, standardised through similar over-wrap.

4.5.11 Statistical analysis

The type of statistical analysis of data used depended on each stage of the project, the experimental design, and the experimental plan. The methods for each stage are briefly outlined below.

<u>Defining pressure limits for colour stability and preliminary shelf life data (Stage 1)</u>

Data was subjected to one-way analysis (ANOVA) using Genstat 15th edition. The effects of pressure treatment (pressure) and treatment time (time), and the interactions (pressure x time) was determined, with a blocking structure of kill date/animal/process date on pH, L*, a*, b*, ΔE, drip loss, cook loss, peak force (PF), initial yield (IY) and peak force minus initial yield (PF-IY). Statistical differences between means for the microbiological testing was determined by one-way analysis of variance, Tukey's method, using Minitab18. Differences from day 2 to day 40 were compared using the 95% CI of the difference in means.

<u>Verification of optimum HPP conditions for colour stability at pilot scale and initial shelf life studies (Stage 2)</u>

For each measurement, a linear mixed model of analysis of variance (ANOVA) was applied to identify the individual sources that significantly impacted the individual trait. The statistical program SAS

(version 9.4, SAS Institute Inc., Cary, NC, USA) was used to perform the analyses. The model can be described as follows:

Trait = mean + pressure + week+ week*pressure + sex + side + cold side weight + P8 fat + ossification + animal + error

Animal variation was fitted as a random effect, and the fixed effects included the main effects of pressure treatment (three levels), sampling week (five or six sampling times), their first-order interactions, sample side, animal sex, and three covariates – cold side weight, P8 fat, and ossification. The reason for including the last three covariates in the model was to account for the initial sample variations, i.e. animal variation.

Shelf life stability and sensory assessment of HPP fresh meat stored at two temperatures (Stage 3)

Initially, the mixed model of ANOVA using the SAS Proc Mixed model (version 9.4, SAS Institute Inc., Cary, NC, USA), was applied for each trait. In each model, pressure, temperature, week, their 2-way and 3-way interactions and hot body weight were fitted as fixed effects, and animal (i.e. carcass ID) as a random effect. As expected, there was no significant animal difference that contributed to the meat attributes. Consequently, the simple linear model of ANOVA in the SAS program (Proc GLM) was applied for each trait. That is, the model consisted of fixed effects of pressure, temperature, week, and their 2- and 3-way interactions. The estimates of the predicted least squared means and the corresponding standard deviations, LSD values for three main effects and their significance levels on each meat attribute were produced.

The sensory data was transferred into Excel (Microsoft) and statistical analysis used the linear mixed model procedure in GenStat (16th Ed). Treatment (HPP or control) and Time (storage at 0, 4, 8 12 and 16 weeks) and interaction between Treatment and Time (Tr*T) were tested as fixed effects. MSA-MB (MSA marbling score) was also tested as an additional fixed effect. Carcass number and panelist number were added as random factors in the model.

Impact of HPP on sliced, steak portions (Stage 4)

A total of 19 attributes, including five meat quality, two microbiology, four visual assessment and eight colour variables, was used for the statistical analysis. For each individual attribute, a linear model of analysis of variance, using the GLM model of the SAS program (SAS Institute Inc., Cary, NC, USA, version 9.4), was applied to examine the effects of HPP treatment, storage day, interaction between pressure treatment and storage day, and the retail-display-day on the individual measurement. Since the values of retail-display-day varied among the treatment groups or storage day, to properly evaluate its effect on individual attributes, it was nested within pressure, or nested within storage day, or nested within the combination of pressure by storage day during the analyses. The predicted means and standard deviations of the means, and LSD values for the main effects, are presented. Microbial data was assessed using Minitab 18 (Minneapolis, USA). Significant differences were determined by means of one-way analysis of variance (Tukey's method).

5.0 PROJECT OUTCOMES AND DISCUSSION

5.1 Defining pressure limits for colour stability and preliminary shelf life data (Stage 1)

It is well-known and well-documented in the literature that modifications to muscle colour occur with the application of certain magnitudes of high pressure (Carlez et al., 1995b, Bak et al., 2019, Ma and Ledward, 2013). High pressure (100–600 MPa, 3°C, 5 min) applied to beef sirloin resulted in increased L* and b* values, with these authors suggesting a pressure threshold at 300 MPa and higher. There was no significant change to a* values (Csehi et al., 2016). Jung et al. (2003) applied high pressure (50–600 MPa, 10°C, 20–300 s) to beef *biceps femoris* and found that a* and ΔE increased with increasing pressure up to about 350 MPa. An increase in all colour parameters (L*, a*, b*) of beef *longissimus dorsi* was reported when pressure was applied at 80–100 MPa at ambient temperature for 20 min, with the a* value showing the largest differences (Cheah and Ledward, 1997). These authors suggested that these colour measurements reflected differences in the rate of metmyoglobin formation.

In this study, increasing pressure resulted in significant (P<0.001) increases in L* values (Figure 1) of beef striploin muscle. The L* values for the control and 100 MPa treated samples were similar (about 35); however, the L* values for all samples treated with pressures of 200 MPa and higher (about 50) were significantly (P<0.05) higher than the control. This indicated that as the pressure was increased the meat became 'lighter' in colour, which is confirmed by the images in Figure 2.

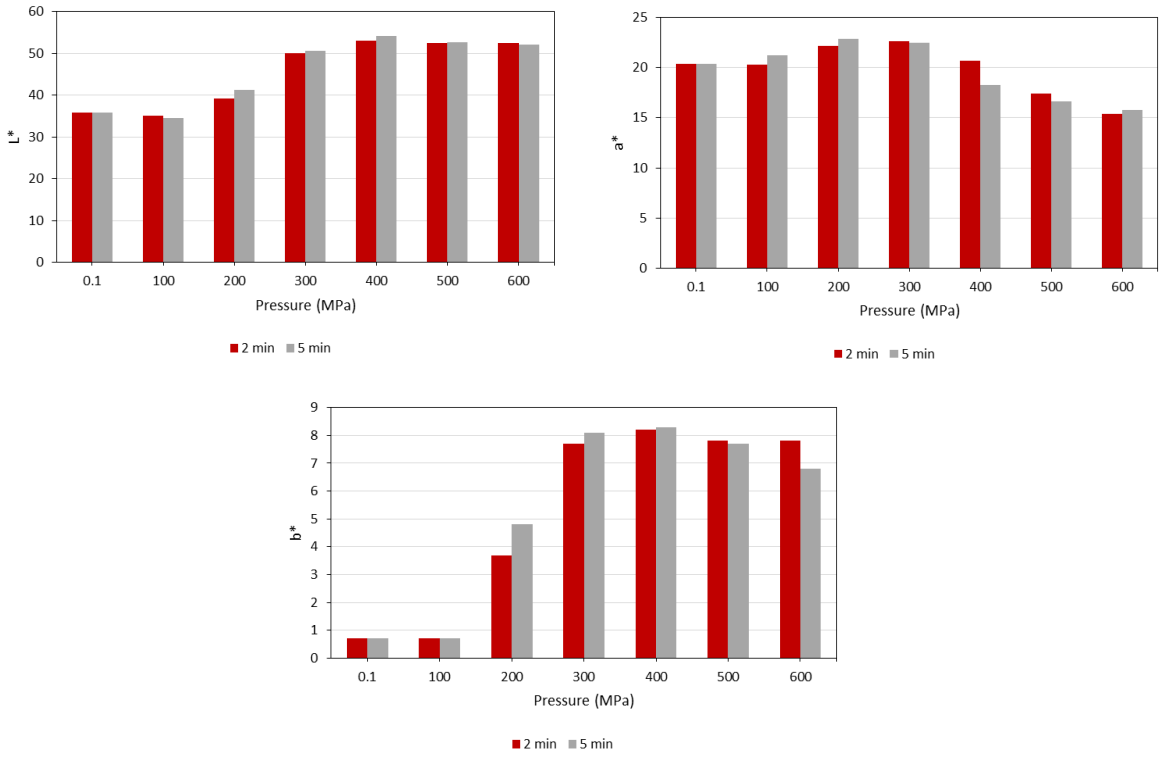

Figure 1. Colour parameters (L*, a* and b*) of beef striploin muscle after treatment (Control, 0.1 MPa vs 100, 200, 300, 400, 500, 600 MPa HPP at ambient temperature for 2 and 5 min) using a Minolta CR-400 (n=6).

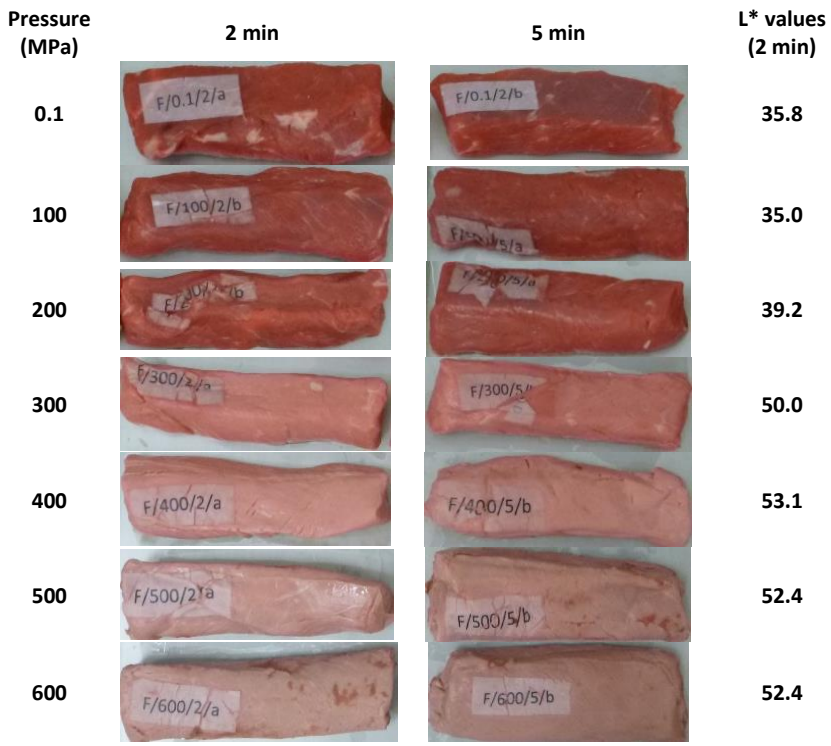

Figure 2. Representative images and L* values (2 min pressure duration) of beef longissimus lumborum after high pressure treatment at ambient temperature and 1 h bloom time – 0.1MPa (control - untreated) and pressure treated (100, 200, 300, 400, 500, 600 MPa) for 2 and 5 min.

Redness (a*) significantly (P<0.001) increased in striploin samples with the application of 200 and 300 MPa pressure and decreased (P<0.001) when treated with higher pressures of 500 and 600 MPa, compared to the control samples (Figure 1). Pressure applied at 100 MPa did not affect the b* value in striploin samples compared to the control (Figure 1). However, pressures of 200 MPa and higher significantly (P<0.001) increased b* values compared to the control, and the b* values of samples treated with pressures of 300 MPa and higher were significantly (P<0.001) higher than at 200 MPa (Figure 1). There was no difference in b* values with pressures of 300 to 600 MPa. There were no significant effects of treatment time or the interaction of pressure and treatment time on L*, a* or b* values.

From the HPP conditions used in this study, pressures above 300 MPa resulted in beef striploin muscle that was lighter/paler, and less red than untreated control samples. The threshold/optimum pressure applied to beef striploin to maintain a 'fresh-like' appearance was between 200–300 MPa. These results agree with other reports in the literature for beef muscle treated with high pressure (Csehi et al., 2016, Jung et al., 2003, Cheah and Ledward, 1997).

It must be noted, that these colour assessments are based on objective colour measurements using a Minolta colorimeter. Holman et al. (2016) reported some preliminary research in correlating objective colour assessments with consumer acceptability and found that only L* and b* values related to consumer opinions of beef acceptability and listed some minimum and maximum values for L*, a* and

b*. Subsequently, these researchers conducted further experimental work with larger numbers of replicates and suggested that redness (a* value) provided the most simple and robust prediction of beef colour acceptability and that an a* value of equal to or greater than 14.5 was considered acceptable (95% acceptance) (Holman et al., 2017). Consequently, an informal sensory assessment for colour was made for further experiments along with the objective colour measurements.

For the analysis of the indicative shelf life of HPP samples, the control samples from day 0 and day 2 were combined. The mean log_{10} cfu/cm^2 and standard deviation for each treatment group and each day is shown in Table 2. At day 2, there was a significant (P<0.01) reduction of TVC on 250 and 300 MPa treated samples compared to 0.1 and 200 MPa treated samples (Figure 3). At 40 d storage at -0.5±1°C, there was no significant (P>0.05) difference in TVC between any treatments, although the mean TVC of 250 and 300 MPa treatment groups was slightly higher than the 0.1 and 200 MPa groups (Figure 3). However, the overall growth recorded after 40 d was greater for 250 and 300 MPa (3.15 and 3.75 log_{10} cfu/cm^2) compared to 0.1 and 200 MPa (2.14 and 2.33 log_{10} cfu/cm^2) (Table 2).

Indicative microbiological testing using the methods and HPP conditions described, suggests that pressures of 250–300 MPa resulted in a significant reduction of TVC at 2 d compared to the control and 200 MPa. However, at 40 d storage, there was no difference in TVC between any of the treatments.

Table 2. Total viable count (TVC, log_{10} cfu/cm^2, mean + standard deviation) of each treatment group at day 2 and day 40 of storage. Control samples are combined for day 0 and day 2.

Pressure (MPa)	Day 2	Day 40	Difference (95 % CI)
0.1	3.34 ± 0.21	5.48 ± 0.55	2.14 (1.53 – 2.75)
200	3.31 + 0.09	5.64 ± 0.26	2.33 (2.05 – 2.61)
250	2.82 ± 0.22	5.97 ± 0.35	3.15 (2.72 – 3.58)
300	2.27 ± 0.11	6.02 ± 0.40	3.75 (3.32 – 4.18)

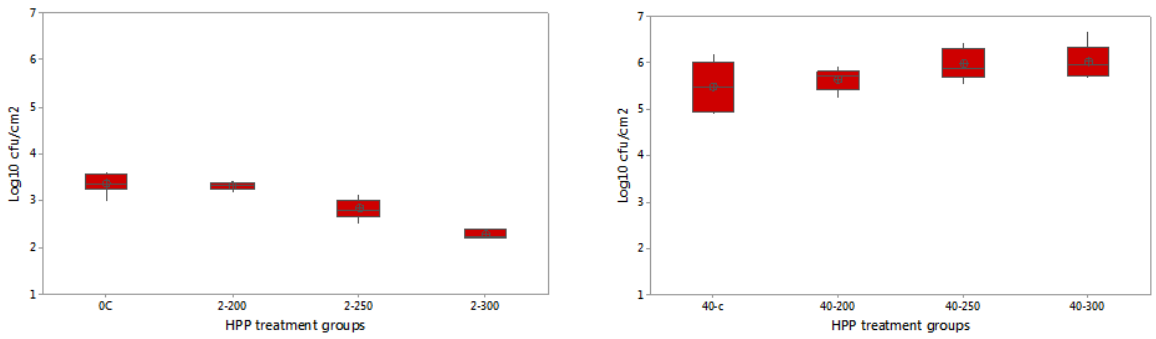

Figure 3. Boxplot of day 2 (left) and day 40 (right) from all treatment groups. The box represents 25 and 75% of the level with the median marked with a line and the mean marked with a cross and connected across treatment groups by a black line. The whiskers extend to the last data-point that is ≤ or ≥ 1.5 times the interquartile range.

In this stage of the project, other meat quality attributes such as pH, yield (moisture loss) and texture (objective measurement) were assessed to see the impact of these HPP conditions on the quality of the processed meat. The impacts are reported briefly below and for specific details on the data collected, please refer to Project 2017-1056 Milestone 2 Report.

Both pressure and treatment time significantly (P<0.001) increased the pH of the striploin samples. It is well documented that the application of pressure at ambient or chilled temperatures results in an increase in the pH of red meat, chicken and seafood (Angsupanich and Ledward, 1998, Macfarlane et al., 1980-1981, McArdle et al., 2010). This increase in pH after HPP has been attributed to a decrease in available acidic groups because of conformational changes associated with protein denaturation.

All pressures applied (100–600 MPa), irrespective of treatment time, significantly increased the drip loss in beef striploin. However, there was no difference in drip loss in samples treated with pressures in the range of 100–500 MPa but drip loss in samples treated with 600 MPa had significantly higher moisture loss than at any other pressure. There was no effect of treatment time or the interactions of pressure and time on drip loss.

Pressure only (i.e. irrespective of treatment time) had a significant (P<0.001) effect on the objective texture parameters of peak force (PF), initial yield (IY) and peak force minus initial yield (PF-IY). The peak force values of samples treated at 100 and 200 MPa were like the control sample. Pressure applied at 300 and 400 MPa resulted in peak force values lower than the control, indicating more tender meat, whereas higher peak force values (i.e. tougher meat) were measured with pressure applied at 500 and 600 MPa. The IY values followed a similar trend to the PF values and indicates that the effect on the texture of HPP meat was due to modification to the myofibrillar component of the muscle. The only significant effect on the connective tissue component of the muscle (i.e. the PF-IY value) was seen with the application of pressure at 600 MPa (P<0.001).

5.2 Verification of optimum HPP conditions for colour stability at pilot scale and initial shelf life studies (Stage 2)

The colour (L*, a* and b*) of the striploin steaks across all treatments (0.1, 200 and 250 MPa) and storage time points (0, 4, 8, 12, 16 and 20 weeks) is displayed in Figure 4.

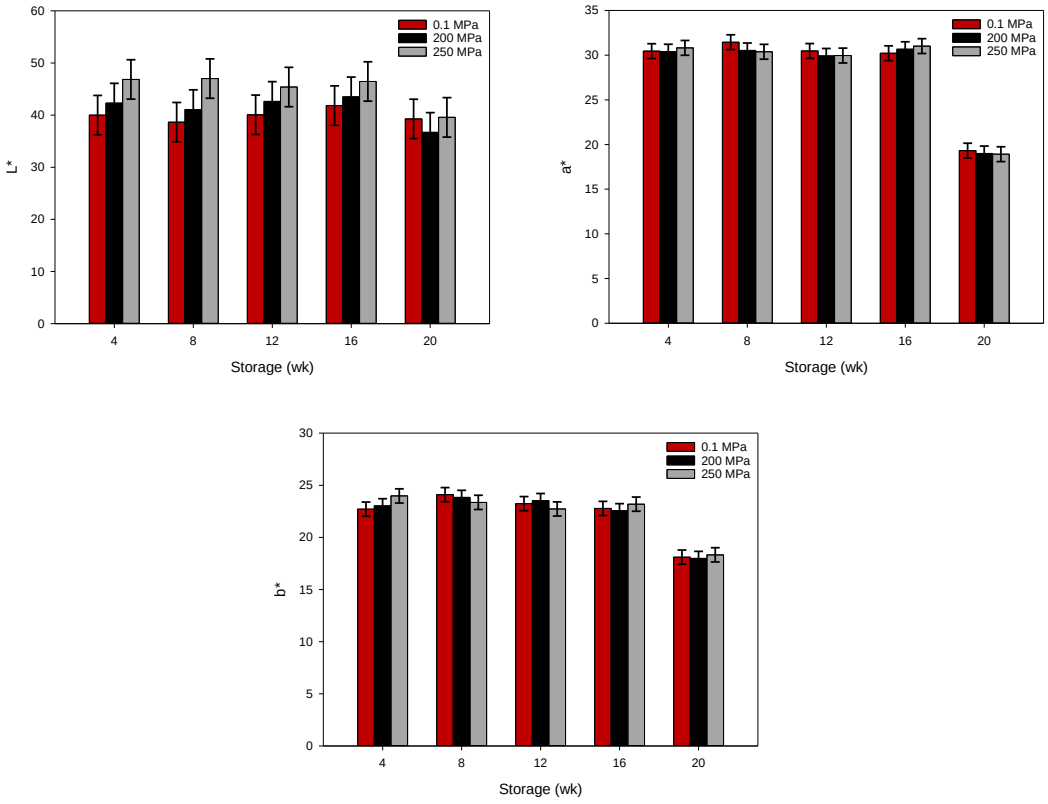

Figure 4. Effect of high pressure processing (HPP) at 0.1, 200 and 250 MPa and storage for 0, 4, 8, 12, 16 and 20 weeks at -1°C on surface colour of beef striploin muscle. Top left, lightness (L*); top right, redness (a*); and bottom, yellowness (b*).

The 250 MPa pressure-treated striploins were distinctly lighter (P<0.0001) over most of the storage weeks, especially compared to controls, with the 200 MPa treatment being intermediate between these treatments (Figure 4, top left). As this trend was not mirrored in redness or yellowness (P>0.05) (Figure 4, top right and bottom, respectively), this indicates some non-pigment colour changes occurred in the 250 MPa treated sample. This increase in lightness was likely due to structural alterations promoting light scattering within the meat and were likely induced by the HPP procedure and would be permanent, as reviewed previously by Hughes et al. (2014). As the 200 MPa treatment did not appear to show such a dramatic increase in lightness, these results indicate some threshold of lightness had been reached at 250 MPa. It is known that a 'whitening' occurs between 200–350 MPa (Carlez et al., 1995a), but from our results this effect appeared to occur between 200–250 MPa and could be related to the extended aging period. All storage time points (4 to 20 weeks) showed that the 250 MPa samples had a lighter appearance, compared to the control and 200 MPa treatment,

indicative of a threshold that likely modified the structure and promoted light scattering.

Across the storage period, samples at week 20 had a distinctly different colour and had lower scores for redness and yellowness, regardless of treatment (Figure 4, top right and bottom, with images shown in Figure 5). Between 4 to 16 weeks, the redness and yellowness were similar, but dropped at the week 20 time point. At week 20, these colour parameters were not only lower, but also more variable. It is likely some dramatic alterations, probably oxidative, to the myoglobin molecule occurred, causing this distinct difference in appearance (Bak et al., 2017). As all the treatments showed a similar trend, it seems unlikely that HPP treatment was favorable for reducing this browning.

In summary, HPP treatment at 250 MPa promoted lightness of the samples across all storage weeks, likely because of modification to structural proteins.

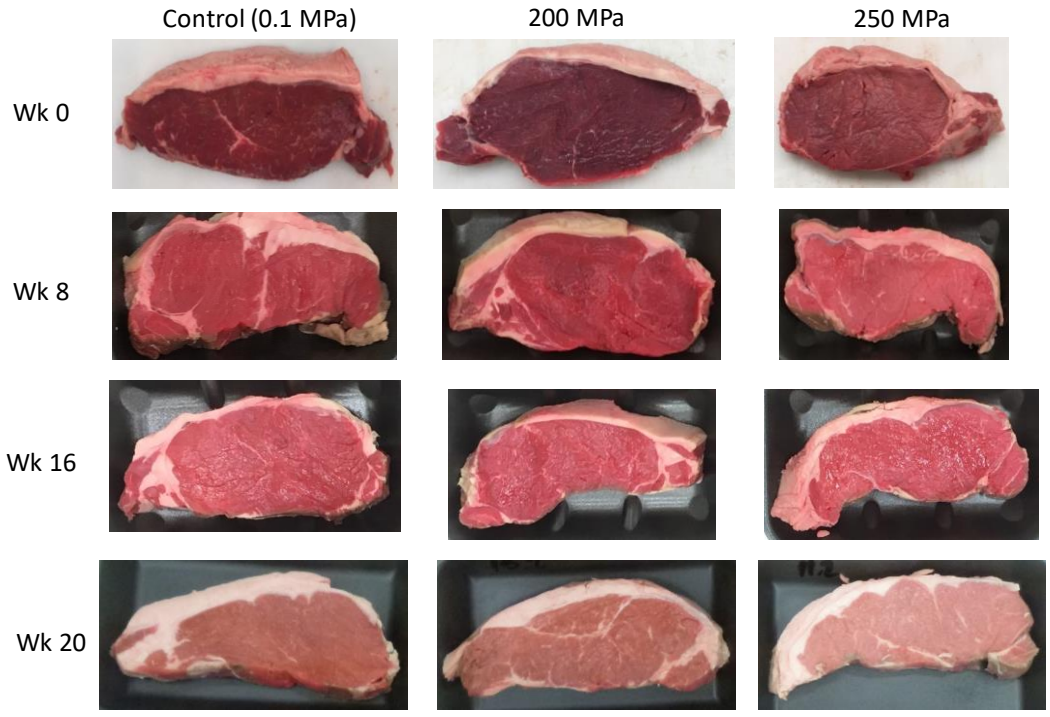

Figure 5. Representative photographs of steaks from pressure-treated (200, 250 MPa at ambient temperature for 2 min) and control (untreated) beef striploins stored at -1°C for 0, 8, 16 and 20 weeks.

It is well documented that HPP can be used for preservation and extension of shelf life of many food products (Rastogi et al., 2007, Raghubeer, 2017, Hayman et al., 2004). The inactivation of spoilage micro-organisms and food-borne pathogens has been demonstrated at higher pressure levels than used in this study, i.e. >300 MPa (Bak et al., 2012, Farkas and Hoover, 2000). The microbiology results are shown in Figure 6, and as expected, showed a marked increase in populations over the 20 week storage period (P<0.0001). As previously reported in striploins, there was an initial low count period (week 0), an exponential growth phase (0–8 weeks) and a stationary plateau (12–20 weeks), and is

consistent with previous studies at -1°C (Small et al., 2012, McPhail et al., 2012). Similarly, at 20 weeks, the LAB counts rarely progressed to 7 log_{10} cfu/cm^2 and further validates the recommendations of long aged storage of vacuum packed beef primals beyond 20 weeks (Meat and Livestock Australia, 2014). The LAB counts at 20 weeks ranged from 4.90 to 6.48 log_{10} cfu/cm^2, with one sample having a high value of 8.93 log_{10} cfu/cm^2. At 20 weeks, there was no difference between treatments (P>0.05), but overall, all except one striploin had LAB counts <6.5 log_{10} cfu/cm^2.

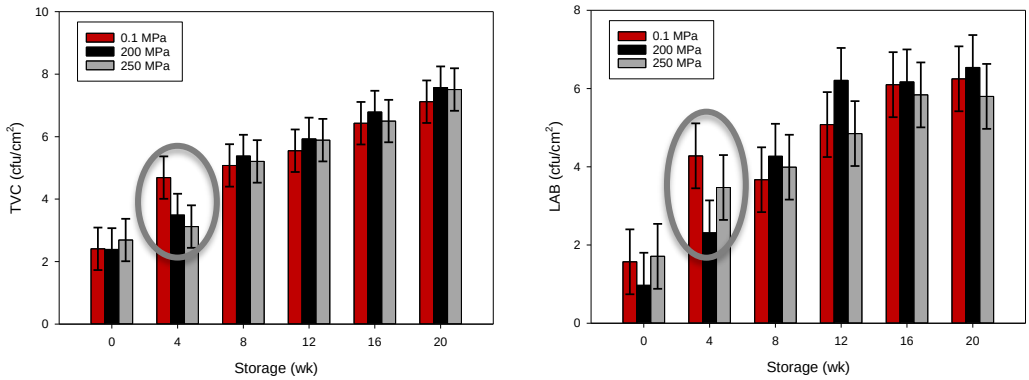

Figure 6. Effect of high pressure processing (0.1, 200 and 250 MPa) and storage (0, 4, 8, 12, 16 and 20 weeks) at -1°C on microbiology of four 10 cm^2 surface slices from beef striploins. Left, total viable counts (TVC); and right, lactic acid bacteria (LAB). Data extracted at week 4 (circled) is given in Table 3.

There were no differences (P>0.05) in either LAB or TVC populations between treatments at any storage time point, but the pressure-treated samples did have a larger variation at week 4 (circled in Figure 6). A summary of the range of values at week 4 is given in Table 3. These results indicate that storage of pressure-treated striploins for around 4 weeks may have some potential benefits to shelf life. Further investigation around this earlier time point could explore potential benefits of HPP on microbial populations.

There was no difference in the *E.coli*/coliform populations with pressure treatment or storage period, or the interaction of these (data not shown).

Table 3: Effect of high pressure processing (0.1, 200 and 250 MPa) after 4 weeks storage at -1°C on lactic acid bacteria (LAB) and total viable counts (TVC) from beef striploins (four 10 cm^2 surface slices).

Pressure (MPa)	LAB log_{10} CFU/cm^2	TVC log_{10} CFU/cm^2
0.1	3.33–5.14	3.54–5.34
200	1.10–3.54	2.00–5.34
250	1.40–3.63	1.40–3.74

Like Stage 1, other meat quality attributes were assessed. In addition, visual assessment of the meat was conducted, using a panel of 5–10 consumers. For specific details on the data collected for these attributes, please refer to Project 2017-1056 Milestone 3 Report.

As in Stage 1, both pressure and storage time significantly (P<0.0001) increased the pH of the striploin samples. The application of pressure, irrespective of storage period, significantly (P<0.0001) increased the drip loss in beef striploin portions. An increase in drip loss indicates a reduced ability of the meat to hold/bind water. Other reports have shown that at lower pressures (e.g. 200 MPa), the water-holding capacity (WHC) was not affected or was lower than untreated samples in beef striploin (Marcos et al., 2010) and beef eye round (Kim et al., 2007), respectively. Although close to significance (P=0.058), the drip loss of control (0.1 MPa) samples increased with storage time up to 12 weeks, but this trend was not apparent with the HPP samples. Cook loss was similar (P>0.05) regardless of pressure treatment. Pressure only, irrespective of storage period, had a significant effect on the objective texture parameters of peak force (PF) (P=0.0007) and initial yield (IY) (P<0.0001) of beef striploin portions. The peak force of beef striploin portions decreased with increasing pressure applied, indicating more tender meat than the control. The IY data followed a similar trend to the PF values. Storage period also had a significant effect (P<0.0001) on PF, IY and PF-IY values. Values for both PF and IY decreased with increasing time of storage, whereas PF-IY values increased with storage period. These results indicate that the effect on the texture of HPP meat, as well as tenderisation due to ageing, was due to modification to the myofibrillar component of the muscle. These results also suggest that HPP did not denature the enzymes responsible for proteolysis, hence resulting in tenderisation over the storage period. Reports of other research investigating the use of HPP at low or ambient temperatures for tenderisation of meat have been contradictory. Kim et al. (2007) found that the peak force of beef eye round muscle was slightly higher when pressure was applied at 100 MPa for 5 min at 15°C but decreased with 300 MPa. On the other hand, pressures applied up to 400 MPa at temperatures between 10 and 20°C was found to increase the toughness and hardness of beef muscles (striploin, outside flat) (Ma and Ledward, 2004, Jung et al., 2000).

The HPP treatment had slightly lower scores, as assessed by a consumer panel, for meat colour and fat colour at various storage weeks (P<0.001), which appeared to negatively impact acceptability. At 200 MPa, this was less pronounced compared to 250 MPa. Across most storage weeks, scores for meat colour and acceptability in the 250 MPa treatment had the lowest scores for these attributes. This is likely due to consumers viewing the steaks at this level of pressure less favorably and indicates lower pressures should be applied, if a fresh-like appearance is to be maintained. Alternatively, using darker coloured meat (dark-cutting meat) at the 250 MPa treatment could be useful to promote lightness, as preliminary evidence indicates HPP could be used as an intervention method to improve the colour of dark meat (Hughes, unpublished). Interestingly, consumer acceptability and intent to purchase scores were still reasonable after the full 20 weeks storage. At weeks 8, 12 and 20, the 250 MPa treatment had the highest intent to purchase scores, even though meat colour and acceptability scores were lower, indicating consumers were still pleased with the product and more inclined to purchase the steaks. Thus, consideration must be given to how the steaks were compared between treatments at any one time point. Overall, it is likely that 250 MPa may have been too high a pressure to keep the fresh-like appearance of the meat.

5.3 Shelf life stability and sensory assessment of HPP fresh meat stored at two temperatures (Stage 3)

The main effects on TVC were storage time and temperature, with limited effect of the pressure treatment. There was no strong interaction between the variables of pressure treatment, storage time and storage temperature. Similar results were found for both the main effects on LAB counts and the interactions of the variables on the LAB counts.

Within each storage temperature, there was a significant (P<0.01) difference in both TVC and LAB counts at 0 and 4 weeks storage compared to all other storage weeks. At longer storage times (8–20 weeks), there was no significant (P>0.05) difference in either TVC or LAB across storage time.

Within each storage time (excluding 0 time), temperature consistently affected the TVC and LAB with significantly (P<0.001) higher counts at 4°C compared to -0.5°C, except for 12 weeks storage. While still significant (P=0.036), the difference between counts at 4°C and -0.5°C was less at 12 weeks storage compared to all other weeks.

There was no significant (P>0.05) effect of pressure treatment on TVC or LAB counts across storage time or temperature. This microbial data taken overall suggests that a pressure treatment of 200 MPa does not adversely affect the microbial counts obtained from this product. Microbial counts increase over storage time, and storage at -0.5°C will limit the microbial counts compared to storage at 4°C. Optimal temperature control has been shown to be important for control of microbial growth (Frank et al., 2019).

Table 4. Total viable counts (TVC, $\log_{10}$ cfu/cm^2) of beef striploin primals treated by HPP (0.1 and 200 MPa), followed by storage (0–20 weeks) at -0.5 and 4°C.

Pressure		0.1 MPa		200 MPa	
Temperature		**-0.5°C**	**4°C**	**-0.5°C**	**4°C**
Storage week	**0**	2.30	1.63	2.05	1.60
	4	4.80	6.41	4.36	5.69
	8	6.50	8.27	6.83	8.51
	12	6.59	7.30	6.27	7.35
	16	6.81	7.38	5.86	8.00
	20	6.70	8.03	7.15	7.87

The mean TVC and LAB for all time zero samples were 2.12 (Table 4) and 1.45 (Table 5) log_{10} cfu/cm^2 respectively, which were within the published ranges stated in AMPC project 2016-1059 (Frank et al., 2019). TVC's are generally expected to increase slowly to approximately 6–7 log_{10} cfu/cm^2 after 20 weeks storage. In this study, TVC exceeded this range when the product was stored at 4°C at 8 weeks or longer. At the ideal storage temperature of -0.5°C, there were higher than expected TVC and LAB counts compared to the control samples stored at -1°C in the Frank et al., (2019) study. Unusually, TVC and LAB counts continued to increase throughout the storage time (Table 3Table 4 and Table 5).

Table 5. Log_{10} cfu/cm^2 of lactic acid bacteria counts (LAB) of beef striploin primals treated by HPP (0.1 and 200 MPa), followed by storage (0–20 weeks) at -0.5 and 4°C.

Pressure		0.1 MPa		200 MPa	
Temperature		-0.5°C	4°C	-0.5°C	4°C
Storage week	0	1.32	1.25	1.37	1.85
	4	3.58	5.45	3.97	3.89
	8	5.94	7.44	6.53	7.89
	12	6.31	6.83	6.06	6.90
	16	6.39	6.87	5.65	7.70
	20	7.28	7.85	6.92	7.70

Representative images of control and HPP meat samples at each storage period at -0.5°C and 4°C are shown in Appendix 3. In addition, Table 6 shows the colour values measured for control and HPP samples, stored at -0.5°C and 4°C for up to 20 weeks. Temperature of storage had little effect (P>0.05) on the colour of the meat. Pressure-treated meat at 200 MPa showed an increase in lightness and yellowness compared to controls (0.1 MPa). Interestingly, this was not accompanied by any increase in redness or any myoglobin forms, suggesting a non-pigment mechanism was responsible. Thus, it is likely that pressure promoted structural alterations that increased light scattering in the meat, but this would need further validation. However, subtle differences (P=0.004) in hue were apparent, with HPP resulting in increased hue values.

The duration of storage showed significant (P<0.05) differences for all colour parameters, with the main effect observed in lightness and yellowness, where values increased with storage. This is like the trend observed in pH and drip loss values with storage. This was also reflected in the brown metmyoglobin levels and red oxymyoglobin levels, indicating myoglobin oxidation had occurred, but was minimal. These colour results are consistent with previous 20 week shelf life trials (Hughes et al., 2015).

Table 6. Effect of HPP (control, 0.1 MPa and 200 MPa) and storage (0, 4, 8, 12, 16 and 20 weeks) at -0.5°C and 4°C on surface colour parameters of beef striploin muscle steaks.

		Temperature -0.5 °C		Temperature 4 °C							
Colour	Storage	Pressure (MPa)		Pressure (MPa)		Pressure		Temperature		Week	
parameter	(wk)	0.1	200	0.1	200	LSD	*P*-value	LSD	*P*-value	LSD	*P*-value
Lightness	0	36.6	41.7	39.0	42.5	1.09	<0.0001	1.09	0.118	1.88	<0.0001
	4	41.7	42.9	40.8	44.3						
	8	41.4	43.2	39.1	41.0						
	12	44.4	46.9	40.8	47.1						
	16	41.3	42.2	39.8	41.9						
	20	42.9	46.0	41.5	43.1						
Redness	0	29.1	31.8	29.2	30.9	0.44	0.298	0.44	0.468	0.77	0.008
	4	30.1	31.3	29.7	29.8						
	8	29.7	29.0	30.5	29.7						
	12	29.0	29.8	30.0	28.2						
	16	30.4	30.5	30.1	30.0						
	20	29.0	29.1	29.5	29.2						
Yellowness	0	22.1	26.2	22.4	24.9	0.45	0.021	0.45	0.083	0.80	<0.0001
	4	24.3	25.3	23.3	23.9						
	8	23.4	22.8	23.9	23.4						
	12	22.2	23.8	22.7	21.5						
	16	23.5	22.8	22.4	22.5						
	20	21.9	22.1	22.5	22.0						
Chroma	0	36.6	36.8	41.1	39.7	0.61	0.093	0.61	0.199	1.06	0.001
	4	38.7	37.7	40.2	38.1						
	8	37.9	38.8	36.9	37.8						
	12	36.5	37.6	38.2	35.4						
	16	38.4	37.5	38.1	37.5						
	20	36.4	37.1	36.5	36.5						
Hue	0	37.1	39.5	37.6	38.9	0.26	0.004	0.26	0.022	0.44	<0.0001
	4	38.9	38.9	38.1	38.7						
	8	38.2	38.2	38.1	38.2						
	12	37.5	38.6	37.2	37.3						
	16	37.7	36.8	36.7	36.9						
	20	37.0	37.2	37.4	37.0						
OMb	0	77.7	82.8	79.2	83.6	1.02	0.084	1.02	0.687	1.76	<0.0001
	4	84.1	82.4	82.1	83.6						
	8	82.4	81.5	82.0	81.1						
	12	78.1	81.4	77.8	77.9						
	16	78.0	77.2	76.3	78.0						
	20	76.2	76.8	78.1	76.5						
MMb	0	14.4	15.4	14.7	12.5	0.70	0.699	0.70	0.844	1.20	<0.0001
	4	15.1	15.7	15.8	14.1						
	8	14.5	13.3	15.5	14.7						
	12	15.5	17.6	15.6	13.9						
	16	16.1	17.2	18.4	19.0						
	20	15.6	14.5	14.2	15.7						
DMb	0	7.9	2.5	6.1	4.0	1.10	0.395	1.10	0.817	1.92	<0.0001
	4	1.1	2.5	2.9	3.1						
	8	4.3	5.2	2.4	5.6						
	12	6.4	3.0	6.7	8.2						
	16	5.9	5.6	5.4	3.0						
	20	8.2	8.7	7.7	7.8						

Over the 20 week storage period, irrespective of treatment or storage temperature, the pH, drip loss and cooking loss of the samples increased (P<0.0001) and is consistent with previous findings in Milestone 3 and other reports (Hughes et al., 2015, McPhail et al., 2012). In addition, pressure increased (P<0.0001) the pH, and it has been proposed to be due to changes in protein conformation and alterations in the histidine amino acid, possibly via oxidative processes, with others supporting this mechanism (Buckow et al., 2012). The drip loss increased (P<0.05) with pressure treatment, and this could be a result of changes in the water distribution that is associated with structural alterations that occurs with pressure treatment. Although significant, this increase was minimal and was not obvious in packs. For some storage weeks, cook loss increased in HPP samples and may also be a result of water relocation.

Storage temperature had no impact (P>0.05) on any of the meat quality parameters measured, indicating that storage temperature post-treatment did not influence the meat quality attributes over and above the processing treatment. This is similar for pH values, where no obvious difference was apparent (P>0.05).

As expected, the meat became more tender (P<0.0001) with aging, regardless of storage temperature or pressure treatment. This was most evident between 0 and 4 weeks, where peak force values dropped over 10 N. Even at 8–20 weeks storage, these values continued to decrease, which is consistent with an increase in MQ4 scores, juiciness and tenderness observed previously with aged beef striploins (Hughes et al., 2015).

High pressure treatment reduced both peak force (PF) and initial yield (IY) values, indicating meat was more tender and most likely due to a reduction in myofibrillar toughness (P<0.0001). Thus, HPP induced structural changes in the myofibrils which generated a steak that was more tender overall, the mechanism of which has been discussed previously (Buckow et al., 2012). This was evident at most storage weeks and occurred regardless of the meat storage temperature. The non-myofibrillar component of the muscle, as indicated by peak force minus initial yield (PF-IY), did not show this trend and suggests that the HPP tenderisation process occurred at the myofibrillar level. Overall, the tenderness of the beef chilled striploins improved with HPP treatment, regardless of whether the meat was stored at -0.5°C or 4 °C.

There was no effect of HPP, temperature of storage or duration of storage on the lipid stability of beef striploin samples.

In terms of consumer acceptance of the visual appearance of the meat, there was no difference observed between control and HPP samples at any storage week, indicating that the participants could not differentiate between control and pressure-treated steaks. The effect of temperature was apparent for all assessment parameters, where steaks stored at 4°C tended to be rated higher compared to those stored at -0.5°C, particularly at the longer storage times (e.g. 20 weeks). This was also evident for the purchase intent scores.

As expected, over the duration of the trial, all the assessment scores decreased. Week 12 to 16 appeared to mark the point at which consumers were least satisfied with the meat colour, fat colour and consequently acceptability. At storage times of 16 and 20 weeks, the purchase intent scores of all steaks dropped below 50%, highlighting the reduced acceptability due to the appearance of the steaks.

The eating quality was also assessed by trained panelists using MSA protocols (Table 7). The effect of HPP treatment approached significance (*P*=0.077); all HPP treated samples were more tender than controls, except for week 12. The reason for the lack of difference at week 12 is uncertain. Regardless of HPP treatment, tenderness generally increased over time and the level of marbling (MSA-MB) did not affect tenderness ratings. The tenderness appeared to increase up until 16 weeks. Juiciness increased to week 4 and remained similar, with no HPP effect and no influence of marbling level. Flavour liking was higher in HPP samples at week 8 and 12 only (hence Tr * T significant), but higher marbled samples had higher flavour liking scores. Overall liking at week 8 and 12 was higher for HPP treated samples mainly because of improved flavour and maybe tenderness (week 8 only) was also positively affected by the level of marbling (MSA-MB). Overall quality was generally higher for HPP treated samples (approached significance, *P*=0.054) and was also positively affected by MSA-MB. Overall, the data show that the eating quality increased up until 16 weeks. Despite the low number of sample replicates tested at each time point (4, 8, 12 and 16 weeks), some significant improvements in the eating quality of HPP samples was indicated. With a higher number of replicates clearer positive effects of HPP may have been demonstrated.

Table 7. MSA eating quality data as assessed by trained panelists.

Sensory attribute	Treatment	Storage time (wk)					Treatment (Tr)	Storage time (T)	Tr*T	MSA-MB
		0	4	8	12	16				
Tenderness	HPP	42.55	57.39	79.74	66.99	99.23	0.077	**<0.001**	0.352	0.338
	Control	32.94	51.81	60.27	68.81	86.26				
Juiciness	HPP	48.61	62.87	64.16	65.76	65.27	0.203	**<0.001**	0.076	0.141
	Control	56.94	70.26	63.47	55.13	67.66				
Flavour liking	HPP	58.24	68.49	67.83	74.01	59.07	0.615	**0.007**	**0.021**	**0.016**
	Control	63.81	69.39	57.55	61.77	60.79				
Overall liking	HPP	51.65	67.73	70.10	74.75	61.40	0.094	**<0.001**	**0.017**	**0.003**
	Control	54.91	65.56	56.51	62.07	61.86				
Quality	HPP	1.856	2.715	2.444	2.736	2.423	0.054	**<0.001**	0.368	**0.006**
	Control	1.928	2.455	2.090	2.406	2.274				

5.4 Impact of HPP on shelf life and meat quality of sliced, steak portions

The mean TVC and LAB for each combination of pressure, storage day and retail display, are shown in Table 8.

Table 8: Total viable count (TVC, mean log_{10} cfu/cm^2) and lactic acid bacteria (LAB, mean log_{10} cfu/cm^2) for striploin treated with high pressure processing (0.1 MPa and 200 MPa) followed by storage (0, 7, 14 and 21 d at -0.5 °C), and retail display (10 to 14°C). No *E. coli* counts were recorded on any samples.

Pressure (MPa)	Storage day (d)	Retail display (d)	Mean TVC (log_{10} CFU/cm^2)	Mean LAB (log_{10} CFU/cm^2)
0.1	0	0	1.32	1.16
0.1	0	7	8.67	7.84
0.1	7	0	2.16	1.50
0.1	7	5	7.68	6.77
0.1	7	7	8.64	7.70
0.1	14	0	3.37	3.29
0.1	14	4	8.73	7.25
0.1	14	7	9.45	8.85
0.1	21	0	4.41	3.76
0.1	21	4	8.98	8.34
0.1	21	7	9.42	8.91
200	0	0	0.95	1.22
200	0	7	8.88	7.94
200	7	0	1.33	0.79
200	7	5	7.85	7.32
200	7	7	8.34	7.80
200	14	0	4.03	3.01
200	14	4	8.81	7.71
200	14	7	9.16	8.70
200	21	0	5.20	4.04
200	21	4	8.86	7.65
200	21	7	9.10	8.81

Statistical analysis (interaction and main effects plots) for the microbiological data is given in Appendix 4. There was no interaction between pressure, storage and retail display when assessing TVC. The main effects on TVC were from storage time and retail display. Similarly, for the LAB counts, there was no interaction between the variables assessed, and storage and retail display times had the most effect on the counts.

There was no significant effect of pressure (P>0.05) across storage and retail time which suggests no detrimental effect on microbial counts when low pressure (200 MPa) was applied to steaks. Therefore,

for analysis of storage time and retail display time, both 0.1 and 200 MPa treated product were analysed together.

There were significant (P<0.01) increases in both TVC and LAB counts as storage time increased (P<0.01). For each storage time, there was significantly (P<0.001) lower TVC at 0 d retail display compared to 7 d retail display. At 7 and 14 d of storage, there was also a significant difference (P<0.001) between each retail display day tested; 0, 4(or 5, where indicated) and 7. This significant difference between retail storage days 4 and 7 disappears after 21 d storage. This suggests that after 21 d storage, the growth rate of microorganisms on the surface of the steaks may have been faster than on steaks stored for 0, 7 or 14 d.

For each retail display time there were significantly (P<0.01) lower counts after 0 (mean 1.14 $\log_{10}$ cfu/cm^2) and 7 d (mean 1.74 $\log_{10}$ cfu/cm^2) initial storage compared to 14 (3.70 $\log_{10}$ cfu/cm^2) and 21 d (4.81 $\log_{10}$ cfu/cm^2) storage. This suggests that steaks stored for 7 d under the conditions used in this study might be able to produce the same retail display shelf life as measured by microbial counts as those stored for 0 d.

As shown in Figure 7A, compared to the control samples, HPP treatment increased the lightness of beef steaks (P<0.001). This was consistent with redness (Figure 7B), yellowness (Figure 7C) and chroma (Figure 7D) values of HPP treated steaks (P<0.05). This was not the case for hue, and the three forms of myoglobin, where no differences were observed between control and HPP treatment (P>0.05) (data given in Project 2017-1056 Milestone 6 Report). This may have been a result of interference of colour parameters with the microbial load and the visual appearance of spoilage on the surface of the meat.

All meat colour attributes changed with storage time (P<0.05) (Figure 7). For both treatments at all the four time periods (0, 7, 14 and 21 d), the meat colour appeared to peak in redness at 1–2 d retail display and then show a decline afterwards. For storage day 0 samples, large variations in sample colour appeared after day 5 of retail display. In comparison, for the longer storage periods (day 7, 14 and 21), the colour deteriorated much faster (2–3 d of retail display), with large variations in purple deoxymyoglobin and red oxymyoglobin levels observed earlier.

The pH range of the striploin primals after ageing for 14 d at -0.5°C was 5.49–5.63, with an average of 5.54 (se = 0.013). This range is within the normal pH range of aged meat and doesn't indicate the presence of dark-cutting meat. With the application of pressure at 200 MPa, there was a significant (P<0.001) increase in pH compared to the untreated control sample. The increase in pH ranged from 0.02 to 0.09 units, however all pH values were less than 5.60. This is consistent with previous findings in this project (Milestones 2, 3 and 5) and in other published work (Buckow et al., 2013, Sikes et al., 2010, Sikes and Tume, 2014). There was no significant (P>0.05) effect of storage time on the pH of vacuum-packed steaks.

There was no effect (P>0.05) of pressure on any of the texture parameters of beef striploin steaks. Although there was no significant (P>0.05) effect of storage day on the texture of the steaks, the trend was for lower peak force (PF) values with increased storage time at -0.5°C, as would be expected for aged beef striploin. Moisture loss during cooking of the steak samples for texture analysis was not affected (P>0.05) by pressure treatment. However, there was a significant (P<0.05) decrease of cook loss with increased time of storage at -0.5°C, which was more evident between 0 and 7 d storage.

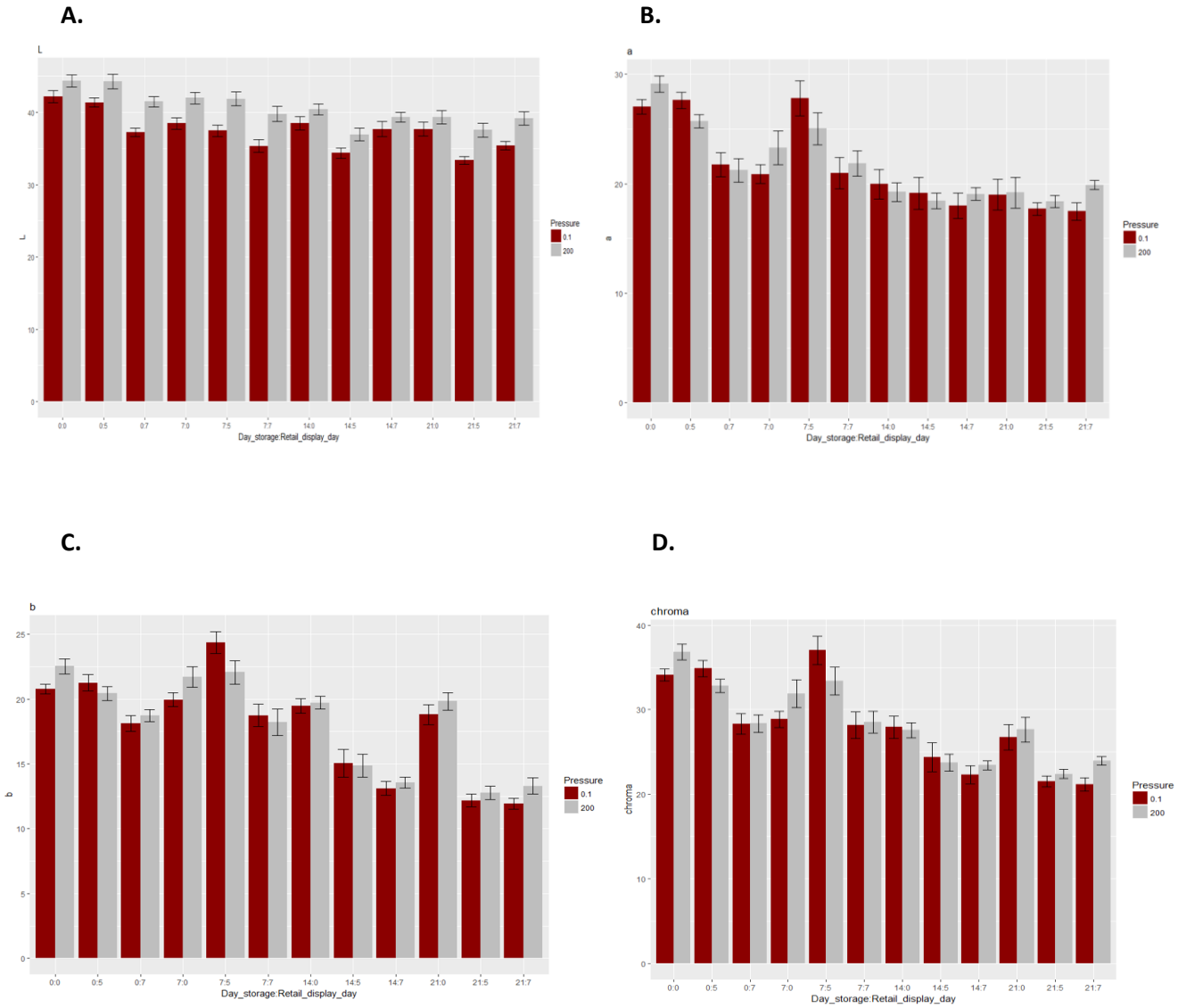

Figure 7. Effect of high pressure processing (0.1 MPa and 200 MPa) and storage (0, 7, 14 and 21 d at -0.5°C, with retail display measurements at 0, 5 and 7 d) on surface colour parameters of beef striploin steaks. A. lightness, L*; B. redness, a*; C. yellowness, b*; and D. chroma. Along the X-axis, the storage time (d) for vacuum-packed display is shown as the first digit, and the second digit is for the retail display day. For example, 0.7 was stored for 0 d in vacuum pack and 7 d retail display.

6.0 CONCLUSIONS/RECOMMENDATIONS

Although HPP is an established processing technology for the shelf life extension of ready-to-eat meat products, the results from this project indicate that the pressure threshold (200 MPa) required to maintain a 'close to fresh' meat appearance does not impact on the microbial populations to provide a beneficial extension of shelf life of fresh meat primals or sliced steak portions, over and above traditionally aged meat. However, these HPP conditions had minimal impact on other meat quality attributes such as tenderness (measured objectively) and yield.

The increase in lightness of muscle that occurred at pressures of 250–300 MPa, combined with no change in redness or yellowness, indicates that non-pigment related changes contributed to the colour stability of the muscle. Therefore, this provides an opportunity to pursue the potential to use HPP as an intervention to improve the colour acceptability of dark-cutting meat.

Consumer assessment of samples showed that visually, consumers could not differentiate between control and pressure-treated samples at any storage point. Additionally, a trained sensory panel assessed pressure-treated samples as having improved eating quality – more tender, with better flavour and a higher overall liking – compared to control samples. So, although these HPP conditions had no effect on shelf life or objective texture, the eating quality was improved. This data from the consumer assessment of pressure-treated samples therefore provides preliminary evidence of consumer perceptions and the likely acceptance of HPP as a food processing technology and the potential success of a pressure-treated meat product in the market place.

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8.0 APPENDICES

8.1 Appendix 1 – An example of the visual assessment conducted at each storage time point, and for the retail display of steaks

Name:_____ Date: _____

0/0

Instructions

PLEASE SCORE ONLY THE SAMPLES LISTED BELOW

Please look at the meat trays and score meat colour, fat colour and overall acceptability according to a scale from 1 (extremely dislike), 5 (neither dislike nor like) to 10 (extremely like) and answer the question: Would you buy this product, Yes or No ?

Steak ID	Meat colour	Fat Colour	Overall Acceptability	Would you buy this product?
	1 (extremely dislike) – 10 (extremely like)	1 (extremely dislike) – 10 (extremely like)	1 (extremely dislike) – 10 (extremely like)	Yes / No
C1				
C2				
C3				
…..				
C10				
P1				
…..				
P10				

8.2 Appendix 2 – Sensory questionnaire

Name _____ Date _____ Booth number _____

Sample ID _____

Tenderness

I_____I_____I

Not tender Very tender

Juiciness

I_____I_____I

Not juicy Very juicy

Liking of flavour

I_____I_____I

Dislike extremely Like extremely

Overall liking

I_____I_____I

Dislike extremely Like extremely

Please rate the quality of the beef sample you have just eaten – You **must** tick only **one**.

Unsatisfactory ( )

Good everyday quality ( )

Better than everyday quality ( )

Premium quality ( )

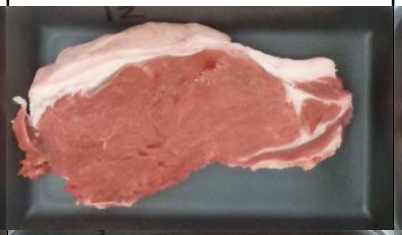
Do you have any other comments regarding this samples? Please detail below.

_____

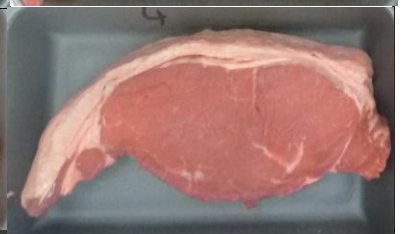
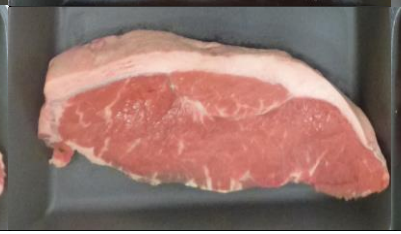
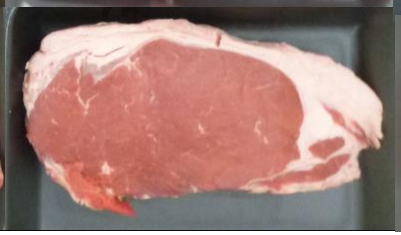
_____

_____

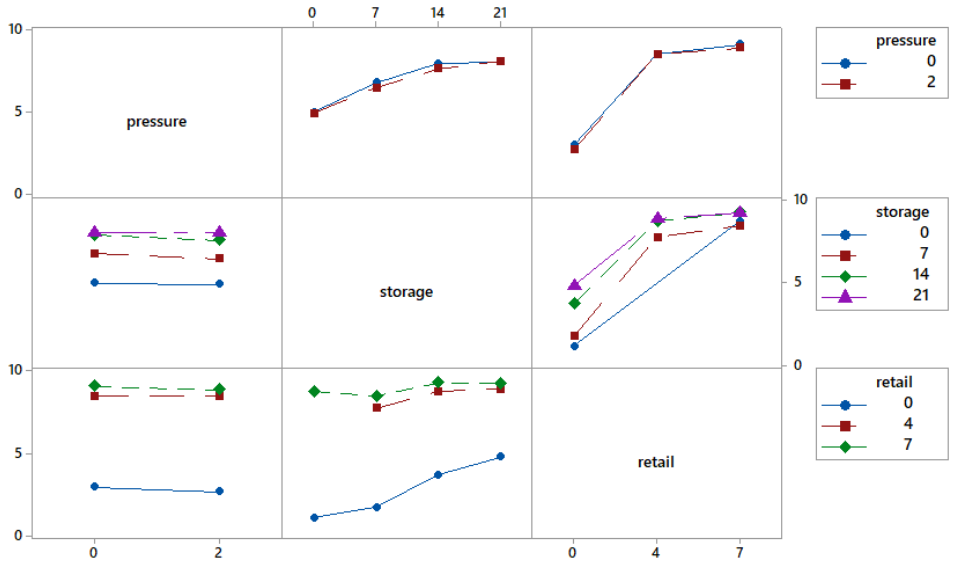
8.3 Appendix 3 – Representative photographs of control and HPP beef striploin samples at each storage period at -0.5 and 4°C (0, 4, 8 weeks)

Pressure / Week	Control (0.1 MPa), -0.5°C	HPP (200 MPa), -0.5°C	Control (0.1 MPa), 4°C	HPP (200 MPa), 4°C
0				
4				
8				

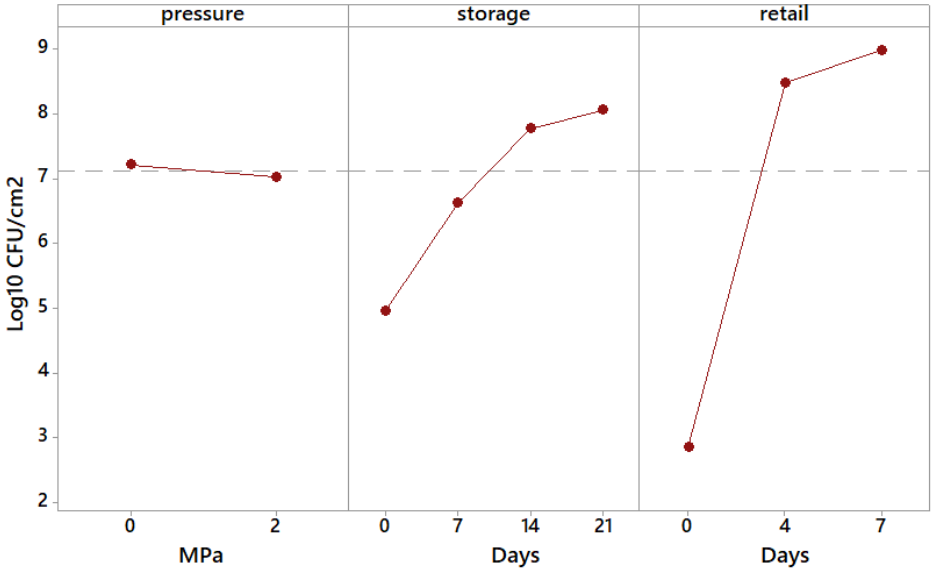
8.3 Appendix 3 Continued – Representative photographs of control and HPP beef striploin samples at each storage period at -0.5 and 4°C (12, 16, 20 weeks)

Pressure / Week	Control (0.1 MPa), -0.5°C	HPP (200 MPa), -0.5°C	Control (0.1 MPa), 4°C	HPP (200 MPa), 4°C
12				
16				
20				

8.4 Appendix 4 – Statistical plots for microbiological data for the effect of HPP on beef striploin pre-cut steak portions



Interaction plot of pressure, storage time and retail display time on total viable count (TVC), using the data means.



Main effects plot of pressure, storage and retail display on total viable count (TVC). The steeper the slope of the line, the greater the magnitude of the effect.