OPTIMISING MEAT QUALITY AND FUNCTIONALITY THROUGH NOVEL PROCESSING INTERVENTIONS – FINAL REPORT

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1.0 EXECUTIVE SUMMARY

Tenderness is recognised as the most important palatability trait for eating satisfaction of meat and, consequently, has a great impact on its value and repeated purchase by consumers. Colour is decisive in fresh meat at display but, once the meat is cooked, this becomes almost irrelevant, and the flavour is of less importance compared to tenderness, provided that no off-flavours are present. In this context, meat processors demand interventions that improve the tenderness of low-value muscles and ensure the consistency of high-value muscles. The development of processing interventions for meat tenderisation is one of the keystones to boost the profitability of the meat industry. Thus, this project focuses on the development and evaluation of novel, post-slaughter processing technologies for rapid tenderisation and increasing value of non-primal meat cuts. Value adding to under-utilised 'tough' muscles will increase the value of the carcass. This project will (1) identify and develop processing interventions for extending and enhancing the quality traits of fresh muscle and (2) evaluate and develop post-slaughter technologies for the development of ready-to-eat (RTE) meat products. The research objectives were:

To investigate novel post-slaughter technologies to 'tenderise' meat of low value or accelerate tenderisation in high value cuts from 'tough' animals;

- Investigate and develop a sustainable Pulsed Electric Field (PEF) processing technology to improve eating quality of meat cuts.
- Evaluate the efficacy of Ultrasound and Shockwave processing technologies to tenderise meat cuts
- Incorporate new science disciplines into meat quality research (confocal microscopy, mathematical modelling, process engineering).

Key Findings

- **PEF** processing (0.25 kV/cm, 100 Hz, 10 µs pulse width, for 30 ms) increased tenderness by 8.5% for beef striploin and by 12-15 % for rump, respectively, after storage at 4 °C for 1 day. There was no effect of PEF treatment on topside muscles. Other quality parameters (drip loss, cook loss and colour) were not affected by the PEF treatments. The sensory evaluation indicated an improvement in juiciness but not in tenderness of PEF treated striploin. The costs of PEF processing under these conditions are estimated to be approximately 7.3 cents per kg of meat.
- **Ultrasound** processing at 40 and 80 kHz for up to 5 min did not significantly affect pH, colour or tenderness of aged (up to 14 days) and cooked brisket meat samples. More intense ultrasound treatments resulted in significant surface discolouration due to overheating of meat cuts.
- **Shockwave** processing (at 35 kV, 1 pulse every 30 mm) of beef striploin muscle increased its tenderness by 12.4%, 8.2%, and 5.8% after ageing for 1, 11, and 21 days. Drip loss, cook loss

and colour of striploins were not significantly affected by the treatment. However, double shockwave treatment had no effect on the texture of the striploin steak size cuts.

Recommendations

- Further research understanding the effect of PEF and shockwave processing on meat structure and underlying mechanisms mediating tenderisation will provide a basis for process and equipment optimisation.
- Scale-up of PEF technology to pilot-scale for processing of whole muscles for market studies and further cost assessment should be performed.
- A better understanding of the effect of specific shockwave conditions on meat structure and its effect on the tenderness of meat is required for a consistent industrial application of the shockwave technology for meat tenderisation.

2.0 INTRODUCTION

The Australian red meat industry has identified a need to value-add to increase profitability of non-premium cuts. If tougher cuts could be tenderised in a quick and safe way, and transformed into tender and healthy products, there would be enormous opportunities for market growth. The implementation of novel processing technologies in the meat industry could result in significant improvements in tenderisation and eating quality thereby providing the industry with a competitive advantage in the area of value-adding.

This project investigated the impact of three emerging processing technologies, such as pulsed electric field (PEF), ultrasound (US) and shockwave (SW), for meat tenderisation along with the study of their effects on important quality traits of beef such as water retention and colour.

PEF is an emerging processing technology, where meat is treated with short high voltage pulses with the aim to modify muscle structure and ultimately achieve tenderisation (Arroyo et al., 2015a; Farnaz Faridnia et al., 2015; Suwandy, Carne, van de Ven, Bekhit Ael, & Hopkins, 2015a, 2015b).

US treatments have also been reported to mechanically disrupt meat tissues due to cavitation (Jayasooriya, Torley, D'Arcy, & Bhandari, 2007). Typically, high power ultrasound at 20 kHz is applied to induce cavitation in the meat tissue. However, application of low frequency ultrasound (i.e. <40 kHz) can result in significant burning of the meat surface and erosion of the sound-emitting sonotrode. Higher frequency US reduces the risk of tissue burning and sonotrode erosion due to less violent cavitation, but attempts to induce textural changes in meat at high frequency (600 kHz) applied in a water bath did not result in any considerable change in meat tenderness (Sikes, Mawson, Stark, & Warner, 2014).

The latest processing technology for meat tenderisation tested in this project is SW technology, also known as hydrodynamic pressure processing. Shockwaves propagate in water with high-energy and travel rapidly through fluids and any objects that are an acoustical match with water. Since meat is composed of 75% water, the pressure wave crosses the meat, and at points where acoustic impedances differ, an energy momentum transfer occurs, which in turn creates mechanical stress that tears the muscle structure. It has been reported that shockwaves can generate high pressure waves of up to 1 GPa in fractions of milliseconds. This produces what could be called a "rupture effect". As a result, the meat is instantaneously softened and an accelerated maturation of the meat can be observed (T. Bolumar & Toepfl, 2016).

3.0 PROJECT OBJECTIVES

- To investigate post-slaughter technologies to ‘tenderise’ meat of low value or accelerate tenderisation in high value cuts from ‘tough’ animals;
- Investigate post-slaughter technologies for new product lines in ready-to-eat (RTE) products;
- Undertake modelling of the effect of the process on meat quality traits;
- Undertake a process-cost analysis of targeted technology; and
- Incorporate new disciplines into meat quality (confocal microscopy, mathematical modelling, process engineering).

4.0 METHODOLOGY

4.1 Sample selection

4.1.1 Pulsed electric field (PEF) processing

Various beef muscles from domestic trade animals were sourced from a local butcher, Tasman Meats, Werribee, Victoria and JBS (Brooklyn, Melbourne). Beef samples were collected in four separate stages (Table 4-1):

(1) 17 Beef brisket muscles (*M. pectoralis profundus*)
(2) 12 Beef topside muscles (*M. semimembranosus*), from both the left and right sides of six domestic trade animals
(3) 12 Beef topsides (*M. semimembranosus*), 12 striploins (*M. longissimus dorsi*) and 12 rumps (*M. gluteus medius*) were sourced from six domestic trade animals
(4) 20 topsides (*M. semimembranosus*) both left and right side from ten animals and 18 striploins (*M. longissimus dorsi*) from both left and right side from nine animals (Table 4-1).

Table 4-1. Beef samples for PEF processing

Stage	Muscle	Animal dentition	No. of animal and sex	Sample size	Average sample weight	Storage period
			Male (M)/ Female (F)	(mm)	(g)	(day)
1	Brisket	0 – 2	10M	65x30x25	52.7 ± 6.4	0, 1, 14
2	Topside	0 – 2	6F	65x30x25	58.1 ± 5.3	1, 14
3	Topside	0 – 4	4M / 1F	65x30x25	61.3 ± 7.7	1, 7, 14
	Striploin	0 – 4	4M / 1F	65x30x25	61.3 ± 7.7	1, 7, 14
	Rump	0 – 4	4M / 1F	65x30x25	61.3 ± 7.7	1, 7, 14
4	Topside	0 – 2	10M	100x100x25	225.4 ± 36.4	1, 7, 14
	Striploin	0 – 2	10M	100x100x25	225.4 ± 36.4	1, 7, 14

The pH of each muscle was measured and muscles with a pH of greater than 5.8 or that were less than 25 mm thick in case of the brisket, were rejected. All muscles were trimmed of excess fat and connective tissue. Various portions, approximately 65 x 30 x 25 mm or 100 x 100 x 25 mm, were cut from each muscle (Figure 4-1) and randomly allocated to PEF treatments (Table 4-2) and ageing periods. Muscle samples were then placed into a treatment chamber designed for PEF treatment of solid foods (Figure 4-2). The pH and weight of each individual sample was recorded and the colour measured on the cut end of each sample. Samples for PEF treatment were covered with plastic wrap and stored at 0 °C until processing, either on the same day or the following day. Samples vacuum packed after PEF treatment and aged at 4 °C.

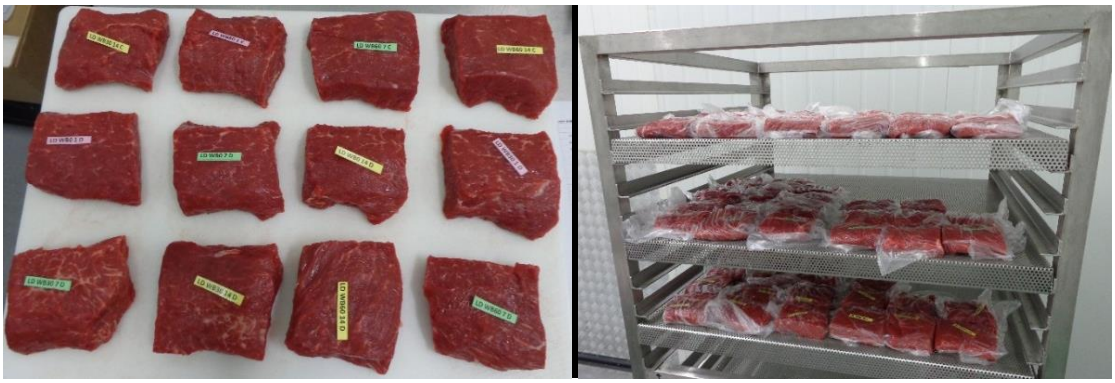


Figure 4-1. Left: An example of random sample allocation to specific PEF treatments. Blocks of cut muscles were randomly allocated to specific PEF treatments and were colour tagged for different storage times (pink = 1 day, green = 7 days, yellow = 14 storage days). **Right**: Samples sealed in plastic bags stored in a chiller (0 °C) until required for processing the following day.

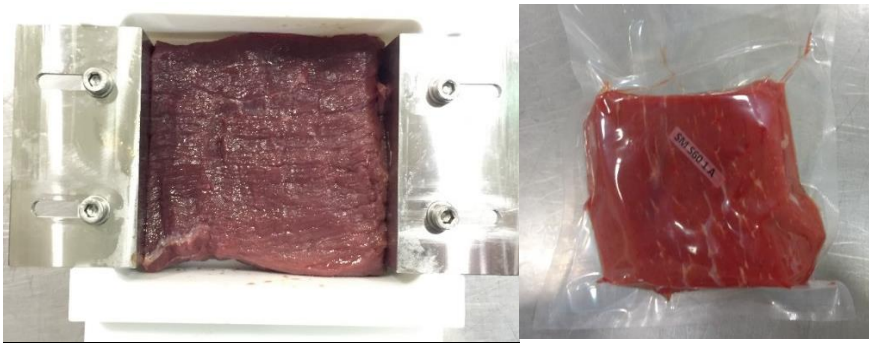


Figure 4-2. Left: Topside meat sample in a modified PEF treatment chamber for treatment of solid foods. **Right**: PEF treated topside meat sample vacuum packaged for ageing storage.

4.1.2 Ultrasound (US) processing

Beef brisket muscles (*M. pectoralis profundus*) were sourced from domestic trade animals (0-2 tooth) from Tasman Meats, Werribee, Victoria. Seven briskets were used for processing on the first processing day and 10 briskets collected for processing on the second processing day. All processing of samples was performed in duplicate with n=5 samples on two separate days.

The pH of each brisket muscle was measured and muscles with a pH of greater than 5.8 or that were less than 25 mm thick, were rejected. The brisket muscles were trimmed of excess fat and connective tissue. Nine portions, approximately 65 x 30 x 25 mm, were cut from each muscle and randomly allocated to US treatments (See Table 4-3, in Section 4.2.2) and three ageing periods (0, 1 and 14 days). The pH and weight of each individual sample were recorded and the colour measured on the cut end of each sample. The weight of the brisket portions ranged from 40 to 70 g, with an average weight of 52 g. Samples for US treatment were vacuum packaged and stored at 4 °C until processing on the same day.

4.1.3 Shockwave (SW) processing

Two target muscles (striploin, *M. longissimus thoracis*; and eye round, *M. semitendinosus*) were treated with shockwaves and the effects on tenderisation, colour and water retention were measured over a period of 21 days of ageing.

The striploin (*M. longissimus thoracis*) and eye round (*M. semitendinosus*) were sourced from six animals (n = 6) of the same breed (German Fleckvieh), age (23 months) and sex (steers), classified as A U 3 per EUROP[1] classification. The pH of the meat ranged between 5.30 and 5.80. The meat was processed when rigor was complete. At least two steaks per animal per each treatment condition was processed.

Two target muscles (striploin and eye round) from both sides of the carcass, right and left, were treated by shock waves. Entire primal cuts of the eye round and steaks from the striploin were trimmed of all visible fat and vacuum packaged (Multivac type C200) in polyamide/ polyethylene bags (Alfo Vakuumverpackungen, Waltenhofen, Germany) prior to processing.

[1] EUROP, European grading system of beef (Allen, 2014), A: uncastrated young male of less than two years old, U: very good conformation, 3: average fat cover.

4.2 Processing conditions

4.2.1 PEF processing conditions

PEF processing conditions were tested in four stages (Figure 4-3). PEF treatment of meat was performed using a Diversified Technologies Power Mod™ 25 kW Pulsed Electric Field System (Diversified Technologies, Inc., Bedford, MA, USA), which consists of a PEF treatment chamber and a modulator cabinet described elsewhere (Buckow, Schroeder, Berres, Baumann, & Knoerzer, 2010) and shown in Figure 4-4 and Figure 4-5.

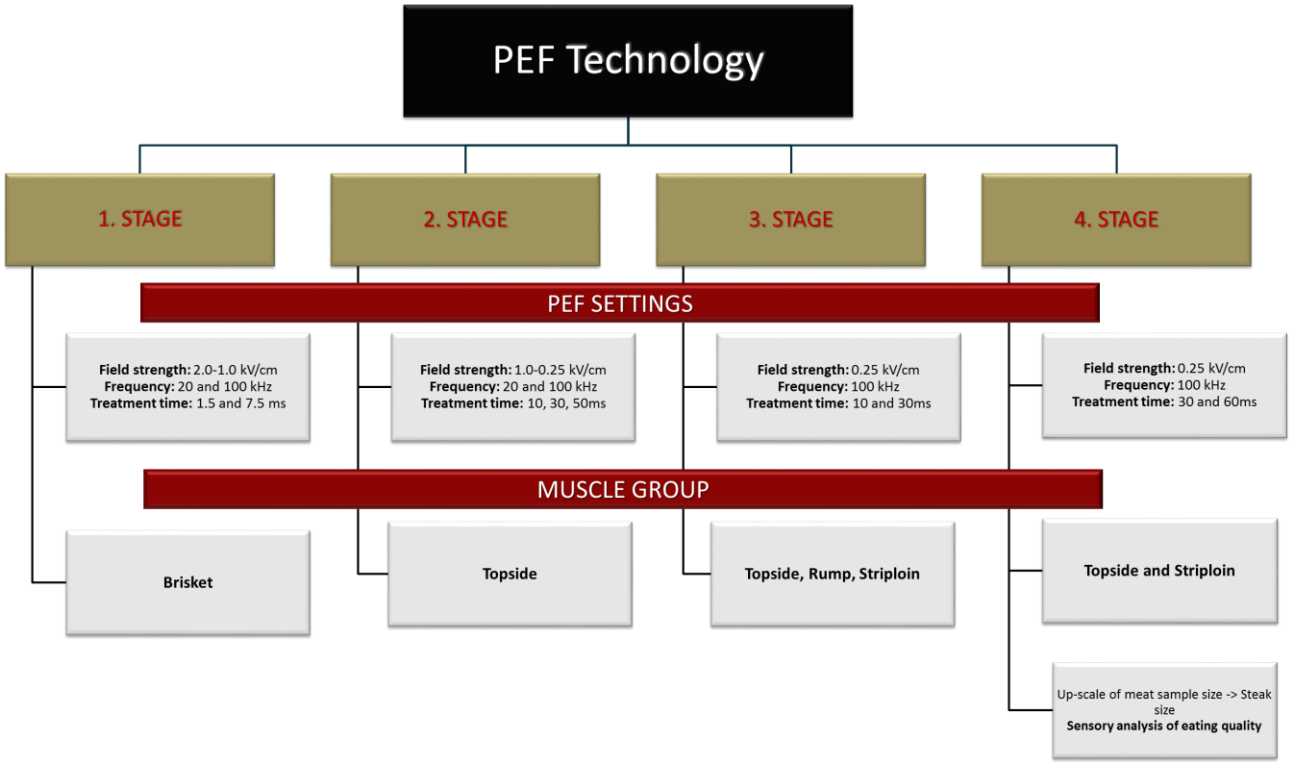


Figure 4-3. Stages of PEF processing conditions.

All meat samples and the PEF treatment chamber were conditioned to approximately 2 °C in an ice slurry prior to processing. The PEF treatment chamber was then dried of excess water. The cut ends of each meat sample were lightly sprayed with cold water prior to PEF processing to ensure good contact between the meat and the electrodes. Samples were placed into the PEF treatment chamber with no air pockets at the surface of the electrodes. Square wave pulses of 10 µs width at peak voltages ranging from 1.5 to 12 kV were applied resulting in several combinations of electrical field strengths from 0.25 to 2 kV/cm (Table 4-2). The pulse repetition rate was set from 10 to 100 Hz depending on treatment conditions (Table 4-2).

Figure 4-4. CSIRO's Power Mod^{TM} 25 kW PEF System with PEF treatment chamber and modulator cabinet (**left**) and modified PEF treatment chamber set-up for processing of meat (**right**).

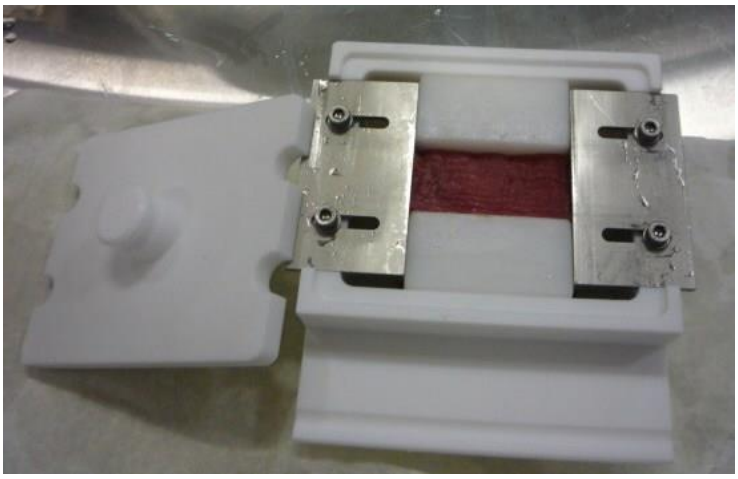


Figure 4-5. Modified PEF treatment chamber for treatment of solid foods.

PEF was applied to the meat for different treatment times resulting in several combinations of energy inputs (Table 4-2). Pulse shape, frequency, peak voltage and electrical currents were recorded with an oscilloscope (#GDS-1102, GW Instek, Taipei, Taiwan) attached to the output ports of the PEF system (Figure 4-6). Processed samples were vacuum packaged, chilled in ice water for 5 min and stored at 4 °C in a chiller for 1, 7 or 14 days before cooking and post-processing evaluation (storage drip loss, cook loss, tenderness, and colour change) or freezing for further sensory assessment.

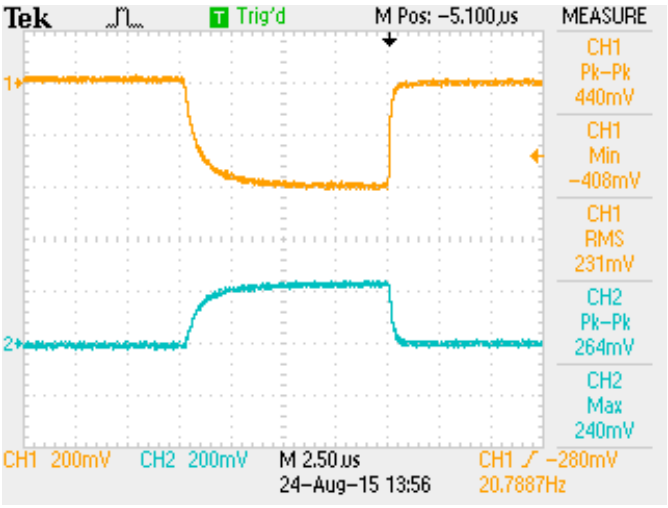


Figure 4-6. A typical pulse applied to the meat during PEF processing at 0.25 kV/cm at 20Hz (blue line = electrical current, orange line = voltage).

The voltage, electrical current and pulse width of the different PEF treatments were monitored using an oscilloscope and used to calculate the energy of the treatments. The energy per pulse (Eq. **4-1**), energy input (Eq. **4-2**) and specific energy (Eq. **4-3**) were calculated using the following equations:

1. Energy per pulse (Wp) = Current x Voltage x Pulse width

Eq. 4-1.

2. Energy input = Wp x number of pulses

Eq. 4-2.

3. Specific energy input = Energy input / weight of the sample

Eq. 4-3.

Table 4-2. PEF treatment conditions applied to meat samples in this study

Stage	Processing conditions			Parameters						
	Field strength	Freq.	Treatment time	Voltage	Current	Pulse width	Wp (Energy per pulse)	No. of pulses	Energy input	Specific energy input
	(kV/cm)	(Hz)	(ms)	(V)	(A)	(µs)	(J/pulse)		(J)	(J/g)
1	2	10	7.5	12000	100.00	10	12.00	750	9000.0	115.0
	2	10	1.5	12000	100.00	10	12.00	150	1800.0	19.0
	1	10	7.5	6000	50.00	10	3.00	750	2250.0	16.0
	1	10	1.5	6000	50.00	10	3.00	150	450.0	8.7
2	1	100	50	6160	56.80	10	3.50	5000	17494.4	300.9
	1	20	50	6160	54.60	10	3.36	5000	16816.8	289.2
	1	100	30	6240	52.60	10	3.28	3000	9846.7	169.4
	1	20	30	6240	51.60	10	3.22	3000	9659.5	166.1
	1	100	10	6320	47.20	10	2.98	1000	2983.0	51.3
	1	20	10	6400	44.00	10	2.82	1000	2816.0	48.4
	0.5	100	50	3136	21.12	10	0.66	5000	3311.6	57.0
	0.5	20	50	3104	22.72	10	0.71	5000	3526.1	60.6
	0.5	100	30	3104	22.08	10	0.69	3000	2056.1	35.4
	0.5	20	30	3168	16.32	10	0.52	3000	1551.1	26.7
	0.5	100	10	3136	18.24	10	0.57	1000	572.0	9.8
	0.5	20	10	3136	17.28	10	0.54	1000	541.9	9.3
	0.25	100	50	1504	9.28	10	0.14	5000	697.9	12.0
	0.25	20	50	1488	9.28	10	0.14	5000	690.4	11.9
	0.25	100	30	1488	9.44	10	0.14	3000	421.4	7.2
	0.25	20	30	1488	11.36	10	0.17	3000	507.1	8.7
	0.25	100	10	1504	8.32	10	0.13	1000	125.1	2.2
	0.25	20	10	1488	9.44	10	0.14	1000	140.5	2.4
3	0.25	100	30	1488	9.44	10	0.14	3000	421.4	7.2
	0.25	100	10	1504	8.32	10	0.13	1000	125.1	2.2
4	0.25	100	30	2384	25.84	10	0.06	3000	1848.1	8.2
	0.25	100	60	2276	22.76	10	0.05	6000	3108.1	13.2

4.2.2 US processing conditions

US processing was performed using a Multisonik-2™ Ultrasonic generator (Blackstone~NEY Ultrasonics, Jamestown, NY, USA). It features a plate transducer with the dimensions of 413 x 517 mm. The maximum output power is approximately 470 W. A Teflon frame was designed and glued onto the ultrasonic transmitter plate and a metal grid was placed on top, so that meat samples could be placed on top of the transmitter plate without direct contact. A sound reflector plate (made of stainless steel) was also used to improve sound reflection in the meat sample.

The application of US at 40 and 80 kHz for up to 6.5 min directly to beef brisket resulted in an increase of temperature on the meat surface of approximately 11 °C/min and discolouration and denaturation

of proteins, giving the meat a cooked appearance. Therefore, in this experiment, the ultrasonic transmitter plate was placed in a cooling water bath (~4 °C) to minimise heat development on the meat surface and in the meat (Figure 4-7).

Table 4-3. US treatments applied to beef brisket muscle

Treatment	Frequency (kHz)	Treatment time (min)	Storage (day)
Control	n/a	n/a	0, 1, 14
Low freq, short time	40	1	0, 1, 14
Low freq, long time	40	5	0, 1, 14
High freq, short time	80	1	0, 1, 14
High freq, long time	80	5	0, 1, 14

Beef brisket samples in vacuum packs were placed in the centre of the ultrasonic transmitter plate (Figure 4-7) and sonicated at 40 and 80 kHz for 1 and 5 min at maximum power setting (470 W) (Table **4-3**). Samples were turned half way through the total processing time to ensure even distribution of of the sound field. After US processing, samples were stored at 4 °C and analysed at each storage period (0, 1, 14 days) for drip loss, pH and colour changes. Tenderness and cook loss at each storage period were evaluated after cooking at 80 °C for 30 min.

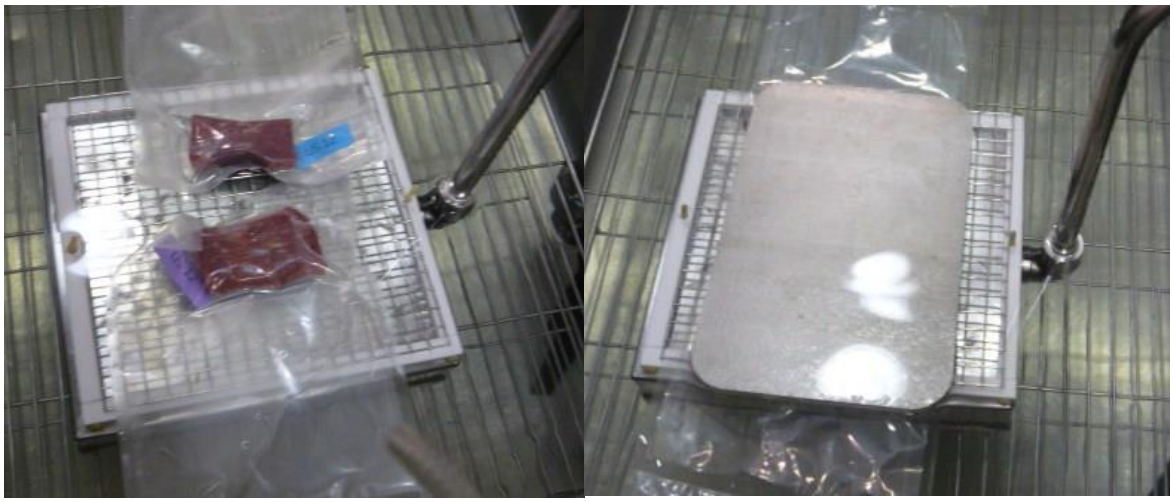


Figure 4-7. Left: Placement of beef brisket samples on ultrasonic transmitter plate; and **Right**: US processing system with sound reflector plate in position.

4.2.3 SW processing conditions

Striploin and eye round muscles (Figure 4-8) meat was submitted to SW treatment in a prototype machine (Figure 4-9) constructed by the German Institute of Food Technologies (DIL, Quakenbrueck, Germany) (Bolumar et al., 2013). Vacuum-packaged steaks and primals were treated by transporting them on a conveyor belt to the treatment area (Figure 4-9). This prototype is based on electrical discharge under water. The system consists of a high voltage power supply, a capacitor bank as well as a high voltage/current switch to discharge the stored electrical energy across the electrodes. By variations in charging voltage and capacity, the energy per pulse can be varied from 36 to 14,400 J per pulse. The treatment intensity can be further adjusted by the number of pulses applied. The following settings were used in the present study: voltage (35 kV) (corresponding to 11025 J per pulse) and distance from meat to spark (20 cm). All the meat samples (striploin steaks and eye round primal cuts), control and shockwave-treated, were taken to the shockwave processing pilot hall and left in a cold room (4 °C), from where they were taken for SW processing when required and put back immediately after processing. The water temperature in the vessel was at ambient temperature (20 °C) and not modified during processing.

Each muscle group was processed in a different way:

- Striploin, a high value muscle, was treated as steaks of 20 mm thickness.
- Eye round, a low value muscle, was treated as whole muscle and then cut into three blocks, one per storage time (1, 11 and 21 days).

Figure 4-8. Left: Striploin muscle cut into steaks of 20 mm thickness. **Right:** Eye round was treated as a whole muscle and then cut into blocks for further ageing.

The vacuum-packaged striploin steaks were subjected to one of three different treatments (n = 6 animals, 2 steaks per animal and treatment condition):

- control (no treatment)
- SW (single shockwave at 35 kV, 1 pulse every 30 mm)

- 2 X SW (double shockwave → (each side treated at 35 kV, 1 pulse every 30 mm) (steaks were introduced twice for shockwave treatment with side up changing between treatments to ensure the treatment of both sides))

The whole vacuum-packaged eye rounds were subjected to one of the following treatments:

- control (no treatment)
- SW (single shockwave at 35 kV, 1 pulse every 30 mm)

After processing, the eye rounds were cut into three pieces each with a length of 100 mm and vacuum-packaged. Each piece was assigned for a different ageing time (i.e. 1, 11 and 21 days) (one per ageing time).

All samples were aged at 4 °C until needed for analysis. The eye rounds were cut into steaks (20 mm thick) on the day of analysis (Figure 4-8). Texture, colour, drip loss, cook loss and microscopy (i.e. confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) was analysed in striploin and eye round samples at 1, 11 and 21 days after processing according to the methods described in section 4.3.

Figure 4-9. Left: The shockwave industrial prototype used in this experiment. **Right:** Meat samples being transported on the conveyor belt to the treatment area.

4.3 Analytical methods

4.3.1 Storage drip loss and cook loss

Storage drip loss and cook loss was calculated as weight difference before and after storage, processing and cooking, respectively.

4.3.2 Colour

The colour of meat samples was measured using a Minolta Colorimeter CR-300 or a CM-600d (Minolta Co., Ltd, Japan), for PEF, US and SW treated samples, respectively. Colour measurements were taken directly on the muscle surface, on the sides of the cut pieces, avoiding areas of visible fat at 8 °C. Raw meat samples were allowed to bloom for 30 min in a chiller at 4 °C prior to each colour measurement. The instrument was equilibrated in a cool room (8 °C) and calibrated with a standard white plate under D65 illumination (Y = 92.0, x = 0.3163, y = 0.3328) before use. Triplicate colour measurements were taken. The final data was represented as redness (a^*), yellowness (b^*) and, and lightness (L^*) values.

4.3.3 pH

Muscle (primal) pH was measured by directly inserting a spear head pH probe and temperature probe, connected to a WP-80 pH-mV-Temperature meter, into the sample. The unit was calibrated with pH 4.00 and pH 7.00 buffer standards at 8 °C. Single pH measurements were conducted per sample.

4.3.4 Mechanical measurements

The tenderness of PEF treated meat was measured using an Instron 5564 fitted with a 500 N load cell (Instron, Norwood, Massachusetts, USA) and a modification of the Warner-Bratzler shear device (L.J. Bratzler, 1932). To be able to compare shear force results from our previous experiments, samples at each storage point were cooked to an internal temperature of 80 °C in a water bath, set at 80 °C, for 60 min. These cooking settings are different from those used to prepare the samples for the sensory descriptive analysis, which was more focused on capturing the realistic eating context of the consumer. After cooking, samples were cooled in an ice slurry for 60 min and then stored at 4 °C for 1 hr prior to texture assessment using the Warner-Bratzler (WBSF) shear force method. The samples were cut into a rectangular shape (15 mm width X 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. 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) was objectively determined using the Bluehill® 3 software (Instron®, Illinois Tool Works Inc., USA), while the initial yield (IY) was determined by the operator as the height of the first peak from the curve. The difference between these measurements (PF-IY) was also calculated. Six determinations were made on each sample and the mean recorded.

In the SW trial conducted at DIL (Germany), samples were cooked for 12 min in an 80 – 82 °C water bath (Brökelmann Beistellkessel type 6080600-KA10) to an internal temperature of 71 °C. Tenderness of shockwave treated and cooked samples was measured using the WBSF procedure. The cooked steaks were left in a chilling chamber (4 °C) till the following day before being cut into 10 mm thick strips using a scalpel. The strips were left at room temperature for 1 hour to equilibrate in order to ensure that all samples were measured at the same temperature. At least five strips for each steak were measured. A total of ten WBSF measurements were carried out and recorded for each steak. The tenderness was measured by a texture analyser Winopal TA-XT 2 (Stable Micro Systems Ltd, Surrey, England) using a standardised Warner–Bratzler shear force blade. The operational settings of the texture analyser were as follows: test mode in compression strength, initial height of cell 30 mm, pre-test speed 1 mm/s, test speed 1.66 mm/s, post-test speed 10 mm/s, travel distance 40 mm and detection of sample 50 g. A 50 kg load cell was used. Maximum shear force is the peak force which is extracted from the texture graphs or TA.XT reports. This value is used to compare the tenderness of the samples.

4.3.5 Microstructural imaging

Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) imaging of the PEF and shockwave treated meat were conducted as described below.

4.3.5.1 Confocal laser scanning microscopy (CLSM)

About 1.5 g of muscle (raw or cooked) was homogenised in 15 ml ice cold Mannitol buffer (380 mM mannitol, 5 mM potassium acetate) using an Ultra-turrax disperser (Ultra Turrax, T25 basic from IKA Labortechnik, Germany) with a 18 mm head at 16,000 rpm for 30 s, followed by 30 s rest, followed by a further 30 s of homogenisation. The Mannitol buffer used for the homogenisation was matched to pH of the muscle sample (pH 5.9) to prevent unwanted structural changes due to pH mismatch. The sample tubes were kept on ice at all times.

Small aliquots of suspended myofibrils were diluted 1:1 with Mannitol buffer and stained with about 10 ppm fluorescein isothiocyanate (FITC). A sample drop was added to a microscope slide and sealed with nail polish. The myofibrils were observed under a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems, Germany). Myofibril sarcomere lengths were measured from the images using Leica LAS AF software.

4.3.5.2 Scanning electron microscopy (SEM)

Random samples (volumetric elements of edge length of 1.5 mm) were taken from the meat, frozen in super-cooled liquid nitrogen and inserted into a cryo-preparation system (Emitech K 1250, France). Free water from the samples was removed by sublimation. Finally, the frozen surface was sputter-coated with gold. The prepared samples were transferred into a SEM (JEOL JSM 6460 LV, Japan) at approximately −180 °C and imaged at 1–30 kV. The generated images were recorded electronically. Longitudinal and cross-sectional orientations of the muscle structure were captured.

4.3.6 Eating quality of PEF-treated beef

Sensory descriptive analysis[2] was used to investigate the impact of PEF treatment times (0, 30 and 60 ms) and storage times (1, 7 and 14 days) on the topside and striploin samples as described below. The following attributes were evaluated during the different stages of mastication: taking a first bite with the front teeth (*springiness, initial resistance, initial juiciness*), during chewing (*chewing down the second half of the sample, juiciness, overall tenderness, strands, chewiness, moistness, connective tissue*) and after swallowing (*residues, fatty mouth coating, metallic feel, tooth wedging, mouth drying*).

4.3.6.1 Cooking and serving of samples

A total of 126 samples was cut from the striploin and topside samples for sensory analysis, to cover two PEF treatment times (30 and 60 ms), one control sample, two different muscle groups (topside and striploin), three storage periods (1, 7 and 14 days) and six animal replications. All the samples were processed at the CSIRO Food Innovation Centre in Werribee, Victoria and then delivered frozen (< -18 °C) to CSIRO North Ryde for sensory testing. A combination of eighteen different samples (various PEF treatment times, and storage times) was obtained (see Table 4-4).

Table 4-4. Description of the treatment conditions of beef steaks used for sensory evaluation for striploin (LD) and the topside (SM) samples

Muscle type	Sample number	Sample name	PEF Treatment time (ms)	Storage time (days)	Sample repetition for evaluations
Topside (SM)	1	SM S0 1	0	1	B, L, G*
	2	SM S0 7	0	7	B, F, H
	3	SM S0 14	0	14	F, M, B
	4	SM S30 1	30	1	D, F, C
	5	SM S30 7	30	7	J, H, E
	6	SM S30 14	30	14	B, A, F
	7	SM S60 1	60	1	K, F, M
	8	SM S60 7	60	7	L, B, M
	9	SM S60 14	60	14	H, C, J
Striploin (LD)	10	LD S0 1	0	1	J, D, K
	11	LD S0 7	0	7	J, M, H
	12	LD S0 14	0	14	C, L , G
	13	LD S30 1	30	1	E, D, C
	14	LD S30 7	30	7	B, E, J
	15	LD S30 14	30	14	E, G, A
	16	LD S60 1	60	1	F, L, M
	17	LD S60 7	60	7	D, M, G
	18	LD S60 14	60	14	K, C, L

* Coding of 6 animal replicates. Muscles were sourced from both left and right side of the animal being coded A – M. Individual samples were randomly assigned to particular processing conditions.

[2] Sensory assessment was conducted by a trained sensory panel. The research was approved by the CSIRO Human Research Ethics Committee.

The preparation of beef steaks for sensory evaluation comprised of the following steps:

Prior to product assessment:

Since the samples were shipped frozen from Werribee, VIC, to North Ryde, NSW, they were placed into a chiller (4 °C) on the day prior to sensory assessment to ensure that the meat samples were completely thawed. To standardise the product evaluation, the three largest and most even samples for each treatment condition and storage point were selected for evaluation.

On the day of assessment:

- Samples were placed on a tray in the sensory kitchen approximately one hour before cooking to allow all steaks to equalibrate to ambient temperature (20 °C). This technique was found to be optimal for cooking since steaks that were directly taken from the chiller took much longer to reach the required internal temperature of 75 °C, for food safety criteria, which resulted in a hard crust being formed on the steak's surface making pieces difficult to bite through.
- Each steak was cut into nine equal pieces (3 x 3 cm) prior to cooking, to ensure that each panellist had a steak piece cooked to the same degree. When cutting each steak, uneven edges or thicker sections were removed to ensure that all individual pieces were of an even size.

To represent a more accurate consumer preparation practice and realistic eating experience, each cut of steak was cut into nine individual pieces, and cooked on a Silex hot plate set to 100 °C. The sample blocks were covered with the hotplate lid and left to cook for 4 min until the internal temperature of each piece had reached at least 75 °C, see Figure 4-10.

Figure 4-10. Cooking the nine individual pieces of beef steak.

Individual pieces were removed from the hotplate and placed into individual 3-digit coded cups and left to 'rest' for 2 min. 'Rested' samples were served to the panellists for evaluation (Figure 4-11). While it was assumed that the PEF treatment would be able to fully and evenly penetrate the whole steak, it was decided that each panellist would receive the same individual piece of steak from the same area

of the larger steak to minimise any potential bias introduced by the degree of steak's cooking or imperfections and unevenness in the steak's structures (sinew, fat, etc.).

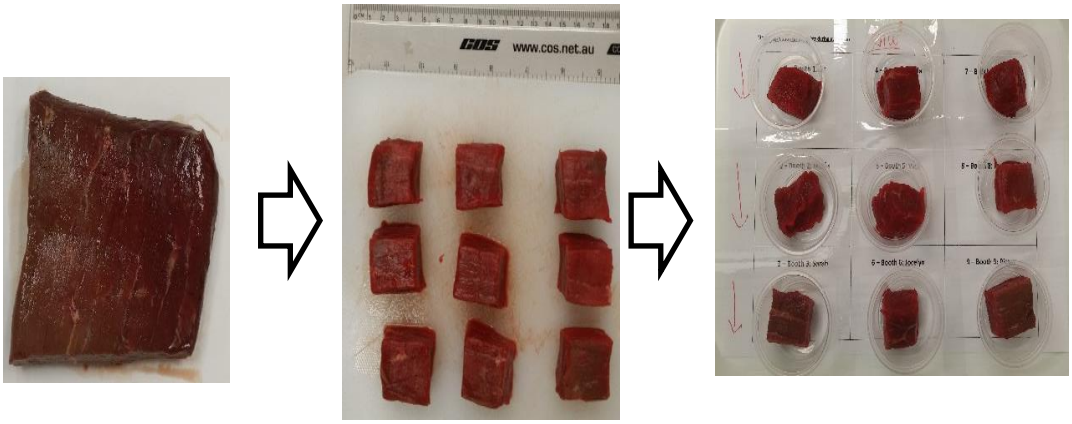


Figure 4-11. Cutting of whole steak into individual pieces and transferring it to individual serving cups in the same order to monitor which panellist received which piece of steak.

4.3.6.2 Descriptive sensory analysis

Descriptive sensory analysis aims to collect objective information about the sensory properties of products to establish comparisons between them. This objective sensory information is provided by a trained descriptive panel that functions like a "human instrument". Descriptive sensory analysis does not provide a judgment on the desirability of product properties or the preference of one sample over another. Acceptance and preference for products can be determined through consumer research.

Descriptive sensory analysis was conducted in August 2016, in the sensory laboratory at CSIRO's North Ryde facilities, designed in accordance with International Standards on Sensory Analysis (ISO 6658:1986). Nine panellists from CSIRO's trained sensory panel (average age of panellists was 51 ± 9 yr) that had previously been screened for sensory acuity and had extensive experience in sensory descriptive analysis on a variety of food products, including red meat, participated in the study. Seven 2 hr training sessions were required to familarize the panel with the samples.Through multiple exposures to the samples and moderated discussions by an experienced panel leader, a consensus vocabulary consisting of 14 attributes was developed which best described the key in-mouth texture and mouth-feel characteristics of the beef steaks (Table 4-5, Table 4-6). In addition, a corresponding standardised method of assessment for evaluating each attribute was developed to ensure that the collected data was objective.

Table 4-5. Descriptive vocabulary of in-mouth texture properties and method of assessment of PEF treated steaks

Method of assessment	Attribute	Definition	Anchors	Related terms
FIRST BITE				
Taking a first bite with the front teeth	***Springiness***	The degree to which the sample returns to its original shape when front teeth are first pushed into the sample	Stay compressed→ quickly returns	bouncy, spongy
	Initial resistance	The effort required to bite through the sample.	Low → High	hard, tough, firm
	Initial Juiciness	Amount of juice released from the sample on the first bite	None → A lot	
DURING CHEWING				
Chewing down the second half of the sample	***Juiciness***	The amount of juice released from the sample on the 4th chew with the molars	None → A lot	
	Overall Tenderness	The overall impression of how easy it is to chew the sample between the molars	Tough → Tender	
	Strands	The amount of strands, long particles in the mouth during breakdown	None → A lot	stringy, fibres
	Chewiness	Amount of work (time and energy) required to chew the sample to a state ready for swallowing (ignore any residual sinew that you need to keep on chewing)	Little → A lot	
	Moistness	The degree to which the mass feels wet when felt with the soft part of the mouth	Dry → Wet	
	Connective tissue	Amount of connective tissue present in the sample	None → Much	sinew, tendon

Table 4-6. After-feel vocabulary and method of assessment for PEF treated beef steaks

Method of Assessment	Attribute	Definition	Anchors	Related terms
AFTER FEEL				
After swallowing	***Residues***	The amount of particles left in the mouth after swallowing	None → A lot	
	Fatty mouth coating	The amount of greasy/oily film left on the lips and mouth surfaces	None → A lot	Cold fat
	Metallic feel	The tingling sensation perceived in the mouth	Low → High	iron, blood
	Tooth wedging	The amount of sample left between the teeth/ molars	None → A lot	
	Mouth drying	The mouth drying sensation perceived 30s after swallowing	Wet → Dry	chalky

The assessment of each sample was broken down into three phases and corresponded to the order in which the samples were consumed. The three phases were: '*first bite'*, '*during chewing*' and '*after-feel*'. For all *'first bite'* attributes, panellists would take one half of the sample and locate the fibre direction of the sample. They would then bite in the sample's fibre direction and not against it. Initially, panellists would push their front teeth into the sample without biting to assess the *springiness* of the sample. They would then bite all the way through the middle of the sample and assess *initial resistance* and *initial juiciness.*

Panellists would then place the remaining half the sample into their mouth and start chewing with their molars to assess the *'during chewing'* characteristics. On the fourth chew they would assess *juiciness.* During chewing, panellists would evaluate the *overall tenderness* and *strands* of the sample, and just before swallowing they evaluated *chewiness, moistness* and *connective tissue.* After swallowing panellists rated *residues, fatty mouth coating, metallic feel* and *tooth wedging,* and thirty seconds after swallowing they assessed *mouth drying*.

All samples were presented sequentially monadic, that is, one at a time, using 3-digit random codes. Sensory attributes were rated on 100 mm unstructured line scales, anchored at 5% and 95%. Assessments were carried out in individual sensory booths under white light, and data was recorded and stored using Compusense sensory data acquisition software (version 5.6, 2004; Compusense Inc., Guelph, Ontario, Canada).

Sensory evaluation was conducted in triplicate over six days with each replicate taking place over two days, i.e. each replicate, nine out of the 18 samples were randomly assessed by all the panellists on the first day and the remaining nine samples were assessed on the second day. On each day of evaluation the samples were assessed using random designs.

Panellists were instructed to cleanse their palate with water and cucumber slices (skin removed) after each sample. They were also provided with a toothpick to remove any residues or particles stuck in the teeth between samples (see sample presentation in Figure 4-12). An inter-stimulus interval of 3 min was imposed due to the cooking time of the samples. A 10 min break after the initial five samples was also imposed to avoid sensory fatigue.

Figure 4-12. Sample presentation of beef steaks to panellists, including palate cleansers.

4.3.7 Statistical analysis

The data of the instrumental meat quality measurements was analysed using a one-way analysis-of-variance (ANOVA) using the 'aov' command using R (R-Core-Team, 2014). The first analysis evaluated the impact that PEF, SW and US treatments had on a number of properties of the meat. Measurements of pH, weight and the colour parameters, were conducted on the samples, before and after application of individual treatments. The ANOVA was performed using time of measurement (before and after processing) and treatment (control, frequency, electric field strength, and treatment time). The second analysis evaluated the impact of storage time on the cook loss and meat tenderness after cooking. The measured parameters were cook loss, initial yield and peak force as well as the colour parameters; L^*, a^* and b^*. The ANOVA was performed using treatment (Control and Treatment time) and storage time (1, 7 and 14 days) as factors. The significance interval was $p < 0.05$.

Another ANOVA was carried out, on the data from the sensory descriptive analysis, for texture and after-feel attributes. Each analysis used individual panellist's scores to determine the effect of experimental design variables on the descriptive sensory attributes (dependent variables). The SPSS statistical software package (version 23, IBM Corp.) was used for all analyses.

1. A two-way ANOVA for sample (n = 18) and Panellist (n = 9) (independent variables)
2. A one-way ANOVA for muscle type (n = 2).
3. For each muscle type separately (topside and striploin), a one-way ANOVA for Treatment Time (n = 3) and Storage Time (n =3)
4. For each muscle type separately (topside and striploin), a two-way ANOVA for Treatment Time (n = 3) and Storage Time (n=3) (independent variables). This was to determine any interaction between treatment and storage times.

For all sensory descriptive analyses, a confidence interval of 5 % was chosen as criterion for statistical significance ($p < 0.05$).

Post-hoc multiple comparison tests were carried out using Fisher's Least Significant Difference (LSD) test as a post-hoc multiple comparison to determine significant differences between sample means. The tests were carried out using XLSTAT (v 2009.4.02, Addinsoft, Paris, France). Results of the test are reported using alphabetical subscript, in the form of *a, b, c, etc.*. The subscripts indicate a statistically significant difference in intensity between two samples.

Principal Component Analysis (PCA) using PanelCheck (v1.4 Beta 4, Nofima, Norway, www.panelcheck.com) was also carried out for all samples in order to visualise the sensory space. PCA is a multivariate statistical analysis technique that summarises the similarities and differences between the samples and sensory attributes and visualises the relationship between them on a two-dimensional bi-plot. The first two dimensions (PC 1 and 2) explain the most of the variance within the data. The PCA

map is a simplification of data in two dimensions. In order to understand absolute similarities and differences between samples, panel mean scores should be looked at.

Selected sample profiles have been presented in the form of “spider plots”. The graphical information displayed in a “spider plot” is useful in order to get an overview of how the storage times compare across the three treatments. The information contained within a “spider plot” is derived from the mean scores for each sensory attribute. Statistically significant attributes that discriminate the samples are indicated with an asterisk.

5.0 PROJECT OUTCOMES & DISCUSSION

5.1 PEF processing

5.1.1 Effect of electric field strength of PEF treatment on meat texture

The effect of PEF treatments at varying electric field strengths on meat tenderness after 1 and 14 days of chilled storage is presented in Figure 5-1. There was no significant improvement in tenderness after PEF treatment at 1 day of chilled storage. In fact, some of the samples were tougher. A similar lack of tenderisation, as measured by shear force, with PEF treatment has also been reported by other authors (Arroyo et al., 2015).

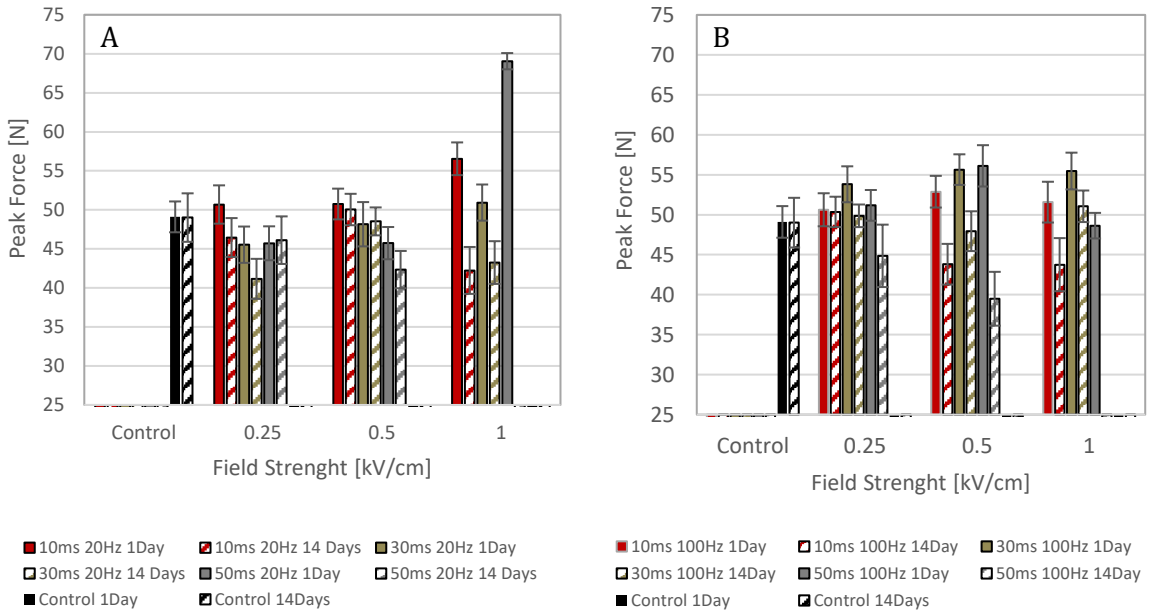


Figure 5-1. Effect of PEF treatment applied to beef topside at different field strengths (0.25, 0.5 and 1.0 kV/cm) and frequencies (**A**: 20Hz and **B**: 100Hz) for different treatment times (10, 30 and 50 ms) and aged for 1 and 14 days on the Warner-Bratzler Shear PF of beef topside. Values are mean ± standard error.

The PEF treatment had a significant effect on meat tenderness after 14 days of chilled storage ($p < 0.001$). Some PEF treated samples (0.25 kV/cm, 20Hz for 30 and 50ms, respectively) did result in a 7% to 16% reduction of the shear force (i.e. improved tenderness) compared to the untreated control after 14 days of storage. PEF treated samples (0.25 -0.5 kV/cm, 100Hz for 50ms, respectively) did result in a 10% to 19.4% reduction of the PF (i.e. improved tenderness) compared to the untreated control after 14 days of storage. As the control did not change in tenderness over time, it appears that the PEF treatment is inducing a tenderisation effect, most likely via induced proteolysis. This tenderisation effect is in agreement with Suwandy, Carne, van de Ven, Bekhit, & Hopkins, 2015a, 2015b, 2015c, but these authors also reported an immediate tenderisation of the meat (after day 1 of storage). These authors reported an increased proteolysis evidenced by an increases in troponin-T and desmin degradation, which is typical for the natural ageing of the meat. In addition, the statistical analysis also

showed an interaction of the tenderness with the frequency of the treatment (20 or 100 Hz) ($p < 0.001$). A low frequency (20 Hz) seems to be more effective for tenderisation after 1 day whereas after 14 days of storage it is not conclusive which frequency (20 or 100 Hz) is more effective.

5.1.2 Effect of PEF treatment on different beef muscles

In this experiment, the PEF treatment was applied at 0.25 kV/cm at a pulse frequency of 100 Hz for 10 or 30 ms. For rump, this treatment reduced the PF, or maximum WBSF, by 8 and 6 N, after one day of storage (Figure 5-2). However, on day 7 and 14, the PEF-treated rump was not always more tender than the control. From a statistical point of view, there was no effect ($p > 0.05$) of PEF treatment on any of the texture parameters (PF, IY, PF-IY) of the rump. Increased storage time of rump resulted in decreased PF ($p < 0.1$) and IY values ($p < 0.05$). There was a significant ($p < 0.01$) synergy between PEF treatment and storage, with PF values for PEF treatments at 1 day storage lower than the control value (Figure 5-2). The 30 ms PEF treatment also resulted in a lower ($p < 0.01$) PF value after 14 days storage. Therefore, it appears that PEF treatments resulted in lower PF values after 1 day storage, but inconsistency in the texture was apparent with increased storage time of rump.

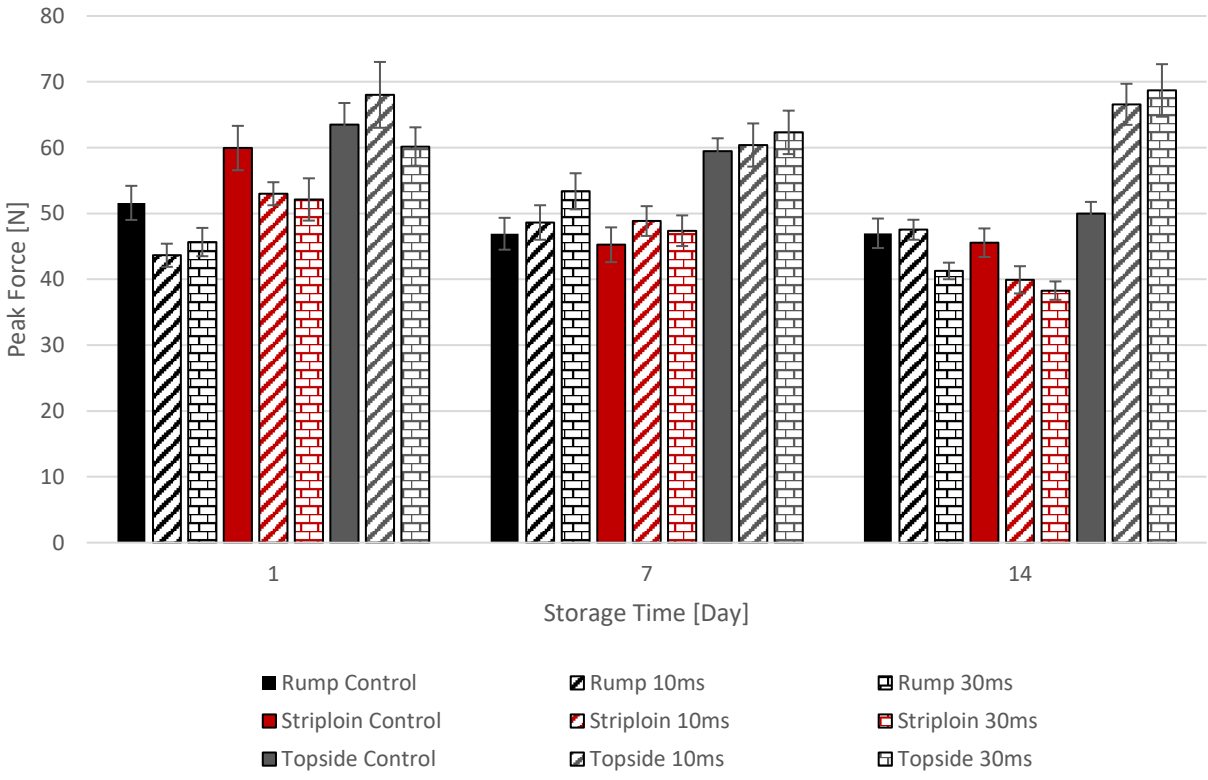


Figure 5-2. Effect of PEF treatment at 0.25 kV/cm (Control, no treatment; 10 ms PEF treatment; 30 ms PEF treatment) on the Warner-Bratzler shear force (peak force) value of beef rump, striploin and topside. Values are mean ± standard error.

For the striploin muscle, both PEF treatments (10 and 30 ms) decreased ($p < 0.1$) the PF value (Figure **5-2**) on day 1 and on day 14, with a larger reduction (16%) for the 30 ms PEF treatment. However, on day 7, the tenderness was similar for all treatment and control. Storage decreased ($p < 0.001$) both PF and IY values of striploin. Using low electric field strengths (approximately 0.3 and 0.55 kV/cm, 3 – 62 kJ/kg), Bekhit, van de Ven, Suwandy, Fahri, and Hopkins (2014) also reported a reduction (19.5%) in PF values of beef striploin. However, other reports that applied PEF treatments (0.2 – 1.4 kV/cm, 3–50 kJ/kg) to beef striploin found no significant improvements in texture (Arroyo et al., 2015b; F. Faridnia, Bekhit, Niven, & Oey, 2014).

PEF treatment applied to topside resulted in no significant change of the PF value on day 1 but a significant increase ($p < 0.01$) in the IY value was observed. Farnaz Faridnia et al. (2015) applied PEF (1.4 kV/cm, 250 kJ/kg, 20 µs pulses) to frozen-thawed beef topside and reported an increase in tenderness after 7 days of storage. Bekhit et al. (2014) reported a 4–19% reduction in PF values of PEF-treated (0.3 and 0.55 kV/cm, 3–62 kJ/kg) topside muscle.

For the control samples of all primal cuts, the expected decrease in PF values (i.e. increase in tenderness) with storage (7 and 14 days) was observed (Figure 5-2). This trend was also seen for both of the PEF treatments for striploin, with PF values decreasing as storage increased (Figure 5-2). However, it is difficult to explain the effects for the rump and the topside muscles. PEF treatments of rump resulted in an increase in PF value on 7 days of storage, however, after 14 days of storage the PF values for both PEF treatments were similar or lower than the control (Figure 5-2). In contrast, PEF treatment (10 ms and 30 ms) of topside resulted in an increase in PF values after 14 days storage (Figure 5-2). These effects are clearly muscle dependent and possible explanations could be due to the different orientation of muscles fibres within each muscle, the composition of the different muscles (e.g. fibre types, connective tissue content), and the amount and activities of the proteolytic enzymes within each muscle.

5.1.3 Effect of PEF treatment on the texture of 'steak size' beef samples

The aim of this experiment was to test the effect of a PEF treatment in a meat sample (topside and striploin) of similar size of a standard steak (100 X 100 X 25 mm). The PEF treatment applied was a field strength of 0.25 kV/cm at a frequency of 100 Hz for 30 and 60 ms.

PEF treatment did not have a significant ($p > 0.1$) effect on the PF value of striploin. Control striploin samples (untreated) had lower PF values than the PEF-treated samples at both treatment times (30 ms and 60 ms) after 1, 7 and 14 days of storage (see Figure 5-3). However, storage time significantly affected the meat texture ($p < 0.05$). Longer storage times resulted in decreased PF ($p < 0.001$) and IY values ($p < 0.001$) (values not shown). Despite the increase in PF of the striploin after PEF treatment, most PF values on day 7 and 14 were very similar within a narrow range of 35-40 N, probably indicating no effect of PEF treatment on striploin tenderness.

PEF treatment for 30 ms applied to the topside muscle resulted in a significant ($p < 0.05$) increase of the PF after 1 day storage, which gradually decreased after 14 days of storage. The higher PEF

treatment time (60 ms) did not have a significant effect after 1 day of storage, however, after day 7 and 14 the PF was lower than the untreated control sample with 15.5% and 3.1% reduction, respectively. Other researchers, Bekhit et al. (2014), showed a 4 to 19% reduction in PF values of PEF-treated (0.3 and 0.55 kV/cm, 3 – 62 kJ/kg) topside muscle. Storage for 7 and 14 days resulted in a lower ($p < 0.1$) IY value for topside than after 1 day of storage but a higher PF-IY value (data not shown).

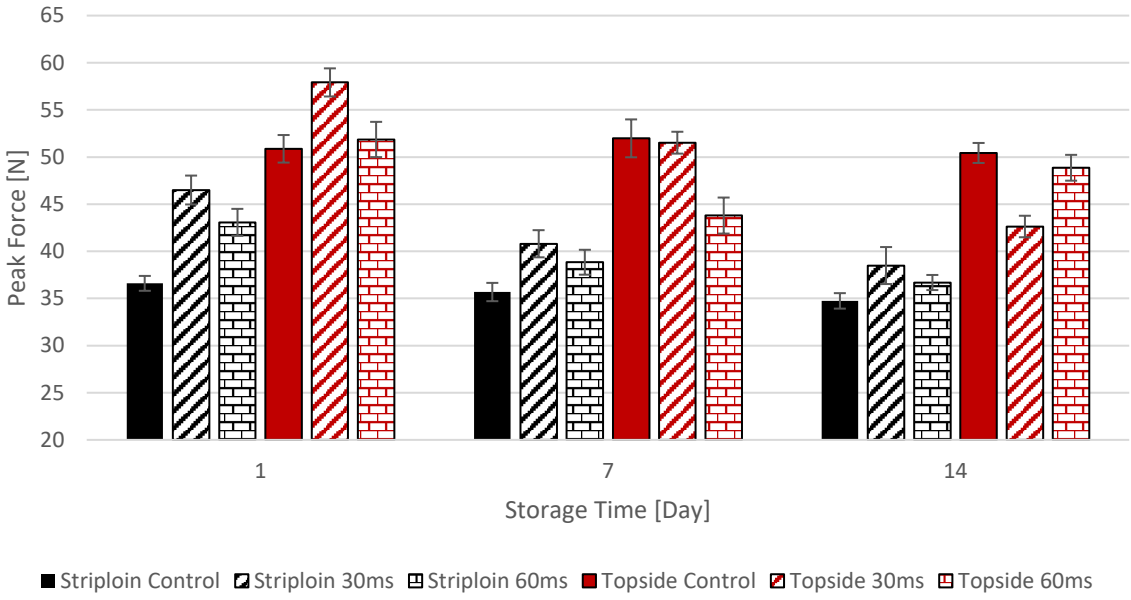


Figure 5-3. Effect of PEF treatment at 0.25 kV/cm (Control, no treatment; 30 ms PEF treatment; 60 ms PEF treatment) on the Warner-Bratzler shear force value of beef striploin and topside. Values are mean ± standard error.

The interaction of PEF treatment time and storage time had no significant ($p > 0.1$) effect on the PF of the striploin and topside. PF values for PEF treatments after 1 day storage were higher than those of the untreated control samples (Figure 5-3) for both muscles but the difference in the rate of PF decrease favoured the topside; i.e. PEF treatment for 30 and 60 ms resulted in a lower ($p < 0.01$) PF value (i.e. more tender) after 7 and 14 days storage compared with the same treatment after 1 day storage.

As in previous experiments, it was difficult to explain the effects of PEF treatment for both primal cuts, striploin and topside. PEF treatments of the striploin resulted in an increase in PF and slowly decreased with storage time. Others authors have also described recently a toughening effect of a PEF treatment (Bekhit et al., 2016, Suwanday et al., 20015b). It seems that these effects were dependent on the particular muscle and feature of the raw meat, and could be due to the different orientation of the fibres within each muscle, the composition of the different muscles (e.g. fibre types, connective tissue content), and the amount and activities of the proteolytic enzymes within each muscle. Overall, the PF values of striploin control (untreated) samples decreased (i.e. increase in tenderness) with storage time (7 and 14 days), whereas the PEF treatment resulted in higher PF values in this trial. The PEF treatment for 30 ms on topside resulted in an increase in PF after 1 day of storage as well, but rapidly

decreased after 7 and 14 days. The PF for the topside control samples did not change significantly ($p > 0.1$) over the storage period (for 1, 7 and 14 d) while PEF treatment accelerated the tenderisation of topside over storage time.

5.1.4 Effect of PEF treatment on meat quality traits

This section discusses the effect of PEF treatments applied in this study on the quality traits of meat, including texture, pH, water retention and colour. These quality traits have to be monitored when applying processes targeting an improvement in tenderness to guarantee that the overall meat quality is not impaired. The PEF treatment did not have a significant impact on pH as expected. During processing, the temperature of the meat increased proportional to the energy input (data not shown). The higher the energy input, the higher the temperature increase. Nevertheless, most of the applied treatments resulted in a relatively low temperature increase (1-13 °C) which can be quickly equilibrated to 4-7 °C once the product is put back into chilled storage. The drip loss was slightly higher in PEF treated meat possibly as a result of the electroporation, especially after treatment at the higher field strength (1 kV/cm) and for the longer treatment times (30 and 50 ms). However, this effect was not statistically significant ($p > 0.1$) and for most of the treatments this additional drip loss was not higher than 0.2 – 0.3% per sample.

The PEF treatment also had no significant effect on any of the colour parameters (data not shown). The applied electrical field strengths, pulse frequencies and treatment times did not have a significant effect on the colour parameters, L^*, a^* and b^*. Therefore, the application of these PEF treatments is advantageous as the raw meat colour is not impaired.

In general, there were no major changes in pH and colour (L^*, a^*, b^*) of fresh or cooked meat after PEF treatment and chilled storage of either 1, 7 or 14 days (data not shown). However, drip loss and cooking loss were slightly higher with longer storage time, independent of the PEF application. Storage time (maturation) had a statistically significant effect on storage drip loss of rump, striploin and topside, and cook loss of rump and striploin. There was a trend, although not statistically significant, for the drip loss to be slightly higher (+ 0.5 – 1%) after 14 days of storage.

5.1.4.1 Microstructure

The microstructure of different, PEF treated, primal cuts was investigated by studying the sarcomere dimensions (length and diameter) of myofibrils by microscopy. This measurement provides an indication of process-induced structure modification and shrinkage that could be responsible for changes in eating qualiy. The sarcomere lengths of each muscle group, rump, striploin and topside, before treatment were 1.55 ± 0.18, 1.72 ± 0.22 and 1.50 ± 0.20 µm, respectively, while the related sarcomere diameters were 1.43 ± 0.25, 1.58 ± 0.29 and 1.47 ± 0.21 µm, respectively.

Neither sarcomere length nor diameter were affected by PEF treatment or storage time. The most significant factor affecting the sarcomere length was the muscle group itself ($p < 0.001$). The comparison of sarcomere length of individual muscle groups subjected to PEF can be seen in micrographs depicted in

Figure 5-4.

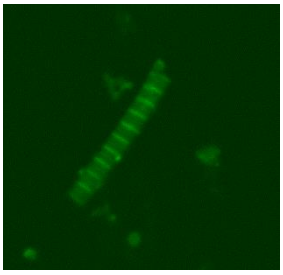
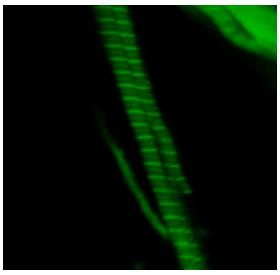
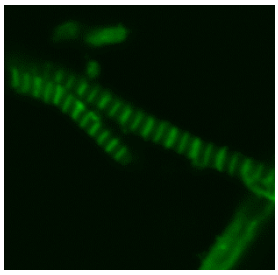
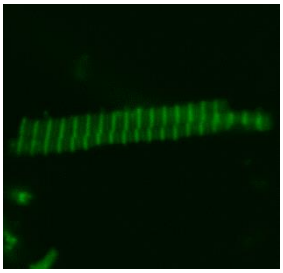
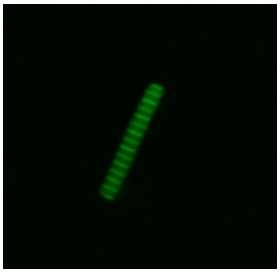
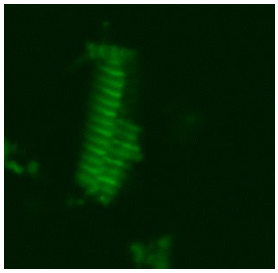
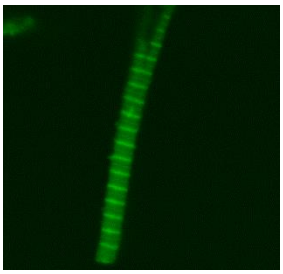
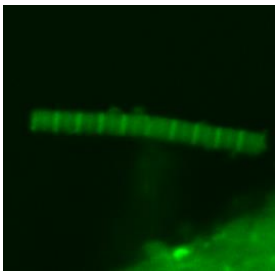
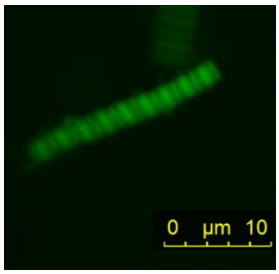
PEF treatment time (ms)	Storage time (Days) 1	7	14
0 (Control)			
10			
30			

Figure 5-4. CLSM micrographs of topside myofibrils (raw samples) subjected to PEF at 0.25 kV/cm (0 ms – control, 10 ms and 30 ms) stored vacuum packaged at 4 °C for 1, 7 and 14 days. No significant differences in the sarcomere length or diameter of muscle fibres could be observed within treatment or storage time. The scale bar applies to all images.

5.1.5 Eating quality of PEF-treated beef. Sensory descriptive analysis

The eating quality of beef samples treated with PEF (0.25 kV/ cm, 10 Hz, 30 and 60 ms) and that of the control (untreated) samples was determined at three different time points (1, 7 and 14 days) using sensory descriptive analysis.

5.1.5.1 Sensory panel assessment of the topside

Sensory descriptive analysis results for the nine topside muscle samples showed that five in-mouth texture attributes: *initial resistance, initial juiciness*, *juiciness, overall tenderness* and *connective tissue* were perceived to significantly discriminate between the PEF treated samples. Whereas none of the *after-feel* attributes were perceived to significantly discriminate between the samples.

The effect of treatment time and storage time on the descriptive sensory attributes are presented as (a) the mean of the three storage times (1, 7, 14 days) for each treatment time (0, 30 and 60 ms); and (b) the mean of the three treatment times for each storage time (1, 7 and 14 days).

Increasing treatment time had a significant effect on *initial juiciness* and *juiciness* for topside muscle samples. It seemed that the application of PEF increased the steaks' perceived *juiciness* over the course of the product consumption (Figure 5-5).

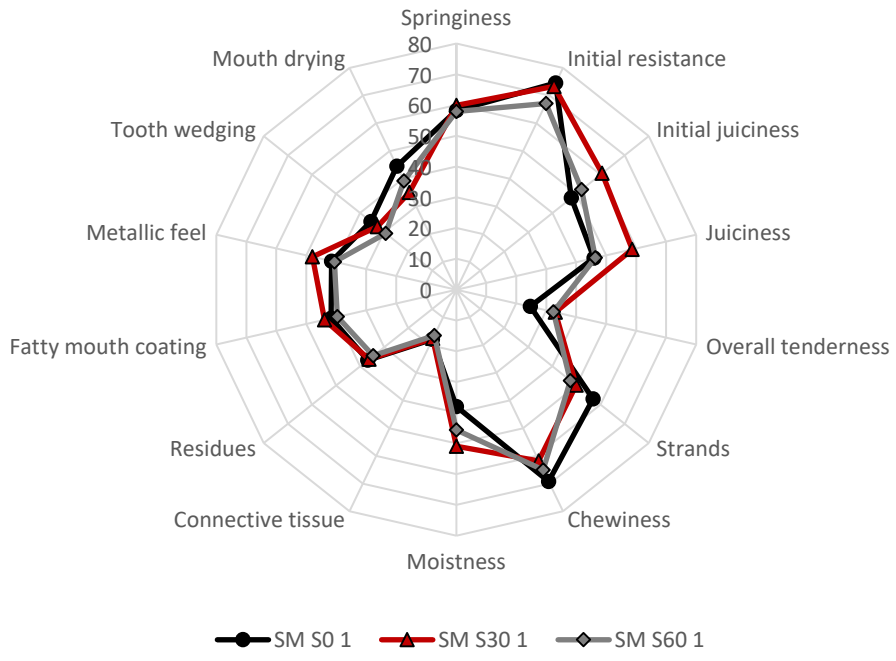


Figure 5-5. Spider plot displaying the differences in texture and after-feel properties of PEF treated and non-treated topside steaks after 1 day of storage (**SM** – topside, **S0, S30, S60** – 0, 30, 60 ms treatment time, **1** day storage time).

Increasing storage time had a significant ($p < 0.05$) effect on *initial resistance* and *overall tenderness*, indicating that the use of PEF could contribute to the steak's increased perceived *tenderness* over the

course of the product consumption. The results also show an effect on connective tissue but this is more likely to be an artefact due to biological variability since the attribute indicates the amount of connective tissue perceived in the sample. This was a characteristic of the meat sample tasted and necessarily independent of treatment or storage time.

There was a significant interaction between treatment time and storage time on *initial resistance*, *overall tenderness* and *chewiness*. Figure 5-6 presents the Principal Components Analysis (PCA) bi-plot of the significantly discriminating attributes for in-mouth texture from the descriptive analysis.

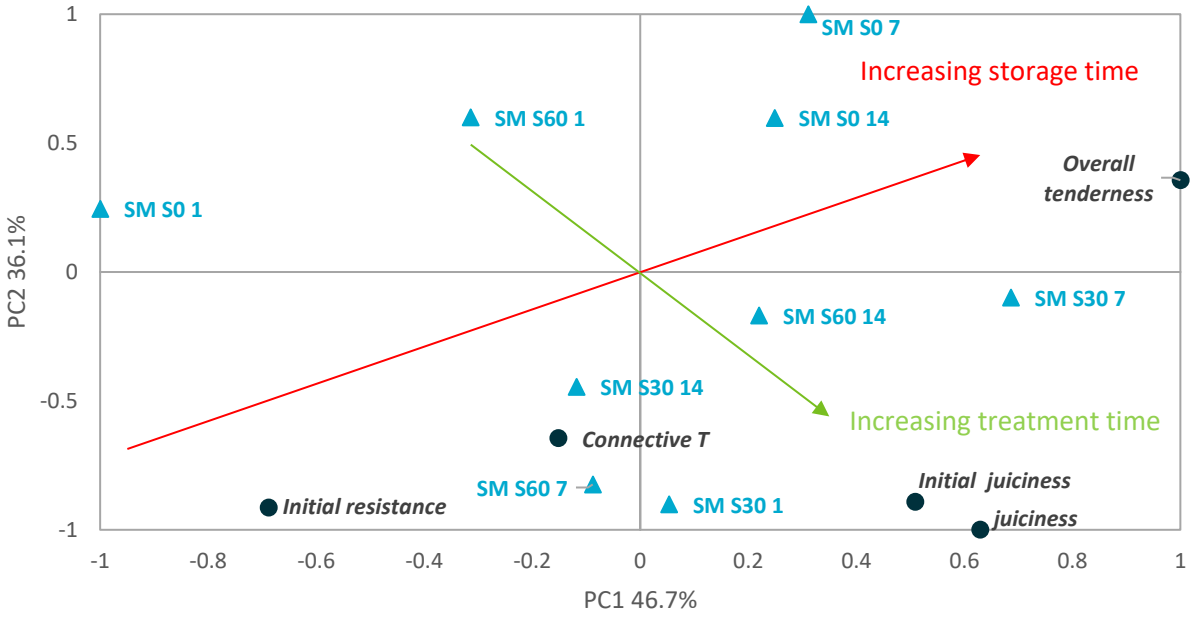


Figure 5-6. PCA bi-plot displaying the two-dimensional sensory space for texture attributes of the PEF treated topside (SM) samples. Only significant ANOVA attributes are plotted.

The first two principal components explain 82.8% of the variance within the sample set of the topside (PC1: 46.7%; PC2: 36.1%, Figure 5-6). This means that the differences between the individual samples are well represented using the first two dimensions of the bi-plot. The largest differences between the samples were determined by the first PC (46.7%).

The first principal component (PC1, as seen in Figure 5-6 from left to right) was associated with *juiciness* and *tenderness* and separated the non-treated sample stored for 1 day which was perceived as being more *resistant,* from the non-treated samples stored for 7 and 14 days, as well as the 30 ms and 60 ms treated samples stored for 7 and 14 days, respectively, which were perceived as being more *juicy* and *tender*.

The second principal component (PC2) explained 36.1% of the variance and was associated with *resistance*, *connective tissue,* and *juiciness* suggesting that the three untreated topside samples, as well as the PEF treated (30 and 60ms), stored for 1 and 7 days, respectively, were more *tender* overall but

less *resistant* and *juicy* than topside samples such as SM S30 1, SM S30 14 and SM S60 7, PEF treated (30 and 60 ms), stored for 1, 14 and 7 days, respectively.

Initial resistance and *overall tenderness* significantly ($p < 0.05$) differed between the non-treated samples stored for 1, 7 and 14 days. The sample stored for 1 day was perceived as significantly more *resistant* on the first bite and less *tender overall* in the chew down than the other two storage samples.

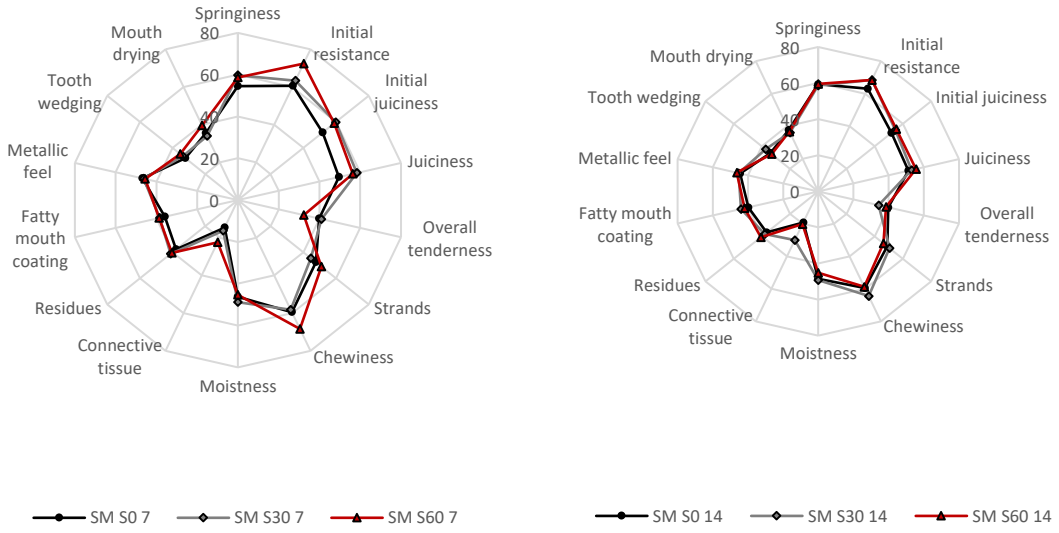


Figure 5-7. Spider plot displaying the differences in texture and after feel properties of PEF treated and non-treated topside steaks after (**Left**) 7 days and (**Right**) 14 days of storage (**SM** – topside, **S0, S30, S60** – 0, 30, 60 ms treatment time, 7 and 14 days storage time).

The three samples that were treated by PEF for 30 ms shared similar characteristics except for *initial resistance* and amount of *connective tissue*. The sample stored for one day was perceived as more resistant to initial compression than the sample stored for seven days. The sample stored for 14 days was perceived as having significantly more connective tissue than the others. The three samples which were treated by PEF for 60ms were perceived to significantly differ only in their *overall juiciness* ($p < 0.05$). The sample stored for only 1 day was perceived as the least *juicy*.

5.1.5.2 Sensory panel assessment of the striploin

Sensory descriptive analysis results of the striploin muscle samples showed that the majority of in-mouth texture attributes including: *springiness* and *initial resistance,* overall *tenderness, strands* and *chewiness* were found to be significant ($p < 0.05$). Whereas the results for the after-feel attributes, showed that none of these attributes were found to significantly differentiate between the samples ($p > 0.05$).

Only the effect of treatment time and storage time on the texture attributes are further discussed (after-feel attributes are not presented as they were not significant). Values are presented as (a) the

mean of the three storage times (1, 7 and 14 days) for each treatment time (0, 30, and 60 ms), and (b) the mean of the three treatment times for each storage time.

Increasing treatment time had a significant ($p < 0.05$) effect on *overall tenderness*, *stands* and *chewiness* for striploin muscle samples evaluated. It seemed that application of PEF to the steaks decreased *tenderness* and increased the amount of *stands* perceived and *chewiness* of striploin samples treated for 30 ms.

Increasing storage time had a significant effect on *initial resistance, overall tenderness, strands* and *chewiness* ($p < 0.05$). It seemed that increasing the PEF treatment time applied to the striploin muscles increased *tenderness* during product mastication, decreased the amount of *strands* perceived during product breakdown and decreased the perceived *chewiness* of the sample.

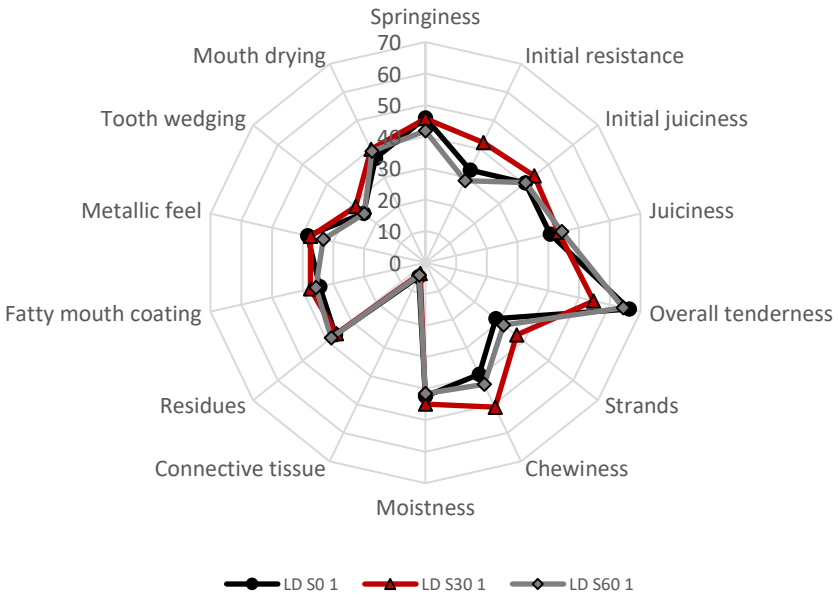


Figure 5-8. Spider plot displaying the differences in texture and after-feel properties of PEF treated and non-treated striploin steaks after 1 day of storage (**LD** – striploin, **S0, S30, S60** – 0, 30, 60 ms treatment time, **1** day storage time).

There was a significant interaction between treatment time and storage time on *springiness* and *initial resistance*.

Figure 5-9 presents the PCA bi-plot of the significantly discriminating attributes. The first two principal components explain 94.5% of the variance within the sample set (PC1: 74.7%; PC2: 19.8%, Figure 5-9). The first principal component (PC1, as seen from left to right in Figure 5-9) was associated with *chewiness* and *tenderness* and separated the 30 ms and 60 ms treated samples stored for 1 and 7 days, as well as the un-treated sample stored for 1 day, from the three 14 day storage samples and the untreated 7 day storage sample. The 14 day storage samples and untreated 7 day storage samples were perceived as being *tender overall*, where the remaining samples were perceived as being *chewy, resistant*, *spring* and having *strands*.

The second principal component (PC2, as seen from bottom to top in Figure 5-9) was associated with *springiness* and *resistance* (first bite) and *chewiness* and *strands* (during chewing) and separated the 30 ms treated samples, stored for one and seven days, and the 60 ms treated sample, stored for one day, which were perceived as being *chewy* and with *strands*, from the 60 ms treated sample stored for 7 days, which was perceived as being more *springy* and *resistant*.

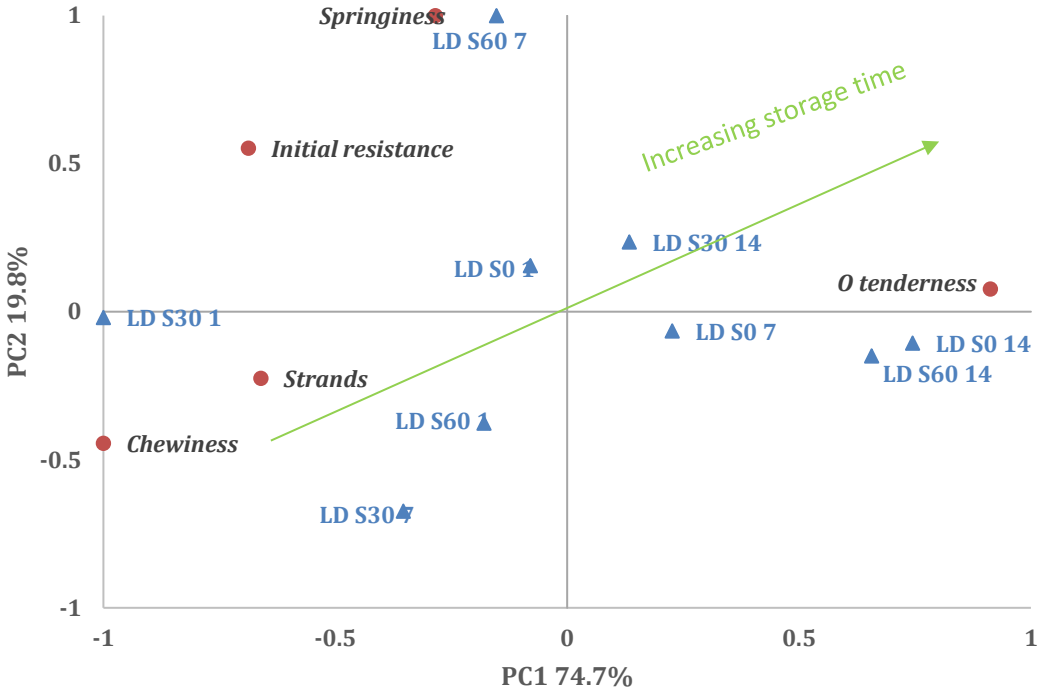


Figure 5-9. PCA bi-plot displaying the two-dimensional sensory space for texture attributes of the PEF treated striploin (LD) samples. Only the significant attributes according to the ANOVA are plotted.

Significant differences were perceived among the non-treated samples for *chewiness.* The 14 day storage sample was perceived as less *chewy* than the remaining two samples. There were significant differences perceived in *springiness, initial resistance,* and *chewiness* between the samples treated by PEF for 30 ms and stored for 1, 7 and 14 days. The largest differences were perceived between the samples stored for 1 day and the samples stored for 14 days. With increasing storage time, the perceived *initial resistance* and *chewiness* decreased while the perceived *tenderness* increased. *Springiness* was affected only by storage time. The samples treated by PEF for 30 ms and stored for 7 days was perceived as the least *springy*.

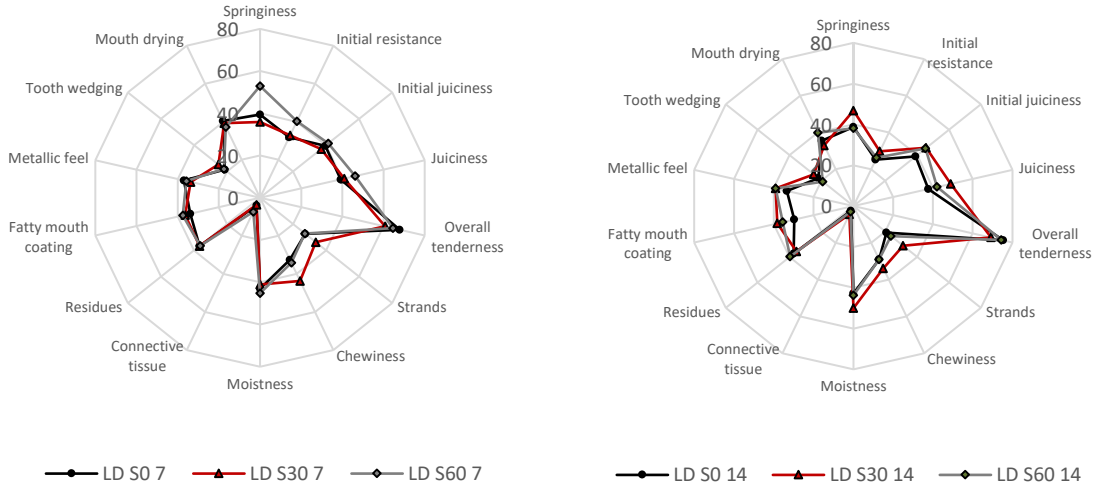


Figure 5-10. Spider plot displaying the differences in texture and after feel properties of PEF treated and non-treated striploin steaks after (**Left**) 7 days and (**Right**) 14 days of storage (**LD** – striploin, **S0, S30, S60** – 0, 30, 60 ms treatment time, 7 and 14 days storage time).

Significant ($p < 0.05$) differences were observed for *springiness, initial resistance, overall tenderness* and *chewiness.* The samples stored for 7 days were perceived as being the most *springy* and more resistant *to initial compression with front teeth*. The sample stored for 1 day was perceived as being the *chewiest* and the sample stored for 14 days was perceived as being the *most tender* overall.

The descriptive analysis results of the texture and after-feel characteristics revealed, that eleven of the fourteen measured attributes (*springiness, initial resistance, initial juiciness, juiciness, overall tenderness, strands, chewiness, connective tissue, fatty mouth coating, metallic feel and toothwedging*) showed significant ($p < 0.05$) perceived textural differences between samples.

5.1.5.3 Comparing sensory panel assessment of the striploin and topside

The largest differences that were perceived between all of the samples were *initial resistance*, *overall tenderness* and *chewiness* regardless of the treatment. Overall, the largest differences were seen between each muscle cut, PEF treated and control. Topside samples were perceived as *springier*, more *resistant* to initial compression, *juicier*, less *tender*, with a larger amount of *strands*, *chewier*, with more *connective tissue*, more *metallic* and *tooth wedging* than striploin samples. Although significant, the difference between topside and striploin muscle was less obvious for *fatty mouth coating.*

Samples from the topside PEF treated for 30 ms and stored for 14 days (SM S30 14) were perceived as having the largest amount of *connective tissue*. This indicates that perceived textural characteristics for this sample might need to be interpreted with caution as the amount of connective tissue might have interfered with the evaluation of other textural characteristics.

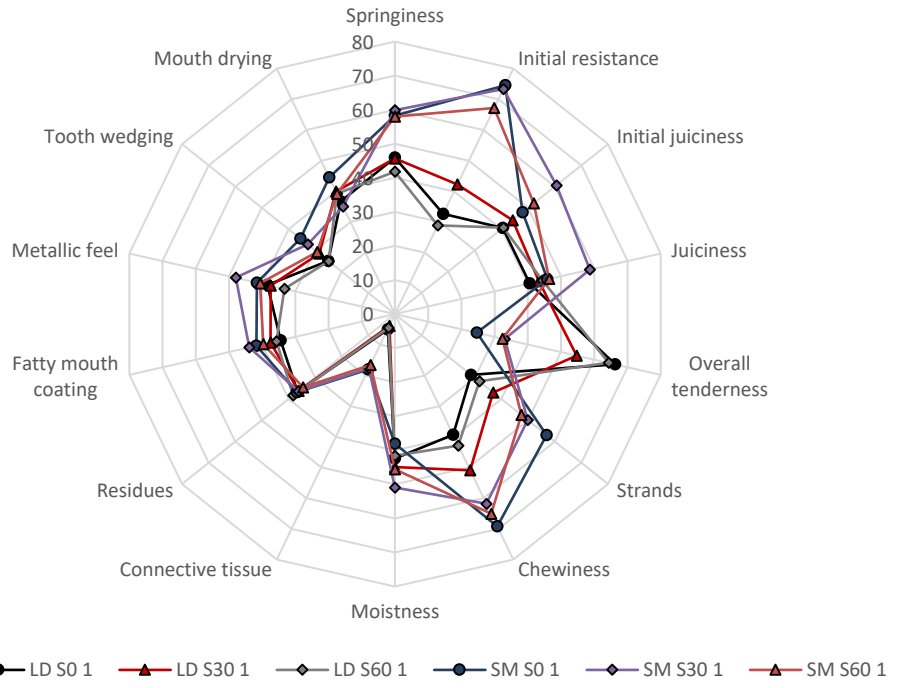


Figure 5-11. Spider plot displaying the differences in texture and after feel properties of PEF treated and non-treated striploin and topside steaks after 1 day of storage (**LD** – striploin, **SM** – topside, **S0, S30, S60** – 0, 30, 60 ms treatment time, **1** day storage time).

The overall sensory assessment showed that eleven of the fourteen texture attributes (*springiness, initial resistance, initial juiciness, juiciness, overall tenderness, strands, chewiness, connective tissue, fatty mouth coating, metallic feel and tooth wedging*) were significantly different between all of samples, i.e. muscles, storage times and treatment times. Muscle samples treated with PEF were perceived as less *tender*, but more *juicy* than untreated control samples. For all significant sensory attributes, PEF treated topside muscle samples were perceived as different from processed striploin samples. PEF treated topside samples were perceived as *springier*, more *resistant* to initial compression, *juicier*, less *tender*, with a larger amount of *strands*, *chewier*, with more *connective tissue*, more *metallic* and *tooth wedging* than striploin samples.

Five of the fourteen texture attributes were found to significantly differ within the topside muscle samples. These were: *initial resistance, initial juiciness, juiciness, overall tenderness* and *connective tissue*. PEF treatment time was perceived to have an effect on *initial juiciness* and *juiciness* and storage time was perceived to have a significant ($p < 0.05$) effect on *initial resistance, overall tenderness* and *connective tissue.* This means that samples treated with PEF were perceived to be the same as untreated samples in relation to tenderness. Five of the fourteen sensory attributes significantly discriminated the striploin muscle samples. These were: *springiness, initial resistance, overall tenderness, strands* and *chewiness.* PEF treatment time was perceived to have a significant effect on *overall tenderness*, *strands* and *chewiness* of the striploin, which was not the case for the instrumentally measured texture data, where PEF treatment did not show any significant difference on the PF value.

The storage time was perceived to have a significant effect on *initial resistance, overall tenderness, strands and chewiness*. Which was similar to the instrumentally measured texture data. The shorter PEF treatment time (30 ms) had the biggest impact on the textural properties of the striploin samples. This resulted in a *chewier* product, which was perceived as less *tender* and containing more *strands*. Increasing storage time increased overall *tenderness*, decreased *initial resistance, strands* and *chewiness*.

Sensory texture data cannot be directly compared to instrumentally measured texture data. Differences between the sensory texture data and instrumentally measured texture data is the result of different cooking regimes used for each method. Samples, which were instrumentally measured were cooked in a water bath to internal temperature of 80 °C for 60 min to ensure comparability between different experiments. Whereas, samples presented to the sensory panel were cooked on a hot Silex plate for 4 min until the internal temperature of each sampled reached at least 75 °C, which was aimed to get a more realistic eating context of the consumer. Therefore results obtained from both methods should be interpreted with care due to the different cooking methods of the samples.

5.1.6 Cost-benefit analysis

5.1.6.1 Introduction

Currently, two to three companies supply industrial-scale, state of the art PEF systems for application in the food industry, with Elea GmbH being the market leader with over 50 systems sold worldwide (Stefan Toepfl, personal communication). Contact details of the main PEF equipment manufacturers are shown in Table 5-1.

Table 5-1. Industrial-scale PEF equipment manufacturer

	Elea GmbH	Diversified Technologies, Inc.
Country	Germany	United States of America
Address	Prof.-von-Klitzing-Str. 7 D-49610 Quakenbrück	35 Wiggins Ave Bedford, MA 01730
Contact	Ph: +49 5431 183 – 442 E: info@elea-technology.com	Ph: +1 781-275-9444 E: info@divtecs.com
Website	www.elea-technology.eu/	www.divtecs.com/

Each equipment manufacturer has several PEF systems on offer, typically ranging from 5 up to 240 kW continuous power supply. Application of industrial-scale PEF systems in the food industry is diverse ranging from fruit juice preservation to texture modification of vegetables and waste water treatments. Most PEF systems are operated in a continuous mode where food is pumped through a set of electrodes where the treatment takes place. PEF treatment of solid foods like potatoes is often performed by submerging and conveying the solids through a water bath where the PEF is applied.

5.1.6.2 Estimation of PEF treatment costs for meat tenderisation

Cost model variables

To estimate the PEF treatment costs for meat tenderisation, a number of process, production, and cost variables were considered and the parameters used as model input variables are shown in Table 5-2. These included: yearly days of production, daily working hours, hours per shift, applied electric field, treatment time, cycle time, treatment chamber volume, product density, costs per unit electricity, costs for spare parts per cycle, factory overheads, labour costs, depreciation period and interest rate. The variables were CSIRO estimates and largely based on experience and personal communications with industrial PEF users and PEF equipment manufactures. Other process and production variables such as total cycle time, electric power demand, hourly product capacity, yearly cycles, yearly total throughput, total labour costs, etc. were direct or indirect functions of other specific model input variables.

The total cycle time is the sum of chamber loading, PEF pulsing time, and chamber unloading. The general assumption was that loading and unloading of the chamber would take 15 s each, and a total of 30 ms PEF pulses would be delivered to the meat within 1 min. Thus, the total cycle time is 1.5 min.

The total labour costs per year were estimated for operators (and engineers) required to effectively operate the PEF system. There were 2 shifts per day of 8 hours each. It was assumed that one operator ($45,000/year) and 0.1 of an engineer ($70,000/year) would be required to effectively operate the system. It is acknowledged that the number of operators can vary depending on the throughput of the PEF system and can be reduced by implementing automated loading and unloading of suitable baskets. In this cost assessment, a PEF treatment chamber volume of 10 L, holding approximately 11 kg of meat, was assumed.

For the cost benefit analysis, the PEF treatment time using 0.25 kV/cm pulses was increased from 10 to 30 ms since better meat tenderisation was found under these conditions.

Table 5-2. Estimated PEF costs factors used in this study

Cost Factors	Variable	Units
Exchange rates [A]		
USD:AUD	1.29	-
EUR:AUD	1.51	-
General factors		
Electric power	0.18	AUD/kWh
Factory overheads	10	%
Depreciation period	10	Years
Interest rate per period	10	%
Production parameters		
Applied Voltage	8,250	V
e-Field strength	25,000	V/m
e-Conductivity	1.2	S/m
Resistive Load	8.0	Ohm
Electrical current	1,031	A
Power required	255	kW
Yearly days of production	312	d/year
Production hours per day	16	h/d
Cycle time	1.5	min
PEF treatment time	30	ms
Treatment chamber volume	10	L
Product density	1.1	kg/L
Labour		
Engineer(s) per shift	0.1	-
Worker(s) per shift	1	-
Annual costs per Engineers	70000	AUD/year
Annual costs per Workers	45000	AUD/year
Hours per shift	8	h
Number of shifts per day	2	-

[A]Exchange rates as of 1 April 2016

PEF processing cost structure analysis

Table 5-3 shows the estimated PEF processing costs per kg of meat using production parameters outlined in Table 5-2. The yearly operating costs for this hypothetical PEF system were $160,332. These costs were largely dependent on costs for labour, depreciation, and factory overheads (Table 5-3). The processing costs per tonne of product was estimated to be approximately $73.

Table 5-3. Cost structure of PEF tenderisation of meat using the estimates of Table 5-2.

Parameter Description	Value	Units
Days of production per annum	312	d
Hours per day	16	h/d
Applied Voltage	8,250	V
e-Field Strength	25,000	V/m
Starting temperature	5	°C
Electrical conductivity	1.2	S/m
Resistive Load	8.0	Ohm
Treatment chamber volume	10	L
Cycle time	1.5	min
PEF treatment time	30.0	ms
Package size	10.0	kg
Product density	1.10	kg/L
Capacity per cycle	0.010	m^3/Cycle
Cycles per annum	199,680	1/a
Capacity per hour	0.400	m^3/h
Production per annum	1,997	m^3/a
Cycles per day	640.0	1/d
Electric power demand	12.762	kWh/m^3 product
Basic capital	188,750	AUD
Installation / Commissioning	7,550	AUD
Freight	7,550	AUD
Total capital	203,850	AUD
Maintenance	0.02	AUD/cycle
Factory overheads	10	% of total cost per year
Depreciation period	10	a
Interest rate per period	10	%
Electric power	4,587	AUD/a
Spare parts	3,994	AUD/a
Labour costs	104,000	AUD/a
Depreciation charge	33,176	AUD/a
Miscellaneous costs	14,168	AUD/a
Sum	160,332	***AUD/a***
Cost per m^3 product:	**80.29**	**AUD/m^3**
Cost per tonne product:	**72.99**	**AUD/t**
Cost per cycle:	**0.803**	**AUD/cycle**

PEF processing costs consisted of the costs for electricity, labour, maintenance, depreciation, and factory overheads. The largest contribution of approximately 67% to the total processing costs was from labour (Table 5-3). Depreciation costs contribute approximately $33,000/year (or 21% of the total

costs) and factory overheads (miscellaneous costs) were in the range of $14,000/year (9% of total costs) due to the fixed factory overhead rate chosen for this study.

Electric energy is used to generate the electric field required for meat tenderisation. The power demand of a PEF system of 10 L treatment chamber volume and capable to deliver 0.25 kV/cm for 30 ms requires approximately 255 kW peak power supply during the treatment (Table 5-2). However, due to the very short treatment time (30 ms) to total power consumption is relatively low (12.7 kWh/m^3 meat) and, thus, the costs for electricity are relatively low ($4587/year) assuming common industry costs for electricity in 2016 in Australia. Wear and tear (e.g. erosion of electrodes or lifespan of switches and capacitors) is expected to be low for this type of system and application. Maintenance costs were estimated to be in the range of 2 cents per cycle or approximately $4,000/year.

Sensitivity analysis

A sensitivity analysis of the cost model offers the possibility of examining the influence of individual process variables on processing costs. Figure 5-12 shows the relationship between important process variables such as PEF chamber filling efficiency, annual production, depreciation period, labour costs and cycle time on the overall PEF processing costs per kg of product ($/kg).

The PEF chamber filling efficiency describes the ratio of product weight (kg) to PEF chamber volume under operating conditions outlined in Table 5-2. In addition to the loading capacity (product weight/usable chamber volume), the filling efficiency (product weight/total chamber volume) also considers the chamber volume that cannot be used for product loading. Depending on the packing of the meat in the chamber, voids possibly remain between pieces. Based on the assumptions made in Table 5-2, the course of the PEF process costs as a function of the chamber filling efficiency can be seen in Figure 5-12A. Processing costs increase substantially when reducing the chamber filling efficiency. A chamber filling efficiency of less than 50% increases the processing cost per kg of product exponentially and, thus, should be avoided.

The applied electric field strength (kV/cm) is a process parameter that largely impacts on process intensity and determines the extent of electrical current flowing through the product during the treatment. As a rough guideline, the electric current, and thus the electric power consumption, increases exponentially with the applied electric field strength. Therefore, it is not surprising that the processing costs increase exponentially with increasing applied electric field strength (Figure 5-12B). However, the increase is only a few cents/kg over a relatively wide range of electric field strengths.

The cycle time of treatments highly affects the throughput of the PEF system and, consequently, the processing costs per unit product. The longer the cycle time the higher the processing costs (Figure 5-12C). PEF costs appear to linearly increase with increasing cycle time. The increase of the cycle time from 1 to 3 min (e.g. because of a longer PEF treatment time) results in an increase of the PEF costs by approximately 10 cents/kg. Obviously, is important to keep PEF ‘down time’ (i.e., the time for loading/unloading of the chamber) appropriately short.

The labour costs, which are often an important process variable in food production, also affect the total processing costs of PEF. PEF costs linearly increase as a function of the labour costs (Figure 5-12D).

It is not surprising that PEF costs per kg of product exponentially increase with decreasing annual production at fixed depreciation costs (Figure 5-12E). For example, a reduction of the annual production from 4000 to 2000 tonnes per year increases the PEF costs by approximately 3 cents per kg product. However, it should be noted that the depreciation period (and thus annual depreciation costs) should be adjusted to the yearly number of PEF cycles (which are dependent on the annual processing hours). This adjustment would flatten the course of the curve and reduce the dependence of processing costs.

The processing costs also show an exponential course as a function of the depreciation period (Figure 5-12F). A reduction of the depreciation period from 10 to 2 years increases the processing costs by approximately 5 cents/kg.

Other processing factors like interest rates or the cost for a kWh of electrical energy linearly increase the total processing costs (dependence not shown). For example, doubling electricity costs from 18 to 36 cents/kWh would only increase processing costs by $0.02 from approximately $0.73 to $0.75 per tonne of product. Thus, the increase of processing costs is relatively small and dependent on the system capital costs and energy demand.

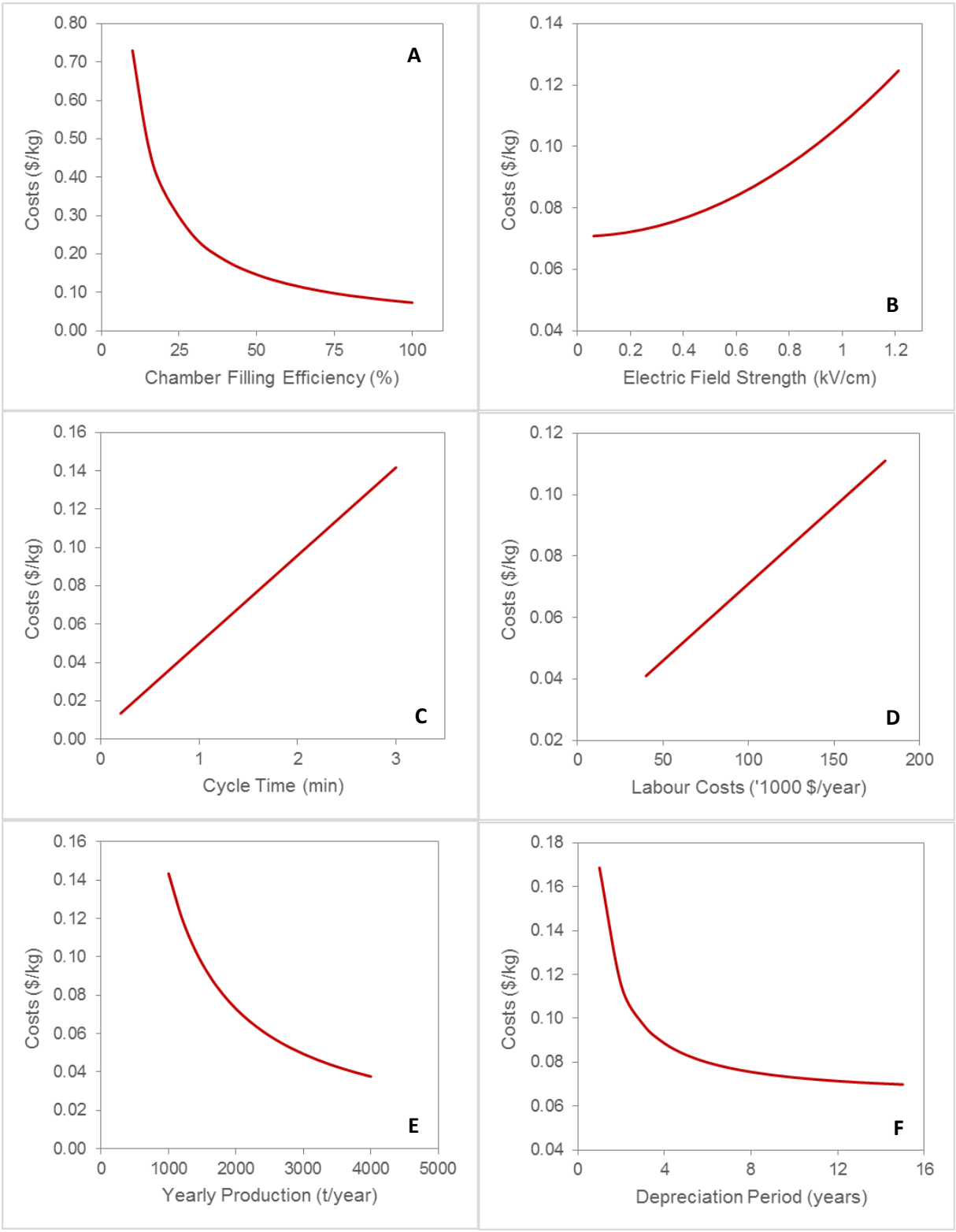


Figure 5-12. Effect of the PEF chamber filling efficiency (A), applied electric field strength (B), cycle time (C), labour costs (D), annual production (E), and depreciation period (F) on the total PEF costs per kg product. All process variables are taken from Table 5-2 and Table 5-3.

5.1.7 Conclusions

The effect of PEF treatment on instrumentally measured texture was muscle-dependent. A PEF treatment time of 30 and 60 ms resulted in increasing peak force (PF) for the striploin after 1, 7 and 14 days (i.e. toughening). In contrast, the same processing conditions applied to the topside resulted in increase of PF after 1 day of storage (i.e. toughening as well) but a decrease of PF after 7 and 14 days of chilled storage (i.e. tenderisation). An improvement in tenderness (16.4% reduction in shear force value) with PEF treatment (0.25 kV/cm, 100 Hz) for 30 ms was observed in topside muscle after 7 day storage at 4 °C. PEF treatment did not significantly ($p > 0.05$) affect cook loss or colour parameters of beef striploin and topside.

PEF processing for meat tenderisation is potentially a commercially viable option for the Australian Meat Industry to improve tenderness of low-value cuts and/or accelerated ageing. The costs of processing are estimated to be approximately 7.3 cents per kg of meat. This is mainly due to low product throughputs associated with a batch treatment chamber design and relatively high labour costs due to manual loading and unloading of meat cuts.

Labour costs for running PEF systems for meat tenderisation make up approximately 66% of the overall processing costs/m³ product. However, labour costs are highly dependent on the degree of automation for loading and unloading of product from the treatment chamber. Today, intelligent loading and unloading systems are offered by manufacturers which can significantly enhance the cost performance of PEF production lines (but requires high capital investments initially). Ultimately, continues PEF treatment systems for solid foods using conveyor belts and flexible electrodes should be developed to increase throughput and minimise labour requirements.

5.2 US processing

5.2.1 The effect of US treatment on meat texture

US treatment of brisket (Figure 5-13) did not appear to have a significant ($p > 0.1$) effect on the PF value overall, even though, the US treated brisket samples (40 kHz, 1min) showed a slight decrease of PF values directly after processing on day 0. The control brisket samples (untreated) had lower PF values than for all US treatment conditions (40/80kHz for 1 or 5 min) after 1 day storage and very similar PF values, after 14 days of storage, for some US treatment conditions (40kHz for 5 min, 80kHz for 1 and 5 min). On the other hand, storage time had a significant ($p < 0.05$) effect on the meat texture. Longer storage times resulted in decreased PF and IY values. Despite the increase in PF of the brisket after US treatment on day 1, all the PF values on day 14 were in a narrow range of 53 – 61N.

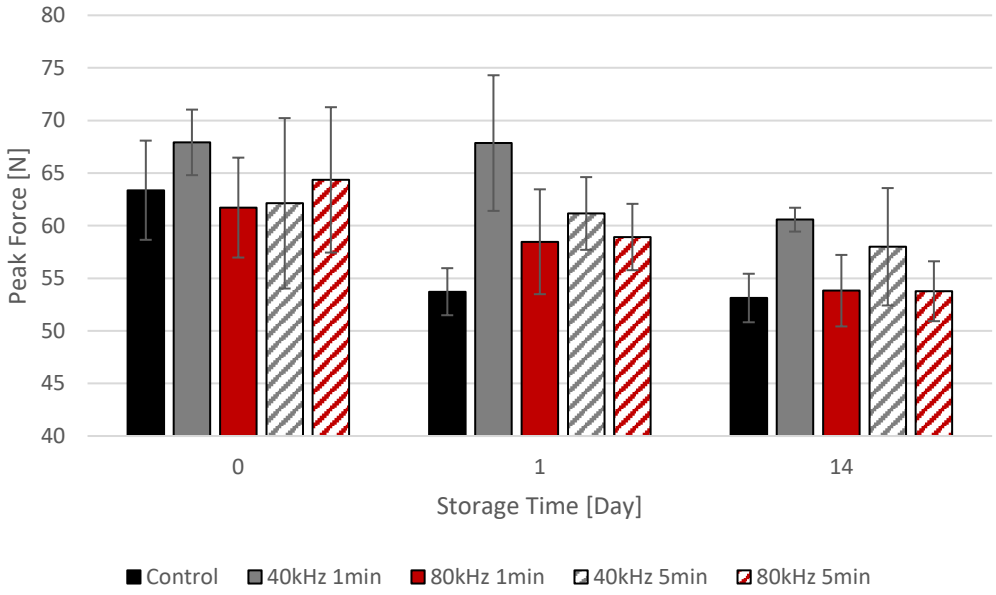


Figure 5-13. Effect of US treatment (40 and 80 Hz applied for 1 and 5 min) on the WBSF value of beef brisket after 0, 1 and 14 days of storage. Values are mean ± standard error.

The interaction of US treatment and storage time had no significant ($p > 0.1$) effect on the PF of the brisket. PF values for US treatments after 1 day storage were slightly higher than those of the untreated control samples for all treatment conditions but the difference in the rate of PF decrease favoured US treatment conditions at higher frequency rates (80 kHz 1 min and 5 min, respectively).

5.2.2 Effect of US treatment on meat quality traits

Figure 5-14 shows the visual appearance of brisket samples after ageing for 14 days following US processing. No obvious discolouration, denaturation or deformation was detected due to US treatment compared to the untreated control samples.

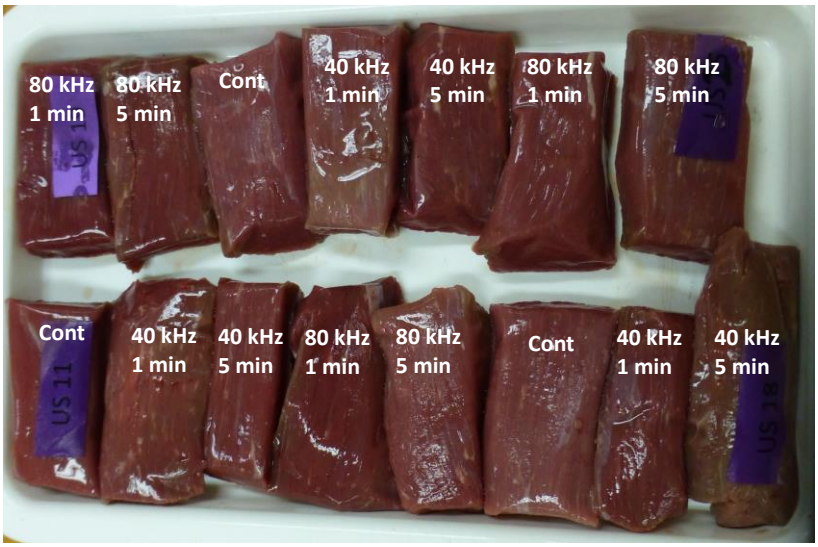


Figure 5-14. Visual appearance of beef brisket muscle and after ultrasound treatment at different intensities and ageing for 14 days at 4 °C.

In addition, statistical analysis of colour changes did not indicate any significant effects of US processing conditions on colour changes. Furthermore, the data did not confirm any significant changes in pH of the treated meat samples, which indicates that US technology does not impair quality parameters of the raw treated meat.

Storage of vacuum packaged brisket samples at 4 °C for up to 14 days had a significant (p<0.001) effect on cook loss, initial yield, peak force and the colour parameters of cooked meat with or without processing intervention. The cook loss, colour parameters L^* and a^*, all increased with storage time and peak force, initial yield and colour parameter b^*, all decreased upon storage.

5.2.3 Conclusion

US treatment at the selected conditions (40 and 80 kHz for 1 and 5 min, respectively) did not significantly affect pH or colour of raw meat or PF of aged (up to 14 days) and cooked brisket meat samples.

5.3 SW processing

5.3.1 The effect of SW treatment on meat texture

The application of a single SW treatment to striploin muscle resulted in a significant ($p < 0.001$) reduction in PF value at all storage times compared to the control: 12.4% at day 1, 8.2% at day 11 and 5.8% at day 21 (Figure 5-15). However, there was no effect of the double SW treatment on the texture of the striploin muscle compared to the control at day 1 and 11 but at day 21 the PF was reduced in SW-treated samples (Figure 5-15).

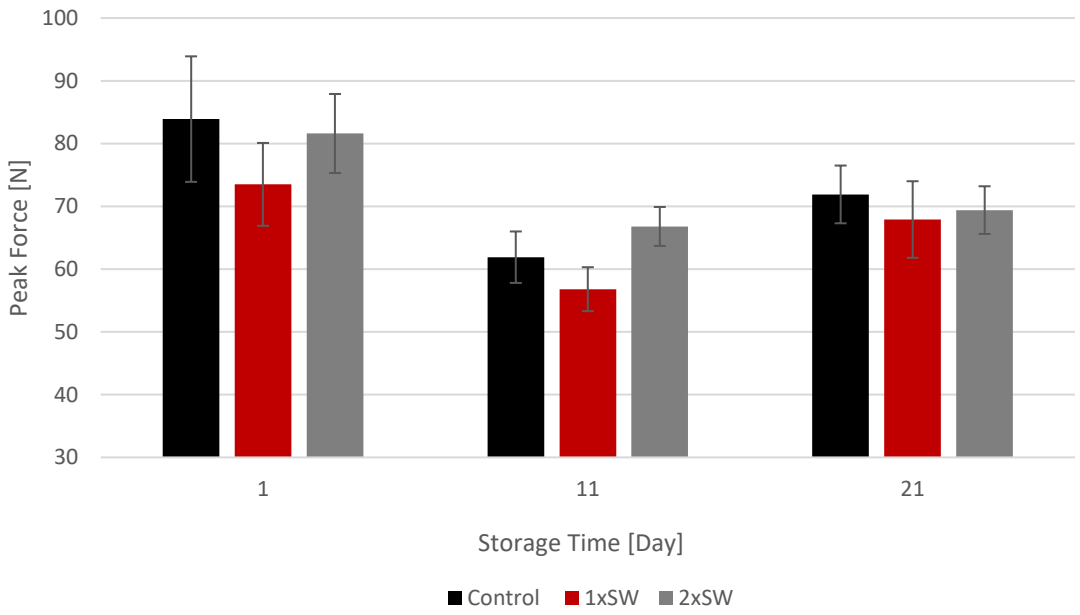


Figure 5-15. Effect of SW treatment (Control, no shockwave treatment; 1 X SW, single shockwave at 35kV, 1 pulse every 30 mm; 2 x SW, each side of the steak treated at 35kV, 1 pulse every 30 mm) on the Warner-Bratzler peak force value of beef striploin muscle. Symbols are means ± standard error.

As expected, the PF value decreased ($p < 0.001$) with storage for 11 days in all striploin samples (Figure 5-15). It is difficult to explain the subsequent increase in PF value at 21 days storage, but this is possibly due to experimental error.

SEM images of cross–sectional and longitudinal muscle sections were obtained to investigate the impact of SW on the muscle microstructure of striploin and eye round (Figure 5-16). In the case of striploin, no microstructural differences could be observed between the control and the SW treated samples (either single or double SW treatment). Furthermore, no remarkable modifications on the muscle microstructure could be detected with ageing time (1, 11 and 21 days). However, some differences were detected in the eye round treated with SW at day 1 of storage (Figure 5-16). A slightly larger intermuscular fibre space was detected in some muscle fibres (indicated with a white arrow in Figure 5-16). Thus, SW treatment may result in alterations to connective tissue as described previously (T. Bolumar, Bindrich, Toepfl, Toldra, & Heinz, 2014; Zuckerman, Bowker, Eastridge, & Solomon, 2013).

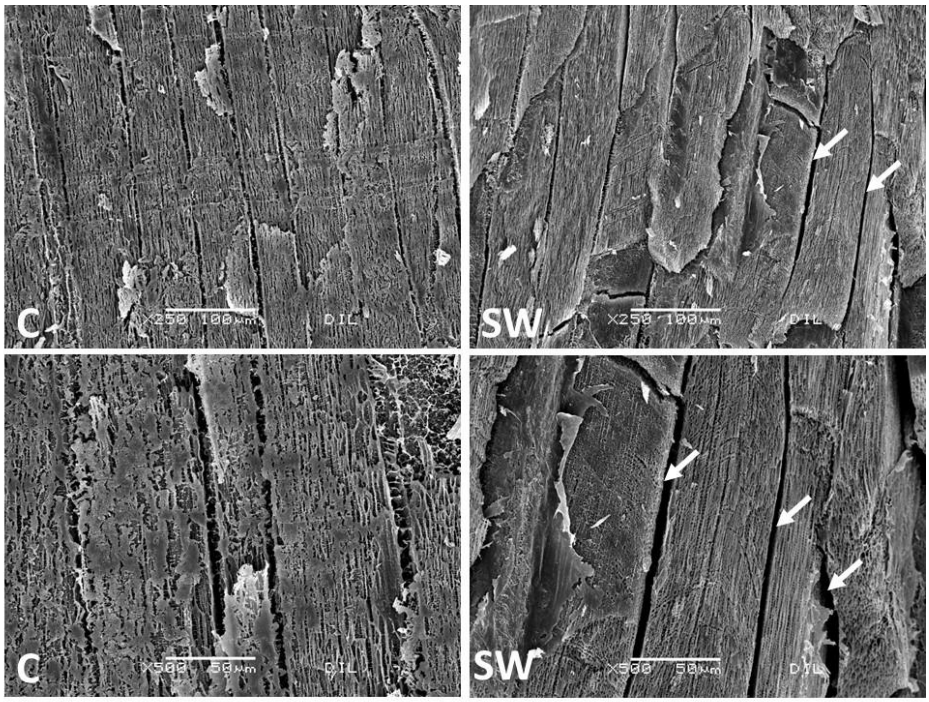


Figure 5-16. Scanning Electron Microscopy (SEM) images of eye round muscle fibre sections. C: control, untreated; and SW, shockwave treatment. Upper panels: 250X magnification, 100 µm scale bar. Lower panels: 500X magnification, 50 µm scale bar.

5.3.2 Effect of SW treatment on meat quality traits

The effect of SW treatment on drip loss and cook loss can be observed in Table 5-4. Similar and consistent results were obtained for both muscles, striploin (Table 5-4), and eye round (data not shown), and for both water binding properties, drip loss and cook loss. Drip loss in both primal cuts was affected by storage time (progressive increase from day 1 to day 14) but not by the application of SW treatment. The same trend was observed for the cook loss which increased with storage time but not with the application of shockwave treatment. Overall, SW treatment did not have an effect on drip loss or cook loss. This is beneficial for its application as a tenderisation method in the industry.

SW treatment did not have a significant effect ($p > 0.1$) on the colour parameters (*L*, a*, b**) of meat at the same time of storage. In contrast, the storage time impacted all colour parameters (*L*, a*, b**) for all muscles (data not shown for the eye round muscle). In general, the lightness (*L**) value of the samples increased with storage time, whereas the redness (*a**) slightly decreased for both striploin and eye round. In conclusion, the SW treatment did not have a significant ($p > 0.1$) effect on the meat colour.

Table 5-4. Effect of SW treatment[A] on drip loss, cook loss and colour parameters (*L*, a*, b**) of beef striploin steaks after storage at 4 °C for 1, 7 and 14 days (mean ± standard error)

Shockwave treatment[B]	Storage time	Drip loss	Cook loss	*L**	*a**	*b**
	(days)	(%)	(%)			
Control	1	3.5 ± 0.2	21.5 ± 1.2	36.8 ± 0.4	14.6 ± 0.3	6.9 ± 0.3
SW	1	3.3 ± 0.4	21.4 ± 1.2	37.3 ± 0.3	14.2 ± 0.2	6.9 ± 0.2
2 X SW	1	3.5 ± 0.5	22.4 ± 1.6	36.9 ± 0.4	14.4 ± 0.2	7.0 ± 0.2
Control	11	6.2 ± 0.6	17.5 ± 0.9	38.4 ± 0.4	16.6 ± 0.2	7.9 ± 0.2
SW	11	6.5 ± 0.5	19.3 ± 0.8	38.6 ± 0.3	15.5 ± 0.2	7.5 ± 0.4
2 X SW	11	6.8 ± 0.9	21.7 ± 1.5	37.8 ± 0.4	15.6 ± 0.3	7.2 ± 0.3
Control	21	7.3 ± 0.7	24.7 ± 0.8	38.7 ± 0.3	14.1 ± 0.3	6.4 ± 0.2
SW	21	6.8 ± 0.8	23.1 ± 1.0	40.0 ± 0.4	14.0 ± 0.4	6.4 ± 0.3
2 X SW	21	5.8 ± 1.5	24.5 ± 1.5	40.7 ± 0.3	13.9 ± 0.3	7.2 ± 0.3
Statistical significance[C]						
Shockwave treatment		n.s	n.s	n.s.	n.s.	n.s.
Storage time		***	***	***	***	***

[A]Control (untreated), single shockwave treatment (SW) and double shockwave treatment (2 X SW).

[B]The shockwave treatment settings were 1 pulse every 30 mm at a voltage of 35 kV.

[C]+$p < 0.1$ * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ n.s. = non-significant

5.3.3 Conclusions

The application of SW processing resulted in a reduction of the PF by up to 10 N (12.4 % reduction) after 1 day of storage, showing that SW as an intervention has great potential for accelerated tenderisation of meat. There was no significant difference in tenderness between SW treated and control (untreated) meat with ageing (11 and 21 days of storage time). The use of SW as an intervention did not significantly affect drip loss, cook loss and colour of beef striploins, indicating the promise of this technology for adoption by meat industry. Further research is required to optimise this intervention for use by industry.

6.0 CONCLUSIONS & RECOMMENDATIONS

Conclusions

PEF processing showed potential for meat tenderisation with improvement of 5 – 10% reduction in PF after 14 days of storage. However, there was no effect on the tenderness and inconsistencies were apparent with the application of PEF treatments to different beef primal cuts such as striploin, topside and rump. Therefore at present, more research is warranted to validate the specific PEF settings to be applied to a specific raw beef cut in order to improve meat quality traits such tenderness.

US processing did not improve the tenderness of beef brisket. Although previous work has demonstrated the potential of this intervention technology for meat tenderisation, the processing conditions required to improve meat tenderness under industrially feasible conditions are still not clear.

SW processing showed potential for accelerated tenderisation of beef striploin steaks with a reduction of 10 N (12. 4 %) in the PF value compared to the control (untreated). SW technology is a non-thermal and non-invasive, and is a promising post-slaughter method for accelerated tenderisation. However, further research is required, particularly to define the treatment conditions for different primal cuts and for the adaptation of SW-resistant packaging materials. CSIRO's Food Innovation Centre in Brisbane has recently installed a SW unit and is going to seek research and development initiatives in partnership with industry to overcome these limitations.

Recommendations

More fundamental research is required to understand the effect of PEF, US and SW processing on meat structure and the underlying mechanisms mediating tenderisation. The Australian Red Meat Industry has an opportunity to fund research in this area and become a world pioneer on the use of novel methods for meat tenderisation which ensure tender meat and maintain Australian's reputation of high quality beef in domestic and export markets.

SW technology is a highly promising technology for meat tenderisation, and investment in shockwave technology could provide the industry with a new method for meat tenderisation. This will improve the quality of Australian beef and could potentially increase the shelf life of meat cuts in domestic and export markets.

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Kai Knoerzer – reviewer

Damien Frank – reviewer

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