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Enhancement of meat quality by pulsed electric field application

Abstract

The effect of PEF on the quality of beef loins (1 day post-mortem) and topsides (1 and 3 day post-mortem) was investigated using a range of treatment intensities. The investigated parameters reported here are purge loss, cooking loss and meat tenderness (shear force) to identify the optimum PEF treatment conditions for each muscle that will be used subsequently for investigating colour and lipid stabilities. The tenderness of the loin samples was found to benefit from PEF treatment (19.5% reduction in the shear force) regardless the electrical input whereas the level of tenderness of the topsides was increased by increasing the treatment frequency (4.1, 10.4 and 19.1% reduction in the shear force at 20, 50 and 90 Hz, respectively). Higher purge loss (%) and lower cooking loss (%) were found in PEF treated samples, but the total losses were similar. It is interesting to observe that the level of SM tenderness improvement was not dependent on the meat post-mortem time which will allows the use of PEF technology without any post-mortem time constraints up until 3 days post-mortem.

The effect of repeat PEF treatment (0, 1, 2 and 3 repeats) under optimal conditions (20µs, 10kV, 90 Hz) on the quality of beef loins and topsides (1 day post-mortem) was investigated. Also, the effect of the meat's pH and fibre direction on the quality of PEF treated beef loins was studied. The investigated parameters were purge loss, cooking loss, meat tenderness (shear force) and colour and lipid stabilities. The tenderness of the loin samples was found to benefit from repeated PEF treatment (on average a 2.5 N reduction in the shear force for every extra PEF treatment). The purge loss of the topsides was significantly ($P = 0.036$) increased by PEF regardless the number of repetitions. Higher cooking loss (%) was found in repeat PEF treated loins, but not the topsides. The redness of the loins and topsides decreased and the hue angle was increased by increasing the PEF repeat treatment. No effect of pH and fibre direction on PEF was found.

Further, the effect of PEF on the quality of hot-boned beef loins (*Longissimus lumborum, LL*) and topsides (M. *Semimembranosus, SM)* was investigated using a range of treatment intensities (20 µs; voltage = 5 and 10 kV; and frequency = 20, 50 and 90 Hz). Also, the effect of repeat PEF treatment (0, 1, 2 and 3 repeats) under optimal conditions (20µs, 10kV, 90 Hz) on the quality of hot-boned beef loins and topsides was investigated. The investigated parameters reported here are purge loss, cooking loss, changes in pH and conductivity, and shear force to identify the optimum PEF treatment conditions for each muscle.

The tenderness of the hot-boned SM samples was found to benefit from PEF treatment (21.6% reduction in the shear force) regardless of the electrical inputs, whereas the shear force level of the LL tended to increase by increasing the treatment frequency. Treated LL muscles tended ($P = 0.08$) to have a higher cooking loss (%) compared with non-treated control (a 1.5% increase in cooking loss (%) was found in treated LL muscles compared with non-treated control), but no effect was found on purge loss (%). Opposite effects were found in SM where within the PEF treated samples there was a tendency toward decreased cooking loss as a result of ageing ($P = 0.08$) and significantly higher purge losses ($P = 0.023$) in PEF treated samples regardless the intensity of treatment. The tenderness of the hot-boned LL samples was found to be negatively affected (higher shear force) from repeated PEF treatment ($P = 0.03$) whereas this treatment resulted in lower ($P = 0.014$) shear force in hot-boned SM at early ageing times (i.e 3 and 7 days of ageing). The purge loss (%) of LL and SM muscles was increased by the number of PEF repeats ($P = 0.004$ and 0.001, respectively). Lower cooking loss was found in LL samples treated by PEF 1x and 2x compared with controls at 3 and 7 days of ageing ($P = 0.028$). No effect for PEF on the cooking loss of hot-boned SM was observed which was consistent with the effect of repeated PEF on cold-boned SM.

Executive Summary

Background

The production of consistently tender meat is required to retain consumer confidence in red meat which is competing with other types of meat that intrinsically do not have toughness problems. Several reports have highlighted the importance of achieving certain pH and temperature levels for maximum aging and tenderness (e.g. pre-rigor temperature at 30°C to achieve pH_{3h} of 6.1 for beef and at 21°C to reach pH 6 for lambs) (Marsh et al., 1987; Thompson et al., 2005). However, given the differences in the glycolytic potential, requirements of different muscles for electrical stimulation and cooling rate of different muscles on the carcase; it is unrealistic to deliver the optimal conditions for tenderness and capture the maximum economical potential of different meat cuts for a whole carcase. A potential stand-alone technology that can be used pre-rigor or post-rigor with multiple functions (enhance cell permeation/electroporation for enhancing tenderization or improve the safety of products by reduction of microbial load) is PEF. This technology could have particular benefits to hot-boned meat by allowing cut specific treatment prior to further processing and is not seen as a replacement for electrical stimulation of carcases, but as an improvement on this technology.

Despite the release of several industry briefs and reports (MIS, 2006; 2010; Midgley and Small, 2006) highlighting the potential benefits of PEF, there are limited studies on the use of PEF in meat processing (Toepfl et al., 2006) and to our knowledge only one recent report on fresh meat quality O'Dowd et al., 2013). PEF improved the microdiffusion of brine solution during ham processing (Toepfl et al., 2006), under mild working conditions enhanced the permeability of cellular components (Jaeger et al., 2008) and can improve the safety of products (Midgley and Small, 2006). Therefore, this technology can potentially accelerate the release of Ca^{++} and μ-calpain early post-mortem as well as stimulate the glycolysis process, all which are required for early proteolysis and establishing maximum tenderisation of meat. The advantage of PEF over other available technologies is the ability to optimize the technology input to different meat cuts and maximize the product quality. This can be translated into quality upgrades for meat cuts that are regarded as less tender and which return low prices. The adaptation of PEF can improve the safety of the final product and reduce the cost associated with carcase handling and conventional chilling regimes.

Research Objectives

- Investigate the effects of several levels of PEF treatments on the quality of meat.
- The impact of meat pH, fibre direction and muscle anatomy on the meat response to PEF treatments.

Key Findings

- Investigate the effects of several levels of PEF treatments on the quality of meat.

1. The tenderness of the loin samples was found to benefit from PEF treatment (19.5% reduction in the shear force) regardless of the electrical input, whereas the level of tenderness of the topsides was increased by increasing the treatment frequency (4.1, 10.4 and 19.1% reduction in the shear force at 20, 50 and 90 Hz, respectively).
2. The tenderness of the loin samples was found to benefit from repeated PEF treatment (on average a 2.5 N reduction in the shear force for every extra PEF treatment).

3. The tenderness of the hot-boned SM samples was found to benefit from PEF treatment (21.6% reduction in the shear force) regardless of the electrical inputs, whereas the level of shear force of the LL tended to increase by increasing the treatment frequency.
4. PEF treatment generally increased the purge loss and decreased the cooking loss. This effect, however, varied depending on the muscle type (LL or SM) and the post-mortem time (cold- or hot-boned).

Conclusions

Pulsed electric field technology can be used to improve the tenderness of cold- and hot-boned topsides and cold-boned loins. The technology appears to be flexible with topsides where the level of SM tenderness improvement was not dependent on the meat post-mortem time (within 3 days post-mortem) which will allows the use of PEF technology without any post-mortem time constraints. This needs to be confirmed with other muscles in combination with understanding what the biochemical basis of the tenderisation is. The technology is independent of the meat pH and fibre direction so it can be applied to meat without any limitations by these two factors.

Contents

1 Background

Meat tenderness is a major eating quality attribute that ensures consumer satisfaction and repeat purchase of red meat. Tenderness is arguably the most important quality attribute of red meat. After the meat is cooked many of the appearance attributes become irrelevant and flavour can be manipulated with other ingredients in the meal or added flavours. The production of consistently tender meat is required to retain consumer confidence in red meat which is competing with other types of meat that intrinsically do not have toughness problems (e.g. poultry) and to maximize the financial gain since tender meat cuts fetch a higher premium than the less tender meat cuts. Several reports have highlighted the importance of achieving certain pH and temperature levels for maximum tenderization rate during ageing and the overall tenderness level achieved (e.g. pre-rigor temperature at 30°C to achieve pH_{3h} of 6.1 for beef and at 21°C to reach pH 6 for lambs) (Marsh et al., 1987; Thompson et al., 2005). However, given the differences in glycolytic potential and cooling rate between muscles in carcases it is impossible to deliver the optimal conditions for tenderness development for each muscle and therefore to capture the maximum economic potential of different meat cuts for a whole carcase. A potential stand-alone technology that can be used pre- or post-rigor with multiple functions (enhance cell permeation/electroporation for enhancing tenderization or improving the safety of products by reduction of microbial load) is pulsed electric field (PEF) technology. Of particular interest are the reported cellular changes in muscle foods after PEF treatment (Gudmundsson and Hafsteinsson, 2001).

Despite the release of several industry briefs and reports (MIS 2010; Midgley and Small 2006) highlighting the potential benefits of PEF, there are limited studies on the use of PEF in meat processing (Töpfl and Heinz, 2007). PEF improved the microdiffusion of a brine solution during ham processing (Töpfl and Heinz, 2007); enhanced permeability of cellular components under mild working conditions (Jaeger et al., 2008); and can improve the safety of products (Midgley and Small, 2006). PEF technology can potentially accelerate the release of Ca^{++} and µ-calpain early post-mortem as well as stimulate the glycolysis process, all which are required for early proteolysis and establishing maximum tenderisation of meat. The advantage of PEF over other available technologies is PEF's ability to optimize the technology input to different meat cuts and maximize the product quality. This can be translated into quality upgrades for meat cuts that are regarded as less tender and which return low prices. The first detailed study on the use of PEF for improving the quality of fresh beef was recently published (O'Dowd et al., 2013). These authors reported no effect of PEF treatment (1.1-2.8 kV cm^{-1}, 5-200 Hz, 12.7-226 kJ kg^{-1}) on the tenderness of beef *M. Semitendinosus* muscle and significant weight loss as a result of PEF treatment. The study used a very small sample size and experimental units (sample = 30g, 3 muscles used) that cannot reflect the effects in a biological system such as meat. Additionally, the tenderness measurement was evaluated immediately after treatment without allowing an ageing time for the endogenous proteases to promote the tenderization process. That study, however, reported structural changes as indicated by fragmentation of myofibrils and electron microscope scanning which suggest potential tenderness improvement on subsequent ageing. Several conventional (physical, chemical and enzymatic) methods can tenderize meat to various levels (Bekhit et al., 2013; 2014a; 2014b). However, PEF may be a promising technology that can modify the structure of biological materials without the side effects encountered with these other methods (e.g. severe structural changes and off flavour development). It also has the advantage that it can be quickly applied (treatment time is in the order of seconds). Therefore, the present project investigated the potential use of PEF for improving the quality of meat at various ageing times from cold- and hot-boned beef muscles [Loins (M.

Longissimus lumborum) and Topsides (M. *Semimembranosus*)] treated by various PEF processing conditions.

2 Project Objectives

- Investigate the effects of several levels of PEF treatments on the quality of cold- and hot-boned beef loins and topsides.
- The impact of meat pH, fibre direction and muscle anatomy on the meat response to PEF treatments.

3 Material and methods

3.1 Pulsed electric field system (PEF) used in the present work

The PEF system (Elcrack- HPV5, DIL, Quakenburck, Germany) was used in batch mode and the meat fibre direction was parallel to the electrodes (Figure 1). An oscilloscope (Model UT2025C, Uni-Trend Group Ltd, Hong Kong, China) was used to monitor the pulse shape used (square wave bipolar). This system has the ability to deliver a wide range of electrical inputs (voltage = 0-25 kV, frequency = 0-1000 Hz and pulse width = 4-32 µs), but the electrical breakdown of the material and electric arching (reflected in the "flashover" count) can limit the range of parameters used.

A B C

Figure 1. Pulsed electric field system (Elcrack- HPV5, DIL, Quakenburck, Germany) used in the present research. A) the front of the instrument showing the control panel, B) the back of the instrument and C) the treatment chamber housed between the electrodes.

3.2 Sample preparation

The samples' description will be provided for each experiment (below). The muscles were trimmed from all visible fat and connective tissue, processed into blocks (average weight in g ± SD is

provided for each experiment below) to fit the PEF treatment chamber (dimensions of 13 × 8 × 5 cm) (Figure 2).

Figure 2. Preparation of meat blocks for treatment. The samples were cut to fit the PEF treatment chamber (dimensions of 13 × 8 × 5 cm) and the conductivity, pH and temperature were measured before and after treatment.

3.3 Experimental plans

The experimental plans consisted of 7 experiments (Figures 3 and 4) to investigate;

1) **Effect of pulsed electric field treatment and aging on the tenderness of beef loins under various levels of PEF.**

Loins (M. *Longissimus lumborum*) were obtained from 6 steers (average carcase weight was 303.4 ± 23.3 kg) raised on pasture and slaughtered by the Alliance Group (Pukeuri plant, Oamaru, NZ). The loins were removed from the carcases at 24h post-mortem. Both the left and right loins were collected from the 6 carcases and, after trimming all visible fat and connective tissue, processed into six blocks (average weight = 346.6 ± 26.5 g) to fit the PEF treatment chamber. The blocks (n = 6 for each carcase) were randomly allocated to 6 treatment combinations; voltages (5 and 10 kV) and frequencies (20, 50 and 90 Hz); plus a non-treated control. The experiment was designed (please see appendix 1 Milestone 3, *PROJECT NO. A.MQA.0005* for the experimental design) so that a block from each carcase was assigned as control and each non-control treatment was not included on one of the carcases only. The treatments were (5 kV and 20 Hz; LLT1), (5 kV and 50 Hz, LLT2), (5 kV and 90 Hz, LLT3), (10 kV and 20 Hz, LLT4), (10 kV and 50 Hz, LLT5), (10 kV and 90 Hz, LLT6) and LL control. The blocks were then sliced into 4 slices that were randomly assigned to 3, 7, 14 or 21 days of storage at 4°C. On the day of the designated storage time, several measurements were carried out (see below) and a subsample was frozen and stored vacuum packed at -40°C for further biochemical analysis. The remaining sections of the samples were frozen and stored at -20°C for tenderness (shear force) measurement which occurred within 1 week of freezing.

2) Effect of pulsed electric field treatment, post-mortem time and ageing on the tenderness of beef topsides under various levels of PEF.

Topsides (M. *Semimembranosus*) were obtained from 6 steers (average carcase weight was 299.2 ± 13.95 kg), raised on pasture and slaughtered by the Alliance Group (Pukeuri plant, Oamaru, NZ). Both the left and right topsides were collected from 6 carcases at 24 h post-mortem and the left topsides were processed/treated at 1 day post-mortem (1-d PM), while the right topsides were processed/treated at 3 day post-mortem (3-d PM). On the day of assigned post-mortem time, the topsides were cut into six blocks (average weight = 362.6 ± 24.9 g) after trimming visible fat and connective tissues. The samples (blocks) from each topside, were randomly allocated to six of the seven treatment combinations for each ageing period, these being voltages (5 and 10 kV) crossed with frequencies (20, 50 and 90 Hz) plus a non-treated control. The experiment was designed so that a block from each carcase was assigned as control and each non-control treatment was not included on one of the carcases only. The treatments were (5 kV and 20 Hz; SM1T1), (5 kV and 50 Hz, SM1T2), (5 kV and 90 Hz, SM1T3), (10 kV and 20 Hz, SM1T4), (10 kV and 50 Hz, SM1T5), (10 kV and 90 Hz, SM1T6) and SM1 control for 1 day postmortem SM samples and (5 kV and 20 Hz; SM3T1), (5 kV and 50 Hz, SM3T2), (5 kV and 90 Hz, SM3T3), (10 kV and 20 Hz, SM3T4), (10 kV and 50 Hz, SM3T5), (10 kV and 90 Hz, SM3T6) and SM3 control for 3 day postmortem SM samples. The meat samples were treated with the fibre direction parallel to the electrodes. The blocks were then sliced into 4 slices that were randomly assigned to 3, 7, 14 or 21 days of storage and subsequently treated as described for the loins above.

3) Effect of repeated pulsed electric field treatment and ageing on the tenderness, colour and lipid stability of beef.

1. Tenderness, purge loss, cooking loss, and conductivity

Loins (*Longissimus lumborum*) and topsides (M. *Semimembranosus*) were obtained from 6 steers (average carcase weight was 275.9 ± 14.0 kg, Grade SP2 = YPS AUSMEAT limited) raised on pasture and slaughtered by the Alliance Group (Pukeuri plant, Oamaru). The loins and the topsides were removed from the carcases at 24h post-mortem. The loins and topsides were randomly collected from the left and right sides of 6 carcases and processed randomly into blocks (average weight = 341.1 ± 33.5 g). The blocks from each muscle were allocated to 4 PEF treatment intensities (20µs, 10kV, 90 Hz) by repeating the treatment once (1x), twice (2x), three times (3x) or no treatment (0). The blocks were then sliced into 4 slices that were randomly assigned to 3, 7, 14 or 21 days of storage at 4°C (please see appendix 1, Milestone 3, *PROJECT NO. A.MQA.0005* for the experimental design). On the day of the designated storage time, several measurements were carried out (see below) and a subsample was frozen and stored vacuum packed at -40°C for further biochemical analysis. The remaining sections of the samples were frozen and stored at -20°C for tenderness (shear force) measurement which occurred within 1 week of freezing.

2. Colour and lipid stability

Loins and topsides were obtained from 6 steers (average carcase weight was 300.5 ± 36.69 kg, Grade P2 = PRS AUSMEAT limited) as described above from the Alliance Group (Pukeuri plant, Oamaru) at 24h post-mortem. The samples were processed as described above for tenderness. The samples at each designated post-mortem time were subsampled for lipid oxidation (TBARS 0 time) and the remaining portion of the samples were used for 9 days of colour display. At the end of the colour display a subsample was obtained to determine the lipid oxidation level at the end of the display time (TBARS end time).

4) The impact of meat pH and fibre direction on the meat response to PEF treatments.

Loins (*Longissimus lumborum*) of 3 different pH range (5.5-5.8, 5.8-6.1 and > 6.1) were obtained from 16 bulls raised on pasture and slaughtered by the Alliance Group (Mataura plant, South Island). The loins were removed from the carcases at 24h post-mortem and processed randomly into blocks as described above for PEF treatment. The blocks were treated either parallel or across to the electric current direction (the experimental design is shown in Appendix 2). The blocks were then sliced into 4 slices that were randomly assigned to 3 and 7 days of storage at 4°C (two slices each ageing time). On the day of the designated ageing, one slice was used for the measurement of purge loss, cooking loss, conductivity, tenderness, and pH. The other slice was used to examine the colour and lipid oxidation stability during simulated display at 4°C for 9 days.

5) Effect of pulsed electric field treatment and ageing on the tenderness of hot-boned beef loins under various levels of PEF.

Loins (*Longissimus lumborum*) were obtained from 6 prime cows (average carcase weight was 277.9 ± 28.3 kg, Grade CP2 = PR AUSMEAT limited) raised on pasture and slaughtered by the Alliance Group (Pukeuri plant, Oamaru). The loins were removed from the carcases at 4h post-mortem. The samples were delivered to the lab within 1 hour and the samples had an average temperature of 24.4 ± 1.3°C during processing. Both the left and right loins were collected from 6 carcases and processed randomly into blocks (average weight = 364.8 ± 26.3 g). The blocks were allocated to 6 treatment combinations; voltages (5 and 10 kV) and frequencies (20, 50 and 90 Hz); and a non-treated control. The blocks were then sliced into 4 slices that were randomly assigned to 3, 7, 14 or 21 days of storage at 4°C (please see appendix 1, Milestone 4, *PROJECT NO. A.MQA.0005* for the experimental design). On the day of the designated storage time, several measurements were carried out (see below) and a subsample was frozen and stored vacuum packed at -40°C for further biochemical analysis. The remaining sections of the samples were frozen and stored at -20°C for tenderness (shear force) measurement which was done within 1 week of freezing.

6) Effect of pulsed electric field treatment, post-mortem time and ageing on the tenderness of hot-boned beef topsides under various levels of PEF.

Topsides (M. *Semimembranosus*) were obtained from 6 prime cows (average carcase weight was 173.0 ± 10.6 kg, Grade CWM = YG AUSMEAT limited) raised on pasture and slaughtered by the Alliance Group (Pukeuri plant, Oamaru). The topsides were removed from the carcases at 4h post-mortem. The samples were delivered to the lab within 1 hour and the samples had an average temperature of 25.5 ± 1.8 °C during processing. The topsides were randomly cut into blocks (average weight = 361.9 ± 33.0 g) and allocated to 6 treatment combinations; voltages (5 and 10 kV) and frequencies (20, 50 and 90 Hz); and a non-treated control. The blocks were then sliced into 4 slices that were randomly assigned to 3, 7, 14 or 21 days of storage (please see appendix 1, Milestone 4, *PROJECT NO. A.MQA.0005* for the experimental design) and subsequently treated as described for the loins above.

7) Effect of repeated pulsed electric field treatment and ageing on the tenderness of hot-boned beef loins and topsides treated at the same level of PEF.

Loins (*Longissimus lumborum*) were obtained from 6 cows (average hot carcase weight was 279.3 ± 28.3 kg, Grade CP2 = PR AUSMEAT limited) and topsides (M. *Semimembranosus*) were obtained from another 6 cows (average hot carcase weight was 279.3 ± 28.3 was 177.9 ± 10.1 kg, Grade CWM = YG AUSMEAT limited). The different muscles were obtained on two consecutive days to accommodate for the logistics of PEF treatment. All animals were raised on pasture and slaughtered by the Alliance Group (Pukeuri plant, Oamaru). The loins and topsides were processed randomly into blocks (average weight was 353.8 ± 31.3 g and 369.3 ± 16.7 g for loins and topsides, respectively) to fit the PEF treatment chamber. The blocks from each muscle were allocated to 4 PEF treatment intensity (20µs, 10kV, 90 Hz) by repeating the treatment once (1x), twice (2x), three times (3x) or no treatment (0). The blocks were then sliced into 4 slices that were randomly assigned to 3, 7, 14 or 21 days of storage at 4°C. On the day of the designated storage time, several measurements were carried out (see below) and a subsample was frozen and stored vacuum packed at -40°C for further biochemical analysis. The remaining sections of the samples were frozen and stored at -20°C for tenderness (shear force) measurement which occurred within 1 week of freezing.

3.4 Measurements

Electrical input

The treatment electrical parameters (pulse electric field strength, pulse peak energy (PPE), pulse peak current (PPC), pulse peak power (PPV), pulse count, resistance, energy, calculated field strength (CSE) and calculated specific energy) are listed in Appendix 2, milestone 2.

pH

The pH of each block was measured before PEF treatment, after PEF treatment and after storage at 4°C for 3, 7, 14 and 21 days of treatment. The pH measurements were undertaken using a combination puncture pH electrode (InLab 427, Mettler-Toledo Process Analytical Inc., Wilmington, MA) attached to a pH-meter (Hanna HI 9025, Hanna Instruments, Woonsocket, RI). The

pH difference from the initial pH (pH before treatment) was calculated at various measurement points.

Temperature

The temperature of the centre of the meat blocks was measured using the combination puncture pH electrode immediately before PEF treatment and after PEF treatment. Additionally the temperature was recorded at several locations (8 locations/block) using a hand held infrared thermometer (Tech imports, Auckland, NZ) due to a temperature gradient found in some of the treatment combinations. The average of the 8 measurements was used for further analysis.

Purge loss percentage

Purge was measured after 3, 7, 14 and 21 days of vacuum packaged storage at 4°C. On the day of designated storage time, the samples were blotted dry using paper towel and weighed and the purge percentage was calculated using the following formula;
Purge loss (%) = 100 – (The weight after storage/Initial weight before storage * 100)

Electrical conductivity σ

The electrical conductivity (mS/cm) of each block was measured immediately before and after the PEF treatment and after 3, 7, 14 and 21 days of vacuum packaged storage at 4°C using a hand held electrical conductivity meter (LF-Star, Matthäus, Pöttmes, Germany). The conductivity measurements were affected by the measurement location and the fibre direction in the meat block. Therefore, four fixed locations per block were measured and the average was used for statistical analysis.

Cooking loss percentage

The samples were thawed overnight at 4°C, weighed and cooked individually in plastic bags immersed in a water bath at 80°C until they reached an internal temperature of 75°C as measured individually using Fluke type K temperature probes attached to Fluke 52 meters. The cooked meat was cooled on ice, patted dry with paper towels and re-weighed. The difference in weight before and after cooking was used to calculate the cooking loss using the formula below;
Cooking loss (%) = 100 – (weight after cooking/ weight before cooking * 100)

Shear force

Shear force was determined as described by Chrystall and Devine (1991) using a MIRINZ tenderometer based on 8 replications for each slice.

Colour stability

Objective colour measurements were obtained for steaks (20 mm thick) placed in polystyrene trays which were covered with O_2 permeable polyvinyl chloride film (O^2 permeability >2000 mL m^{-2} atm^{-1} $24h^{-1}$ at 25°C, AEP FilmPac(Ltd), Auckland, NZ). The steaks were exposed to fluorescent cool light (1,076 lux) and colour measurements were carried out daily over 12 days (1-day PM samples) and 6 days (21 day PM samples) of retail display at 4°C using MiniScan EZ (Hunter Associates Laboratory Inc., Reston, VA). The unit was calibrated using black and white standard plates. Measurements were Hunter L^*, a^* and b^* values and spectral reflectance (400–700 nm) using illuminant *C* and a 10° observer with an aperture size of 3.0cm. The chroma ($C = [a^{*2}+b^{*2}]^{[1/2]}$), hue angle ($HA = \tan^{-1} b^*/a^*$) and browning indexes (630nm/580nm and 630nm-580nm) were calculated.

Lipid oxidation (Thiobarbituric Acid Reactive Substances) Analysis

Thiobarbituric acid reactive substances (TBARS) at day 0 and at the end of the display time (9 days) were determined as reported by Bekhit et al. (2005). Thiobarbituric acid reactive substances were calculated as milligrams of malondialdehyde/kilogram of sample.

3.5 Statistical analyses

Exp 1 & 2.

The data was analysed as a split-split-split plot design with the nested sources of variation being Animals; Samples within Animals; Sub-samples within Samples within Animals; and slices within Sub-samples. Linear mixed models were used to analyse the data. The effects of the independent variables on the shear force ($\log_e$ transformation of Average SF), conductivity change (ConductDiff2), cooking loss (CookLoss), and purge loss % (PurgeLoss) were determined. The full model comprised random effects associated with the experimental design, being Animals; Samples within animals; Sub-samples within samples; and finally random error (slices within Sub-samples). The fixed effects terms comprised effects (main and interactions) associated with initial ageing period prior to treatment (AgeTrt at levels 1 or 3 days for SM muscle only); Frequency x Voltage combinations ({0,0), {20,5}, {50,5}, {90,5}, {20,10}, {50,10}, {90,10}); and Ageing period (3, 7, 14 or 21 days). Also included in the model as covariates were pH1, and for non-control treatments (i.e. Frequency and Voltage combinations other than {0, 0}) covariates PPE, PPV, PPC, CSE, temperature increase due to treatment (TempDiff) and Weight.

The above full model was fitted in two stages involving removal of non-significant terms. In the first stage, the main effects for the Frequency x Voltage combinations (denoted by FV) were constrained to remain in the model irrespective of significance. This constraint was imposed in order to identify those covariates in the model that significantly removed variation in the data after adjusting for FV effects. This was necessary as any of the above covariates other than pH1, could equally be used as an approximate surrogate measure of voltage under the non-control treatments. Non-significant covariates were then removed from the model, and under the second stage of the analysis the effects for the Frequency x Voltage combinations were separated into a linear effects for Frequency and for Voltage; a Control (Ctrl) effect where Ctrl equals “Yes” for Frequency and Voltage combinations {0, 0} and “No” otherwise; deviations from linearity for Frequency; and interactions between these terms. The model was then further simplified to remove non-significant effects.

Exp 3.1 Tenderness, purge loss, cooking loss, and conductivity

The experimental design for a cut (LL or SM) was conducted as a 24 × 4 row-column design (appendix 1 Milestone 3). The Row treatments were set up as a split-plot design, where each of four levels of the repeat applications of PEF treatment (0, 1, 2 and 3 repeats) were randomly assigned to four sub-samples from each the six samples taken from six animals (one sample per animal). The column effects were four ageing treatments (3, 7, 14 and 21 days). Each sub-sample from an animal was sliced into four and the four slices were randomly assigned to the four ageing periods. The full model for the analysis for each trait within a cut (LL or SM) included the following fixed terms; linear ageing effect (Ageing) and effects for the four levels of repeats (0, 1, 2, and 3). These repeats effects are separated into a linear trend (Repeats), a comparison between treated (1, 2 or 3 repeats) versus controls (untreated) samples, and deviations from these two components (FacRepeats). The fixed effects in the full model also included interactions between ageing and each of repeats, treated and FacRepeats and three covariates [pH0 (pH at Time 0); sample weight (SampleWt); and temperature increase during application of repeats]. The latter two covariates are only fitted for treated samples (i.e. Repeats=1, 2 or 3). The random terms in the model included effects for

animals; samples within animals; separate ageing effects for each level (FacAgeing), interactions between FacAgeing and FacRepeats; and finally random error.

The model fitted to the data for each cut can be represented as
trait = baseline + Repeats + Ageing + Repeats:Ageing
+ Treated + FacRepeats + Treated:Ageing + FacRepeats:Ageing
+ pH0 + Treated:SampleWt + Treated:TempIncrease
+ FacAgeing + FacRepeats:FacAgeing + ***Animal + Animal:Sample***
+ error

The terms in bold/italic in the above model are fitted as random effects.

For each trait (DripLoss, CookLoss, pHDiff, ConductDiff and logAvSF) within each Cut (LL and SM) the full model above is fitted in R (R Development Core Team, 2010) using the *asreml* linear mixed modelling package (Butler, 2009). The logAvSF corresponds to the $\log_e$ (Average shear force). The full model was simplified by removing terms not significant at the 0.05 level with the exception of the linear Ageing effect, which was retained even when not significant at the 0.05 level. This is the case because it is probably reasonable to assume that ageing will have an effect on the traits examined, but as there is very limited replication of the ageing effect (2 degrees of freedom for estimating the variation in the ageing effect) this term will generally not be detected as significant.

Exp 3.2 Colour and lipid stability

The seven traits (L, a*, b*, C, h, 630nm/580nm [ratio] and 630nm-580nm) were analysed using a linear mixed model analysis, fitted using the software package asreml (Butler, 2009) under R (R Core Team, 2013). The fixed effects in the full model were: muscles (loin or topside); ageing, display time; ageing x display time; repeats of PEF treatment (0, 1x, 2x and 3x); interaction between linear PEF repeat effects and linear trends with ageing and display time and non-linear PEF repeat effects associated with repeats which may differ across muscles. The random effects in the model associated with each of the following sources: design strata (animal; muscle within animal; repeat level within muscle within animal; and ageing level within repeat level within muscle within animal) and additional random sources of variation (ageing × display time combinations within each muscle; ageing × display, time × repeat combinations within each muscle, and random variation). All random effects were assumed uncorrelated and the additional sources of variation above were modelled to have possible different variation within each muscle.

Exp 4.

Linear mixed model analyses were used to separately analyse the results (purge loss, cooking loss, pH difference [pHdiff], conductivity difference [conductDiff] and shear force). Fixed effects in each model included effects for pHrange (5.5-5.8, 5.8-6.1 and > 6.1), fibre direction (crossed and parallel), treatment (control and PEF treated), ageing (3 and 7 days), and pair-wise interactions between these. Random effects were effects for loins within each pHrange, blocks within each loin within each pHrange, cooking batch and random error. Denoting the response variable as Y, the above model corresponds to:

Y = baseline + (pHRange + fibre + treatment + fibre:treatment + ageing)^2 +
loin:pHRange + loin:pHRange:fibre:treatment + cooking batch + error

Terms in bold/italic are fitted as random.

Exp 5 & 6.

Measured traits [purge loss (PurgeLoss), cooking loss (CookLoss), pH difference due to treatment (pHDiff), conductivity difference due to treatment (ConductDiff), and log the average shear force (logAvSF)] of the two cuts (loin and topside) from experiments were analysed using an identical linear mixed model. The model included a baseline effect; an effect for control versus treated (IsCtrl); differences between voltages (Volt); differences between frequencies (Freq); and difference between Volt x Freq as fixed effects. Also included as fixed effects were a linear aged effect (Aged); separate aged group effects (FAged); and interactions between Aged and FAged and the effects associated with control and Volt x Freq combinations. Finally a fixed effect for sample weight (Wt) was included as a covariate for non-control samples. The random effects were effects for animal; samples within animal (Animal x Trt) and random error.

Exp 7.

Prior to analysis of results for experiment 7 a number of new variates were formed for loin and for topside samples. These are;

- Nrep corresponds to the number of repeat applications. Nrep equals 0 for controls and 1, 2 or 3 for Repeat equals 1, 2 or 3 respectively.
- IsCtrl equals yes for controls and equals no if Repeat equals 1, 2 or 3.
- FAged is also formed having levels Aged=03, Aged=07, Aged=14, Aged=21 for Aged equals 3, 7, 14, 21 respectively.
- CWt equals the mean adjust weight of electrically stimulated sample. CWt is set equal to 0 for Controls
- Trt is basically a relabelled version of Repeat.

Measured traits [purge loss (PurgeLoss), cooking loss (CookLoss), pH difference due to treatment (pHDiff), conductivity difference due treatment (ConductDiff), and log the average shear force (logAvSF)] of the two cuts (loin and topside) were analysed using an identical linear mixed model. This model included, as fixed effects, a baseline effect; an effect for the number of repeats (Nrep); an effect for control versus treated (IsCtrl); and the overall Repeats effects. Also included as fixed effects were; a linear aged effect (Aged); separate aged group effects (FAged); and interactions between Aged and FAged and the effects associated with Nrep, IsCtrl and Repeat combinations. Finally a fixed effect for sample weight (CWt) was included as a covariate for non-control samples. The random effects were effects for animal; samples within animal (Animal x Trt) and random error.

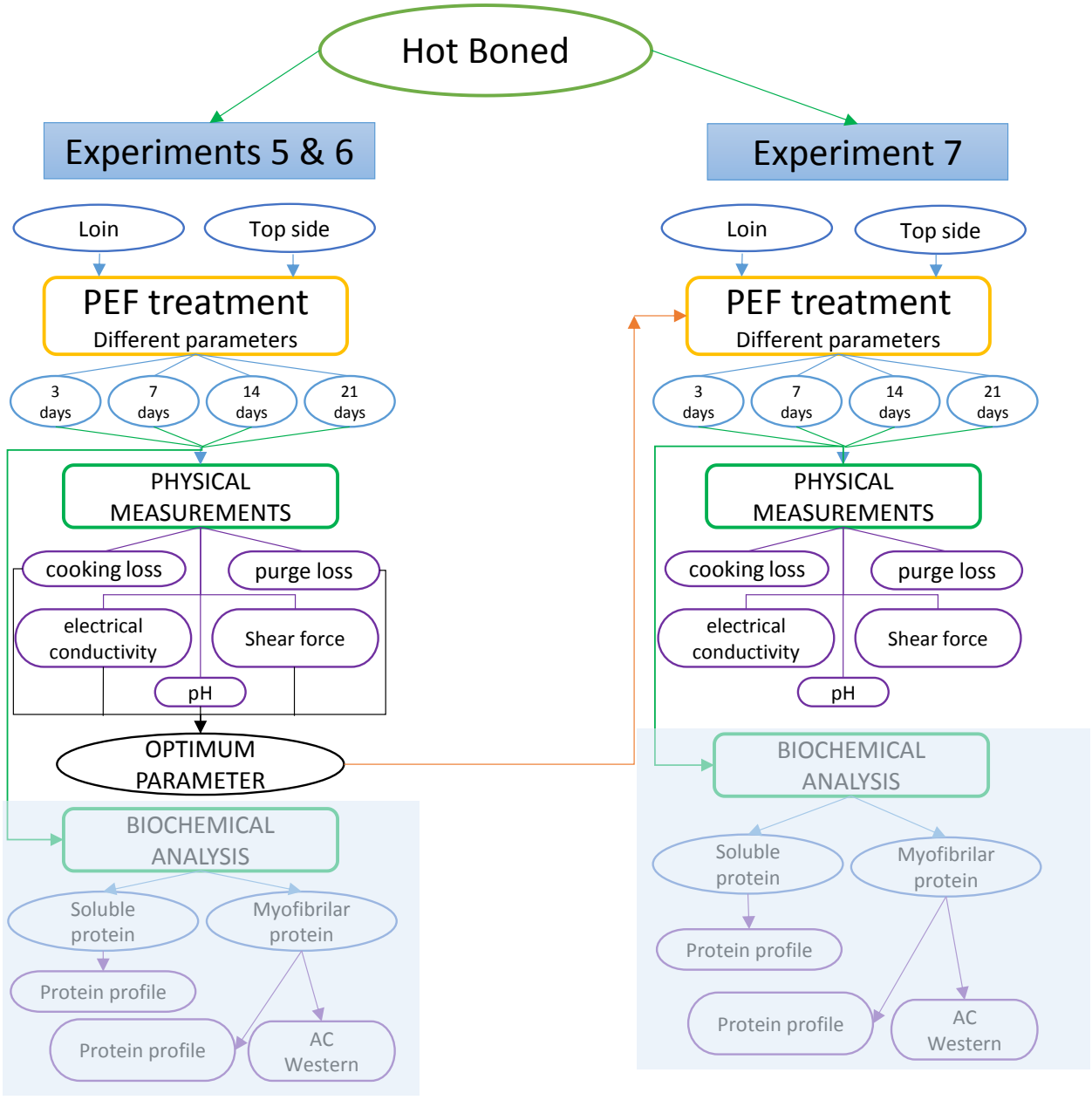

Figure 4. Experimental plans for hot-boned beef loins and topsides. Shaded biochemical analysis section is not done and is not part of this project.

4 Results and Discussion

4.1 Effects of PEF processing on beef tenderness

4.1.1 Cold- boned loins: Effect of PEF treatment at various processing conditions

The tenderness (as measured by the shear force) of beef LL loins was decreased by the PEF treatment regardless of the intensity of treatment compared with the non-treated control (P = 0.002) and ageing period (*P* < 0.001) (Figures 5 & 6).

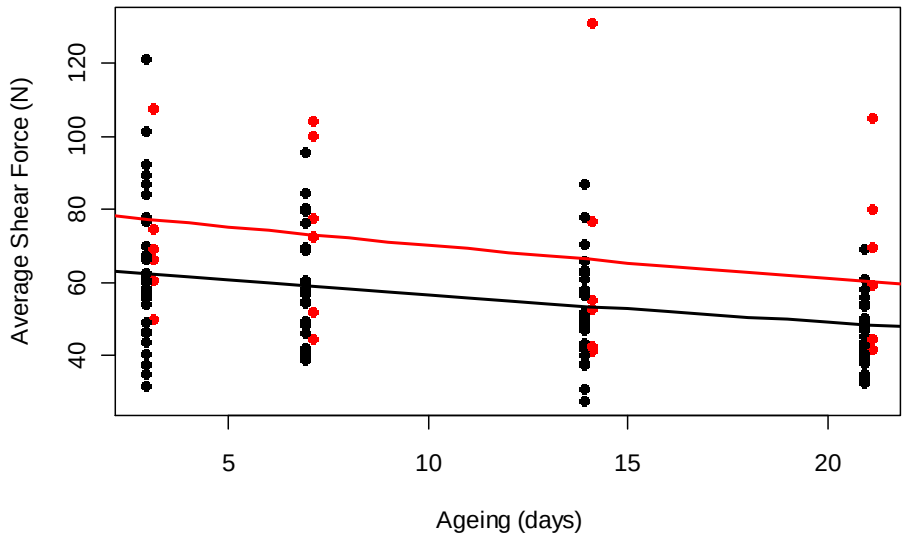

Figure 5: Plot of observed means of Shear force for LL muscles at various Ageing storage times (3, 7, 14 and 21 days) for PEF treated sample (Ctrl:No; black points fitted with black line) and control non-treated samples (Ctrl:Yes; red points fitted with red line).

As the fitted model included the ageing effect on a continuum, it is appropriate to plot the predicted shear force at differing Ageing values as a line (i.e. as a continuum see Figures 5 & 6). Below are presented the predicted shear force values (PV) and associated standard errors (SE) at the observed ageing values (3, 7, 14 and 21) for Controls (Ctrl=Yes) and Treated (Ctrl=No) samples.

Ctrl	Ageing	PV	SE
No	3	62.4	3.8
No	7	59.0	3.4
No	14	53.5	3.0
No	21	48.5	3.0
Yes	3	77.4	6.2
Yes	7	73.2	5.7
Yes	14	66.4	5.1
Yes	21	60.2	4.9

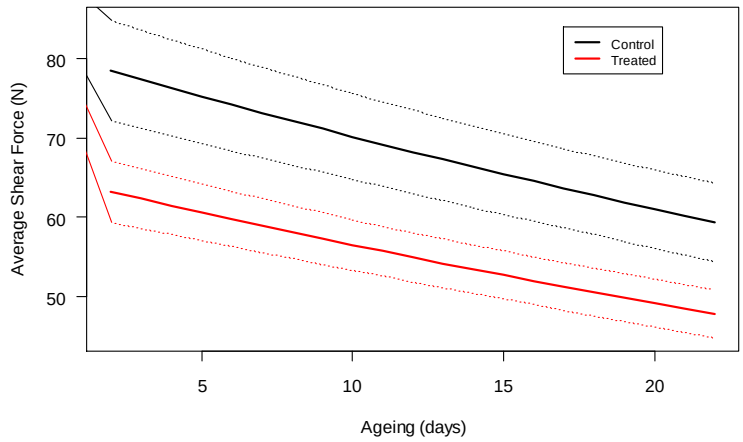

Figure 6: Plot of predicted means for shear force at various ageing storage times (3-20 days) for PEF treated LL muscles (Ctrl:No) and control non-treated samples (Ctrl: Yes). The Std. Errors for predicted values are on average 3.22 (range 3.00, 3.78) and 5.38 (range 4.91, 6.19) for treated and controls respectively.

4.1.2 Effect of repeated pulsed electric field treatment on cold-boned beef LL

Overall, the average shear force was found to decline with ageing (P = 0.058) with an estimated 1.02 N decline in the shear force of the loins for every day of ageing. The repeat of PEF treatment (20 µS, 10 kV and 90 Hz) was found to decrease the shear force of loins by 2.5 N for every extra application of PEF within the four levels used (0, 1x, 2x and 3x). The predicted means for the 4 treatments are shown in Figure 7.

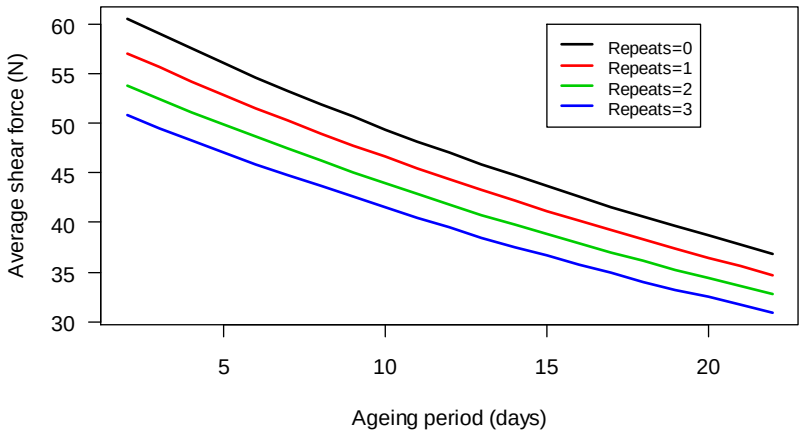

Figure 7: Plot of predicted means for shear force (with 95% confidence intervals) of LL muscles at various ageing storage times (2-22 days) treated with repeated PEF (20µ, 10 kV, 90 Hz) at 1x, 2x and 3x repeat levels and control non-treated samples (0). The Std. Errors for predicted values are on average 6.90 (range 5.43, 8.37), 6.38 (range 5.03, 7.72), and 6.02 (range 4.76, 7.28) for 0, 1 and 2 repeats, respectively.

4.1.3 Hot- boned loins: Effect of PEF treatment at various processing conditions

The average shear force of hot boned beef loins was found to decline with ageing (P = 0.006) and tended to increase at high frequencies (90 Hz) within the PEF treated group (P = 0.07) (Figure 8). None of the other parameters or their interactions had any effects on the shear force. The reduced model used for the statistical analysis included the ageing effect and the random error. The overall shear force was decreased by about 4, 11 and 17 N at 7, 14 and 21 days of ageing, respectively, compared with the average shear force at 3 days of ageing (Figure 9). The response of hot-boned LL to PEF treatment was different from that observed in cold-boned LL where a decrease in the shear force was found with PEF treatment regardless of the intensity of treatment compared with the non-treated control (P = 0.002). This effect was not expected as PEF treatment was expected to release Ca^{2+} and lead to an early activation of calpains. It was noted that the highest PEF intensity used in the present experiment (10 kV and 90 Hz) increased the temperature of the sample to an average of 28.9 ± 2.27 (an average increase of 4.36 ± 1.41 °C) while some of the samples had pH values > 6.0. This maybe caused the development of heat shortening in some of the samples.

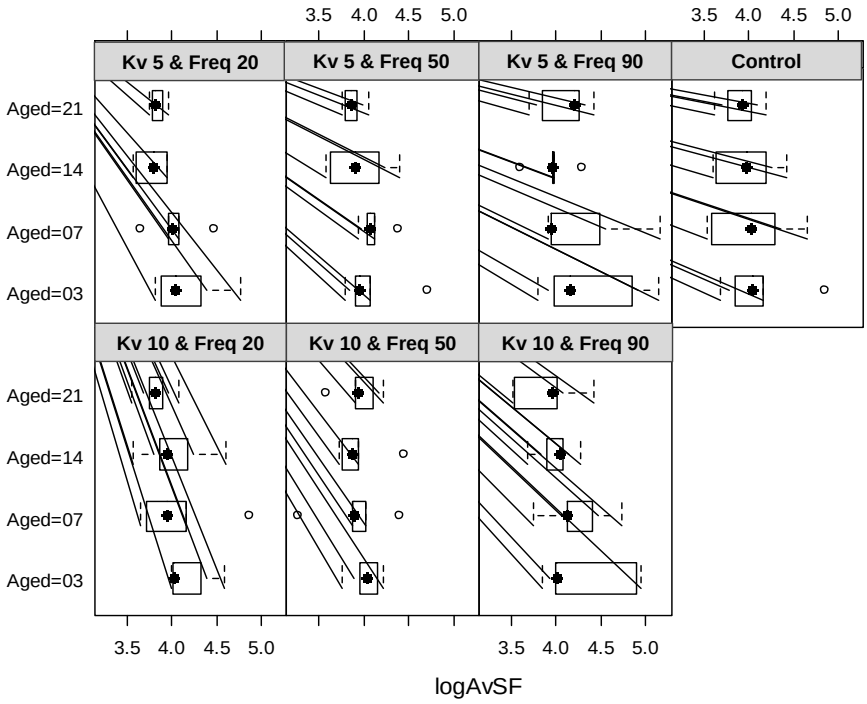

Figure 8: Box-plots for mean log shear force values (logAvSF) for hot-boned beef LL muscle as affected by ageing (3, 7, 14 or 21 days) and treatment (pulse electric field of 5 or 10 Kv and frequency of 20, 50 or 90 Hz).

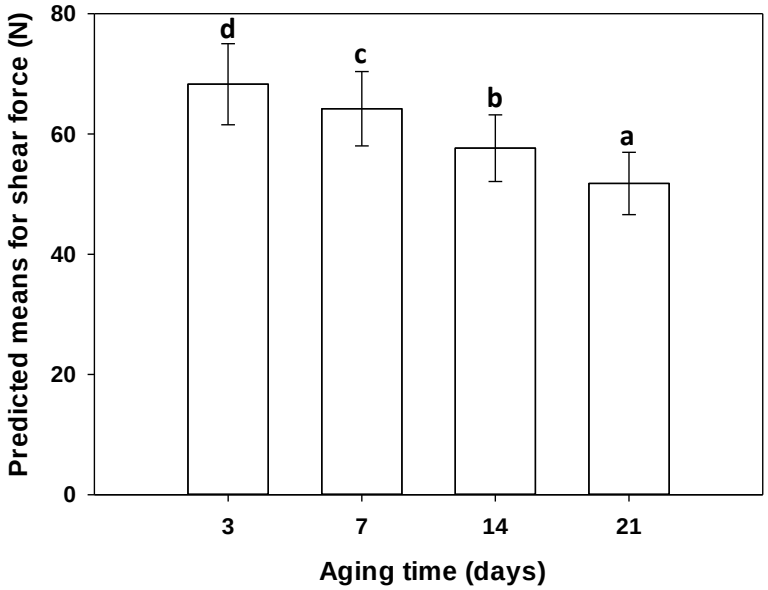

Figure 9: Overall predicted means for shear force of hot-boned beef LL muscles at various ageing storage times (3, 7, 14 and 21 days).

4.1.4 Effect of repeated pulsed electric field treatment on hot-boned beef LL

The shear force of hot-boned LL muscles was decreased by ageing (P = 0.007) and increased by the number of repeated PEF treatment (P = 0.03) (Figures 10 & 11). At the same ageing time, treated samples were estimated to increase in average logAvSF by 0.160 (s.e. = 0.037) for every extra PEF repeat. The interaction between ageing and PEF treatment almost approached a significant level (P = 0.055) suggesting various effects for PEF treatment at different ageing times. Generally, when hot-boned LL was treated once this resulted in lower shear force values compared with 2x and 3x PEF treated samples. The highest treatment intensity (3 times treated) produced meat samples with high shear force values which was higher than all other treatments and controls. Applying PEF treatment (20µs, 10kV, 90 Hz) at 1x, 2x and 3x to hot boned LL muscles resulted in temperature increase by 6.45 ± 2.4, 8.15 ± 0.98 and 13.37 ± 5.20, respectively. The final temperatures were 31.25 ± 3.39, 33.15 ± 1.30 and 38.28 ± 5.23 for 1x, 2x and 3x treated samples, respectively. The high temperature for 3x treatment may cause protein denaturation and reduced proteolysis. Kim et al. (2012) found pre-rigor incubation of beef loins at 38°C induced protein denaturation and limited the extent of µ-calpain autolysis and desmin degradation. The net outcome of the incubation process was an increase in the shear force values and a decrease in the water holding capacity of the meat.

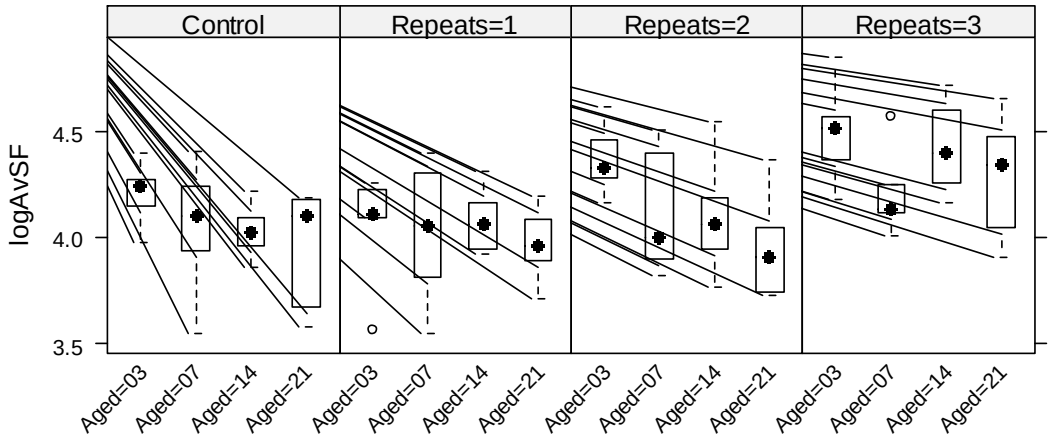

Figure 10: Box- plot of observed means of shear force of hot-boned beef LL muscles at 3, 7, 14 and 21 days of ageing. The muscles were treated with repeated PEF (0, 1x, 2x or 3x).

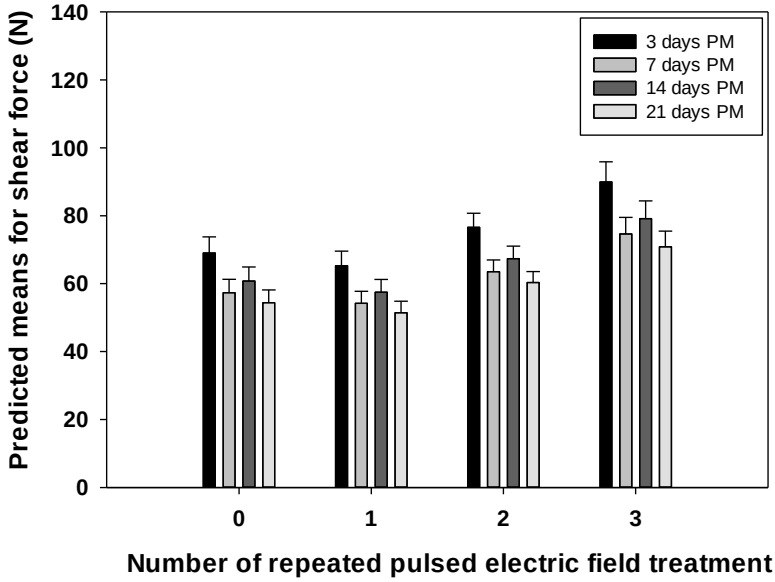

Figure 11: Effect of repeated PEF treatment (20µ, 10 kV, 90 Hz) on the shear force of beef LL samples at various ageing times (3, 7, 14 and 21 days). Use of letters to indicate a difference was ignored for the sake of clarity.

4.1.5 Cold- boned topsides: Effect of PEF treatment at various processing conditions

The shear force of SM muscles was decreased as frequency increased ($P < 0.001$) and as ageing storage time increased ($P < 0.001$). The treatment post-mortem time (1 day vs 3 day post-mortem) did not have any effects on the shear force of SM muscles. The rate of tenderization of SM muscles was similar over the ageing storage time (Figures 12 and 13) and frequency was the main source of variance of shear force at each ageing time.

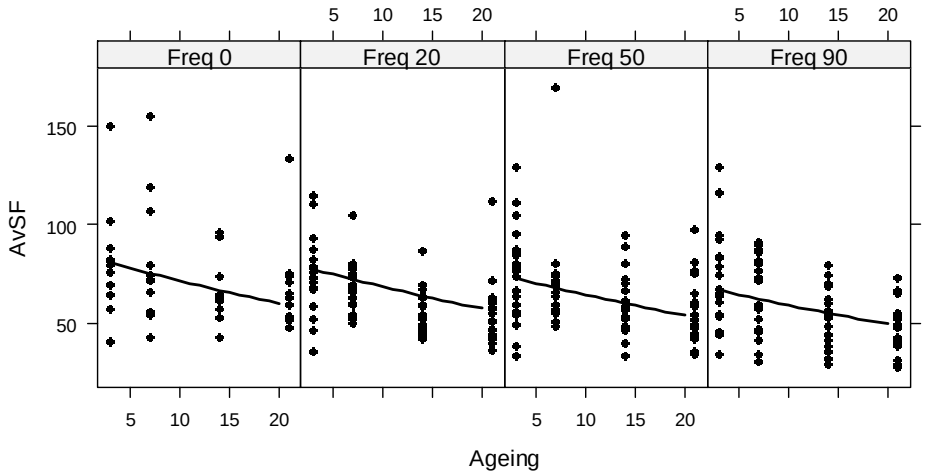

Figure 12: Plot of observed means of shear force at various ageing times (3, 7, 14 and 21 days) for SM muscles treated with pulsed electric field at various frequency levels (0, 20, 50 and 90 Hz). Superimposed on each graph is the line of predicted means for shear force at various ageing times (3, 7, 14 and 21 days).

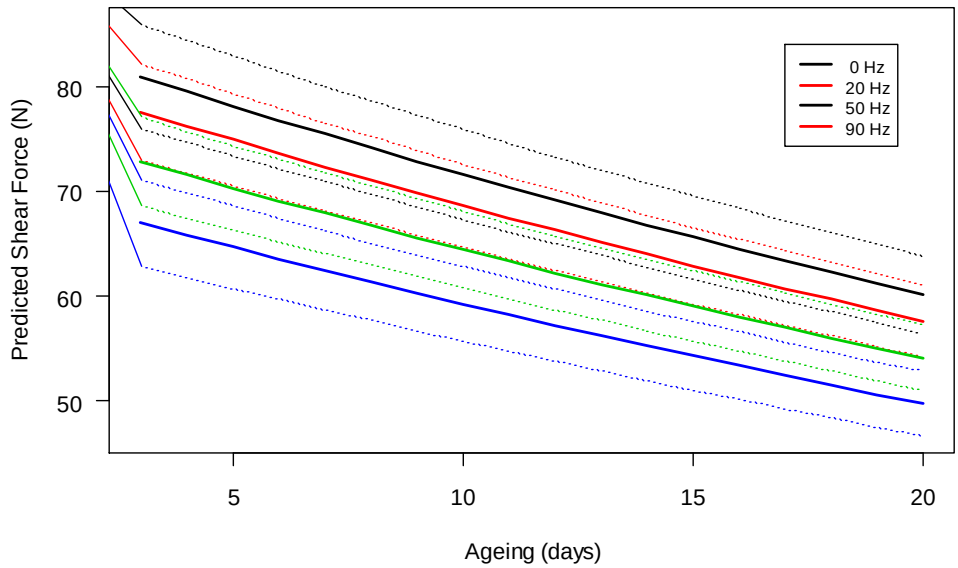

Figure 13: Plot of predicted means of shear force (with 95% confidence intervals) at various ageing times (3-20 days) for SM muscles treated with pulsed electric field at various frequency levels (0, 20, 50 and 90 Hz).

4.1.6 Effect of repeated pulsed electric field treatment on cold-boned beef topsides

Unlike LL muscle; the SM muscle was not affected by repeat PEF treatment ($P > 0.05$). Ageing time (3, 7, 14 or 21 day) had no effect on the MS shear force (Figure 14).

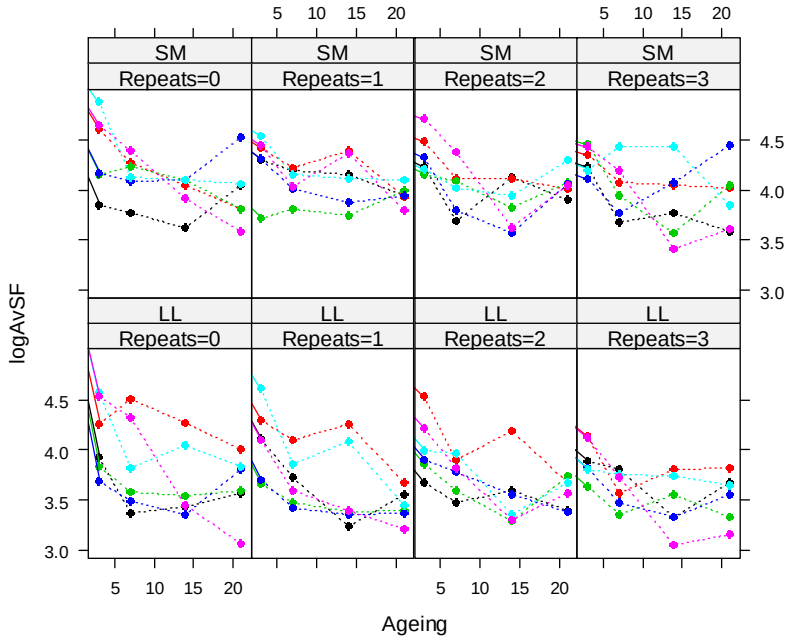

Figure 14: Mean shear force values of LL and MS muscles at 3, 7, 14 and 21 days of ageing treated with repeated PEF (20µ, 10 kV, 90 Hz) at 1x, 2x and 3x repeat levels and control non-treated samples (0). Different lines represent individual animals.

4.1.7 Hot- boned topsides: Effect of PEF treatment at various processing conditions

The shear force of the hot-boned beef SM muscle was decreased by ageing ($P < 0.001$) and by PEF treatment (P = 0.008) regardless of the treatment intensity (Figure 15).

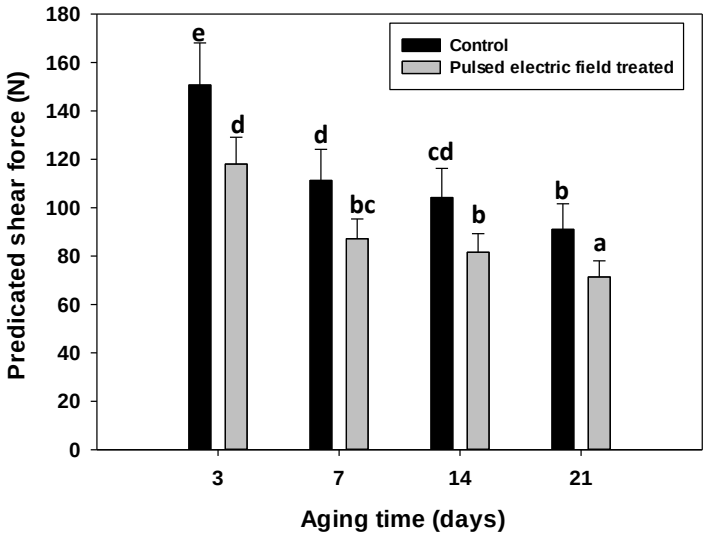

Figure 15: Overall predicted means for shear force of hot-boned beef SM muscles treated by PEF at various ageing storage times (3, 7, 14 and 21 days).

The shear force of this muscle tended to decrease by increasing the frequency within the PEF treated group and by the interaction between separate aging groups (FAged) and voltage (P = 0.084 for both) (Figure 16). These results are in line with those found in cold-boned SM, but the significance level of the treatment was much higher in cold-boned SM muscles.

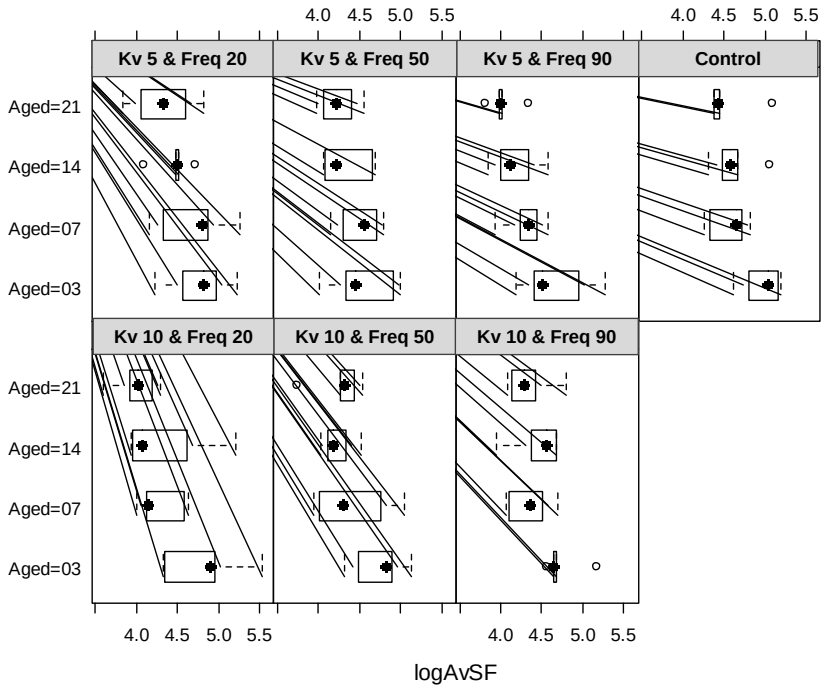

Figure 16: Box-plots for mean log shear force values (logAvSF) for hot-boned beef SM muscle as affected by ageing (3, 7, 14 or 21 days) and treatment (pulse electric field of 5 or 10 Kv and frequency of 20, 50 or 90 Hz).

4.1.8 Effect of repeated pulsed electric field treatment on hot-boned beef topsides

The effects of repeated PEF treatment and ageing on hot-boned SM muscle are shown in figures (17 and 18). The shear force of treated samples was affected by ageing ($P < 0.001$) and there was a treatment by ageing interaction (P = 0.014). In contrast with hot-boned LL muscle, the highest PEF treatment (i.e. 3x) resulted in SM samples with lower shear force at 3 days of ageing compared with 1x and control samples (Figure 17). However, this beneficial effect disappeared during further ageing times. Applying PEF treatment (20µs, 10kV, 90 Hz) at 1x, 2x and 3x to hot boned SM muscles resulted in temperature increases of 1.75 ± 1.12, 5.20 ± 1.12 and 6.72 ± 3.19°C, respectively. The final temperatures were 26.33 ± 0.79, 29.88 ± 1.30 and 31.83 ± 3.55 for 1x, 2x and 3x treated samples, respectively. The maximum temperature was lower than that found with LL muscles and therefore protein denaturation was less likely to occur. Overall, the effect of repeated PEF treatment on hot-boned meat appears to generate an opposite effect to that observed in cold-boned meat.

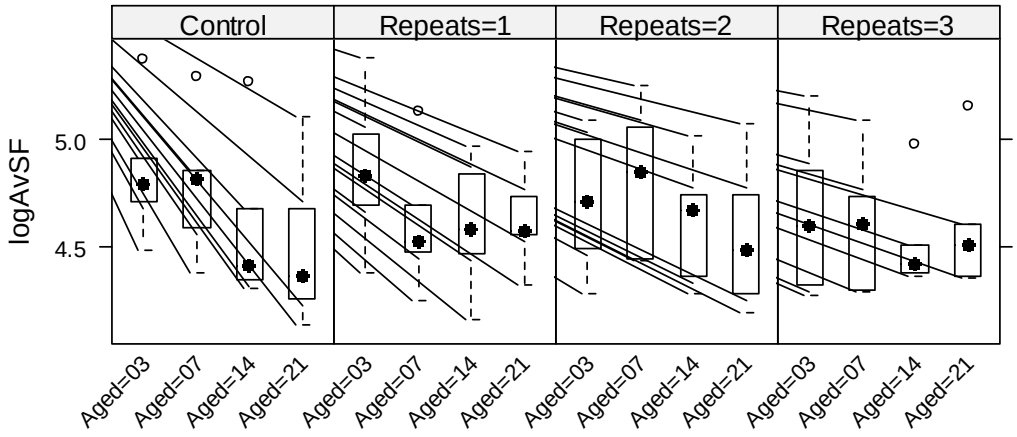

Figure 17: Box-plot of observed means of shear force of hot-boned beef SM muscles at 3, 7, 14 and 21 days of ageing. The muscles were treated with repeated PEF repeated PEF treatment (20µ, 10 kV, 90 Hz).

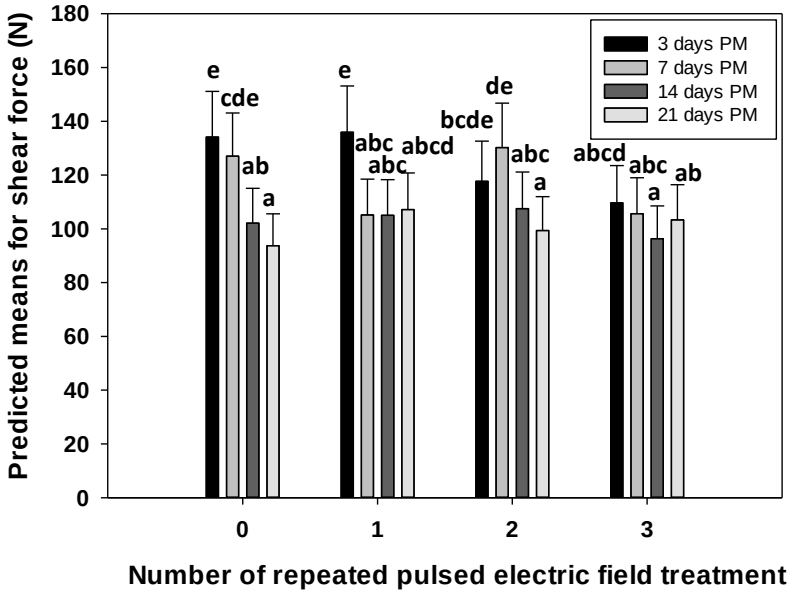

Figure 18: Effect of repeated PEF treatment (20µ, 10 kV, 90 Hz) on the shear force of beef SM samples at various ageing times (3, 7, 14 and 21 days).

In the present study the range of actual electric field strength used (pulse peak voltage divided by the space between the electrodes) was mostly within the range 0.27 to 0.56 kV/cm (Appendix 1) with the exception in the fibre direction and pH experiment where the range was 0.59 to 0.73 kV/cm. The electric field strength was affected by voltage, but was not affected by the pulse

frequency (Table 1A, 9A, Appendix 1). The generation of an effective electric field around the muscle cell can lead to electroporation of the sarcolemma. This consequently can cause a spontaneous physical effect at the cellular level, but more importantly can lead to direct interactions between enzymes and their substrates leading to faster rates of reaction. The physical changes in meat as a result of PEF treatment is shown in Figure 19. Disorganisation of the myofibrilliars structure occurred at low (0.3 kV cm^{-1}, 1 Hz, and 20 µs for 30 sec) and intense (0.6 kV cm^{-1}, 50 Hz, and 20µs for 30 sec) PEF treatment.

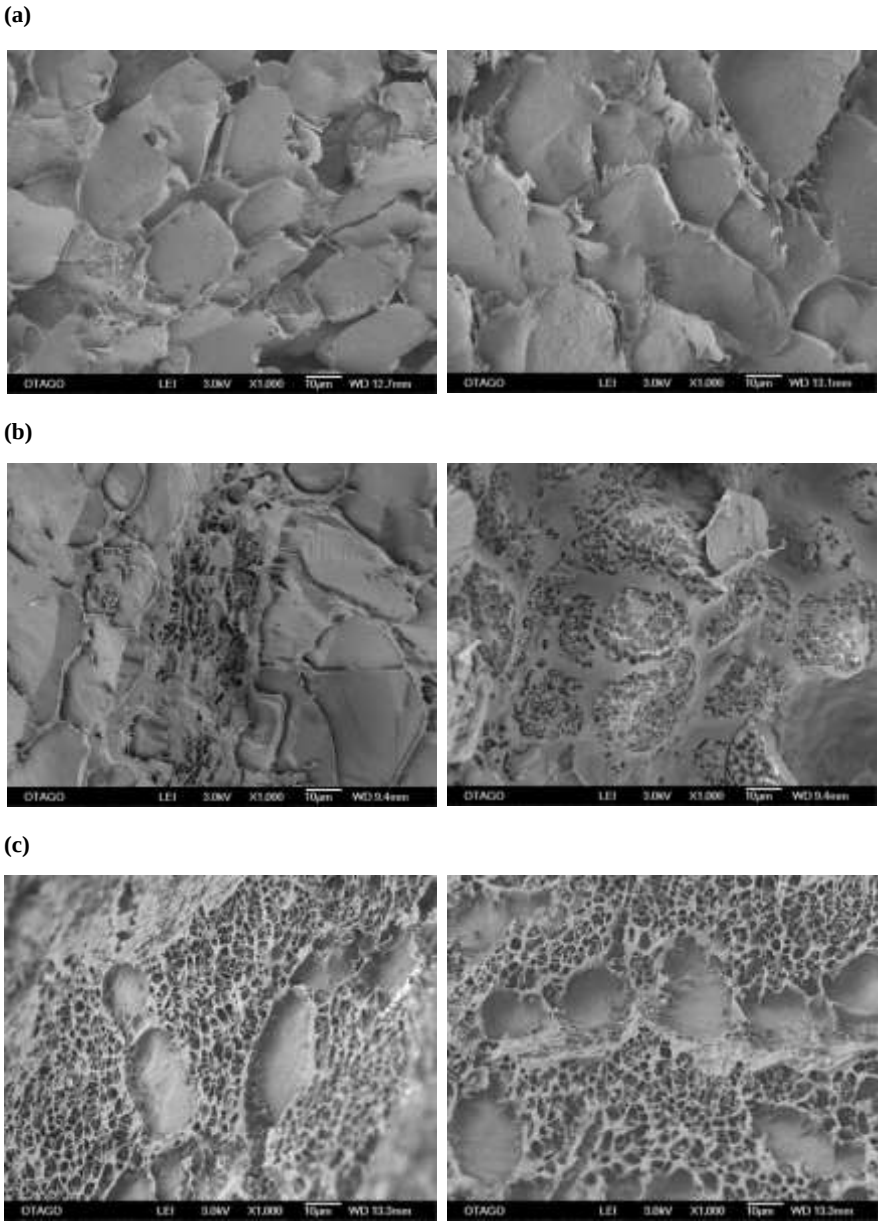

Figure 19. Cryo-SEM micrographs of beef samples: (a) untreated; (b) after a PEF treatment (0.6 kV cm^{-1}, 50 Hz, and 20µs); (c) after a PEF treatment (0.3 kV cm^{-1}, 1 Hz, and 20 µs). Faridnia et al. (2014).

To achieve cell electroporation, the electric field around the cell needs to exceed the critical transmembrane potential and therefore, the electric field strength is regarded as the most important factor that affects PEF treatment. The critical field strength of animal cells was reported to be 0.5 kV/cm (Töpfl 2006), therefore an input voltage of 10 kV will deliver an effective electric field and lead to cell permeabilization. Gudmundsson and Hafsteinsson (2001) reported significant changes in texture and microstructure of fish and chicken at a PEF strength of 1.36 kV/cm and O'Dowd et al. (2013) reported PEF effects on the microstructure of beef (significantly lower particle size and numerically higher myofibrillar index) with a PEF treatment of 1.9 kV/cm, 65 Hz, 250 pulses and pulse width of 20 µs. It is not clear whether the electric fields reported in Gudmundsson and Hafsteinsson (2001) and O'Dowd et al. (2013) (1.36 and 1.9 kV/cm, respectively) were based on the actual pulse peak voltage or based on the theoretical input voltage used (the latter was 0.63 and 1.25 kV/cm, for 5 kV and 10 kV, respectively in our study). The mean conductivity change values were larger at the higher voltage and higher frequency (Table 2A) which is in agreement with the reported increase in conductivity of pork (Töpfl 2006) and beef ST muscles (O'Dowd et al. 2013) upon PEF treatment. The conductivity change of 3 day post-mortem samples was not affected by PEF treatment (Table 2A). The increase in the conductivity after post-mortem ageing was possibly due to endogenous processes leading to cellular damage during the ageing process since conductivity reflects the ease of movement of ionic species at the cellular level which is normally hindered by cell membranes (Lebovka et al. 2002).

The energy density and pulse peak power values were larger with the higher voltage and higher pulse frequency (Tables 1A and 9A) which was translated into a larger temperature change in the samples. The post treatment temperature change was larger with the higher voltage and frequency values (Table 2A). A lower range of temperature change was found in hot-boned LL treated beef (-0.18 – 4.4) compared with cold-boned LL beef (0.4 – 7.7) (Tables 2A and 10A). The change in temperature was not affected by post-mortem time (Table 2A) and was similar for both muscles used in the study. The change in temperature range in the present study was between 0.0 and 8.0°C which was smaller than that observed by O'Dowd et al. (2013) in beef ST samples (ranged from 5 to 30°C depending on the electric field and the frequency used). It is worth noting that while O'Dowd et al. (2013) and our study used the same PEF system, the differences in the treatment chamber, sample size and processing parameters in both studies means that direct comparison of the results cannot be made since the consequences of the treatments on the biological systems (e.g. endogenous enzymes and physical stimuli) are expected to be different.

Low intensity PEF treatment can enhance the mobility of intracellular constituents via permeabilization of plant or animal tissues (Barsotti and Cheftel 1999). This may lead to an early release of cellular material and earlier activation of biochemical events (e.g. proteolysis). However, proteolysis is not an instantaneous process and it will require time for the effect on tenderness to be observed which may explain the importance of using an ageing step, as in the present study. Pulsed electric field is similar to the electrical stimulation technique that is practised by the meat industry to stimulate carcases early postmortem (within the order of minutes from carcase dressing) and therefore some of the mechanisms suggested for improved meat tenderness found in electrically stimulated carcases may be applicable to PEF. Three major mechanisms have been suggested to explain the effects of electrical stimulation on shear force (i) acceleration of the onset of rigor and therefore the prevention of cold induced shortening, (ii) weakening of muscle fibres as a result of the

physical disruption of the sarcomeres caused by severe muscle contractions (Sorinmade et al. 1982; Takahashi et al. 1987), (iii) cellular changes leading to a release of calcium ions leading to an early activation of the calcium activated protease μ-calpain and consequently accelerated proteolysis in electrically stimulated neat (Ducastaing et al. 1985; Lee et al. 2000). These mechanisms have reviewed in detail by Hwang et al. (2003). Meat tenderization is a multifaceted process that involves the degradation of key structural myofibrillar proteins, which are responsible for maintaining the integrity of the myofibrils (Hopkins and Geesink 2009). However, several physical processes (e.g. mechanical tenderization by blade, freeze-thaw) can improve meat tenderness (Hopkins 2004; Bekhit et al. 2013) based on disintegration of the meat structure rather than biochemical events. At this stage, it is not clear what the contribution of these tenderizing systems is to the observed improvement in meat tenderization found in the present study, but it can only be due to the latter two mechanisms as the meat was in rigor at the time of treatment. Another explanation is the action of cathepsins. Much of the focus on cathepsins and their role in meat tenderisation has been directed towards B (EC 3.4.22.1), L (EC 3.4.22.15) and D (EC 3.4.23.5), which are endopeptidases. Cathepsins B and L (cysteine proteases; EC 3.4.22) have also been shown to exhibit exopeptidase activity. Cathepsins are located in the lysosomes (Goll et al. 1983) and thus to play a part in myofibril degradation they must be released from the lysosomes, which is feasible under the conditions created by PEF treatments. This is an area for further investigation.

4.2 Effect of PEF treatment on the purge and cooking losses and conductivity

A summary of the effects of PEF processing on the purge and cooking losses and conductivity is shown in Table 1. A full set of results were reported in milestones 2, 3 and 4. There was an inverse relationship between purge loss and cooking loss when there was an effect for PEF treatment. For example increased purge loss (%) and decreased in cooking loss (%) was found in PEF treated cold-boned loins and topsides samples and hot-boned topsides. An exception for this was found in hot-boned loins and cold- and hot-boned topsides when repeated PEF treatment was used. In these samples, an increased purge loss was found with no corresponding significant reduction in cooking loss. Furthermore, repeated PEF treatment for cold-boned meat resulted in a significant increase in cooking loss, but no effect on purge loss. The purge and cooking losses reflect the availability of free moisture that can be easily removed by several factors (pressure, compression, vacuum or upon heating). This increase in free un-entrapped moisture results from loss of structural or chemical barriers that normally constrain the movement of moisture (e.g. chemical/biochemical binding and cell wall) and therefore it could be concluded that increased purge may be due to physical change in the cellular system or due to denaturation of some proteins. O'Dowd et al. (2013) examined the effect of PEF treatment on the drip loss (%) of beef ST samples, as an indication of the water holding capacity. The authors found an insignificant effect of PEF treatment compared with non-treated controls. It is estimated that about 85% of the muscle moisture is held by the myofibrils (Huff-Lonergan and Lonergan 2005), therefore, the higher purge (%) in our PEF treated samples in the present study indicated changes in the myofibril structure and their ability to contain moisture and may be due to proteolysis which alters the ability of the muscle to retain moisture (Pearce et al. 2011).

4.3 Effect of PEF treatment on colour stability and lipid oxidation

The trends of the colour parameters and the various fixed factors are shown in Appendix 2. Display time was the only factor that had a significant effect on the meat lightness (L*) values ($P < 0.001$). The estimated decline rate of L* is 0.0077 (SE 0.0015)/ hour of display. The redness of the meat (a*-values) was significantly affected by muscle, ageing, display time and their interactions ($P < 0.05$). The redness of the meat was decreased by increasing the PEF repeat (P = 0.04) with an estimated decline of 0.22 (SE = 0.10) in a*-value for every additional PEF repeat treatment. The yellowness component of the colour (b*- values) were affected by the display time and the interactions of display time, muscle and ageing. There was no effect for repeat PEF treatment on b*-values. The chroma (C-values) and browning indexes (630nm/580nm and 630nm-580nm) showed a similar trend to yellowness (b*-values) whereas the hue (h- values) exhibited a similar trend as redness (a*-values). The predicted means for h- values as affected by the repeat of PEF treatment is shown in figure 20. The results suggest slight negative effect for PEF treatment on the colour of fresh beef. A recent review on interventions for meat tenderization (Bekhit et al. 2014) reported a negative effect for meat tenderization techniques on the colour of fresh meat due to reduced integrity of cellular structure and direct interactions between various biochemical components which lead to increased oxidation (e.g. lipid and pigments).

For lipid oxidation, there was no effect for PEF treatment ($P > 0.05$) and only the interaction between ageing x display time influenced lipid oxidation (P = 0.06, Figure 21).

Table 1. Effects of various PEF processing conditions, repeat PEF treatment at optimal conditions (10kV, 90 Hz, 20µS), and fibre direction and pH on purge and cooking losses and conductivity of cold- and hot-boned beef loins and topsides.

Treatment	Experiment	Purge loss (%)	Cooking loss (%)	Conductivity	Milestone
Cold-boned loins	1	↑ with the frequency of PEF treatment (P = 0.002), the frequency × voltage (P = 0.003) and frequency × ageing (P = 0.002)	↑ cooking losses in control samples compared with PEF treated samples ($P < 0.001$) and this effect was significantly higher as the ageing period increased for control samples ($P < 0.001$)	Increased conductivity as a result of PEF treatment. Less change in PEF treated samples during ageing	2
Cold-boned topsides	2	↑ with PEF treatment regardless the intensity of treatment ($P = 0.001$)	↑ cooking losses in control samples compared with PEF treated samples ($P < 0.001$).	Increased conductivity as a result of PEF treatment. Less change in PEF treated samples during ageing compared with control	2
Repeated PEF treatment cold-boned loins	3	No effect	↑ with PEF treatment ($P < 0.05$).	No effect	3
Repeated PEF treatment cold-boned topsides	3	topside samples was significantly (P = 0.036) increased by PEF treatment regardless the number of PEF repetition	No effect	Less change in PEF treated samples during ageing compared with control	3
Effect of pH and fibre direction	4	No effect	No effect	No effect	3
Hot-boned loins	5	No effect	Treated LL muscles tended (P = 0.08) to have a higher cooking loss (%) compared with non-treated control	No effect	4
Hot-boned topsides	6	PEF treatment regardless the intensity of treatment (P = 0.023). On average an increase of 1.2% in purge loss was resulted from PEF treatment.	Within the PEF treated samples, there was a tendency toward decreased cooking loss as a result of ageing (P = 0.08).	No effect	4
Repeated PEF treatment hot-boned loins	7	The purge loss (%) was increased by PEF treatment (P < 0.001), number of PEF repeats (P = 0.004)	Control samples tended to have higher cooking loss (P = 0.08). This was significant for 1x and 2x treated at 3 and 7 days of ageing	No effect	4
Repeated PEF treatment hot-boned topsides	7	Purge loss (%) ↑by increasing the ageing time (P < 0.001), number of PEF repeats (P = 0.001) and their interaction (P = 0.003).	No effect	At the same ageing time, control on average 1.15 units less conductivity difference than PEF treated samples,	4

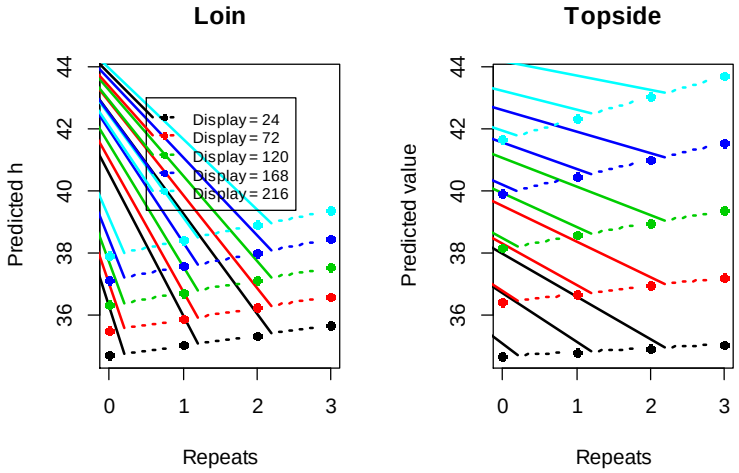

Figure 20. Predicted means of hue (h-value) in beef loins (LL muscle) and topsides (SM) treated with repeated (0, 1x, 2x and 3x) PEF treatment (20µ, 10 kV, 90 Hz).

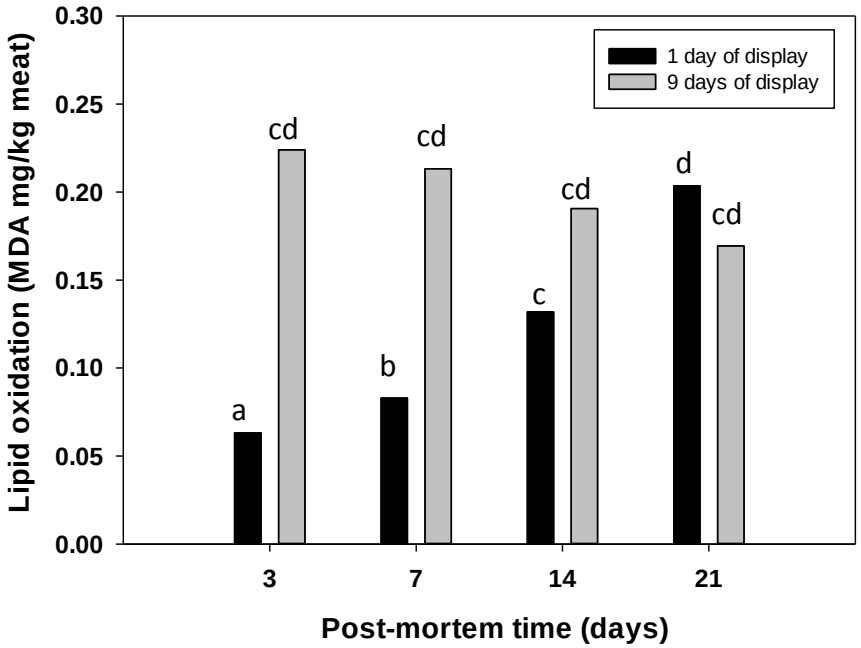

Figure 21. Predicted means of lipid oxidation values (Malondialdehyde mg/kg meat) in beef loins (LL muscle) and topsides (SM) treated with repeated (0, 1x, 2x and 3x) PEF treatment (20µ, 10 kV, 90 Hz).

The above results collectively indicate a positive impact on the tenderness of beef loins and topsides with about a 19% increase in tenderness due to PEF treatment, but response varied according to the muscle (hot-boned loins are more sensitive to heat generated during PEF treatment and a negative impact on tenderness resulted). The colour of the fresh beef was affected negatively, but not lipid oxidation by PEF treatment. PEF systems that are used for experimentation are those that can treat small samples (≅ 30 g meat) and medium size samples (≅ 350 g meat) used by O'Dowd et al. (2013) and in the present project, respectively which are all batch systems. A larger batch system that has been used with ham legs (treatment chamber was 25 L) in a study by Töpfl (2006). The potential of up scaling the technology has been presented by the manufacturer (DIL Quakenburck, Germany) and a few concepts for commercial continuous meat treatment have been proposed (Figure 22).

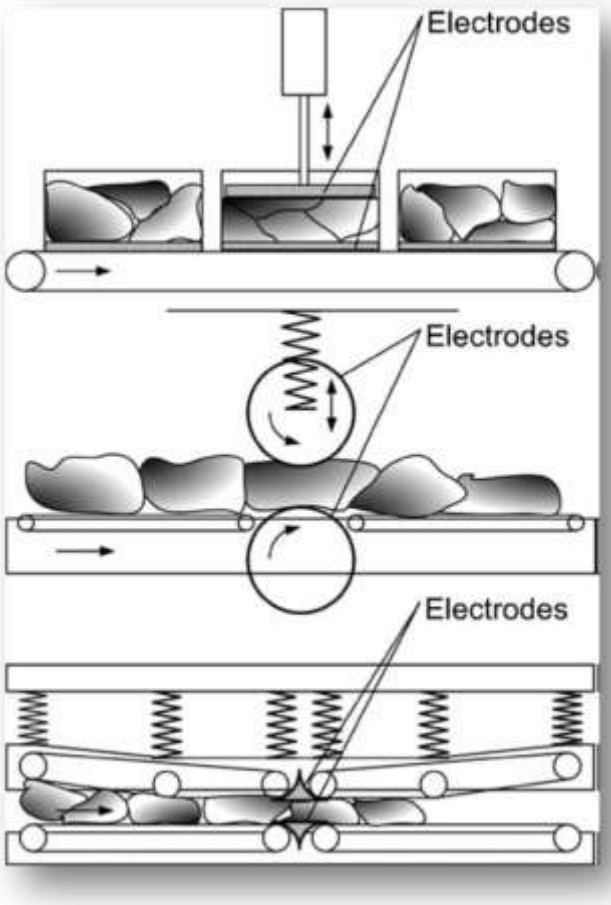

Figure 22. Design concepts for a continuous system for the treatment of meat whole cuts.

5 Success in Achieving Objectives

The results from the executed experiments indicate a promising potential to tenderize meat using pulsed electric field treatment (PEF) with a 19% reduction in the shear force of beef m. *Longissimus lumborum* (LL) and m. *semimembranosus* (SM) muscles. Differences in the muscle response to PEF were observed as the reduction in SM shear force was dependent on the treatment

frequency, but this was not observed in the LL. The results from the executed experiments also indicated a promising potential to gain further meat tenderness using repeated PEF treatment (20µ, 10 kV, 90 Hz) with beef m. *Longissimus lumborum* (LL), but not m. *semimembranosus* (SM) muscles. The repeated PEF however, appears to have a negative impact on the colour stability of meat. The PEF treatment was not affected by the pH and fibre direction of the LL muscles. The results obtained in the present project also indicated that LL and SM muscles respond differently to electrical input and this response can vary depending on whether the muscles were pre-rigor (hot-boned) or post-rigor (cold-boned). The technology appears to be beneficial for hot-boned SM, but not for LL as the latter muscle seems to be more susceptible to heat generation and protein denaturation.

6 Impact on Meat and Livestock Industry- now & in five years time

The project confirmed the potential use of PEF technology to improve the tenderness of cold- and hot-boned beef loins and topsides by about 20%. Therefore the research provides the industry with another technology that can be used to improve the tenderness of meat and add value to the less tender meat cuts. Also the project identified some differences in the response of the two cuts to the same PEF treatment and possibly differing PEF processing conditions can be applied for maximum tenderness gain. The slight, but significant negative effect on fresh meat colour may see most benefits to be gained from this technology in meat that is vacuum packed, destined to foodservices or markets where the product has quick turnover. An advantage of this technology is the potential of use at late post-mortem times (i.e. 3 days post-mortem in topsides) as well as early post-mortem time (e.g. hot-boned topsides) while still gaining a positive impact on the meat tenderness which can give some flexibility for the meat industry in applying this technology.

7 Conclusions

- Pulsed electric field technology can be used to improve the tenderness of cold- and hot-boned topsides and cold-boned loins.
- The technology appears to be flexible with topsides where the level of SM tenderness improvement was not dependent on the meat post-mortem time which will allows the use of PEF technology without any post-mortem time constraints. This needs to be confirmed with other muscles in conjunction with what is the biochemical basis for the tenderizing effect.
- Meat tenderized with this technology may need to be branded as “high yield product” since lower cooking loss is found in meat treated with PEF. This is not real improvement in moisture retention, but is due to higher purge loss during packaging and storage.
- The technology is independent of the meat pH and fibre direction which it can be applied to meat without any limitations to these two factors.

8 Recommendations and Future Opportunities

The present project reveals several research opportunities that could benefit the meat industry through optimisation of the use of PEF to tenderise meat cuts and understand the mechanism of action which will enable better control over the process and facilitate further opportunities for

optimization. Also, evaluation of the treated product is a very important step to confirm the acceptability and any improvements obtained through PEF processing.

- Given the known differences in the muscle biochemistry, the possible use of PEF to tenderize topsides at 1 or 3 days post-mortem cannot be generalized to other meat cuts. This need to be confirmed experimentally.
- Explore the mechanism of action for the observed tenderizing effect of PEF to determine the nature of the process (i.e. whether it is a biochemical, physical process or both).
- The use of PEF improved the tenderness of cold-boned LL and SM muscles and improved the tenderness of hot-boned SM, but not LL muscle. It appears that applying PEF to hot-boned LL muscles generates more heat and renders the muscle more susceptible to heat denaturation. It would be of interest to examine the response of the muscles to PEF at range of fixed temperatures and study the impact of such treatments on the meat proteolysis process. This would enable a clear understanding for the boundary limits for PEF use for different muscles.
- An important aspect of any meat tenderization process is to be able to produce a highly acceptable product. Therefore, it is recommended to carry a consumer sensory study on PEF treated meat and compare it to non-treated control.

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11 Appendix 1.

Table 1A: Pulsed electric field processing parameters used in Experiments 1 and 2 (section 3.3).

Treatment*	IV (kV)	PF (Hz)	PN	PW (μs)	PPP (kW)	PPC (A)	EFS (kV/cm)	ED (kJ/kg)
LL Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LLT1	5.00	20	607	20	100.8± 2.5	44.6± 3.5	0.34± 0.12	3.1± 0.4
LLT2	5.00	50	1529	20	115.2± 1.6	46.3± 1.9	0.31± 0.01	8.6± 1.1
LLT3	5.00	90	2726	20	123.0± 1.9	47.1± 2.1	0.33± 0.01	17.4± 0.8
LLT4	10.0	20	606	20	429.4± 1.3	102.2± 2.9	0.53± 0.01	12.6± 1.4
LLT5	10.0	50	1528	20	468.2± 7.2	104.9± 2.5	0.56± 0.01	35.8± 1.3
LLT6	10.0	90	2724	20	471.2± 10.5	109.0± 1.0	0.54± 0.01	62.2± 4.3
SM1 Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SM1T1	5.00	20	607	20	98.8± 1.6	46.2± 1.5	0.27± 0.01	3.4± 0.2
SM1T2	5.00	50	1529	20	111.2± 4.3	47.8± 1.7	0.29± 0.01	9.4± 1.0
SM1T3	5.00	90	2726	20	119.0± 4.4	48.5± 1.4	0.31± 0.02	18.6± 2.1
SM1T4	10.0	20	606	20	408.0± 16.9	103.6± 3.2	0.51± 0.02	14.3± 0.7
SM1T5	10.0	50	1528	20	445.8± 21.8	107.5± 3.1	0.52± 0.03	37.7± 2.3
SM1T6	10.0	90	2724	20	451.6± 11.7	113.1± 2.9	0.50± 0.03	64.7± 4.9
SM3 Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SM3T1	5.00	20	607	20	103.2± 1.6	45.0± 0.5	0.29± 0.01	3.6± 0.2
SM3T2	5.00	50	1529	20	116.0± 2.0	47.4± 2.7	0.31± 0.02	9.4± 1.0
SM3T3	5.00	90	2726	20	121.4± 1.7	49.6± 1.8	0.31± 0.01	17.8± 2.0
SM3T4	10.0	20	606	20	432.2± 10.3	102.9± 3.3	0.52± 0.03	14.2± 1.1
SM3T5	10.0	50	1528	20	473.2± 13.5	105.3± 4.1	0.56± 0.04	40.7± 2.8
SM3T6	10.0	90	2724	20	475.0± 10.4	109.1± 2.2	0.55± 0.02	73.2± 6.4

* LL = Loins (M. *Longissimus lumborum*), SM = Topsides (M. *Semimembranosus*), IV = Input voltage, PF = Pulse frequency, PN = Pulse number, PW = Pulse width (μs), PPP = Pulse peak power, PPC = Pulse peak current, EFS = Electric field strength, ED = Energy density

Table 2A: Changes (post treatment – pre-treatment) in average beef temperature, conductivity and pH under different pulsed electric field treatments as described in Experiments 1 & 2 (section 3.3).

		LL			SM1			SM3		
Input voltage (kV)	Pulse frequency (Hz)	ΔT	Δσ	ΔpH	ΔT	Δσ	ΔpH	ΔT	Δσ	ΔpH
5.00	20	0.4± 0.7	0.8± 0.7	0.02± 0.09	1.0± 0.4	0.4± 0.4	0.00± 0.06	1.2± 1.1	0.6± 0.5	0.03± 0.04
5.00	50	1.7± 0.7	0.1± 2.4	-0.03± 0.07	1.6± 0.5	0.4± 0.6	0.03± 0.04	1.6± 0.8	0.9± 1.5	0.00± 0.02
5.00	90	2.7± 0.6	0.9± 0.3	-0.01± 0.03	2.4± 0.2	0.6± 0.6	-0.05± 0.05	2.7± 1.6	0.4± 0.4	-0.04± 0.04
10.0	20	2.4± 0.8	-0.2± 0.9	0.01± 0.13	1.9± 0.7	0.6± 0.6	-0.03± 0.02	2.3± 0.3	0.3± 0.2	0.00± 0.04
10.0	50	4.3± 1.6	2.7± 1.3	-0.04± 0.03	4.0± 0.6	0.7± 0.9	-0.03± 0.02	5.2± 1.3	0.8± 0.9	0.01± 0.04
10.0	90	7.7± 1.9	2.2± 1.3	-0.03± 0.07	6.8± 1.7	1.9± 2.5	-0.03± 0.02	8.0± 1.6	0.6± 0.1	-0.02± 0.04

LL = 24 h postmortem M. *Longissimus lumborum* SM1 = 24 h postmortem M. *Semimembranosus* SM3 = 72 h postmortem M. *Semimembranosus*

ΔT = Change in Temperature Δσ = Change in Conductivity ΔpH = Change in pH

Table 3A: Pulsed electric field processing parameters used in Experiment 3.1.

Treatment	PPP x1	PPP x2	PPP x3	Δ x1-x2	Δ x2-x3	Δx1-x3
LL Control						
LLT1x	476.5±9.4					
LLT2x	478.3±13.8	464.2±12.0		-14.2±2.1		
LLT3x	480.3±15.2	467.0±14.4	455.7±14.3	-13.3±0.8	-11.3±1.2	-24.7±1.6
SM Control						
SMT1x	481.2±8.4					
SMT2x	480.7±11.3	468.0±11.2		-12.7±0.5		
SMT3x	477.5±11.5	464.7±11.4	454.2±16.0	-12.8±0.8	-10.5±0.5	-23.3±0.8
Treatment	PPC x1	PPC x2	PPC x3	Δ x1-x2	Δ x2-x3	Δx1-x3
LL Control						
LLTx1	105.7±2.5					
LLTx2	105.0±3.3	108.6±2.8		3.6±0.5		
LLTx3	104.3±4.2	108.1±3.4	111.6±3.7	3.8±0.9	3.8±0.9	7.3±2.0
SM Control						
SMTx1	104.9±2.5					
SMTx2	105.1±3.0	108.3±2.6		3.2±0.4		
SMTx3	105.8±3.1	109.0±2.7	111.4±2.4	3.2±0.4	2.4±0.3	5.6±0.7
Treatment	EFS x1	EFS x2	EFS x3	Δ x1-x2	Δx2-x3	Δx1-x3
LL Control						
LLTx1	0.56±0.02					
LLTx2	0.57±0.04	0.53±0.03		-0.04±0.01		
LLTx3	0.58±0.04	0.54±0.03	0.52±0.03	-0.04±0.02	-0.02±0.01	-0.06±0.02
SM Control						
SMTx1	0.58±0.02					
SMTx2	0.57±0.03	0.54±0.02		-0.03±0.01		
SMTx3	0.56±0.03	0.54±0.02	0.51±0.02	-0.03±0.02	-0.03±0.01	-0.05±0.01
Treatment	ED x1	ED x2	ED x3	Δ x1-x2	Δ x2-x3	Δx1-x3
LL Control						
LLTx1	50.3±4.8					
LLTx2	55.3±7.4	52.7±7.1		-2.6±1.2		
LLTx3	52.4±6.8	50.3±6.3	49.8±5.9	-2.1±1.3	-0.5±1.0	-2.6±0.6
SM Control						
SMTx1	47.2±2.2					
SMTx2	47.7±2.6	46.2±2.8		-1.5±1.3		
SMTx3	46.8±3.4	45.4±3.2	44.4±2.7	-1.3±2.6	-1.0±2.8	-2.3±1.3

Table 4A: Changes (post treatment – pre-treatment) in average beef temperature, conductivity and pH under different pulsed electric field treatments as described in Experiment 3.1 (section 3.3).

		LL				SM	
Treatment	Δ T	Δ σ	Δ pH	Treatment	Δ T	Δ σ	Δ pH
Control	-	-	-	Control	-	-	-
Tx1	8.5±1.7	1.7±0.7	-0.05±0.01	Tx1	7.9±1.0	2.2±0.9	-0.03±0.04
Tx2	12.6±3.1	3.9±2.3	-0.02±0.01	Tx2	13.0±0.9	2.3±1.5	-0.02±0.05
Tx3	16.2±2.7	3.7±1.8	-0.01±0.03	Tx2	9.4±1.6	2.0±1.4	-0.04±0.04

The abbreviations are as stated in Table 1A above.

Δ = Change in the measured property, x1, x2 and x3 = one, two and three PEF repeated treatments

Table 5A: Pulsed electric field processing parameters used in Experiment 3.2

Treatment	PPP x1	PPP x2	PPP x3	Δ x1-x2	Δ x2-x3	Δx1-x3
LL Control						
LLTx1	474.0±10.8					
LLTx2	476.0±10.2	462.0±10.3		-14.0±1.5		
LLTx3	471.5±12.8	456.5±13.2	444.7±13.2	-15.0±2.0	-11.8±1.9	-26.8±3.8
SM Control						
SMTx1	475.8±3.3					
SMTx2	484.8±6.2	471.0±6.3		-13.8±0.8		
SMTx3	478.0±7.4	465.0±7.4	543.8±0.2	-13.0±0.9	-11.2±0.8	-24.2±1.6
Treatment	PPC x1	PPC x2	PPC x3	Δ x1-x2	Δ x2-x3	Δx1-x3
LL Control						
LLTx1	108.9±3.0					
LLTx2	108.2±2.6	111.8±2.4		3.5±0.6		
LLTx3	109.6±3.3	113.0±2.9	115.6±2.6	3.4±0.6	2.6±0.4	6.0±1.1
SM Control						
SMTx1	108.3±0.8					
SMTx2	105.5±1.9	109.2±1.6		3.7±0.3		
SMTx3	107.5±2.0	110.8±1.8	113.4±1.7	3.3±0.3	2.6±0.2	5.9±0.5
Treatment	EFS x1	EFS x2	EFS x3	Δ x1-x2	Δx2-x3	Δx1-x3
LL Control						
LLTx1	0.5±0.03					
LLTx2	0.6±0.03	0.5±0.02		-0.04±0.01		
LLTx3	0.5±0.03	0.5±0.03	0.5±0.03	-0.03±0.01	-0.03±0.01	-0.05±0.01
SM Control						
SMTx1	0.5±0.01					
SMTx2	0.6±0.04	0.5±0.01		-0.03±0.03		
SMTx3	0.6±0.02	0.5±0.01	0.5±0.01	-0.03±0.01	-0.03±0.0	-0.06±0.01
Treatment	ED x1	ED x2	ED x3	Δ x1-x2	Δ x2-x3	Δx1-x3
LL Control						
LLTx1	47.6±4.4					
LLTx2	48.2±3.2	46.4±3.3		-1.8±1.2		
LLTx3	45.7±2.7	45.0±3.4	43.6±3.5	-0.7±0.7	-1.4±1.0	-2.2±1.1
SM Control						
SMTx1	46.5±1.2					
SMTx2	50.3±5.7	50.6±5.0		-2.7±3.3		
SMTx3	47.6±2.6	46.0±2.7	44.6±3.0	-1.6±1.3	-1.3±0.6	-2.9±1.0

Table 6A: Changes (post treatment – pre-treatment) in average beef temperature, conductivity and pH under different pulsed electric field treatments as described in Experiment 3.2 (section 3.3).

		LL				SM	
Treatment	Δ T	Δ σ	Δ pH	Treatment	Δ T	Δ σ	Δ pH
Control	-	-	-	Control	-	-	-
Tx1	8.05±1.09	10.9±4.3	-0.06±0.03	T1	7.7±2.0	1.4±0.7	-0.03±0.04
Tx2	12.72±1.67	15.6±4.6	-0.07±0.05	T2	12.5±3.0	3.1±1.8	-0.08±0.03
Tx3	16.05±2.20	18.6±5.1	-0.08±0.05	T3	15.6±2.1	3.1±1.5	-0.07±0.04

Abbreviations are as in Table 4A above

Table 7A: Pulsed electric field processing parameters used in Experiment 4 (section 3.3).

pH Range	Fibre	PPP	PPC	EFS	ED
5.5-5.8	crossed	488.7±7.5	104.3±2.5	0.59±0.02	19.2±1.3
5.8-6.1	crossed	494.5±9.2	101.7±3.2	0.61±0.03	17.8±1.8
6.1+	crossed	486.3±18.4	104.5±5.9	0.58±0.06	17.3±1.7
5.5-5.8	parallel	497.2±9.3	101.6±3.1	0.61±0.03	16.2±1.5
5.8-6.1	parallel	494.8±7.8	102.2±2.9	0.61±0.03	19.3±1.3
6.1+	parallel	507.8±8.30	97.0±3.1	0.73±0.16	18.7±4.0

Table 8A: Changes (post treatment – pre-treatment) in average beef temperature, conductivity and pH under different pulsed electric field treatments as described in Experiment 4 (section 3.3).

pH Range	Fibre	Δ Temperature	Δ Conductivity	Δ pH
5.5-5.8	crossed	12.2±5.6	2.8±0.9	-0.05±0.08
5.8-6.1	crossed	11.5±4.2	2.3±0.8	-0.06±0.02
6.1+	crossed	10.7±2.8	2.1±0.6	-0.06±0.03
5.5-5.8	parallel	9.5±2.9	2.3±0.8	-0.07±0.03
5.8-6.1	parallel	12.5±3.7	1.8±0.8	-0.06±0.04
6.1+	parallel	9.9±2.3	1.5±0.2	-0.10±0.02

Abbreviations are as in Table 1A above.

Table 9A: Pulsed electric field processing parameters used in Experiments 5&6 (section 3.3).

Treatment	IV (kV)	PF (Hz)	PN	PW (µs)	PPP (kW)	PPC (A)	EFS (kV/cm)	ED (kJ/kg)
LL Control	-	-	-	-	-	-	-	
LLT1	5	20	606	20	99.6±4.4	50.0±3.4	0.28±0.03	3.3±0.2
LLT2	5	50	1528	20	113.0±4.7	46.2 3.5	0.31±0.03	9.4±0.7
LLT3	5	90	2726	20	118.4±2.7	51.4±1.8	0.29±0.02	17.3±1.1
LLT4	10	20	606	20	397.4±7.9	108.5±5.7	0.46±0.03	12.6±0.6
LLT5	10	50	1527	20	438.8±13.3	106.4 ± 6.5	0.51±0.03	39.9±3.9
LLT6	10	90	2724	20	440.8±25.6	112.7±5.7	0.50±0.05	66.5±8.2
SM Control	-	-	-	-	-	-	-	
SMT1	5	20	607	20	102.6±1.9	41.9±2.3	0.31±0.03	3.2±0.2
SMT2	5	50	1529	20	115.4±2.2	43.2±3.6	0.34±0.03	9.8±1.3
SMT3	5	90	2726	20	122.6±1.5	43.6±3.1	0.35±0.03	18.7±2.0
SMT4	10	20	606	20	437.2±18.17	100.8±5.4	0.55±0.05	15.4±2.8
SMT5	10	50	1527	20	468.2±19.5	105.5±5.9	0.56±0.05	42.2±6.5
SMT6	10	90	2724	20	471.0±6.4	110.0±1.7	0.54±0.02	70.2±4.2

Table 10A: Changes (post treatment – pre-treatment) in average beef temperature, conductivity and pH under different pulsed electric field treatments as described in Experiments 5 & 6 (section 3.3)

	LL				SM		
Treatment	Δ Temperature	Δ conductivity	Δ pH	Treatment	Δ Temperature	Δ conductivity	Δ pH
Control	-	-	-	Control	-	-	-
T1	-0.18±0.7	1.12±1.1	-0.03±0.03	T1	-0.70±1.4	3.5±3.0	-0.03±0.07
T2	-0.58±1.3	1.5±1.2	-0.05±0.04	T2	0.10±0.8	2.1±2.6	-0.07±0.10
T3	0.4±1.5	2.4±2.7	-0.09±0.08	T3	1.68±1.7	1.4±0.5	-0.05±0.13
T4	0.88±0.8	3.1±2.6	-0.05±0.11	T4	1.24±1.3	1.3±1.9	-0.07±0.11
T5	2.72±2.2	3.0±2.1	-0.13±0.14	T5	2.48±1.7	2.4±0.3	-0.02±0.06
T6	4.4±1.4	2.2±1.7	-0.11±0.13	T6	8.06±2.4	3.2±2.2	-0.08±0.07

Abbreviations are as in Table 1A above.

Table 11A: Pulsed electric field processing parameters used in Experiment 7 (section 3.3)

Treatment	PPP x1	PPP x2	PPP x3	Δ x1-x2	Δ x2-x3	Δx1-x3
LL Control						
LLTx1	441.3±5.3					
LLTx2	437.2±10.9	423.2±9.3		-14.0±1.7		
LLTx3	443.7±11.5	429.0±10.6	417.5±10.0	-14.7±1.4	-11.5±1.6	-26.2±3.0
SM Control						
SMTx1	462.3±18.4					
SMTx2	440.8±13.2	451.3±12.3		10.5±1.4		
SMTx3	452.2±16.3	461.3±14.8	468.7±14.1	9.2±1.6	73.±1.0	16.5±2.6
Treatment	PPC x1	PPC x2	PPC x3	Δ x1-x2	Δ x2-x3	Δx1-x3
LL Control						
LLTx1	115.8±1.1					
LLTx2	116.5±2.0	119.0±1.6		2.6±0.5		
LLTx3	114.9±2.2	117.8±2.1	119.9±1.8	2.9±0.3	2.1±0.3	5.0±0.5
SM Control						
SMTx1	121.8±1.7					
SMTx2	122.5±0.5	122.5±0.5		0.0±0.6		
SMTx3	122.2±0.8	121.7±1.2	121.3±0.0	-0.5±0.5	-0.3±0.5	-0.8±0.4
Treatment	EFS x1	EFS x2	EFS x3	Δ x1-x2	Δx2-x3	Δx1-x3
LL Control						
LLTx1	0.475±0.47					
LLTx2	0.471±0.02	0.444±0.02		-0.03±0.01		
LLTx3	0.483±0.02	0.456±0.02	0.435±0.02	-0.03±0.01	-0.02±0.01	-0.05±0.01
SM Control						
SMTx1	0.330±0.02					
SMTx2	0.350±0.01	0.338±0.01		-0.01±0.0		
SMTx3	0.341±0.01	0.331±0.02	0.327±0.01	-0.01±0.01	0.0±0.01	-0.01±0.01
Treatment	ED x1	ED x2	ED x3	Δ x1-x2	Δ x2-x3	Δx1-x3
LL Control						
LLTx1	70.5±8.8					
LLTx2	66.7±6.6	63.2±5.5		-3.6±1.8		
LLTx3	68.6±6.2	66.3±6.1	65.0±6.6	-2.2±1.5	-1.4±1.4	-3.6±0.7
SM Control						
SMTx1	17.8±1.0					
SMTx2	19.0±0.4	18.4±0.6		-0.6±0.4		
SMTx3	17.9±1.7	17.5±1.3	17.8±0.6	-0.3±0.7	0.2±1.0	-0.1±0.3

Table 12A: Changes (post treatment – pre-treatment) in average beef temperature, conductivity and pH under different pulsed electric field treatments as described in Experiment 7 (section 3.3)

	LL				SM		
Treatment	Δ T	Δ σ	Δ pH	Treatment	Δ T	Δ σ	Δ pH
Control	-	-	-	Control	-	-	-
Tx1	6.5±2.4	1.3±1.6	-0.08±0.11	T1	1.8±1.1	1.5±0.7	-0.03±0.04
Tx2	8.2±1.0	3.6±2.7	-0.07±0.05	T2	5.2±1.1	4.5±0.7	0.15±0.4
Tx3	13.4±5.2	5.0±4.6	0.02±0.08	T3	6.7±3.8	3.0±1.4	-0.04±0.04

Abbreviations are as in Table 4A above.

Appendix 2

Effect of repeat PEF treatment and ageing time on the colour parameters of LL and MS beef muscles.

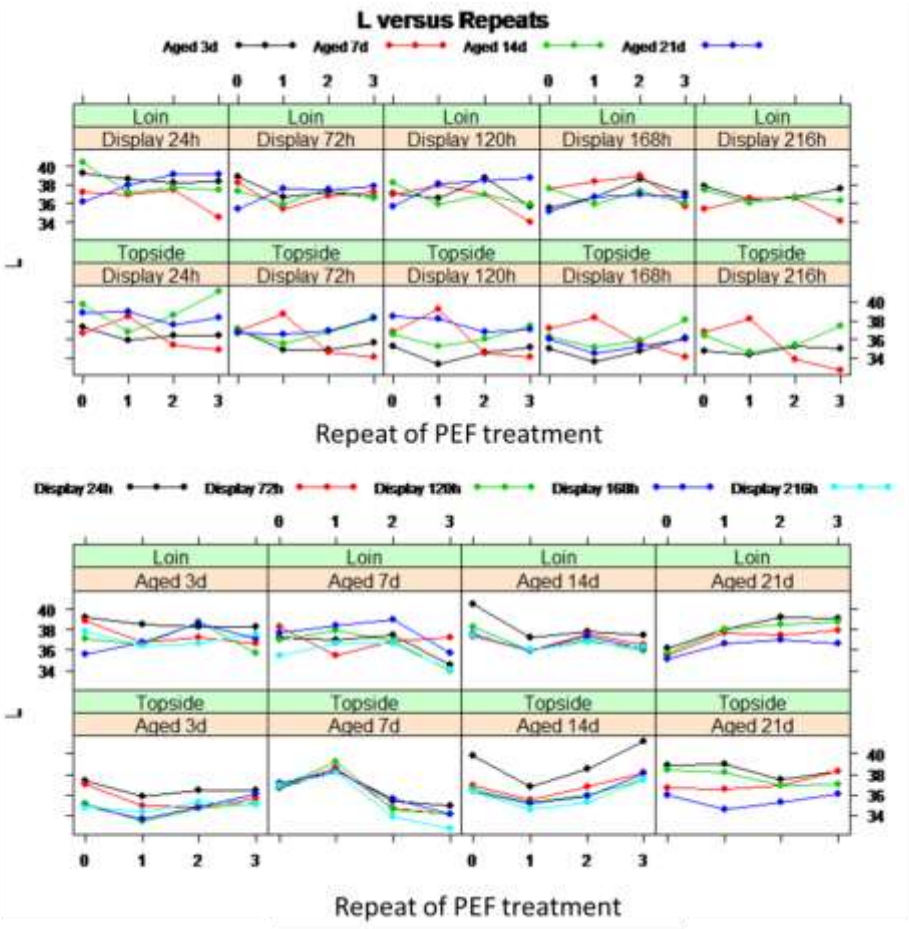

Figure 1A. Effect of repeated (0, 1, 2 or 3) pulsed electric field on the lightness value (L) of loin and topsides aged for 3, 7, 14 and 21 days post-treatment.

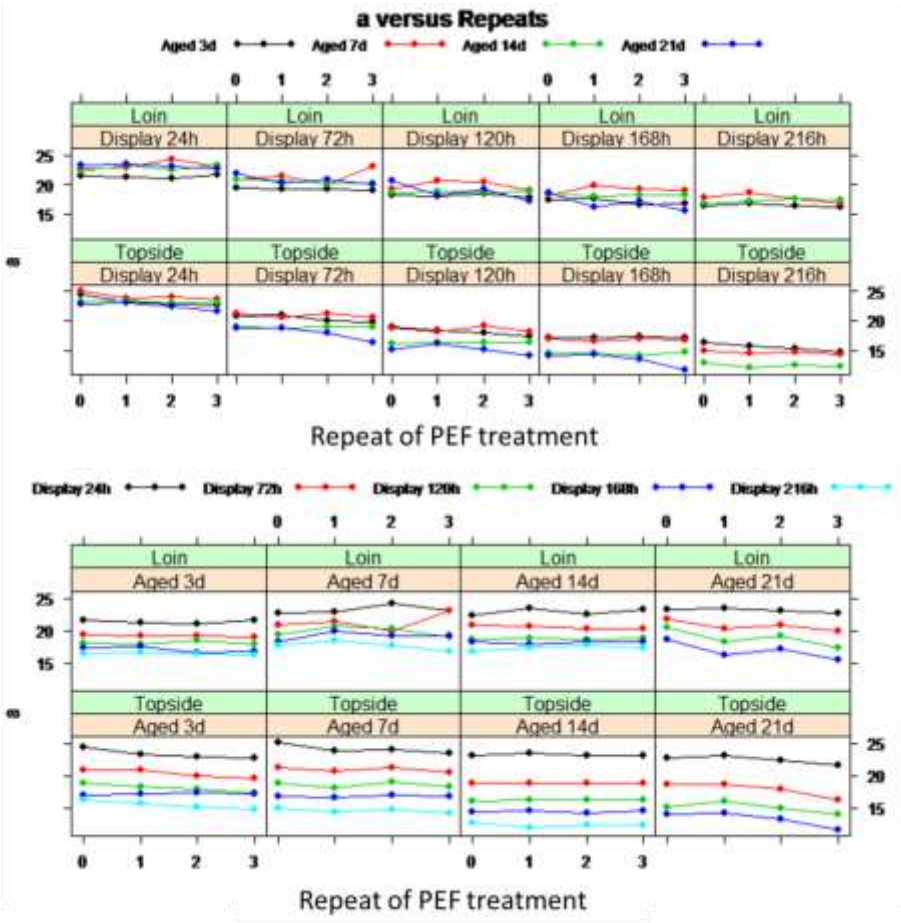

Figure 2A. Effect of repeated (0, 1, 2 or 3) pulsed electric field on the redness value (a) of loin and topsides aged for 3, 7, 14 and 21 days post-treatment.

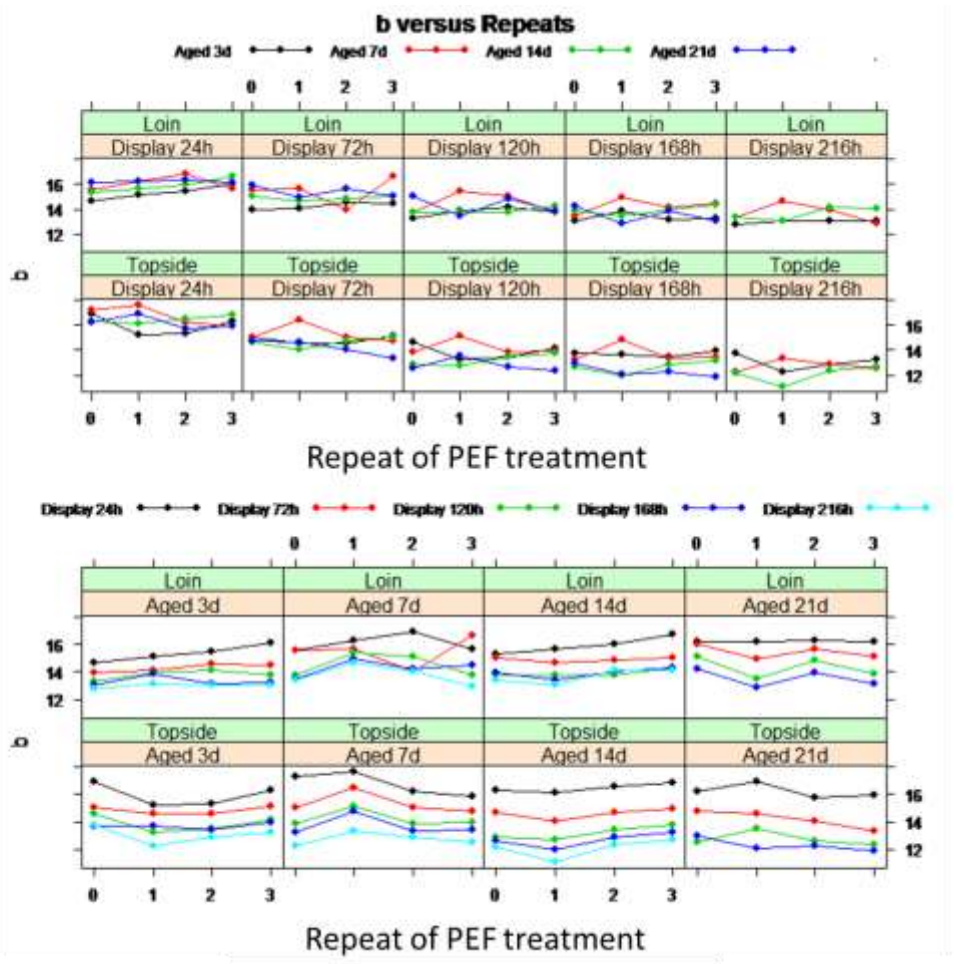

Figure 3A. Effect of repeated (0, 1, 2 or 3) pulsed electric field on the yellowness value (b) of loin and topsides aged for 3, 7, 14 and 21 days post-treatment.

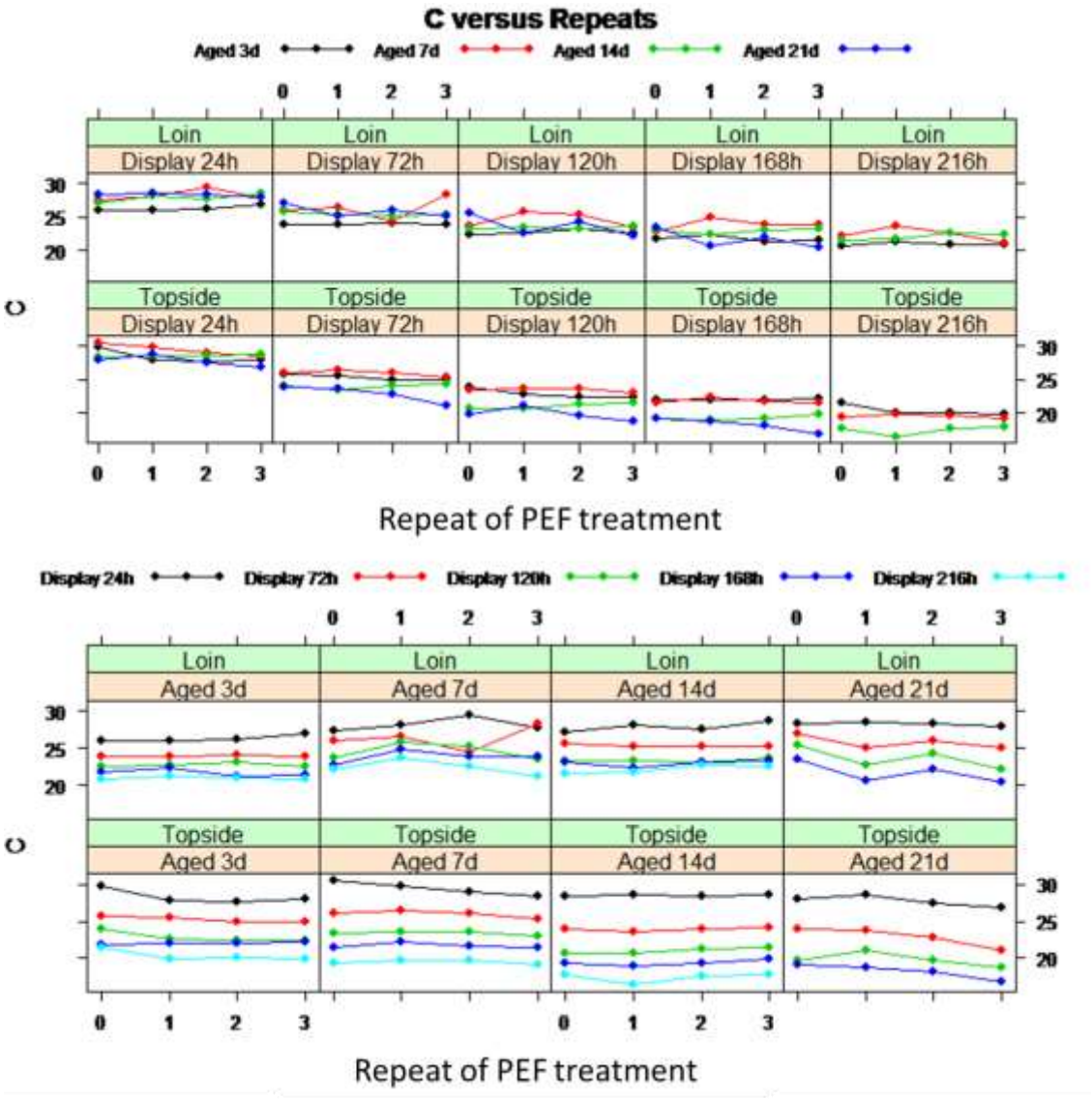

Figure 4A. Effect of repeated (0, 1, 2 or 3) pulsed electric field on the Chroma value (C) of loin and topsides aged for 3, 7, 14 and 21 days post-treatment.

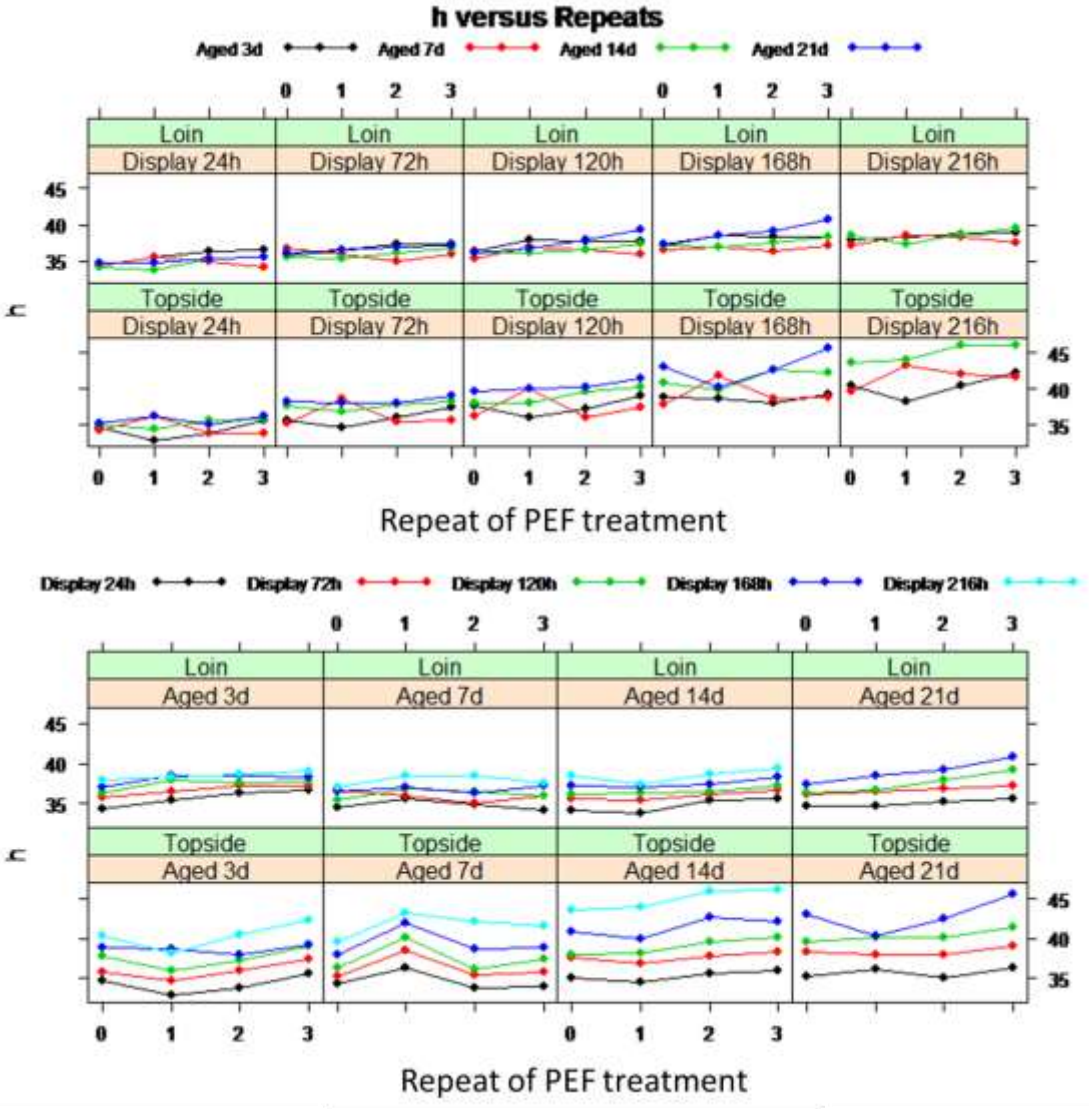

Figure 5A. Effect of repeated (0, 1, 2 or 3) pulsed electric field on the hue value (h) of loin and topsides aged for 3, 7, 14 and 21 days post-treatment.

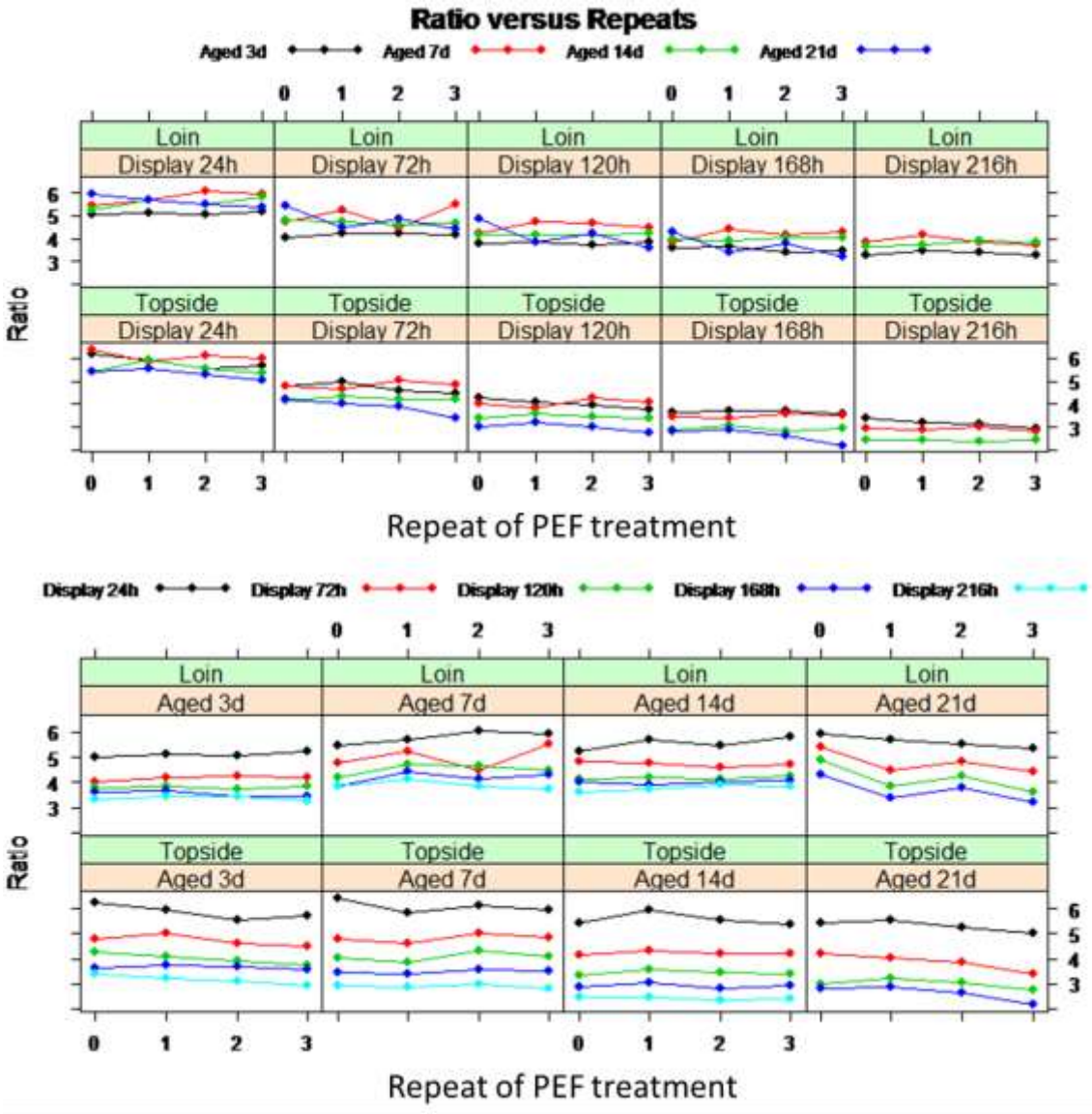

Figure 6A. Effect of repeated (0, 1, 2 or 3) pulsed electric field on the browning index ratio (Ratio) of loin and topsides aged for 3, 7, 14 and 21 days post-treatment.