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Effect of repeated pulsed electric field treatment on the quality of hot-boned beef loins and topsides

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ABSTRACT

The effects of repeated (1×, 2×, 3×) pulsed electric field (PEF) treatment (10 kV, 90 Hz, 20 μs) on the quality of beef loins and topsides were evaluated, including the tenderness, purge loss, cooking loss, myofibrillar protein profile and post-mortem proteolysis. Both muscle types were obtained from each of 12 steers and were processed within 6 h. After PEF treatment samples of both muscle types were subjected to 3, 7, 14 and 21 days ageing. The 1× and 2× PEF treatments had no effect on the tenderisation of hot-boned beef *Musculus longissimus lumborum* muscle for all ageing treatment times, while the 3× treatment reduced the tenderness. The opposite effect was observed in hot-boned beef *Musculus semimembranosus* muscles where the 3× treatment produced meat with lowest shear force at 3 days post-treatment time, but this beneficial effect disappeared with longer ageing times. An increase in proteolysis of troponin T was seen to the largest extent with 1× PEF treatment and decreased with every extra application of treatment.

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1. Introduction

There are several quality attributes of fresh meat that significantly influence the initial purchasing and subsequent repurchasing decisions of a consumer. Colour is one of the most important commercial attributes of meat which is normally used as an indicator of freshness and wholesomeness of retailed fresh meat (Castigliego, Armani, & Guidi, 2012; Mancini & Hunt, 2005). On the other hand, tenderness has been well accepted as the most important quality attribute for repurchasing decisions of meat products (Bolumar, Enneking, Toepfl, & Heinz, 2013). Although many methods (physical, chemical and enzymatic) have been reported for improving meat tenderisation, these methods often produce side effects such as severe structural changes (e.g. mushy texture), microbial contamination and off-flavour development (Bekhit, Carne, Ha, & Franks, 2014a; Bekhit, Hopkins, Geesink, Bekhit, & Franks, 2014b).

Pulsed electric field (PEF) is a stand-alone technology that has the potential to improve meat tenderisation by enhancing cell permeation and consequently increasing proteolysis that contributes to tenderisation (Bekhit, van de Ven, Suwandy, Fahri, & Hopkins, 2014c; Suwandy, Carne, van de Ven, Bekhit, & Hopkins, 2015a). PEF has the potential for optimising the technology input to different meat cuts, thereby optimising the product quality and allowing the quality upgrade of less tender meat cuts. Moreover, PEF has the potential to modify the structure of biological materials without the side effects encountered with the conventional methods mentioned above. PEF was found to increase the tenderness of cold-boned beef *Musculus longissimus lumborum* and *Musculus semimembranosus* as well as increasing post-mortem proteolysis as evident by the increase in troponin and desmin degradation (Suwandy et al., 2015a). During hot-boning, muscles are removed from the carcass in a pre-rigour state and due to the absence of the skeletal framework hot-boned beef is more susceptible to contraction and shortening than cold-boned beef and thus generally would be tougher than cold-boned beef (Troy, 2006; Troy & Kerry, 2010; White, O'Sullivan, Troy, & O'Neill, 2006). One of the benefits of hot-boning over cold-boning is that with the former each muscle is separated from the carcass in the pre-rigour state, thus allowing the use of different intensities of PEF treatment to be applied to the muscles to achieve optimal results due to muscle manipulation prior to the onset of rigour. This study aimed to investigate the effect of repeated (1×, 2× and 3×) PEF treatment (voltage of 10 kV and frequency of 90 Hz), followed by ageing for various periods of time, on the tenderness of hot-boned beef *M. longissimus lumborum* (LL) and *M. semimembranosus* (SM) muscles.

2. Materials and methods

2.1. Meat

Loins (*M. longissimus lumborum*) and topsides (*M. semimembranosus*) were obtained from 12 steers (six steers with average weight 279.3 ±

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28.3 kg, prime beef grade and six steers with average weight 177.9 ± 10.1 kg, manufacturing beef grade loin and topsides, respectively) raised on pasture and were slaughtered by the Alliance Group (Pukeuri plant, Oamaru, New Zealand). The carcasses were electrically stimulated [square mono wave, 80 V, 25 s, pulse width of 68 ms and at a pulse rate per second of 15 pps]. Muscles were removed from carcasses at 4 h post-mortem and processed within 2 h.

2.2. Antibodies

The cardiac troponin T antibody developed by Jim Jung-Ching Lin was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242. The secondary antibody used was Pierce antibody (goat anti-mouse) (product no. 31430), Thermo Scientific, Auckland, New Zealand.

2.3. PEF treatments and subsequent meat ageing

The PEF system (Elcrack-HPV5, DIL, Quakenbruck, Germany) was used in batch mode, and the meat fibre direction was parallel to the electrodes. An oscilloscope (Model UT2025C, Uni-Trend Group Ltd., Hong Kong, China) was used to monitor the pulse shape used (square wave bipolar). The PEF 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). The samples had an average temperature of 24.8 ± 1.4 °C and 24.5 ± 1.0 °C for loins and topsides, respectively at the time of treatment. Both muscles were cut into seven blocks of 13 × 8 × 5 cm (average weight of 354 ± 31 g and 369 ± 17 g for loins and topsides, respectively). The blocks were randomly allocated to 3 levels of PEF treatment, at 10 kV and 90 Hz, repeats of 1×, 2× and 3× and a non-treated control. PEF treatments were applied in batch mode and the treatment time was 30 s. After treatment, the blocks were then cut into 4 equal pieces that were weighed, vacuum packed (VP) and randomly assigned to 4 different ageing storage times (3, 7, 14, or 21 days). Each sample was stored at 4 °C until the designated ageing time was reached. Weight, temperature, pH and electrical conductivity of each block were measured directly before and after treatment. The blocks were sampled for biochemical analyses and for tenderness measurements. The samples were frozen and maintained at −40 °C.

2.4. Measurements

2.4.1. Electrical input

The treatment electrical parameters (pulsed electric field strength, pulse peak energy, pulse peak current, pulse peak power, pulse count, resistance, energy, calculated field strength and calculated specific energy) were obtained and recorded from the PEF instrument for each treatment. The energy density was calculated as described by Zhang, Barbosa-Canovas, and Swanson (1995) and O'Dowd, Arimi, Noci, Cronin, and Lyng (2013) using the following equation;

$$Q = \frac{V^2 t}{Rv}$$

Here Q is the energy density (kJ/kg), V is the voltage (Volts), t is the treatment time (t = number of pulses × pulse duration in μS), R is the resistance (Ohms), and v is the weight of the sample (g).

2.4.2. pH

The pH was measured using a puncture pH electrode InLab 427 (Mettler-Toledo Process Analytical Inc., Wilmington, MA) attached to a Hanna HI 9025 (Hanna Instruments, Woonsocket, RI). The pH of each block was measured directly before and after PEF treatment and after storage at 4 °C for 3, 7, 14, and 21 days of treatment. The pH difference from the initial pH before treatment was calculated at various measurement points.

2.4.3. Temperature

The temperature of the centre of the meat block was measured using a puncture pH electrode directly before and after PEF treatment. Additionally the temperature was recorded at several locations (8 locations/block) using a hand held infrared thermometer, as a temperature gradient was detected in some of the treatment combinations. The average of the 8 measurements was used for further analysis.

2.4.4. Electrical conductivity σ

The electrical conductivity (mS cm^{-1}) of each block was measured directly before and after PEF treatment and after VP storage at 4 °C using a hand held electrical conductivity metre. Electrical conductivity of the block differed between locations and was dependent on the fibre direction in the meat block. Four fixed locations per block were measured and averaged.

2.4.5. Purge loss percentage

Purge was measured after 3, 7, 14, and 21 days of VP storage at 4 °C. On the designated storage time, samples were blotted dry using a paper towel and weighed. Purge loss percentage was calculated using the following formula:

Purge loss (%) = 100 × (1 − weight after storage / initial weight before storage).

2.4.6. Cooking loss

The samples were thawed overnight at 4 °C, weighed and cooked individually in plastic bags immersed in a water bath at 80 °C until the internal temperature reached 75 °C as measured individually using a temperature probe. Each sample was cooled immediately in an ice bath, blotted dry with paper towels and weighed. The difference in weight before and after cooking was used to calculate the cooking loss using the formula below:

Cooking loss (%) = 100 × (1 − weight after cooking / weight before cooking).

2.4.7. Shear force

Shear force was determined as described by Chrystall and Devine (1991). The cooked meat sample was sliced along the muscle fibre axis to produce 8 subsamples with a 1 × 1 cm cross section. Each subsample was sheared using a MIRINZ tenderometer with a wedge shaped tooth and the peak shear force value was obtained.

2.5. Myofibrillar protein extraction

Myofibrillar protein fractions were prepared according to the procedure described by (Han, Morton, Bekhit, & Sedcole, 2009). A 1.00 ± 0.01 g sample was cut from each meat subsample and then cut into small pieces. A 5 μl aliquot of a phenylmethanesulfonylfluoride (PMSF) solution (17.42 mg of PMSF dissolved in 50 μl of ethanol and then the volume was made to 1 ml with Milli-Q water) and 5 ml homogenisation buffer (100 mM KCl, 2 mM $MgCl_2$, 2 mM EGTA, 1 mM NaN_3, 20 mM Na_2HPO_4, 20 mM NaH_2PO_4, pH 6.8) were added to the meat sample. The mixture was homogenised for 1 min to achieve fine particles. The mixture was centrifuged (CPR centrifuge, Beckman Coulter, Inc., California, USA) at 2800 × g at 4 °C for 10 min. A 5 μl aliquot of a PMSF solution, 5 μl 1% Triton X-100, and 5 ml washing buffer (100 mM NaCl, 5 mM NaN_3) were centrifuged 3200 × g for 10 min at 4 °C. The resultant supernatant was discarded and 5 ml of an SDS sample buffer composed of 8.2 ml Milli-Q water and 1.25 ml stacking Tris buffer (0.5 M Tris–HCl, pH 6.8, containing 0.3 g SDS, and 0.5 ml β-mercaptoethanol) was added. The sample was homogenised for 30 s, heated at 90 °C for 5 min, centrifuged at 3200 × g at 25 °C for

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