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Effect of pulsed electric field on the proteolysis of cold boned beef *M. Longissimus lumborum* and *M. Semimembranosus*

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ABSTRACT

The effects of pulsed electric field (PEF) and ageing (3, 7, 14 and 21 days) on the shear force, protein profile, and post-mortem proteolysis of beef loins (*M. Longissimus lumborum*, LL) and topsides (*M. Semimembranosus*, SM) were investigated using a range of pulsed electric field treatments [voltages (5 and 10 kV) and frequencies (20, 50, and 90 Hz)]. PEF treatment decreased the shear force of beef LL and SM muscles by up to 19%. The reduction in the shear force in the LL was not affected by the treatment intensity whereas the reduction in the SM was dependent on PEF frequency. PEF treated beef loins showed increased proteolysis, both early post-mortem and during subsequent post-mortem storage reflected by increased degradation of troponin-T and desmin. The most prominent troponin-T degradation was found in samples treated with 5 kV–90 Hz, 10 kV–20 Hz at day 3 and day 7 post-treatment in addition to 10 kV–50 Hz in subsequent post-treatment times. The degradation of desmin in PEF treated beef loins increased with ageing time.

1. Introduction

Meat is an important source of micronutrients (e.g. iron, selenium, zinc, vitamins A and B12) and is a rich source of high quality protein. Meat has been consumed widely around the world as a staple food and its consumption is important for optimal human growth and development (Biesalski, 2005; Higgs, 2000; Pereira & Vicente, 2013). Tenderness is considered to be the most important quality attribute for repeat purchasing decisions by the consumer (Bolumar, Enneking, Toepfl, & Heinz, 2013) since other eating quality attributes such as flavour and juiciness can be manipulated by the addition of ingredients during meal preparation.

Pulsed electric field technology demonstrated great potential in liquid foods in relation to the inactivation of pathogenic microorganisms, spoilage microorganisms or enzymes associated with quality and safety issues in these foods (Elez-Martinez, Sobrino-Lopez, Soliva-Fortuny, & Martin-Belloso, 2012; Vega-Mercado et al., 1997). Recently, several studies have emerged investigating the use of PEF in solid foods with the aim of modifying their structure for various reasons (e.g. extraction of bioactive compounds or change of physical properties of plant material). However, there have only been very limited studies investigating the application of PEF in muscle foods (O'Dowd, Arimi, Noci, Cronin, & Lyng, 2013). The application of PEF to meat may have multiple functions such as enhancing cell permeation by electroporation and consequently tenderness as well as possibly reducing the microbial load which would improve the shelf-life and the safety of the product (Jaeger, Balasa, & Knorr, 2008; Töpfl et al., 2007). Although there are limited studies on the effect of PEF on red meat, there are several possibilities in which PEF technology can accelerate the release of enzymes and the glycolysis process that are needed for early proteolysis generating optimum conditions for meat tenderisation. PEF technology offers the ability to optimize the technology input to provide optimum conditions for various meat cuts and quality upgrade for less tender meat cuts. The use of PEF for improving the quality of fresh beef *M. Semitendinosus* muscle was investigated by O'Dowd et al. (2013). The authors reported structural changes in the samples treated by PEF (1.1–2.8 kV cm^{-1}, 5–200 Hz, 12.7–226 kJ kg^{-1}) but there was no effect on the tenderness level of the beef *M. Semitendinosus* muscle. The study investigated the immediate effect of PEF treatment and did not consider the effect of ageing on tenderness. A recent study by Bekhit, van de Ven, Suwandy, Fahri, and Hopkins (2014) found a significant increase in the tenderness of beef *Longissimus lumborum* and *Semimembranosus* muscles due to PEF treatment (combinations of voltages (5 and 10 kV) and frequencies (20, 50 and 90 Hz)), but the mechanism of tenderization was not reported. Therefore, the present study aimed to investigate the effect of PEF treatment on the sarcoplasmic and myofibrillar proteins in LL and SM muscles as well as the proteolysis of the protein in beef LL during ageing.

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2. Materials and methods

2.1. Meat

Loins (*M. Longissimus lumborum*) and topsides (*M. Semimembranosus*) were obtained from two different animal groups, each comprising 6 Hereford steers that had been raised on pasture. The animals were slaughtered by the Alliance Group (Pukeuri plant, Oamaru, New Zealand). The carcases were of grade P2 (prime steers with fat cover 3–10 mm) and the average hot carcass weights were 303.4 ± 23.3 kg and 299.2 ± 13.95 kg, for the loin and the topside animal groups respectively. The loins from both sides were excised at 24 h post-mortem, vacuum-packed (VP) and treated within 6 h. The topsides from both sides were excised at 24 h post-mortem. The left topsides were treated at 24 h post-mortem similar to the loins while the right muscles were treated at 3 day post-mortem to examine the effect of PEF treatment on meat at different post-mortem times.

2.2. PEF treatments

The samples were cut into blocks of 13 × 8 × 5 cm and randomly allocated to 7 treatment combinations; voltages (5 and 10 kV) and frequencies (20, 50 and 90 Hz) plus a non-treated control. The pulsed electric field treatment was carried out using Elcrack-HPV5 (DIL, Quakenburck, Germany) in batch mode as described in Bekhit et al. (2014) and the meat fibre direction was parallel to the electrodes. The PEF system consisted of a power generator, treatment chamber and 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 were sliced into 4 pieces, weighed, vacuum packed and randomly assigned to 4 post-treatment ageing times (3, 7, 14, or 21 days). The samples were stored at 4 °C during ageing.

2.3. pH

The pH was measured for each block directly before and after PEF treatment and for each sub-sample after the allocated storage time at 4 °C (i.e. 3, 7, 14 or 21 days). The pH difference from the initial pH before treatment was calculated at various measurement points.

2.4. Shear force

The shear force was determined by the MIRINZ tenderometer test after cooking the samples individually in plastic bags immersed in a water bath at 80 °C until they reached an internal temperature of 75 °C (10–14 min) as described in Bekhit et al. (2014) and the values are reported in Newtons.

2.5. Sarcoplasmic and myofibril protein extraction

Sacroplasmic and myofibrillar protein fractions were separated after PEF treatment and ageing according to the procedure described by (Han, Morton, Bekhit, & Sedcole, 2009). A 1.00 ± 0.01 gramme of meat was excised from each subsample and was cut into small pieces. A 5 μl aliquot of phenylmethylsulfonyl fluoride (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 1 ml aliquot of supernatant (sarcoplasmic extract) was transferred to a clean tube and stored at −20 °C until further processing. A 5 μl aliquot of PMSF solution, 5 μl 1% Triton X-100, and 5 ml washing buffer (100 mM NaCl, 5 mM NaN_3) were added to the remaining pellet. The mixture was homogenised for 30 s and centrifuged at 3200 ×*g* for 10 min at 4 °C. The resultant supernatant was discarded and 5 ml of SDS sample buffer (8.2 ml MilliQ-water, 1.25 ml stacking Tris buffer, 0.3 g SDS, 0.5 ml of β-mercaptoethanol) were added. The sample was homogenised for 30 s, heated at 90 °C for 5 min, and then centrifuged at 3200 ×*g* at 25 °C for 5 min. The supernatant was transferred to a clean tube and the extraction was repeated twice to make up a total volume of 15 ml supernatant (which was called the myofibrillar fragmentation index, MFI, protein extract). MFI protein extract was stored at −20 °C until further processing.

2.6. 1D-SDS-PAGE gel electrophoresis

1D-SDS-PAGE was used to examine the meat myofibril and sacroplasmic profiles. Myofibrillar and sarcoplasmic protein profiles were obtained according to the procedure described by Ha (2012) with modification. The myofibril and sarcoplasmic protein extracts were thawed and 30 μl aliquots were transferred to 600 μl microfuge tubes. 15 μl Milli-Q water, 17.1 μl BOLT™ sample buffer (4×) and 6.75 μl BOLT™ reducing agent (10×) were added to each extract to make a stock sample. The stock samples were heated at 90 °C for 5 min. A 15.3 μl aliquot of each stock sample was loaded onto a 15 well BOLT™ 4-12% Bis-Tris gel. Electrophoresis was performed in BOLT™ MES SDS running buffer (1×) at 164 V for 34 min at room temperature (21 °C ± 2.0). The gels were washed three times in Milli-Q water for 5 min each time and stained overnight in 20 ml Invitrogen SimplyBlue™ SafeStain with gentle shaking, or were further processed for western blotting. Stained gels were de-stained with Milli-Q water and scanned.

2.7. Western blotting

Prepared gels were briefly washed in Towbin buffer (standard recipe for desmin transfer: 6.6 g Tris, 28.8 g glycine, 10% (v/v) methanol, made to a total volume of 2 l; for troponin transfer Tween detergent was also added: 6.6 g Tris, 28.8 g glycine, 10% (v/v) methanol and 0.05% (v/v) Tween 20 to a total volume of 2 l). Proteins were electro-transferred to nitrocellulose membrane using a Hoefer electroblot cell at 300 mA for 3 h with cold tap water (12 °C) circulated cooling. The electroblotted gels were stained overnight in 20 ml Invitrogen SimplyBlue™ SafeStain with gentle shaking. After electro-transfer nitrocellulose membranes were briefly washed with Milli-Q water prior to background blocking that was performed by adding 20 ml of 5% (w/v) non-fat milk powder in Tris-buffered saline (TBS) solution containing 0.1% (v/v) TWEEN 20 for 3 h with gentle shaking. Background block solution was discarded and 10 ml milk solution containing primary Ab (either 10 μl troponin or 5 μl desmin) was added to the nitrocellulose membrane and left overnight with gentle agitation at room temperature. Membranes were washed three times in TBS for 10 min each time. A 10 ml fresh milk solution containing 1 μl of secondary Ab was added and incubated for 3 h with gentle shaking at room temperature. Membranes were briefly washed three times in Milli-Q water. The membrane was washed in TBS solution twice for 30 min to remove background from the membrane. A 2.5 ml aliquot of ECL solution 1 [2.5 mM luminol, 1.36 mM p-coumaric acid, 1.5 M Tris (pH 6.8), Milli-Q water] and ECL solution 2 [2.5 ml of 1.5 M Tris (pH 6.8), containing 5 μl of 20% H_2O_2], Milli-Q water] were added to the membrane briefly and detected using a FUJI imager LAS-3000.

2.8. Statistical analysis

The results, including shear force on the $\log_e$ transformed scale, for SM and LL were analysed separately using linear mixed model (LMM) methods. Included in each LMM as random terms were effects associated with the split-plot nature of the experimental designs, these being