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Effect of pulsed electric field treatment on hot-boned muscles of different potential tenderness

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ABSTRACT

In this study, the effect of pulsed electric field (PEF) treatment and ageing on the quality of beef M. *longissimus lumborum* (LL) and M. *semimembranosus* (SM) muscles was evaluated, including the tenderness, water loss and post-mortem proteolysis. Muscles were obtained from 12 steers (6 steers for each muscle), removed from the carcasses 4 hour postmortem and were treated with pulsed electric field within 2 h. Six different pulsed electric field intensities (voltages of 5 and 10 kV × frequencies of 20, 50 and 90 Hz) plus a control were applied to each muscle to determine the optimum treatment conditions. Beef LL was found to get tougher with increasing treatment frequency whereas beef SM muscle was found to have up to 21.6% reduction in the shear force with pulsed electric field treatment. Post-mortem proteolysis showed an increase in both troponin and desmin degradation in beef LL treated with low intensity PEF treatment (20 Hz) compared to non-treated control samples.

1. Introduction

Hot-boning is the process of removing muscles from the carcass prior to chilling, which normally occurs within 90 min post-slaughter (Troy & Kerry, 2010). Hot-boning was originally developed to reduce energy usage and space during chilling storage since the removal of excess fat and bones prior to storage allows the muscle to be subdivided into smaller pieces which will conserve space as well as afford a faster cooling time (Bolumar, Enneking, Toepfl, & Heinz, 2013; Kastner, Henrickson, & Morrison, 1973; Troy & Kerry, 2010). The most concerning disadvantage of hot-boning is the production of tougher meat. Regardless of the chilling rate; hot-boning processes produce a tougher meat than cold-boning (Troy, 2006; White, O'Sullivan, Troy, & O'Neill, 2006). In hot-boning, muscles are removed from the carcass in a pre-rigour state and hence are more susceptible to contraction and shortening, due to the absence of the skeletal framework, producing a tougher meat (Troy, 2006; Troy & Kerry, 2010; White et al., 2006). Mechanical stretching methods such as SmartStretch™/Smartshape™ and Tenderbound have been developed to reduce the toughness of hot-boned beef (Hwang & Thompson, 2001; Sørheim & Hildrum, 2002). The application of these methods was found to be beneficial in improving the sarcomere lengths of hot-boned beef muscles. However, it was observed that Tenderstretch™ only improves the shear force of some muscles (LL and SM) and that other muscle types (*semitendinosus* and *psoas major*) were not affected (Bouton, Fisher, Harris, & Baxter, 1973; Hostetler, Landmann, Link, & Fitzhugh, 1970; Hostetler, Link, Landmann, & Fitzhugh, 1972). The same concerns are equally applicable to the application of electrical stimulation to the whole carcass in that not all muscles are affected equally since different muscles have different glycolytic behaviour under different conditions (Olsson, Hertzman, & Tornberg, 1994). Therefore, the use of pulsed electric field (PEF) technology might be of benefit since it is a stand-alone technology that can be applied to different muscles, pre-rigour or post-rigour. Moreover, hot-boning allows each muscle to be pre-rigour separated from carcasses. In this way an optimal intensity treatment can be applied to different types of muscles and achieve optimal results.

This study aimed to investigate the potential use of PEF to improve the quality of hot-boned beef from different muscles (LL and SM). Several PEF treatment parameters (5 or 10 kV at frequencies of 20, 50, or 90 Hz) were used, including a non-treated control, and several properties were measured over several post-mortem times in order to determine the potential for improving the quality of different muscles. In addition muscle proteins were extracted and displayed by SDS-PAGE and post-mortem proteolysis was evaluated by analysis of proteolysis of troponin and desmin.

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2. Materials and methods

2.1. Meat

Loins (*M. longissimus lumborum*, LL) and topsides (*M. semimembranosus*, SM) were obtained from two separate lots of 6 steers (average carcass weight was 279.9 ± 28.3 kg, prime beef grade and 177.9 ± 10.1 kg, manufacturing beef grade for loin and topside steers, respectively) raised on pasture and were slaughtered by the Alliance Group (Pukeuri plant, Oamaru, New Zealand). Both left and right loins and the topsides were removed from the 6 carcasses at 4 hour post-mortem and processed within 2 h.

2.2. Antibodies

Monoclonal anti-desmin antibody D1033 was from Sigma-Aldrich Corporation (St Louis, MO, USA). 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.

2.3. PEF treatments

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 pulsewidth = 4–32 μs). The samples had an average temperature of 24.4 ± 1.3 °C and 25.5 ± 1.8 °C for loins and topsides, respectively. Loin and topside muscles were each randomly cut into six blocks of 13 × 8 × 5 cm (average weight of 364.8 ± 26.3 g and 361.9 ± 33.0 g for loins and topsides, respectively). The six blocks for each muscle were allocated to six of the seven treatment combinations; voltages (5 and 10 kV cm^{-1}) × frequencies (20, 50 and 90 Hz) and a non-treated control. A separate voltage × frequency treatment was omitted for testing on each muscle from each animal. PEF treatments were applied and after treatment the blocks were cut into 4 equal pieces that were weighed, vacuum packed (VP) and randomly assigned to 4 different 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.

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;

$$Q = \frac{V^2 t}{R\nu}$$

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

2.4.2. pH

The pH of each block was measured directly using a Hanna pH electrode and meter (model HI 98150) calibrated at ambient temperature 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 at 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 averages of the 8 hand held measurements were used for later analyses.

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 meter. Electrical conductivity of the block differed between locations and was dependent on the fibre direction in the meat block. Conductivity at four locations per block was measured and the averages were used for further statistical analysis.

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 x (1-Weight after storage / initial weight before storage)

The meat samples were then frozen until cooking, which started 2 days after the last sampling time point (i.e. 21 days post-mortem).

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 x (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 (in kPa) was obtained. The values obtained were converted to Newtons using the following equation:

Shear force (N) + ((0.2035 x shear force in kPa) - 2.2945) x 9.8.

2.5. Myofibrillar protein extraction

Myofibrillar protein fractions were prepared according to the procedure described by Han, Morton, Bekhit, and Sedcole (2009). A 1.00 ± 0.01 g sample was cut from each meat subsample and was cut into small pieces. A 5 μl aliquot of a PMSF solution (17.42 mg of PMSF dissolved in 50 μl of ethanol and then the volume was made to 1 ml with