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Effect of freezing as pre-treatment prior to pulsed electric field processing on quality traits of beef muscles

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ABSTRACT

The purpose of this research was to study the effects of freezing as pre-treatment prior to pulsed electric field (PEF) on the quality of beef *semitendinosus* muscles. Fresh and frozen-thawed samples were treated using square -wave bipolar pulses at electric field strength 1.4 kV/cm, pulse width 20 μs, frequency 50 Hz and total specific energy 250 kJ/kg. PEF caused significant microstructural changes of meat tissue compared to freezing. Combined freezing–thawing and PEF resulted in improved tenderness indicated by reduced shear force, but not PEF alone. PEF significantly increased purge loss but not cooking loss. A two log-unit increase in aerobic microbial counts during log phase of frozen-thawed PEF-treated samples was positively associated with increased purge loss. PEF itself did not affect the ratios of polyunsaturated/saturated fatty acids and omega 6/omega 3 nor the free fatty acid profiles. Freezing with and without PEF greatly affected the volatile profile of meat.

Industrial relevance: PEF treatment provides an alternative to mechanical, thermal and enzymatic cell disintegration of animal raw materials, providing a short duration (milliseconds) and energy efficient treatment. In this study, the possible relationship between freezing prior to PEF and changes in beef tissue microstructure that influence storage stability and safety was investigated. The results of this study contribute toward understanding how PEF induced changes in beef microstructure influence the important quality attributes of beef.

1. Introduction

Pulsed electric field (PEF) is a non-thermal food processing technology that permeabilizes cell membranes by delivering high-voltage brief pulses (μs) through a food product placed between two conductive electrodes (Puértolas, Luengo, Álvarez, & Raso, 2012). The application of PEF processing has been demonstrated to induce changes in the structure and texture of meat, potentially improving its functional properties or aiding in the development of new products (Topfl & Heinz, 2007). Therefore, PEF technology could be applied as a relatively new method for cell disintegration (Knorr et al., 2013). Studies on the effect of electropermeabilization on protein-based foods such as fish and meat are limited and different experimental setups and processing parameters make them difficult to compare. PEF treatment of duck meat or beef and subsequent curing storage for 12 h has previously been shown to increase tenderness (Töpfl, 2006). PEF (3.5 kV/cm, 20 Hz, 5 s) treatment of beef *triceps brachii* muscles has also been reported to decrease the average maximum shear force to cut meat by 21.5% and hence enhanced tenderness (Lopp & Weber, 2005). In contrast, other studies have shown that tenderness of beef *semitendinosus* muscles is either not affected by PEF (1.1–2.8 kV/cm, 5–200 Hz, 12.7–226 kJ/kg) (O'Dowd, Arimi, Noci, Cronin, & Lyng, 2013), or PEF treatment causes a decrease tenderness after cooking (Hoffmann et al., 2009). Recently, we have shown that tenderness and colour stability of beef *longissimus thoracis* muscles are not affected by PEF (0.2–0.6 kV/cm, 1–50 Hz, 20 μs) treatments and a subsequent vacuum ageing (Faridnia, Bekhit, Niven, & Oey, 2014). The apparent discrepancy may be associated with differences in the experimental conditions such as the use of different processing parameters of PEF treatment, treatment chamber, sample size, muscle types and sample preparation prior to PEF treatment such as freezing and thawing before PEF which complicate the interpretation of previous experiments. Due to these contradictory findings demonstrating PEF impacts on meat, it is apparent that further studies are required to investigate the effects of freezing as pre-treatment and PEF on animal tissues.

In addition to tenderness, other quality parameters of meats should be considered since biochemical reactions involved in post-mortem process influence the generation of volatile compounds through enzymatic oxidation of unsaturated fatty acids, and further interactions with proteins, peptides and amino acids (Huang & Ho, 2001). Fatty acids

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in muscle tissue affect meat quality, including its tenderness, colour, lipid stability, odour and flavour (Wood et al., 2004) and are essential to determine the nutritional value of meat. Since PEF treatment changes the cell permeability, it makes the meat component such as lipids more susceptible to oxidation or facilitates the reaction between enzymes and their substrates. It could induce alteration of fatty acid composition and volatile profile of the meat and ultimately influence meat shelf life. Up to now, no/limited study on the effect of PEF on these quality traits of meat has been conducted.

Therefore, the purpose of this research was to study the effects of freezing as a pre-treatment prior to PEF treatment on the quality traits of *semitendinosus* (ST) beef muscle. In this study, *semitendinosus* muscle was selected because it is retailed as a relatively low value steak due to its high connective tissue content and this muscle is more resistant to thermal, mechanical, chemical, and enzymatic method interventions to increase the tenderness of muscles (Istrati, Vizireanu, & Dinică, 2012) in contrast to *longissimus* muscle. In this study, the treatment effects on tenderness, microstructure, microbial shelf life, fatty acid composition, lipid oxidation, and volatile profile after cooking were evaluated.

2. Materials and methods

2.1. Raw materials and sampling procedure

Beef *semitendinosus* (ST) muscles from 9 animals (mean cold carcasses weight of 245–285 kg) were obtained at 24 h post-mortem from a local commercial slaughter-house (Silver Fern Farms Ltd., Finegand Plant, Balclutha). Upon arrival, all visible fat was removed by trimming and for each animal, each muscle was divided into two halves. The first half of the muscle was further divided into two portions; one was immediately PEF treated ('fresh-PEF' (P)) and the other retained as a 'fresh-control' (C)). The second half was vacuum packed in polyethylene plastic bags (~500 g) and immediately frozen at −18 °C in a digitally temperature controlled thermostat freezer with static flow cold air temperature of −20 °C for 7 days before PEF treatment (the samples from this half are called 'frozen-thawed PEF' (FP) and 'frozen-thawed control' (FC)). The frozen samples were thawed overnight at 4 °C prior to PEF treatment.

On the day of PEF treatment, the muscles for both control and PEF treatment were cut parallel to the fibre direction into triangular pieces using a guided chopping board fitted with a tailored-made stainless steel triangular blade. The form and dimension of the triangular blade were the same as those of PEF batch treatment chamber (6 cm height × 4 cm width × 6 cm length). The weight of the sample was ~70 g for each piece and the fibre direction was arranged so as to be perpendicular to the electric current.

2.2. Pulsed electric field (PEF) treatments

The beef samples were processed in a pilot plant scale PEF system (Elcrack-HVP 5, DIL Quakenbruck, Germany) using a batch mode configuration. The selection of PEF processing parameters was determined based on the resulting visual quality of samples immediately after PEF treatment and the stability of current delivery (no electric arching) during PEF treatment. Our preliminary work showed that the visual quality of samples was severely affected after the treatment at electric field strength of 1.7−2 kV/cm (the edges of the meat were cooked). Therefore, the operating variables used in this experiment were as follows: constant pulse width of 20 μs, electric field strength of 1.4 kV/cm, constant frequency of 50 Hz, pulse number of 1032, and total specific energy input of 250 kJ/kg. Pulse shape (square wave bipolar) was monitored on-line with an oscilloscope (Model UT2025C, Uni-Trend Group Ltd., Hong Kong, China) during treatment. The pulsed electrical energy, also known as specific input energy (W_{spec}) applied to meat samples at square-wave pulse was calculated according to Zhang, Barbosa-Cánovas, and Swanson (1995) using Eq. (1).

$$\text{Specific energy input},\ W_{spec}\left(\frac{kJ}{kg}\right) = \frac{V^2 \times (n\tau)}{R \times W} \quad (1)$$

V is the pulse peak voltage (in kV), n is the number of pulses applied (dimensionless), τ is the pulse width of square pulses (in microsecond), R is the effective load resistance (in ohm) and W is the weight of sample (in kilogramme) to be treated in the PEF treatment chamber. The temperature of samples before and after treatment was monitored using a temperature logger (Grant Squirrel SQ800, Cambridge, UK) and type T thermocouples. The initial temperature was maintained at 4 °C and the pH of each meat sample was measured by inserting a calibrated pH probe (HANNA HI 98140, Woonsocket, USA) directly into the meat. Duplicate pH and temperature readings were taken for each sample before and after PEF treatment. A hand held meat conductometer (LF-STAR, R. Mathäus, Germany) was used to determine the change in electrical conductivity (σ) of beef samples prior to and after treatment, by inserting the twin probes directly into the samples at three different positions.

After treatment, all beef samples for shear force measurement, purge loss, and cooking loss, were vacuum packed in polyamide polyethylene bags and stored at 2 °C for 7 days. After assigned storage time, samples for volatile compounds and fatty acids profile were snap-frozen in liquid nitrogen and stored at –80 °C for less than a month until analysed. In total, 6 independent samples coming from different animals were used (n = 6).

For microbiology testing and lipid oxidation determination, the beef samples after PEF treatment were placed in a sterile stomacher bag (15 × 23 cm, 0.102 mm thick, 710 ml capacity) and stored at 4 °C for different predefined ageing times (up to 16 days). The sampling for microbiology test and lipid oxidation was taken every 2 days of storage (n = 4).

2.3. Study on changes in physical properties of meat

2.3.1. Determination of purge loss

The samples were weighed immediately after PEF treatment (initial weight). On the day of sampling, the meat samples were removed from the vacuum packed bags, blotted dry with paper towels and weighed (final weight). The purge loss was calculated using Eq. (2) and expressed as %.

$$\text{Purge loss}\ (\%) = [(\text{Initial weight} - \text{Final weight})/\text{Initial weight}] \times 100 \quad (2)$$

2.3.2. Determination of cooking loss and shear force

The cooking loss was determined by weighing samples before and after cooking in a water bath set to 80 °C. Thermocouples were inserted to the centre of each meat sample to monitor the temperature during cooking. After reaching the desired internal temperature of 75 °C, the samples were immediately cooled down in an ice-water bath, blotted dry and weighted. The loss of weight due to cooking was calculated as cooking loss and is expressed as a percentage of the original sample weight.

For shear force measurement, the same sample used for the cooking loss test was employed. After cooling, 10 × 10 × 25 mm cross section samples (ten replicates for each sample) were cut parallel to the fibre direction and the peak shear force (kgF) was determined, perpendicular to the fibre direction using a MIRINZ Tenderometer (Chrystall & Devine, 1991).

2.3.3. Electron microscopy analysis using TEM

The microstructure of meat samples was evaluated using Transmission Electron Microscopy (TEM). For TEM observation, samples were