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Effect of Pulsed Electric Field treatments at various stages during conditioning on quality attributes of beef *longissimus thoracis et lumborum* muscle

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ABSTRACT

Beef *longissimus thoracis et lumborum* (LTL) muscle was used to evaluate the effect of PEF treatments (1.4 kV/cm, 10 Hz, 20 μs, 300 and 600 pulses) on meat quality attributes (weight loss, colour, cook loss and texture) and its evolution at various stages during ageing (2, 10, 18 and 26 days post-mortem). The length of meat ageing before and after PEF application exerted no influence on weight loss, colour and cook loss. Results also demonstrated that PEF treatments applied at different times post-mortem (2, 10, 18 and 26 days) showed a tendency towards reducing toughness of beef samples but that the application of PEF did not affect the tenderization process provided by ageing itself. 60% of the sensory panellists scored PEF treated samples as tender (≥6.0 points out of 9.0) whereas only 27.5% did so for untreated samples.

1. Introduction

It is well known that consumers' perception of meat and meat products is a critical issue for the meat industry because it directly impacts on its profitability (Boleman et al., 1997). Research into meat eating quality revealed that tenderness, juiciness, flavour and overall palatability remain the most sought after attributes by consumers, though tenderness is deemed the most important (Miller, Carr, Ramsey, Crockett, & Hoover, 2001; Troy & Kerry, 2010) as it strongly influences the re-purchase intent of consumers and their choice of particular meat cut (Boleman et al., 1997). Therefore, it is a real challenge for the meat industry and the scientific community to deliver products with standardized and guaranteed tenderness (Verbeke et al., 2010).

Post-mortem ageing is a common practice in the meat industry to improve the consistency of meat tenderness (Sitz, Calkins, Feuz, Umberger, & Eskridge, 2006; Tatum, Belk, George, & Smith, 1999), however, the duration of post-mortem ageing, as well as the individual muscle, makes a large difference to its final toughness (Harris & Shorthose, 1988; Tornberg, 1996). For beef, traditional ageing or conditioning is slow and therefore represents a major cost to the meat industry as it requires refrigerated storage for extended periods of time (≤28 days). Nonetheless, several intervention techniques may be applied post-mortem to increase beef tenderness by physically disrupting its structure. Well established techniques include aitchbone hanging, blade/needle tenderisation and electrical stimulation (Bolumar, Enneking, Toepfl, & Heinz, 2013). Besides, controlling chilling regimes and pH decline of beef carcasses, the infusion or injection of carcasses with calcium chloride as an activator of the calpain system (Koohmaraie, Whipple, & Crouse, 1990), or the injection of proteolytic enzymes of plant origin such as papain and bromelain or animal origin such as porcine pancreatin (Pietrasik, Aalhus, Gibson, & Shand, 2010) can also accelerate post-mortem ageing and improve ultimate beef tenderness.

More recent research has focused on the application of novel processing techniques to post-rigour muscles as means of tenderizing or accelerating post-mortem ageing of meat. Ultrasound (US), high hydrostatic pressure (HHP) and shockwaves produced by detonating explosives or electrical discharge are examples of such technologies. US treatment of meat has produced inconsistent effects on meat tenderness, with some ultrasound treatments producing no effect on tenderness, whilst others decreased or increased tenderness (Jayasooriya, Bhandari, Torley, & D'Arcy, 2004). In post-rigour meat, it has been reported that tenderization can only be achieved after long (20–30 min) exposure to 150–200 MPa at 55–60 °C. Cold pressurisation (above 150 MPa, <10 °C) has been reported to have the disadvantage of inducing discolouration and a cooked meat appearance in red pork and beef muscle, precluding its sale as fresh meat (Simonin, Duranton, & de Lamballerie, 2012). Shockwave technology based on the use of explosives emerged in the early seventies and subsequent systems have been developed in the USA but the commercial implementation has been very limited because of the technical challenges in terms of equipment development, potential contamination with residues from the

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explosion and safety issues for operators (Solomon, Sharma, & Patel, 2011). The available literature related to the tenderization of meat using electrically-generated shockwaves under water is much more limited and its development is currently in-progress (Bolumar et al., 2013). Another emerging technology suggested for meat processing is Pulsed Electric Fields (PEF). PEF involve the application of microsecond pulses of a high-strength electric field through a material located between two electrodes and is often classed as a "non-thermal" treatment though some elevation in product temperature can occur. The effect of an electric field on cells has been explained by the dielectric breakdown theory, where the external electric field induces an electric potential over the membrane causing reversible or irreversible permeabilization of membranes of both eukaryote and prokaryote cells (Ho & Mittal, 1996). PEF is an emerging food processing technology, which has been widely investigated in terms of its potential for industrial pasteurization of liquid food such as milk, fruit juices and liquid whole egg (Elez-Martínez, Sobrino-López, Soliva-Fortuny, & Martín-Belloso, 2012), but also has commercial potential in different processes of the food industry (increasing juice yield, softening tissues like potatoes and extraction of compounds). However, limited studies are available on muscle food. Based on the electroporation of biological membranes, it has been proposed that a similar effect could occur in muscle fibres and affect various quality parameters (Bekhit, van de Ven, Suwandy, Fahri, & Hopkins, 2014; Gudmundsson & Hafsteinsson, 2001; O'Dowd, Arimi, Noci, Cronin, & Lyng, 2013; Töpfl, 2006). Gudmundsson and Hafsteinsson (2001) who studied the effect of PEF on microstructure of chicken meat observed that low field strength PEF treatments (1.36 kV/cm, 40 pulses, 2 μs pulse width, "room temperature") caused a reduction in cells' size but without visible gapping. In addition, Töpfl (2006) reported an improvement of water binding characteristics of pork meat indicated by swollen, sponge-like tissue structure, an enhanced micro-diffusion of brine and improved water binding due to interaction between protein/salt/phosphate during cooked ham processing. As well a more porous tissue structure in cod was observed after PEF.

Due to the limited studies on the use of PEF for muscle food processing such as beef, this study was designed to analyse the effect of PEF treatments on beef quality throughout the conditioning period. The quality attributes investigated included weight loss, cook loss, instrumental colour and tenderness. Samples treated at different stages of ageing (2, 10, 18 and 26 days post-mortem) have been compared so as to assess the physical effect of PEF at that specific times of conditioning. The evolution during ageing (up to 26 days post-mortem) of the quality attributes of samples exposed to PEF on day 2 has been also assessed in order to consider any biochemical change during conditioning resulting from the application of PEF early post-mortem.

2. Material and methods

2.1. Beef samples and experimental plan

Six beef *longissimus thoracis et lumborum* (LTL) muscles (~7 kg) from steers under 30 months were obtained at 48 h post-mortem from a local meat supplier (Kepak Group, Clonee, Dublin, Ireland) with a four-week interval between the delivery of each muscle. Immediately after reception, the muscle was cut into 4 pieces and randomly assigned one piece for each day of treatment, i.e. 2, 10, 18 and 26 days post-mortem. Pieces for treatment on days 10 (~2 kg), 18 (~1.5 kg) and 26 (~1 kg) were immediately vacuum-packed in individual polyamide/polyethylene Exovac-73 bags (Euroflex, Ireland) (Webomatic packaging system, Model No. 021ODC681, Bochum, Germany) and stored at 4 °C (Fig. 1).

For studying the effect of post-PEF treatment time, on day 2 post-mortem the piece for day 2 (~2.5 kg) was trimmed of all visible fat and cut into 24 strips (~30 g/piece, 2 × 2 × 6 cm as described in Section 2.2) and randomly assigned: 16 samples were PEF-treated and 8 samples kept as controls (untreated). Of these, only 6 strips (4 PEF-treated and 2 controls) were immediately analysed and the 18 remaining pieces were individually vacuum-packed, stored at 4 °C and analysed on days 10, 18 and 26 (6 each time: 4 PEF-treated and 2 controls). For studying the effect of pre-PEF treatment time, on days 10, 18 and 26 post-mortem the corresponding meat pieces were cut into 6 strips (~30 g/piece, 2 × 2 × 6 cm). Of these, 4 strips were PEF-treated and 2 were kept as controls and all were immediately analysed (Fig. 1).

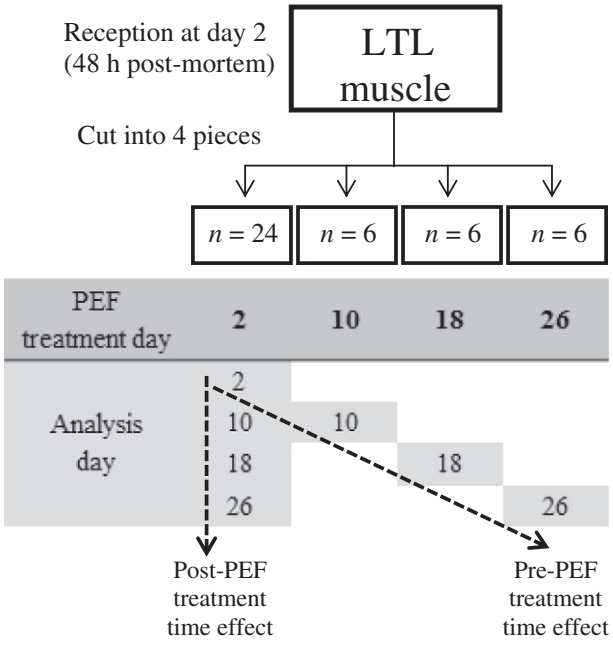

Fig. 1. Schematic illustration of the experimental design for each muscle.

2.2. PEF treatments

Batch PEF treatments were performed in a laboratory scale PEF system (Elcrack-HVP5, DIL, Quakenbrück, Germany). The system was monitored using a digital oscilloscope (Tektronix, TDS 2012, Beaverton, OR, USA). The 2 × 2 × 6 cm meat strips were accommodated in the treatment chamber which consisted of two parallel stainless steel electrodes with a sample-electrode contact area of 4 cm^2 and a cross section width of 6 cm using Teflon (PTFE) as insulating material. 300 and 600 square waveform pulses of 20 μs width at electrical field strength of 1.4 kV/cm (8400 V) with a frequency of 10 Hz were used. This moderate electric field strength was chosen attending to previous studies (Gudmundsson & Hafsteinsson, 2001; O'Dowd et al., 2013). Total specific energy intake (kJ/kg) by the beef strips (considering density ≈ 1042 kg/m^3, constant conductivity ≈ 2.7 mS/cm) was estimated according to Zhang, Barbosa-Cánovas, and Swanson (1995) with the following equation:

$$\text{Total specific energy} = \frac{V^2 \tau \sigma A}{d} \times \frac{n}{m} \quad (1)$$

where V is the input voltage (V), τ is the pulse width (s), σ is the conductivity of meat (S/cm), A is the electrode area (cm^2), d is the gap (cm), n is the number of pulses (dimensionless) and m is the mass of the meat strip (kg). Total specific energy was approximately 25 kJ/kg for the 300-pulse and 50 kJ/kg for the 600-pulse treatment. Each time, two samples were kept as controls (untreated samples), two were subjected to a 300-pulse PEF treatment and two to a 600-pulse PEF treatment. Temperature was monitored before and after PEF treatment with a thermocouple (Thermo

B + B Thermo-Technik, Donaueschingen, Germany) and the temperature rise recorded.

2.3. Meat quality attributes

Following the PEF treatment or the assigned ageing period (Fig. 1), beef samples were assessed for weight loss, colour Hunter *Lab*, cook loss, and Warner–Bratzler shear force.

2.3.1. Weight loss and storage loss assessment

Sample weight was measured before (W_i) and immediately after PEF (W_f) using a digital scale (Model No. TE 313S, Sartorius Mechatronics Ireland Ltd., Dublin, Ireland). Weight loss (%) due to the PEF treatments was expressed as a percentage of the original weight (Eq. (2)). Following the assigned ageing time (t), the storage loss (%) was determined by dividing the weight difference before (W_f) and after storage (W_t) by the weight before storage (W_f) (Eq. (3)).

$$\text{Weight loss } (\%) = \frac{W_i - W_f}{W_i} \times 100 \tag{2}$$

$$\text{Storage loss } (\%) = \frac{W_f - W_t}{W_f} \times 100 \tag{3}$$

2.3.2. Colour measurements

The surface colour before and after PEF treatments or after the storage period was measured by a reflectance spectrophotometer (Minolta CR-400, Minolta UK Ltd., Milton Keynes, UK) and the coordinates *L* (lightness), *a* (redness/greenness) and *b* (yellowness/blueness) recorded. Colour readings were taken on two random surface points on each sample which were previously left to stand for 20 min in light and ambient temperature.

2.3.3. Cook loss assessment and Warner–Bratzler shear force determination

After measuring the colour, samples were vacuum-packed and placed in a pre-heated water bath at 80 °C (Model No. GD100, Grant Instruments Ltd., Cambridge, UK). A thermocouple was inserted into the core of a spare meat sample to monitor the temperature rise. Once the core temperature reached 71 °C, samples were removed from the water bath and cooled to 4 °C in ice water. Cooked samples were then weighted, vacuum-packed and kept refrigerated overnight at 4 °C before texture measurements were performed. Cook loss (%) was determined by dividing the weight difference before and after cooking by the weight before cooking. For the Warner–Bratzler shear force (WBSF) determination, samples were cut into two 1 × 1 × 3 cm strips and placed with fibres running at right angle to the flat blade of the texture analyser (Instron Universal Testing machine, Model No. 5544, Instron Corporation, High Wycombe, UK). The maximum shear force (N) was recorded using a 500 N load cell at a cross head speed of 50 mm/min.

2.4. Sensory analysis

For the sensory analysis, a LTL muscle 48 h post-mortem was cut into 60 pieces (2 × 2 × 6 cm strips): 20 samples were PEF treated (1.4 kV/cm; 600 pulses of 20 μs; 10 Hz) and 40 untreated samples were used as controls. All samples were vacuum-packed and stored for 10 days at 4 °C. After storage, samples were cooked in an electric convection oven, preheated at 180 °C, until a final internal temperature of 71 °C. Three samples (one PEF treated and two controls cut into 1 × 1 × 3 cm strips) were labelled with three digit random numbers and presented cold in white plates for evaluation. The consumer panel consisted of 20 untrained people balanced for age and gender. Using a 9-point hedonic scale, members scored each sample for tenderness (1 = very tough, 3 = tough, 5 = intermediate, 7 = tender, 9 = very tender) and odour (1 = dislike, 5 = neither like nor dislike, 9 = like).

2.5. Statistical analysis

The effects of the pre-PEF treatment time *or* the post-PEF treatment time (T), the number of pulses (p) and their interaction on meat quality traits were assessed using the general linear model (GLM) procedure of SPSS, version 20.0, with the animal (A) as random effect. The model is as follows:

$$Y_{ijk} = \mu + T_i + p_j + A_k + (Tp)_{ij} + e_{ijk} \tag{4}$$

where Y_{ijk} is the observation of the dependent variables; μ is the overall average; T_i is the fixed effect of time (pre- *or* post-PEF treatment); p_j is the fixed effect of the number of pulses (0, 300, 600); A_k is the random effect of animal; $(Tp)_{ij}$ is the interaction effect of time and number of pulses, and e_{ijk} is the aleatory error associated with the observation. Tukey post-hoc test was applied to compare the mean values and differences were considered significant if $P \leq 0.05$. Mean values and the 95% confidence intervals (*CI*) are reported in figures and the standard errors of the mean (*SEM*) in tables. For the sensory analysis, a *t*-test was performed with the same software and differences were considered significant if $P \leq 0.05$.

3. Results and discussion

Although PEF processing is defined as a "non-thermal" technology, some of the PEF energy input is transformed into heat during process. In the present study, two different PEF treatments were studied which differed in the number of pulses applied, 300 or 600, which induced a temperature rise in beef samples of 7.7 °C (*SD* = 1.2) and 14.5 °C (*SD* = 1.8), respectively, which is consistent with the theoretical values (8 and 16 °C, respectively, considering a beef LTL muscle specific heat capacity ≈ 3.2 kJ/kg). As the samples were kept under refrigeration (~4 °C) before performing the PEF treatments, these raises in temperature did not affect the appearance of meat samples.

To the knowledge of the authors, only one recent study (Bekhit et al., 2014) reports the effect of PEF on beef loins (*longissimus lumborum*, LL) and topsides (*semimembranosus*, SM) quality attributes over the ageing period (up to 21 days post-mortem) when applied on day 1 (for LL) and on days 1 and 3 post-mortem (for SM). The rationale for conducting this study was to gain knowledge of the possible physical effect on beef *longissimus thoracis et lumborum* quality attributes of PEF treatments applied at specific times post-mortem: 2, 10, 18 and 26 days (pre-PEF treatment time effect); and the evolution of these attributes during conditioning as a result of the application of PEF on day 2 post-mortem (post-PEF treatment time effect). The experiment did not intend to compare pre-PEF ageing with post-PEF ageing and combinations of pre- and post-PEF treatment times (e.g. samples pre-aged for 10 days, exposed to PEF and allowed 8 days for further ageing) did not exhibit any beneficial or detrimental effect on meat quality attributes, and have been therefore omitted from the manuscript.

3.1. Pre-PEF treatment time effect

In this section, the effect of the time previous to the PEF treatment (pre-PEF treatment time) on meat quality attributes (weight loss, colour, cook loss and texture) was studied by comparing the untreated samples (controls) with those samples treated by PEF on days 2, 10, 18, and 26 post-mortem. Therefore, for all samples, analyses were performed immediately after PEF treatments.

Fig. 2 illustrates the weight loss in beef LTL muscle caused by the application of PEF treatments (300 or 600 pulses of 20 μs; 1.4 kV/cm; 10 Hz). As can be seen, weight loss always occurred regardless of the pre-PEF treatment time; although no direct relationship between

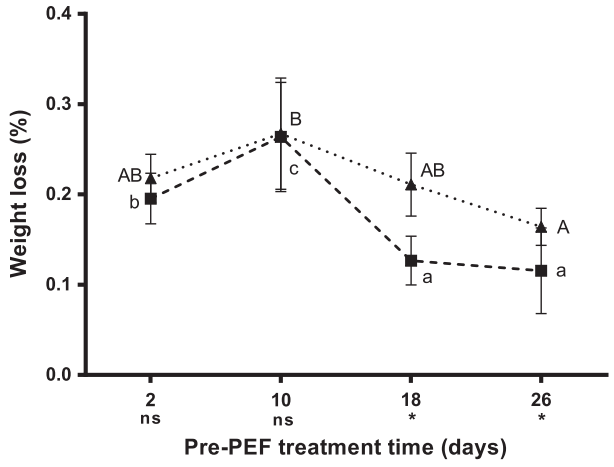

Fig. 2. LTL muscle weight loss (%) caused by PEF treatments (■ 300 pulses; ▲ 600 pulses) applied during meat ageing (2, 10, 18, and 26 days post-mortem). Data are means and 95% *CI* (error bars). Different letters for each PEF treatment indicate significant differences ($P \leq 0.05$) between mean values. Symbols at each treatment day indicate significant (*, $P \leq 0.05$) or non-significant differences (ns, $P > 0.05$) between mean values.

ageing time and weight loss was observed as the higher losses were observed at day 10. In terms of PEF treatment intensity, the 600-pulse treatment only involved significantly higher losses than the 300-pulse treatment at days 18 and 26 ($P \leq 0.05$). Nonetheless, weight loss due to the PEF application only accounted for a maximum of 0.28% of total weight, which can be considered to have minimal commercial significance. An approximately 1.5% weight loss following PEF treatments (2.8 kV/cm; 300 pulses of 20 μs; 5 Hz; 225.8 kJ/kg) was previously observed in 72 h post-mortem beef *semitendinosus* (ST) muscle by O'Dowd et al. (2013). Also Bekhit et al. (2014) observed a 1.3% increase in purge loss (%) for 1-day post-mortem beef SM muscles exposed to PEF (0.27–0.56 kV/cm, 20–90 Hz, 20 μs) and aged for 3 days when compared to untreated samples. Although loss values reported by these authors were significantly higher, we can attribute these differences to the different PEF conditions applied and the muscles assayed (LTL vs ST or SM). This weight loss due to the application of PEF treatments has been attributed to the puncturing of the cell membranes which causes leakage of cell fluids (Gudmundsson & Hafsteinsson, 2001).

The effect of PEF on meat colour was also tested, as the visual appearance of meat, in particular its colour, is the main factor affecting acceptability at retail (Moore, 1990; Sapp, Williams, & McCann, 1999). Besides, post-mortem ageing has been reported to affect the biochemical processes determining meat colour stability (Feldhusen, Warnatz, Erdmann, & Wenzel, 1995; Ledward, 1985; Tang et al., 2005). Table 1 includes the mean values of the colour attributes measured as lightness (L), redness (a) and yellowness (b) during the ageing period for both PEF-treated and untreated (control) samples. As can be seen for the controls, only L values remained constant throughout the ageing period ($P > 0.05$). The fact that lightness was not affected by ageing is in agreement with those data of King, Shackelford, Kalchayanand, and Wheeler (2012) with beef *longissimus* steaks (13th rib) aged for 14 days. However, a gradual increase of L values with the ageing period has been also previously reported for vacuum-packed beef *longissimus thoracis* (LT) muscle (Boakye & Mittal, 1996; Joseph & Connolly, 1977). These authors attributed this increase in lightness throughout ageing to increased retention of oxygen in the outer layers of freshly cut meat since the oxygen-utilizing enzymes in the deeper tissues became progressively inactivated with the passing of ageing time. By contrast, a and b values significantly increased with time ($P \leq 0.05$). For instance, a and b values increased from 16.4 and 5.4 at 2 days post-mortem to 20.4 and 7.7 at 26 days post-mortem, respectively (Table 1). Beriain, Goñi, Indurain, Sarriés, and Insausti (2009) also observed that colour coordinates CIE a^* and b^* for beef LT muscle increased between 24 h and 14 days, having attributed the increase of a values through storage to an increase in oxymyoglobin concentration in the muscle surface (Byrne, Troy, & Buckley, 2000). Regarding the evolution of colour parameters for the PEF treated samples, Table 1 shows no statistically significant differences ($P > 0.05$) for any of them along the ageing period and between treatments at each ageing time. In contrast to this data, O'Dowd et al. (2013) found that lightness of 72 h beef ST PEF-treated samples (1.9 kV/cm; 250 pulses of 20 μs; 65 Hz; 83.6 kJ/kg) was lower than that of controls, and attributed this decrease to the temperature induced by PEF (an increase of 22 °C), as colour is less stable at elevated temperatures (Jeremiah & Gibson, 2001); but in agreement with data here presented, a and b values were unaffected by the PEF treatment. The fact that PEF treatments at the intensities here used have no detrimental effect ($P > 0.05$) on L, a, and b values when comparing the PEF-treated samples with the controls, represents a valuable outcome and an advantage over other technologies such as HHP which induces drastic changes in the colour of the meat when applied in conditions that could permit tenderization, thus preventing its commercialization as fresh (Simonin et al., 2012).

It is known that cooking denatures the muscle proteins, which directly influences their structural characteristics (Tornberg, 2005) and thereby the water distribution of the meat (Bertram, Kohler, Böcker, Ofstad, & Andersen, 2006). Such structural changes lead to substantial loss of juice, i.e. cook loss, in the range of 15 to 35% of which 90% is generally water (Pearce, Rosenvold, Andersen, & Hopkins, 2011). However, the amount of cook loss is highly dependent on the cooking method (heating rate, time and end-point temperature) and the characteristics of the raw material (moisture, protein and fat content, pH and

Table 1
Effect of pre-PEF treatment time on lightness (L), redness (a) and yellowness (b) of beef LTL muscle samples subjected or not to PEF treatments. Different letters in the same row indicate significant differences among mean values ($P \leq 0.05$); Sig, significance level; ns, non significant.

	Pulses	Pre-PEF treatment time (days)								Sig
		2		10		18		26		
		Mean	*SEM*	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*	
L	0	31.09	0.79	33.97	1.49	33.56	0.55	34.16	0.50	ns
	300	31.94	0.71	32.48	0.46	32.89	0.31	32.84	0.45	ns
	600	31.23	0.69	32.29	0.47	32.56	0.50	32.99	0.72	ns
	Sig	ns		ns		ns		ns		
a	0	16.41 a	0.56	19.28 ab	1.09	19.75 ab	0.70	20.35 b	1.04	$P \leq 0.05$
	300	17.29	0.95	17.91	1.07	19.30	1.06	18.89	0.79	ns
	600	16.19	1.18	18.17	1.48	18.99	1.21	19.52	0.84	ns
	Sig	ns		ns		ns		ns		
b	0	5.36 a	0.25	7.20 b	0.48	7.11 b	0.27	7.66 b	0.49	$P \leq 0.05$
	300	5.44	0.27	6.12	0.59	7.01	0.37	6.91	0.44	ns
	600	5.18	0.53	6.33	0.67	6.57	0.61	7.30	0.38	ns
	Sig	ns		ns				ns		

dimensions) (Aaslyng, Bejerholm, Ertbjerg, Bertram, & Andersen, 2003; Jeremiah, Dugan, Aalhus, & Gibson, 2003). In this study, vacuum-packed beef LTL samples were cooked in a water bath up to 71 °C core temperature after PEF treatments and cook loss assessed. Results regarding the weight loss of PEF treated samples following cooking are shown in Fig. 3. As can be seen, ageing time had no effect on the cook loss for control and 300 pulses-PEF treated samples ($P > 0.05$), accounting for a mean value of 22.6%. Cook loss values for those samples treated with 600 pulses, by contrast, were significantly lower on day 2 (17.2%, $SD = 4.1$) ($P \leq 0.05$), and were progressively increasing with time up to equate the cook loss values of controls and 300 pulses-PEF treated samples on day 26 ($P > 0.05$). This suggests that under our experimental conditions PEF treatment did not damage muscle fibres, connective tissue or thermally affected the myofibrillar proteins that might enhance the movement of water out of the muscle. The fact that cook loss for control samples remained almost constant throughout the ageing period contrasts with data reported by Bertram, Whittaker, Shorthose, Andersen, and Karlsson (2004) and Boakye and Mittal (1993) who observed an increase in cooking losses in beef LTL and LT, respectively, with ageing up to 16 days, which was found to correspond to structural changes during ageing responsible for the binding of water within the cooked meat based on the reduction of NMR T2 relaxation times by Bertram et al. (2004). Nonetheless, the improvement in water-binding properties of PEF treated beef strips (600 pulses) at earlier ageing is a fact that should be studied in depth.

Texture or tenderness is the predominant quality determinant and is probably the most important organoleptic characteristic of red meat (Koohmaraie, 1989). Moreover, the improvement of meat tenderness upon ageing is a generally accepted event. Thus our results demonstrated that, as expected, the WBSF values at 2 days post-mortem for untreated samples (initial toughness) were the highest values and diminished during the following eight days (10 days post-mortem) by 22 N (Fig. 4). A further extension in the ageing period did not entail any reduction in WBSF values as no statistically significant differences were obtained in WBSF values for days 10, 18 and 26 ($P > 0.05$). Although ageing improves meat tenderness, it should be considered that storage of meat during this period has economic consequences. For that reason, there is interest in developing methods for giving consistent and rapid improvements in meat tenderisation (Bertram et al., 2004). According to the results presented in Fig. 4, it was observed that although mean values for PEF treated samples (300 or 600 pulses) were slightly lower, there were no statistically significant differences

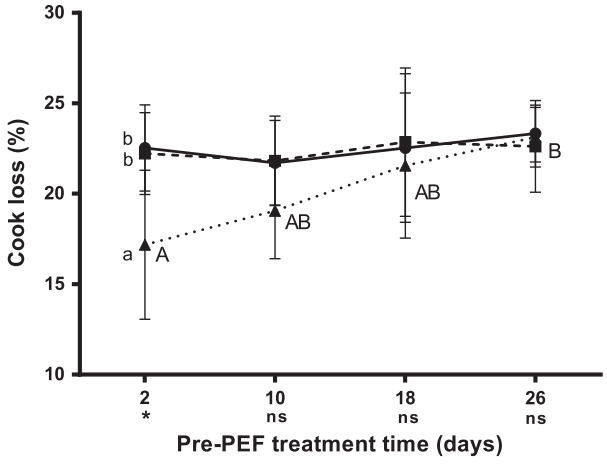

Fig. 3. Effect of pre-PEF treatment time on cook loss (%) for LTL muscle samples subjected or not to PEF treatments. Non-treated samples (●) and PEF treated samples (■ 300 pulses; ▲ 600 pulses). Data are means and 95% *CI* (error bars). Different letters for each PEF treatment indicate significant differences ($P \leq 0.05$) among mean values. Symbols at each treatment day indicate significant (*, $P \leq 0.05$) or non-significant differences (ns, $P > 0.05$) among mean values.

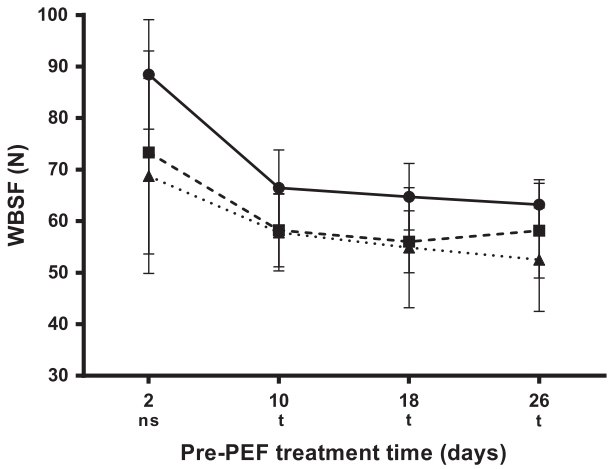

Fig. 4. Effect of pre-PEF treatment time on Warner–Bratzler Shear Force values (N) for LTL muscle samples subjected or not to PEF treatments. Non-treated samples (●) and PEF treated samples (■ 300 pulses; ▲ 600 pulses). Data are means and 95% *CI* (error bars). Symbols at each treatment day indicate a tendency (t, $P \leq 0.10$) or non-significant differences (ns, $P > 0.05$) among mean values.

($P = 0.1197$) between them and control samples on day 2, which is due to the larger *CI*. Interestingly, a tendency ($P \leq 0.10$) was observed for those PEF treated samples on days 10 ($P = 0.0836$), 18 ($P = 0.0987$) and 26 ($P = 0.0989$) whilst no differences were found between samples treated with 300 or 600 pulses ($P > 0.05$), i.e. under our experimental conditions PEF treatments applied on day 2 and onwards appeared to tenderize beef LTL strips. In previous work, O'Dowd et al. (2013) reported that the texture of 72 h post-mortem treated beef ST muscle was not influenced by PEF (1.9 kV/cm; 250 pulses of 20 μs; 65 Hz; 83.6 kJ/kg). The discrepancy between both studies may be ascribed to the different PEF treatments applied (25 or 50 kJ/kg vs 83.6 kJ/kg), or to the structural differences between muscles (LTL vs ST). According to Dransfield (1977), tenderness in the "*longissimus dorsi*" (LD) muscle would not be influenced to the same extent by the connective tissue component as total collagen and heat soluble collagen contents are lower and higher than the ST, respectively. Furthermore, although LD shear force is not a good indicator of toughness in other muscles of the carcass and that of ST is close to the median value for all muscles of the carcass, studies of toughness using ST can potentially be confounded by sampling effects as location along the whole ST muscle makes a significant difference to the measurement of shear force (Harper, 1999; Shackelford, Wheeler, & Koohmaraie, 1997). Moreover, our current understanding of meat toughness has been constructed largely from experimental studies on LD muscle because it has relatively high commercial value and is easily sampled on the carcass (Harper, 1999). Another study performed on turkey breast meat demonstrated that a PEF treatment had no effect on texture (WBSF values) either in fresh (1.2 kV/cm; 300 pulses of 20 μs; 10 Hz) or frozen (2.1 kV/cm; 300 pulses of 20 μs; 10 Hz) meat, attributing this lack of effect in PEF being not strong enough to induce physical disruption of meat fibres in order to affect texture (Eslami et al., unpublished results).

3.2. Post-PEF treatment time effect

The previous section has dealt exclusively with the effect of PEF on meat quality attributes at specific times post-mortem. In this section, the effect on meat quality attributes of PEF treatments with subsequent ageing was assessed. All PEF treatments were applied 48 h post-mortem (day 2), and measurements of storage loss, colour, cook loss and WBSF were taken immediately and on days 10, 18 and 26 post-mortem, i.e. 0, 8, 16 and 24 days of post-PEF treatment, respectively. It is important to mention that values for untreated samples (controls) in this section are not coincident with controls in the previous one – except for those

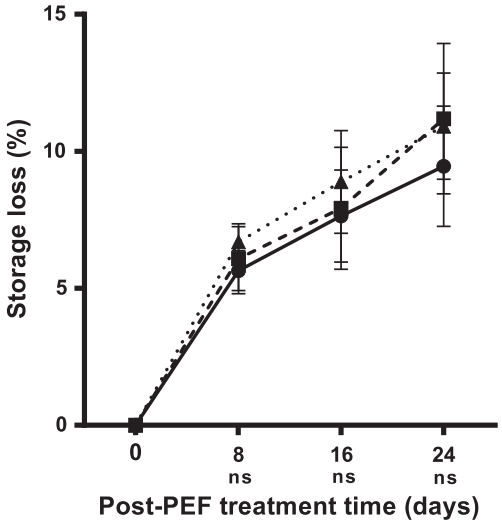

Fig. 5. Effect of post-PEF treatment time on storage loss (%) of LTL muscle samples subjected or not to PEF treatments. Non-treated samples (●) and PEF treated samples (■ 300 pulses; ▲ 600 pulses). Data are means and 95% *CI* (error bars). At each treatment day, ns indicates non-significant differences ($P > 0.05$) among mean values.

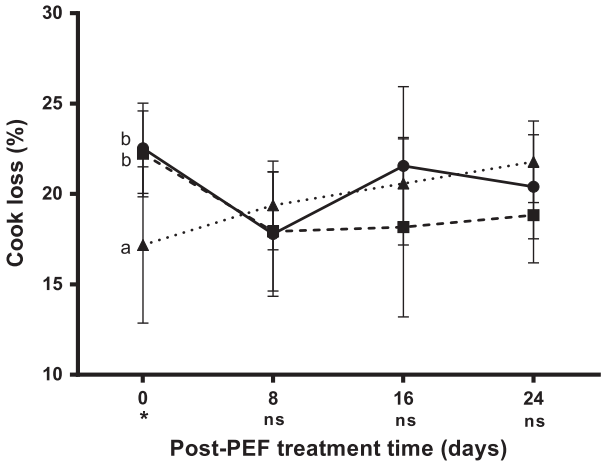

Fig. 6. Effect of post-PEF treatment time on cook loss (%) for beef LTL muscle samples subjected or not to PEF treatments. Non-treated samples (●) and PEF treated samples (■ 300 pulses; ▲ 600 pulses). Data are means and 95% *CI* (error bars). Different letters for each PEF treatment indicate significant differences ($P \leq 0.05$) among mean values. Symbols at each treatment day indicate significant (*, $P \leq 0.05$) or non-significant differences (ns, $P > 0.05$) among mean values.

of day 2 (0 time post-PEF treatment) – because, in this case, all samples were cut into 2 × 2 × 6 cm strips on day 2 and vacuum-packed individually for ageing whereas in the previous section samples were cut into strips on each specific day of treatment.

The weight loss on account of the PEF treatments is the same for all samples as they were all PEF-treated on day 2 post-mortem, with values ranging from 0.20% ($SD = 0.03$) to 0.22% ($SD = 0.03$) for 300- and 600-pulse treatments ($P > 0.05$), respectively.

Mean values for percentage storage loss – which indicates the weight loss of samples due to the storage time under refrigeration (4 °C) – are shown in Fig. 5. Expelled water progressively increased when increasing the time and no statistically significant differences ($P > 0.05$) were found between untreated and PEF-treated samples (300 and 600 pulses). Thus, meat samples lost approximately 6%, 8% and 10% of weight after 8, 16 and 24 days of storage under vacuum-packed conditions. Values here recorded are in concordance with those of Seideman, Smith, Carpenter, Dutson, and Dill (1979), who reported weight loss of beef *longissimus* steaks (from the 13th thoracic–3rd lumbar vertebrae region) of 2 cm in thickness of 6.0%, 7.67% and 8.89% after 7, 14 and 21 days of storage under vacuum-packed conditions at 1–3 °C. During the ageing process, the increase in exudate losses can be described as the natural progressive release and drainage of intra-cellular fluid (Offer & Knight, 1988a,b); and the fact that PEF treatments did not result in an extra weight loss during storage time may be an indication of no volume changes of myofibrils caused by the PEF treatment.

Table 2 shows that the application of PEF on day 2 did not imply any change in *L*, *a*, and *b* value evolution along the ageing period, as no statistically significant differences ($P > 0.05$) were found between untreated and PEF-treated samples at each post-PEF treatment time. These results suggest that changes in colour during ageing are not produced by the PEF treatment but for the conditioning process itself. In addition, whereas *a* and *b* values after 10, 18 and 26 days were significantly lower than on day 2 ($P \leq 0.05$), lightness did not seem to be affected.

Despite cook loss values for samples treated with 600 pulses were significantly lower on day 2 ($P \leq 0.05$), as stated in the previous section, no differences ($P > 0.05$) were here found on cook loss for the PEF-treated samples (300 and 600 pulses) over the storage period, which indicates that PEF treatments did not affect the cook loss of samples over ageing (Fig. 6). On the contrary, Bekhit et al. (2014) observed that untreated beef LL and SM muscles had higher cook losses compared with PEF-treated samples (0.27–0.56 kV/cm, 20–90 Hz, 20 μs) regardless of the post-treatment ageing time (3, 7, 14 or 21 days post-mortem), which was attributed to a lower free moisture available in PEF-treated samples as these had higher purge loss values. In agreement with the previous section, a decrease in WBSF values within the first 10 days

Table 2
Effect of post-PEF treatment time on lightness (*L*), redness (*a*) and yellowness (*b*) of beef LTL muscle samples subjected or not to PEF treatments. Different letters in the same row indicate significant differences among mean values ($P \leq 0.05$); Sig, significance level; ns, non significant.

	Pulses	Post-PEF treatment time (days)								Sig
		0		8		16		24		
		Mean	*SEM*	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*	
L	0	31.09	0.79	31.05	1.20	32.33	0.99	32.49	1.10	ns
	300	31.94	0.71	30.51	1.09	30.48	0.78	31.31	1.07	ns
	600	31.23	0.69	30.62	0.83	31.95	1.07	31.36	0.73	ns
	Sig	ns		ns		ns		ns		
a	0	16.41 b	0.56	10.86 a	0.64	11.60 a	0.46	10.84 a	0.71	$P \leq 0.05$
	300	17.29 b	0.95	11.24 a	0.35	10.50 a	0.71	10.54 a	0.63	$P \leq 0.05$
	600	16.19 b	1.18	11.55 a	0.42	11.22 a	0.69	11.47 a	0.60	$P \leq 0.05$
	Sig	ns		ns		ns		ns		
b	0	5.36 b	0.25	2.97 a	0.43	2.75 a	0.26	3.06 a	0.69	$P \leq 0.05$
	300	5.44 b	0.27	2.66 a	0.43	2.47 a	0.41	3.39 a	0.48	$P \leq 0.05$
	600	5.18 b	0.53	2.69 a	0.38	2.46 a	0.49	2.62 a	0.47	$P \leq 0.05$
	Sig	ns		ns		ns		ns		

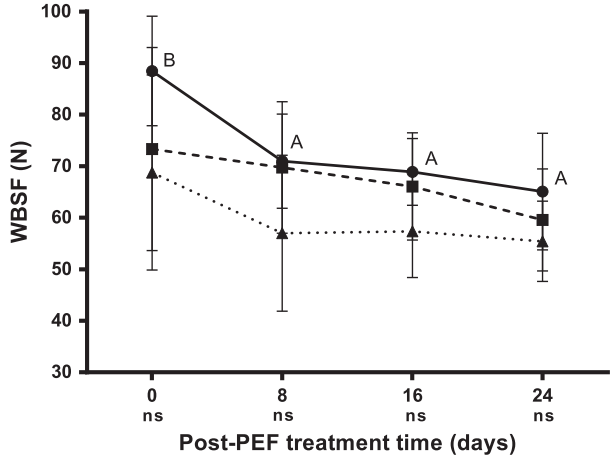

Fig. 7. Effect of post-PEF treatment time on Warner–Bratzler Shear Force values (N) for beef LTL muscle samples subjected or not to PEF treatments. Non-treated samples (●) and PEF treated samples (■ 300 pulses; ▲ 600 pulses). Data are means and 95% *CI* (error bars). Different letters for each PEF treatment indicate significant differences ($P \leq 0.05$) among mean values. At each treatment day, ns indicates non-significant differences ($P > 0.05$) among mean values.

post-mortem was similarly observed for untreated samples, whereas further storage did not involve any additional decrease in this value (Fig. 7). Regarding WBSF measurements for PEF-treated samples, mean WBSF values were slightly diminishing over the ageing period although no statistically significant differences were found ($P > 0.05$) — we assume due to the largest *SD*. Besides, results in Fig. 7 showed that PEF treatments applied in this study (1.4 kV/cm; 300 or 600 pulses of 20 μs; 10 Hz) caused no detrimental effect in tenderness along the post-PEF treatment time as WBSF mean values were lower than for the controls although no statistically significant differences were found ($P > 0.05$). Similary, Bekhit et al. (2014) reported that tenderness of beef LL and SM muscles benefited from PEF. Whereas shear force values for 1-day post-mortem LL samples decreased with PEF regardless of the post-treatment ageing, values for 1 or 3-day post-mortem SM samples decreased as ageing storage time increased.

3.3. Sensory analysis

Finally, a sensory analysis was performed using an untrained consumer panel to assess their perception of PEF-treated beef samples (1.4 kV/cm; 600 pulses of 20 μs; 10 Hz). No information was provided to panellists on sample treatments prior to consumption. According to the scores for odour, the panellists (n = 20) did not detect any differences between PEF treated and untreated samples ($P > 0.05$), with mean values of 5.7 ($SD = 1.6$) and 5.9 ($SD = 1.6$), respectively. Interestingly, tenderness for PEF treated samples had a mean value of 5.6 ($SD = 1.5$) whereas untreated samples had a mean of 4.6 ($SD = 1.5$) ($P \leq 0.05$). In this case, 60% panellist scored PEF treated as tender (≥6 points out of 9) whereas only 27.5% did for untreated samples. To the knowledge of the authors no previous sensory analyses have been reported on meat treated by PEF, even though presented data here demonstrates that PEF application on beef samples apparently has no impact on consumer's sensory acceptance.

4. Conclusion

Results presented indicated that the PEF treatments applied (1.4 kV/cm; 300 or 600 pulses of 20 μs; 10 Hz) resulted in no detrimental effect on cook loss, storage loss and colour regardless of the length of ageing before PEF application (2, 10, 18 or 26 days post-mortem) and of the length of ageing after PEF application (0, 8, 10 or 24 days). Furthermore, although the application of PEF resulted in an immediate, but low weight loss (~0.3%), this fact remains insignificant taking into account that, whatever the length of meat ageing before PEF application, the exudate after cooking remained constant or even was reduced in the PEF treated samples (600 pulses) on day 2 post-mortem. Moreover, PEF treatments applied at different times post-mortem (2, 10, 18 and 26 days) showed a tendency on reducing toughness of beef samples, but the application of PEF did not affect the tenderization process provided by ageing itself as WBSF values at different times post-treatment (0, 8, 16 and 24 days) was not affected by an early application of PEF (on day 2). This opens a possibility for studying PEF application to meat at a relatively early stage in the conditioning period (e.g. 48 h post-mortem). Finally, an untrained panel of 20 people scored similar odour but better texture marks for the PEF treated samples.

Conflict of interest

The authors have no conflict of interest to declare.

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References

Aaslyng, M.D., Bejerholm, C., Ertbjerg, P., Bertram, H. C., & Andersen, H. J. (2003). Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. *Food Quality and Preference*, *14*, 277–288.

Bekhit, A. E. D., van de Ven, R., Suwandy, V., Fahri, F., & Hopkins, D. L. (2014). Effect of pulsed electric field treatment on cold-boned muscles of different potential tenderness. *Food and bioprocess technology*, http://dx.doi.org/10.1007/s11947-014-1324-8.

Beriain, M. J., Goñi, M. V., Indurain, G., Sarriés, M. V., & Insausti, K. (2009). Predicting *Longissimus dorsi* myoglobin oxidation in aged beef based on early *post-mortem* colour measurements on the carcass as a colour stability index. *Meat Science*, *81*, 439–445.

Bertram, H. C., Kohler, A., Böcker, U., Ofstad, R., & Andersen, H. J. (2006). Heat-induced changes in myofibrillar protein structures and myowater of two pork qualities: A combined FT-IR spectroscopy and low-field NMR relaxometry study. *Journal of Agricultural and Food Chemistry*, *54*, 1740–1746.

Bertram, H. C., Whittaker, A. K., Shorthose, W. R., Andersen, H. J., & Karlsson, A. H. (2004). Water characteristics in cooked beef as influenced by ageing and high-pressure treatment — An NMR micro imaging study. *Meat Science*, *66*, 301–306.

Boakye, K., & Mittal, G. S. (1993). Changes in pH and water holding properties of *longissimus dorsi* muscle during beef ageing. *Meat Science*, *34*, 335–349.

Boakye, K., & Mittal, G. S. (1996). Changes in colour of beef *M. longissimus dorsi* muscle during ageing. *Meat Science*, *42*, 347–354.

Boleman, S. J., Boleman, S. L., Miller, R. K., Taylor, J. F., Cross, H. R., Wheeler, T. L., et al. (1997). Consumer evaluation of beef of known categories of tenderness. *Journal of Animal Science*, *75*, 1521–1524.

Bolumar, T., Enneking, M., Toepfl, S., & Heinz, V. (2013). New developments in shockwave technology intended for meat tenderization: Opportunities and challenges. A review. *Meat Science*, *95*, 931–939.

Byrne, C. E., Troy, D. J., & Buckley, D. J. (2000). Postmortem changes in muscle electrical properties of bovine *M. longissimus dorsi* and their relationship to meat quality attributes and pH fall. *Meat Science*, *54*, 23–34.

Dransfield, E. (1977). Intramuscular composition and texture of beef muscles. *Journal of the Science of Food and Agriculture*, *28*, 833–842.

Elez-Martínez, P., Sobrino-López, A., Soliva-Fortuny, R., & Martín-Belloso, O. (2012). Pulsed Electric Field processing of fluid foods. In P. J. Cullen, B. K. Tiwari, & V. P. Valdramidis (Eds.), *Novel thermal and non-thermal technologies for fluid foods* (pp. 63–108). London: Elsevier Inc.

Feldhusen, F., Warnatz, A., Erdmann, R., & Wenzel, S. (1995). Influence of storage time on parameters of colour stability of beef. *Meat Science*, *40*, 235–243.

Gudmundsson, M., & Hafsteinsson, H. (2001). Effect of electric field pulses on microstructure of muscle foods and roes. *Trends in Food Science & Technology*, *12*, 122–128.

Harper, G. S. (1999). Trends in skeletal muscle biology and the understanding of toughness in beef. *Australian Journal of Agricultural Research*, *50*, 1105–1129.

Harris, P. V., & Shorthose, W. R. (1988). Meat texture. In R. Lawrie (Ed.), *Developments in meat science* (pp. 245–296). London, UK: Elsevier Inc.

Ho, S. Y., & Mittal, G. S. (1996). Electroporation of cell membranes: A review. *Critical Reviews in Biotechnology*, *16*, 349–362.

Jayasooriya, S. D., Bhandari, B. R., Torley, P., & D'Arcy, B. R. (2004). Effect of high power ultrasound waves on properties of meat: A review. *International Journal of Food Properties*, *7*, 301–319.

Jeremiah, L. E., Dugan, M. E. R., Aalhus, J. L., & Gibson, L. L. (2003). Assessment of the chemical and cooking properties of the major beef muscles and muscle groups. *Meat Science*, *65*, 985–992.

Jeremiah, L. E., & Gibson, L. L. (2001). The influence of storage temperature and storage time on color stability, retail properties and case-life of retail-ready beef. *Food Research International*, *34*, 815–826.
Joseph, R. L., & Connolly, J. (1977). The effects of suspension method, chilling rates and post-mortem aging period on beef quality. *International Journal of Food Science & Technology*, *12*, 231–247.
King, D. A., Shackelford, S. D., Kalchayanand, N., & Wheeler, T. L. (2012). Sampling and aging effects on beef longissimus color stability measurements. *Journal of Animal Science*, *90*, 3596–3605.
Koohmaraie, M. (1989). The role of endogenous proteases in meat tenderness. *Proceedings — Annual Reciprocal Meat Conference of the American Meat Science Association*, *41*, 89–100.
Koohmaraie, M., Whipple, G., & Crouse, J.D. (1990). Acceleration of postmortem tenderization in lamb and Brahman-cross beef carcasses through infusion of calcium-chloride. *Journal of Animal Science*, *68*, 1278–1283.
Ledward, D. A. (1985). Post-slaughter influences on the formation of metyyoglobin in beef muscles. *Meat Science*, *15*, 149–171.
Miller, M. F., Carr, M.A., Ramsey, C. B., Crockett, K. L., & Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. *Journal of Animal Science*, *79*, 3062–3068.
Moore, V. J. (1990). Factors influencing frozen display life of lamb chops and steaks: Effect of packaging and temperature. *Meat Science*, *27*, 91–98.
O'Dowd, L. P., Arimi, J. M., Noci, F., Cronin, D. A., & Lyng, J. G. (2013). An assessment of the effect of pulsed electrical fields on tenderness and selected quality attributes of post rigour beef muscle. *Meat Science*, *93*, 303–309.
Offer, G., & Knight, P. (1988a). The structural basis of water-holding in meat. Part 1: general principles and water uptake in meat processing. In R. Lawrie (Ed.), *Developments in meat science* (pp. 63–171). London: Elsevier Inc.
Offer, G., & Knight, P. (1988b). The structural basis of water-holding in meat. Part 2: Drip loss. In R. Lawrie (Ed.), *Developments in meat science* (pp. 173–243). London: Elsevier Inc.
Pearce, K. L., Rosenvold, K., Andersen, H. J., & Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts of fresh meat quality attributes — A review. *Meat Science*, *89*, 111–124.
Pietrasik, Z., Aalhus, J. L., Gibson, L. L., & Shand, P. J. (2010). Influence of blade tenderization, moisture enhancement and pancreatin enzyme treatment on the processing characteristics and tenderness of beef *semitendinosus* muscle. *Meat Science*, *84*, 512–517.
Sapp, P. H., Williams, S. E., & McCann, M.A. (1999). Sensory attributes and retail display characteristics of pasture- and/or grain-fed beef aged 7, 14 or 21 days. *Journal of Food Quality*, *22*, 257–274.
Seideman, S.C., Smith, G. C., Carpenter, Z. L., Dutson, T. R., & Dill, C. W. (1979). Modified gas atmospheres and changes in beef during storage. *Journal of Food Science*, *44*, 1036–1040.
Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (1997). Repeatability of tenderness measurement in beef round muscles. *Journal of Animal Science*, *75*, 2411–2416.
Simonin, H., Duranton, F., & de Lamballerie, M. (2012). New insights into the high-pressure processing of meat and meat products. *Comprehensive Reviews in Food Science and Food Safety*, *11*, 285–306.
Sitz, B.M., Calkins, C. R., Feuz, D.M., Umberger, W. J., & Eskridge, K. M. (2006). Consumer sensory acceptance and value of wet-aged and dry-aged beef steaks. *Journal of Animal Science*, *84*, 1221–1226.
Solomon, M. B., Sharma, M., & Patel, J. R. (2011). Hydrodynamic pressure processing of meat products. In H. Q. Zhang, G. V. Barbosa-Cánovas, V. M. Balasubramaniam, C. P. Dunne, D. F. Farkas, & J. T. C. Yuan (Eds.), *Nonthermal processing technologies for food* (pp. 98–108). IFT Press, Wiley-Blackwell.
Tang, J., Faustman, C., Hoagland, T. A., Mancini, R. A., Seyfert, M., & Hunt, M. C. (2005). Post-mortem oxygen consumption by mitochondria and its effects on myoglobin form and stability. *Journal of Agricultural and Food Chemistry*, *53*, 1223–1230.
Tatum, J.D., Belk, K. E., George, M. H., & Smith, G. C. (1999). Identification of quality management practices to reduce the incidence of retail beef tenderness problems: Development and evaluation of a prototype quality system to produce tender beef. *Journal of Animal Science*, *77*, 2112–2118.
Töpfl, S. (2006). *Pulsed Electric fields (PEF) for permeabilization of cell membranes in food- and bioprocessing — Applications, process and equipment design and cost analysis.* (PhD Thesis). Berlin University of Technology.
Tornberg, E. (1996). Biophysical aspects of meat tenderness. *Meat Science*, *43*, 175–191.
Tornberg, E. (2005). Effects of heat on meat proteins — Implications on structure and quality of meat products. *Meat Science*, *70*, 493–508.
Troy, D. J., & Kerry, J. P. (2010). Consumer perception and the role of science in the meat industry. *Meat Science*, *86*, 214–226.
Verbeke, W., Van Wezemael, L., de Barcellos, M.D., Kügler, J. O., Hocquette, J. F., Ueland, Ø., et al. (2010). European beef consumers' interest in a beef eating-quality guarantee: Insights from a qualitative study in four EU countries. *Appetite*, *54*, 289–296.
Zhang, Q., Barbosa-Cánovas, G. V., & Swanson, B. G. (1995). Engineering aspects of pulsed electric field pasteurization. *Journal of Food Engineering*, *25*, 261–281.