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The effect of electro-hydrodynamic shockwaves on the quality of striploin and brisket beef muscles during long-term storage

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ABSTRACT

Shockwaves generate instantaneous high pressures, which could affect meat shelf-life or quality. This study assessed microbiological counts, pH, drip, cook and moisture loss and texture of striploin (*longissimus lumborum*) and brisket (*pectoralis profundus*) treated with electrical shockwave (25 kV, 8 pulses) and subsequently stored (−0.5 °C) for 0, 4, 8, 12, 16 and 20 weeks. Shockwave did not affect total viable counts ($p>0.05$), with all samples considered microbiologically acceptable (<7 $\log_{10}$ CFU/cm^2) after 20 weeks. Shockwave reduced the peak force of striploin by 14.4% (5.8 N) ($p<0.001$). However, for brisket, there was no effect of shockwave on texture ($p>0.05$). Shockwave × storage time increased moisture losses in striploin ($p<0.01$) and brisket ($p<0.01$) at week 0 but this decreased over subsequent storage weeks. Shockwave technology did not affect meat shelf-life and has potential for beef tenderisation.

1. Introduction

Meat tenderness is an important quality parameter which contributes to the overall sensory acceptance of an individual meat cut and can impact the monetary value of the product (Liu & Zhang, 2020). It is affected by many intrinsic and extrinsic factors such as species, genotype, stress, connective tissue content and cross-linking as well as ultimate pH and age, amongst others and reviews on the detailed mechanisms are available (Devine, 2014; Klont, Brocks, & Eikelenboom, 1998; Purchas, 2014; Thompson et al., 2006). Tenderness can also vary between muscles within a carcass depending on their locomotive or support role (Nair, Canto, Rentfrow, & Suman, 2019) and this, in part, can be attributed to the intrinsic muscle fibre type (Klont et al., 1998). Several post-slaughter interventions exist to improve tenderness and consistency, with a common technique being ageing of meat, since it provides time and environmental conditions for endogenous proteolytic enzymes to disrupt the integrity of the myofibrillar and cytoskeletal protein structures (Bhat, Morton, Mason, & Bekhit, 2018). Ageing processes can be divided into two forms; dry ageing, and wet or vacuum-ageing. Dry ageing is commonly applied to a whole carcass or primal without packaging in controlled environmental conditions (temperature, humidity, air flow) to allow for enzymatic tenderisation and flavour formation within the sterile interior of the meat (Kim, Kemp, & Samuelsson, 2016). Wet ageing is a more common practice, whereby meat primals or sub-primals are aged in vacuum packaging for 7 to 21 days (Dashdorj, Tripathi, Cho, Kim, & Hwang, 2016). Vacuum packaging is also favourable for extended shelf-life with consumer sensory analysis supporting improvements in eating quality and tenderness (particularly up to 140 days or 20 weeks) (Hughes, McPhail, Kearney, Clarke, & Warner, 2015). Other studies have also shown that microbial counts for striploins and cube rolls can remain <7 $\log_{10}$ colony forming units per cm^2 (CFU/cm^2) for up to 30 weeks when stored in optimum conditions (−0.5 °C and vacuum packaged) (Small, Jenson, Kiermeier, & Sumner, 2012). This allows the meat to age within the distribution network in transit to its point of sale (Devine, 2014).

The complexity of meat tenderness, along with the variability between and within animals, has resulted in interest in post-slaughter processing technologies that could potentially improve the rate and consistency of tenderness, while improving shelf-life. Several novel processing technologies have been investigated for tenderisation such as pulsed electric field (PEF) (O'Dowd, Arimi, Noci, Cronin & Lyng, 2013), high-pressure processing (HPP) (Sikes & Tume 2014) and ultrasound

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(US) (Barekat & Soltanizadeh, 2018; Peña-Gonzalez, Alarcon-Rojo, Garcia-Galicia, Carrillo-Lopez, & Huerta-Jimenez, 2019). A meta-analysis by Warner et al. (2017) on the tenderising effects of PEF, HPP, US, shockwave and Smartstretch™/Pivac® revealed that the technologies can have effects on meat tenderness through different mechanisms, depending on the technology, such as physical muscle disruption, enhanced proteolysis or muscle protein solubilisation.

Shockwave technology, or hydrodynamic pressure processing (HDP), is considered a potential meat tenderisation technique through the generation of instantaneous high pressure in a rapid rise-time (Bolumar & Toepfl, 2016; Sikes & Tobin, 2021). As the pressure wave is generated in water, it will propagate through any material that is an acoustic match for water, e.g. meat which is ~75% water (Bolumar, Enneking, Toepfl, & Heinz, 2013; Solomon, Long, & Eastridge, 1997). However, when it reaches a point of acoustic impedance, energy transfer occurs which is hypothesised to cause mechanical rupture of the material (Bolumar & Toepfl, 2016). Shockwave for the tenderisation of meat has been studied using two methods for pressure generation. Firstly, Solomon et al. (1997) demonstrated that detonating explosives under water can tenderise meat but this was not regarded as commercially feasible due to safety concerns. Later, it was demonstrated that a potentially safer method by electrical discharge underwater (electro-hydrodynamic shockwave) had the ability to tenderise poultry (Claus et al., 2001). A meta-analysis which collated studies of shockwave on meat from various species and muscles suggested that explosive shockwaves could reduce peak shear force (PSF) of meat by 17.7 N and electrical shockwave significantly reduced PSF by 7.5 N (Warner et al., 2017). Few studies exist on the effect of shockwave on microbial inactivation in meat and the results are conflicting (Bolumar & Toepfl, 2016). For example, it has been reported that explosive shockwave can achieve up to a 4.5 $\log_{10}$ CFU reduction in ground beef which was stored aerobically (5 °C) for 14 days (Williams-Campbell & Solomon, 2002), while other studies suggest no effect on coliform bacteria and aerobic plate counts in explosive shockwave treated pork loins (Moeller et al., 1999).

The effect of electrical shockwave processing on the microbial load during long aged shelf-life of beef primals remains unknown and we hypothesise that eletcro-hydrodynamic shockwaves could affect meat shelf-life and/or quality. The objective of this study was to assess the effect of electrical shockwave on the quality and microbial counts of vacuum-packaged beef striploin and brisket muscle during long-term storage of up to 20 weeks.

2. Materials & methods

2.1. Sample preparation

Two separate studies were conducted on two different muscles: one high value muscle which commonly undergoes ageing and can have inconsistent tenderness (striploin) and another lower value muscle with high connective tissue content (brisket). Striploin (*longissimus lumborum)* and brisket, point end deckle off (*pectoralis profundus*) from 18 grain-fed carcasses (0–2 dentition, 227.8–295.9 kg hot carcass body weight, Table 1 in Supplementary document) were collected from a local meat processing plant in South East Queensland at 24 h post-mortem and only muscles of normal pH (5.5–5.67) were used. Striploins (left and right sides of 12 animals) were collected and each striploin was cut into 3 portions (130 × 100 × 50 mm, w x l x d), yielding a total of 72 samples. Brisket muscles were obtained from the same 12 animals as the striploin muscles, and from an additional 6 animals (left and right sides of 18 animals) and each brisket was cut into 2 portions (130 × 100 × 30 mm, w x l x d) giving a total of 72 samples. Samples were vacuum packed in shockwave resistant bags (Ultra High Abuse Barrier Bag 100 μm, Cryovac, Sealed Air Food Care, Qld, Australia) and stored at −0.5 °C for 24 h until processing.

2.2. Sample processing

Two muscles (striploin and brisket) were treated as separate experiments. The experimental design contained two treatments (control or shockwave) and six storage points (0, 4, 8, 12, 16 or 20 weeks), resulting in a 2 × 6 factorial design (12 different treatments). The striploin samples were shockwave treated at 48 h post-mortem, while brisket samples were treated the following day (72 h post-mortem). The samples were randomised with respect to treatment such that the experimental design resulted in 6 replicates per treatment. Each replicate sample was treated independently, one at a time. Processing was conducted using a commercial prototype system (Shockwave, DIL German Institute of Food Technologies, Quakenbrueck, Germany) which generated shockwave using electrical discharges under approx. 500 L of water (Fig. 1A).

The treatment involved placing the vacuum packed sample directly in the area of impact, as determined in previous works (McDonnell et al., 2019a), under the emitting head, located 13 cm from the spark (shockwave origin) with fat side down and fibre direction parallel to the conveyor within the tank (Fig. 1B). The treatment consisted of 25 kV for 8 pulses in stationary mode as chosen from preliminary studies (McDonnell et al., 2019b). The duration of treatment was approx. 5 min from loading to unloading. The discharge lasted 1 s and a total of 8 discharges were applied. The water remained at 22 °C throughout processing. The temperature of an independent set of samples was assessed to determine if the shockwave contributed to any increase in temperature in the samples. The temperature was measured at 3 points (centre and edges) in the samples by a direct insertion thermometer (Checktemp1, Hanna Instruments, VIC, Australia). The sample temperature increased from 3.7 ± 0.4 to 5.5 ± 1.0 °C after treatment and could be due to the 5 min treatment duration since no temperature increases in meat due to electrically-generated shockwaves have been previously reported (Warner et al., 2017). After treatment, the samples were chilled at −0.5 °C for the allocated storage time (0 to 20 weeks, with week 0 samples stored overnight). At each storage time point, all of the replicate samples from both striploin and brisket (control and shockwave treated) from each carcass, were removed from the chiller, cut and processed (10 °C) for microbiological and meat quality assessment.

2.3. Microbiological analysis

Four surface slices (× 10 cm^2) comprising two subcutaneous fat and two lean portions of meat were excised from each primal. Surface slices from the same sample were combined with 0.85% saline (100 mL) in sterile bags and homogenised for 1 min. An aliquot of each sample was decimally diluted in 0.85% saline and plated onto Petrifilm Aerobic Count (AC) plates (3M Microbiology, Minnesota, USA) for determining total viable counts (TVC) and *E. coli*/Coliform (EC) count plates (3 M) for determining *E. coli*/Coliform counts. Parallel dilutions were also prepared in de Man, Rogosa, Sharpe (MRS) broth (Thermo Fisher, Australia) and plated onto AC plates for enumerating lactic acid bacteria (LAB). AC plates were incubated at 25 ± 1 °C for 96 ± 3 h; EC plates were incubated at 35 ± 1 °C for 24 ± 2 h and LAB plates were incubated anaerobically at 25 ± 1 °C for 120 ± 3 h. Colonies on EC were enumerated following the manufacturer's instructions for AOAC 998.08. Samples with counts of zero (no colony forming units) were arbitrarily assigned a value of half the limit of detection (i.e. 0.1 $\log_{10}$ CFU/cm^2 for TVC and EC and 1.10 $\log_{10}$ CFU/cm^2 for LAB).

2.4. Quality assessment

2.4.1. pH measurement

The pH of the samples was measured using a TPS WP-80 pH meter with a polypropylene spear-type gel electrode (IJ 44) and temperature probe (TPS Pty Ltd., Brisbane, QLD, Australia). Calibration was performed using pH 4.00 and pH 7.00 buffers equilibrated to the sample temperature.

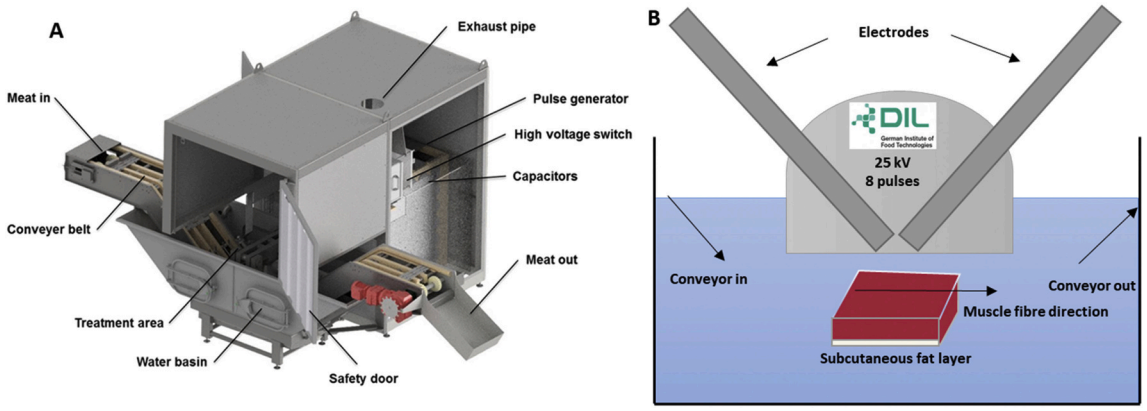

Fig. 1. (A.) The electrical discharge shockwave unit developed by DIL German Institute of Food Technologies adapted from Aganovic, Bolumar, Töpfl, & Heinz, (2021) and (B) diagram of experimental set-up. Although arrows indicate conveyor direction, this is for orientation purposes only. The unit was operated in batch mode, meaning the sample was placed directly into the treatment area in stationary mode.

2.4.2. Drip loss, cook and total moisture loss

All samples were weighed before treatment and after storage to determine the impact of treatment and storage on drip loss. Drip loss was calculated as a percentage of the difference between the original weight and the post-treatment weight. Cook loss was analysed on samples (5 × 10 × 3 cm) taken from the centre of treated meat, that had been cooked to an internal temperature of 72 °C. This was completed by holding the meat (41 min at 75 °C) in a circulating water bath using a digitally controlled heater with a temperature variation of +/− 0.5 °C. The cooking protocol was determined in preliminary trials by inserting the thermocouples into meat. After cooking, samples were immediately cooled in an ice bath for 20 min and then stored at 5 °C overnight prior to texture measurement. The cook loss was calculated as the difference in weight between raw and cooked samples, presented as a percentage of the initial weight. Total moisture loss was calculated by adding the drip loss and the cook loss.

2.4.3. Texture measurement

Texture measurements were carried out using a Lloyd LS 2.5 with a 500 N load cell (Lloyd Instruments, West Sussex, United Kingdom) and a modified Warner-Bratzler shear device (Bouton & Harris, 1972; Bouton, Harris, & Shorthose, 1971). The cooked and chilled samples were cut into rectangular shapes (15 mm × 6.7 mm, giving a cross-sectional area of 1 cm^2) and at least 25 mm long to enable secure clamping of the sample into the holder. A straight blade with a thickness of 0.64 mm was attached to an overhead clamp and pulled up through the muscle fibres, perpendicular to the fibre direction, at a speed of 100 mm/min. The maximum peak force (PF) and initial yield (IY) were determined using Nexygen Plus V3.0 software (Lloyd Instruments, West Sussex, United Kingdom). The difference between these measurements (PF-IY) was also recorded. At least six measurements were made on each sample and the mean recorded.

2.5. Statistical analysis

For each individual trait, a mixed model of analysis of variance (ANOVA) fitting treatment, storage week and their interaction as fixed effects and animal as a random effect, was applied to investigate the impact of shockwave treatment and the length of storage time on the microbial counts and meat quality attributes. The PROC Mixed model with the REML estimation method in the SAS Program (version 9.4, SAS Institute Inc., Cary, NC, USA; 2002–2012) was used for the analysis. Type 3 of ANOVA tests of fixed effects and their interaction, and the estimated least-square means (LSM) and standard errors for individual fixed effects were then produced. In addition, the detailed results about significance tests of pair-wise comparisons between the levels of individual fixed effects (i.e. differences of LSM) were also generated, with a significance level of 0.05.

3. Results & discussion

3.1. Microbial analysis

The TVC was similar for shockwave treated and untreated control samples, with the mean count of all sample/treatment combinations, at all timepoints, considered to be microbiologically acceptable when applying a cut-off of 7 $\log_{10}$ CFU/cm^2 (Fig. 2A-D). The similar trendlines of LAB and TVC across the storage trial suggests that the microbial population was mostly comprised of lactic acid bacteria and consistent with previous findings observed in striploin stored under similar conditions (Frank et al., 2019; Small et al., 2012). For example, vacuum packed striploins and cube rolls stored at −0.5 °C for 210 days (30 weeks) have shown microbiological counts that rarely progressed to 7 $\log_{10}$ CFU/cm^2 and that samples remained organoleptically acceptable for at least 26 weeks (Small et al., 2012).

Visual inspection of packs post-treatment did not reveal any damage to this particular brand of packaging and the high LAB concentration indicated that vacuum integrity was maintained throughout shockwave treatment and storage. This demonstrates that the selected packaging was sufficient to withstand shockwaves, which has presented challenges for the application of the technology to meat in the past (Warner et al., 2017). While the mean count at each timepoint was comparable to previous similar studies, there were some instances of large variations (2–3 $\log_{10}$ CFU/cm^2) between replicate samples. Metagenomics tools could be used in future studies to gain a clearer understanding of the microbiological population structure of samples and identify sub-populations associated with increased bacterial growth rates.

E. coli, often used as an indicator of process control, was absent from all samples, except for one control sample (week 4 brisket), which was at a very low level (0.4 $\log_{10}$ CFU/cm^2). The lack of *E. coli* and low initial bacterial counts indicates control of plant hygiene, typical of Australian meat production systems (Small et al., 2012). It also demonstrates that the meat used in this study is microbiologically consistent with that used in previous studies which achieved extended shelf-life under the same storage conditions (Small et al., 2012). The comparable results between control and treated samples across storage indicate that shockwave did not affect microbial populations after treatment (day 0) or their ability to grow (weeks 4–20). Others have demonstrated <1 $\log_{10}$ CFU reduction of pre-inoculated *Escherichia coli* O157:H7 (EHEC) ground beef treated with explosive (100 g) HDP analysed at day 0 and, although the reduction was statistically significant, <1 $\log_{10}$ CFU reduction was impractical without other hurdles in place (Podolak, Solomon, Patel, &

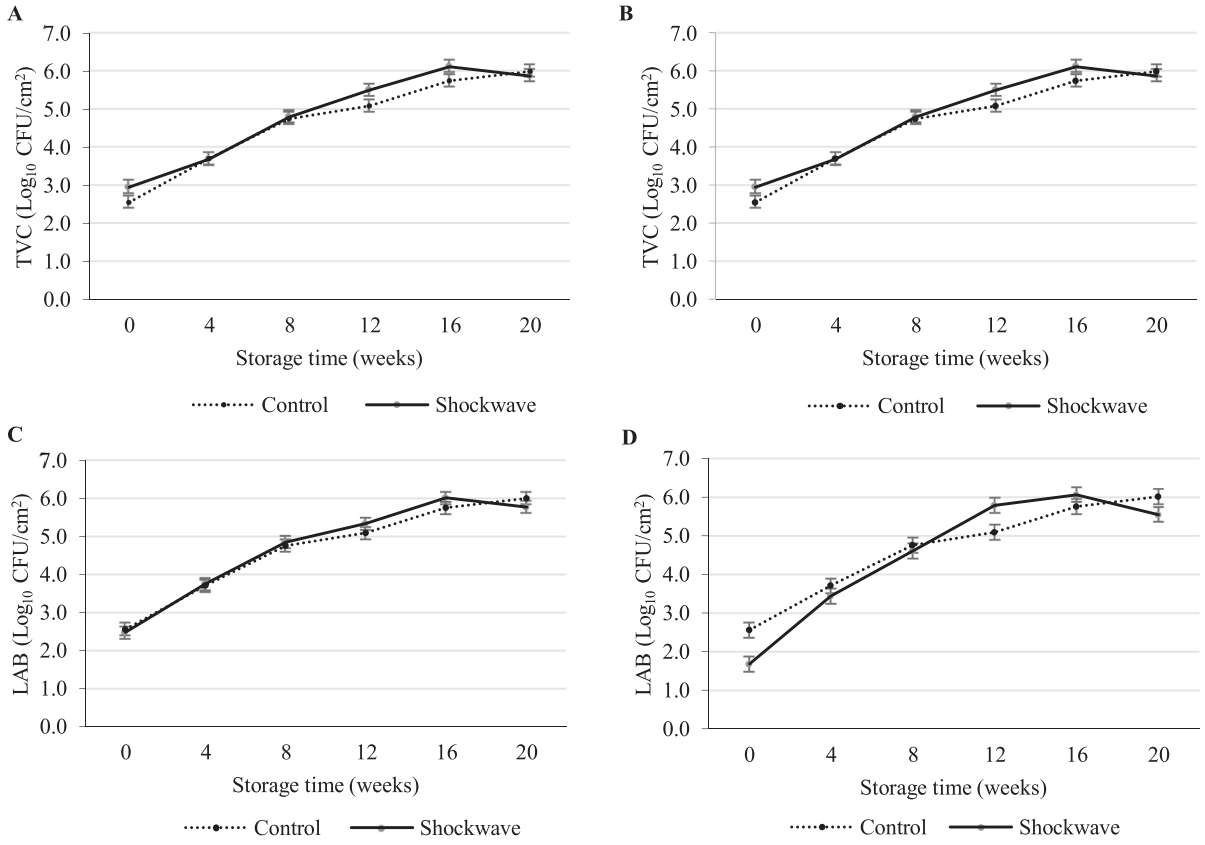

Fig. 2. Predicted least-square means of total viable counts (TVC) colony forming units per cm^2 (Log_{10} CFU/cm^2) for striploin (A) and brisket (B) samples and Lactic acid bacteria (LAB) counts for striploin (C) and brisket (D) samples – control and shockwave (25 kV, 8 pulses) over a 20-week storage period. Error bars indicate standard error.

Liu, 2006). Few studies have assessed the effects of shockwave on microbial counts of meat over periods of storage. Williams-Campbell and Solomon (2002) suggest that the reduction becomes larger over time with day 0 HDP treated ground beef achieving a 1.5–2.0 log_{10} CFU reduction which increased to 4.5 log_{10} CFU reduction after 14 days storage. However, the majority of studies show little or no effect of shockwave on microbial inactivation in meat (Bolumar & Toepfl, 2016; Moeller et al., 1999; Podolak et al., 2006). Overall, based on the TVC and LAB results for the striploin and brisket, the shockwave treatment did not cause any adverse effects to shelf-life and demonstrated a similar trend to the controls.

3.2. Quality assessment

3.2.1. pH

For both striploin and brisket, pH was not significantly affected by treatment. However, there was a significant increase in pH over time, from 5.43 to 5.47 at Day 0 to 5.63 to 5.64 at week 20 (Table 1), consistent with other long aged shelf-life experiments showing increases in pH (Hughes et al., 2015). The increase in pH of meat during long aged shelf-life experiments is believed to be associated with proteolysis (either autolytic or microbial) which results in the release of basic compounds and a subsequent elevated pH. It is noted that peak pH was observed at week 12 for both brisket and striploin samples. It is hypothesised that the samples evaluated in week 12 likely originated from animals with higher starting pH rather than any alternative physiological, biochemical or microbiological effect. Williams-Campbell and Solomon (2002) reported no difference in the pH of minced and stewing pieces of beef following treatment with explosive shockwave when analysed at day 0 and, while a difference was reported following 14 day storage, this was attributed to aerobic bacteria, which is not comparable to this present work where storage was under vacuum.

3.2.2. Texture

For striploin, peak force was affected by shockwave treatment ($p<0.001$) and storage time ($p<0.0001$), but not the interaction (Table 2). Although the interaction between storage time and treatment was not significant, Fig. 3 shows that the initial reduction in PF was maintained throughout storage for striploin with the mean difference of 5.8 N (14.4% lower peak force) over full storage trial. However, although the brisket had an initial reduction (10%) in PF at week 0, the trend was not maintained throughout storage and there was no effect of treatment ($p>0.05$) nor interaction with storage (Table 2, Fig. 3). Considering the difference between muscle tenderness (e.g. tender to

Table 1
pH (predicted least square means ± standard error) of striploin and brisket -control and shockwave (25 kV for 8 pulses) over a 20-week storage period.

	Treatment		Storage (weeks)						Treatment x storage
Muscle	Control	Shockwave	0	4	8	12	16	20	
Striploin	5.59 ± 0.01	5.56 ± 0.01	5.43 ± 0.02^{e}	5.52 ± 0.02^{d}	5.56 ± 0.02^{c}	5.70 ± 0.02^{a}	5.59 ± 0.02^{b}	5.63 ± 0.02^{b}	N.S.
Brisket	5.60 ± 0.01	5.62 ± 0.01	5.47 ± 0.01^{d}	5.56 ± 0.01^{c}	5.63 ± 0.01^{b}	5.71 ± 0.01^{a}	5.66 ± 0.01^{b}	5.64 ± 0.01^{b}	N.S.

[a-e] Superscripts indicate a significant difference between storage times ($p<0.0001$); N.S. = not significant.

Table 2
Texture analysis (predicted least square means ± standard error) for striploin and brisket samples - control and shockwave (25 kV for8 pulses) over a 20-week storage period.

Muscle	Treatment		Storage (weeks)					Treatment x storage
	Control	Shockwave	0	4	8	12	16	20	
Striploin									
Peak Force	40.32 ± 1.33^{a}	34.51 ± 1.35^{b}	54.05 ± 1.79^{a}	36.72 ± 1.72^{b}	34.99 ± 1.72^{bc}	32.87 ± 1.72^{bc}	33.62 ± 1.72^{bc}	32.21 ± 1.73^{c}	N.S.
IY	37.37 ± 1.52^{a}	30.56 ± 1.54^{b}	51.74 ± 1.93^{a}	33.51 ± 1.87^{b}	31.9 ± 1.87^{bc}	29.28 ± 1.87^{bcd}	29.8 ± 1.87^{cd}	27.57 ± 1.88^{d}	N.S.
PF-IY	3.03 ± 0.39^{a}	4.02 ± 0.39^{b}	2.31 ± 0.54^{c}	3.21 ± 0.52^{bc}	3.22 ± 0.52^{bc}	3.60 ± 0.52^{abc}	4.15 ± 0.52^{ab}	4.66 ± 0.52^{a}	N.S.
Brisket									
Peak Force	49.94 ± 1.47	48.19 ± 1.47	58.69 ± 1.90^{a}	45.15 ± 1.90^{b}	44.03 ± 1.90^{bc}	45.89 ± 1.90^{c}	48.49 ± 1.90^{c}	52.17 ± 1.90^{c}	N.S.
IY	34.12 ± 1.16	31.14 ± 1.16	49.67 ± 1.80^{a}	34.27 ± 1.80^{b}	28.23 ± 1.80^{c}	26.22 ± 1.80^{c}	29.64 ± 1.80^{bc}	27.75 ± 1.80^{c}	N.S.
PF-IY	15.57 ± 1.02	17.31 ± 1.02	9.56 ± 1.63^{d}	11.43 ± 1.63^{dc}	15.63 ± 1.63^{bc}	19.51 ± 1.63^{ab}	18.45 ± 1.63^{b}	24.02 ± 1.63^{a}	N.S.

$^{a-d}$ Superscripts indicate a significant difference between treatment for peak force (PF) ($p<0.001$), initial yield (IY) ($p<0.0001$), PF-IY ($p<0.05$) and storage times ($p<0.0001$) N.S. = not significant; PF-IY = peak force - initial yield.

very tender) can be 0.7 kg (equivalent to 7 N), this is an improvement in tenderisation (Sullivan & Calkins, 2011). This initial tenderising effect was also seen in the preliminary studies (McDonnell, Fitzgerald, et al., 2019a). Other authors have also reported an effect of HDP and ageing on tenderness but no interaction, whereby a 23% reduction in Warner-Bratzler Shear Force (WBSF) was found in shockwave treated beef striploin at 0, 5 and 8 days (Bowker, Fahrenholz, Paroczay, Eastridge, & Solomon, 2008).

While PF is the total force required to shear the sample, other information can be extrapolated from the deformation curve. Initial yield (IY) and peak force minus initial yield (PF-IY) can be considered as indicators of the respective contribution from the myofibrillar and connective tissue proteins to meat toughness (Sikes, 2014). In the case of striploin, both PF-IY and IY were significantly affected by shockwave treatment ($p<0.0001$, 0.05, respectively) and storage time ($p<0.0001$, 0.05, respectively) but not the interaction (Table 2). The difference between the control and treated striploin samples for PF and IY values, were 5.81 N and 6.81 N, respectively, suggesting that the difference in overall texture measurement was largely determined by the muscle's myofibrillar component. Thus, from this measurement alone, shockwave treatment appears to affect the myofibrillar proteins rather than the connective tissue proteins and/or other muscle proteins, such as sarcoplasmic and cytoskeletal proteins. Previous studies have suggested that the tenderising effects of shockwave result from physical disruption of the muscle (Zuckerman, Bowker, Eastridge, & Solomon, 2013) and action of proteolytic enzymes, such as calpastatin and calpain, thereby having a similar effect as age-related proteolysis (Bowker et al., 2008). Bolumar, Bindrich, Toepfl, Toldrá, and Heinz (2014) found an 18% reduction in WBSF of beef loin steaks and while microstructural changes suggest physical disruption, no changes to proteolytic enzymes (cathepsins and peptidases) were found. The current findings support the theory of muscle fibre disruption but future studies with analysis points in a narrower range (1–21 days), would be useful to elucidate the mechanisms of tenderisation and the possibility of accelerated ageing.

It must be noted that the experiments were treated independently per muscle. However, when considering the mechanistic actions of shockwave, the brisket muscle showed less difference in IY between treatments compared to the striploin, and so no significant difference was observed for any texture attribute under these processing conditions, suggesting that some underlying differences exist between these muscles when considering the effect of shockwave treatment. Previous studies have also found that the effect of shockwave on meat tenderness is both muscle and species dependent for which the reasons are unclear (Warner, Claus, Huynh, Lee, & Ha, 2019). For example, improvements for WBSF in beef silverside and loin have been reported as 4.8–24.8% and 18.1% respectively, but no changes in PF of pork topside and silverside were found after applying electrical shockwave with different number of pulses being applied (Bolumar et al., 2013). In the current study, fibre differences between these muscles exist, with brisket known to have nearly double the incidence of type I muscle fibres compared to striploin (Totland & Kryvi, 1991). There is also some preliminary evidence to suggest other muscles (*biceps femoris*) that also have a higher incidence of type I fibres, appear to be less susceptible to shockwave induced improvements in shear force, compared to other muscles (*semitendinosus, semimembranosus*) with more type IIA and type IIX fibre types (Warner, Claus, Huynh, Lee, & Ha, 2019). This suggests that higher levels of type I fibres may reduce the tenderisation effect induced by shockwave processing but this needs further exploration. In addition, brisket has a higher connective tissue content, a higher proportion of type III collagen and more heat-stable crosslinks compared to striploin. As alterations to the endomysial collagen fibril network have been discussed as a tenderisation mechanism (Bolumar et al., 2014), however further investigation of the effects of shockwave on muscles with differing connective tissue characteristics is warranted. Therefore, more detailed studies on the mechanistic tenderisation actions of electrical shockwave, considering fibre type, accelerated ageing and

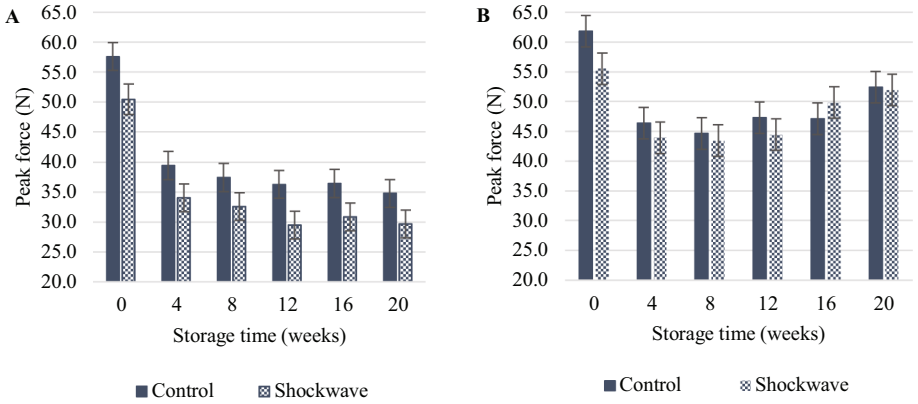

Fig. 3. Peak force (N) predicted least square mean for (A) striploin and (B) brisket samples - control and shockwave (25 kV for 8 pulses) over a 20-week storage period (where the interaction of storage time and treatment was not significant ($p>0.05$). Error bars indicate the standard error.

ultrastructural changes, are required.

3.2.3. Drip, cook and moisture loss

Drip loss is an important quality attribute because it can affect the yield of the cut while also affecting the perceived eating quality and product appearance by consumers (Warner, Ferguson, Cottrell, & Knee, 2007). In both muscles, there was a trend for increased drip loss in samples at week 0 which often reversed or became similar (Fig. 4), resulting in a treatment effect, whereby shockwave treated striploin and brisket demonstrated 0.65% ($p<0.05$) and 0.5% ($p<0.05$) less drip than the controls ($p<0.05$) as a mean value over the total storage period (Table 3). When considering time points, it should be noted that at week 0, shockwave treated samples did not have more than 1% increase in drip loss compared to control samples and ageing resulted in similar drip.

Nonetheless, it is important to also consider cook loss and total moisture losses as overall water-holding capacity of the meat has an effect on perceived juiciness and is directly related to the structural aspects of the muscle (Hughes, Oiseth, Purslow, & Warner, 2014; Warner et al., 2007). Cook loss data showed a similar trend to drip loss, with shockwave resulting in increased cook loss on day 0 and the treatment effect was only significant in brisket, which had 1.6% more cook loss on average in shockwave-treated samples than the control ($p<0.01$, Table 3). The total moisture loss increased over the storage time ($p<0.0001$) and was significantly affected by the interaction of treatment and storage time for striploin ($p<0.01$) and brisket ($p<0.01$). As can be observed in Fig. 5, shockwave resulted in increased moisture losses at the beginning of storage but, for subsequent storage weeks, it became the same between treatments or on some storage weeks, the control exhibited more moisture losses than the shockwave treated sample.

The effect of shockwave on drip and cook loss have been reported in the past, but to our knowledge, no studies have assessed this either as total moisture loss or assessed the changes during long-term storage. Bowker, Schaefer, Grapperhaus, and Solomon (2011) found a 2–3% increase in cooking loss when beef loin steaks were treated with electrical shockwave but the interaction between storage (0–7 days) and shockwave treatment was not significant. Similarly, Moeller et al. (1999) reported ~2% increased cook loss in shockwave-treated pork loins, contributing to lower perceived juiciness by a sensory panel. In contrast, other authors have reported no significant effect of shockwave on cook loss (Liu et al., 2006; Schilling, Marriott, Wang, & Solomon, 2003). Ha, Dunshea, and Warner (2017) conducted a meta-analysis of twelve shockwave studies assessing the effect of the technology on cook loss and colour of meat. It was found that, on average, shockwave increased cook loss by 0.6% compared to the control. Furthermore, the magnitude was similar between aged and unaged meat (−0.931 and −0.517, respectively) and explosive or electrical shockwaves (−0.639 and − 0.891, respectively). Our findings support this meta-analysis where the relationship between ageing and cook loss is considered in comparison to the mechanisms of shockwave. It has been hypothesised that increased cook loss after ageing is due to proteolysis resulting in protein fragments and water which is more easily expelled during cooking (Purslow, Oiseth, Hughes, & Warner, 2016). Zuckerman et al. (2013) analysed shockwave treated beef *semimembranosus* by scanning electron microscopy and suggested the loss was due to fragmented collagen fibrils separated from the connective tissue matrix. The recent study of Chian et al. (2021) also suggested that changes to the secondary structure of connective tissue proteins by shockwave can result in a lower denaturation temperature and as a result, this could lead to

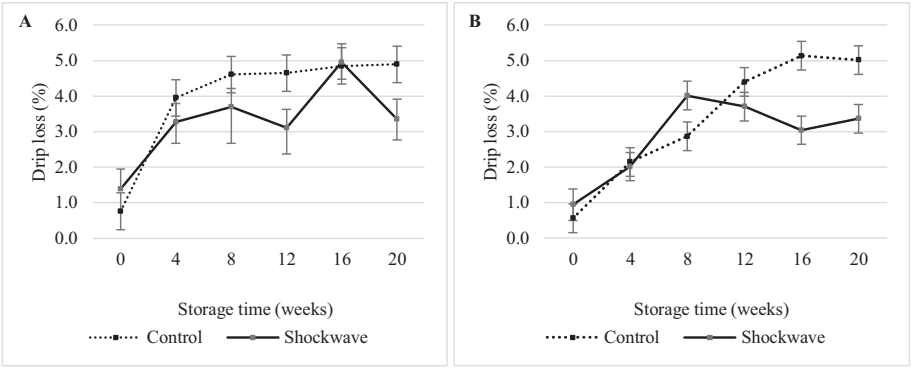

Fig. 4. Drip loss (%) predicted least square mean for (A) striploin ($p>0.05$) and (B) brisket samples ($p<0.01$) – control and shockwave (25 kV, 8 pulses) over a 20-week storage period. Error bars indicate the standard error.

Table 3
Drip, cook and total moisture losses (%) (predicted least square means ± standard error) for striploin and brisket samples - control and shockwave (25 kV, 8 pulses) over a 20-week storage period.

	Treatment		Storage (weeks)					Treatment x storage
Muscle	Control	Shockwave	0	4	8	12	16	20	
Striploin									
Drip Loss	3.95 ± 0.21^{a}	3.30 ± 0.22^{b}	1.07 ± 0.38^{c}	3.61 ± 0.36^{b}	4.15 ± 0.36^{ab}	3.88 ± 0.36^{ab}	4.90 ± 0.36^{a}	4.12 ± 0.38^{ab}	N.S.
Cook Loss	26.71 ± 0.52	27.40 ± 0.52	27.05 ± 0.60^{ab}	26.23 ± 0.58^{b}	26.29 ± 0.58^{b}	27.77 ± 0.58^{a}	27.42 ± 0.58^{a}	27.59 ± 0.59^{a}	N.S.
Moisture Loss	30.54 ± 0.59	30.82 ± 0.60	28.11 ± 0.67^{c}	29.85 ± 0.66^{b}	30.44 ± 0.66^{b}	31.65 ± 0.66^{a}	32.32 ± 0.66^{a}	31.72 ± 0.68^{a}	$p<0.01$*
Brisket									
Drip Loss	3.35 ± 0.17^{a}	2.85 ± 0.17^{b}	0.75 ± 0.3^{c}	2.08 ± 0.28^{b}	3.44 ± 0.28^{a}	4.05 ± 0.28^{a}	4.09 ± 0.28^{a}	4.19 ± 0.28^{a}	$p<0.01$**
Cook Loss	29.2 ± 0.39^{a}	30.8 ± 0.39^{b}	27.67 ± 0.56^{c}	29.37 ± 0.56^{b}	30.75 ± 0.56^{ab}	30.08 ± 0.56^{ab}	30.83 ± 0.56^{ab}	31.18 ± 0.56^{a}	N.S.
Moisture Loss	32.58 ± 0.4^{a}	33.67 ± 0.41^{b}	28.66 ± 0.61^{c}	31.43 ± 0.58^{b}	34.16 ± 0.58^{a}	34.10 ± 0.58^{a}	34.96 ± 0.58^{a}	35.42 ± 0.58^{a}	$p<0.01$*

* See Fig. 5 for values; ** See Fig. 4 for values $^{a-c}$ Superscripts indicate a significant difference between treatments for drip loss ($p<0.05$), cook loss ($p<0.01$), moisture loss ($p<0.05$) and storage times ($p<0.0001$), N.S. = not significant.

shrinkage of connective tissues at a lower temperature leading to increased water expulsion as indicated by a significantly larger extracellular space area. Similarly, microstructural changes to the muscle fibre bundles and increased endomysial space have been observed in electrical shockwave treated (36 kV, 1 pulse) beef loins (Bolumar et al., 2014).

The results of this present study suggest that microstructural changes induced by shockwave at week 0, could be similar to microstructural changes induced by the ageing process as moisture losses from initial shockwave treated samples and the controls from week 4 were similar (Fig. 5). It would be useful to explore this in future studies with microstructural studies and/or the application of low-field nuclear magnetic resonance, as this could identify changes in water distribution, i.e. extra-myofibrillar and intra-myofibrillar. This could elucidate if the effect of shockwave on water distribution at week 0 is indeed similar to water distribution in an aged non-shockwave treated control at week 4. Water molecules are considered to be bound in the meat matrix in either of three different ways: water which is tightly bound to macromolecules, water that is entrapped within myofibrils (intra-myofibrillar) and that in the free form that is loosely held between myofibrils and can be easily expelled (extra-myofibrillar) (Bertram, Purslow, & Andersen, 2002). It seems likely that microstructural changes to the meat matrix induced by the shockwave may have caused free water to be relocated and consequentially expelled as drip in packaging, and further removed during the cooking process, early in the storage process (week 0). This may impact on the overall mechanism of shockwave tenderisation, as water plays a key role in the interaction of structural integrity, denaturation temperatures and moisture/cook loss (Hughes et al., 2014). Further research into the structural mechanisms for water losses and the impact on perceived eating quality would be of interest, particularly for consumer acceptance. From a commercial perspective, the findings that the initial moisture losses from shockwave are similar to moisture losses during ageing could be of significance.

4. Conclusions

This study provides new information on the effect of electrical shockwave on the long-term storage of beef. Shockwave treatment did not have any effect on microbial counts of vacuum-packaged beef striploins and brisket over a storage period of 20 weeks at −0.5 °C. The mean total viable counts were similar across samples at each timepoint, with all samples considered microbiologically acceptable after 20 weeks of storage, demonstrating that application of shockwave technology does not change the microbiological acceptability of beef. Shockwave significantly increased the tenderness of striploin samples by 14.4% over the storage period, demonstrating its potential for reduced ageing time which should be investigated further. In brisket, there was an initial reduction in peak force by 10.1% at week 0 (24 h after treatment), but this effect was not significant nor maintained during storage (up to 20 weeks), thus confirming that shockwave treatment was more effective for tenderisation of striploin than brisket. This varying effect of shockwave on different muscles requires further investigation, with these results indicating that shockwave affected myofibrillar proteins more than those present in the connective tissue. The effect of shockwave on tenderness was muscle dependent and electrical shockwave requires further processing optimisation to have a similar impact resulting from explosive shockwave. Of other quality attributes, shockwave also had some initial effects on moisture loss, but these were not more than those induced by ageing alone, when considering moisture losses over storage. Overall, this work demonstrates the use of shockwave as a promising tenderisation technique for some muscles, which does not affect shelf-life. Future work could focus on: (1) process optimisation; (2) elucidating the microstructural changes responsible for tenderisation and the related effect on water mobility; and (3) sensory evaluation to evaluate the consumer acceptance of a meat's quality attributes after shockwave treatment.

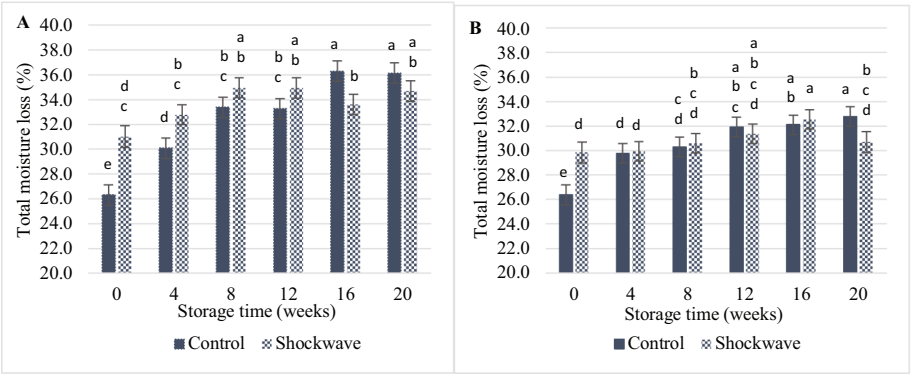

Fig. 5. Total moisture loss (%) predicted least square mean for (A) striploin ($p<0.01$) and (B) brisket ($p<0.01$) samples – control and shockwave (25 kV, 8 pulses) over a 20-week storage period. Letters a-e indicated a significant difference between treatment × storage time. Error bars indicate the standard error.

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Declaration of Competing Interest

None.

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