Accepted Manuscript

Very fast chilling modifies the structure of muscle fibres in hot-boned beef loin

Anita L. Sikes, Robin Jacob, Bruce D'Arcy, Robyn Warner

PII: S0963-9969(16)30625-1
DOI: doi: 10.1016/j.foodres.2016.12.027
Reference: FRIN 6556

To appear in: *Food Research International*

Received date: 2 September 2016
Revised date: 11 December 2016
Accepted date: 24 December 2016

Please cite this article as: Anita L. Sikes, Robin Jacob, Bruce D'Arcy, Robyn Warner , Very fast chilling modifies the structure of muscle fibres in hot-boned beef loin. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Frin(2016), doi: 10.1016/j.foodres.2016.12.027

Very fast chilling modifies the structure of muscle fibres in hot-boned beef loin

Anita L. Sikes[1,2*], Robin Jacob[3], Bruce D'Arcy[2] and Robyn Warner[4,a]

[1]*CSIRO Agriculture and Food, 39 Kessels Road, Coopers Plains, Queensland 4108, Australia*

[2]*The University of Queensland, School of Agriculture and Food Sciences, St Lucia, Queensland 4072, Australia*

[3]*Department of Agriculture and Food WA, South Perth, Western Australia 6151, Australia*

[4]*CSIRO Agriculture and Food, 671 Sneydes Road, Werribee, Victoria 3030, Australia*

[a] *Present address: Faculty of Veterinary and Agricultural Science, The University of Melbourne, Parkville, Victoria 3010, Australia*

* Corresponding author, A.L. Sikes, CSIRO Agriculture and Food, PO Box 745, Archerfield, Queensland, Australia, 4108

E-mail address: anita.sikes@csiro.au

ABSTRACT

The aim of this study was to gain a better understanding of the tenderisation mechanism associated with very fast chilling (VFC) of beef muscle. Hot-boned striploins from 36 carcasses were allocated to a treatment (control, delayed or immediate chilling below 0°C), and each striploin was divided into three equal portions and allocated to a time post-mortem (2, 5 or 14 d). Immediate chilling resulted in lower peak force values at 2 d post-mortem and lower particle size after 5 d post-mortem. Both chilling treatments significantly improved the WHC by reducing drip loss and cooking loss. Sarcomere lengths were not affected by chilling treatments, although fragmentation and cleavage of muscle fibres were evident along the fibre length, possibly contributing to the reduction in peak force values at 2 d post-mortem. Both delayed and immediate chilling resulted in a higher pH at 4 and 24 h post-mortem, and colour parameters were modified. These results suggest that VFC has potential for accelerating tenderisation early post-mortem by a combination of biochemical and biophysical effects.

Keywords: very fast chilling; beef; tenderness; hot-boning; pH decline; structure

1. Introduction

Very fast chilling (VFC) has several potential advantages, including the reduction of cooling times, increased rate of product turnover and decreased shrink and evaporative losses compared to contemporary chilling rates. Conventional chilling is a lengthy, energy-expensive process (Janz, Aalhus, & Price, 2001) and increases evaporative weight loss (Bowater, 1997; Joseph, 1996). Very fast chilling or rapid

chilling was first defined as the chilling of muscle from about 40°C to below 0°C within five hours of stunning (Joseph, 1996).

Fast chilling generally leads to cold shortening of unrestrained muscles and consequent toughening of cooked meat, even after ageing (Locker & Hagyard, 1963; Marsh & Leet, 1966). This led to the generally accepted '10/10 rule of thumb' that muscle should not be cooled below 10°C within 10 hours of slaughter (Bendall, 1973). In addition, Jeacocke (1977) found that cold shortening occurred in pre-rigor muscle when temperatures were less than 10°C. Marsh and Leet (1966) also showed that holding pre-rigor muscle at 15°C (conditioning) prevents shortening and cold-induced toughening.

The initial report by Joseph (1996) showed that VFC did not impact tenderness compared to conventionally chilled muscle, and there was a lack of consistency reported in the studies on the effect of the treatment on texture. There have been several reports supporting this lack of effect of VFC on tenderness (Redmond, McGeehin, Sheridan, & Butler, 2001; Van Moeseke, De Smet, Claeys, & Demeyer, 2001). In contrast to these earlier observations, some studies on VFC of meat show enhanced eating quality compared to meat from carcasses that are chilled slowly (Beltran, Jaime, Lopez-Lorenzo, & Roncales, 1986; Bowling, Dutson, Smith, & Savell, 1987; Jacob et al., 2012; Jaime, Beltrán, Ceña, López-Lorenzo, & Roncalés, 1992; Sheridan, 1990).

Colour is an important indicator of the quality of fresh meat and can influence the purchase decision of consumers. The chilling rate and the rate of pH decline post-mortem, as well as ultimate pH, have been shown to influence meat colour following the chilling process (Jacob & Thomson, 2012; Young, Priolo, Simmons, & West, 1999). Rapid chilling or VFC of carcasses or sides has been reported to result in

darker meat colour in beef (Aalhus, Robertson, Dugan, & Best, 2002; Bowling et al., 1987), bison (Janz, Aalhus, & Price, 2002) and lamb (Kahraman et al., 2014). Therefore, the potential impact on meat colour is an important consideration when meat chilling conditions are changed.

Several mechanisms have been suggested for the tenderisation of meat as a direct result of VFC applied to pre-rigor meat, including; prevention of sarcomere shortening by physical restraint provided by a hard crust on the outer surface, accelerated protease activity due to early release of calcium from the sarcoplasmic reticulum (SR) (Davey & Garnett, 1980; Jaime et al., 1992; Joseph, 1996), and rapid metabolism interfering with the actomyosin rigor bond (Warner et al., 2015). Sheridan (1990) showed that VFC resulted in a fast glycolysis rate that led to a rapid pH decline, while Roncales (1998) hypothesised that VFC caused physical damage to the myofibrillar structure resulting in increased tenderness. Jacob et al. (2012) showed that sarcomere shortening is prevented independently of external restraint when temperature and time specifications were optimised for VFC. Warner et al. (2015) suggested that the increased production of inosine 5-monophosphate (IMP) in VFC muscle caused a weakening of the actin-myosin bridge, affecting the tenderness. In spite of these suggested hypotheses, the mechanism of tenderisation involved when using VFC is still not fully understood, although it is unlikely to be due to acceleration of the protease activity seen with conventional chilling.

Delaying VFC with a 15°C conditioning period until the commencement of rigor should minimise cold shortening and allow comparison of the effects of VFC to conventionally chilled muscle, independent of sarcomere shortening. Therefore, the aim of this research was to determine if VFC led to an improvement in tenderness and improved eating quality of beef striploins and to investigate its mechanism of action,

given the expectation that unrestrained meat should shorten. This was achieved by comparing an immediate chilling (IC) treatment commenced at 1 h post-mortem with another where chilling treatment was delayed by a conditioning period at 15°C (delayed chilling, DC).

2. Materials and Methods

2.1 Experimental design

The experiment was a 3x3 factorial design consisting of three chilling treatments (Table 1), and three post-mortem time periods: 2, 5 and 14 d. There were 12 different slaughter dates over a three week period, with all treatments applied on each slaughter date, and one striploin per treatment per replicate.

2.2 Muscles

One striploin (*M. longissimus lumborum*, LL) muscle was collected from the left side of each of 36 beef carcasses at Teys Australia, Beenleigh, Qld. The carcasses were from cows (6–8 tooth), ranging in weight from 204 to 336 kg HSCW (Hot Standard Carcass Weight) (mean=262 kg), and were electrically stimulated post-dressing (low voltage electrical stimulation, 350 V, 15 Hz, 30–45 s). The intention within the design was to collect three striploins from three different animals on each slaughter date from within the same mob (mob number being the identity of the consignment). This was not the case on two slaughter dates: on one occasion, two striploins were collected from animals within the same mob and the third from a different mob; on another slaughter date, striploins were collected from animals from three different mobs.

2.3 Sample preparation

Pre-rigor striploins were collected from 24 animals (hot-boned), while a further 12 muscles from separate animals were tagged after slaughter and collected the following

day in the boning room (controls, cold-boned). The time from slaughter to carcass tagging and hot boning ranged from 41 to 76 min (mean of 47 min). Prior to chilling treatments, the fat layer and cap muscle (*M. spinalis dorsi*) were removed from the striploin to ensure even chilling, and each muscle was allocated to a treatment (DC or IC) and cut into thirds, approximately 120x120x60 mm. This cutting technique was also used for cold-boned (control) striploins. Each piece of striploin (0.80 to 1.72 kg, average weight 1.19 kg) was then randomly allocated to a time post-mortem (2, 5 or 14 d) and sealed into laminated vacuum bags (VAC LS pouches, multilayer, coextruded film, PA and PE resins, 70 μm; Bemis Flexible Packaging Australasia, Brisbane).

2.4 Preliminary fast chilling trials

In this study, the target was not a desired chilling rate per se, but was designed to chill to the freezing plateau of meat, approximately -1 to -1.5°C, in the centre (thermal and geometric) of the sample. This temperature was also chosen as it was shown by Jacob et al. (2012) that for VFC to be successful, the critical temperature is below 0°C. Preliminary trials were conducted to optimise the developed fast chilling system to achieve the required chilling conditions. This involved the insertion of a thermocouple (0.2 mm Type T copper-constantan wire connected to a Grant Squirrel Series 1250 data logger) into the centre (length, width and depth) of the striploin sample, after which the sample was vacuum-packed with the thermocouples sealed inside the bag. Samples were lowered into the cooling medium (propylene glycol in water, 45% v/v, at -20°C) and temperature was logged (real-time temperature monitoring) until the centre of the meat sample reached -3°C. After removal from the water bath (Julabo Labortechnik GmbH, Seelbach, Germany; F38-ME), the thermocouple junctions were located and the cross-sectional dimensions of the samples at these points were

measured. Food Product Modeller™ (FPM v 2.0, MIRINZ, AgResearch Limited, Hamilton, New Zealand) was used to develop a chilling model that simulated the chilling process, in order to predict the chilling times for samples of different sizes, initial temperatures and cooling medium temperatures relative to those tested. A suitable generic shape was selected in FPM, and the sample dimensions, thermocouple location and fluid temperature were inserted into the model. The heat transfer coefficient was adjusted until the model predicted the cooling rate at the thermocouple location with reasonable accuracy. Using this model, a good prediction of the sample centre cooling rate was achieved (Figure 1A).

2.5 Chilling treatments

2.5.1 Control

Carcasses were conventionally chilled (0–3°C air temperature for 16 h with spray chilling) and the striploin removed from the carcass 24 h post-mortem (approximately). Each striploin was then cut into thirds, packed into vacuum bags, and allocated to a time post-mortem (as described in Section 2.3).

2.5.2 Immediate chilling (IC)

Striploins were removed from the carcass on the slaughter floor, vacuum-packed and cooled to a core temperature of approximately -1.5°C by immersion in a propylene–glycol water bath, set at -20°C. The core temperature was identified by real-time temperature monitoring using inserted thermocouples, as described in Section 2.4.

2.5.3 Delayed chilling (DC)

Striploins were removed from the carcass on the slaughter floor, vacuum-packed and placed in a second Julabo water bath at 15°C. The time spent in the 15°C water bath was intended to be the time required for the core temperature of the sample to reach

15°C (±1°C), but in practice, this was often the time required for the concurrent IC treatment to complete the cycle in the -20°C water bath (about 1 h). The samples were transferred from the 15°C water bath to the chilling water bath (-20°C), and chilled according to the protocol used for the IC treatment (Section 2.5.2).

2.6 Storage time post-mortem

Samples were stored in a chiller with an air temperature of -1.5°C for the designated times post-mortem (2, 5 and 14 d post-mortem).

2.7 Moisture loss

The drip loss for each sample stored in a vacuum bag was calculated on a weight loss basis. The intact packs at each time point were weighed prior to opening, and the weight of the empty bag was subtracted from the initial weight. The moisture loss was expressed as a percentage of the initial weight of the sample.

Cook loss of samples was determined prior to texture measurement. Samples were weighed prior to cooking and were reweighed after cooking (70°C for 60 min) and blotting dry. Cooking loss was expressed as the initial weight minus the final weight, and expressed as a percentage of the initial weight.

2.8 pH measurement

The superficial freezing of muscle samples during the two chilling protocols necessitated the use of two methods for measuring pH: probe and homogenisation. Table 2 outlines the pH method used for samples from each treatment at each time point.

Probe measurements of muscle pH were made with a digital pH meter (TPS WP-80, Springwood, Australia) fitted with a combination electrode (Ionode IJ44, Tennyson, Australia; glass body with a spear tip) with temperature compensation.

Subsamples taken after the fast chilling process was completed were frozen in dry ice and stored at -80°C for pH measurement by homogenisation. A frozen muscle sample (0.5 g) was homogenised (Ultra Turrax T25, IKA, Germany) at 20,500 rpm for 15 s in 5 mL of an ice-cold solution containing sodium iodoacetate (5 mM) and potassium chloride (150 mM), pH 7.0 (Bendall, 1975). The pH of the homogenate was measured with a digital pH meter.

2.9 Sarcomere length

Sarcomere lengths were measured using a helium-neon gas laser diffraction technique on unfixed portions taken from frozen (-20˚C) samples. The laser has a wavelength of 635 nm, and was used as the light source to obtain diffraction patterns from muscle fibre samples held between glass microscope slides. Sarcomere length was determined from the diffraction pattern displayed on a frosted screen (Bouton *et al.* 1973). Sarcomere length (μm) was calculated from the average distance (mm) of the inner and outer diffraction bands from the centre of the screen.

2.10 Peak force measurement

At each time point post-mortem, a subsample was frozen at -20°C for analysis. Subsamples were thawed overnight at 5°C, cooked in a water bath at 70°C for 60 min, chilled and stored at 5°C, prior to analysis. The method used for peak force (PF) measurement was a modification of the Warner-Bratzler shear force (WBSF) textural method (Bratzler, 1932; Warner, 1928). Details of blade type, sample thickness, shape and fibre orientation are given in Bouton, Harris, and Shorthose (1971) and Bouton and Harris (1972). All measurements were made on a Lloyd Instruments LRX Materials testing instrument fitted with a 500 N load cell (Lloyd Instruments Ltd., Hampshire UK). Six subsamples having a rectangular cross-section of 15 mm wide by

6.7 mm deep (1 cm^2 cross-sectional area) were cut from all samples, with the fibre orientation parallel to the long axis, and at right angles to the shearing surface. The force required to shear through the clamped subsample with a 0.64 mm thick blade, pulled upward at a speed of 100 mm/min at right angles to fibre direction, was measured as peak force (PF, N) (Bouton & Harris, 1972; Bouton et al., 1971). Warner-Bratzler shear force was not used as the force measurement as a Warner-Bratzler blade was not used in this measurement and sample dimensions were different. The mean of the peak force for the six subsamples was recorded.

2.11 Colour measurement

At 2 and 14 d post-mortem, the muscle samples were removed from vacuum bags and cut in half perpendicular to the backbone, and steaks (approximately 25 mm) were cut for colour measurement. Two steaks were placed cranial side facing up onto black snopak foam trays (205x130x12 mm) (Vadals, Brisbane, Australia) and wrapped with Glad-Wrap®. Muscle samples were bloomed (5°C for 60 min, in light conditions) and triplicate colour measurements (L*, a* and b* values) and spectral scan from 400–700 nm were conducted at room temperature (approximately 25°C) using a Hunterlab Miniscan EZ 45/0 LAV (Hunter Associates Laboratory Inc., Reston, VA, USA). The aperture was 5 cm, illuminant A, and 10° standard observer was used. The Hunterlab was calibrated at room temperature (15–20°C) using white (L=100) and black (L=0) calibration tiles. Steaks remained on the black trays during measurement. Where required, standardisation of the Hunterlab was conducted through Glad-Wrap®, similar to the sample wrapping. The redness value was calculated as the ratio of the percentage of light reflectance at 630 nm and the percentage of light reflectance at 580 nm (R630/R580) (AMSA, 2012).

2.12 Structural modifications

For visualising structural changes using light microscopy, subsamples (approximately 1 cm^3) were taken from the centre of each control and treated muscle sample (n=4), cryosectioned (10 μm), and stained using Bouin's fixative, Orange G (muscle fibres)

and aniline blue (collagen). Sections were viewed with a light microscope (Nikon Eclipse E200, Japan) and images were obtained with a Tucsen 5.0 MP camera (Tucsen Digital Imaging Technology, China). The spaces between muscle fibres were measured with the length measurement function of an image analysis software (ImagePro® Premier 9.1, 2015, Media Cybernetics).

Sub-samples (approximately 10x15x15 mm) from each treatment at each time point post-mortem were selected for transmission electron microscopy (TEM) analysis. Samples were fixed in glutaraldehyde and osmium tetroxide followed by embedding in an araldite/Epon resin mixture. The samples were ultrathin-sectioned to 90 nm thickness, mounted on copper grids, and stained with uranyl acetate and lead citrate. The images were taken using a Philips CM120 transmission electron microscope at 120 kV.

2.13 Particle size analysis

Samples taken at each time point post-mortem were measured for particle size according to the method of Karumendu, Van de Ven, Kerr, Lanza, and Hopkins (2009). Approximately 1 g of muscle (cut along the fibre direction) was homogenised at 16,000 rpm in 15 mL of ice-cold phosphate buffer (25 mM potassium phosphate, pH 7.0, 0.1 M KCl, 1 mM EDTA), using an Ultra-Turrax® T-25 (10 mm diameter shaft, IKA Labortechnik, Staufen, Germany). The samples were homogenised twice for 30 s, with the sample being held on ice between homogenisation bursts. The homogenate was filtered through nylon mesh (1 mm^2) and left on ice until tested for particle size using a laser diffraction particle size analyser (Malvern Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK). The refractive index for the muscle homogenate was set at 1.46. The volume mean diameter D[4,3], referred to as mean particle size (μm), is presented in this study.

2.14 Statistical analysis

Statistical analyses were carried out using Genstat 15th edition (http://www.vsni.co.uk/software/genstat). A linear mixed model using REML was fitted to the data (peak force, drip loss, sarcomere length, cook loss, colour parameters, particle size). The fixed effects were treatment*time post-mortem and the random effects, kill date/mob number/animal. Five samples were removed from the data due to high residual values (1 from control, 1 from IC and 3 from DC); these either had a pH_u > 5.8 and/or high peak force values. As pH measurements were recorded using two different methods, a separate analysis was done on this data. Measurements of pH using both the probe and homogenisation methods were recorded for the DC treatment at 3 h post-mortem. Initially a t-test was performed to test for significance between the methods, followed by analysis using a repeated measures model, with one fixed effect being treatment, and time presented as a variate and a factor.

3. Results

3.1 Temperature profiles

The chilling model developed from the FPM was used to verify that the chilling conditions would achieve the required chilling temperature (-1.5C) within a certain time (less than 5 h post-mortem), irrespective of the size of the sample (Figure 1).

Several definitions have been used for the temperature-time conditions for VFC. In this work, a target temperature, rather than a defined chilling rate, was used – a final core muscle temperature of ~ -1.5°C. The average time for striploins to reach the target temperature for the IC treatment was 88 min, with a range of 55–138 min. For the DC treatment, striploins were conditioned at 15°C for an average of 94 min (range 60–149 min), followed by an average of 58 min (range 40–79 min) in the water bath

to reach the target temperature. The variable times taken to reach the target temperature within each chilling treatment would have been dependent on the thickness of the striploin samples, as well as the initial temperature of the muscle before chilling, i.e. ~ 37°C for the IC treatment and 15°C for the DC treatment. During this chilling process, freezing was initiated in all chilled samples.. Typical temperature-time profiles for the DC and IC treatments are shown in Figures 1B and 1C, respectively. All chilling treatments were completed in less than 4 h from hot-boning, and less than 5 h from slaughter.

3.2 pH decline and ultimate pH (pH_u)

The pH of the samples measured using the probe and homogenisation methods for the DC treatment at 3.08 h post-mortem was not significantly (P=0.34) different. Therefore, the mean pH measurements of both methods for this treatment and time were used in the repeated measures analysis.

The pH of the conventionally chilled (control) striploins declined from the start of chilling to 24 h after slaughter (Figure 2. Both the DC and IC treatment groups had slower pH declines than the conventionally chilled treatment group (Figure 2). Even though there were some missing data points as a result of superficial freezing of samples, there were significant differences in the pH measured at different times post-mortem. At 3 h post-mortem, the pH of the conventionally chilled striploin samples was significantly (P<0.001) lower than that of the DC samples. At 4 h post-mortem, the pH of the conventionally chilled samples was significantly (P<0.001) lower than both the DC and IC samples, with no difference between the pH of the DC and IC samples at this time point. This difference in pH was also observed at 24 h post-mortem, with both chilling treatments having a significantly (P<0.001) higher pH (pH 5.64 for DC, pH 5.63 for IC) compared to the conventionally chilled striploins (on the

carcass) (pH 5.49). This significantly (P<0.001) higher pH was also evident at 2, 5 and 14 d post-mortem (Figure 3).

3.3 Sarcomere length

Sarcomere lengths were similar (P>0.05) between treatments but there was an effect of post-mortem time and also an interaction between chilling and time (P<0.05) (Table 3). Sarcomere lengths of samples stored for 14 d were significantly (P=0.031) shorter than those stored for 2 and 5 d. At 2 and 5 d post-mortem, sarcomere lengths of both the DC muscle samples and IC samples were significantly (P=0.05) shorter than the control samples (Table 3). However, at 14 d post-mortem, there was no significant (P>0.05) difference between any of the samples. Mean sarcomere lengths for each chilling treatment were above 1.8 μm, suggesting that no cold-shortening of muscle had occurred. With samples from both chilling treatments having sarcomere lengths above 1.8 μm, the toughness (increased peak force value) of the cooked meat would not appear to be explained by cold-shortening.

3.4 Peak force measurement

Muscles subjected to the IC treatment tended to have lower (P=0.051) peak force values at 2 d post-mortem (55.1 N compared to 61.5 N for conventionally chilled striploins) (Figure 4). With increasing time post-mortem, the peak force of striploins was significantly (P<0.001) reduced The peak force values for the IC treatment were similar (P>0.05) to the control striploins at 5 and 14 d post-mortem. There was no significant (P>0.05) interaction between treatment and time post-mortem on peak force values.

The initial yield (IY) value, which is attributed to the contribution of myofibrillar proteins to the overall texture of meat, showed a significant (P<0.001) decrease as the

time post-mortem increased (Table 3), and although not significant, there was a trend for an interaction between time post-mortem and fast chilling (P=0.069). For the IC, the initial yield declined from 2 to 14 d post-mortem. For the control and DC, the initial yield was similar at 2, 5 and 14 d post-mortem.

The impact of the connective tissue on meat texture is reflected in the peak force minus initial yield (PF-IY) value. Both chilling treatments resulted in significantly (P=0.006) lower PF-IY values compared to the conventionally chilled striploins, suggesting some degradation of the connective tissue during chilling (Table 3). Conversely, as the time post-mortem increased for the control samples, the PF-IY value increased but this was not evident for either fast chilling treatment (P=0.061). This is difficult to explain but could purely be a factor of the calculation of the PF-IY value and is dependent on the magnitude of the PF and IY values.

3.5 Colour measurement

There were main effects of time post-mortem (P=0.002) but not chilling treatment on L^* value (Table 4). L^* value increased with increased post-mortem time for each treatment, and L^* value was higher for control than both chilling treatments, and fort IC than DC, at both post-mortem times.

For a^* and b^* values, there were treatment and time effects but no interaction (Table 4). Both a^* and b^* values were lower (P<0.001) for chilled samples compared to the control treatment. Samples were more red (higher a^* value) and more yellow (higher b^* value) at 14 d than at 2 d post-mortem.

There was a significant effect of treatment (P=0.024) on redness (R630/R580) but no post-mortem time or interaction effects (Table 4). Samples were less red (R630/R580)

for both chilling treatments compared to the control, and this difference was greater at 14 d than at 2 d post-mortem.

3.6 Moisture loss

Chilling, time post-mortem, and an interaction between chilling and time had a significant (P<0.001) effect on the moisture lost during storage of vacuum-packed muscles (Table 3). Drip loss from the control samples was 0.24%, 0.52% and 1.15% at 2, 5 and 14 d post-mortem, respectively, and increased (P<0.001) during storage. Conversely, drip loss from both the fast chilling treatments was much lower than control treatments at each time point and did not increase with time post-mortem (P>0.05).

The loss of water in samples due to cooking in a water bath at 70°C for 1 h was lower (P=0.003) in both the IC and DC treatments compared to conventional chilling at each time point post-mortem (P=0.003; Table 3).

3.6 Structural modifications

From the light micrographs, the cross-section of the control muscle at 2 d post-mortem showed a regular shape and distribution of muscle fibres surrounded by connective tissue (Figure 5A), with few gaps between fibres. At 14 d post-mortem, a greater number of gaps between fibres are evident and the connective tissue appears to be more aggregated (Figure 5D, arrow). In the images of the DC samples, fragmentation of the fibres is evident, which is more obvious at 14 d post-mortem (Figure 5E, arrow) compared to 2 d post-mortem (Figure 5B, arrow). Freezing damage is more obvious in the IC samples Formation of holes or spaces due to ice crystal formation are evident within the muscle fibres (Figure 5C, arrow). There appears to be no difference in fragmentation of muscle fibres with storage at 2 or 14 d

(Figures 5C, 5F). It is also evident from the transverse sections of the muscle samples that there are considerably more holes or spaces in the muscle fibres in the IC samples (Figure 5C, right hand image) than the DC samples (Figure 5B, right hand image).

It should be noted that the assessment of the spaces between the fibres of the light microscopy images using the image analysis software was quite difficult. In some images, the spaces between the fibres and the spaces within the fibres were hard to distinguish from each other. At 2 d post-mortem, the spaces between the fibres tended to be larger for the IC samples (30.1 ± 4.69 μm, mean ± SEM) compared to the control (19.6 ± 2.72 μm) and DC samples (22.9 ± 8.06 μm) but differences between treatment means were not significant (P>0.05).

The TEM images of the control muscle at 2 d post-mortem shows the regular pattern and normal structures, with intact myofibrils (Figure 6A, 6C). At 14 d post-mortem, control samples showed fractures in the Z-line, as well as fragmentation at the junction of the Z-line and I-band (Figure 6B, arrow). In DC samples at 2 d post-mortem, intact myofibrils were evident (Figure 6C), with wide I-bands and little overlap between A- and I-bands. At 14 d post-mortem, the DC samples showed some fracturing of the Z-line (Figure 6D). Samples at 2 d post-mortem from the IC treatment showed some misalignment of the sarcomeres and the banding pattern was less regular. We postulate that this is due to ice crystal pressure occurring during the application of immediate chilling to pre-rigor samples. The A- and I-bands are clearly visible and the Z-lines are deformed but undamaged, although some longitudinal splits are evident (Figure 6E, arrows). At 14 d post-mortem storage of IC samples (Figure 6F), fragmentation along the fibre is evident, and the appearance of amorphous material is observed. Intact Z-lines are visible but thickening of the Z-line is evident (Figure 6F).

3.7 Particle size analysis

Mean particle size was significantly impacted by both treatment (P<0.001) and time post-mortem (P=0.001) but there was no interaction effect (Figure 7). Mean particle size was lower for IC samples than both control and DC samples, and the biggest effect was at 5 d post-mortem.

4. Discussion

Very fast chilling has been reported to result in a high degree of variability in tenderness (Joseph, 1996). Compared to control and DC, IC striploins had lower peak force values but only at 2 d post-mortem. This suggests accelerated tenderisation occurred as a result of VFC early post-mortem, and confirms previous studies that the timing of VFC is critical (Jacob et al., 2012). Subzero temperatures must be reached during chilling before rigor occurs, for this tenderisation process to occur.

The peak force values of the IC striploins at 5 and 14 d post-mortem were no different to the conventionally chilled striploins. This again confirms that VFC presumably, has no influence on proteolysis during ageing, as previously described by Jacob et al. (2012).

The *M. longissimus thoracis et lumborum* muscle is well-documented to cold-shorten when exposed to a cold environment after excision in a pre-rigor condition (Locker, 1960), and this cold-shortening leads to consequent toughening of cooked meat, even after ageing (Marsh & Leet, 1966; Marsh, Woodhams, & Leet, 1968). Jeacocke (1986) suggested this extensive muscle contraction is triggered by an increased calcium release from the sarcoplasmic reticulum at low temperatures. However, in our study, there was no difference in sarcomere lengths between treatments, and the mean value for all treatments was 2.0 μm. Although the sarcomere lengths of the control

samples were longer at 2 and 5 d post-mortem, it must be noted that the measurements were done on separate samples from the same muscle and therefore, some variability in the measurement is possible. Toughening due to cold-shortening did not occur regardless of the deboning temperature; hot-boned for both chilling treatments compared to cold-boned for the control. Any differences in texture between IC and DC would therefore have been due to an effect related to the timing of chilling in relation to slaughter and/or the difference in initial temperatures at the start of chilling (i.e. around 30°C for IC and 15°C for DC) which would result in different rates of chilling.

The freezing of lamb carcasses within 4 h post-mortem resulted in surface hardening which was thought to prevent cold-shortening (Davey & Garnett, 1980). Janz et al. (2002) reported that lean bison carcasses after 4 or 6 h chilling at -35°C had longer sarcomere lengths compared to conventionally chilled carcasses. In the absence of significant sarcomere shortening, Janz et al. (2001) suggested that any disparity in peak force values might be the result of impaired activity of endogenous proteolytic enzymes under the extreme cold conditions. However, Li et al. (2012) showed that superficial freezing of excised striploins from Chinese Yellow cattle cooled in air at -21°C to achieve a 0°C core temperature, did not prevent cold-shortening. In the present study, although the sarcomere lengths of the striploins in the control, DC and IC treatments were similar at 2 d post-mortem, the IC treatment resulted in a lower peak force value than the DC and control treatments. Therefore, an additional mechanism other than the prevention of cold-shortening by surface hardening is suggested to contribute to the tenderisation in the VFC striploins at 2 d post-mortem.

As with most biochemical reactions, the lowering of chilling temperature of muscle in the range of 40 to 0°C decreases enzyme activity, hence the rates of chemical

reactions, including glycolysis, and the rate of pH decline slows during cooling (Lawrie & Ledward, 2006). However, with pre-rigor muscle, when the temperature is lowered from about 0 to -3°C, an increase in the reaction rate and hence, an increase in pH decline occurs, and this has been suggested to be a result of freeze concentration. At the initiation of freezing, the formation of ice crystals reduces the volume of liquid water, increases the concentration of dissolved substrate, thereby increasing the reaction rate independent of enzyme activity (Dransfield, 1998).

As there are missing data points early post-mortem in this study, it is difficult to compare the change in pH between treatments up to 3 h post-mortem. However, at 4 and 24 h, the pH of striploins from both fast chilling treatments was higher than the conventionally chilled control striploins, indicating a decreased rate of pH decline in the fast chilled treatments compared to the control over the 24 h period; striploin pH at 24 h post-mortem for all samples was <5.7. This decreased rate of pH decline is consistent with other VFC studies (Janz et al., 2001; Li et al., 2012; Van Moeseke et al., 2001; Zhu, Gao, & Luo, 2011), however, in these studies, the chilling temperature achieved was 0°C or above. In the reports by Zhu et al. (2011) and Van Moeseke et al. (2001), the pH of VFC samples at 5 h post-mortem was higher than control samples but this difference was not observed at 24 h post-mortem. In another VFC study where beef carcasses were blast-chilled below 0°C (-35°C giving -1°C at 5 h post-mortem), Aalhus et al. (2002) showed a slower rate of pH fall and higher pH at 3, 5, 7 and 10 h post-mortem in striploin muscles.

In contrast, there have been several documented studies showing a faster pH decline with exposure of muscles to lower temperatures (10 to -3°C) (Jeacocke, 1977; Jolley, Honikel, & Hamm, 1981; Winger, Fennema, & Marsh, 1979), and in a few VFC studies (Jacob et al., 2012; Sheridan, McGeehin, & Butler, 1998). In the study of

Sheridan et al. (1998), although lamb carcasses were chilled in air at -20°C, sub-zero temperatures were not achieved. This resulted in a faster decline in pH in the *M. longissimus thoracis* muscle up to 12 h post-mortem in the VFC carcasses compared to conventional chilling but a higher ultimate pH was observed. Honikel, Roncalés, and Hamm (1983) showed that in beef muscle, the rate of pH fall is slower only when carcasses are cooled at temperatures above 6–8°C. Below this temperature, an accelerated pH fall is observed in the first 3–4 h post-mortem. They explained that the severe muscle contraction (or cold-shortening) was attributed to this biochemical event. Jeacocke (1977) had also previously suggested that cold-shortening and increased glycolytic rate occur simultaneously. Therefore, it could be argued that in the absence of cold-shortening, the glycolytic rate should not be accelerated. This supposition was not supported by the work of Jacob et al. (2012), who showed that even without cold-shortening, a faster rate of pH decline occurred in lamb striploins exposed to VFC conditions, and this was accompanied by an improvement in texture. One hypothesis proposed by Warner et al. (2015), is that IMP is in higher concentration in pre-rigor VFC muscle, so may substitute for ATP on the myosin head and allow the myosin head to dissociate from actin, thus breaking the cross-bridge. Bendall (1973) reported that a slower rate of fall in pH occurs during the early stages of post-mortem metabolism when ATP is being resynthesised from creatine phosphate, and a noticeable increase in the rate of pH fall when resynthesis through this mechanism failed. Changes in metabolism occurred within 1.5 and 5.5 h post-mortem with VFC, with higher levels of glycolytic intermediate metabolites being measured (Warner et al., 2015); therefore the effects of VFC on pH decline, Ca^{2+} flux and metabolism warrants further investigation.

As the rate and extent of pH decline was different in the two chilling treatments, differences in the colour parameters could also be expected (Renerre, 1990). Colour in meat is determined by the concentration and chemical state of myoglobin, as well as achromatic factors, such as light scattering properties (Krzywicki, 1979). In this study, chilling treatments resulted in meat that was less red (R630/R580) and darker in colour compared to the control. Changes in R630/R580 represents a colour change due to either oxygenation or oxidation of myoglobin. Lower R630/R580 for treated samples relative to the control suggests that fast chilling resulted in less redness. Jacob and Thomson (2012) speculated that decreased R630/R580 in rapidly chilled lamb muscles, with no change in lightness (as found in this study), was due to a slower bloom due to colder temperatures keeping oxygen consumption by mitochondria active for longer. Mitochondrial activity decreases with time so a greater effect at 14 d could be consistent with an effect on mitochondria (Bendall & Taylor, 1972). Fast chilling also results in less protein denaturation (MacDougall, 1982; Sammel et al., 2002), and in this study, moisture loss from fast chilled striploins was considerably lower than conventionally chilled samples. Differences in the amount of free water, combined with structural modifications caused by fast chilling (as evidenced from microscopy images and particle size analysis) could influence the light reflectance and scattering properties and hence, the colour quality of the meat. The colour changes with very fast chilling treatment could therefore be due to changes in the status of myoglobin (a higher oxygen consumption rate and/or an inhibition of the formation of oxymyoglobin) or changes in achromatic properties of the muscle, such as light scattering and reflectance, or a combination of both.

The difference in the peak force values at 2 d post-mortem between the DC and IC may be explained by the microscopy images. The IC samples at 2 d post-mortem

tended to have larger spaces between fibres than the control and DC treatments. The transverse images also show that more holes occurred in the fibres from the IC treatment than with the DC treatment. There was more physical disruption with IC than with DC, in evidence in the TEM images. This disruption was along the fibre length rather than across the fibre, which would indicate that it was due simply to expansion of the spaces between fibres rather than the fibres fragmenting under tension from contraction (Roncales, 1998). The density of water reaches a maximum at 4°C and then declines with temperature, and subsequently, ice formation occurs (http://www.simetric.co.uk/si_metric.htm). This therefore provides some evidence that the mechanism for tenderisation using VFC was due to the expansion of water between fibres, with the timing of VFC perhaps influencing this through the effects of osmotic pressure on the freezing temperature (Farouk, Kemp, Cartwright, & North, 2013). Although no specific studies were conducted to investigate the effect of VFC on proteolysis, SDS-PAGE analysis showed no differences in the degradation of major muscle proteins with chilling treatments (results not shown). Also, the change of microstructure as shown in the microscopy images, is unlikely due to differences in proteolysis since the major fragmentation occurred along the fibres only.

Particle size analysis using the method of Karumendu et al. (2009) has been developed as an alternate method for assessing myofibrillar fragmentation, and hence as an indicator of proteolysis, with smaller values indicating greater myofibrillar structure disintegration. However, in studies investigating the effect of freezing on proteolysis of beef (Nakai, Nishimura, Shimizu, & Arai, 1995), and earlier method development using particle size analysis for determining myofibrillar fragmentation (Hopkins, Littlefield, & Thompson, 2000), it has been suggested that physical disruption caused by freezing was more likely the reason for differences between

fresh and frozen muscle than increased proteolysis. Therefore, in this study, as there was no evidence of changes in proteolysis between conventionally chilled and fast chilled samples, the differences in mean particle size was most likely due to modification of the muscle structure due to the chilling process. For all treatments, the microscopy images showed that there was more damage at 14 d than at 2 d post-mortem. Correspondingly, the mean particle size was smaller at 14 d than at 2 d post-mortem for all treatments. Mean particle size of IC samples were smaller than the control and DC samples, particularly at 5 d post-mortem. The particle size analysis reflects the information obtained from the microscopy analysis and therefore supports the theory that tenderisation due to very fast chilling is most likely due to structural modification of the muscle.

The high ultimate pH at 2 d post-mortem of the fast chilled muscles would be expected to impact on the ability of the muscle to retain water and therefore influence drip and cooking losses. In the present study, this was indeed the case. Chilling treatments had a significant impact on moisture loss during storage and cooking. Drip losses from rapidly chilled striploins were considerably lower than the conventionally chilled striploins. This difference is probably due to the slower rate of pH decline and higher ultimate pH of the very fast chilled samples compared to the control. Also, reabsorption of water by muscle proteins which may take place during thawing would be affected by the ice-fibre interfacial area (Bevilacqua, Zaritzky, & Calvelo, 1979). In addition, an increase in intracellular osmotic pressure possibly caused by the splitting of bonds between the myofibrillar protein aggregates and the breakdown of sarcoplasmic proteins to amino acids, could also account for this enhanced water-holding capacity in the chilled samples. This supports the lack of cold-shortening of chilled muscles under these conditions, as it is known that loss of water from muscles

may be increased by calcium-induced shrinkage of myofibrils and the extent of shrink during rigor development (Marsh, Cassens, Kauffman, & Briskey, 1972). Therefore, cold-shortened muscles exhibit increased drip loss early post-mortem. This is also summarised by Honikel, Kim, Hamm, and Roncales (1986), who stated that it is not temperature that is responsible for drip loss but the change in sarcomere length produced by temperature.

In our study, a combination of factors, and not any one in particular, appear to be involved in the mechanism for tenderisation of beef striploin using VFC. Prevention of cold-shortening in both chilling treatments did not alone account for the reduction in peak force values at 2 d post-mortem in the IC treatment. Structural damage from expansion of water between fibres and ice crystal formation appears to be the difference between the DC and IC treatments, and breakage and cleavage along the fibres is postulated to be responsible for the reduced shear force values at 2 d post-mortem. The impact of VFC on pH decline, ATP hydrolysis and Ca^{2+} release warrants further investigation as it appears that the rate of pH decline, as well as the ultimate pH, could be significant modulators of tenderisation using VFC.**5. Conclusions**

The work reported in this study showed that IC resulted in lower peak force values at 2 d post-mortem. Despite early pre-rigor temperatures being low in fast chilling treatments, all sarcomere lengths were similar between treatments. Visualisation of the muscle samples using microscopic methods and particle size analysis suggests that very fast chilling affected muscle structure. The two chilling processes used in this study, DC and IC, also resulted in different rate and extent of pH declines compared to the control, as well as differences in colour parameters. This indicated that the rate of pH decline and the ultimate pH were significant modulators of tenderisation using VFC, and that the relationship between pH–temperature and tenderness was different for VFC, suggesting that conventional pH–temperature benchmarks do not apply to VFC.

Acknowledgements

The authors acknowledge co-funding provided by Meat and Livestock Australia (MLA) (A.MQT.0053) +for this work, and the support provided by Teys Australia, Beenleigh staff is greatly appreciated and endorsed. The authors would also like to thank Neil McPhail, Janet Stark, Joanne Hughes for their contributions to this study.

References

Aalhus, J.L., Robertson, W.M., Dugan, M.E.R., & Best, D.R. (2002). Very fast chilling of beef carcasses. *Canadian Journal of Animal Science, 82*, 59-67.

AMSA. (2012). Meat Colour Measurement Guidelines. Illinois, USA: American Meat Science Association.

Beltran, J.A., Jaime, I., Lopez-Lorenzo, P., & Roncales, P. (1986). Effect of early conditioning temperature on tenderness and aging of lamb meat *32nd European Meeting of Meat Research Workers* (Vol. 1, pp. 157-159). Ghent, Belgium.

Bendall, J.R. (1973). Postmortem changes in muscle. In G. H. Bourne (Ed.), *The Structure and Function of Muscle* (Vol. 3, pp. 227-309). New York: Academic Press.

Bendall, J.R. (1975). Cold-contracture and ATP-turnover in the red and white musculature of the pig, *post mortem*. *Journal of the Science of Food and Agriculture, 26*(1), 55-71.

Bendall, J.R., & Taylor, A.A. (1972). Consumption of oxygen by the muscles of beef animals and related species. II. Consumption of oxygen by post-rigor muscle. *Journal of the Science of Food and Agriculture, 23*(6), 707-719.

Bevilacqua, A.E., Zaritzky, N.E., & Calvelo, A. (1979). Histological measurements of ice in frozen beef. *International Journal of Food Science & Technology, 14*(3), 237-251.

Bouton, P.E., & Harris, P.V. (1972). A comparison of some objective methods used to assess meat tenderness. *Journal of Food Science, 37*, 218-221.

Bouton, P.E., Harris, P.V., & Shorthose, W.R. (1971). Effect of ultimate pH upon the water-holding capacity and tenderness of mutton. *Journal of Food Science, 36*, 435-439.

Bowater, F. (1997). Economies of meat chilling and freezing *Proceedings of the Institute of Refrigeration* (pp. 1-11). London, England.

Bowling, R.A., Dutson, T.R., Smith, G.C., & Savell, J.W. (1987). Effects of cryogenic chilling on beef carcass grade, shrinkage and palatability characteristics. *Meat Science, 21*(1), 67-72.

Bratzler, L.J. (1932). *Measuring the tenderness of meat by means of a mechanical shear*. (Master of Science Thesis), Kansas State College, KA, USA.

Davey, C.L., & Garnett, K.J. (1980). Rapid freezing, frozen storage and the tenderness of lamb. *Meat Science, 4*(4), 319-322.

Dransfield, E. (1998). Very fast chilling in beef: Muscle to meat. In E. Dransfield, P. Roncales, R. L. Joseph & D. J. Troy (Eds.), *Concerted Action CT94-1881 Very Fast Chilling in Beef* (Vol. 2. Muscle to Meat). Bristol, UK: University of Bristol.

Farouk, M.M., Kemp, R.M., Cartwright, S., & North, M. (2013). The initial freezing point temperature of beef rises with the rise in pH: A short communication. *Meat Science, 94*(1), 121-124.

Honikel, K.O., Kim, C.J., Hamm, R., & Roncales, P. (1986). Sarcomere shortening of prerigor muscles and its influence on drip loss. *Meat Science, 16*(4), 267-282.

Honikel, K.O., Roncalés, P., & Hamm, R. (1983). The influence of temperature on shortening and rigor onset in beef muscle. *Meat Science, 8*(3), 221-241.

Hopkins, D.L., Littlefield, P.J., & Thompson, J.M. (2000). A research note on factors affecting the determination of myofibrillar fragmentation. *Meat Science, 56*(1), 19-22.

Jacob, R.H., Rosenvold, K., North, M., Kemp, R.M., Warner, R.D., & Geesink, G.H. (2012). Rapid tenderisation of lamb *M. longissimus* with very fast chilling depends on rapidly achieving sub-zero temperatures. *Meat Science, 92*(1), 16-23.

Jacob, R.H., & Thomson, K.L. (2012). The importance of chill rate when characterising colour change of lamb meat during retail display. *Meat Science, 90*(2), 478-484.

Jaime, I., Beltrán, J.A., Ceña, P., López-Lorenzo, P., & Roncalés, P. (1992). Tenderisation of lamb meat: Effect of rapid postmortem temperature drop on muscle conditioning and aging. *Meat Science, 32*(4), 357-366.

Janz, J.A.M., Aalhus, J.L., & Price, M.A. (2001). Blast chilling and low voltage electrical stimulation influences on bison (Bison bison bison) meat quality. *Meat Science, 57*(4), 403-411.

Janz, J.A.M., Aalhus, J.L., & Price, M.A. (2002). Meat quality of bison (*Bison bison bison*) longissimus thoracis et lumborum following very fast chilling. *Canadian Journal of Animal Science, 82*(3), 327-337.

Jeacocke, R.E. (1977). The temperature dependence of anaerobic glycolysis in beef muscle held in a linear temperature gradient. *Journal of the Science of Food and Agriculture, 28*(6), 551-556.

Jeacocke, R.E. (1986). The mechanism of cold shortening. In I. I. o. Refrigeration (Ed.), *Recent advances and developments in the refrigeration of meat by chilling: Meeting of IIR Commission C2* (Vol. Section 1, pp. 39-43). Bristol, UK: International Institute of Refrigeration.

Jolley, P.D., Honikel, K.O., & Hamm, R. (1981). Influence of temperature on the rate of post-mortem metabolism and water-holding capacity of bovine neck muscles. *Meat Science, 5*(2), 99-107.

Joseph, R.L. (1996). Very fast chilling of beef and tenderness. A report from an EU concerted action. *Meat Science, 43*(Supplement 1), 217-227.

Kahraman, T., Issa, G., Bingol, E.B., Dumen, E., Goksoy, E.O., & Buyukunal, S.K. (2014). Effect of rapid chilling and pelvic suspension on meat quality of *longissimus dorsi* muscle of lamb. *Kafkas Universitesi Veteriner Fakultesi Dergisi, 20*(5), 697-701.

Karumendu, L.U., Van de Ven, R., Kerr, M.J., Lanza, M., & Hopkins, D. L. (2009). Particle size analysis of lamb meat: Effect of homogenization speed, comparison with myofibrillar fragmentation index and its relationship with shear force. *Meat Science, 82*, 425-431.

Krzywicki, K. (1979). Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. *Meat Science, 3*(1), 1-10.

Lawrie, R.A., & Ledward, D.A. (2006). *Meat Science*. Cambridge, England: Woodhead Publishing.

Li, K., Zhang, Y., Mao, Y., Cornforth, D., Dong, P., Wang, R., Zhu, H., & Luo, X. (2012). Effect of very fast chilling and aging time on ultra-structure and meat quality characteristics of Chinese Yellow cattle M. Longissimus lumborum. *Meat Science, 92*(4), 795-804.

Locker, R.H. (1960). Degree of muscular contraction as a factor in tenderness of beef. *Journal of Food Science, 25*(2), 304-307.

Locker, R.H., & Hagyard, C.J. (1963). A cold shortening effect in beef muscles. *Journal of the Science of Food and Agriculture, 14*, 787-793.

MacDougall, D.B. (1982). Changes in the colour and opacity of meat. *Food Chemistry, 9*(1), 75-88.

Marsh, B.B., Cassens, R.G., Kauffman, R.G., & Briskey, E.J. (1972). Hot boning and pork tenderness. *Journal of Food Science, 37*(1), 179-180.

Marsh, B.B., & Leet, N.G. (1966). Studies in meat tenderness. III. The effects of cold shortening on tenderness. *Journal of Food Science, 31*(3), 450-459.

Marsh, B.B., Woodhams, P.R., & Leet, N.G. (1968). Studies in meat tenderness. 5. The effects of tendnerness of carcass cooling and freezing before the completion of rigor mortis. *Journal of Food Science, 33*(1), 12-18.

Nakai, Y., Nishimura, T., Shimizu, M., & Arai, S. (1995). Effects of freezing on the proteolysis of beef during storage at 4°C. *Bioscience Biotechnology and Biochemistry, 59*(12), 2255-2258.

Redmond, G.A., McGeehin, B., Sheridan, J.J., & Butler, F. (2001). The effect of ultra-rapid chilling and subsequent ageing on the calpain/calpastatin system and myofibrillar degradation in lamb *M. longissimus thoracis et lumborum*. *Meat Science, 59*, 293-301.

Renerre, M. (1990). Factors involved in the discoloration of beef meat. *International Journal of Food Science & Technology, 25*(6), 613-630.

Roncales, P. (1998). Very fast chilling of beef: Muscle to meat. An introductory review. In E. Dransfield, P. Roncales, R. L. Joseph & D. J. Troy (Eds.), *Concerted Action CT94-1881 Very Fast Chilling in Beef* (Vol. 2. Muscle to Meat, pp. 1-6). Bristol, UK: University of Bristol.

Sammel, L.M., Hunt, M.C., Kropf, D.H., Hachmeister, K.A., Kastner, C.L., & Johnson, D.E. (2002). Influence of chemical characteristics of beef inside and outside semimembranosus on color traits. *Journal of Food Science, 67*(4), 1323-1330.

Sheridan, J.J. (1990). The ultra-rapid chilling of lamb carcasses. *Meat Science, 28*(1), 31-50.

Sheridan, J.J., McGeehin, B., & Butler, F. (1998). Effects of ultra-rapid chilling and electrical stimulation on the tenderness of lamb carcass muscles. *Journal of Muscle Foods, 9*(4), 403-417.

Van Moeseke, W., De Smet, S., Claeys, E., & Demeyer, D. (2001). Very fast chilling of beef: effects on meat quality. *Meat Science, 59*(1), 31-37.

Warner, K.F. (1928). Progress report of the mechanical test for tenderness of meat. *Proceedings of the American Society for Animal Products, 21*, 114-116.

Warner, R.D., Jacob, R.H., Rosenvold, K., Rochfort, S., Trenerry, C., Plozza, T., & McDonagh, M.B. (2015). Altered post-mortem metabolism identified in very fast chilled lamb *M. longissimus thoracis et lumborum* using metabolomic analysis. *Meat Science, 108*(0), 155-164.

Winger, R.J., Fennema, O., & Marsh, B.B. (1979). Rate of pH decline in beef muscle stored at above- and below-freezing temperatures. *Journal of Food Science, 44*(6), 1681-1685.

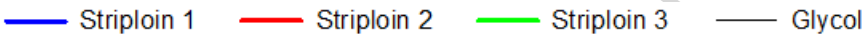
Young, O.A., Priolo, A., Simmons, N.J., & West, J. (1999). Effects of rigor attainment temperature on meat blooming and colour on display. *Meat Science, 52*(1), 47-56.

Zhu, L., Gao, S., & Luo, X. (2011). Rapid chilling has no detrimental effect on the tenderness of low-voltage electrically stimulated *M. longissimus* in Chinese bulls. *Meat Science, 88*(3), 597-601.

Legend to figures

Fig. 1 A. Data from the preliminary trial showing actual (measured) and predicted chilling rates (from Food Product Modeller) at the centre of a 66 mm thick beef striploin muscle, suspended in a water bath set to -20°C (45% propylene glycol).

B. Typical delayed chilling (DC) temperature-time profile at the centre of a 60 mm (approximately) beef striploin, suspended in a water bath set to -20°C (45% propylene glycol), after conditioning at 15°C.



C. Typical immediate chilling (IC) temperature-time profile at the centre of a 60 mm (approximately) beef striploin, suspended in a water bath set to -20°C (45% propylene glycol); initial muscle temperature was around 30°C.

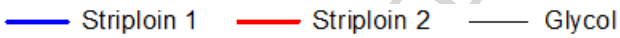
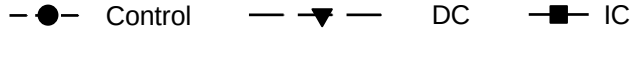


Fig. 2 The effect of chilling treatments (Control, conventionally chilled; DC, delayed chilling, hot-boned muscle held at 15°C prior to chilling; and IC, immediate chilling, hot-boned muscle chilled in glycol water bath at -20°C) and time post-mortem (0.8, 1,1. 3.1, 4.3 and 24 h) on the pH of beef striploins. Values are predicted means with the l.s.d. bar representing the least significant difference (P<0.05) for comparing means for the interaction of treatment and time.

Fig. 3 The effect of chilling treatments (Control, conventionally chilled; DC, delayed chilling, hot-boned muscle held at 15°C prior to chilling; and IC, immediate chilling, hot-boned muscle chilled in glycol water bath at -20°C) and time post-mortem (2, 5,

14 d) on the pH of beef striploins. Values are predicted means with the l.s.d. bar representing the least significant difference (P<0.05) for comparing means.

Fig. 4 Effect of chilling treatments (Control, conventionally chilled; DC, delayed chilling, hot-boned muscle held at 15°C prior to chilling; and IC, immediate chilling, hot-boned muscle chilled in glycol water bath at -20°C) and time post-mortem (2, 5, 14 d) on texture (PF, peak force) of beef striploin. Values are predicted means with the l.s.d. bar representing the least significant difference (P<0.05) for comparing



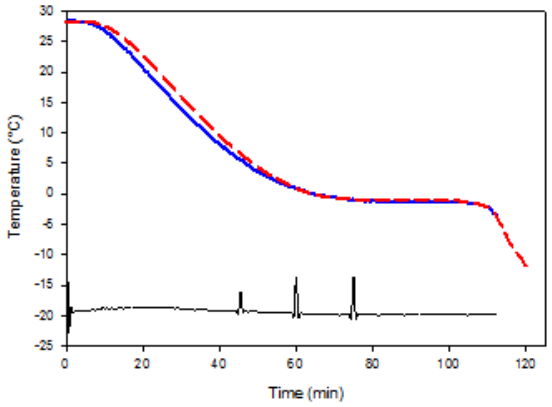
means.

Fig. 5 Light microscopy images of beef striploin samples after chilling treatments. Control – conventionally chilled; DC, delayed chilling – hot-boned muscle held at 15°C prior to very fast chilling; IC, immediate chilling – hot-boned muscle chilled in glycol water bath at -20°C. Left hand images, longitudinal sections; Right hand images, transverse sections. A. Control, 2 d post-mortem; B. DC, 2 d post-mortem; C. IC, 2 d post-mortem; D. Control, 14 d post-mortem; E. DC, 14 d post-mortem; F. IC, 14 d post-mortem. Scale bar = 100 μm.

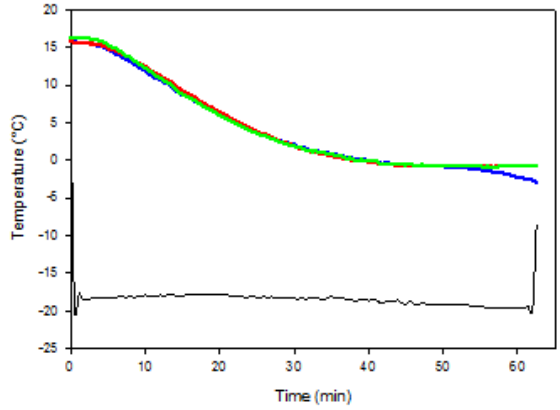
Fig. 6 Transmission electron microscopy (TEM) images of beef striploin samples after chilling treatments. Control – conventionally chilled; DC, delayed chilling – hot-boned muscle held at 15°C prior to very fast chilling; IC, immediate chilling – hot-boned muscle chilled in glycol water bath at -20°C. Left hand images, longitudinal sections scale bar = 200 nm, right hand images, transverse sections scale bar = 3 μm. A. Control, 2 d post-mortem; B. Control, 14 d post-mortem; C. DC, 2 d post-mortem; D. DC, 14 d post-mortem; E. IC, 2 d post-mortem; F. IC, 14 d post-mortem. S, sarcomere; A, A-band; I, I-band; Z, Z-line.

Fig. 7 The effect of chilling treatments (Control, conventionally chilled; DC, delayed chilling, hot-boned muscle held at 15°C prior to chilling; and IC, immediate chilling, hot-boned muscle chilled in glycol water bath at -20°C) and time post-mortem (2, 5, 14 d) on mean particle size (μm) of beef striploin samples. Values are predicted means with the l.s.d. bar representing the least significant difference (P<0.05) for comparing means.

A



B



C

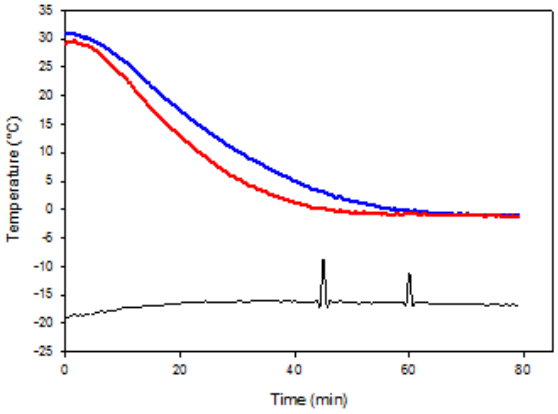


Figure 1

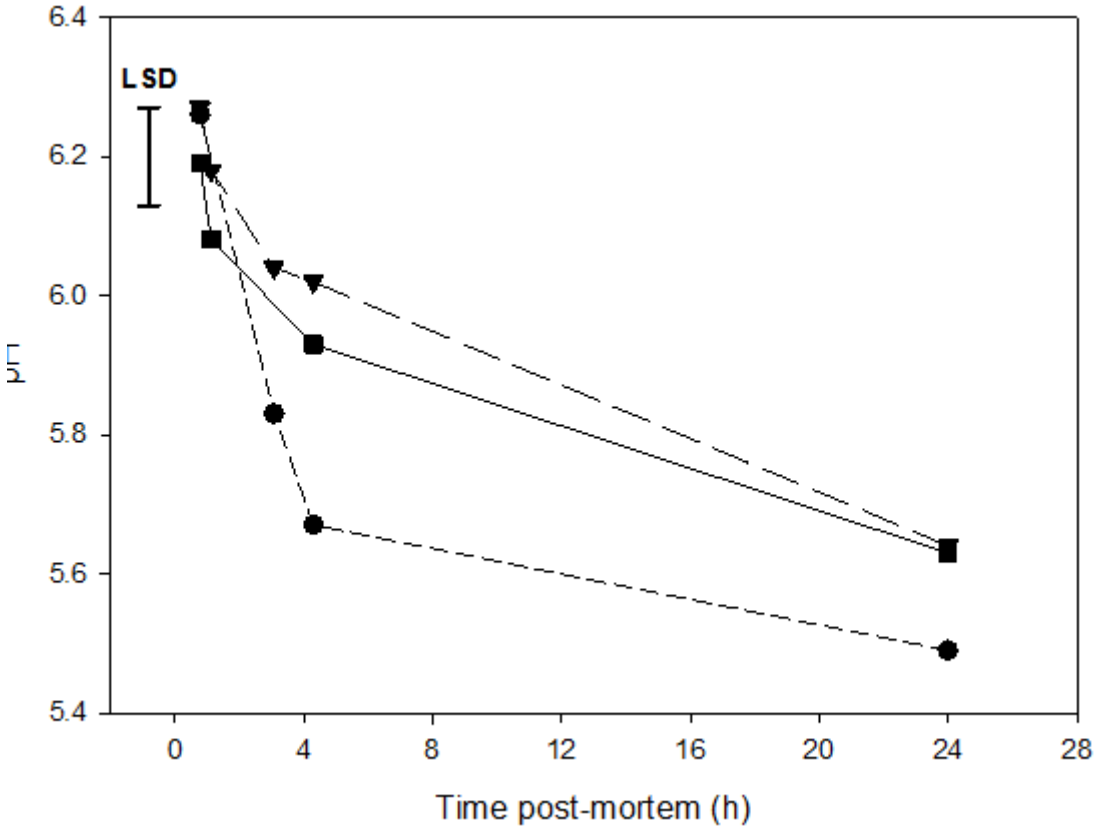


Figure 2

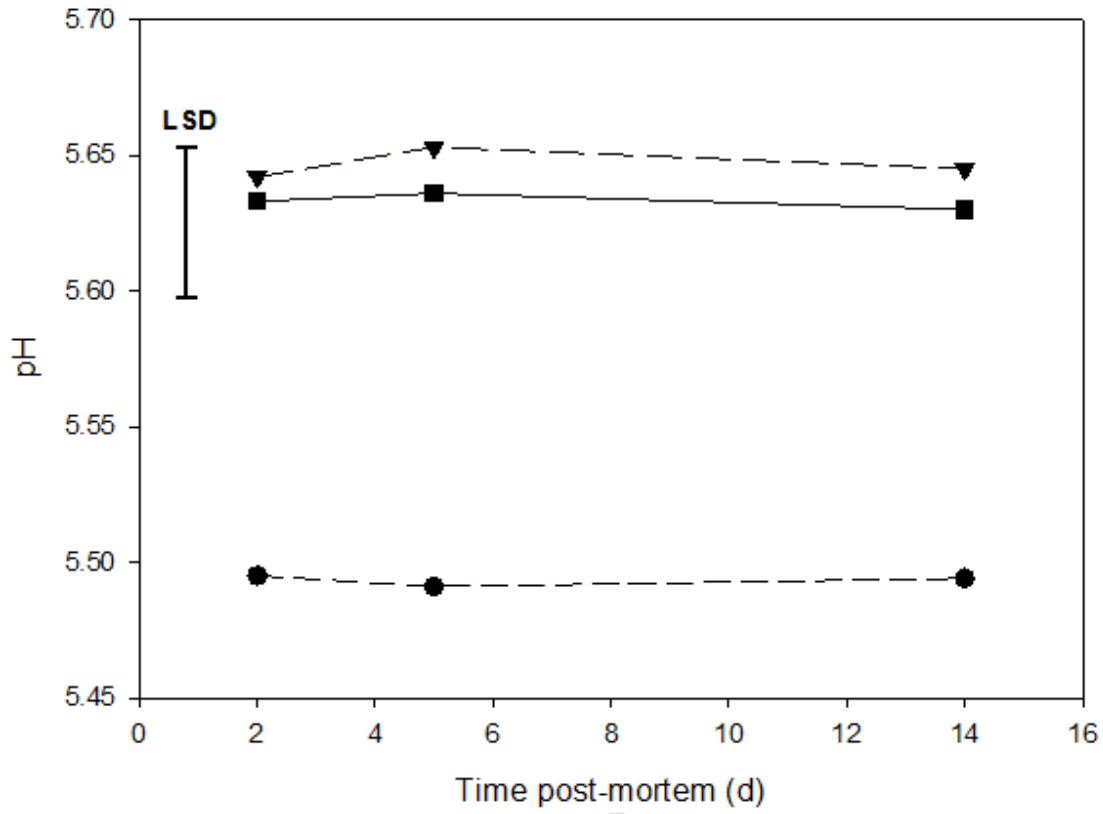


Figure 3

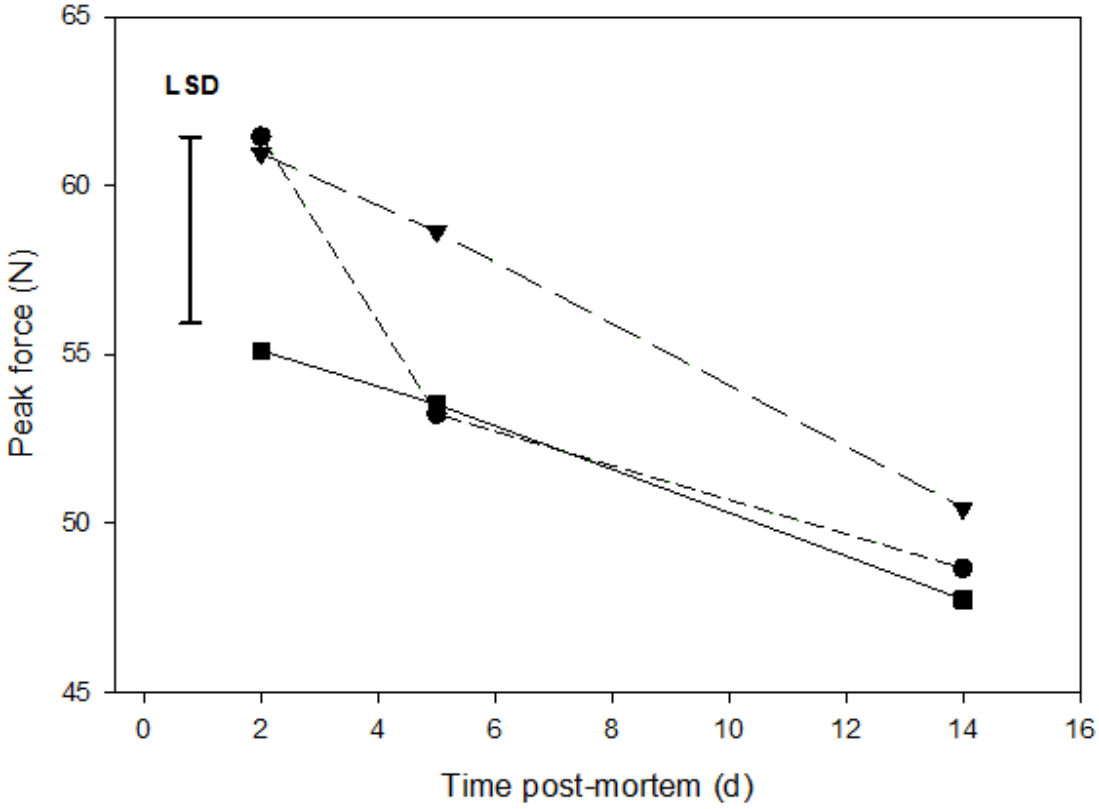


Figure 4

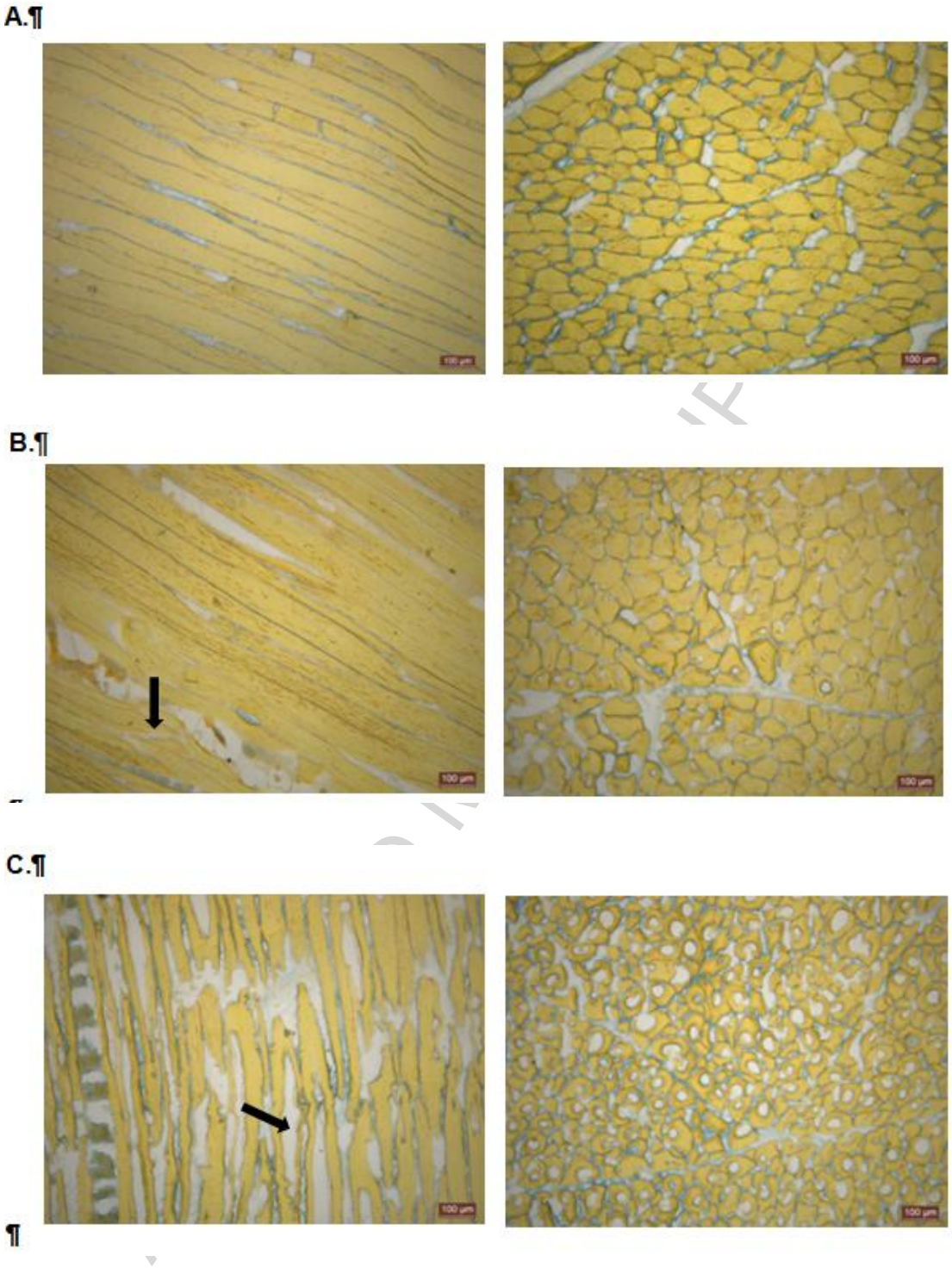


Figure 5

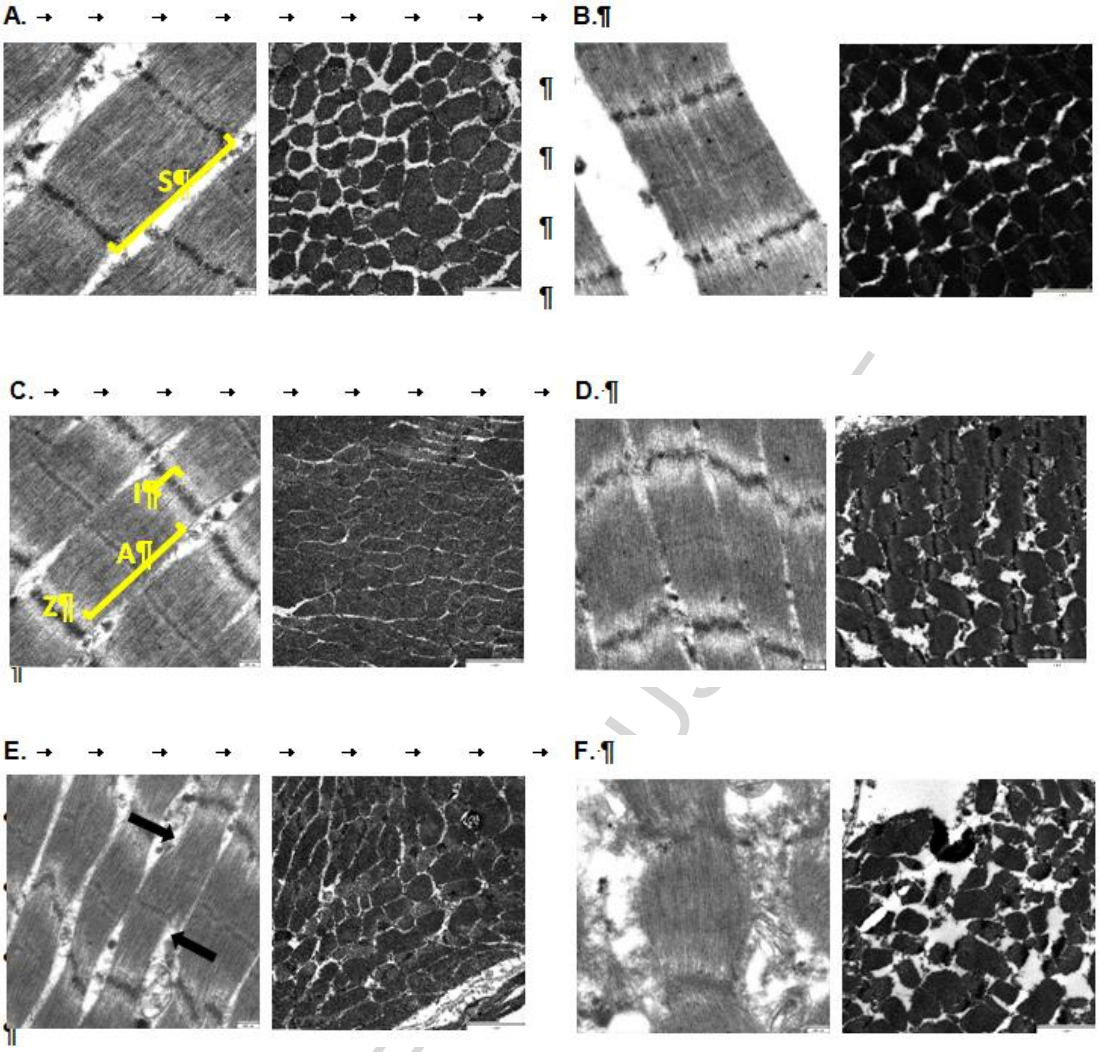


Figure 6

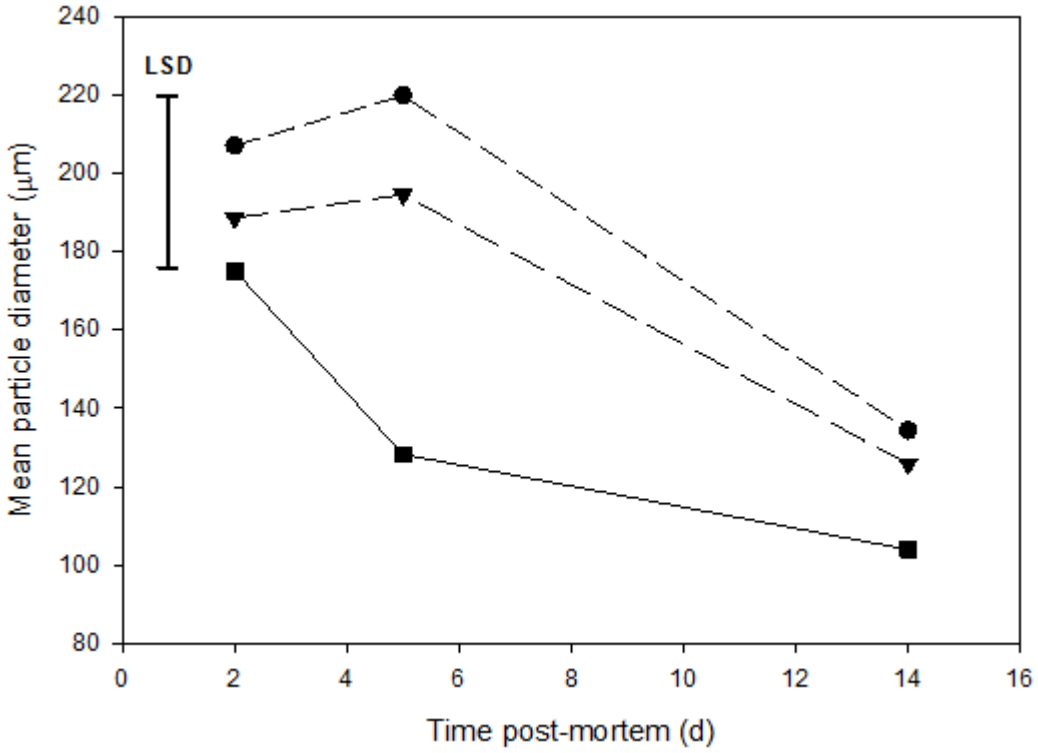


Figure 7

Tables

Table 1: Definition of control and chilling treatments.

Treatment	Conditions
Control	Conventionally chilled through normal processing
Delayed chilling (DC)	Hot-boned muscle placed in 15°C water bath until centre reached 15°C; then placed in -20°C glycol water bath until internal temperature of < -1°C
Immediate chilling (IC)	Hot-boned muscle placed in -20°C glycol water bath until internal temperature reached < -1°C

Table 2: Description of method of pH measurement for each treatment at each time point.

Time post-mortem (h)	Treatment		
	Control	**Delayed chilling**	**Immediate chilling**
0.78	probe[1]	probe	probe
1.13	nm[2]	probe	probe
3.08	probe	probe, homogenised[3]	nm
4.30	probe	homogenised	homogenised
24	probe	homogenised	homogenised

[1] probe – pH measured with a digital pH meter fitted with a spear tip electrode.
[2] nm - not measured.
[3] homogenised – frozen subsample homogenised in iodoacetate solution, pH 7 and pH of homogenate measured with a digital pH meter.

Table 3: Effect of chilling treatment (Treatment, T; Control, conventionally chilled; delayed chilling, DC, conditioned at 15°C prior to chilling at -20°C; immediate chilling, IC, chilled at -20°C) and time post-mortem (Ti pm; 2, 5 and 14 days) on meat quality attributes (PF, peak force; IY, initial yield; PF-IY, peak force minus initial yield; sarcomere length; drip loss and cook loss) of beef striploin. Values are least squares means and the average l.s.d. (least squares difference at $P<0.05$) is given.

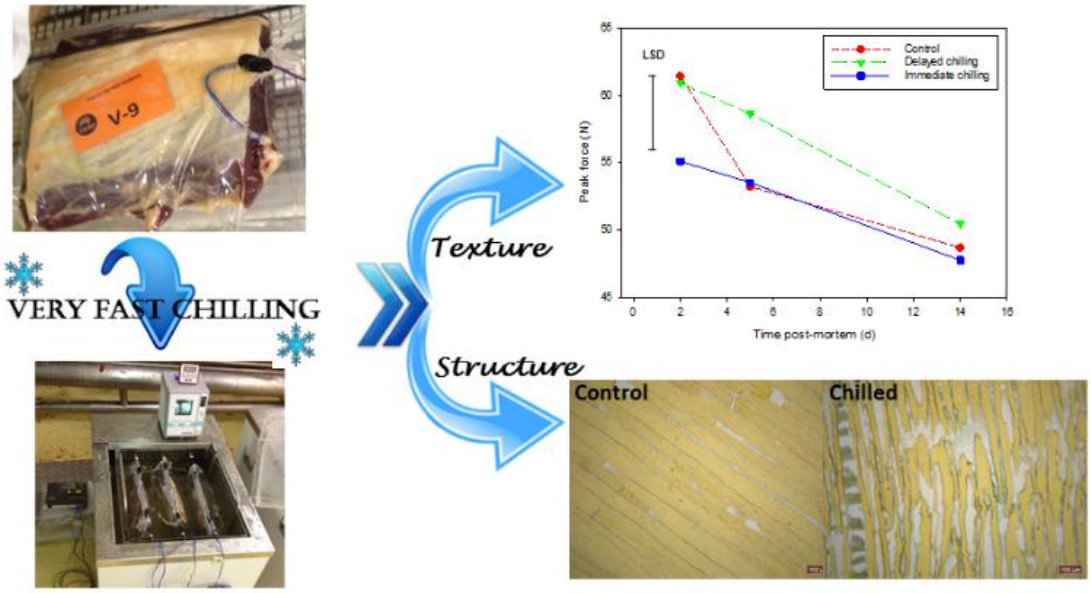
Attribute	Treatment	Ageing times (d)			*P*-value			l.s.d. (avg)
		2	5	14	T	Ti pm	T*Ti pm	(*P*=0.05)
PF (N)	Control	61.45	53.23	48.68	0.051	<0.001	0.336	5.516
	DC	60.95	58.65	50.46				
	IC	55.12	53.53	47.76				
IY (N)	Control	53.84	44.27	35.20	0.241	<0.001	0.069	5.388
	DC	51.74	49.43	42.77				
	IC	50.55	47.15	39.53				
PF-IY (N)	Control	8.12	9.46	13.98	0.006	0.040	0.061	3.680
	DC	9.05	9.05	7.53				
	IC	5.11	6.98	8.76				
Sarcomere length (μm)	Control	2.13	2.11	1.88	0.255	0.031	0.050	0.145
	DC	1.99	1.94	2.01				
	IC	2.02	2.01	1.92				
Drip loss (%)	Control	0.237	0.522	1.154	<0.001	<0.001	<0.001	0.1660
	DC	0.042	0.043	0.075				
	IC	0.027	0.020	0.022				

Cook loss (%)	Control	25.50	25.35	25.47	0.003	0.240	0.795	1.822
	DC	23.09	23.70	24.31				
	IC	22.90	22.97	24.15				

Table 4: Effect of chilling treatment (Treatment, T; Control, conventionally chilled; delayed chilling, DC, conditioned at 15°C prior to chilling at -20°C; immediate chilling, IC, chilled at -20°C) and time post-mortem (Ti pm; 2, 5 and 14 days) on surface colour attributes (L^*, lightness; a^*, redness; b^*, yellowness; Redness, R630/R580 ratio) of beef *M. longissimus lumborum* (LL) measured after 60 min blooming at 5°C. Values are least squares means and the average l.s.d. (least squares difference at $P<0.05$) is given.

Attribute	Treatment	Time post-mortem (d)		*P*-value			l.s.d. (avg)
		2	14	T	A	T*A	(*P*=0.05)
L*	Control	34.8	37.8	0.091	0.002	0.706	2.63
	DC	32.6	34.3				
	IC	34.1	36.2				
a*	Control	28.4	31.5	<0.001	0.001	0.538	2.13
	DC	25.7	27.4				
	IC	26.5	28.2				
b*	Control	21.1	23.2	<0.001	0.021	0.377	1.69
	DC	18.5	19.3				
	IC	19.6	20.3				
Redness	Control	7.78	8.08	0.024	0.999	0.765	1.074
	DC	7.07	6.99				

	IC	7.03	6.81		



Graphical abstract

Highlights

- Beef striploins were treated with very fast chilling (VFC) conditions and assessed for textural and structural modifications
- VFC of hot-boned striploin resulted in lower shear force values at 2 d post-mortem
- VFC improved WHC of hot-boned striploins
- VFC affected muscle structure
- A different rate and extent of pH decline was observed in VFC striploins