THE RATE OF VERY FAST CHILLING ALTERS THE POST-MORTEM TENDERISATION OF BEEF *LONGISSIMUS LUMBORUM*

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

[1]CSIRO Agriculture and Food, Coopers Plains, QLD, Australia

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

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

[4]Faculty of Veterinary and Agricultural Science, The University of Melbourne, Parkville, VIC, Australia

*Corresponding author email: anita.sikes@csiro.au

Abstract – A study was undertaken to investigate the effect of chilling medium on tenderisation of beef loins chilled with a very fast chilling (VFC) regime. Hot-boned muscles from 16 carcasses were allocated to: control (held at 15°C for 24 h); dry ice (-78.5°C for 50 min); and liquid immersion bath (-20°C for 35 min). Liquid immersion chilling resulted in more tender meat (peak force) compared to the control, whereas dry ice chilled muscle was tougher. There was no effect of either chilling treatment on sarcomere length or cook loss. TEM showed that control samples had regular Z-line alignment and dry ice chilled samples had larger spaces between fibrils than controls. Liquid immersion chilled samples had misaligned and fragmented fibrils. Both chilling treatments resulted in a slower pH decline than the control. These results suggest that the chilling media influenced the chilling rate and subsequent tenderisation of beef loin muscle.

Key Words – beef, pH decline, structure, tenderness.

I. INTRODUCTION

The chilling of carcasses after slaughter is critical when considering meat quality. Rate of chilling influences factors such as sarcomere length, proteolytic activity and glycolysis, and is therefore important for textural outcomes. Chilling is also important to achieve a desirable shelf-life, as temperature influences the growth rate and genus of microbial populations. Very fast chilling (VFC) has potential economic benefits compared to conventional chilling but variable meat quality results have been reported [1] and hindered industry adoption. The rate of VFC is defined as a muscle core temperature reaching -2°C to 0°C within 5 h of slaughter [1]. Several studies have demonstrated that achieving the temperature and time specifications within the VFC regime are critical for accelerating tenderisation early post-mortem (PM) [2, 3]. However, factors such as the prevention of muscle shortening due to crust freezing, the modification of metabolism (rate of pH decline) and structural modifications have to be considered. The aim of this study was to determine if different chilling media (dry ice and liquid immersion bath) influence the chilling rate and the texture of beef muscle.

II. MATERIALS AND METHODS

Pre-rigor *M. longissimus lumborum* (LL) muscles were collected from 16 beef carcasses on 8 slaughter dates from 14 mobs of animals. The fat layer and cap muscle (*M. spinalis dorsi*) were removed. Each muscle was cut into 3 portions (40x90x100 mm) and randomly allocated to one of 3 treatments: control, wrapped in cling wrap and held in an incubator at 15°C for ~24 h; dry ice chilling, vacuum packed, placed in dry ice for 50 min; and liquid immersion chilling, vacuum packed, immersed in 45% propylene glycol in water (v/v) at -20°C for 35 min. Preliminary work determined these times for each chilling method, based on the muscle core reaching a temperature at the middle of the freezing plateau (~ -1 to -1.5°C) (Fig. 1). Muscles were collected within 40 min of slaughter and subjected to both chilling treatments within 60 min of slaughter. Subsamples (~1 g) were taken for pH measurement at 0.25, 1, 3, 6 and ≥24 h PM, immediately frozen in liquid nitrogen and stored at -80°C for subsequent analysis. After chilling treatments, muscle samples were stored at 5°C for 24 h prior to analysis for sarcomere length (µm), texture (peak force, PF, [N]), cook loss (%), and structural analysis (microscopy). Muscle pH was measured using the iodoacetate homogenisation method [4]. Sarcomere lengths were measured using the laser diffraction technique [5]. Texture (PF) was assessed after cooking to an internal temperature of 72°C using the modified Warner-Bratzler shear force method [6, 7]. Cooking loss was determined from the weights before and after cooking and expressed as a percentage of the initial weight. Subsamples (~10x15x15 mm) from all treatments from one animal were selected for transmission electron microscopy (TEM) analysis to visualise any structural modifications. Samples were prepared and viewed as described by Sikes *et al.* [3]. One way analysis of

variance (ANOVA) was carried out using treatment as the main effect and a block structure of Day/Mob/Animal for sarcomere length, peak force and cook loss. For pH, the interaction between treatment and time PM was also analysed using one way ANOVA.

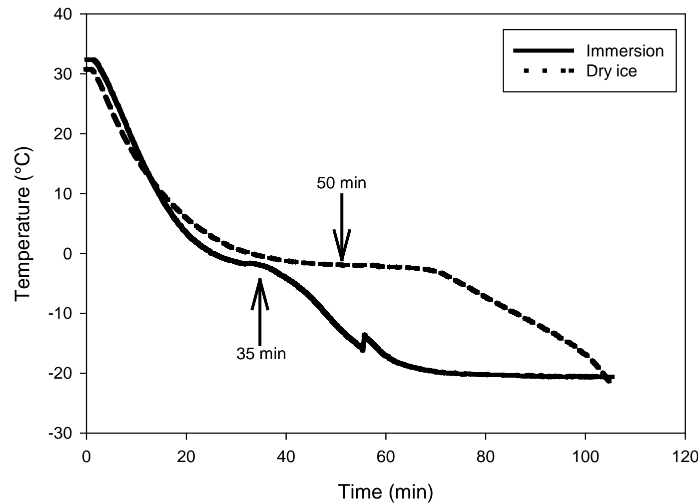

Figure 1. Temperature-time profiles identifying the chilling times using the two chilling media, liquid immersion chilling (immersion) and dry ice chilling (dry ice).

III. RESULTS AND DISCUSSION

Mean sarcomere lengths of LL muscles ranged from 1.8–1.9 µm, with no effect of chilling treatment (*P*>0.05) (Table 1). Thus, there was no indication of any cold shortening in the samples. This was possibly due to the formation of a frozen crust on the surface of the chilled muscle that prevents shortening of muscle fibres [8].

Table 1 Meat quality attributes at 24 h PM of beef *longissimus lumborum* muscle after different chilling methods. Values are ANOVA means and the least significant differences (LSD) and *P*-values from comparison tests are given (n=16).

Attribute	Control	Dry ice [A]	Liquid immersion [B]	*P*-value	LSD
Sarcomere length (µm)	1.82	1.91	1.85	0.329	0.127
PF (N)	78.6	90.6	67.9	<0.001	5.43
Cook loss (%)	21.8	18.8	21.2	0.253	3.84

[A] chilled for 50 min to reach core temperature of -1°C.
[B] chilled for 35 min to reach core temperature of -1°C.

Both chilling treatments reached pH 6 later than the non-chilled treatment (Fig. 2). Interestingly, at 1 h PM, there was a slight increase in pH in both of the chilling treatments, as reported by Jacob et al. [2], before the pH continued to decline with time. The pH of the liquid immersion chilled sample (6.47±0.051 sed) was higher (*P*=0.037) than the control (6.33±0.051 sed) at 1 h PM. The pH of samples from both chilling treatments was higher (*P*=0.037) at 3 and 6 h PM than the control samples, however there were no differences between any of the treatments at 24 h; the mean pH for all treatments was 5.7–5.8 (Fig. 2).

Enzyme activity reduces in the temperature range of 40 to 0°C [9]. However, at temperatures in the range of 0 to -3°C, 'freeze concentration' of solutes increases reaction rates due to an increase in substrate concentration and increases pH decline in this temperature range [10]. In this study, the pH at 3 and 6 h PM was higher in both fast chilling treatments compared to the control, indicating a slower rate of pH decline in the fast chilled treatments at these times PM. This is consistent with other VFC studies where the temperature was lowered to subzero temperatures [3, 11]. An accelerated pH fall at temperatures below 6–8°C has been associated with cold shortening of muscle with conventional

cooling rates [12, 13] due to a reduction in the concentration of adenosine triphosphate (ATP). So in the absence of cold shortening, the pH decline could be expected to accelerate relative to the control, as was observed.

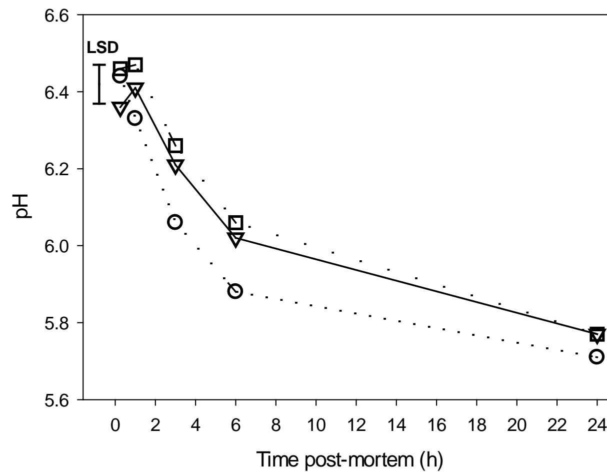

Figure 2. Effect of treatment on pH decline of beef *M. longissimus lumborum*. Control (circles), dry ice chilling (triangles), liquid immersion chilling (squares). Values are means with the LSD bar representing the least significant difference ($P<0.05$) for comparing means for the interaction of treatment and time.

Chilling using dry ice produced very tough meat (PF values, Table 1), with a PF value of 91 N. This was tougher (higher PF value, $P<0.001$) than both the control (79 N) and the liquid immersion chilled LL muscles (68 N). The liquid immersion chilled LL muscles were more tender ($P<0.001$) than the control samples. The cook losses for the LL samples were similar (~19–22%, $P>0.05$), regardless of treatment. The toughening in the dry ice chilled samples was not due to cold shortening as sarcomere lengths were similar for all treatments, including the control. Also, the change in pH over time was similar for fast chilled treatments. Therefore, any differences in texture would have been related to structural changes observed between treatments.

In the TEM images of the control samples, the fibrils were straight and parallel and separated from each other by narrow spaces. The sarcomeres were clearly bordered by Z-lines (Fig. 3A). Fibrils in muscles exposed to dry ice chilling were separated from each other by larger spaces than in the control sample (Fig. 3B). Shrinkage appears to have occurred in some fibrils. This pattern possibly results from water expanding due to phase change between the fibre bundles. At some locations, the fibrils were torn by longitudinal fissures (arrow) and elongated throughout the sarcomeres; however, the structural elements of the sarcomeres were clearly recognisable. In electron micrographs from muscles subjected to liquid immersion chilling, the fibrils were positioned parallel to each other and close together; they were occasionally separated by small gaps (Fig. 3C). Misalignment of the sarcomeres was evident and the banding was less regular. Longitudinal fissures were seen along some fibres and fragmentation along the fibre was also evident (arrows).

The mode of fast chilling (dry ice or liquid immersion), and hence the rate of chilling, therefore had an impact on the extent of modification to the muscle structure. Although the temperature of the medium in the liquid immersion bath was set at -20°C and the surface temperature of dry ice was -78.5°C, the conduction and heat transfer was faster in the liquid immersion bath as it needed less time to reach the freezing plateau (Fig. 1) [14].

IV. CONCLUSION

This VFC study showed that LL muscle chilled by immersion in a water bath resulted in tender meat at 24 h PM. However, fast chilling in dry ice produced tougher meat than the control. The two methods of chilling did not result in

cold shortening and the rate of pH decline was similar. Electron microscopy indicated that the two methods of chilling affected modifications to the muscle to different extents. This suggests that structural changes are the reason why the rate of temperature reduction is important in the application of VFC for improved texture.

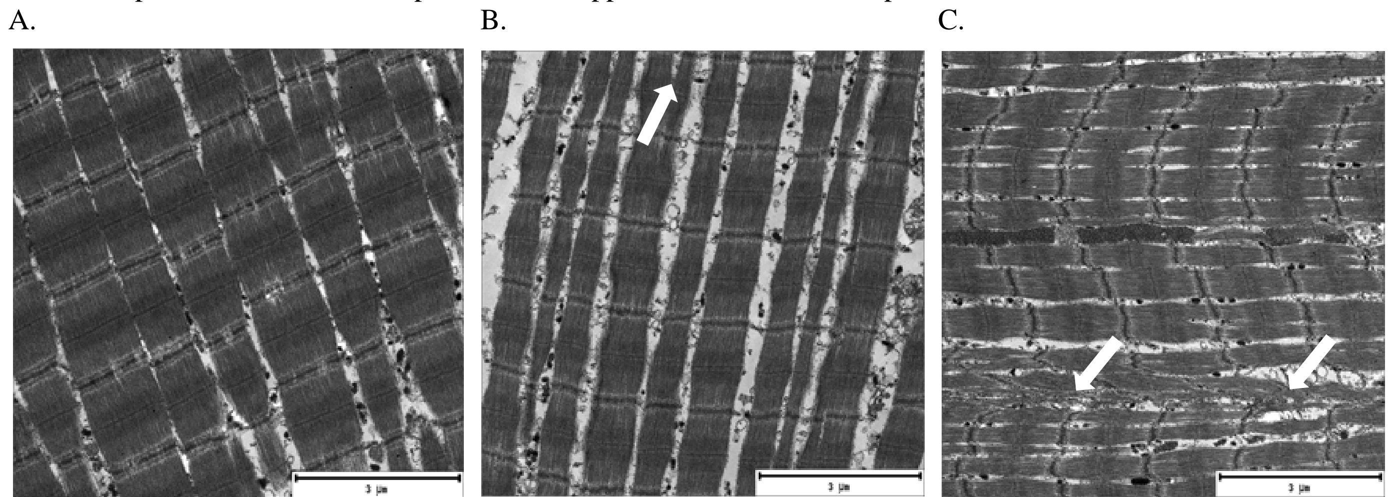

Figure 3. Transmission electron microscopy (TEM) images of beef *M. longissimus lumbrum* samples after chilling treatments. A. Control; B. dry ice chilling; C. water bath chilling. Longitudinal sections, scale bar = 3μm.

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