EFFECTS OF ELECTRICAL STIMULATION AND PRE-RIGOR CONDITIONING TEMPERATURE ON AGEING POTENTIAL OF HOT-BONED BEEF MUSCLE

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Abstract – The objective of this study was to determine the impact of 3 hrs *pre-rigor* holding temperatures and low voltage electrical stimulation (ES), on the activities of small heat shock proteins (sHSP) and μ-calpain and tenderness of bull beef. Paired loins (*M. longissimus lumborum*) from 13 bulls were hot-boned within 40 min of slaughter, immediately electrically stimulated and subjected to various holding temperatures (5°C, 15°C, 25°C and 35°C) for 3 hrs and the rate of muscle pH decline, sarcomere length, shear force, and proteolysis of muscle proteins were measured. ES-35°C and ES-25°C samples had lower shear force value at 14 days of ageing when compared to other samples. The combination of ES and *pre-rigor* holding temperature (25°C) resulted in longer sarcomere length compared to non-ES. μ-Calpain activity – as indicated by autolysis – was highest in ES-35°C samples. Desmin, troponin-T and sHSP degradation were highest for ES-35°C samples. The results of this study show that the combination of ES with 3 hrs *pre-rigor* holding temperature at 35°C improved the tenderness of bull beef.

Key Words – electrical stimulation, meat quality, *pre-rigor* holding temperature, proteolysis.

I. INTRODUCTION

Meat tenderness is one of the most important quality attributes affecting consumers' eating satisfaction and repeated purchasing decision. Varying *pre-rigor* environments generated by the application of electrical stimulation and/or *pre-rigor* chilling conditions influence the rate of glycolysis and subsequent pH decline in *post-mortem* muscles (1). The complex interaction of pH and temperature decline in *pre-rigor* muscle has a significant role in meat tenderization by influencing proteolytic enzyme activity, particularly μ-calpain (1). The combined effect of low voltage electrical stimulation (ES) with *pre-rigor* temperature conditioning at 30°C for 3 hrs *post-mortem* resulted in the fastest pH drop to 6.0 compared to at 2 and 16°C (2).

The specific role of small heat shock proteins (sHSP) in *ante mortem* cell system has been well studied (3); however, their role in *post-mortem* protein degradation and meat quality has not been fully elucidated. It was speculated in recent studies that the up-regulation of sHSP in muscle may be associated with a decrease in the ageing-potential of intermediate pHu (ultimate pH between 5.8 to 6.19) beef (3, 4). Also, intermediate pHu bull beef with the highest level of αβ-crystallin at 3 hrs *post-mortem*, had the highest shear force values throughout 1 week of ageing (5). This suggests a possible involvement of sHSP in the meat tenderization process mainly early *post-mortem*. Hence, we hypothesize that the influence of *pre-rigor* pH and temperature on ageing-potential of bull beef will depend on the presence of intact sHSP and μ-calpain activity. To our knowledge, the effects of sHSP coupled with μ-calpain under various pH/temperature conditions of early post-mortem beef have never been examined. This study was designed to determine the impact of *pre-rigor* pH/temperature on the activities of sHSP and μ-calpain and the ageing-potential of bull beef.

II. MATERIALS AND METHODS

Raw materials and processing

Thirteen bulls ~ 24-month old) were slaughtered at the AgResearch Ruakura Abattoir, Hamilton, New Zealand over five slaughter days. All the bulls were captive bolt stunned pre-slaughter. No electrical inputs (immobilization or electrical stimulation) were applied during the slaughter process in order not to confound the effect of ES. Initial pH (pH_{40min})

was recorded and the *M. longissimus lumborum* (LL) from the left side of the carcass was immediately stimulated, ES for 30 seconds after hot-boning (frequency = 14.28 Hz, pulse width = 7.5 milliseconds, peak voltage = 90 volts). Immediately after stimulation, pH was recorded once again. The LL from the right side of the carcass was not electrically stimulated (NES). Each LL was cut transversely into quarters of approximately equal sizes, each sub-sample was placed in a plastic bag and randomly subjected to one of four *pre-rigor* holding temperatures by submerged in either a 5°C, 15°C, 25°C or 35°C water baths for 3hrs. A temperature thermocouple was inserted into each loin section to monitor continuous drop in temperature. At the end of 3 hrs of holding temperature, the muscle samples were taken from the plastic bags, pH measured, and further sampled for biochemical analysis (sHSP and calpains). The samples were transferred to the AgResearch laboratory where they were vacuum packed and aged at 1°C for 24, 48 hrs, and 14 days *post-mortem*. Muscle samples for biochemical analyses and sarcomere length were taken at 24 and 48 hrs *post-mortem,* respectively.

pH

pH of the loin samples was measured in duplicate by inserting a calibrated pH probe (Hanna HI99163 pH meter with a FC232D combined pH/ temperature probe, HANNA instruments, Rhode Island, USA) directly into the muscle at 40 min (before and after stimulation), 3 hrs, 6 hrs, 24 hrs, 48 hrs and 2 weeks *post-mortem,* respectively.

Sarcomere length measurement

The 48 hrs *post-mortem* samples (approximately 1 g) for sarcomere length were chopped finely and placed into a new centrifuge tube and to this 10ml of 0.25M sucrose (85.57g/L) were added. Samples were then homogenized using the Ultra-Turrax T25 at 8000 rpm for 10 pulses. Sarcomere lengths were measured under a phase contrast microscope and images taken for analysis. Ten pictures were taken with at least 10 sarcomeres present in each picture (6).

Shear force

The loin cuts were cooked in a water bath set at 99°C to an internal temperature of 75°C (measured by 12 channel Digisense Thermocouple Thermometer). After cooling, 10 mm x 10 mm cross section samples were cut and sheared using MIRINZ Tenderometer. Ten replicates were measured for each sample. The results were expressed as shear force (kgF) (4).

Western blot

Whole muscle protein extraction, gel sample preparation and Western blotting (desmin, troponin-T, μ calpain, sHSP [αβ-crystallin sHSP20, sHSP20] and HSP70) were performed as described previously (4, 7).

Data analysis

All statistical analysis was performed using the REML directive of GenStat (8). The pH fall was analyzed using ANOVA where the animal (beef) ID and side of the carcass was included as blocking variables including all possible two-way and three-way interactions. Shear force and sarcomere length were analyzed using ANOVA with sample ID included as a blocking variable and temperature, ES and their interaction as explanatory variables. Least squares means for each attribute were separated using least significant differences (F test, $P < 0.05$).

III. RESULTS AND DISCUSSION

pH decline

ES resulted in an immediate fall in pH as expected (Table 1) probably due to accelerated glycolysis (9). Muscle pH declined soon after electrical stimulation (ΔpH 0.43 pH), and continued until the ultimate pH was reached. The rate of pH decline reached ΔpH 1.37 pH units at 220 min *post-mortem* for ES-35°C samples and continued to be low when compared to the rest of the samples. This observation corroborates earlier findings (2).

Sarcomere length

Sarcomere length measured at 48 hrs *post-mortem* was significantly influenced by both electrical stimulation and *pre-rigor* holding temperature (Table 2). Sarcomere length was longer for ES-25°C and ES-35°C samples compared to the other samples ($P < 0.05$). The 5°C samples cold shortened probably due to rapid temperature decline *pre-rigor* (10). Also, these samples had higher shear force values (Table 2) and pH_{3h}.

Similar findings were reported (11) in which strong associations between sarcomere length and shear force values were found.

Shear force

The ES treatment applied to the hot-boned loin samples resulted in lower shear values at 48 hrs *post-mortem*, when compared to the non-stimulated samples (Table 2). The 3 hrs *pre-rigor* holding temperature also had significant effect on the shear force values, where ES-35°C samples had most tender meat when compared to the other ES and NES counterparts. A similar trend was observed in the shear force values at 14 days *post-mortem*, where ES samples were significantly tender when compared to the NES samples. Similarly, ES-35°C and ES-25°C samples were significantly lower in the shear force values when compared to the other samples. This outcome is explained by the faster myofibrillar protein and sHSP degradation and the earlier activation of proteolytic enzyme such as μ-calpain (presence of high 78 kDa), lesser expression of sHSP coupled with lesser expression of intact myofibrillar proteins (desmin and troponin-T) and longer sarcomere length, which altogether could have resulted in the lower shear force values in ES-35°C and ES-25°C samples when compared to the other samples (3, 5).

Table 1 Effect of ES and *pre-rigor* holding temperature (for 3hrs) on rate of pH fall of bull beef LL samples.

	pH fall (average)							
Time post mortem	ES				NES			
	5°C	15°C	25°C	35°C	5°C	15°C	25°C	35°C
before ES	7.1	7.1	7.1	7.1	7.2	7.2	7.2	7.2
after ES	6.6	6.6	6.6	6.6	7.2	7.2	7.2	7.2
3 hrs	6.5	6.4	6.1	5.7	7.1	7.0	6.9	6.2
6 hrs	6.3	6.2	5.9	5.6	6.8	6.7	6.8	5.8
24 hrs	5.8	5.8	5.8	5.6	6.2	6.0	6.0	5.7
48 hrs	5.6	5.6	5.7	5.6	5.7	5.6	5.6	5.6
14 days	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5

ES – electrical stimulation; NES: non electrical stimulation, n=13; overall SED = 0.06. Treatment effect [ES vs NES] (P < 0.001); temperature effect (P < 0.001); time effect (P < 0.001); Interaction effects [Treatment.Temperature (P = 0.22)]; [Treatment.Time (P < 0.001)] [Temperature.Time (P < 0.001)] [Treatment.Temperature.Time (P = 0.52)].

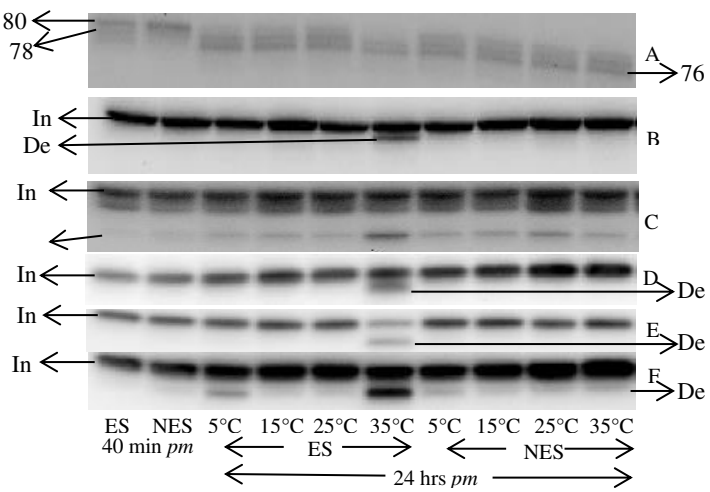

Figure1. Representative Western blot depicting (A) μ-calpain, (B) desmin, (C) troponin-t, (D) αβ-crystallin (E) sHSP20, (F) sHSP27 of whole muscle extraction of the beef loins. ES = electrical stimulation, NES = Non electrical stimulation, *pm* = *post-mortem*, 80 = 80kDa, 78 = 78kDa, 76 = 76kDa, In = intact bands, De = degraded bands.

Table 2 Effect of ES and *pre-rigor* holding temperature (for 3hrs) methods on shear force and sarcomere length of beef LL samples.

Trait	ES				NES			
	5°C	15°C	25°C	35°C	5°C	15°C	25°C	35°C
SF[A]	21.4	18	15.3	12.6	26.7	26.2	23.4	20.2
SF[B]	17.1	14	9.6	8.7	24.2	19.4	18.4	16.3
SL[C]	1.3	1.4	1.7	1.7	1.2	1.4	1.5	1.6

ES – electrical stimulation; NES – non electrical stimulation, n=13. [A]Shear force (48 hrs); Treatment effect [ES vs NES] (P < 0.001); temperature effect (P = 0.001); Interaction effect (P = 0.30, SED = 1.24). [B]Shear force (2 weeks). Treatment effect [ES vs NES] (P < 0.001); temperature effect (P < 0.001); Interaction effect (P = 0.13, SED = 1.00). [C]Sarcomere length; Treatment effect [ES vs NES] (P < 0.001); temperature effect (P < 0.001); Interaction effect (P = 0.01, SED = 0.04).

Myofibrillar protein and sHSP analysis

Pre-rigor temperature (35°C) and ES influenced the μ-calpain autolysis, desmin, troponin-T, αβ-crystallin, HSP20, HSP27 and HSP70 degradation based on the qualitative image analysis of the Western blot (data not shown). ES-35°C samples (at 24 hrs *post-mortem*) showed no 80kDa μ-calpain subunit, but only intermediate (78 kDa) and fully autolyzed (76 kDa) μ-calpain sub-units indicating that calpain was activated in a faster rate when compared to the other treatments (Figure 1).

Similarly, ES-35°C samples had less intact myofibrillar proteins and more degraded proteins (desmin and troponin-T) and less intact and more degraded sHSP (αβ-crystallin, HSP20, and HSP27) when compared to other samples (Figure 1). The extent of desmin and troponin-T degradation is well-known as an indicator of meat tenderization (12). This outcome is in agreement with a recent publication (4) in which a significant correlation between the degradation of sHSP and myofibrillar proteins in beef samples (degraded sHSP27 and degraded desmin) were found (3, 4).

IV. CONCLUSION

The outcome of this study indicates holding *pre-rigor* bovine muscle at 35°C for 3 hrs *post-mortem* resulted in early activation of μ-calpain, prevented sarcomere shortening, and degraded sHSP and myofibrillar proteins. In conclusion, this study demonstrated that holding *pre-rigor* bovine muscle at ES-25°C and ES-35°C for 3 hrs *post-mortem* accelerated the ageing of beef compared to at 5°C and 15°C. Further research studies are required to validate these findings in the commercial setting, where other electrical inputs are used in stunning and immobilization.

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