EARLY ACTIVATION OF μ-CALPAIN COULD LIMIT AGEING POTENTIAL OF OVINE *M. LONGISSIMUS*

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Abstract – The objectives of this study were to determine effects of the slaughter processing and different ageing/freezing storage conditions on biochemical and quality attributes of lamb loins. Forty-eight lamb carcasses from two plants (A and B) were monitored for temperature and pH decline for the 24 hours *post mortem*. Then, both loins (*M. longissimus*) were excised from the carcasses and were randomly allocated to four different ageing (-1.5°C) and/or freezing (-18°C) treatments for a total storage period of 9 weeks. After storage, shear force, drip loss and Western-blot assays (1 day and 9 weeks) were conducted. The carcasses from Plant B had slower temperature and rapid pH decline rates than the carcasses from Plant A. Western blot analysis showed that the loins from Plant B appeared to have more autolyzed subunits of μ-calpain and troponin-T degradation than the loins from Plant A at 1 d *post mortem*. However, after aged/frozen storage, Plant B loins had higher shear force, more drip loss and less desmin degradation than Plant A loins ($P < 0.05$). These results suggest that too early activation of μ-calpain could adversely affect the ageing potential and subsequent tenderness development of loins during ageing.

Key Words – μ-Calpain, Proteolysis, *Pre rigor*

I. INTRODUCTION

In general, fresh aged meat is considered a higher quality product having more reliable tenderness and less drip loss than frozen-thawed meat, and thus attains higher prices than frozen meat [1]. However, recent studies found that differences in meat quality attributes including tenderness and colour stability between aged and frozen/thawed meat can be narrowed if the meat is sufficiently aged prior to freezing [2-5].

Different *pre rigor* conditions created by application of electrical stimulation and/or different chilling regimes influence the rate of glycolysis and subsequently pH decline in muscle early *post mortem* [6]. Furthermore, different pH decline rates in early *post mortem* muscle affect the activity of μ-calpain, a major proteolytic enzyme contributing to protein degradation, which can eventually influence the tenderness development process [7-9]. Therefore, it is crucial to understand and compare how different slaughter processes including different electrical stimulation inputs and chilling regimes at different packing plants influence the rates of pH/temperature decline in lamb carcasses, and subsequently how these differences impact tenderness development of aged-then-frozen stored meat. Hence, the objectives of this study were to evaluate 1) the effect of the slaughter processes of two slaughter plants and 2) the effect of ageing prior to freezing on biochemical and meat quality attributes of lamb loins stored for an extended period (9 weeks).

II. MATERIALS AND METHODS

A. Raw materials and processing

Twenty-four lamb carcasses (approximately 4-month-old; average hot-carcass weight 18 kg) from two slaughter plants – Plant A and B (total 48 lamb carcasses) were monitored for temperature and pH decline rate for the first 24 hours *post mortem*. The pH decline rate was measured by inserting a calibrated pH probe (Testo 205 pH meter with combined temperature and pH insertion probe, Lenzkirch, Germany) directly into the loin eye between the 11th and 12th rib of carcass. At 24 hours *post mortem*, both loins (*M. longissimus dorsi*) were excised, vacuum packed and then transported in cold chilly bins to AgResearch, Hamilton. The loins were randomly allocated to four different ageing (-1.5 °C) and/or freezing (-18 °C) treatments in groups of 12 for a total storage period of 9 weeks: 1) frozen 9 weeks (A0F9); 2) aged 2 weeks then frozen 7 weeks (A2F7); 3) aged 3 weeks then frozen 6 weeks (A3F6); and 4) aged 9 weeks (never frozen; A9F0). After 9 weeks, the frozen loins were thawed overnight at -1.5 °C, and cut into two 6-cm thick

cuts for shear force and drip loss measurements and a thin cut (0.5 cm) for biochemical analysis.

B. Shear force

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

C. Drip loss

Drip loss was measured on the day of sample preparation after 9 weeks of storage by following the procedure of Honikel [11].

D. Western blot

Whole muscle protein sample preparation, protein assay and Western-blot assay for μ-calpain autolysis and desmin degradation of loin samples from 1 day and 9 weeks *post mortem* were conducted by the procedures described by Kim et al. [12].

E. Data analysis

The experimental design was split plot, where the whole-plot was for lamb carcasses from the two slaughter plants (Plant A and B), and the sub-plot was for the loins assigned for the four different ageing/freezing treatments with a balanced random assignment from each side of a carcass. All statistical analysis was done using the REML directive of GenStat. Least squares means for each attribute were separated (F test, $P < 0.05$) by using least significant differences.

III. RESULTS AND DISCUSSION

In general, the lamb carcasses from Plant B had a slower temperature decline rate than the carcasses from Plant A during the first 24 hours of carcass chilling period. Particularly at 1 hour *post mortem*, the carcasses from Plant B were about 7°C higher than those from Plant A (27°C and 20°C, respectively). Further, much faster pH decline of the carcasses from Plant B compared to the carcasses from Plant A was observed up to 12 hours *post mortem* (Fig. 1; $P < 0.05$). The different pH decline rate is likely due to different electrical stimulation regimes between the two plants [13] – Plant A utilizes a mid-voltage stimulation system (300V maximum, 1-166Hz adjustable frequency and 45 seconds), whereas Plant B adopts a high-voltage stimulation system (1138V at approximately 60Hz and contact duration of less than 90 seconds). In addition, the higher carcass temperature of Plant B during the initial chilling period could induce more accelerated glycolysis resulting in more rapid pH decline compared to the carcasses from Plant A [14].

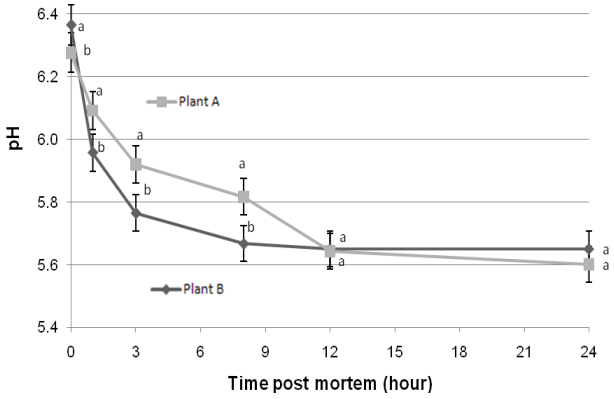

Fig. 1. The pH decline rate of lamb carcasses from the two packing plants for 24 hour of *post mortem* measured at the loin between the 11th and 12th rib of each carcass. Least square means lacking a common superscript letter (a-b) indicate differences ($P < 0.05$).

The Western blot analysis of μ-calpain autolysis at 24 hours *post mortem* showed that the loins from Plant B appeared to have more autolyzed subunit of μ-calpain (76kDa) than the ones from Plant A, in which a presence of intact μ-calpain (80kDa) and intermediate autolyzed subunit (78kDa) was apparent (Fig. 2A). This finding indicates early activation and faster autolysis of μ-calpain of the loins from Plant B compared with the ones from Plant A. Further, more troponin-T degradation products from the loins from Plant B were observed at 24 hour *post mortem* (Fig. 2B). Autolysis of μ-calpain early *post mortem* has been suggested to be associated with earlier activation of μ-calpain and consequently earlier degradation of some myofibrillar proteins [9]. The current results clearly demonstrated that the loins from Plant B had a greater extent of μ-calpain autolysis and subsequently more myofibrillar degradation

products compared to the loins from Plant A at 24 hours *post mortem*.

The different extent of μ-calpain autolysis and protein degradation between the two plants could be attributed to the different chilling temperature and pH decline rates of the lamb carcasses as illustrated above. *Pre rigor* temperature and pH decline (particularly within 3 hours *post mortem*) play a crucial role in μ-calpain activity and subsequently proteolysis and tenderness development [15, 16]. The higher *pre rig*or temperature and rapid pH decline of lamb carcasses from Plant B compared to Plant A could create a more favorable condition for μ-calpain activation and faster autolysis.

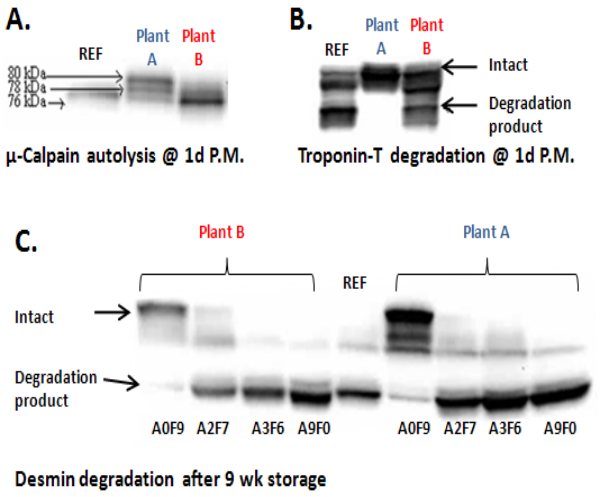

Fig. 2. Representative Western blot of whole muscle protein extractions of lamb *M. longissimus lumborum* from the two plants (A and B) after 1 day or 9 weeks of aged (-1.5 °C)/frozen (-18 °C) storage treatments: 1) frozen 9 weeks (A0F9); 2) aged 2 weeks and frozen 7 weeks (A2F7); 3) aged 3 weeks and frozen 6 weeks (A3F6); and 4) aged 9 weeks (never frozen; A9F0). **A**: μ-calpain autolysis at 1 day; **B**: troponin-T degradation at 1 day; **C**: desmin degradation after 9 weeks storage; REF: a reference lamb loin sample aged for 14 days.

However, too early activation and/or faster autolysis of μ-calpain early *post mortem* could result in an early exhaustion of its proteolytic activity and consequently losing the ageing potential of meat [7, 8]. In the current study, the Western blot assay for desmin for the loins after 9 weeks of storage (aged and/or frozen) supported the above hypothesis (Fig. 2C and Table 1). The aged/frozen loins from Plant B had more intact desmin than the counterparts from Plant A indicating less proteolysis had taken place in the loins from Plant B during the ageing period possibly due to its adverse effect of early activation of μ-calpain and subsequent early reduction of ageing potential.

The shear force values of the loins measured after 9 weeks of storage corresponded well to the results of Western-blot analysis (Table 1). A significant Treatment by Plant interaction was found in shear force values of the loins (Table 1). There was no difference ($P > 0.05$) in shear force values between the plants for the frozen only (A0F9) loins, which indirectly suggests no initial animal variation between the plants. However, as ageing progressed the tenderness improved more rapidly in the loins from Plant A than in the loins from Plant B confirming that the loins from Plant B had inferior ageing potential over the loins from Plant A as shown in the Western-blot analysis (Fig. 2C and Table 1).

The loins frozen on day 1 (A0F9) had significantly higher shear force values than the other three treatments. However, ageing for either 2 or three weeks prior to freezing (A2F7 and A3F6) resulted in similar ($P > 0.05$) shear force values to the loins aged for 9 weeks (A9F0). No further benefit, beyond 2 weeks of ageing prior to freezing, was found in shear force when comparing A2F7 and A3F6. This observation confirms the previous studies of positive effects of the aged/frozen treatment in tenderness development [2-5].

Table 1. Effects of different ageing (-1.5 °C)/freezing (-18 °C) treatments* on shear force (kgF), intact desmin (unitless ratio) and drip loss (%) of loins from each plant.

Trait	Shear force		Intact desmin		Drip loss	
	Plant A	Plant B	Plant A	Plant B	Plant A	Plant B
A0F9	5.1^{x}	5.6^{x}	5.9^{x}	6.3^{x}	2.6^{ax}	5.8^{b}
A2F7	2.8^{ay}	3.8^{by}	1.4^{y}	2.3^{y}	2.5^{ax}	5.8^{b}
A3F6	2.6^{ay}	3.8^{by}	1.0^{ay}	2.5^{by}	2.1^{ay}	5.8^{b}
A9F0	2.4^{y}	3.3^{y}	0.5^{y}	0.6^{z}	1.2^{az}	5.9^{b}
SED	0.5	0.5	0.6	0.8	0.2	0.2

Least square means within each trait lacking a common superscript letter in the same row (a-b) and column (x-z) indicate differences ($P < 0.05$). *1) frozen 9 weeks (A0F9); 2) aged 2 weeks and frozen 7 weeks (A2F7); 3) aged 3 weeks and frozen 6 weeks (A3F6); and 4) aged 9 weeks (never frozen; A9F0).

The drip loss data showed that the loins from Plant B had more drip loss than the loins from Plant A regardless of the aged/frozen treatments ($P < 0.05$; Table 1). Further, within Plant A, ageing for 3 weeks prior to freezing (A3F6) significantly decreased drip loss of the loins compared to the frozen only loins suggesting that ageing for 3 weeks prior to freezing can improve water-holding capacity of lamb loins.

IV. CONCLUSION

The results from the present study suggest that different slaughter processing conditions, such as different electrical inputs and chilling rates of plants, could influence the ageing potential of lamb loins by affecting the rate and extent of proteolysis and subsequently impact the efficacy of the aged/frozen treatment on tenderness development. In addition, this study confirms that ageing prior to freezing would result in superior tenderness values compared to loins frozen without ageing.

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