CAUSE OF MUSCLE SHORTENING, PROTEOLYSIS AND WB-SHEAR FORCE IN BEEF LONGISSIMUS AND SEMITENDINOSUS

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Background

pH/temperature interactions during rigor development are significant determinants of proteolytic activity of the calpain system (Koohmaraie, 1996) and the manifestation of cold shortening and rigor toughness. Tornberg (1996), considered that meat toughness in the early postmortem period was the sum of physical and enzymatic effects. Sarcomere length was a significant determinant of shear force for cold shortened beef longissimus muscle, while that was not always the case for muscle which had entered rigor mortis at high temperature (Hertzman et al., 1993; Olsson et al., 1994; Simmons et al., 1997). Wheeler and Koohmaraie (1999) showed that proteolysis of post-mortem muscle during chiller ageing was independent of sarcomere length, which suggests that proteolysis during rigor development could possibly ameliorate the adverse effects of physical shortening on tenderness. These studies indicated that meat toughness in cold shortened muscle was associated with shortened sarcomeres and reduced proteolysis during the early post-mortem period, while heat-induced meat toughness was more a function of acceleration and autolysis of the calpain enzymes (Hwang and Thompson 2001). The relative contribution of proteolysis and sarcomere lengths to tenderness was examined in cold shortened and rigor toughened samples where all samples had been held at constant temperature regimes for 24h.

Objectives

This study investigated the relative importance of rate of proteolysis and sarcomere length on WB-shear force in beef *Mm. longissimus* and *semitendinosus* exposed to extremes in temperature, from soon after slaughter to 24h post-mortem.

Materials and Methods

Animal, experimental design and treatment: Eight Hanwoo steers (685 ± 17 kg) were sampled from the Korean National Livestock Research Institute feeding program. Within 30 min of slaughter, the *Mm. longissimus thoracis et lumborum* (LD) and *semitendinosus* (ST) were removed from the carcass and transported to the laboratory, where they were divided into three portions (each 4 cm in length), and allocated into one of three temperature treatments (5, 15 or 36°C) in a latin square design. Samples were placed in re-sealable polyethylene bags and incubated in air-based 5, 15, or 36°C incubators for 24 hrs. After the incubation period, muscle temperatures were equalized by placing the bags in running water (ca. 18°C) for 30 min, prior to preparation for WB-shear force and sarcomere length measurements.

Measurement: Core sample temperatures were logged (Thermo Recorder, TR-50C, Japan) in the geometrical centre of the samples at 5 minute intervals. pH was monitored using a portable needle-tipped combination electrode (NWKbinar, pH-K21, Germany) at approximately 15 min intervals until the muscle was judged to have reached ultimate pH 6.0, and thereafter hourly. Sarcomere length was measured using a Helium-Neon laser diffraction technique on fixed muscle according to the method of Cross et al. (1980). WB-shear force was measured on steaks cooked at 70°C water bath for 60 min. and determined according to Wheeler et al. (2001). Immuno-detectable Troponin-T was determined at 0, 1, 3, 6, 12 and 24 h incubation times according method described by Geesink et al (2001) using monoclonal anti-Troponin-T 1:2000 (Clone JLT-12, Sigma).

Results and Discussion

Table 1 shows the effects of incubation temperatures on changes in pH and temperature of samples during the incubation time, and WB-shear force and sarcomere length at 24 h for the LD and ST muscles. Both muscles showed a similar temperature decline at each incubation temperature. Rate of pH decline, measured at both 3 and 6 hours showed significant effects of both muscle and temperature. The ST had a faster rate of pH fall than the LD muscle and this rate accelerated at the higher temperatures. This suggests that at the higher temperatures glycolytic rate was a largely a function of muscle temperature, but at the lower temperatures, fibre type tended to be the main driver. Incubation at 15°C resulted in the longest sarcomeres, which agreed with previous studies by Lockyer and Hagyard (1963). Previous studies have suggested that tenderization starts when the pH approaches 6.2 (Devine and Graafhuis, 1995) and that proteolysis was independent of sarcomere length (Wheeler and Koohmaraie, 1999). The results for time taken to reach pH 6.2 and therefore the initiation of proteolysis, would suggest that proteolysis would have commenced at a similar time in the LD 5°C and 15°C samples, and LD 36°C, SM 5°C and SM 15°C samples. However the results from the gels showed that the degradation rate of Troponin-T was greatest in the 36°C>15°C>5°C treatments for both muscles, and there was no obvious differences between muscles (Figure 1). When temperature at pH 6.2 was taken into account the faster degradation for the higher temperature was expected, as Koohmaraie, (1996) considered that μ-calpain activity was largely a function of the pH/temperature effects. Similarly the rate of u-calpain autolysis increased at the higher incubation temperatures(data not shown). These results again confirmed the importance of the interaction between chilling rate and pH decline in the initiation of proteolysis.

It has been defined that cold and rigor shortening occurs when pre-rigor muscle was exposed to temperature below 10°C or higher than 30°C with ATP available (e.g. pH above 6.2), respectively (Bendall, 1973). The experimental design achieved extremes in rigor temperature with samples from both muscles approaching or achieving the cold shortening and rigor toughening windows. The impact of these extreme conditions at rigor was reflected in shortened sarcomeres at both the 5 and 36°C temperature treatments. However in spite of the similarity in sarcomenre length the 5°C treatment resulted in significantly higher WB-shear force (i.e. 5 kg). This implied that the time of initiating tenderization process and temperature during the period was an important determinant for meat tenderness at 24 h (Dransfield et al., 1994). In addition, proteolysis was obviously more important than sarcomere length in determining meat tenderness in the early post-mortem period. Indeed, degradation rate of Troponin-T mirrored WB-shear force score for both muscles. Tender meat at 24 h resulted from the higher rigor temperature does not mean the most tender meat after extended ageing as both Simmons et al., (1996) and Hwang and Thompson, (2001) demonstrated that high rigor temperature which resulted in tender meat in the early ageing period was due largely to early activation of the calpain system, and that the benefits were eroded with extended ageing times. The current study demonstrated that length of tenderization early postmortem and temperature during the period was considerably important on meat tenderness compared to sarcomere length. It indicated that meat toughness linked to cold-shortening was more associated with enzymatic tenderization mechanism than shortened sarcomeres. In addition, the current results suggested that pH/temperature profile early postmortem, or rate of proteolysis needs to be taken into account when

sarcomere length and WB-shear force are related, and sarcomere length for fast glycolysing muscle was not significant factor influencing meat tenderness early postmortem period as shown by (Smulders et al., 1990).

Conclusions

With a similar sarcomere length between cold and heat shortened muscles, WB-shear force was approximately 5 kg higher for cold shortened muscles and that was related to duration of tenderization and temperature during the period. This suggested that early postmortem proteolysis was more important in determining meat tenderness in the early post-rigor period than sarcomere length. In addition meat toughness related to cold shortening is likely more largely affected by endogenous enzymatic tenderization mechanism rather than physical shortening. The current study indicated that protelysis factor needs to be accounted when relationship between sarcomere length and WB-shear force are established.

References

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Table 1. Least square means and F ratio of the mixed model for pH, temperature, WB-shear force and sarcomere length as a function of incubation temperature and muscle type

	m. longissimus			m. semitendinosus			Av. Se	Model terms		
	5°C	15°C	36°C	5°C	15°C	36°C		Muscle	Temp	Temp × muscle
pH at 3 h	6.68^{a}	6.66^{a}	6.12^{b}	6.32^{b}	6.32^{b}	5.92^{c}	0.07	13.9^{**}	49.2^{***}	0.72
pH at 6 h	6.34^{a}	6.32^{a}	5.69^{b}	6.02^{c}	6.02^{c}	5.57^{b}	0.076	8.24*	61.94***	1.93
Temperature at 3 h (°)	13.9^{a}	16.1^{b}	37.3^{c}	14.8^{ab}	16.8^{b}	37.5^{c}	0.78	0.71	538.3^{***}	0.10
Temperature at 6 h (°)	8.4^{a}	15.5^{b}	37.2^{c}	8.8^{a}	15.5^{b}	37.1^{c}	0.50	0.07	1726***	0.19
Time to pH 6.2 (h)	8.0^{a}	7.4^{a}	3.1^{bc}	5.0^{b}	4.4^{b}	2.0^{c}	0.89	5.95*	17.35***	1.20
Temperature at pH 6.2 (°)	7.7^{a}	15.0^{b}	37.4^{c}	13.9^{b}	15.8^{b}	37.4^{c}	0.82	9.97**	672.7***	9.42**
Shear force (kg)	9.3^{a}	5.9^{b}	4.2^{cd}	9.6^{a}	5.4^{bd}	4.9^{c}	0.48	0.1	106.4^{***}	1.43
Sarcomere length (μm)	1.52^{a}	1.76^{b}	1.45^{a}	1.52^{a}	1.76^{b}	1.42^{a}	0.06	0.01	19.8^{***}	0.05
df[φ]								1/14	2/26	2/26

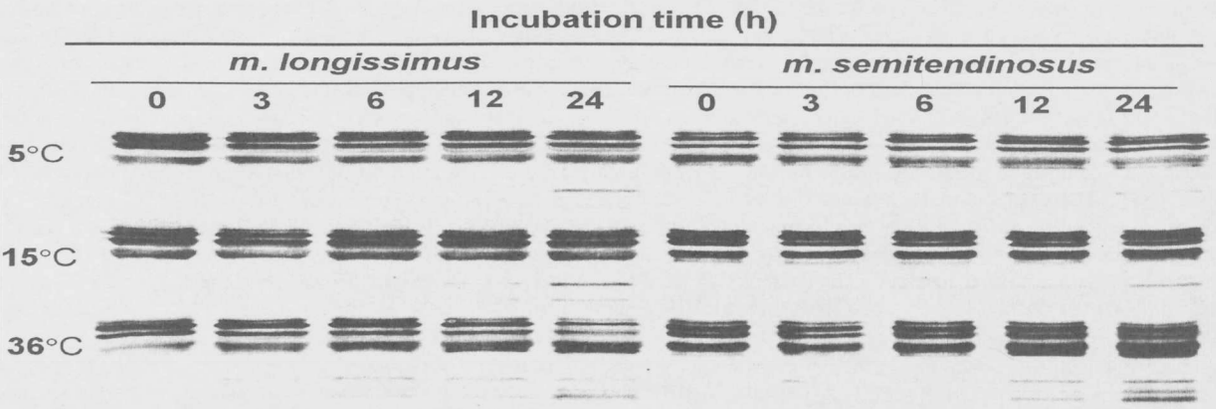

Figure 1. Degradation of Troponin-T as a function of incubation time for longissimus and semitendonosus