



Role of Calpain-2 in the Tenderization of Extended Aged Beef

M. J. Colle* and M. E. Doumit

Animal and Veterinary Science, University of Idaho, Moscow, ID, USA

Keywords: aging, beef, calpain
Meat and Muscle Biology 1(2):61

doi:10.221751/rmc2016.058

Objectives

Our objectives were to determine the effect of post-mortem aging (2, 3, 4, 14, 28, and 42 d) on calpain-1 and -2 activity in beef *longissimus lumborum* (LL) and *semimembranosus* (SM).

Materials and Methods

Carcasses were fabricated at 24 h postmortem. At 48 h postmortem, strip loin (IMPS 180) and top (inside) round (IMPS 168) from USDA Choice carcasses ($n = 12$) were purchased from AB Foods (Toppenish, WA) and transported to the University of Idaho Meat Science Laboratory. The LL and SM were removed from their respective wholesale cuts and samples from the anterior portion of the LL and proximal portion of the SM were removed and vacuum packaged until sampling on d 2, 3, 4, 14, 28, and 42 postmortem. Samples were frozen in liquid nitrogen and stored at -75°C until calpain analysis. One gram of each sample was homogenized at 12,000 rpm on ice 3 times for 15 s with 15 s cooling between bursts in extraction buffer (3 mL; 100 mM Tris, 10 mM EDTA, 10 mM DTT, pH 8.3). The homogenate was pipetted into two 2 mL microcentrifuge tubes, then centrifuged for 30 min at $8800 \times g$ at 4°C . The supernatant was aliquoted and stored at -75°C until calpain analysis. Calpain-1 and -2 activities were determined utilizing casein zymography. A d 0 sample from the *sternoccephalicus* was collected 10 min postmortem from a steer harvested at the University of Idaho Meat Science Laboratory. This sample served as the reference standard on each gel. Polyacrylamide gels (12.5%; 75:1 acrylamide to bisacrylamide) containing 0.2% casein were poured and then pre-run at 100 V for 15 min in an ice water bath with running buffer (25 mM Tris, 1 mM DTT,

192 mM glycine, 1 mM EDTA, pH 8.3) before loading samples. Sample buffer (10 μL ; 150 mM Tris, 20% glycerol, 10 mM DTT, 0.02% BPB, pH 6.8) was added to the supernatant (40 μL). Samples were loaded (20 μL) and gels were run at 100 V for 6 h in an ice water bath. Gels were then incubated in buffer (50 mM Tris, 10 mM DTT, 4 mM CaCl_2 , pH 7.5) at room temperature with slow shaking for 1 h (2 changes of buffer) followed by 16 h of incubation in the same buffer at room temperature. Gels were stained in Coomassie Blue R-250 (BioRad) for 1 h and destained or 3 h. Clear bands indicating calpain activity were quantified by inverting the image and then comparing the density of each band to the reference standard on each gel utilizing a Gel Doc (BioRad). Autolysis was used as an indicator of calpain activation. Data were analyzed using the Mixed Model procedure of the Statistical Analysis System (SAS Inst., Inc., Cary, NC) and significance was determined at $P \leq 0.05$.

Results

No native or autolyzed calpain-1 was detectable in either muscle following 14 d of aging. Native calpain-2 activity decreased ($P < 0.001$), while autolyzed calpain-2 activity increased ($P < 0.001$) with longer aging for both LL and SM. Our results indicate that calpain-1 is active early postmortem, while calpain-2 was activated in a majority of muscles by d 14 of aging. Calpain-2 activity persists until at least 42 d postmortem in both the LL and SM.

Conclusion

In conclusion, both calpain-1 and calpain-2 may contribute to postmortem improvement in beef tenderness, with calpain-1 being responsible for myofibrillar proteolysis early postmortem and calpain-2 contributing to tenderization during extended aging.