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Strategies to improve beef tenderness by activating calpain-2 earlier postmortem

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ABSTRACT

Our objectives were to determine the effect of post rigor calcium chloride injection or freezing on 1) sarcoplasmic calcium concentration and calpain-2 activity of *beef longissimus lumborum* (LL) and *semimembranosus* (SM) steaks aged 1, 4, and 14 days post-treatment and on 2) Warner-Bratzler shear force, water holding capacity, and consumer acceptability of LL and SM steaks aged 4 and 14 days post-treatment. Free calcium levels in the calcium, frozen, and control steaks averaged 1256, 127, and 121 μM for the LL and 1520, 120, and 111 μM for the SM, respectively. Measurable LL native calpain-2 activity was lower in calcium and frozen steaks than control steaks ($P < 0.01$), while SM native calpain-2 activity was lowest in calcium steaks and intermediate in frozen steaks ($P < 0.01$). LL calcium steaks were more tender ($P = 0.04$) than control steaks. In conclusion, calcium chloride injection and freezing activate calpain-2 earlier postmortem in both muscles and calcium injection improves LL tenderness.

1. Introduction

The effect of wet aging for 2, 14, 21, 42, and 63 days on retail color stability, microbial growth, Warner-Bratzler shear force, and consumer acceptability of strip loin (*longissimus lumborum*), top round (*semimembranosus*), top sirloin (*gluteus medius*), and bottom round (*biceps femoris*) steaks was recently evaluated (Colle et al., 2015 and 2016). The *longissimus lumborum* (LL) showed improvement in tenderness from day 2 to 14, but remained relatively consistent thereafter for all traits evaluated by the consumer panelists. Interestingly, the *semimembranosus* (SM) continues to improve for 42 days after carcass fabrication. Panelist scores for overall acceptability, tenderness, and juiciness of the SM showed steady improvement up to 42 days of aging. The continuous improvement of tenderness and juiciness was unexpected. Proteolysis, or breakdown, of myofibrillar proteins during extended aging may explain the improved tenderness by causing less resistance to shearing or chewing.

Calpains are enzymes that breakdown proteins. Autolysis of calpains coincides with degradation of myofibrillar proteins and therefore improved tenderness (Geesink, Kuchay, Chishti, & Koohmaraie, 2006). Currently, most of the postmortem tenderness improvement of beef is attributed to calpain-1 (Geesink et al., 2006; Koohmaraie & Geesink, 2006; Koohmaraie, Seideman, Schollmeyer, Dutson, & Crouse, 1987). However, recently we have shown that calpain-2 is activated postmortem in both the strip loin and top round (Colle & Doumit, 2017). Since calpain-1 activity is nearly gone by day 4 postmortem, the activation of calpain-2 is likely involved in the continued tenderness improvement in the top round. The lack of significant improvement in strip loin tenderness after day 14 is probably because the strip loin is already very tender by day 14 when the calpain-2 is activated. The 2010/2011 National Beef Tenderness Survey found the minimum postfabrication aging time was 2 days for both the strip loin and top round, additionally the percentage of strip loins and top rounds aged < 14 days was 36.2 and 46.6%, respectively (Guelker et al., 2013). Activation of calpain-2 early postmortem would ensure a more tender product as early as 2 days post-fabrication.

One strategy to activate calpain-2 early postmortem is calcium activated tenderization (CAT). Wheeler, Crouse, and Koohmaraie (1992) found that in beef *longissimus dorsi* (LD) injected with a 0.3 M calcium chloride solution to 110% of the green weight on day 1 postmortem, calpain-2 activity was 47% less in the calcium injected steaks than uninjected steaks after 7 days of aging. These authors noted an improvement in tenderness in LD steaks injected with calcium chloride. Likewise, Pringle, Harrelson, West, Williams, and Johnson (1999) showed that in beef LD injected with 2.2% calcium chloride to 105% of the green weight at 24 h postmortem, calpain-2 activity was 77% less in

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injected LD than uninjected LD at 48 h postmortem. Both Wheeler et al. (1992) and Pringle et al. (1999) determined calpain activity by using a column chromatography to separate calpains followed by casein hydrolysis in solution to determine activity. This method allows for the quantification of calpain-2 activity by determining the caseinolytic units per 50 g of tissue, but is unable to distinguish between native and autolyzed calpain-2. Casein zymography is able to distinguish between native and autolyzed calpain-2 and would allow for a better understanding of the effect of CAT on calpain-2 activity (Geesink et al., 2006; Veiseth, Shackelford, Wheeler, & Koohmaraie, 2001). Interestingly, Pringle et al. (1999) found CAT improved Warner-Bratzler shear force (WBSF) of the strip loin and top sirloin but not the top round. On the contrary, Wheeler, Koohmaraie, and Crouse (1991) found hot-boned beef top round injected with a 0.3 M calcium chloride solution to 110% of the green weight had lower WBSF values than control throughout aging. However, these authors did not evaluate calpain activity. Additionally, Wheeler et al. (1991 and 1992) and Pringle et al. (1999) did not use a sensory panel to determine tenderness of the calcium treated steaks.

Freezing is another potential strategy to activate calpain-2 earlier postmortem. Shanks, Wulf, and Maddock (2002) showed that freezing steaks at − 16 °C resulted in a more tender product than steaks aged for the same time but never frozen. Crouse and Koohmaraie (1990) found that aging after being frozen at − 30 °C for 27 days led to improved WBSF values. They note postmortem proteolysis may be enhanced by freezing meat before aging. They attribute the increase in proteolysis to a loss of calpastatin activity but not calpain activity during frozen storage. Wheeler et al. (1992) found that freezing LD steaks for 7 days and then aging the steaks for 7 days did not significantly change the calpain-2 activity compared to steaks not frozen and aged for 7 days. However frozen steaks did have a decreased WBSF value. Using casein zymography will allow for differences in native and/or autolyzed calpain-2 activity to be quantified in steaks that have been frozen. We anticipate that freezing will cause the sarcoplasmic reticulum to release enough calcium to active calpain-2.

We are not aware of research that examines calpain-2 activity with casein zymography after CAT or short term frozen storage. Both the strip loin and top round are excellent candidates for accelerating tenderization because of the high percentage of these subprimals aged 14 days or less (Guelker et al., 2013). Additionally, the top round seems to be a logical candidate for CAT because of its high initial toughness and ability to tenderize over 42 days of aging (Colle et al., 2016). Our objectives were to determine the effect of post rigor calcium chloride injection or post rigor freezing on 1) sarcoplasmic calcium concentration and calpain-2 activity of beef strip loin and top round steaks aged 0, 1, 4, and 14 days post-treatment and on 2) Warner-Bratzler shear force, water holding capacity, and consumer acceptability of beef strip loin and top round steaks aged 4 and 14 days post-treatment.

2. Materials & methods

2.1. Human subject participation in consumer panel

The University of Idaho Institutional Review Board certified this project as Exempt.

2.2. Product procurement

Forty eight hours postmortem, strip loin (IMPS 180) (n = 12) and top (inside) round (IMPS 168) (n = 12) from the right side of USDA Select carcasses were purchased from a commercial slaughter facility and transported to the University of Idaho Meat Science Laboratory.

2.3. Preparation of product

The *longissimus lumborum* (LL) and *semimembranosus* (SM) were removed from their wholesale cuts for treatment and subsequent analysis. These muscles (n = 12 LL and n = 12 SM) were cut into 3 sections. Each section was randomly assigned to one of the three treatments (control (CO), calcium chloride injection (CA), or freezing (FR)).

On day 2 postmortem the CO sections were cut into 4, 2.54 cm-thick steaks and vacuum sealed. The CA sections were injected to 105% of their green weight with a 2.2% calcium chloride solution, vacuum tumbled (20 min), cut into 4 steaks and then vacuum sealed. The FR sections were cut into 4 steaks, vacuum sealed, and frozen (− 20 °C) on day 2 and on day 4 were thawed at 4 °C. Two steaks from each treatment were designated for cooking and WBSF measurement. The other two steaks were designated for sensory analysis. Each steak was weighed prior to vacuum packaging and after aging to determine purge. Steaks were aged at 0 °C until completion of their assigned aging time. Samples from each treatment were removed and frozen in liquid nitrogen on days 0, 1, 4, and 14 post-treatment and subsequently stored at − 75 °C until determination of calcium concentration and calpain-2 activity.

2.4. Warner-Bratzler shear force

On days 4 and 14 post-treatment, steaks designated for WBSF were cooked and WBSF was conducted following the procedure in Colle et al. (2016).

2.5. pH

pH was measured following the procedure in Colle et al. (2016).

2.6. Sensory panel

Consumer panel analysis was conducted following Colle et al. (2016) with minor modifications. On days 4 and 14 post-treatment, steaks designated for consumer acceptability were vacuum packaged and frozen at − 20 °C until the sensory panel was conducted. Two consumer panels were conducted (n = 72 panelists per muscle) to evaluate cooked steaks from the designated treatments. Four 1.27-cm × 1.27-cm × steak thickness cubes were obtained from each steak. Using an incomplete block design panelists sampled 4 samples from the 3 treatments and 2 aging periods. Demographics of consumer panelists are shown in Table 1.

2.7. Calcium concentration

Calcium concentration was determined following a procedure by Hopkins and Thompson (2001) with minor modifications. Samples that had been stored at − 80 °C were weighed (2 g) and placed in a − 20 °C freezer at least 36 h prior to calcium measurement. Following equilibration to − 20 °C samples were placed in a refrigerator (4 °C) for 20 min before being finely diced, placed on ice, and then centrifuged (Sorvall RT1 Centrifuge, Thermo Scientific, Waltham, MA) at 30,000*g* at 5 °C for 40 min. 250 µl aliquots of the supernatant were mixed with 5 µl of Calcium Ion Strength Adjuster (Mettler Toledo, Woburn, MA). These samples were then incubated in a water bath at room temperature for 5 min. Calcium concentration was determined using a calcium selective electrode (PerfectION combination Ca^{2+}, Mettler Toledo, Woburn, MA) attached to a portable ion meter (Seven2Go pro, Mettler Toledo, Woburn, MA). The electrode was allowed to soak in a 1×10^{-2} calcium solution at least 1 h prior to calibration and reading samples. A calibration curve was created prior to each run. Calcium standards containing 8000 µM, 2000 µM, 1000 µM, 500 µM, 100 µM, and 50 µM calcium were made from Calcium ISE standard 1000 mg/l as Ca^{2+} (Mettler Toledo, Woburn, MA).