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Investigation of tenderness mechanisms in calcium-enhanced muscle

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INVESTIGATION OF TENDERNESS MECHANISMS IN CALCIUM-ENHANCED MUSCLE

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Summary

We explored the mechanism(s) of calcium-induced tenderization in calcium-enhanced beef muscle. At 72 hours postmortem, we injected (9% by weight) beef strip loins (n=15) with 0, 0.05, 0.1, 0.2, or 0.4 M calcium chloride (CaCl_2) with and without 0.05 M zinc chloride (ZnCl_2), and they were then aged until 15 days postmortem. Warner-Bratzler shear force peak values indicated that addition of ZnCl_2 drastically inhibited tenderization; however, enhancement with CaCl_2 still tended to reduce shear values ($P=0.07$; 0.55 kg) when ZnCl_2 was present. In the absence of ZnCl_2 , the 0.2 and 0.4 M CaCl_2 treatments were 18.9 and 32.1% more ($P<0.05$) tender than the no CaCl_2 treatment. These results suggest that both calcium-activated enzymatic activity and a non-enzymatic salting-in effect contributed to tenderization of calcium-enhanced muscle. However, the enzymatic mechanism reduced toughness 2.9 to 7.5 fold more than the non-enzymatic mechanism. Calcium-activated enzymatic degradation appears to be the major tenderization mechanism, and non-enzymatic salting-in of calcium ions appears to be a minor tenderization mechanism, even at high calcium concentrations.

Introduction

Muscle food research conducted during the past three decades showed that enhancement of fresh skeletal muscle with calcium ions resulted in the weakening and fragmentation of the myofibrillar component, thereby inducing meat tenderization. The majority of

research suggests that the mode of tenderization is through the activation of calcium-dependent proteases occurring in skeletal muscle. In contrast, other research utilizing protease inhibitors suggests a non-enzymatic salting-in calcium effect causing protein solubilization. Chloride salts of calcium, barium, and magnesium destabilize proteins by increasing the electrostatic interactions between protein molecules and ionic solutions, thereby increasing protein solubility. With scientific evidence supporting both theories, both mechanisms possibly occur simultaneously in post-rigor muscle. In addition, these mechanisms have been investigated almost exclusively on normal calcium concentrations in postmortem muscle. Data are limited on the tenderization mechanisms at work in calcium-enhanced muscle. Therefore, the objective of this research was to inject a wide range of calcium concentrations into muscle with and without zinc ions (inhibitor of calpain enzyme activity) to determine if calcium induced tenderization is the result of calpain activity, a salting-in effect, or both.

Experimental Procedures

We selected beef strip loin subprimals (n=15) from USDA Standard carcasses from the fabrication line of a commercial processor and transported them at 0°C (32°F) to the Kansas State University Meat Laboratory. At 72 hours postmortem, a 1-inch-thick steak was cut from the center of each loin section and cooked immediately for Warner-Bratzler shear force evaluations. The remainder was cut transversely into four equal loin sections. We randomly allocated the 60 loin sections to one

of the following enhancement treatments, each in distilled water: (1) no CaCl_2 ; (2) 0.05 M CaCl_2 ; (3) 0.1 M CaCl_2 ; (4) 0.2 M CaCl_2 ; (5) 0.4 M CaCl_2 ; (6) 0.05 M ZnCl_2 ; (7) 0.05 M CaCl_2 + 0.05 M ZnCl_2 ; (8) 0.1 M CaCl_2 + 0.05 M ZnCl_2 ; (9) 0.2 M CaCl_2 + 0.05 M ZnCl_2 ; and (10) 0.4 M CaCl_2 + 0.05 M ZnCl_2 . The pH of the solutions was 7.36, 9.08, 9.66, 9.91, 9.95, 6.75, 6.73, 6.62, 6.49, and 6.10, respectively. We injected (9% by weight) loin sections with their respective treatment, then vacuum packaged and stored them at 1°C (34°F) until 15 days postmortem. At 15 days postmortem, we cut one 1-inch-thick steak from each loin section and cooked it immediately for shear force evaluation. We cooked steaks to an internal temperature of 70°C (158°F) on an electric belt grill set at 117°C (242°F), cooled them in a refrigerator for 24 hours at 1°C (34°F), then removed eight round cores (0.5 inch diameter) from each steak parallel to muscle fiber orientation. We sheared cores once through the center by a V-notch Warner Bratzler shear attachment connected to an Instron Universal Testing Machine and recorded the peak force required to shear each core.

Results and Discussion

All treatments containing ZnCl_2 had higher shear force ($P<0.05$) than those without ZnCl_2 (Figure 1). Within ZnCl_2 treatments, peak force decreased numerically when exogenous CaCl_2 was added. Treatments with ZnCl_2 and CaCl_2 tended to have lower shear values ($P=0.07$; 0.55 kg) than the ZnCl_2 only treatment. For treatments without ZnCl_2 , peak force values decreased ($P<0.05$) as CaCl_2 concentration increased; treatments with CaCl_2 at 0.2 or 0.4 M were more tender ($P<0.05$) than the no CaCl_2 treatment.

Data in Figure 2 illustrate the reduction in Warner-Bratzler shear force from 72 hours (pre-enhancement) to 15 days postmortem (12 day post-enhancement) as a function of calcium concentration, regardless of level of calcium enhancement. Addition of ZnCl_2 resulted in less than 1.0 kg of reduction in Warner-Bratzler shear force. In comparison, enhancement without ZnCl_2 resulted in Warner-Bratzler shear force reductions of 1.5 to 3.4 kg. These data clearly illustrate that calcium-enhancement accelerates postmortem tenderization and that zinc retards tenderization. Similar to peak force values, treatments with ZnCl_2 and CaCl_2 tended ($P=0.06$; 0.60 kg) to have a larger reduction in shear values during aging than the ZnCl_2 only treatment, suggesting that minor non-enzymatic (salting in) tenderization occurs when exogenous calcium is added.

Non-enzymatic salting-in mechanism was responsible for approximately 30% of the improvement in tenderness from 72 hours to 15 days, whereas calcium-induced enzymatic activity was responsible for approximately 70% of the improvement. Results from adding the combination of ZnCl_2 and CaCl_2 to muscle suggest that the salting-in mechanism is responsible for small improvements in tenderness, but this is less than the improvement from activation of calcium-dependent proteases.

Postmortem tenderization of muscle is a complex process. From our experiment, we conclude that at least two mechanisms contribute to tenderization in calcium-enhanced muscle. When longissimus muscle is injected with calcium ions at 72 hours postmortem, calcium-activated enzymatic activity and a non-enzymatic salting-in mechanism account for about 70% and 30%, respectively, of tenderization at 15 days postmortem.

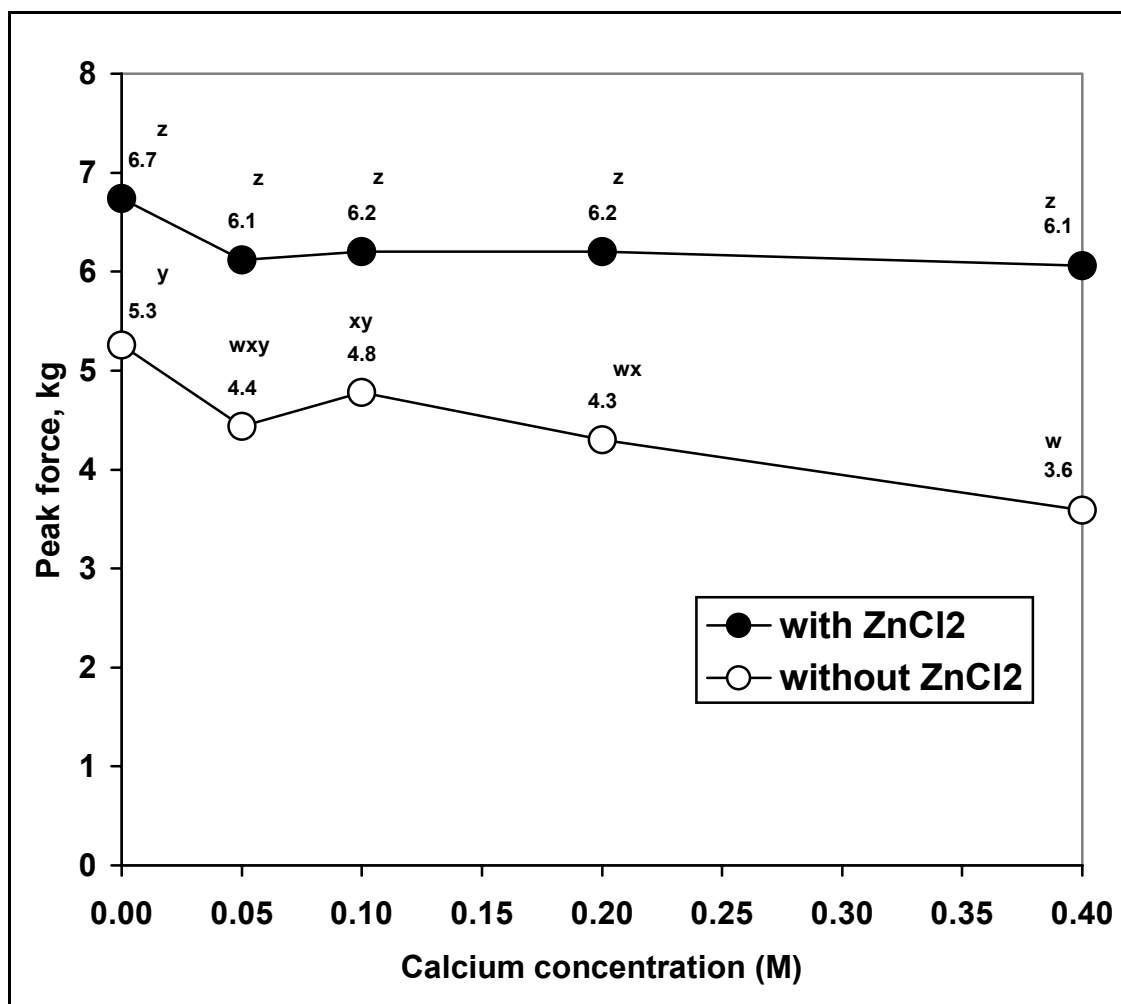


Figure 1. Warner-Bratzler peak shear force values of beef longissimus muscle enhanced with CaCl₂ with and without ZnCl₂ (SE=0.3). Means without a common superscript letter differ ($P<0.05$).

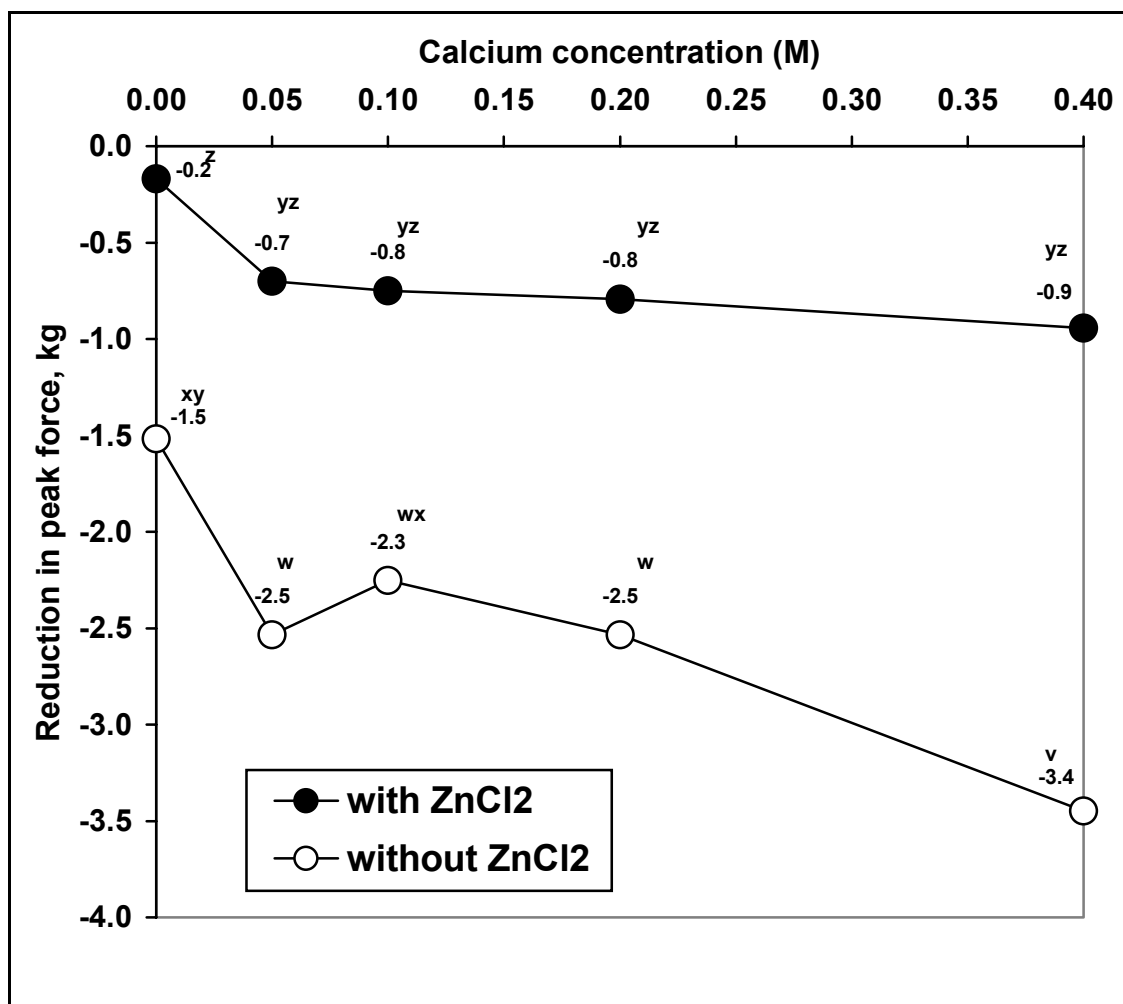


Figure 2. Reduction in Warner-Bratzler shear force values from 72 hours to 15 days post-mortem of beef longissimus muscle enhanced with CaCl₂ with and without ZnCl₂ (SE=0.4). Means without a common superscript letter differ ($P<0.05$).