# PROTEIN DENATURATION OF BEEF DEEP SEMIMEMBRANOSUS MUSCLE NEGATIVELY AFFECTS MEAT TENDERNESS BY LIMITING PROTEIN DEGRADATION AND µ-CALPAIN AUTOLYSIS

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*Abstract*— **In two experiments, this study tests the hypothesis that protein denaturing conditions during the conversion of muscle to meat in beef deep semimembranosus (SM) will negatively influence meat tenderness by decreasing the extent of µ-calpain autolysis and concomitant decreases in postmortem proteolysis. In experiment 1, the SM was removed from beef carcasses (n=10) at 24 h postmortem, packaged in high-oxygen modified atmosphere (HiOx-MAP; 80% $O_2$, 20% $CO_2$), and displayed for 7 d at 1°C. The pH, protein denaturation, µ-calpain autolysis, and degradation of desmin and troponin-T were determined. In experiment 2, ten beef cattle were slaughtered, and temperature decline of deep SM (DSM) and superficial SM (SSM) was monitored for 24 h of chilling period. At 24 h postmortem, the SM was removed, packaged in vacuum, and stored for 9 d at 1°C. Then, the SM steaks were repackaged in HiOx-MAP, and further displayed for 7 d d at 1°C. Star probe measurements were taken for steaks from each storage and display period to determine instrumental tenderness differences of DSM and SSM. DSM had a significantly slower temperature decline rate than SSM during chilling. Location did not affect pH at 24 h postmortem. DSM had higher ($P < 0.05$) protein denaturation values than SSM. Western blotting for µ-calpain autolysis revealed that at 48 h postmortem DSM maintained unautolyzed µ-calpain subunit (80-kDa), whereas SSM had mostly autolyzed subunits (78- and 76-kDa). The western blotting of desmin and troponin-T also found that DSM had less ($P < 0.05$) protein degradation than SSM throughout display time. The star probe determined that DSM had significanlty lower tenderness values than SSM throughout storage and display periods. These results confirm our hypothesis that increased protein denaturation of DSM results in minimal proteolysis by negatively affecting µ-calpain activity, which subsequently leads to decrease instrumental meat tenderness values.**

*Index Terms*— **Protein denaturation, Proteolysis, Semimembranosus; Tenderness**

## I. INTRODUCTION

Beef cuts from the semimembranosus (SM) muscle often appears a two-toned color, with a paler color in the deep SM (DSM) than superficial SM (SSM). Because of the size, thickness, and location of the SM, the DSM has a slower chill rate and more rapid pH decline than SSM, which can result in exhibiting different qualities within that muscle. Tarrant (1977) reported a higher temperature (above 30 °C) and a more rapid pH decline (below 6) in DSM than SSM early postmortem. As a result, the high temperature and low pH condition of DSM negatively affects muscle protein functionality, which results in less metmyoglobin reducing ability, water-holding capacity, and color stability (Sammel, Hunt, Kropf, Hachmeister, Kastner & Johnson, 2002; Tarrant & Mothersill, 1977).

The high temperature/low pH condition can influence the µ-calpain activity and autolysis, which consequently affects postmortem proteolysis and ultimate tenderization (Barbut et al., 2008; Melody, Lonergan, Rowe, Huiatt, Mayes & Huff-Lonergan, 2004). Therefore, we hypothesized that the protein denaturing conditions created during the conversion of muscle to meat in the DSM would negatively influence postmortem proteolysis and consequently meat tenderness by decreasing the extent of µ-calpain autolysis. Although several studies showed the two-toned color phenomenon within the SM, none examined how different muscle locations within the SM might affect µ-calpain activity and postmortem proteolysis. Therefore, the objective of this study was to determine the influence of biochemical characteristics of beef DSM and SSM on µ-calpain autolysis, postmortem protein degradation, meat tenderness development.

## II. MATERIALS AND METHODS

### *A. Raw materials and processing*

Two consequtive experiments were conducted to determine the influence of biochemical characteristics of beef DSM and SSM on µ-calpain autolysis, protein degradation, and meat tenderness development. In experiment 1, ten

semimembranosus (SM) muscles were obtained at 1 d postmortem from market weight beef cattle (n=10; A-maturity; quality grade - Low Choice). After trimming subcutaneous fat, the SM was removed. Steaks (2.54 cm thick) were cut from the anterior portion of the SM, and were placed in preformed trays. Trays were packaged to high oxygen-modified atmosphere (HiOx-MAP; 80% $O_2$/20% $CO_2$, Praxair, Inc. Specialty Gases; Cahokia, IL) by using Multivac C500 (Koch Supplies Inc., Kansas City, MO). Packages were displayed for 7 d at 1 °C under continuous fluorescent natural white light of 2150 ± 50 lux intensity. After each designated display time (d 1 or d 7), samples were removed from the packages and separated to deep portion (DSM; the medial inner 1/3 closest to the femur) and superficial portion (SSM; the lateral outer 1/3 closest to the surface of the carcass). Both portions of the SM (whole DSM and SSM respectively) were minced, frozen in liquid nitrogen, pulverized and stored at -80 °C until used for the chemical and biochemical analyses. In expermiment 2, ten beef cattle (A-maturity; quality grade - low Choice) were slaughtered at the Iowa State University Meat Laboratory. The postmortem temperature decline of DSM and SSM during chilling was monitored using a temperature logger for 24 h. At 24 h postmortem the SM was removed, cut into a steak (2.54 cm thick), vacuum packaged, and stored at 1 °C for 9 d. After storage, steaks were placed in preformed trays, repackaged to HiOx-MAP, and further displayed for 7 d (designated as d 16 – 17 days postmortem). Steaks from d 1, 9, and 16 were frozen at -20 °C in vacuum bags until used for the instrumental tenderness measurement.

*B. pH determination*

The pH measurements for both the DSM and SSM from d 1 and d 7 of display in experiment 1 were measured by a glass penetration probe at two different locations per sample using a Hanna 9025 pH/ORP meter (Hanna Instruments, Woonsocket, RI), and were averaged for each portion at each time point.

*C. Protein denaturion*

Protein denaturation was determined by measuring sarcoplasmic protein extractability following procedures described by Ockerman and Cahill (1968). High transmission values for percent turbidity (at 600 nm) of the sample solution indicate less soluble protein and higher protein denaturation (Ockerman & Cahill, 1968).

*D. Western blotting*

Western blotting to determine μ-calpain autolysis, desmin degradation, and troponin-T degradation for both the DSM and SSM samples (d 1 and d 7) in experiment 1 was conducted using procedures described by Kim, Huff-Lonergan, Sebranek, and Lonergan (2010). Protein bands were detected using a chemiluminescent detection kit (ECL Plus, Amersham Pharmacia Biotech). The density of the immunoreactive bands were quantified by densitometry using ChemiImager 5500 (Alpha Innotech, San Leandro, CA) and Alpha Ease FC software (v. 2.03; Alpha Innotech). The proportions of the μ-calpain catalytic subunit present as the intact 80 kDa, intermediate 78 kDa, and autolyzed 76 kDa band were each calculated.

*E. Star probe*

In experiement 2, star probe analysis of steak samples on d 1 and d 9 of storage, and d 16 of display was measured (Lonergan et al., 2007). Steaks were cooked on clamshell grills to an internal temperature of 71 ºC, which was monitored using thermocouples (Omega Engineering, Inc. Stamford, CT). A circular, five-pointed star probe attachment was used to determine the amount of force necessary to compress the sample to 20% of the initial sample height. Each steak was compressed three times with a circular, five-pointed star probe attached to an Instron Universal Testing Machine (Model 1122, Instron, Norwood, MA) and the average of the three values was calculated to determine the overall value.

*F.* Statistical analysis

Data were analyzed using the Mixed Model procedure of SAS for ANOVA (SAS, 2007). Type-3 tests of fixed effects for muscle location, display time, and their interaction, and random effects for animal and animal by location were analyzed. Least squares means for all traits of interest were separated using least significant differences generated by the PDIFF option.

## III. RESULTS AND DISCUSSION

*A. Ultimate pH and temperature decline*

The DSM had significantly slower temperature decline rate than SSM during chilling period (Figure 1). The temperature of DSM was more than 10 °C higher than that of SSM at 5 h postmortem. The pH at d 1 (DSM = 5.54, SSM = 5.50, SE = ± 2) and d 7 (DSM = 5.56, SSM = 5.53, SE = ± 2) of display was not affected ($P > 0.05$) by the location within the SM. In this study, the rate of pH delcline in the DSM and SSM within 24 h postmortem during

chilling was not monitored. However, Sammel et al. (2002) determined a more rapid pH decline in the DSM from above 6.5 to 5.6 by 3 h postmortem, while SSM maintained pH of above 6 until 8 h postmortem. The slow chill rate in DSM results in accelerated glycolysis and rapid pH decline (Sammel et al., 2002; Tarrant & Mothersill, 1977), which consequently could affect biochemical differences within the SM, eventually resulting in meat quality variations.

*B. Protein denaturation*

Protein extractability measurements based on the percent transmission values revealed that DSM had greater ($P < 0.05$) protein denaturation than SSM regardless of display time (DSM = 76.2%, SSM = 10.7%, SE = ± 4.9%). The high transmisson values of DSM indicate lower amounts of extracted proteins because of protein denaturation that occurred in the muscle prior to extraction. Increased protein denaturation in the DSM was also reported by Sammel et al. (2002). They determined higher transmission values from traditionally boned DSM; however, the hot-boned (and more rapidly chilled) DSM exhibited less denatured protein, confirming that the high temperature/rapid pH decline rate resulted in protein denaturation of DSM and could be minimized by the accelerated chilling technique. The consequences of protein denaturation in the DSM can be linked to poor water holding capacity and perhaps decreased tenderness (Sammel et al., 2002).

*C. μ-Calpain autolysis and protein degradation*

The extent of autolysis can be estimated by calculating the percent of the total detected μ-calpain large subunit present as the autolysis products (78- and 76 kDa). At 1 d of display (48 h postmortem), the extent of autolysis was greater in the SSM (94 % of the detected large subunit detected as autolysis products) than the DSM (67%; Figure 2A). Furthermore, the DSM portion still maintained a greater ($P < 0.05$) percentage (26.4%) of unautolyzed 80-kDa catalytic subunit of μ-calpain than that of the SSM portion (1.8%) at d 7 of display (Figure 2A). The determined effect of location on protein denaturation indicates that conditions existed in the DSM that inactivated μ-calpain. Rapid pH decline during the early postmortem period has been shown to result in a pronounced protein denaturation and decreased μ-calpain activity (Claeys, De Smet, Demeyer, Geers & Buys, 2001), which could influence early postmortem proteolysis and subsequent postmortem tenderization and water holding capacity (Melody et al., 2004). The μ-calpain results are consistent with the observation that there was less ($P < 0.05$) degraded desmin and less ($P < 0.05$) troponin-T degradation products in the DSM (Table 1 and Figure 2B, 2C). Desmin degradation in the DSM increased after 7 d of display time, but the extent of desmin degradation was still less ($P < 0.05$) than that observed in the SSM at d 7 (Table 1). The 30-kDa troponin-T product of DSM at d 1 did not change with additional aging time, whereas there was a significant increase in degradation of troponin-T in SSM after 7 d of display time. Since desmin and troponin-T are known substrates of μ-calpain (Huff-Lonergan, Mitsuhashi, Beekman, Parrish, Olson & Robson, 1996), the rate of postmortem degradation of desmin and troponin-T is associated with tenderness development (Huff-Lonergan et al., 1996).

*D. Strar probe analysis*

The star probe measurement revealed a significant tenderness difference between DSM and SSM throughout storage and display periods. The DSM always had greater ($P < 0.05$) star probe values (indicating lower tenderness) than SSM regardless of postmortem time periods (Table 1). This observed tenderness variation within SM could be attributed to the limited degradation of myofibrillar proteins and μ-calpain autolysis, which are influenced by the protein denaturation due to the high temperature and previously observed rapid pH decline during the conversion of muscle to meat.

## IV. CONCLUSION

Results from these experiments demonstrate that differences in meat quality across locations in the SM are associated with protein denaturation and protein degradation. The results confirm our main hypothesis that increased protein denaturation in the DSM results in decreased proteolysis by slowing μ-calpain autolysis subsequently leading to decreased meat tenderness. Implementation of efficient chilling techniques to prevent protein denaturation in the interior of the SM should improve consistency and quality of fresh beef cuts from the top round. Furthermore, any investigations dealing with SM should consider location differences within SM in meat quality and biochemical characteristics to remove known sources of variation.

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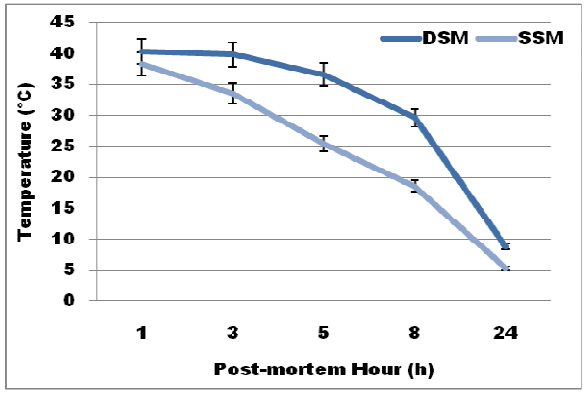


Figure 1. Postmortem temperature decline of DSM and SSM of beef carcasses during chilling period

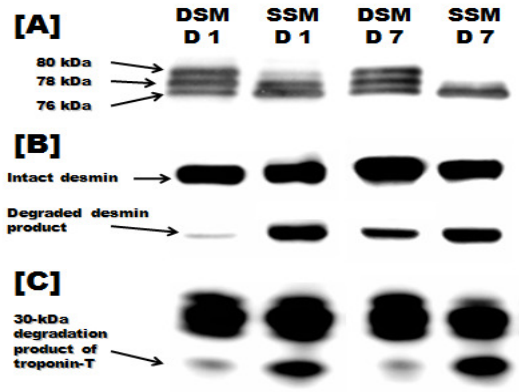


Figure 2. Representative western blot depicting μ-calpain autolysis [A], desmin [B], and troponin-T [C] of deep (DSM) and superficial (SSM) portion of beef semimembranosus steaks packaged in a high-oxygen modified atmosphere and displayed for 1 d and 7 d at 1 °C

Table 1. Least squares means for degraded desmin and troponin-T product (experiment 1), and star probe (experiment 2) of deep (DSM) and superficial (SSM) portion of beef semimembranosus during storage and display period

| Trait | Degraded desmin (Unitless) | | 30 kDa degradation product of Troponin-T (Unitless) | | Star probe (kg) | | |
|---|---|---|---|---|---|---|---|
| | D 1 | D 7 | D 1 | D 7 | D 1 | D 9 | D 16 |
| DSM | 0.26ax | 0.64bx | 0.25ax | 0.36ax | 6.93x | 7.44x | 7.20x |
| SSM | 0.89ay | 1.15by | 0.56ay | 1.04by | 6.24y | 6.47y | 6.22y |
| SEM | 0.13 | 0.13 | 0.10 | 0.10 | 0.31 | 0.31 | 0.31 |

Means in a row with different superscripts (a-b) within each trait are different ($P < 0.05$).
Means in a column with different superscripts (x-y) are different ($P < 0.05$).