Degradation of Filamin in Aged Pork Loins Classified by High and Low Star Probe Values

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Amanda C. Outhouse, Graduate Research Assistant, Department of Animal Science; Kelsey B. Carlson, Graduate Research Assistant, Department of Animal Science; Chris A. Fedler, Assistant Scientist, Department of Food Science and Human Nutrition; Elisabeth Huff-Lonergan, Professor of Animal Science; Kenneth J. Prusa, Professor of Food Science and Human Nutrition; Steven M. Lonergan, Professor of Animal Science; Iowa State University, Ames, Iowa

Summary and Implications

Filamin is necessary to maintain the integrity of muscle structure. Postmortem degradation of filamin results in loss of organizational structure at the Z-disk and improves meat tenderness. Intact and degraded filamin was successfully identified in aged pork loin. The removal of amino acids to form this degradation product is likely the first cleavage of intact filamin observed in postmortem muscle. A significant decrease in abundance of intact filamin in low star probe (SP) value samples demonstrates that degradation of filamin varies when aging period and pH are similar. Filamin degradation was significantly different in SP groups, suggesting that filamin proteolysis and formation of degradation products may have an impact on the SP values of aged pork loin.

Introduction

Postmortem degradation of structural proteins plays a large role in the development of meat tenderness. Because consumers use tenderness as a key indicator of pork quality, it is important to understand how degradation of different proteins influences tenderness. Filamin is a large protein that binds actin in many different cell types. It is located at the periphery of Z-disks in a variety of muscle types. Because of its role in muscle structure, filamin degradation could influence meat tenderness. The objective of this research was to determine the abundance of intact filamin in aged pork loins with high and low star SP values. Star probe is used as an instrumental measurement of tenderness. We hypothesized that greater proteolysis of filamin would be observed in aged pork loins that exhibit lower SP values.

Materials and Methods

Pork loins (n = 159) were vacuum packaged and aged for nine to eleven days. Following cooking to 68º C, cook loss, Instron star probe, and sensory characteristics were determined. Samples for proteolytic evaluation were selected to represent highest (n = 12; mean = 7.75 kg) and lowest (n = 12; mean = 4.95 kg) SP values in the entire population. Selected samples were within defined ranges of pH (5.54 – 5.86), marbling score (1.0 – 3.0), and lipid content (1.61 – 3.37 %).

Immunoblots were used to determine the abundance of intact filamin in samples from high and low SP value groups. Gradient acrylamide TEA-Tricine gels were loaded with whole muscle protein samples. Gels were run for approximately 360 volt×hours. An internal protein sample reference was generated from an aged pork longissimus muscle. Primary antibody concentration of 1:1,000 α-filamin and secondary antibody concentration of 1:20,000 goat anti-rabbit were used. Immunoblots were quantified using densitometry. Spots from gels were selected for mass spectrometry analysis to confirm the identity of intact and degraded filamin.

One-dimensional SDS-PAGE gels were prepared to confirm the identity of the high molecular weight protein filamin. Preparative, continuous gels (8%, 18cm x 16 cm, 1.5 mm thick; filtered reagents) were loaded with 200 and 225 µg of protein and run for 2,200 volt×hours. Gels were stained using filtered Colloidal Coomassie Blue Stain (1.7% ammonium sulfate, 30% methanol, 3% phosphoric acid, and 0.1% Coomassie G-250) and destained with filtered ddH_2O.

Protein bands (an intact band and large degradation product of filamin) were excised from the gels and sent to the Iowa State University Protein Facility. Spots were digested with trypsin using Genomic Solutions Investigator ProGest automated digester (Chelmsford, MA), separated by liquid chromatography, and analyzed by tandem mass spectrometry (MS/MS) using a Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, Rockford, IL). Resulting raw peptide fragment patterns were compared to the database search program MASCOT database search (MatrixScience, London, UK) using Uni-Prot to identify proteins. The peptides identified were compared to the peptide sequence of full-length filamin (*homo sapiens,* accession # Q14315).

Results and Discussion

Intact filamin was 30% less abundant in low SP value samples than high SP value samples (Figure 1. $P < 0.01$). The mass spectrometry analysis confirmed the identification of filamin. In addition, the identity of the filamin degradation product (12 kDa smaller than the intact filamin) was confirmed. The analysis of the intact filamin band demonstrated that peptides were dispersed evenly throughout the entire protein, displaying good coverage and successful identification of intact and degraded filamin. The band recognized as the degradation product of filamin

contained peptides throughout the entire protein with the exception of the carboxyl terminal end (amino acids 2616-2725), indicating that the degradation product is missing this portion of the protein. It is possible that this small change in filamin could influence tenderness of aged pork.

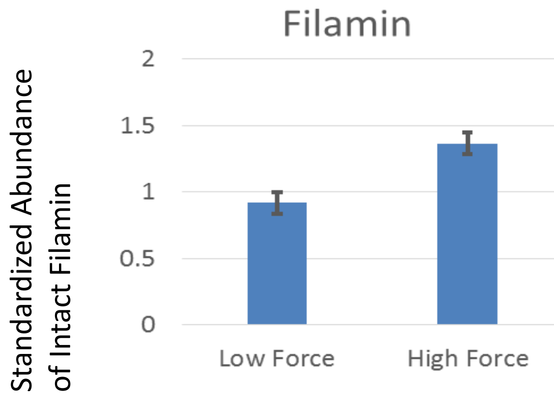

Figure 1. Abundance of intact filamin is greater in the high force (and thus less tender) pork loin.