

# Proteolysis Influences Tenderness of Aged Pork Loins

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### Summary and Implications

Aged pork loins were selected to have similar ultimate pH, color, lipid content, and purge. The star probe values (kg) obtained from these loins were evaluated and loins were sorted into either a low star probe (LSP) group or a high star probe (HSP) group. Loins were evaluated for autolysis of calpain-1 and degradation of troponin-T, desmin, and titin. Results showed calpain-1 was completely autolyzed in all samples. LSP pork loins exhibited more degradation of troponin-T, desmin, and titin, demonstrating proteolysis influences measured tenderness in aged pork.

### Introduction

Tenderness is a fundamental component of pork quality. There is significant variation in tenderness presented to consumers. Currently, there is no method to rapidly differentiate between tough and tender pork products in processing facilities. Degradation of muscle proteins is known to influence measured tenderness in meat. Therefore, this study examined differences in degradation of structural proteins in samples selected for extreme star probe differences. Better understanding of proteomic differences in pork loins with extreme tenderness differences could help identify protein markers to differentiate between tough and tender pork products.

### Materials and Methods

Commercial pork loins (n = 159) were collected from Duroc-sired crossbred pigs and aged for 9 to 11 days. Chops (2.54 cm thick) were collected from loins and data were collected for ultimate pH, visual color and marbling, Hunter L, a, b color, sensory, star probe (kg), and total lipid. Loins that had extreme star probe measurements were selected if in range for ultimate pH (5.54 – 5.86), visual marbling score (1.0 – 3.0), and total lipid (1.61 – 3.37%). These ranges were selected because previous research shows ultimate pH and lipid content can influence measured tenderness in fresh pork products. Selected samples were grouped into either a LSP (n = 12) or HSP (n = 12) group. Whole muscle protein samples were prepared from the selected samples.

Proteolytic data were collected using SDS-PAGE and Western blots. Calpain-1 was evaluated for autolysis. The proteins troponin-T, desmin, and titin were examined for degradation differences between LSP and HSP samples. All samples used for analysis with SDS-PAGE and Western blots were run in duplicate. Data were analyzed using the mixed procedure in SAS (version 9.4), with a fixed effect of star probe force and random effect of gel.

### Results and Discussion

Samples from both classification groups had similar ultimate pH, color, and loin and chop purge. The LSP group had an average star probe force of 4.95 kg, whereas the HSP group had an average star probe force of 7.75 kg. The appearance of the 76-kDa product of calpain-1 demonstrated this protease was completely autolyzed in samples from both classification groups. This shows calpain-1 was potentially active in samples. There were extreme proteolytic differences between LSP and HSP pork loins. LSP samples exhibited 26% less abundant intact (37-kDa) troponin-T and over 100% more abundant 37-kDa degradation bands and 27 – 30-kDa products of troponin-T ( $P < 0.01$ , Table 1). Intact desmin (55-kDa) was found to be 59% less abundant in LSP samples compared to HSP samples. Consequently, desmin degradation bands (38-kDa) were approximately 255% more abundant in LSP samples ( $P < 0.01$ , Table 2). Samples from both classification groups exhibited titin degradation, as determined from visual analysis of stained SDS-PAGE gels. Interestingly, some HSP samples showed some intact titin still remaining the whole muscle protein fraction (Figure 1).

Results demonstrate that even when ultimate pH, color, and purge are not different between samples, there can still be extreme differences in measured tenderness. Samples in the LSP group had significantly more degradation of proteins, demonstrating that proteolysis plays a large role in tenderness of aged pork loins.

**Table 1. Proteolytic data for of troponin-T, desmin, and in aged pork loin whole muscle samples. Samples were analyzed using densitometry, comparing measured protein bands to corresponding bands of an internal reference samples.**

<i>Item</i>	<i>Low Star Probe</i> ( <i>n = 12</i> )		<i>High Star Probe</i> ( <i>n = 12</i> )		<i>P-value</i>
	Least	Standard	Least	Standard	
	Squares Mean	Error	Squares Mean	Error	
<b>Troponin-T</b>					
37-kDa Intact Band	0.94	0.05	1.27	0.05	<0.01
30-kDa Degradation Band	1.37	0.07	0.65	0.07	<0.01
Degradation Products (27-30-kDa)	1.25	0.06	0.60	0.06	<0.01
<b>Desmin - Whole Muscle</b>					
55-kDa Intact Band	0.28	0.06	0.68	0.06	<0.01
38-kDa Degradation Band	1.10	0.10	0.31	0.10	<0.01

**Figure 1. Representative SDS-PAGE stained gel showing titin degradation in aged loin whole muscle pork samples. Samples in low and high star probe groups were evaluated for presence of the intact titin band (T1) and the degraded titin band (T2). Low star probe (LSP) and high star probe (HSP) samples are labeled.**

