

# SARCOPLASMIC PROTEIN PROFILE AS A PREDICTOR OF AGED PORK LOIN TENDERNESS

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## I. INTRODUCTION

Predicting pork tenderness is challenging due to the variety of quality factors that influence tenderness. Tenderness is modified by a multitude of factors including lipid content [1], pH [2], collagen content [3], fiber type [4], and protein degradation [5]. Pork loins of differing tenderness have been shown to have variations in structural proteins such as desmin [6]. The objective of this study was to document protein profile differences of pork loins aged 1-day postmortem based on high or low Star Probe (SP) values at 21 days (d) postmortem. Identifying early postmortem tenderness biomarkers is critical for quality-based pork marketing.

## II. MATERIALS AND METHODS

Pork loins (total n=12) were categorized by differences in SP measurements at 21 d postmortem from a larger group of twenty pork loins. Loins were sorted into Low SP (LSP) (n=6), (SP value below 5.80 kg at 21 d postmortem), and High SP (HSP) (n=6), (SP value above 7.00 kg at 21 d postmortem). Samples were aged 1 or 21 d for fresh quality data collection. At completion of each day of aging, chops were evaluated to determine percent purge, Hunter L, a, and b value, pH, color and marbling score [7], SP, cook loss and whole muscle intact desmin. Longissimus muscle sarcoplasmic protein extracts (10 mg/mL) were prepared from loin samples aged 1 d for 2-dimensional difference in gel electrophoresis (2D-DIGE). Sarcoplasmic protein (50 µg) extracts from each sample of each SP group were labeled alternatively with CyDye3 or CyDye5. A pooled reference sample containing equal amounts of all samples in this study was labeled with CyDye2. Labeled protein samples were prepared for running on immobilized pH gradient (IPG) strips (11-cm pH 3 to 10). After rehydration, strips were run in the first dimension (separating proteins by isoelectric point) on an Ettan IPGphor isoelectric focusing system. Strips were run for a total of 11,500 Volt-hours. Strips were equilibrated for second dimension separation using a 12.5% polyacrylamide SDS-PAGE gels (approximately 2,500 Volt-hours). DeCyder 2D software version 6.5 was used to analyze differences in spot abundance between Star Probe groups. 1 mg of reference sample was used on 12.5% polyacrylamide gels (18 by 16 cm and 1.5 mm thick; filtered reagents) to pick individual protein spots for identification. Spots were picked from two individual gels and protein identity was determined using liquid chromatography mass spectrometry.

## III. RESULTS AND DISCUSSION

SP value was significantly ( $P<0.01$ ) lower in the LSP group compared to the HSP group at each day of aging. Chop purge was significantly lower in the LSP group compared to the HSP group at 21 d aged but did not differ between categories at 1 d aged. pH was significantly greater in the LSP group compared to the HSP group at each day of aging. Marbling score was significantly greater in the LSP group compared to the HSP group at each day of aging. Cook loss was significantly lower in the LSP group compared to the HSP group at 21 d aged but did not differ between categories at 1 d aged. Intact desmin ratio was significantly lower in the LSP group compared to the HSP group at 21 d aged but did not differ between categories at 1 d aged. Color score, Hunter L, a, and b score were not different between SP groups. Quality data from each SP group is summarized in Table 1. Table 2 summarizes the identify of proteins that tend to be more abundant in the less tender aged pork.

Table 1. Summary of pork loin quality features categorized by SP group.

Item	LSP (n=6)		HSP (n=6)	
Days Aged	1	21	1	21
Star Probe (kg)	7.65 <sup>a</sup>	5.72 <sup>x</sup>	10.26 <sup>b</sup>	8.76 <sup>y</sup>
Purge (%)	0.14 <sup>a</sup>	2.41 <sup>x</sup>	0.16 <sup>a</sup>	4.54 <sup>y</sup>
pH	5.82 <sup>a</sup>	5.86 <sup>x†g=</sup>	5.76 <sup>b</sup>	5.76 <sup>y</sup>
Marbling Score	2.0 <sup>a</sup>	2.5 <sup>x</sup>	1.3 <sup>b</sup>	1.7 <sup>y</sup>
Cook Loss (%)	22.40 <sup>a</sup>	18.71 <sup>x</sup>	19.71 <sup>a</sup>	22.22 <sup>y</sup>
Intact Desmin Ratio <sup>1</sup>	1.16 <sup>a</sup>	0.38 <sup>x</sup>	1.32 <sup>a</sup>	0.93 <sup>y</sup>

\* <sup>a, b</sup> Means with different superscripts within rows at 1 days aged are significantly different within classification ( $P < 0.05$ ).

\* <sup>x, y</sup> Means with different superscripts within rows at 21 days aged are significantly different within classification ( $P < 0.05$ ).

<sup>1</sup> Ratio indicates abundance of intact desmin within samples at 1 d postmortem compared to an intact desmin from a day 0 reference sample present on each gel.

Table 2. Summary of identified proteins associated with aged pork loin SP classification.

Protein	Ratio <sup>1</sup>	P-value
Aldehyde Dehydrogenase	-2.14	0.14
Pyruvate Kinase	-1.84	0.16
Creatine Kinase	-1.64	0.12
Glyceraldehyde-3 Phosphate Dehydrogenase	-2.37	0.11
Glyceraldehyde-3 Phosphate Dehydrogenase	-1.59	0.15
Triose Phosphate Isomerase	-1.54	0.13
Triose Phosphate Isomerase	-1.52	0.13
Adenylate Kinase	-2.08	0.08

\* <sup>1</sup> Ratio indicates spot abundance differences between low and high star probe samples. Negative values represent less abundant in low star probe group. Positive value represents more abundant in low star probe group.

#### IV. CONCLUSION

The results of the current experiment demonstrate that pork quality features were significantly different between SP groups at each aging period. Protein profile differences showed a tendency for greater potential for glycolytic metabolism in HSP samples. Collectively, these observations support the hypothesis that metabolic and proteomic profile in perimortem and/or postmortem sarcoplasm can impact aged pork tenderness.

#### ACKNOWLEDGEMENTS

Partial funding from the Iowa Agricultural and Home Economics Experiment Station project number 3721. Appreciation is expressed to Danika Miller and Madeleine Kiepora for assistance with data collection.

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