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Determination of Protein Markers for Beef Tenderness in U.S. Select Beef

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Objectives

Beef tenderness is a primary quality feature that defines the consumer's perception of the product quality. Therefore definition of chemical characteristics that contribute to variation in tenderness within a specific USDA grade can help refine our understanding of meat tenderness. The objective of this experiment was to identify novel protein markers for tenderness in *Longissimus dorsi* sarcoplasmic fractions utilizing proteomics and a valuable library of beef samples.

Materials and Methods

Steaks (USDA Select grade) used in this trial were classified as low shear force (LSF, n = 12; 11.3 ± 1.6 kg) and high shear force (HSF, n = 12; 34.3 ± 4.7 kg), according to their slice shear force (SSF, kg) measurements at 14 d postmortem. Western blotting of whole-muscle samples was used to determine troponin-T degradation (indicator of overall proteolysis). Sarcoplasmic protein samples from d 2 postmortem were prepared for two-dimensional difference in gel electrophoresis (2D-DIGE). Samples from LSF and HSF were sorted according to SSF values and were matched to a pooled internal reference (representative of all samples). Protein samples were loaded (75 µg) on 24 cm strips, pH 6 to 9, and focused in immobilized pH gradient in the first dimension. For the second dimension the strips were run on 12.5% acrylamide gels (n = 12). After 2D-DIGE, protein spots found to be different in relative abundance between HSF and LSF samples, and with other proteins of interest, were identified using Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (LC-MS/MS). Immunoblots were performed to confirm protein identity.

Results

The LSF samples exhibited greater troponin-T degradation (as evidenced by abundance of a 30 kDa fragment) than the HSF samples (LSF ratio to reference was 1.8, HSF ratio to reference was < 0.01; $P < 0.01$). A total of 31 spots showed a significant difference ($P < 0.1$) in relative abundance across groups (HSF vs. LSF). Among these, 18 spots were identified as 8 different proteins (Table 1). Proteins that were more abundant in the LSF samples are metabolic enzymes involved in the glycolytic pathway (glyceraldehyde-3-phosphate dehydrogenase, fructose bisphosphate aldolase A). Among the six proteins that showed greater abundance in the HSF samples, four belong to the oxidative energy metabolism pathways (malate dehydrogenase, creatine kinase M-type, myoglobin, carbonic anhydrase 3), one is involved in redox pathways (peroxiredoxin-1) and another one in protein turnover (polyubiquitin). The greater abundance of spots corresponding to oxidative metabolism enzymes suggests that aerobic metabolism may decrease potential for postmortem proteolysis.

Conclusion

The results demonstrate that variation in tenderness within U.S. Select Top loin steaks is due in large part to variation in proteolysis. The variation in tenderness and proteolysis in these defined groups may be partially explained by a greater abundance of metabolic proteins involved in oxidative metabolism. This project was funded in part by grants from the Beef Checkoff (Denver, CO) and the Iowa Beef Industry Council (Ames, IA). The scholarship for the first author was granted by CNPq-Brazil.

Table 1. Differentially abundant proteins in high and low slice shear force groups

Protein	% Coverage	Ratio (LSF/HSF)	*P*-value
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	26.73	1.98	0.060
Fructose-bisphosphate aldolase A	62.36	1.79	< 0.050
Polyubiquitin (Fragment)	21.22	–1.18	0.100
Malate dehydrogenase, mitochondrial	63.31	–1.18	< 0.050
Creatine kinase M-type	70.87	–1.19	0.080
Myoglobin	99.35	–1.29	0.095
Carbonic anhydrase 3	79.12	–1.56	< 0.010
Perociredoxin-1	70.85	–1.24	0.055