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PROTEOMIC COMPARISON OF PORK LOIN MUSCLE EXTRACTS AND EXUDATE

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I. INTRODUCTION

The advancements in proteomic tools, instrumentation, and data analysis have greatly improved access to conduct larger proteomic experiments. The meat industry could benefit from specific biological markers indicative of product quality, like meat tenderness. Such a marker could more rapidly evaluate many samples with less sample preparation, minimal handling, and fewer destructive assays, like shear force measurements. These markers could also give companies a new phenomics parameter for genetic selection. Recent work suggests protein markers from low-ionic-strength soluble extracts of aged pork have the potential for such predictive capability. However, muscle exudate, or purge, is an ideal target for nondestructive sampling. Therefore, the objective was to compare similarities in identified proteins and protein abundance between two protein fractions of pork loins selected for early postmortem pH. It was hypothesized that structural and cytoskeletal protein differences observed in prior studies would be similarly observed and differentially abundant between pH categories and protein fractions.

II. MATERIALS AND METHODS

Pork loins were evaluated and selected based on pH at 1 d postmortem in a commercial plant [1]. Two balanced categories (n = 25/category) were generated to establish samples with differences in meat quality: Low pH (5.59–5.71) and High pH (5.90–6.46). Individual chops were aged for 1, 7, or 14 d postmortem, and frozen (-80°C) after their respective aging periods. Two separate proteomic experiments were conducted, where each pork loin sample and aging period was analyzed using two different starting protein fractions: 1) proteins extracted from muscle tissue in a low-ionic strength buffer (50 mM Tris-HCl [pH 8.5], 1 mM EDTA) and 2) proteins collected from muscle exudate. Briefly, proteins soluble in a low-ionic-strength buffer were extracted from powdered muscle tissue samples [2]. Muscle exudate samples were clarified by centrifugation and diluted 1:7 (vol/vol) in a low-ionic-strength buffer. All samples within protein fractions were diluted to equivalent protein concentrations before proteomic analysis. Proteins were reduced, alkylated, and digested with trypsin. Within each protein fraction, tryptic peptides were labeled with Tandem Mass Tag (TMT) 10plex reagents (90110, Thermo Scientific). A pool of all samples within the protein fraction was labeled with a TMT11-131C (A37724, Thermo Scientific) reagent to serve as an internal reference. Peptides were separated by liquid chromatography over 4.5 h and introduced into a Q-Exactive Hybrid Quadrapole-Orbitrap mass spectrometer (Thermo Scientific) [2]. The resulting .raw files were converted to .mzML using msConvert and analyzed with FragPipe (v. 22.0) using default settings against a *Sus scrofa* UniProt reference proteome .fasta file (22,673 sequences). Peptide spectrum match files were summarized and analyzed using MSstatsTMT (v. 2.14.2) with default settings. Proteins in < 60% of samples at each time point were excluded. Fitted mixed models were used to evaluate pairwise comparisons of pH category within protein fraction and time point. Proteins were considered significantly different at a Benjamini-Hochberg false discovery rate (FDR) < 0.05 and $\log_2$ fold difference > |0.3|.

III. RESULTS AND DISCUSSION

The High pH category was characterized by a trained panel as more ($P < 0.05$) tender, juicy, less ($P < 0.05$) chewy, and had lower ($P < 0.01$) instrumental star probe tenderness at each day postmortem [1]. Variation in water holding capacity was observed, where the High pH category had less ($P < 0.01$) cook loss and drip loss than the Low pH category [1]. This current work focused on using a population of pork loins divergent in meat quality parameters to define the protein differences between these groups. Figure 1 illustrates that 189 proteins were similarly identified across all time points between the two protein fractions. 49, 50, and 87 proteins were differentially abundant (FDR < 0.05) in the low ionic strength protein extracts between the High and Low pH categories at 1, 7, and 14 d postmortem, respectively. Similarly, 41, 24, and 46 proteins were differentially abundant (FDR < 0.05) in the muscle exudate between the High and Low pH categories at 1, 7, and 14 d postmortem, respectively. Proteins, including titin, filamin C, obscurin, troponin-I, -C, -T, desmin, and alpha actinin 2 and 3, were greater in abundance at 14 d postmortem in the muscle protein extracts in the High pH category, which was more tender based on instrumental star probe values. These observations validate previously reported differential proteins in aged pork loin based on meat quality categories [2]. This work expands on prior works by evaluating proteomic changes over time in the low-

ionic strength extract, where at 1 d postmortem, filamin C, desmin, and obscurin were significantly greater in the High pH category. Intriguingly, many of these same structural and cytoskeletal proteins were observed and were greater in abundance in the High pH category at each day postmortem in the muscle exudate. Indeed, prior reports of titin, troponin-C, -I, -T, nebulin, and filamin C [3] have been observed in pork muscle exudate samples. Among this current work's most novel and fundamental observations is identifying 8 proteins consistently differentially abundant in protein fractions and all time points. For these individual proteins, 14-3-3 protein gamma, alpha-crystallin B chain, calsequestrin, four and a half LIM domains 1, heat shock protein beta 1, LIM domain binding 3, myosin binding protein C2, and obscurin, within themselves the $\log_2$ fold difference across protein fractions and time points was all directionally the same in comparisons between the High and Low pH categories. This discovery at the protein level demonstrates that these specific proteins could be among the best targets to assess to rapidly predict the ultimate meat quality.

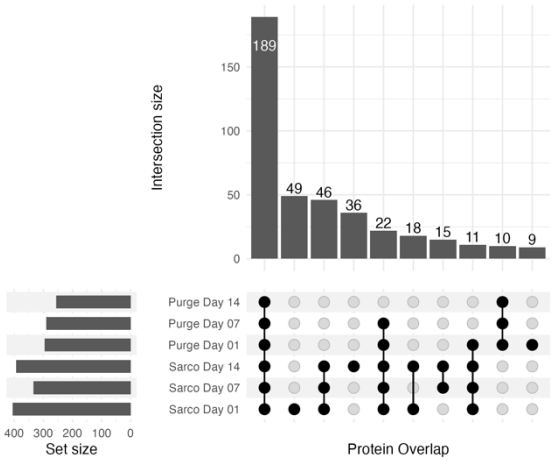


Figure 1. Comparison of identified proteins between protein fractions over postmortem time points.

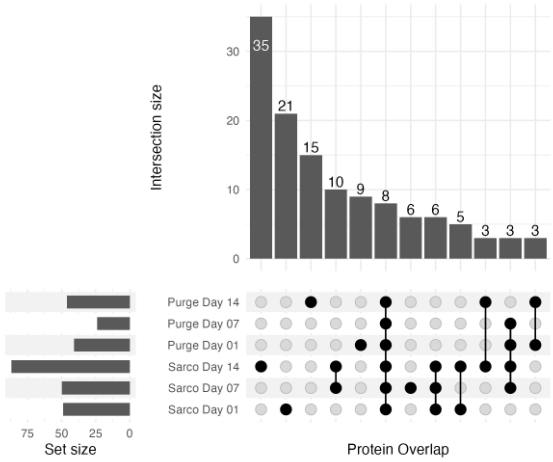


Figure 2. Comparison of significantly different proteins between protein fractions over postmortem time points.

IV. CONCLUSION

Selecting pork loins based on early postmortem pH resulted in two categories of pork loins, where the High pH category had pork chops rated more favorably by the trained sensory panel. Instrumental star probe tenderness was also lower in High pH samples, and water holding capacity was higher in High pH samples. Altogether, the meat quality phenotype was different, which allowed for the investigation of protein-level differences in these pork loin samples. This work validated prior published work of similar pork loin protein extracts and extended these observations across the postmortem aging period and in two protein fractions. The consistency of differences in specific proteins demonstrates the potential utility of these proteins to be markers of higher quality pork loins. Quantifying alpha-crystallin B chain, calsequestrin, four and a half LIM domains 1, or obscurin could help classify pork loins early postmortem by their ultimate eating quality. Collectively, these data identify and illustrate robust protein markers of pork loin quality that persist within the muscle tissue and muscle exudate over the aging period.

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