Desmin and Peroxiredoxin-2 are Potential Biomarkers for Pork Tenderness

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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. Two-dimensional difference in gel electrophoresis (2D-DIGE) was used to identify differences in sarcoplasmic proteomes between LSP and HSP groups. Metabolic, stress response, structural, and regulatory protein spots were identified to be significantly different between classification groups. The structural protein desmin was identified to be more abundant in LSP samples and the stress response protein peroxiredoxin-2 was identified to be more abundant in HSP samples. Because these protein were identified as being significantly different between classification groups in the soluble, sarcoplasmic fraction of samples, these proteins are potential candidates to be biomarkers to differentiate between tough and tender aged pork loins.

Introduction

Consumers, purveyors, and chefs continue to be presented with inconsistent fresh pork quality, specifically pork tenderness. Currently, there is no method to rapidly differentiate between tough and tender fresh pork in processing facilities. Pork loins that exhibit differences in measured tenderness have shown differences in abundance of metabolic, stress response, structural, and regulatory proteins. Rapidly identifying proteins that differ between tough and tender pork is fundamental to identify biomarkers that can be used to differentiate between tough and tender fresh 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 they were also within the following ranges for the following traits: 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 showed 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.

Sarcoplasmic protein samples were prepared from the selected loins to be used for 2D-DIGE. Proteins are separated by charge and size using this method. In order to have a direct in-gel comparison between LSP and HSP samples, samples were labeled with fluorescent dyes that excite at different wavelengths. DeCyder statistical software (version 6.5) was used to identify significantly different spots between classification groups. Identities of these protein spots was determined using mass spectrometry. In order to confirm the identity of the proteins from 2D-DIGE and mass spectrometry, sarcoplasmic protein samples were prepared for SDS-PAGE and Western blot analysis. Samples from both classification groups were run in duplicate for 2D-DIGE, SDS-PAGE, and Western blots. Data for SDS-PAGE and Western blots 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/chop purge. The average star probe for the LSP samples was 4.95 kg and the average star probe force for the HSP samples was 7.75 kg. These values demonstrate there was an extreme difference in instrumental tenderness between classification groups. There was a total of 102 protein spots significantly different between LSP and HSP groups in the sarcoplasmic protein fraction ($P < 0.01$). Of these spots, 16 of the most prevalent were picked for identification. The protein spots identified included metabolic, stress response, structural, and regulatory proteins.

The structural protein desmin was found to be 90% more abundant in LSP samples (Figure 1). After further analysis of the peptides identified from this protein spot, it was found that only a fragment of desmin (rod portion) was identified. This demonstrates desmin was potentially degraded into the sarcoplasmic protein fraction. This is a novel result because desmin is typically thought to be associated with the insoluble myofibrillar protein fraction due to the role it plays with structure of myofibrils. The stress response protein peroxiredoxin-2 (two spots identified) was found to be 37% and 51% more abundant in

HSP samples (Figure 1). Peroxiredoxin-2 plays a role in protecting cells from oxidative stress.

Protein spot identities were confirmed with SDS-PAGE and Western blots. Western blots confirmed the presence, size, and abundance of these proteins in the sarcoplasmic protein fraction (Figure 2). The sarcoplasmic protein fraction is soluble and is found in meat purge. The ease of solubility of these proteins, coupled with the extreme differences in abundance between LSP and HSP samples, demonstrates the potential for use of desmin and peroxiredoxin-2 as biomarkers to differentiate between tough and tender pork samples.

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Figure 1. Representative 2D-DIGE gel of the 16 protein spots identified from the sarcoplasmic protein fraction of aged pork loins. Desmin and Peroxiredoxin-2 are labeled.

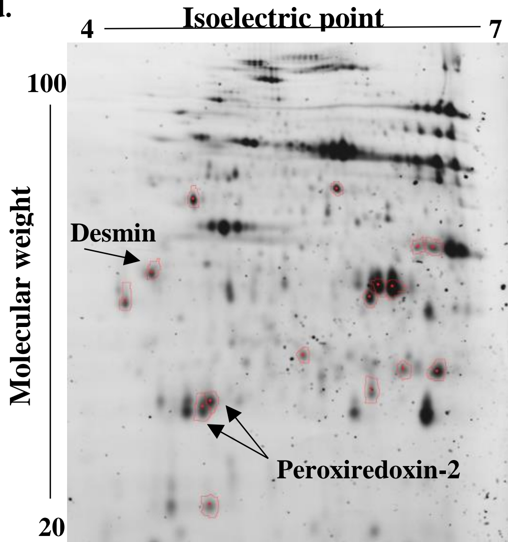
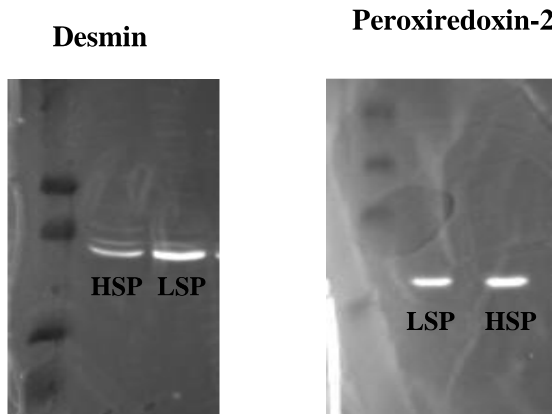


Figure 2. Representative Western blots images of desmin and peroxiredoxin-2 from the sarcoplasmic protein fraction of aged pork loins. Western blots were used to confirm protein identity, size, and abundance from protein spots identified from 2D-DIGE and mass spectrometry. Low star probe (LSP) and high star probe (HSP) samples are labeled.



Acknowledgements