RESEARCH REPORT

PORK QUALITY

pork checkoff

Title: New Insights on Cellular Events and Gene products to Predict Pork Quality **19-083**

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Industry Summary:

The production of high-quality pork is essential to the food and agriculture industries' success and sustainability. Tenderness of fresh pork is among the most critical attributes determining consumer satisfaction of fresh pork and, therefore, the product's value. Consumers assign value based on experienced quality, which is primarily influenced by tenderness. In the retail, food service, and export markets, a significant variation in pork tenderness is experienced, and this variation detracts from the product value. Continued and sustained market presence requires a consistent product quality. Therefore, defining the quality before delivery is necessary for initial and ongoing market access. Controlling and predicting fresh pork quality is exceptionally challenging because, despite years of research, we still do not fully understand the biology of early postmortem muscle that governs major quality features, including tenderness and water-holding capacity. The only way the research community and the food industry can develop reliable pork quality indicators is to understand factors that determine quality.

Pork tenderness improves through the degradation of myofibrillar proteins in postmortem muscle. Postmortem proteolysis of myofibrillar proteins is well documented during meat aging, primarily by the calpain family of proteases. Degradation of myofibrillar proteins can occur at variable rates and occasionally to a minimal degree, resulting in less tender meat. One source of proteolysis variation can be the rate and extent of tissue and protein oxidation. Peroxiredoxins are a family of antioxidant proteins ubiquitously expressed in cells. Their function is to reduce reactive oxygen species (ROS), primarily hydrogen peroxide. We hypothesized that variation in these antioxidant proteins could vary across pigs and phenotypes and, therefore, explain variation in pork quality. Specifically, we asked how abundance and state of peroxiredoxin-2 and peroxiredoxin-6 are linked to fresh pork loin tenderness differences.

The results demonstrate that peroxiredoxin-2 is involved in postmortem changes, specifically proteolysis, linked to pork's tenderness. The decrease in abundance of peroxiredoxin-2 shows that it is degraded or altered in some way during aging. Less abundant PRXD-2 in aged pork was observed in more tender pork with more significant desmin degradation. Importantly, abundance and oxidation of peroxiredoxin-2 are linked to swine growth performance with a greater aboundnce and oxidation linked to less efficient growth and less tender pork. A new observation that can help us understand the nature of the metabolism is that the oxidation states of PRXD-2 change during aging. This question has never been investigated, and the results show that some forms of PRXD-2 are more resilient during aging, which could explain less proteolysis. The next question is to define the molecular nature of the novel band 3 of non-reduced PRXD- to determine its role in proteolysis and definition of fresh pork tenderness.

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

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Key Findings:

- Greater abundance of the antioxidant protein Peroxiredoxin-2 in aged pork loin indicates less proteolysis and less tender pork.
- The abundance of Peroxiredoxin-2 changes at a variable rate during aging based on tenderness classification.
- There is a transition in oxidation state of Peroxiredoxin -2 during postmortem aging.
- Abundance of Peroxiredoxin-6 did not change during postmortem aging.
- Antemortem conditions that alter Peroxiredoxin-2 status and abundance do not change overall protein oxidation in pork longissimus muscle.

Keywords: Pork, tenderness, proteolysis, postmortem aging, protein oxidation, peroxiredoxin-2, peroxiredoxin-6.

Scientific Abstract: This should be a scientific description limited to one page in length to describe your project and its results.

Pork tenderness improves through the degradation of myofibrillar proteins in postmortem muscle. One source of proteolysis variation can be the rate and extent of tissue and protein oxidation. Peroxiredoxins are a family of antioxidant proteins ubiquitously expressed in cells. Their function is to reduce reactive oxygen species (ROS), primarily hydrogen peroxide. Because PRXD-2 is known to have several oxidation states, we hypothesized that the oxidation state of PRXD-2 could also change with postmortem storage and impact fresh pork tenderness. We also hypothesized that these variations in PRXD-2 reflect variation in fresh pork proteolysis and tenderization. Peroxiredoxin-6 does not exhibit differences in oxidation state, but very little is known about how it changes with postmortem storage. We have hypothesized that PRXD-6 abundance throughout aging can change and reflect differences in tenderness phenotype.

Because PRXD-2 is known to have several oxidation states, we hypothesized that the oxidation state of PRXD-2 could also change with postmortem storage and impact fresh pork tenderness. The variations in PRXD-2 are hypothesized to affect or at least reflect variation in fresh pork proteolysis and tenderization. Peroxiredoxin-6 does not exhibit differences in oxidation state, but very little is known about how it changes with postmortem storage. Fresh pork loins were collected at 1 d postmortem. Pork chops (2.54 cm) were fabricated and aged for 1, 8, 14, or 21 d postmortem. After aging, chops were cooked to 68°C, and instrumental tenderness was measured using the star probe. Star probe values were used to classify chops into high (HSP; star probe > 7.0 kg, n=6) and low (LSP; star probe < 5.8 kg, n=6) star probe groups based on 21 d analysis. Whole muscle proteins were extracted and prepared for desmin degradation. Sarcoplasmic proteins from the *longissimus dorsi* from each aging time were solubilized in ice-cold, low-ionic strength buffer, and samples with and without a reducing agent were prepared for immunoblot analysis. In a separate experiment, PRXD-2 abundance and profile and protein carbonylation were determined longissimus muscle samples from pigs treated to create differences in oxidative stress (selection for residual feed intake and disease challenge (n=6 per treatment combination)). PRXD-6, calpain autolysis, and desmin western blots were executed with reduced samples. Reduced and non-reduced PRDX-2 abundance and profile were determined with immunoblot assay.

The LSP group had lower star probe values than the high star probe group at each day of aging and more desmin degradation early postmortem. Abundance of PRXD-6 was not different across aging time or tenderness classification. In contrast, PRXD-2 decreased with aging time and showed a more significant decrease in the LSP group, indicating that it is degraded or altered during the aging process at a different rate in the tender group. A different pattern of non-reduced PRXD-2 during storage showed one species of PRXD-2 (band 3) was more abundant in the aged pork loin from the tough (high star probe) pork. This observation provides insight that this form of the protein should be more reliable in predicting pork tenderness.

The abundance of both forms of PRXD-2 (reduced and non-reduced) was affected by the RFI line. Infection status did not affect these forms of PRXD-2. Infection status nor RFI line influenced protein carbonylation in the pork loin. Antioxidant proteins like PRXD-2 are involved in postmortem changes, specifically proteolysis, linked to pork's tenderness. A new observation is that the oxidation states of PRXD-2 change during aging. The results show that some forms of PRXD-2 are more resilient during aging, which could explain less proteolysis. The link between growth performance and PRXD-2 abundance implies a connection of PRXD-2 to muscle metabolism and pork

quality. The next question is to define the molecular nature of the novel band 3 of non-reduced PRXD-2 to determine its role in proteolysis and definition of fresh pork tenderness.

Introduction:

Pork tenderness improves through the degradation of myofibrillar proteins in postmortem muscle. Postmortem proteolysis of myofibrillar proteins is well documented during meat aging, primarily by the calpain family of proteases. Degradation of myofibrillar proteins can occur at variable rates and occasionally to a minimal degree, resulting in less tender meat. One source of proteolysis variation can be the rate and extent of tissue and protein oxidation. Peroxiredoxins are a family of antioxidant proteins ubiquitously expressed in cells. Their function is to reduce reactive oxygen species (ROS), primarily hydrogen peroxide.

Peroxiredoxins exist as a homodimer with two identical subunits involved in the active site of the protein. The catalytic cycle of peroxiredoxins involves a peroxidatic cysteine residue, which is oxidized to a sulfenic acid. The peroxidatic cysteine can be further oxidized to a sulfinic acid or inactivated to a sulfonic acid. In postmortem skeletal muscle, peroxiredoxins, specifically peroxiredoxin-2 (PRDX-2) and peroxiredoxin-6 (PRDX-6) are hypothesized to play a critical role protecting against oxidative damage by ROS. Peroxiredoxin-2 (PRDX-2) was more abundant in aged pork that was less tender (Carlson et al. 2017). A greater abundance of PRXD-6 was more abundant in tough beef than tender beef (XXX). Reactive oxygen species, such as hydrogen peroxide, contribute to tissue and protein oxidation (Phaniendra et al., 2015). The active site of calpain can be oxidized, inhibiting calpain's proteolytic capability, limiting the development of meat tenderness (Lametsch et al., 2008). Peroxiredoxin-2 may limit calpain oxidation and consequently improve meat tenderness. However, the relationship between PRDX-2 and meat tenderness during postmortem aging are not well understood.

These observations are consistent with the detrimental effect protein oxidation appears to have on fresh meat tenderness. Because PRXD-2 is known to have several oxidation states, we hypothesized that the oxidation state of PRXD-2 could also change with postmortem storage and impact fresh pork tenderness. We also hypothesized that these variations in PRXD-2 reflect variation in fresh pork proteolysis and tenderization. Peroxiredoxin-6 does not exhibit differences in oxidation state, but very little is known about how it changes with postmortem storage. We have hypothesized that PRXD-6 abundance throughout aging can change and reflect differences in tenderness phenotype. Based on our preliminary observations and the literature, the following objectives were addressed.

Objectives:

Objective 1: To determine the extent to which abundance and diversity of peroxiredoxin-2 and peroxiredoxin-6 correspond to documented differences in fresh pork technical and sensory quality.

Objective 2: To determine the extent to which abundance and diversity of peroxiredoxin-2 and peroxiredoxin-6 change during postmortem aging and how these changes are linked to fresh pork quality.

Objective 3: To understand the extent to which technical and sensory quality are explained by endogenous oxidation of sarcoplasmic and myofibrillar proteins that we know occurs.

Materials & Methods

Objectives 1 & 2

Twenty pork loins, previously described (Schulte et al., 2019), were sorted based on 21 d aged pork loin star probe values to obtain high and low star probe groups. Previously, pairs of loins were collected from a commercial harvest facility at 1 d postmortem and transported to the Iowa State University Meat Laboratory for fabrication (Schulte et al., 2019). Chops were aged 1, 8, 14, or 21 d and quality data were collected as previously described (Schulte et al., 2019). Loins with high (star probe>7.0 kg) and low (star probe<5.8 kg) star probe values at 21 d postmortem were chosen to represent extreme differences in star probe value. Marbling score and pH value parameters were set as inclusion criteria to further identify sample experimental groups.

Loin marbling scores at 21 d aging ranged from 1.0 to 3.0. Loin pH at 21 d aging ranged from 5.69 to 5.93. This 21 d aging classification criteria narrowed the sample set to a balanced experiment of high (n=6) and low (n=6) star probe categories.

Frozen meat containing only the *longissimus dorsi* (100 g) was homogenized in liquid nitrogen. Samples from each aging time (0.5 g) were homogenized and whole muscle protein extracts were completed using 10 mM sodium phosphate, pH 7.0, and 2% SDS (wt/vol) as described by Carlson et al. (2017a).

Frozen meat containing only the *longissimus dorsi* (100 g) was homogenized in liquid nitrogen. Samples from each aging time (3 g) were homogenized and sarcoplasmic proteins were extracted (4°C; 50 mM Tris-HCl and 1 mM EDTA, pH 8.0.) Protein concentration was adjusted to 4 mg protein/mL in each sample using low ionic strength extraction buffer, 500 µL of Wangs tracking dye (3 mM EDTA, 3% [wt/vol] SDS, 30% [vol/vol] glycerol, 0.01% [wt/vol] pyronin-Y, 30 mM Tris HCl, pH 8.0) and 100 µL of mercaptoethanol (reduced samples) or 100 additional µL of tracking dye (non-reduced samples). All prepared samples were vortexed, then reduced samples were heated to 50°C for 20 min. Non-reduced samples were not heated. All samples were stored at -80°C until further use. Reduced samples were used for calpain autolysis, PRXD-6, and reduced PRXD-2. Non reduced samples were used specifically for the non-reduced PRXD-2 western blots.

Desmin degradation in the whole muscle protein extracts (1, 8, 14, and 21 d aged) reduced PRXD-2, PRXD-6 (1, 8, 14, and 21 d aged) as well as calpain-1 autolysis at 1 d postmortem were determined using one-dimensional SDS-PAGE gel electrophoresis as described by Carlson et al. (2017a, b). The non-reduced PRXD-2 sample was also resolved on an SDS-PAGE gel with the exception that the sample did not have any reducing agent. A reference (longissimus muscle, 0 d aging) was used for desmin analysis, and a 1 d reference sample was used for peroxiredoxin-2 analysis. SE 260 Hoefer Mighty Small II electrophoresis units (Hoefer, Inc., Holliston MA) were used to run 15% gels for desmin, troponin-T, and peroxiredoxin-2 analysis, and 10% gels for calpain-1 autolysis. Peroxiredoxin-6 abundance was determined using a monoclonal antibody (anti-PRDX6; AB1333, AbCam. Reduced and non-reduced PRDX-2 and reduced intact desmin were determined using monoclonal rabbit anti-peroxiredoxin-2 antibody (ab109367; ABCam, Cambridge, UK) and polyclonal rabbit anti-desmin antibody (ISU), respectively, and normalized by a reference sample on each gel. Calpain autolysis was evaluated using a monoclonal mouse anti-calpain-1 (MA3-940, Thermo Scientific, Rockford, IL) and proportion of the large subunit detected as an autolyzed catalytic subunit was determined. Results were analyzed using PROC MIXED of SAS 9.4 with fixed effects of days aging and classification.

Objective 3 Determination of how Peroxidoxin-2 content affects overall protein oxidation.

Pigs from different residual feed intake (RFI) selection lines were used to generate muscles exhibiting differences in oxidative stress. More efficient pigs (with low RFI) show less muscle oxidation and ROS generation by mitochondria in muscle. A health challenge was also utilized with the RFI pigs to add another possible source of oxidative stress. The pigs used in this objective were a subset of a larger project (Helm et al., 2018a,b). All animals in this study were handled following the Iowa State University Institutional Animal Care and Use Committee (IACUC #6-16-8298-S). Six littermate pairs of low RFI and six littermate pairs of high RFI barrows from the eleventh generation of the Iowa State University RFI Project were randomly selected from a larger set of pigs (24 selected from 100). At 50±7kg in weight, littermate pairs were split and randomly assigned to individual pens across two separate rooms in the same barn, resulting in six high RFI and six low RFI pigs in each room. Two rooms were used (health challenged and control).

On 0 days post-inoculation (dpi), barrows in the health-challenged room were inoculated with *Mycoplasma hyopneumoniae* and *Lawsonia intracellularis*, while those in the control room were inoculated with a sham. For the respiratory inoculation, *Mycoplasma hyopneumoniae* was dosed in a 10 mL inoculum (strain 232, containing 10^5 color-changing units/mL) through an intra-tracheal gavage. For the enteric challenge, pigs were intra-gastrically gavaged with 40 mL of *Lawsonia intracellularis* inoculum (2 mL gut homogenate, containing 2×10^7 *Lawsonia intracellularis* organisms). Inoculums were prepared at the Iowa State University Veterinary Diagnostic Laboratory (Ames, Iowa). Pigs in the control room were inoculated with a sham. The result was a 2 x 2 factorial design with four experimental groups, including low RFI control, low RFI infected,

high RFI control, and high RFI infected (n=24 total, n=6 per group) (Helm et al., 2018b). Criteria for confirmation of challenge included daily observation, sera antibody at dpi 21, and lesion scoring at dpi 21. MhLI pigs were confirmed positive for both pathogens (Helm et al. 2018b). On 21 dpi (projected peak infection), pigs were euthanized using a captive bolt stunning device followed by exsanguination. Following euthanasia, longissimus muscle was immediately removed, trimmed to remove adipose and connective tissue, and homogenized in liquid nitrogen. Samples were held at -80°C until protein extraction.

Carbonyl content of sarcoplasmic proteins was determined according to Reznick and Packer (1994). All samples were run in triplicate. Samples from the sarcoplasmic protein fraction were diluted to 6 mg/ml concentration using 1mM EDTA, 50 mM $NaHPO_4$ pH 7.4 at 4°C. One mL of 6 mg/ml sample was incubated with 4 ml of 10 mM 2, 4-Dinitrophenylhydrazine (DNPH) in 2.5 M HCl. A second 1 ml of 6 mg/ml sample was added to 4 ml of 2.5 M HCl to serve as a control. All samples were vortexed and incubated in the dark at 22°C for 30 minutes, vortexed again, and incubated at the same temperature for an additional 30 minutes. After incubation, the reaction was stopped with the addition of 5 ml of 20% (w: v) trichloroacetic acid and placed in an ice bath for 10 minutes before being centrifuged at 3100 ×g for 10 minutes at 4°C. Following centrifugation, the supernatant was discarded, and the pellet was washed in 10% (w: v) trichloroacetic acid before being vortexed and centrifuged at 3100 ×g at 4°C. After rinsing, the collected protein was s dissolved entirely. All DNPH treated samples were read using an Ultrospec 3000 spectrophotometer (Pharmacia Biotech, Piscataway, NJ) at 365nm. Protein content was determined from control (no DNPH) samples using a Bradford assay (BioRad Laboratories, Hercules, CA). Total carbonyl concentration (nmoles/mg protein) was calculated using the molecular extinction coefficient for DNPH.

Results

Table 1 Summary of fresh pork loin quality attributes, proteolysis of whole muscle protein fraction desmin, sarcoplasmic protein fraction calpain-1 autolysis, peroxiredoxin-2, and peroxiredoxin-6 in pork Longissimus dorsi (LM) of selected star probe groups and aging time.

Item	Low Star Probe Group (n=6)					High Star Probe Group (n=6)					*P*-Value	
Days Aged	**1**	**8**	**14**	**21**	**SEM**	**1**	**8**	**14**	**21**	**SEM**	**Days Aged**	**Category**
Star Probe (kg)[1]	7.64^{ax}	5.50^{bx}	5.86^{bx}	5.72^{bx}	0.57	10.26^{ay}	8.59^{by}	8.30^{by}	8.76^{by}	0.60	<0.01	<0.01
pH[2]	5.82^{abx}	5.79^{b}	5.86^{ax}	5.86^{ax}	0.02	5.76^{ay}	5.73^{a}	5.78^{ay}	5.76^{ay}	0.02	0.06	<0.01
Intact Desmin[3]	1.16^{a}	0.58^{b}	0.47^{bx}	0.38^{bx}	0.12	1.32^{a}	0.81^{b}	0.81^{by}	0.93^{by}	0.13	<0.01	<0.01
Peroxiredoxin-2[4]	1.01^{a}	0.65^{bx}	0.73^{b}	0.58^{bx}	0.06	1.00^{a}	0.95^{aby}	0.78^{bc}	0.77^{cy}	0.06	<0.01	<0.01
Autolyzed Calpain-1 (%)[5]	53.67	-	-	-	9.14	57.83	-	-	-	9.14	-	0.75
Peroxiredoxin-6[6]	1.17	1.11	1.02	1.15	0.09	1.08	0.99	0.87	1.07	0.10	0.86	.76

[1] A five-point star probe attachment fitted with an Instron was used to assess the force needed to compress a chop to 20% of its original height (Schulte et al. 2019).
[2] pH measurements were taken at the center of each chop.
[3] Ratio of the densitometry units of the degraded 30-kDa band of the sample compared to the 30-kDa band of the reference sample.
[4] Ratio of the densitometry units of the intact 22-kDa band of the sample compared to the 22-kDa band of the reference sample.
[5] Percentage indicates the percent of autolyzed calpain-1 as a total of calpain-1 in each sample.
[6] Ratio of the densitometry units of the intact Peroxiredoxin-6 band of the sample compared to the intact Peroxiredoxin-6 band of the reference sample.

[a, b, c, d] Means within star probe group with different superscripts are significantly different (P<0.05).[4] [x, y] Means within rows and day of aging with different superscripts are significantly different (P<0.05).

Figure 1. Representative western blots of non-reduced and reduced peroxiredoxin-2 during aging.

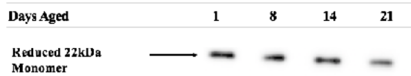


Figure 2. Reduced peroxiredoxin-2 (22 kDa) during postmortem aging based on tenderness classification

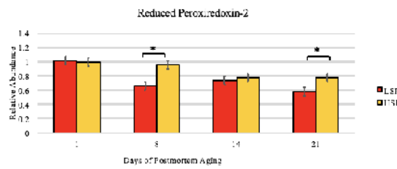
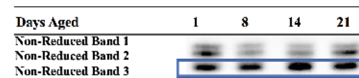
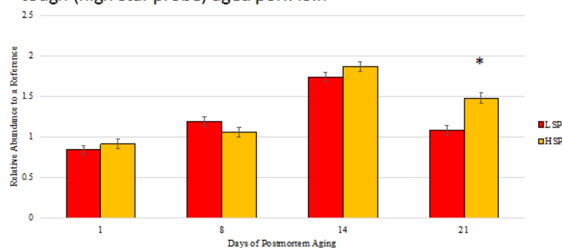


Figure 2. Representative western blot of non-reduced peroxiredoxin-2 classified by days aging.



The results of objectives 1 and 2 are summarized in Table 1. It is confirmed that chops in the low star probe group (Tender) had lower star probe values than the high star probe group at each day of aging. In general, aging eight days resulted in an improvement of star probe value in each group. However, tenderness did not improve with extended aging. These observations are confirmed with the desmin degradation data that showed greater desmin degradation (and thus proteolysis) in the tender group. This defines a very good sample set to investigate the contribution of antioxidant proteins PRXD-2 and PRXD-6 to variation in tenderness. The abundance of PRXD-6 was not different across aging time or tenderness classification. In contrast, PRXD-2 did decrease with aging time and showed a greater decrease in the low star probe group, indicating that it is degraded or altered during the aging process at a different rate in the tender group. This confirms our earlier observations (Carlson et al. 2017) that PRXD-2 is less abundant in tender aged pork loin. Peroxiredoxin-2 western blots (reduced) are shown in Figure 1 and Figure 2. Here we see that abundance of PRXD-2 is not different in tenderness groups early postmortem, but is in aged pork.

Figure 3.Non-reduced peroxiredoxin-2 band 3 is more abundant tough (high star probe) aged pork loin



We hypothesized that the form of PRXD-2 could help explain the contribution of PRXD-2 to proteolysis and tenderization. Figure 2 shows a western blot of PRXD-2 in the non-reduced state. Clearly, this is a different pattern than reduced PRXD-2 shown in Figure 1, where we see only one band. We observed that the lowest band (band 3) in figure 3 was the most resilient PRXD-2 non-reduced band. It became clear that this form of PRXD-2 is more abundant in the aged pork loin from the tough (high star probe) pork. This observation provides insight that this form of the protein should be more reliable in predicting pork tenderness.
The abundance of both forms of PRXD-2 (reduced and non-reduced) was affected by the RFI line (Table 2). There was a greater abundance of reduced PRXD-2, and the band corresponding to band 3 in Figure 2. Infection status did not affect these forms of PRXD-2. Infection status nor RFI line influenced protein carbonylation in the pork loin.

Table 2. Comparisons of RFI line*infection status combinations with main effects of residual feed intake (RFI) line and infection status (IS), and interactions of RFI line and Infection Status on peroxiredoxin-2 (Prdx-2) features, and protein carbonylation of the longissimus skeletal muscle for RFI line* infection status.

	Treatment					P-value[1]		
Trait	LRFI Control	HRFI Control	LRFI MhLI	HRFI MhLI	SEM	RFI Line	Infection Status	RFI Line* Infection Status
n	6	6	6	6				
Total Prdx-2[2]	0.55^{a}	0.65^{ab}	0.61^{a}	0.69^{b}	0.058	**0.035**	0.269	0.893
Prdx-2 Non-reducing Gel Band 3	0.90^{ab}	1.03^{bc}	0.87^{a}	1.11^{c}	0.051	**0.0006**	0.620	0.277
Carbonyl Content, nM/mg protein	7.34^{a}	9.61^{a}	9.44^{a}	7.71^{a}	1.16	0.801	0.923	*0.064*

[a,b,c]Means with differing subscripts in the same row indicate a significant difference (P≤0.05).

[1]Significant statistical differences (p≤0.05) are **bolded**. Statistical trends (0.10>p>0.05) were *italicized*
[2]Relative protein content was determined using western blotting by comparing the densitometry of sample protein bands to a reference sample of 0 days aged sarcoplasmic extract protein sample. Thus, the observed data are a ratio of sample to reference.
[3]Comparisons were made through a ratio of the second, faster-migrating protein band, and all immunoreactive protein in sample lane

Discussion:

The production of high-quality pork is essential to the food and agriculture industries' success and sustainability. Tenderness and water binding capacity of fresh meat are among the most critical attributes determining consumer satisfaction of fresh pork and, therefore, the product's value. Consumers assign value based on experienced quality, which is primarily influenced by tenderness. In the retail, food service, and export markets, a significant variation in pork tenderness is experienced, and this variation detracts from the product value. Continued and sustained market presence requires a consistent product quality. Therefore, defining the quality before delivery is necessary for initial and ongoing market access. Controlling and predicting fresh pork quality is exceptionally challenging because, despite years of research, we still do not fully understand the biology of early postmortem muscle that governs major quality features, including tenderness and water-holding capacity. The only way the research community and the food industry can develop reliable pork quality indicators is to understand factors that determine quality.

Using experiments to define the profile of the proteins in fresh pork, we discovered new knowledge to advance the quality and consistency of fresh pork. We compare the profile of the antioxidant protein profile of fresh pork loins of known quality differences, thereby empowering us to identify the types of proteins that are routinely linked to variations in fresh pork quality and sensory value. The completed work is a systematic assessment of how the antioxidant and metabolic features of the proteins in muscle impact the conversion of muscle into high-quality fresh pork. The results are novel. They represent an in-depth look at two previously unexplained factors that are moderators of metabolism and their effects on the processes that impact meat quality. The outcome of these experiments will provide means for farmers, animal scientists, and food scientists to improve the value of fresh pork for the domestic and global markets.
Experiments in objective 3 are important because they demonstrate that PRXD-2 does vary with different genetic lines and, therefore, could be controlled. Importantly, PRXD-2 was more abundant in the less efficient

line, and that line has already been shown to generate more ROS (Grubbs et al. 2014) and oxidized protein (Cruzen). Neither attempt to demonstrate differences in oxidative stress (RFI or Infection) resulted in differences in these forms of PRXD-2 or carbonylation of protein. This is likely due to the emphasis of many metabolic mechanisms to maintain homeostasis.

The results demonstrate that antioxidant proteins like PRXD-2 are involved in the postmortem changes, specifically proteolysis, linked to tenderness in pork. The decrease in abundance of PRXD-2 shows that it is degraded or altered in some way during aging. Less abundant PRXD-2 in aged pork was observed in more tender pork with more significant desmin degradation. Another new observation is that the oxidation states of PRXD-2 change during aging. This question has never been investigated, and the results show that some forms of PRXD-2 are more resilient during aging, which could explain less proteolysis. Oxidation of calpain proteinases inhibits proteolysis. The oxidized PRXD-2 might also indicate how much proteolysis can occur. The next question is to define the molecular nature of the novel band 3 of non-reduced PRXD-2 (seen in Figure 2) to determine its role in proteolysis and definition of fresh pork tenderness.

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