



Fresh Pork Loin pH Influences Meat Quality and the Presence of Desmin Degradation Products

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Abstract: This study aimed to test the hypothesis that pork loin pH at 1-day postmortem influences fresh pork quality and degradation of desmin. Fresh pork loins from a commercial facility were evaluated based on 1-day postmortem pH value and ultimately classified as high/normal (5.90–6.46, $\bar{x}$ = 6.09; n = 25) and low/normal (5.59–5.71, $\bar{x}$ = 5.67; n = 25). Instrumental color and drip loss were determined on 1-day postmortem chops (2.54 cm). The remaining chops were individually vacuum packaged, aged 1, 7, or 14 d postmortem at 4°C, and then frozen until further analyses. Intramuscular lipid and moisture content was determined through proximate analysis. Instrumental star probe tenderness was evaluated on cooked chops (68°C) from each aging time. Trained sensory panel evaluation and cooking loss were determined on cooked, 14-d postmortem chops. Western blot analysis examined the presence of desmin degradation products within low ionic strength extracts of *longissimus thoracis*. Based on the pH categories, star probe tenderness, drip loss, cook loss, palatability traits, meat color measurements, and the presence of desmin degradation products were significantly different, whereas intramuscular lipid content was not different. High/normal pH chops had lower L values at 1-d (P < 0.01) and 14-d postmortem (P < 0.01). Drip loss was greater (P < 0.01) in 1-d low/normal pH chops, as was cooking loss at each aging time (P < 0.01). The trained sensory panel rated high/normal pH loins as juicier (P < 0.01), more tender (P < 0.01), and less chewy (P < 0.05). High/normal pH chops had lower star probe values compared to low/normal pH chops at 1 (P < 0.01), 7 (P < 0.01), and 14 (P < 0.01) d postmortem. High/normal pH chops exhibited a greater proportion of samples containing desmin degradation products within the low ionic strength extract at each aging time (P < 0.01). Variation in 1-d postmortem pH contributes to differences in measurable quality characteristics of fresh pork loin and impacts the presence of desmin degradation products in low ionic strength extracts.

Key words: desmin, fresh meat quality, pork loin, proteolysis, ultimate pH

Meat and Muscle Biology 9(1): 18426, 1–13 (2025)

doi:10.22175/mmb.18426

Submitted 13 November 2024

Accepted 25 March 2025

Introduction

Consumers value the tenderness of fresh pork products (Norman et al., 2003; Moeller et al., 2010; Miller, 2020); however, measuring tenderness is intensive and costly at a large scale, resulting in inconsistencies in product quality within the retail case. Consumers cannot visually determine the eating experience of pork products; therefore, defining alternative means to classify and identify higher-quality pork products would be of value to consumers and

pork producers. Such approaches have begun to focus on the connection between specific biomolecules, namely proteins and metabolites, to pork quality traits (Ramanathan et al., 2023; Gagaoua et al., 2024). The multifaceted nature of factors (Rosenvold and Anderson, 2003; Scheffler and Gerrard, 2007) influencing pork quality has posed a significant challenge in the search for a simple, convenient measurement.

The rate and extent of postmortem pH decline can considerably affect fresh pork color (Brewer et al., 2001; Fernández-López et al., 2004;

Boler et al., 2010; Ramanathan et al., 2020), water-holding capacity (Bendall and Wismer-Pedersen, 1962; Huff-Lonergan and Lonergan, 2005; Cheng and Sun, 2008) and tenderness (Watanabe et al., 1996; Melody et al., 2004; Bee et al., 2007; Huff-Lonergan et al., 2010; Kim et al., 2014; Lomiwes et al., 2014; Zuber et al., 2021). Directly measuring these meat quality attributes would help assign different levels of quality. However, meat quality measurements can be challenging to collect consistently, and the feasibility of measuring pH in a commercial setting, especially within a commercial setting across multiple locations. Thus, various proxy measurements, including pH, have been proposed as alternatives to directly measuring meat quality. Although the rate and extent of pH decline are important in the development of ultimate meat quality, the feasibility of capturing nuanced variations of pH in a commercial setting while maintaining calibration and accurate records remains challenging. Biological markers could more efficiently and consistently distinguish pork loin quality, as changes in specific, relevant biomolecules likely reflect the progression of meat quality development in ways other measurements do not. Specific biomolecules, including proteins, peptides, and metabolites, have been previously evaluated to establish relationships to phenotypic traits. The potential development of a rapid antigenic test to screen the presence or absence of a biological indicator, like a protein, could rapidly and accurately screen many samples simultaneously. A robust understanding and relationship of protein changes associated with phenotypic traits, like pork loin quality, are first needed.

Proteolysis of myofibrillar and cytoskeletal proteins, including desmin, by the endogenous protease calpain-1, has widely been considered a key mechanism responsible for disruption to myofibrillar organization that results in tenderness (Taylor et al., 1995; Geesink et al., 2006; Bhat et al., 2018). The degradation of desmin is highly related to pork tenderness (Melody et al., 2004; Carlson et al., 2017b; Schulte et al., 2020) and water-holding capacity (Zhang et al., 2006; Bee et al., 2007; Zeng et al., 2017). Zuber et al. (2021) found that pork loins classified as normal pH (5.53–5.67) exhibited more desmin degradation and also had significantly lower star probe values than pork loins classified as low pH (5.38–5.45). Intriguingly, desmin degradation products have been detected in the low ionic strength soluble protein fraction of pork chops of different quality measures (Carlson et al., 2017a; Johnson et al., 2023a; Johnson et al., 2024).

Given that desmin is known to be degraded in early postmortem environments (Melody et al., 2004), desmin degradation products may serve as a reliable protein marker within the soluble protein fraction of meat.

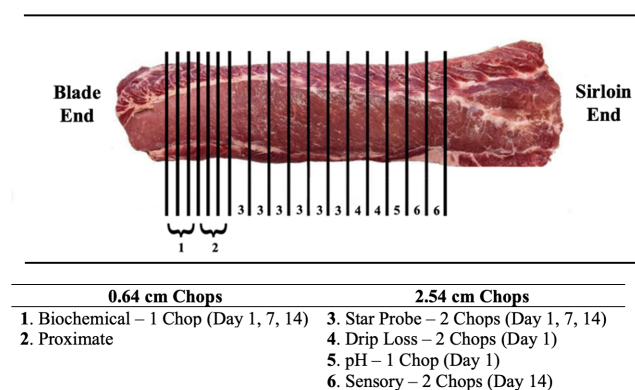
Therefore, the first objective of this study was to define how early postmortem pH categories affect the aged pork loin phenotype. Subsequently, this work sought to evaluate desmin degradation products and their potential to be used as markers of pork loin quality. It was hypothesized that a greater 1-day postmortem pH would result in more desirable quality attributes of fresh and aged pork loin and a greater abundance of degraded desmin within the low ionic strength soluble protein fraction.

Materials and Methods

No live animals were used in the current study; therefore, approval from the Institutional Animal Care and Use Committee was not needed. The sensory analysis protocol was approved by the Iowa State University Institutional Review Board (IRB ID: 21-046-00).

Collection of pork loin quality data

Fresh pork loins were evaluated from the fabrication line of a commercial processing facility the day after harvest over the course of an hour. The predetermined selection criteria for inclusion in this study included loins with a 1-day pH value (Hanna HI905, Hanna Instruments, Woonsocket, RI, USA) less than 5.7 (low/normal pH, $n = 25$; 5.59–5.71; $\bar{x} = 5.67$) or greater than 5.9 (high/normal pH, $n = 25$; 5.90–6.49; $\bar{x} = 6.09$) to represent variation in early postmortem pH. Selected loins were vacuum packaged and transported on ice to the Iowa State University Meat Laboratory (Ames, IA, USA) on the same day. Each loin was fabricated into chops containing only the *longissimus thoracis* (LT) muscle on the same day as collection from the plant (see Figure 1 for loin fabrication layout). Chops from each loin were then trimmed of external fat and connective tissue. Chops that were not used immediately were vacuum packaged, stored at 4°C, and frozen at the end of their respective aging times. During fabrication, the pH of a 1-day chop (2.54 cm) from each loin was measured using a pH meter (Hanna HI905, Hanna Instruments, Woonsocket, RI, USA). The pH meter was calibrated using commercial pH 4 and 7 buffers. Calibration was maintained by checking accuracy between each chop using pH 7 buffer within a range of (6.95–7.05). Color was measured following a 20-



¹ Pork loin graphic was adapted from Schulte et al. (2019), with permission from the authors.

Figure 1. Fresh pork loin fabrication and sample analysis designation.

min bloom period after removing chops from vacuum packaging. Hunter *L*, *a*, and *b* values were measured at the center of the surface of 1 and 14-day aged chops using a Konica Minolta Chroma Meter (Konica Minolta, Ramsey, NJ, USA) with a D65 light source, 50-mm aperture, and 2° observer angle (King et al., 2022). Hue angles of 1- and 14-d aged chops were calculated as (arctangent [*b/a*]) (King et al., 2022).

Drip loss

Two fresh 1-d chops (2.54 cm) from each loin were used to determine drip loss on the same day as loin collection and fabrication. Excess moisture on the chop surface was blotted off before weighing. Chops were then weighed, placed in sealed individual plastic bags, and stored at 4°C for approximately 24 h. Chops were then removed from the bags and reweighed to calculate drip loss using the equation: $[(\text{Initial wt. (g)} - \text{Final wt. (g)}) / \text{Initial wt. (g)}] \times 100$ (Melody et al., 2004). The average drip loss of the chops from each loin was used in the analysis.

Instrumental tenderness analysis

Two cooked chops (2.54 cm) from each aging period were used for instrumental tenderness measurement. Before cooking, chops were thawed at 4°C (approximately 24 h) in the vacuum bag. Chops were cooked to an internal temperature of 68°C using Ninja Foodi grills (AG302, SharkNinja, Needham, MA, USA). Cooking loss was calculated using the equation: $[(\text{raw wt. (g)} - \text{cooked wt. (g)}) / \text{raw wt. (g)}] \times 100$ (Carlson et al., 2017b). Chops were cooled at room temperature (approximately 22°C) for 20 min prior to instrumental tenderness measurement. An Instron (Model# 5566, Norwood, MA, USA) was

equipped with a 5-point star probe attachment to measure the force (kg) required to puncture, shear, and compress a cooked chop to 20% of its original height (Huff-Lonergan et al., 2002). Three replicate compressions were made on each chop and averaged to determine a final star probe value (Schulte et al., 2019).

Collection of sensory data

Two 14-d aged chops (2.54 cm) from each loin were used for sensory analysis. Before cooking, chops were thawed at 4°C (approximately 24 h). Chops were cooked to an internal temperature of 68°C as described for instrumental tenderness. Following cooking, 1 chop was cut into 1.5-cm cubes and immediately served to panelists for evaluation. A trained sensory panel (*n* = 3, Iowa State IRB ID: 21-046-00), all with at least 3 y of training and continuous evaluation experience, evaluated tenderness, juiciness, chewiness, pork flavor, and off-flavor on a 10-point categorical scale (Carlson et al., 2017b). Lower scores indicated a lesser degree of a given characteristic, whereas higher scores indicated a greater degree of a given characteristic in a sample. For tenderness, (1 = tough; 10 = tender). For juiciness, (1 = dry; 10 = very juicy). For chewiness, (1 = minimal chewiness; 10 = high chewiness). For pork flavor, (1 = low pork flavor; 10 = high pork flavor). For off-flavor, (1 = no detectable off-flavors; 10 = high presence of off-flavors).

Proximate analysis

Pork loin chops (0.64 cm) containing only the LT muscle from each loin were individually frozen in liquid nitrogen and pulverized for use in proximate analysis. Moisture content was determined using the CEM SMART 6 system (Official Method 2008.06, AOAC International, 2019), and fat content was determined using the CEM ORACLE system (Official Method 2008.06, AOAC International, 2019) (CEM Corporation, Matthews, NC, USA). All analyses were conducted in triplicate and averaged.

Protein extraction and sample preparation

Extraction of proteins soluble in low ionic strength buffer was done as described by Johnson et al. (2023a). Briefly, frozen pork loin chops (0.64 cm) containing only the LT muscle (~200 g) from each loin and aging period were separately diced, homogenized, uniformly powdered in liquid nitrogen, and stored at –80°C. Powdered muscle samples (~1.5 g) were mixed with 4.5 mL of ice-cold low ionic strength extraction buffer

(50 mM Tris-HCl [pH 8.5] and 1 mM ethylenediamine-tetraacetic acid [EDTA]), homogenized using a Polytron PT 3100 (Polytron, Lucerne, Switzerland), clarified by centrifugation, and subsequently filtered through cheesecloth. The protein concentration of the supernatant was determined using a Bradford Colorimetric Assay Kit (#5000002, Bio-Rad Laboratories, Hercules, CA, USA), and samples were adjusted to a final protein concentration of 4 mg/mL using ice-cold low ionic strength extraction buffer, 0.5 vol Wang's tracking dye (3 mM EDTA, 3% [wt/vol] sodium dodecyl sulfate [SDS], 30% [vol/vol] glycerol, 0.001% pyronin Y, 30 mM Tris-HCl [pH 8.0]), and 0.1 vol of 2-mercaptoethanol. Samples were heated at 50°C for 15 min in a dry heating block, vortexed, and stored at -80°C until use. Consistency of sample protein concentrations was confirmed by loading 40 µg of each sample in a random order onto 10 by 10 cm 15% polyacrylamide separating gels overlaid with a 5% stacking gel containing 10 lanes as previously described (Johnson et al., 2023a). Following electrophoresis, gels were stained overnight (approximately 15–20 h) with Colloidal Coomassie Blue stain according to Merrill (1990) with slight modifications (Carlson et al., 2017b). Gels were destained with distilled, deionized water, and changed 3 times in 30-min increments. Protein samples were visually assessed to confirm similar protein loading equivalents.

SDS-polyacrylamide gel electrophoresis and running conditions

One-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting were used to determine the abundance of desmin degradation products within the low ionic strength extract of post-mortem muscle at each aging time. Gel systems and running conditions were utilized as described by Johnson et al. (2023a). 15% polyacrylamide gels overlaid with a 5% stacking gel containing 10 lanes were loaded with 50 µg of protein from the prepared 4 mg/mL gel samples. The first lane contained Precision Plus Protein All-Blue Pre-Stained Protein Standards (Bio-Rad Laboratories, Hercules, CA, USA). A 14-d post-mortem reference sample of low ionic strength soluble protein was prepared, and 20 µg protein was loaded into the fifth lane of every gel. Proteins were separated using SE 260 Hoefer Mighty Small II Vertical Electrophoresis units (Hoefer, Inc.) at a constant 120 V for approximately 360 V/h. The running buffer consisted of 25 mM Tris, 192 mM Glycine, 2 mM EDTA, and 0.1% (wt/vol.) SDS.

Transferring conditions and western blot analysis

Western blot analysis was performed as described by Schulte et al. (2019). Following electrophoresis, proteins were transferred to polyvinylidene difluoride membranes (0.2 µm, Millipore Corporation, Billerica, MA, USA) using TE-22 Mighty Small Transphor Units (Hoefer, Inc.) at a constant rate of 90 V for 90 min at 4°C. Membranes were blocked for 45 min at room temperature in a PBS-Tween solution [80 mM Na₂HPO₄, anhydrous, 20 mM NaH₂PO₄, 100 mM NaCl, 0.1% [vol/vol] polyoxyethylene sorbitan monolaurate (Tween-20)] containing 5% [wt/vol] non-fat dry milk. After blocking, polyclonal rabbit anti-desmin (Iowa State University, Huff-Lonergan et al., 1996a) was diluted 1:40,000 in PBS-Tween and incubated with membranes overnight (approximately 15–20 h) at 4°C. Membranes were subsequently washed 3 times for 10-min intervals using PBS-Tween and incubated for 1 h at room temperature with goat anti-rabbit-horseradish peroxidase-conjugated (31460, Thermo Scientific, Rockford, IL, USA) secondary antibody diluted 1:20,000 in PBS-Tween. After incubation, membranes were washed 3 times for 10-min intervals using PBS-Tween. Desmin was detected using a chemiluminescent detection kit (ECL Prime, GE Healthcare, Piscataway, NJ, USA) and captured and analyzed using a ChemiImager 5500 (Alpha Innotech, San Leandro, CA, USA) and Alpha Ease FC Software (v 3.03 Alpha Innotech). Analysis of desmin degradation was performed as described by Carlson et al., 2017b. Separately, the densitometry of the 2 desmin degradation products (~38-kDa, ~37-kDa) was compared with the corresponding degradation bands of the internal reference used on all blots and quantified as a comparative ratio (Figure 2). All samples were completed at least in duplicate with a desired coefficient of variation of less than 20%. The desmin western blot data were subsequently classified as a binary variable based on the presence of detectable desmin degradation products.

Statistical analysis

All data were analyzed using R Statistical Software (v 4.2.1, R Core Team, 2023). All data were analyzed separately by aging time, as aging time was confounded with chop location within a loin. A comparative group design was employed, where an individual loin ($n = 50$) was considered the experimental unit. A one-way analysis of variance was performed with a fixed effect of pH category for quality measurement

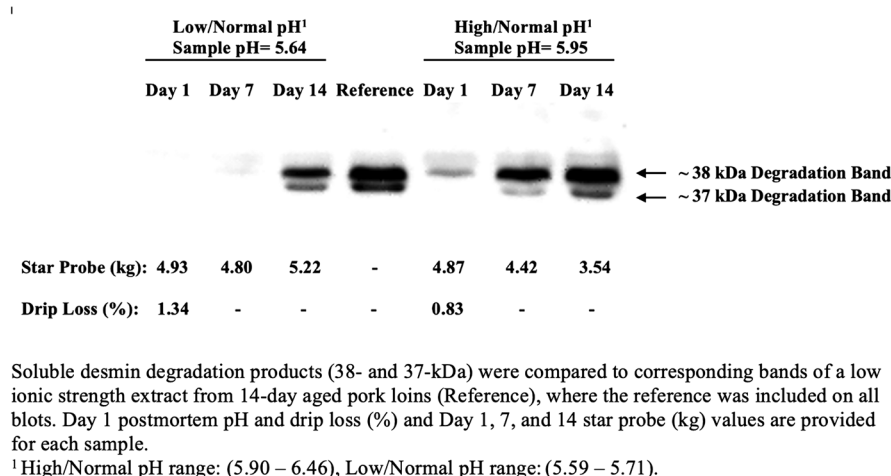


Figure 2. Representative western blot of desmin degradation products in low ionic strength soluble extracts from pork loins classified as low/normal or high/normal based on 1-d postmortem pH.

data using the *car* (v. 3.1-2) package. The analysis of sensory data included a fixed effect of pH category and a random effect of the panelist in the model. Estimated marginal means were obtained for all data using the *emmeans* (v. 1.8.9) package. Comparisons of means were performed using Tukey's test with a Kenward-Roger degrees of freedom adjustment. The binary desmin degradation product variable was analyzed using a chi-squared test of independence with continuity correction using the *prop.test* function in the *stats* package (R Core Team, 2023). The significance level was determined at $P < 0.05$.

Results

Fresh pork loin quality

Fresh pork loins were categorized as high (5.90–6.46; $\bar{x}$ = 6.09) and low (5.59–5.71; $\bar{x}$ = 5.67) based on 1-d postmortem pH values. All loins were evaluated and screened for quality defects, and no loins in either pH category exhibited attributes associated with dark, firm, and dry or pale, soft, and exudative quality defects. Thus, the pH categories were termed “high/normal” and “low/normal” to reflect the absence of these quality defects. Fresh pork loin quality attributes of both pH categories are summarized in Table 1.

Hunter *L* value was lower in high/normal pH chops at day 1 ($P < 0.01$) as well as day 14 ($P < 0.01$). Low/normal chops exhibited a higher ($P = 0.014$) Hunter *a* value on day 1 (13.2 vs. 12.6); however, after 14 d of aging, high/normal pH chops exhibited a higher ($P < 0.01$) *a* value compared with low/normal pH chops

(13.7 vs. 13.1). Hunter *b* value was higher in low/Normal pH chops at day 1 ($P < 0.01$), but was not significantly different ($P = 0.13$) between the 2 pH categories at day 14. The hue angle was higher in low/normal pH chops on day 1 ($P < 0.01$) and day 14 ($P < 0.01$).

Intramuscular lipid content was not different ($P = 0.36$) between pH categories. Intramuscular moisture content was greater ($P = 0.028$) in high/normal pH chops compared with low/normal pH chops (74.1% vs. 73.5%; Table 1). Drip loss was greater ($P < 0.01$) in low/normal pH chops compared with high/normal pH chops on day 1 (Table 2). Cooking loss was less ($P < 0.01$) in high/normal pH chops compared to low/normal pH chops at each aging time (Table 2).

Instrumental tenderness and trained sensory panel analysis

Results from sensory panel analysis of 14-d aged chops from each pH category are summarized in Table 3. Chops from the high/Normal category demonstrated lower star probe values compared to low/normal chops on day 1 ($P < 0.01$), day 7 ($P < 0.01$), and day 14 ($P < 0.01$) (Table 2). Sensory analysis results show that chops from the high/normal pH category had greater scores for juiciness ($P < 0.01$) and tenderness ($P < 0.01$), lower chewiness scores ($P = 0.012$), greater pork flavor ($P < 0.01$), and less off-flavor ($P < 0.01$).

Western blot analysis of desmin degradation

One-dimensional SDS-PAGE and western blot analyses confirmed that soluble desmin degradation products were present within the low ionic strength soluble protein fraction of postmortem muscle

Table 1. Summary of fresh pork loin chop quality traits from pork loins classified as high/normal or low/normal based on 1-d postmortem pH and analyzed separately by aging time

	High/Normal pH Category ¹	Low/Normal pH Category ¹	SEM	P Value
Moisture (%) ²	74.1 ^a	73.5 ^b	0.18	0.028
Lipid (%) ³	2.8	3.0	0.20	0.36
Day 1				
Hunter <i>L</i>	44.3 ^b	49.5 ^a	0.55	<0.01
Hunter <i>a</i>	12.6 ^b	13.2 ^a	0.16	0.014
Hunter <i>b</i>	3.4 ^b	4.4 ^a	0.15	<0.01
Hue angle	15.1 ^b	18.6 ^a	0.48	<0.01
Day 14				
Hunter <i>L</i>	49.5 ^b	53.2 ^a	0.55	<0.01
Hunter <i>a</i>	13.7 ^a	13.1 ^b	0.16	<0.01
Hunter <i>b</i>	4.7	5.1	0.17	0.13
Hue angle	18.9 ^b	21.3 ^a	0.61	<0.01

^{a,b}Means with different superscripts within rows indicate a significant difference between means within pH category ($P < 0.05$).

¹High/Normal pH range: (5.90–6.46), low/Normal pH range: (5.59–5.71).

²Determined using the CEM SMART 6 system (Official Method 2008.06, AOAC International).

³Determined using the CEM ORACLE system (Official Method 2008.06, AOAC International). Hunter *L*, *a*, and *b* determined with Minolta Chroma Meter with D65 light source, 50-mm aperture, and 2° observer.

Hue angle = [arctangent (*b/a*)].

SEM = standard error of the mean.

(Figure 2) and that the presence or absence of detectable degradation products was significantly different between high/normal and low/normal pH categories (Table 4). The low/normal pH category had detectable desmin degradation products in 3, 0, and 10 samples of the 25 samples on days 1, 7, and 14 postmortem, respectively. The high/normal pH category had detectable desmin degradation products in 15 samples on days 1 and 7 and 22 samples on day 14 postmortem. At each aging time, there was a difference ($P < 0.01$) in the proportion of samples containing soluble desmin degradation products between the 2 pH categories. The proportion of samples containing desmin degradation products at day 1 was 60% in the high/normal versus 12% in the low/normal (95% CI 21–75%). The proportion of samples containing desmin degradation products at day 7 was 60% in the high/normal versus 12% in the low/normal (95% CI 37–83%). At day 14, the proportion of samples with detectable desmin degradation products was 88% in the high/normal versus 40% in the low/normal category (95% CI 21–75%).

Table 2. Pork loin chop drip loss, instrumental star probe, and cook loss measurements from pork loins classified as high/normal or low/normal based on 1-d postmortem pH and analyzed separately by aging time

	High/Normal pH Category ¹	Low/Normal pH Category ¹	SEM	P Value
Day 1				
Drip Loss (%) ²	0.53 ^b	2.46 ^a	0.14	<0.0001
Star Probe (kg) ³	5.11 ^b	5.88 ^a	0.19	<0.01
Cook Loss (%) ⁴	17.3 ^b	21.1 ^a	0.53	<0.01
Day 7				
Star Probe (kg) ³	4.90 ^b	6.14 ^a	0.19	<0.0001
Cook Loss (%) ⁴	17.0 ^b	21.8 ^a	0.51	<0.01
Day 14				
Star Probe (kg) ³	4.07 ^b	5.18 ^a	0.16	<0.0001
Cook Loss (%) ⁴	17.0 ^b	21.0 ^a	0.64	<0.01

^{a,b}Means with different superscripts within rows indicate a significant difference between means within pH category ($P < 0.05$).

¹High/normal pH range: (5.90–6.46), low/normal pH range: (5.59–5.71).

²Drip loss % = [(initial weight [start] – final weight [end])/initial weight] × 100.

³An Instron fitted with a 5-point star probe attachment was used to assess the force needed to compress a chop to 20% of its original height (Carlson et al., 2017a).

⁴Chops were cooked to an internal temperature of 68°C on Ninja Foodi Grills. Cook loss % = [(raw weight – cooked weight)/raw weight] × 100.

SEM = standard error of the mean.

Table 3. Trained sensory panel scores of cooked 14-d aged pork loin chops from loins classified as high/normal or low/normal based on 1-d postmortem pH

	High/Normal pH Category ¹	Low/Normal pH Category ¹	SEM	P Value
Juiciness	7 ^a	6 ^b	0.2	<0.001
Tenderness	8 ^a	6 ^b	0.2	<0.0001
Chewiness	4 ^b	5 ^a	0.2	0.012
Pork Flavor	6 ^a	5 ^b	0.5	<0.0001
Off-Flavor	1 ^b	2 ^a	0.3	<0.0001

^{a,b}Means with different superscripts within rows indicate a significant difference between means within pH category ($P < 0.05$).

¹High/normal pH range: (5.90–6.46), low/normal pH range: (5.59–5.71).

²Attributes were evaluated by a trained sensory panel ($N = 3$) using a 10-point scale, whereby lower scores indicate a lower degree of a characteristic, and higher scores indicate a higher degree of a characteristic.

SEM = standard error of the mean.

Discussion

Variation in the quality of fresh pork products is a pertinent challenge to the U.S. pork industry. Identification of markers within the low ionic strength soluble protein fraction of meat indicative of pork quality

Table 4. The proportion of samples with detectable desmin degradation products in the low ionic strength soluble extracts from pork loins classified as high/normal or low/normal based on 1-d postmortem pH

Soluble Desmin	High/Normal pH Category ¹	Low/Normal pH Category ¹	95% CI of Difference	P Value
Day 1 Present	15 (60%)	3 (12%)	[21%–75%]	<0.0001
Total	25	25		
Day 7 Present	15 (60%)	0 (0%)	[37%–83%]	<0.0001
Total	25	25		
Day 14 Present	22 (88%)	10 (44%)	[21%–75%]	<0.01
Total	25	25		

¹High/normal pH range: (5.90–6.46), low/normal pH range: (5.59–5.71).

attributes, such as water-holding capacity (Johnson et al., 2023a) and tenderness (Carlson et al., 2017a; Schulte et al., 2020), could eventually be used to predict final product quality. Proposed protein markers found within this protein fraction could then potentially be detected through rapid, nondestructive analyses that utilize the exudate of fresh meat. Such methods would not only preserve the integrity of the product but also introduce the possibility of sorting products according to expected quality for marketing purposes, producing financial incentives for producers and a consistent product for consumers.

Toward that aim, this study generated 2 distinct categories of fresh pork loins that were divergent in 1-d postmortem pH, which were used to evaluate meat quality, sensory attributes, and desmin degradation profiles in the low ionic strength soluble protein fraction. It was hypothesized that variation in 1-d pH would significantly impact quality and sensory attributes and desmin degradation and that these differences would persist across 14 d of postmortem aging. These results demonstrate that the pork loin phenotype is affected by differences in early postmortem pH. The difference in the presence of desmin degradation products was apparent at 1-d postmortem, illustrating that these protein fragments could be reliable indicators of higher-quality pork loins.

Instrumental color

Meat color is often indicative of quality and is a crucial component of consumers' decisions to purchase fresh meat products (Brewer and McKeith, 1999; Ramanathan et al., 2021). In the current study,

high/normal pH chops exhibited lower Hunter *L* and *b* values and a lower hue angle at 1 and 14 d postmortem compared to low/normal pH chops. Lower hue angle values in high/normal pH chops at days 1 and 14 of aging may suggest less discoloration than in low/normal pH chops. The results of the current study are in agreement with prior studies that found instrumental color differences in pork exhibiting differences in 24-h postmortem pH. Pork with a higher pH (5.70–6.10; Kim et al., 2016) or high/normal pH (5.75–6.04; Boler et al., 2010) was darker in color, as evidenced by lower CIE and Minolta *L** values. Similarly, pork classified as normal pH (5.53–5.67) at 14 d postmortem had lower Hunter *L* and Hunter *b* values compared with low pH chops (Zuber et al., 2021). Kim et al. (2016) reported that pork with a pH below 5.70 at 24 h postmortem had higher CIE *a** and *b** values compared with pork with a pH between 5.7 and 6.1 and above 6.1. The results of the current study support the general observations that show that a higher ultimate pH is often associated with darker pork.

Drip loss and cook loss

High/Normal pH chops in the current study possessed less drip loss than low/Normal pH chops at 1-day postmortem (Table 2). Cooking loss was greater in low/Normal pH chops than in high/Normal pH chops (Table 2) on each day of postmortem aging. These observations are consistent with results from Johnson et al. (2023a), who found that pork chops with an average 24 h pH of 5.77 had less purge loss (0.33% vs. 1.19%), exhibited a darker color, lower star probe values, and greater sensory tenderness scores than chops with an average 24 h pH of 5.63. Kim et al. (2016) reported that pork with a 24 h pH below 5.7 had more drip loss than pork with a higher 24 h pH from 5.7 to 6.1. Previous studies have reported more cooking loss in lower-pH pork (Dall Aaslyng et al., 2003) and that cooking loss decreased as pH increased (Boler et al., 2010). Higher cooking loss of low/normal pH chops in the present study could be attributed to a lower ultimate pH caused by a greater extent of postmortem pH decline and a lesser degree of proteolysis.

Drip loss, while considerably associated with the rate and extent of pH decline, has also been strongly linked to proteolysis of myofibrillar proteins and drip channel formation (Melody et al., 2004; Huff-Lonergan and Lonergan, 2005; Zhang et al., 2006; Bee et al., 2007; Qian et al., 2020). Specifically, it has been found that the degradation of cytoskeletal

proteins such as integrin and desmin can influence the formation of drip channels in fresh pork (Lawson, 2004; Zhang et al., 2006). Zhang et al. (2006) demonstrated that degradation of integrin was associated with more drip loss in fresh pork, while more desmin degradation was associated with less drip loss in pork loin chops, emphasizing that degradation of proteins within the muscle cell can generate different quality phenotypes than degradation of proteins at the sarcolemma. The proposed mechanism for this observation highlights that intact desmin is involved in the transfer of lateral shrinkage of myofibrils that occurs postmortem and causes water to be expelled (Zhang et al., 2006). Therefore, degradation of desmin would aid in lessening the extent of this shrinkage, allowing for greater intracellular space to retain water.

Desmin is the most abundant intermediate filament protein in skeletal muscle. It is involved in the lateral linkage of adjacent myofibrils at the Z-disk and the connection of peripheral myofibrils to the muscle cell membrane, or sarcolemma (Clark et al., 2002; Capetanaki et al., 2007). Disruption in the linkages created by desmin between individual myofibrils and myofibrils to the sarcolemma is thought to prevent or limit the shrinking of the entire muscle during rigor development that could ultimately lead to drip channel formation (Offer and Cousins, 1992; Huff-Lonergan and Lonergan, 2005; Qian et al., 2020). Additionally, disruption of these linkages has been hypothesized to allow an increase in the swelling of myofibrils with water that has entered the muscle cell from the extracellular compartment (Kristensen and Purslow, 2001; Zeng et al., 2017). In the current study, it can be proposed that chops from high/normal pH loins experienced a more favorable postmortem extent of pH decline that may have enabled greater proteolysis of cytoskeletal proteins such as desmin (Table 4), resulting in less drip loss and cook loss (Table 2).

Instrumental star probe tenderness and sensory analysis

Tenderness has been regarded as the most important attribute of the consumer eating experience (Offer et al., 1989; Verbeke et al., 1999; Warner et al., 2020). Understanding the intrinsic factors of postmortem muscle that contribute to tenderness development is critical for producing consistent, high-quality pork products. Consumers have previously associated pork with a higher pH with more desirable quality and sensory attributes (Moeller et al., 2010; Miller, 2020). Low ultimate pH is associated with increased concentrations

of lactate and H^+ , along with a greater amount of released free water (Briskey and Wismer-Pedersen, 1961; Sayre et al., 1963). Choe et al. (2008) found pork muscle with high glycogen and lactate to exhibit an accelerated rate of postmortem glycolysis, paler surface color, greater drip loss, and a greater extent of protein denaturation. Similar results were found by Zuber et al. (2021), whereby normal pH samples (pH 5.53–5.67) were found to have less pyruvate and lactate than low pH samples (pH 5.38–5.45) and were generally of greater quality in terms of color, tenderness (star probe), drip loss, and cooking loss. The higher sensory off-flavor scores of low/normal pH chops could be explained by a greater accumulation of lactate and H^+ , leading to a higher incidence of sour flavors than high/normal pH chops. The incidence of sour flavors in low/normal pH chops could potentially be attributed to a greater concentration of lactic acid. The high/normal chops had a greater percent moisture than the low/normal chops, yet no significant difference in the percent of intramuscular lipid was detected between high/normal and low/normal pH chops (Table 1; $P = 0.36$). Lipid content between pH categories was not significantly different and was unlikely to contribute to quality differences between the categories. Thus, differences observed in sensory attributes and tenderness across pH categories are likely not due to lipid content but instead a result of other factors, such as proteolysis.

Proteolysis of myofibrillar and cytoskeletal proteins is thought to be the primary contributor to the tenderization of fresh meat products, primarily driven by the calpain family of proteinases. Calpains are a class of calcium-dependent cysteine proteases (Goll et al., 2003) whose activity is responsible for the postmortem degradation of meat proteins (Koochmaraie, 1994), of which desmin is a known substrate for calpain (Huff-Lonergan et al., 1996a; Huff-Lonergan et al., 1996b; Melody et al., 2004; Carlin et al., 2006; Carlson et al., 2017b; Zeng et al., 2017; Bhat et al., 2018; Qian et al., 2020). Lower-pH environments, especially near and below 6.0, negatively impact the activity of calpains (Maddock et al., 2005; Carlin et al., 2006; Maddock et al., 2024). Maddock et al. (2005) and Carlin et al. (2006) observed that calpain-1 activity was greatest at pH 6.5 when compared with pH 6.0 or 7.5, which was hypothesized to be a result of a slower autolytic inactivation in both studies. Based on the results of the current study, it is likely that high/normal pH chops maintained a cellular environment that would be more favorable for optimal calpain activity compared to low/normal pH chops, which could ultimately explain the differences

in sensory and instrumental tenderness measurements in the current study. Other observations consistent with pH category differences may also provide an explanation for differences, including water-holding capacity and filament spacing, given that the high pH chops had less drip and cook loss.

Soluble desmin degradation products

Two distinct desmin degradation products, approximately 38- and 37- kDa, were detected within the low ionic strength soluble protein fraction in the current study (Figure 2). The differential proportion in the presence of desmin degradation products between high/normal and low/normal pork chops at each aging time was significantly different (Table 4). A greater proportion of samples within the high/normal pH category exhibited the presence of desmin degradation products than the low/normal pH category. The difference in proportion between these pH categories of desmin degradation products is likely attributed to differences in the activation and autolysis of calpain-1. Calpain-1 activity is influenced by the rate and extent of pH decline (Melody et al., 2004; Bee et al., 2007; Pomponio et al., 2010). The pH differences observed at 1-day postmortem would likely differentially alter calpain activity and autolysis, yet even earlier post-mortem pH measurements and calpain assays would be necessary to confirm.

The low ionic strength soluble protein fraction of skeletal muscle comprises many classes of proteins. Proteins that form a fundamental part of the myofibril's architecture and its connection to the sarcolemma through the costamere are typically expected to be soluble in high-ionic-strength buffers; however, recent studies have consistently shown that some of these proteins, along with their degradation products, also appear in the low ionic strength soluble protein fraction (Carlson et al., 2017a; Schulte et al., 2020; Zuber et al., 2021; Johnson et al., 2023a). This shift in protein solubility, likely due to degradation, and their differential presence and abundance according to pork quality traits strongly suggest that these products may serve as robust and efficient markers for rapidly predicting the ultimate quality of pork loin. The altered solubility of these proteins, likely degradation products, and differential presence and abundance based on pork quality traits illustrate that these products could be ideal markers to predict the ultimate pork loin quality more rapidly and consistently. For example, a degradation product like this, if present in the purge, could be used to gain insight into the extent of proteolysis of

structural proteins without damaging the product. The current results suggest that the presence or absence of desmin in this fraction (given the detection method) would be useful in predicting fresh pork tenderness.

Myofibrillar and myofibrillar-associated proteins are considered to be salt-soluble, becoming soluble in solutions >0.3 M ionic strength (Huff-Lonergan et al., 1996a; Huff-Lonergan et al., 1996b; Carlin et al., 2006; Chen et al., 2017; Johnson et al., 2023b). Thus, fully intact desmin is not expected to be found within the low ionic strength soluble protein fraction of post-mortem muscle. Recent evidence supports the observation that upon proteolytic degradation of desmin, specific and unique desmin degradation products are generated, which have distinct chemical and physical properties from the intact desmin parent molecule that result in these degradation products being soluble under low ionic strength environments (Carlson et al., 2017a; Johnson et al., 2023a; Johnson et al., 2024). More specifically, the abundance of low ionic strength soluble desmin degradation products was significantly greater in pork chops classified as more tender by instrumental star probe measurements (Carlson et al., 2017a). Furthermore, desmin was greater in abundance in low ionic strength soluble extracts from aged pork chops classified as lower purge loss versus higher purge loss using proteomic techniques (Johnson et al., 2023a). Taken together, these results from different populations of pork loins support the notion that protein degradation products, namely generated through calpain proteolytic degradation, are specific products regarding their mass, solubility, and likely other chemical and physical properties.

The structure of intact desmin (55-kDa) consists of an N-terminal head, C-terminal tail, and coil-coiled central rod domain (Henderson et al., 2017). Two monomers of desmin form dimers and arrange in a parallel formation, then form tetramers in an antiparallel orientation that ultimately comprise desmin filament (Henderson et al., 2017; Claeysen et al., 2024). Through the use of proteomics, it was identified that the desmin degradation product (34-kDa) within the soluble protein fraction observed in Carlson et al. (2017a) corresponded to the rod domain of desmin. Considering the structure of intact desmin and findings from Carlson et al. (2017a), who used mass spectrometry to identify soluble peptides in aged pork loin, it is reasonable to suggest that the soluble desmin degradation products (~38-kDa, ~37-kDa) in the present study also corresponded to the central rod domain of desmin, indicating that this portion became soluble following its release from the head and tail domains.

The solubility of muscle proteins can be affected by pH, temperature, and myofibrillar fragmentation, as demonstrated by Wismer-Pedersen, 1959; Sayre and Briskey, 1963; and Feng et al., 2020. Pork, which exhibited a rapid pH decline, was shown to result in a loss of protein solubility in 0.6 M KCl (Wismer-Pedersen, 1959). The solubility of sarcoplasmic and myofibrillar proteins has also been found to be significantly reduced in pork with low pH (5.30–5.60) at high temperatures (<35°C) at the onset of rigor (Sayre and Briskey, 1963); however, it is important to note that 24 hr pH was reported to have only a minor effect on protein solubility (Sayre and Briskey, 1963). Melody et al. (2004) discovered that porcine *psaos major* muscle exhibited a more rapid decline in pH and also exhibited earlier degradation of intact desmin. This was thought to be so due to earlier autolysis of calpain-1, which, in turn, lowered the Ca²⁺ required for activation (Goll et al., 2003; Li et al., 2004; Melody et al., 2004). Pomponio et al. (2010) concluded that porcine *longissimus dorsi* muscle experiencing a rapid decline pH (3 h pH < 6.0) resulted in reduced activity and increased autolysis of calpain-1 compared to pork with an intermediate (6.0 < 3 h pH > 6.30) or slow pH decline (3 h pH > 6.30). Lomiwes et al. (2014) reported that calpain-1 autolysis and activation occurred more rapidly in bovine muscle with a higher pH (≥6.2) and was delayed in muscle with a lower pH (≤5.79). In agreement with Lomiwes et al. (2014), Maddock et al. (2005) and Carlin et al. (2006) found greater activity of calpain-1 and slower autolysis at pH 6.5 in porcine muscle. Although the rate of pH decline is unknown in the present study, this measurement would have been useful for explaining the meat quality, sensory, and low ionic strength soluble desmin differences. It can be hypothesized that high/normal pH chops (5.71–6.46) allowed for greater calpain activity, resulting in a greater proportion of high/Normal chops having desmin degradation products in the low ionic strength soluble protein fraction. Perhaps low/normal pH chops exhibited a lesser proportion of detectable desmin degradation products due to an earlier loss of calpain-1 activity in lower-pH conditions, thus diminishing the amount of proteolysis that could have occurred throughout postmortem storage.

Conclusions

Fresh pork quality development continues to be a complex and multifaceted process that is not entirely understood. In this study, the variability in early

postmortem pH resulted in differences in pork meat quality, including color, water-holding capacity, palatability, and tenderness. Furthermore, the pH category impacted the proteolytic activity within high/normal pH chops. Specifically, the average star probe value of high/normal pH chops was approximately 1 kg lower compared with low/normal pH chops on each day of aging. High/normal pH chops exhibited a greater proportion of soluble desmin degradation products on each day of aging. The degradation of desmin and the presence of the degradation products in the low ionic strength fraction is likely related to lower star probe values, lower sensory chewiness scores, and greater sensory tenderness scores. As differences in desmin degradation products were apparent at 1 d postmortem, these results inform the search for robust candidate protein markers to predict final fresh pork loin quality.

Conflict of Interest

The authors declare no real or perceived conflicts of interest.

Acknowledgments

The authors would like to extend thanks to Jay Stowater, Dr. Edward Steadham, and Leah Reeve, as well as the staff at the Iowa State University Meats Laboratory for their assistance in pork loin fabrication and data collection. The authors also appreciate the statistical consultation provided by Dr. Juan Steibel. Partial funding for this project was provided by the Iowa Agricultural and Home Economics Experiment Station project no. IOW04121 and the Iowa Pork Producers Association.

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