Meat and Muscle Biology™

Influence of postmortem aging and post-aging freezing on pork loin quality attributes

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Abstract: The objectives were to determine 1) the interaction between aging duration and post-aging freezing on pork loin quality attributes and 2) the relationship between pork loin star probe (SP) and Warner-Bratzler shear force (WBS). Loins from 20 carcasses were collected 1 d postmortem. Chops ($n = 9$; 2.54-cm thick) were fabricated from each loin and vacuum packaged. Four chops from each loin were aged at 4°C for 1, 8, 14, and 21 d and immediately evaluated (Fresh). Four adjacent chops were aged (1, 8, 14, and 21 d), frozen for 14 d, and thawed for evaluation (Frozen). An additional chop was used for evaluation of sarcomere length, intact desmin, and troponin-T degradation. Purge, objective color, pH, subjective color and marbling score, cook loss, SP, and WBS were evaluated at each aging period. Desmin and troponin-T degradation, and sarcomere length were measured on fresh samples at each day of aging. Post-aging freezing had no significant impact on SP, WBS, pH, and subjective color or marbling score at any aging period. Fresh chop purge increased at each day of aging ($P < 0.01$). Post-aging freezing resulted in greater purge at 1, 8, and 14 d aging ($P < 0.01$). Fresh chop cook loss was greater than post-aging freezing chop cook loss at 14 and 21 d aging ($P < 0.05$). Across all aging periods and treatments, SP was correlated ($r = 0.85$; $P < 0.01$) with WBS. Fresh chop SP and WBS decreased from 1 to 8 d aging but was not different after 8 d aging. The abundance of intact desmin decreased ($P < 0.01$) between 1, 8, and 14 d aging. Troponin-T degradation increased ($P < 0.01$) with each aging period. Sarcomere length was not different across aging periods ($P > 0.05$). Aging, without freezing, for 14 or 21 d did not improve SP or WBS observed at 8 d, corresponding with changes in desmin degradation.

Introduction

Tenderness is one of the most important traits consumers use to determine the overall eating quality of fresh pork. This complex trait can be impacted by factors such as postmortem proteolysis, sarcomere length, and collagen content (Koohmaraie et al., 2002). Postmortem aging of fresh pork loin is a practice implemented to improve tenderness through proteolysis of myofibrillar, cytoskeletal, and intermediate filament proteins (Wheeler et al., 2000; Melody et al., 2004; Carlson et al., 2017). However, the benefit of aging pork loin for periods greater than 14 d has not been well documented.

Freezing, a storage practice, is one of the oldest and most common methods to increase the safety and shelf life of meat products. Freezing and thawing, however, can negatively impact product quality compared to meat that is never frozen (Leygonie et al., 2012a). Major meat quality attributes affected by the freezing and thawing process include water loss (Vieira et al., 2009; Leygonie et al., 2012a), protein denaturation causing discoloration (Añón and Calvelo, 1980), as well as oxidation of lipids and proteins (Estévez, 2011).

The impact of freezing and thawing meat for different periods of time has been investigated (Kim et al., 2011a, 2015, 2018; Leygonie et al., 2012a;

Coombs et al., 2017). Results from these studies have determined that aging prior to freezing will negate some of the negative factors associated with the process of freezing and thawing. Additionally, freezing prior to aging will result in less improvement in meat tenderness (Lagerstedt et al., 2008). The interaction of applying both postmortem aging for different periods of time and post-aging freezing practices in pork has not been investigated to determine the combined impact of these practices.

Star probe (SP) and Warner-Bratzler shear force (WBS) are 2 different instrumental tenderness measurement methods. Many studies have used these measurements separately to determine tenderness values and their relationship to trained sensory panels (Huff-Lonergan et al., 2002; Melody et al., 2004; Arkfeld et al., 2015; Richardson et al., 2017). Observations of SP and WBS in fresh and post-aging freezing pork will help to further define the relationship of these instrumental tenderness measurement methods.

The relationship of SP and WBS in fresh and post-aging freezing pork has not been investigated. It was hypothesized that 1) aging for 21 d would improve instrumental tenderness values, 2) post-aging freezing would negatively impact pork color values and water holding capacity compared with fresh pork, and 3) data would support that SP and WBS measurements are related instrumental tenderness measurements. Therefore, the objectives of this study were to document the aging response of fresh pork over 21 d, determine the impact of post-aging freezing on pork quality attributes across different aging periods, and determine the relationship between SP and WBS force values under fresh and post-aging freezing conditions.

Materials and Methods

Pigs were harvested at a commercial processing facility under the inspection of the United States Department of Agriculture and a pair of loins were collected after inspection, so Institutional Animal Care and Use approval was not sought.

Pork loin collection and fabrication

Paired sides of fresh pork loins of similar genetics (Duroc sired crossbreds), management, diets, harvest, and chilling methods were collected 1 d postmortem from 20 carcasses harvested at a commercial processing facility. Loins were vacuum packaged and transported to the Iowa State University Meat Laboratory (Ames, IA)

for fabrication 1 d postmortem. Each pair of loins were fabricated into 9 chops after removal of the sirloin end (approx. 10 cm; Fig. 1). Loin chops (2.54 cm; $n = 8$), containing only the longissimus muscle, were trimmed of external fat and connective tissue. Chops were vacuum packaged prior to aging. Four chops from each pair of loins were aged for 1, 8, 14, and 21 d at 4°C and immediately evaluated at the conclusion of the prescribed aging period (Fresh; Fig. 1). Four adjacent chops were aged (1, 8, 14, and 21 d at 4°C), individually placed on racks, frozen (–29°C) post-aging for 14 d and then thawed (2°C, 22 h) for quality evaluation (Frozen; Fig. 1). Quality evaluations of frozen chops occurred at 15, 22, 28, and 35 d postmortem. The final chop was divided into 4 equal sections (~100 g) and vacuum packaged. These samples followed the aging period of that loin to mimic each aging period. Following aging, the sample was frozen (–80°C) for protein extraction and analysis. Loin side for each set of aging times (1 and 8 or 14 and 21) was randomly assigned.

Quality data analysis

At completion of aging or freezing, chop purge was collected by weighing the chop and the package with the purge in the package. Chop purge was calculated using the following formula: [(weight of package with purge – weight of package without purge)/chop weight] × 100. Chop pH was measured using a Hanna HI9025 pH meter (Hanna Instruments, Woonsocket, RI). The pH meter was calibrated using pH 4 and 7 buffers at room temperature (20°C). Accuracy of calibration of pH was checked before each measurement using pH 7 buffer (6.95 to 7.05 pH range). Chops were removed from refrigeration and allotted 15 min to bloom at room temperature (~22°C). Subjective color and marbling scores were assessed using the National Pork Board 6-point and 10-point scale standard pictures, respectively (Color: 1 = pale pinkish gray to white; 6 = dark purplish red; Marbling: 1 = 1.0% intramuscular fat; 10 = 10.0% intramuscular fat; NPPC, 1999). Hunter L, a, and b values were measured on each chop at the center of the chop surface using a Minolta Chroma Meter with a D65 light source, 50 mm aperture, and 2° observer angle (CR-410; Konica Minolta Sensing Americas Inc., Ramsey, NJ; AMSA, 2012). Hue angle and chroma values were calculated using the following equations: hue angle = arctangent (b/a) and chroma = $(a^2 + b^2)^{1/2}$ (AMSA, 2012). Each chop was cooked to an internal temperature of 68°C on clamshell grills (Cuisinart, East Windsor, NJ). Cook loss was calculated by using the following formula: [[raw chop weight – cooked chop
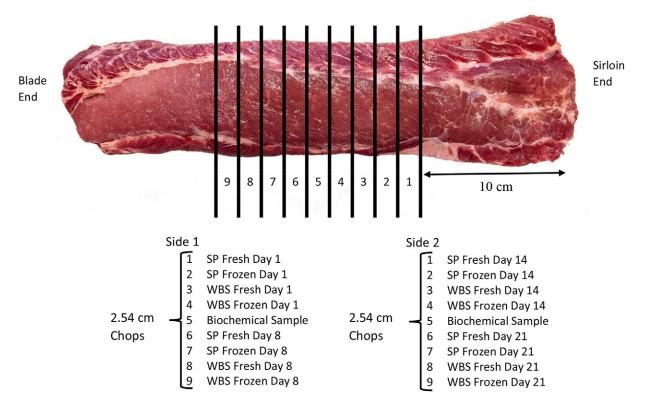
Figure 1. Chop fabrication layout for Star Probe (SP) and Warner-Bratzler Shear force (WBS) Analysis.

weight)/raw chop weight] × 100. Purge; pH; visual color and marbling scores; Hunter L, a, and b values; and cook loss measurements were averaged for both chops of each treatment at each aging period.
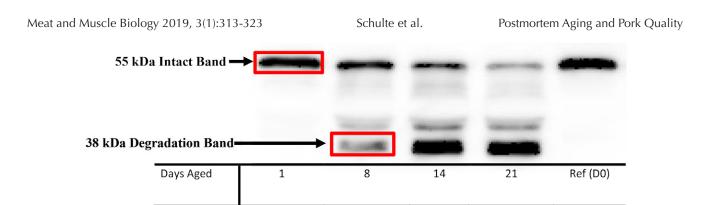
Instrumental tenderness analysis

Star probe punctures and compresses the chop to 20% of its original height. Value from this analysis are similar to the nature of chewing (Huff-Lonergan et al., 2002). An Instron (Instron, Norwood, MA) fitted with a five-point star probe attachment was used to measure instrumental tenderness on 1 cooked chop per treatment per day of aging (Arkfeld et al., 2015; Carlson et al., 2017). Three replicate compressions were made on each chop and averaged for a final instrumental tenderness value. Adjacent chops were cooked in the same manner and analyzed with a WBS force attachment to an Instron (Instron, Norwood, MA). Three 1.27-cm diameter cores were removed from each chop for analysis. Cores were removed parallel to the muscle fibers (Wheeler and Koohmaraie, 1994). Two samples were excluded for analysis at 21 d postmortem due to not reaching endpoint temperature before analysis of WBS.

Whole muscle protein extraction

Frozen meat (longissimus dorsi; 100 g) was homogenized in liquid nitrogen. Samples from each aging time (fresh; 0.5 g) were homogenized and whole muscle protein extracts were completed as described by Carlson et al. (2017). Consistency of protein concentrations were assured using 15% SDS-PAGE gels and Colloidal Coomassie blue staining (1.7% ammonium sulfate, 30% methanol, 3% phosphoric acid, and 0.1% Coomassie G-250).

Running conditions

Desmin and troponin-T degradation were determined using one-dimensional SDS-PAGE gel electrophoresis as described by Carlson et al. (2017). Protein was extracted from porcine longissimus dorsi (aged 0 and 7 d) with the identical protocol to generate a reference sample (4 mg/ml of protein) that was included in 1 well on each gel. A d 0 reference was used for desmin analysis and a d 0/7 mixed reference sample was used for troponin-T analysis. SE 260 Hoefer Mighty Small II electrophoresis units (Hoefer, Inc., Holliston, MA) were used to run 15% gels.

Figure 2. Representative Western blot of intact and degraded desmin in pork Longissimus dorsi (LM) whole muscle samples aged over time (1, 8, 14, and 21 d aging). Intact bands (55 kDa) and degradation bands (38 kDa) were compared to corresponding bands of a 0 d aged pork LM sample (Ref).

Transfer conditions

At completion of running the SDS-PAGE gels, the gels were transferred to polyvinylidene difluoride membranes with pore sizes of 0.2 μm as described by Carlson et al. (2017). Membranes were soaked in methanol for less than 1 min for activation prior to transferring. Gels were transferred using a TE-22 Mighty Small Transhpor unit (Hoefer, Inc.). The unit ran at a constant voltage of 90 V for 90 min at 4°C.

Western Blot analysis

Western Blot analysis was conducted as described by Carlson et al. (2017). Following transfer, the membrane was incubated for 60 min at 22°C in PBS-Tween mixed with 5% non-fat dry milk. Desmin primary antibody was added to the blot at a dilution of 1:40,000 polyclonal rabbit anti-desmin antibody produced at Iowa State University (Huff-Lonergan et al., 1996; Carlson et al., 2017) with PBS-Tween. Troponin-T primary antibody was added to separate blots at a dilution containing of 1:10,000 using monoclonal mouse anti-troponin-T primary antibody (T6277, JLT-12; Sigma-Aldrich, St. Louis, MO) with PBS-Tween. Blots were incubated in primary antibodies overnight at 4°C. After incubation with primary antibody, blots were washed with PBS-Tween 3 times for 10 min. Secondary antibodies were diluted with PBS-Tween and incubated with each blot for 1 h at room temperature. Secondary antibody dilution for desmin and troponin-T blots contained 1:20,000 goat anti-rabbit-HRP antibody (31460; Thermo Scientific, Rockford, IL; for desmin blots) and 1:5,000 goat anti-mouse HRP antibody (32430; Pierce) for troponin-T blots. Following incubation with secondary antibodies, blots were washed with PBS-Tween 3 times for 10 min. A chemiluminescent detection kit (ECL Prime, GE Healthcare, Piscataway, NJ) was used to detect proteins. Blots were imaged and analyzed using a ChemiImager

5500 (Alpha Innotech, San Leandro, CA) and Alpha Ease FC software (v 3.03, Alpha Innotech). Using the internal reference on each blot, the intensity of the 55 kDa intact desmin band and 30 kDa troponin-T degradation product was quantified as a comparative ratio of the sample protein band to the internal reference protein band on each gel. All western blots were completed in at least duplicate with a coefficient of variance less than 20%. See Fig. 2 and 3 for a representative Western blot of desmin and troponin-T analysis, respectively.

Sarcomere length

Sarcomere length determination was made using the helium-neon laser diffraction method as described by Cross et al. (1981). Small aliquots of powdered tissue (approx. 0.5 g; $n = 6$ per sample) were placed on microscope slides. Approximately 150 ML of 0.2 M sucrose in 0.1 M $NaHPO_4$ buffer was added to each aliquot prior to determining sarcomere length. Six sarcomere laser diffraction patterns were recorded per aliquot on paper, for a total of 36 sarcomere lengths per sample. Diffraction patterns were scanned into JPEG (Joint Photographic Experts Group) images, and Image Pro (Media Cybernetics, Inc., Rockville, MD) software was used to measure the distance between primary diffraction bands and calculate sarcomere length using the equation reported by Cross et al. (1981).

Statistical analysis

Each individual chop was the experimental unit. All quality data (cook loss; pH; purge; subjective color and marbling; Hunter L, a, and b; hue angle; chroma; SP; and WBS values [$n = 160$] per measurement except for WBS [$n = 158$]) measurements were analyzed using the MIXED procedure of SAS (v.9.4; SAS Inst. Inc.,
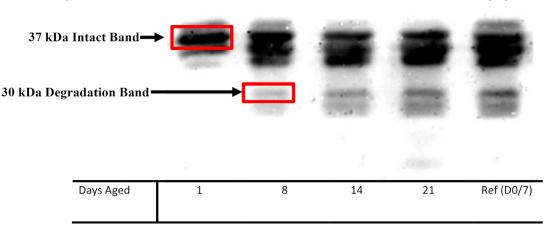
Figure 3. Representative Western blot of intact and degraded troponin-T in pork Longissimus dorsi (LM) whole muscle samples aged over time (1, 8, 14, and 21 d aging). Intact bands (37 kDa) and degradation bands (30 kDa) were compared to corresponding bands of a mixed 0/7 d aged pork LM sample (Ref).

Cary, NC). Fixed effects included days aged and treatment (Fresh or Frozen) for all quality data measurements. Carcass was used as a random effect in all models.

Intact desmin ($n = 80$) and troponin-T degradation ($n = 80$) product were analyzed using the MIXED procedure of SAS (v9.4; SAS Inst. Inc.) with a fixed effect of days aged. Gel was used as a random effect in the model. Sarcomere length measurements ($n = 80$) were analyzed using the MIXED procedure of SAS (v.9.4; SAS Inst. Inc.). Sarcomere length analysis fixed effects included days aged. Carcass was used as a random effect in all models. Least squares means and standard errors were reported for all measured attributes. Least squares means were separated using pdiff procedure of SAS (v.9.4; SAS Inst. Inc.). Significance levels were denoted with a $P < 0.05$.

Pearson correlations were generated using PROC CORR. Correlations were considered lowly correlated at $r \leq 0.35$, moderately at $0.36 \leq r \leq 0.67$, and highly if $r \geq 0.68$ (Taylor, 1990). Fisher's Z Transformation was performed to determine correlation comparisons between treatments and across d aging within treatments. Correlations and correlation comparisons were considered significant when $P < 0.05$. Fisher's Z Transformation was not used to compare negative correlations to positive correlations.

Results and Discussion

Freezing meat is a practice used to prolong the shelf-life of meat that has been practiced for thousands of years. However, freezing and thawing whole muscle cuts has significant impacts on meat quality attributes, specifically moisture loss (Leygonie et al., 2012b). The results of the current experiment demonstrate the impact of post-aging freezing of pork loins at different aging periods. Table 1 summarizes the effects of aging and post-aging freezing on meat characteristics.

Instrumental tenderness

Post-aging freezing had no significant impact on SP or WBS values compared with fresh chops that were evaluated immediately after aging (Table 1; $P > 0.05$). Chop SP and WBS values were greatest at 1 d aging than any other aging period regardless of treatment. These results align with previous data from beef, pork, and ovine studies (Kim et al., 2011a, 2011b, 2015, 2018) that demonstrate freezing meat prior to aging does not allow product to improve in tenderness value if cooked immediately after thawing frozen product. Star Probe and WBS values decreased significantly from 1 to 8 d aging ($P < 0.01$). Star Probe value was not different at 8, 14, and 21 d aging ($P > 0.05$) regardless of treatment whereas WBS values decreased in frozen chops from 14 to 21 d aging ($P < 0.01$). This change in WBS value from 14 to 21 d aging is in alignment with other studies that observed decreased WBS values from the freezing and thawing process (Lagerstedt et al., 2008; Kim et al., 2015).

Post-aging freezing tended ($P < 0.08$) to result in lower WBS values in samples aged 21 d. Freezing induced decrease in WBS value may be caused by ice crystal formation between myofibrils (Leygonie et al., 2012b). From the literature and these results, it is understood that post-aging freezing does not significantly impact tenderness values if product is aged prior to freezing. Additionally, chops must be aged prior to

Table 1. Summary of effects of aging and post-aging freezing on pork loin chop ($n = 20$) quality characteristics

| | Treatment | | | | | | | | | P-value | | |
| | Fresh | | | | Frozen | | | | | | | |
Days aged	1	8	14	21	1	8	14	21	SEM	Days aged	Treatment	Days aged * treatment
SP, kg[1]	8.44a	6.58b	6.33b	6.54b	7.92a	6.22b	6.30b	5.82b	0.37	< 0.01	0.12	0.82
WBS, kg[2]	5.62a	3.83b	3.62b	3.31b	6.19a	3.36bc	4.30b	2.53c	0.34	< 0.01	0.94	0.06
Purge, %[3]	0.15dx	1.31cx	2.23bx	2.93a	1.81cy	2.56by	3.83ay	3.09b	0.21	< 0.01	< 0.01	< 0.01
pH[4]	5.88a	5.82a	5.89a	5.86a	5.82b	5.90ab	5.96a	5.93a	0.04	0.09	0.08	0.11
Color score[5]	3.4a	3.0b	2.8bc	2.7c	3.1a	2.8b	2.7bc	2.5c	0.11	< 0.01	0.01	0.89
Marbling score[6]	1.9	2.0	1.9	2.2	2.2	2.3	2.2	2.2	0.17	0.73	0.05	0.82
Cook loss, %[7]	20.73a	18.25b	20.18ax	19.62abx	21.98a	18.41b	18.37by	16.88by	0.64	< 0.01	0.06	< 0.01
Hunter L[8]	43.88c	48.62b	48.83ab	50.03a	44.94c	49.20b	49.17by	50.96a	0.47	< 0.01	0.03	0.87
Hunter a[8]	11.96bx	13.66a	13.92a	13.73a	13.13by	13.86a	13.78a	14.00a	0.18	< 0.01	< 0.01	< 0.01
Hunter b[8]	2.49bx	3.71a	3.79ax	3.60ax	3.40cy	3.86b	4.29ay	4.59ay	0.13	< 0.01	< 0.01	< 0.01
Hue angle[9]	11.62bx	15.14a	15.21ax	14.67ax	14.46by	15.55b	17.29ay	18.20ay	0.59	< 0.01	< 0.01	0.02
Chroma[10]	12.27bx	14.19a	14.46a	14.23ax	13.60by	14.42a	14.47a	14.77ay	0.17	< 0.01	< 0.01	< 0.01
Intact desmin[11]	1.18a	0.64b	0.50c	0.50c	–	–	–	–	0.06	< 0.01	–	–
Degraded Troponin-T[12]	0.00d	0.40c	0.74b	1.08 a	–	–	–	–	0.09	< 0.01	–	–
SL, μm[13]	1.83	1.81	1.82	1.82	–	–	–	–	0.03	0.84	–	–

[a–d] Means with different superscripts within rows are significantly different within treatments ($P < 0.05$).

[x,y] Means with different superscripts within rows and days aged are significantly different between treatments ($P < 0.05$).

[1]A five-point Star Probe (SP) attachment fitted with an Instron was used to assess force needed to compress a chop to 20% of its original height (Carlson et al. 2017).

[2]Warner Bratzler Shear Force (WBS) attachment fitted with an Instron was used to assess force needed to shear through cored samples (Huff-Lonergan et al. 2002).

[3]Percent chop purge = [(weight of package with purge- weight of package without purge)/chop weight] × 100.

[4]pH measurements were taken at the center of each chop.

[5]National Pork Board standards, 6-point scale (1 = pale pinkish gray/white; 6 = dark purplish red).

[6]National Pork Board standards, 10-point scale (1 = 1% intramuscular fat; 10 = 10% intramuscular fat).

[7]Chops were cooked to an internal temperature of 68°C on clamshell grills. Percent cook loss = [(raw chop weight – cooked chop weight)/raw chop weight] × 100.

[8]Hunter L, a, and b determined with Minolta Chroma Meter with D65 light source, 50 mm aperture, and 2° observer.

[9]Hue angle values were calculated using the following equation: arctangent (b/a) (AMSA, 2012).

[10]Chroma values were calculated using the following equation: $(a^2+b^2)^{1/2}$ (AMSA, 2012).

[11]Ratio of the densitometry units of the intact 55 kDa band of the sample compared to the 55 kDa band of the reference sample.

[12]Ratio of the densitometry units of the degraded 30 kDa band of the sample compared to the 30 kDa band of the reference sample.

[13]Sarcomere length (SL) was determined using helium-neon laser diffraction (Cross et al., 1981).

freezing to allow for tenderization if chops will not be aged post-freezing.

Fresh SP value decreased by 23% from 1 to 21 d aging across all fresh samples ($P < 0.05$). Frozen SP value decreased by 27% from 1 to 21 d aging across all frozen samples ($P < 0.05$), indicating a similar response to its fresh counterparts. Fresh WBS value decreased by 41% from 1 to 21 d aging across all fresh samples ($P < 0.05$) whereas frozen WBS value decrease by 59% from 1 to 21 d aging across all frozen samples ($P < 0.05$), indicating a possible consideration for a different response to aging by the frozen treatment. The demonstrated numerical difference in percentage change between fresh and frozen WBS values from 1 to 21 d aging is consistent with previous research demonstrating that post-aging freezing resulted in decreased WBS values of pork loins (Kim et al., 2018). Those researchers determined that this lesser WBS value from post-aging freezing steer longissimus muscle was not due to increased protein degradation but most likely loss of structural integrity caused by the formation of ice crystals between degraded myofibrils as proposed by Leygonie et al. (2012b).

Moisture loss

Previous reports have demonstrated that freezing and thawing contributed to decreased water holding capacity. The loss of water binding is caused by multiple factors including protein denaturation and protein modifications as well as disruption of the muscle fiber

structure (Leygonie et al., 2012b). The current results demonstrate that fresh chop purge significantly increased at each d aging (Table 1; $P < 0.01$). Post-aging freezing chop purge also increased from 1 to 8 and 8 to 14 d aging ($P < 0.01$) but decreased from 14 to 21 d aging ($P < 0.01$). Additionally, frozen chop purge was greater than fresh chop purge in samples aged 1, 8, and 14 d ($P < 0.01$) but were not different at 21 d aging ($P > 0.05$). As expected, fresh chops had the least purge. This observation is consistent with previous research demonstrating that decreased water holding capacity is observed when chops are frozen (Lagerstedt et al., 2008; Kim et al., 2015; Coombs et al., 2017). Additionally, chop purge increased through aging of fresh chops and generally increased in frozen chops as demonstrated by others examining beef loin muscle (Lagerstedt et al., 2008; Vieira et al., 2009). Increased purge and water loss in general may be due to protein denaturation, chilling rate, proteolysis during aging, and protein oxidation (Kristensen and Purslow, 2001; Rowe et al., 2004; Huff-Lonergan and Lonergan, 2005).

Several studies have demonstrated that freezing can impact cook loss (Vieira et al., 2009; Leygonie et al., 2012a; Kim et al., 2015). Weight lost during cooking is believed to originate from the melting of fat and the denaturation of proteins that are bound to water causing subsequent cooking losses of both fat and moisture (King et al., 2003; Vieira et al., 2009). However, the current results demonstrate that fresh chops had greater cook loss than post-aging freezing chop cook loss at 14 (Table 1; $P < 0.03$) and 21 ($P < 0.01$) d aging. These results are not consistent with past observations identifying significantly greater cook loss in frozen steaks compared with fresh steaks (Vieira et al., 2009; Kim et al., 2011a; Kim and Kim, 2017). This difference could be due to the experienced purge loss. It is possible that increased purge loss during aging and freezing could decrease the total moisture available to be lost during cooking in the post-aging freezing samples. The current results also demonstrate that post-aging freezing chops aged 1 d ($P < 0.05$) had the greatest percent cook loss compared with all other frozen chops. This result is also inconsistent with other studies which demonstrated that steaks frozen without aging demonstrated lesser cook loss than steaks that were allowed to age and then were frozen (Kim et al., 2015). This difference is likely due to the observation of less purge in the d 1 post-aging freezing chops.

Color analysis

Fresh chop L value increased from 1 to 8 d aging ($P < 0.01$) but did not change after 8 d aging ($P > 0.05$). Post-aging freezing chop L value also increased from 1 to 8 d but also increased from 14 to 21 d aging ($P < 0.01$). Chops demonstrated lesser redness values at 1 d aging ($P < 0.01$) than any other aging period considered. Fresh chop b value increased from 1 to 8 d aging ($P < 0.01$) but was not different after 8 d aging ($P > 0.05$). Post-aging freezing chop b value increased from 1 to 8 d and 8 to 14 d aging ($P < 0.01$) but did not differ between 14 and 21 d aging ($P > 0.05$). As expected, chops became lighter, more red, and more discolored over time, indicating loss of color stability which could be caused by a large array of factors such as pH, muscle source, lipid oxidation, oxidation of myoglobin, and mitochondrial activity (Mancini and Hunt, 2005). Meat color stability is also impacted by storage time (Harsh et al., 2018), loss of water (Kim et al., 2018), and freezing rate (Kim et al., 2018).

Fresh chops aged 1 d had lower a values ($P < 0.01$) than their frozen counterparts that were aged 1 d ($P < 0.01$). Post-aging freezing also resulted in greater b value compared with fresh chop b value at 1, 14, and 21 d ($P < 0.01$) aging. Decreased redness and lesser discoloration were expressed in fresh chops at 1 d aging compared to frozen chops aged 1 d. It is well known that freezing and thawing reduces blooming ability and color stability (MacDougall, 1982). It has been previously demonstrated that steaks from beef and lamb as well as chops from pork longissimus muscle subjected to post-aging freezing demonstrated greater discoloration compared with fresh chops (Kim et al., 2011b, 2015, 2018). This increase in discoloration in the post-aging freezing chops is most likely due to increased susceptibility of myoglobin to oxidation (MacDougall, 1982; Kim et al., 2011b). Contrary to previous results of other studies, the results of this study demonstrate decreased redness in the fresh chops compared with the post-aging freezing chops at 1 d aging. This could be due to the blooming ability (MacDougall, 1982) and the mitochondrial reducing ability of the samples (Tang et al., 2005). Fresh samples may have had mitochondria that were continuing to respire, thus decreasing the blooming ability of myoglobin due to a lower partial pressure of oxygen within chops aged 1 d. This can be compared to chops which experienced post-aging freezing at 1 d aging. These chops may have experienced damage to the mitochondria through the freezing and thawing process, thus impacting mitochondrial functionality and ultimately the blooming ability of myoglobin (Vestergaard et al., 2000).

The rate of freezing and confounding factors such as pH and myoglobin concentration can have a significant impact on color stability. In a study by Kim et al. (2018), fast-freezing pork loin sections after aging resulted in greater redness and greater discoloration compared with loin sections that were frozen slowly. The current results demonstrated differences in a and b values at 1 d aging between fresh and frozen samples ($P < 0.01$) which may be impacted by the freezing method used. However, this difference in a and b values is also impacted by decreases in metmyoglobin reducing agent activity and alterations of mitochondrial function (Kim et al., 2011b).

Post-aging freezing had no significant effect on pH, subjective color score and marbling score, or Hunter L value at any aging period (Table 1; $P > 0.05$). Post-aging freezing was not expected to impact marbling due to no significant changes in the fat content of the chops throughout the freezing period. Previous research has demonstrated small, but significant impacts of post-aging freezing on quality attributes of pH and color scores (Kim et al., 2011b, 2018; Leygonie et al., 2012b; Kim and Kim, 2017). Kim et al. (2018) demonstrated that pork longissimus sections which were aged for 19 d, frozen, and then thawed maintained greater pH values than sections that were only frozen or frozen, thawed, and then aged (Kim et al., 2018). In a similar study examining the effects of aging and freezing/thawing sequence on beef biceps femoris and gluteus medius, freezing and thawing of muscles decreased the pH regardless of the sequence of freezing and thawing (Kim and Kim, 2017). Kim et al. (2011b) observed that freezing for 9 wk reduced pH values in sheep longissimus muscle compared to post-aging freezing of steaks and wet aging of steaks (Kim et al., 2011b). Differences in Hunter L values and visual color scores were not observed between fresh and post-aging freezing treatments. Since samples were stored in opaque boxes during storage, this result was expected.

Correlation of star probe and Warner-Bratzler shear force

Previous studies have shown a moderate correlation between SP and a trained sensory panel tenderness score (Huff-Lonergan et al., 2002; Lonergan et al., 2007). These results of the current experiment demonstrate that SP and WBS generate correlated measurements and they can be utilized to assess texture regardless of aging time or conditions. The relationship between SP and WBS value across all days aging and all treatments is shown in Fig. 4. Across

all aging periods and treatments ($n = 158$), SP value was highly correlated ($r = 0.85$; $P < 0.01$) with WBS values. Fresh SP and WBS correlations across all days aging ($n = 80$; $r = 0.89$) were not significantly different ($P = 0.13$) than post-aging freezing correlations ($n = 78$; $r = 0.83$) across all aging periods. Within aging periods, fresh SP and WBS values were trending ($P = 0.06$) to be more highly correlated at 8 d aging ($n = 20$; $r = 0.93$) than 1 d aging ($n = 20$; $r = 0.78$) but did differ from 14 ($n = 20$; $r = 0.89$) or 21 d aging ($n = 20$; r = 0.92). Post-aging freezing SP and WBS values were significantly more highly correlated ($P < 0.01$) at 14 d aging ($n = 20$; $r = 0.93$) than 8 ($n = 20$; $r = 0.76$) or 21 d aging ($n = 18$; $r = 0.65$). The results suggest that the relationship between SP and WBS is weaker at 21 d aging when pork chops are frozen after aging.

Sarcomere length

During the conversion of muscle to meat, contraction of muscles and shortening of sarcomeres occurs impacting tenderness of whole muscle products. The extent and rate of shortening is impacted by many factors, one of those being temperature. This study demonstrated no significant changes in sarcomere length across days aging ($P > 0.05$) in the fresh chops. This suggests that improvement of WBS or SP during aging is not due to changes in sarcomere length during aging. However, sarcomere length was correlated with WBS ($r = –0.46$) and SP ($r = –0.43$) across all d aging ($P < 0.01$). At 1, 8, 14, and 21 d aging, sarcomere length was correlated with WBS ($r = –0.60, –0.56, –0.71,$ and $–0.51$) and SP ($r = –0.53, –0.60, –0.55,$ and $–0.47$), respectively. Across all days aging, correlation values were not significantly ($P > 0.05$) different between sarcomere length and WBS or SP values. These results do propose a consistent sarcomere length contribution to textural measurements of fresh pork.

Postmortem protein degradation

A large array of biochemical changes occurs during the conversion of muscle to meat influencing meat quality attributes such as water holding capacity and tenderness. Postmortem protein degradation is one factor that has a significant impact on the development of meat tenderness and meat's ability to hold water (Taylor et al., 1995; Melody et al., 2004; Carlson et al., 2017). Desmin is an intermediate filament protein that connects the myofibril with other myofibrils and integrates surrounding organelles (Clark et al., 2002). Troponin-T is part of the troponin complex which functions to modu-
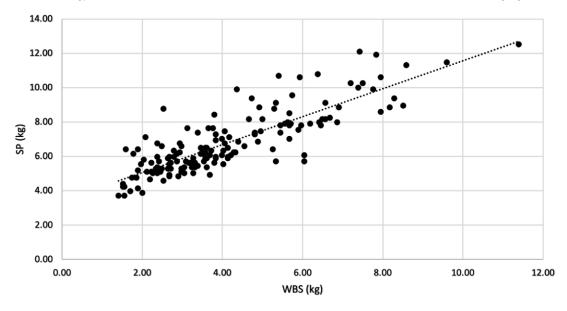
Figure 4. Linear regression showing Star Probe (SP) and Warner-Bratzler Shear Force (WBS) values of Longissimus Dorsi (LM) chops across all days aged and treatments ($n = 158$). Coefficient of Determination and slope for star probe values are denoted (y = 0.8143x + 3.4416; $R^2 = 0.7136$).

late actin and myosin cross bridging (Clark et al., 2002). Greater degradation of these proteins has been linked to pork loin with lower star probe values (Carlson et al., 2017). Fresh samples used in this study were aged for 1, 8, 14, and 21 d to assess the development of pork tenderness throughout different aging periods to determine optimum time for tenderness development. Abundance of intact desmin and troponin-T degradation product is summarized in Table 1. The results show that abundance of intact desmin decreased from 1 to 8 and 8 to 14 d aging ($P < 0.01$) but was not different at 21 d aging ($P > 0.05$) compared with 14 d aging. Troponin-T degradation product was not detected at 1 d postmortem in any sample. Abundance of troponin-T degradation product significantly increased at each d postmortem ($P < 0.01$). Additionally, intact desmin decreased by 56% from 1 to 21 d aging across all fresh samples ($P < 0.05$). Intact desmin and troponin-T were highly correlated ($P < 0.01$) to SP ($r = 0.61$ and -0.62, respectively) and WBS ($r = 0.66$ and -0.67) across all d aging in the fresh samples. Within day correlations revealed that intact desmin was not significantly correlated with WBS ($r = 0.26$; $P = 0.26$) or SP ($r = 0.70$; $P = 0.09$;) at 1 d aging. Troponin-T degradation product was not detected in any sample aged 1 d, so no correlations were computed. In contrast, troponin-T degradation was moderately and highly correlated with WBS at 8, 14, and 21 d postmortem ($r = -0.73$, -0.64, and -0.77, respectively) and with SP at 8, 14, and 21 d postmortem ($r = -0.70$, -0.69, -0.79, respectively) but Fisher's Z transformation revealed that the correlations to troponin-T degrada-

tion were not different across days ($P > 0.05$). Similarly, intact desmin was moderately and highly correlated at 8, 14, and 21 d postmortem with WBS ($r = 0.45$, 0.55, 0.82, respectively) and SP ($r = 0.46$, 0.58, 0.73, respectively). Correlation values at 8, 14, and 21 d were not different from each other ($P > 0.05$) but intact desmin correlations to SP and WBS at 8, 14, and 21 d were all significantly greater than what was determined at 1 d aging ($P < 0.01$). However, intact desmin and troponin-T were weakly correlated to sarcomere length ($r = -0.20$; $P = 0.08$ and $r = 0.24$; $P = 0.04$, respectively) across all day aging. This weak relationship demonstrates that in this experiment, sarcomere length did not contribute to variation in access of proteinases to substrates in different locations in the muscle cell and myofibril.

Degradation of desmin and troponin-T has consistently shown to account for differences seen in instrumental tenderness values of pork muscles (Wheeler et al., 2000; Melody et al., 2004; Carlson et al., 2017). Degradation of desmin protein can ultimately alter myofibril alignment and connection to conjoining structures and resulting in differences in tenderness. Degradation of troponin-T could demonstrate weakening of the complex formed between actin and myosin or skeletal muscle protein degradation overall. These results demonstrate the corresponding relationship between both instrumental tenderness measurements and protein degradation data. These results also demonstrate protein degradation, in general, is consistent with SP and WBS value decline across aging periods.

Conclusions

Aging fresh pork loin for 8 d showed a 22 and 32% improvement in SP and WBS values, respectively. The current results suggest that aging 14 or 21 d did not result in improved SP or WBS values compared 8 d aging. Degradation of desmin and troponin-T were, in general, consistent with instrumental tenderness measurements across days aging, demonstrating that desmin degradation to 14 d and troponin-T degradation to 21 d are key components of fresh pork tenderness. Sarcomere length did not change during the postmortem aging process. Protein degradation data were numerically more highly correlated with instrumental tenderness measurements later postmortem when compared with sarcomere length, suggesting protein degradation may have a greater impact on tenderness values later postmortem. Furthermore, SP and WBS values are highly correlated instrumental tenderness measurements regardless of post-aging storage conditions used in this study. These results demonstrate that post-aging freezing did not have a significant impact on pork quality features of color and marbling score, cook loss, and instrumental tenderness measurements. Lastly, freezing pork at 1 d postmortem will not allow products to improve in SP or WBS values so a recommended best practice is aging pork prior to freezing.

Acknowledgements

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