Meat and Muscle Biology™

Influence of Aging Temperature and Duration on Spoilage Organism Growth, Proteolytic Activity, and Related Chemical Changes in Vacuum-Packaged Beef *Longissimus*

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Abstract: The objective was to evaluate the influence of vacuum-packaged aging temperature, duration, and their interaction on spoilage organism growth, proteolytic activity, and resulting beef tenderness. Paired strip loins were collected from 60 USDA Low Choice beef carcasses ($n = 60$), then assigned a storage temperature (−2°C, 0°C, or 4°C). Loins were portioned into half loins and assigned to an aging duration (14, 28, 42, or 56 d) and vacuum packaged. Loins were aged in commercial upright refrigerators. Half-loin packages, at their respective aging duration, were aseptically opened and cut surface swabbed for microbial analysis before fabrication into 2.54 cm strip steaks ($n = 5$). Steaks assigned to slice shear force (SSF) were cooked to 71°C. A raw steak was used for microbial, proteolytic, and volatile analyses. Two-way interactions were observed for all spoilage organisms ($P < 0.001$). Aging for 42 and 56 d at −2°C produced lower microbial counts compared to individual aging durations at 4°C ($P < 0.05$). Loins aged for 14 d at 4°C had increased desmin and troponin-T degradation compared to aging for 14 at −2°C and 0°C ($P < 0.05$). Loins aged at 4°C produced more tender steaks compared to −2°C and 0°C ($P = 0.001$). Steaks aged for 42 and 56 d possessed the lowest SSF values ($P < 0.05$). Aging for 56 d at 4°C produced the greatest amount of total free amino acids ($P < 0.001$). Two-way interactions were observed for 7 compounds (alcohols, aldehydes, carboxylic acids, ketones, and sulfur-containing compounds; $P < 0.05$). Aging for 56 d at 4°C had the greatest ethanol concentration ($P < 0.05$). These data indicate aging at 4°C increases the rate of proteolysis and subsequent tenderness development and flavor precursor accumulation. However, extended aging at 4°C resulted in increased microbial counts. Many traits peaked at 42 d of aging.

Key words: wet-aging, beef, spoilage organisms, proteolysis, volatiles, amino acids
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Introduction

Postmortem aging is a common management practice in the beef industry to enhance beef palatability, primarily tenderness. During aging, several biochemical mechanisms intrinsically tenderize beef (Huff Lonergan et al., 2010; Kemp et al., 2010). Proteolytic enzymes degrade the myofibrillar structures, namely structural and regulatory proteins. Through postmortem aging and proteolysis, important beef flavor precursors (free amino acids, sugars, and peptides) are made available (Koutsidis et al., 2008; Foraker et al., 2020; Vierck et al., 2020).

Wet-aging is a method of postmortem aging in which vacuum-packaged beef is held in refrigerated temperatures for a period of time. The 2015 National Beef Tenderness Survey reported that the average retail postfabrication aging time was 25.9 d with a range of 6 to 102 d, and in foodservice, average postfabrication aging time was 35.1 d with a range of 3 to 91 d

(Martinez et al., 2017). While aging improves and ensures tenderness, beef flavor can deteriorate with excessive aging time (Lepper-Blilie et al., 2016; Evers et al., 2020; Foraker et al., 2020). Deterioration of beef flavor occurs through increases in lipid oxidation and microbial growth giving rise to volatile compounds that are perceived as off-flavors (Shahidi and Pegg, 1994; Casaburi et al., 2015; Frank et al., 2020). These volatile compounds are also responsible for the characteristic odor associated with opening vacuum packaging in aged product. This has been coined as "confinement odor" and has been characterized as sour, cheesy, acid, and winey (Ercolini et al., 2011; Small et al., 2012; Casaburi et al., 2015). Measuring volatile compound production in raw steaks would provide insight into the extent of lipid oxidation and microbial growth.

Meat begins to freeze at approximately −3.33°C; therefore, −2°C is the minimal temperature for an aging cooler (Aberle et al., 2012). Conversely, 4°C borders on the food safety danger zone (4.44°C to 60°C), which is the range in temperature in which bacteria grow rapidly. As a result, beef aging temperature is most typically between −2°C and 4°C. Elevated temperatures have been found to increase proteolytic activity (Whipple et al., 1990; King et al., 2003). However, these studies used postmortem carcass chilling as a model. King et al. (2009) reported increased desmin degradation when subprimals were aged at 3.3°C compared to −0.5°C. Moreover, storage temperature has been shown to influence microbial growth in vacuum-packaged subprimals. The shelf life of vacuum-packaged subprimals has been suggested to be 100 d if stored at −1°C (Small et al., 2012). Chai et al. (2017) reported storage temperature influenced bacterial growth curves, where colder temperatures extended the lag phase, subsequently extending shelf life.

Based on available literature, there is a need to understand the influence of storage temperature and duration on shelf life and proteolytic activity during aging. Therefore, the objective of this work is to evaluate the influence of wet-aging temperature, duration, and the interaction of these factors on spoilage organism growth, proteolytic activity, and the release of beef flavor precursors.

Materials and Methods

Product selection, subprimal aging, and fabrication

Paired beef strip loins (Institutional Meat Purchasing Specifications #180) were selected from A maturity, USDA Low Choice carcasses free of quality defects 5 d postmortem ($n = 60$). Trained Texas Tech University personnel collected yield and quality grade data, including preliminary and adjusted yield grade; ribeye area; kidney, pelvic, and heart fat; lean and skeletal maturity; and marbling score. Strip loins were collected in 2 collection trips due to space limitations in the commercial upright refrigerators. Following each collection, strip loins were transported under refrigeration (0°C to 4°C) to the Gordon W. Davis Meat Laboratory in Lubbock, TX. Paired loins from each carcass was assigned to an aging temperature environment (−2°C, 0°C, or 4°C). Paired strip loins were halved, and each half loin was randomly assigned to an aging duration (14, 28, 42, or 56 d). Aging treatments were applied 5 d postmortem. Loins were aged in their respective commercial upright refrigerator (ESF1, Everest Refrigeration, Compton, CA). Refrigerator temperatures and defrost cycles were monitored continuously with remote temperature recorders (Multitrip temperature recorders, Temprecord, Auckland, New Zealand). Defrost cycles occurred every 6 h and lasted approximately 30 min. Mean temperatures and standard deviations are presented in Table 1. At each aging duration interval, loins were fabricated into 2.54 cm steaks ($n = 5$), vacuum packaged, and then frozen at −20°C until subsequent analysis.

Spoilage microorganism enumeration

Lactic acid bacteria (LAB), *Enterobacteriaceae* (EB), mesophilic aerobic plate counts (M-APC), and psychrotrophic aerobic plate counts (P-APC) spoilage organisms were measured at each aging interval. Prior to fabricating steaks, the vacuum packaging was aseptically opened to expose the cut surface of the *longissimus lumborum*. The cut surface was swabbed using a sterile 50 cm^2 template and prehydrated buffered peptone water (BPW) swabs (EZ Reach swabs, World Bioproducts, Bothell, WA). Research personnel sanitized the knife with 70% ethanol solution and changed gloves between each loin. Samples were then transported to the Texas Tech University Experimental Sciences Building (Lubbock, TX) for microbial

Table 1. Refrigerator temperature means and standard deviations

Temperature Treatment	Mean	Standard Deviation
−2°C	−1.56	±1.60
0°C	0.66	±1.25
4°C	4.40	±1.03

analyses. Sample sponges were stomached for 30 s at 230 rpm (Seward Stomacher 400C, Seward, Bohemia, NY). Serial dilutions were performed as necessary using sterile BPW tubes. P-APC spoilage organisms were enumerated using Petrifilms (3M, Maplewood, MN). Each sample was plated in duplicate. Once plated, samples were incubated at 7°C for 10 d. Following incubation, duplicate plates with the most appropriate dilution, decided using the 3M Interpretation Guide, were counted using a Petrifilm reader (3M). LAB, EB, and M-APC spoilage organisms were enumerated using the TEMPO system (bioMérieux, Marcy-l'Étoile, France). Glass vials with dehydrated culture media were filled with either 3.0 or 3.9 mL of autoclaved water, then vortexed. Either 1 mL or 100 µL of sample was pipetted into the vials, then vortexed. TEMPO cards, or plates, were filled with sample media solution using a TEMPO filler. Once all sample cards were filled, samples were incubated. LAB samples were incubated for 44 to 48 h at 35°C in a microaerophilic environment. EB and M-APC samples were incubated for 22 to 28 h at 35°C. Following incubation, samples were counted using a TEMPO reader. All data were transformed to $\log_{10}$ colony-forming units (CFU)/50 cm^2 for statistical analysis and reporting. The detectable limit was 0.30 $\log_{10}$ CFU/cm^2.

Raw sample homogenization

Frozen raw steaks were tempered for 24 h at 2°C to 4°C. Raw steaks were trimmed of external fat, connective tissue, and accessory muscles; cut into cubes; flash frozen with liquid nitrogen; and homogenized (Blixer 3 Food Processor, Robot Coupe, Jackson, MS). Raw homogenates were stored in labeled bags (Whirl-Pak Standard 13-oz bag, Whirl-Pak, Madison, WI) at −80°C until subsequent analyses.

Free amino acid analysis

Free amino acid analysis was conducted using the modified methods of Koutsidis et al. (2008). For water soluble extraction, 3 g of raw, homogenized sample was weighed into a 50 mL conical tube. Ten milliliters of autoclaved, cold, double-distilled water was added to each sample. Samples were shaken for 10 min, then centrifuged at 29,900 × *g* for 33 min at 4°C. The supernatant was decanted, then an additional 5 mL of water was added to the remaining pellet. Samples were re-extracted as previously described, then the 2 extracts were combined. The combined supernatant was filtered through a 0.2 µm disc filter (Thermo Fisher Scientific, Waltham, MA). Free amino acids were derivatized using 100 µL of the aqueous extract from the combined supernatant and an EZ:Faast amino acids kit (Phenomenex, Torrance, CA). Amino acids were determined using gas chromatography–mass spectrometry in electron impact mode with a 3:1 split ratio (6890A; 5975B, Agilent, Santa Clara, CA). Derivatives were separated using a Zebron ZB-AAA capillary column (10 m × 0.25 mm; 0.25 µm film thickness, Phenomenex). Amino acids were quantified (millimole per kilogram of initial wet sample) using a 3-level calibration curve based on response and concentration ratios between an internal standard (Norvaline) and external authentic standards.

Automated Western blots

Protein degradation was measured using an automated capillary-based immunoassay Western blot system (Wes Instrument, ProteinSimple, San Jose, CA). Desmin and troponin-T were proteins of interest. Frozen raw powdered muscle samples were shipped overnight to the Roman L. Hruska U. S. Meat Animal Research Center (Clay Center, NE). One gram of powdered sample was homogenized with 10 mL Tris-ethylenediamine tetraacetic acid (EDTA) buffer (50 mM Tris, 10 mM EDTA: pH 8.3) for 20 s using a Polytron PT-2100. A 0.5 mL aliquot of homogenate was immediately transferred into a 1.5 mL microcentrifuge tube. Five hundred microliters of 2X Treatment Buffer (0.125 M Tris, 4% sodium dodecyl sulfate, 20% glycerol; pH 6.8) was added and samples were then vortexed. Samples were heated in a 50°C water bath for 20 min. Samples were mixed with a Pipetman fixed with a pipette tip that had the tip snipped off, aspirated and dispensed multiple times (to shear the stringy and viscous nucleic acids), then reheated for 5 min. Samples were centrifuged (Centrifuge 5415 C or 5418, Eppendorf, Enfield, CT) for 20 min at 16,000 × *g*. Protein concentration was determined using the Thermo Fisher Scientific/Pierce Micro-BCA Protein Assay Reagent (Thermo Fisher Scientific). The supernatant was diluted 1:5 (10 µL sample supernatant + 40 µL buffer) with 1X Treatment Buffer (2X treatment buffer diluted with water). An Immulon 1B microtiter plate (Thermo Fisher Scientific) was used for the assay. A standard curve of 5 concentrations (0, 0.5, 1, 2, 4 mg/mL) of bovine serum albumin (BSA) was used to calculate protein concentration. Ten microliters of sample or BSA standard was added to the assigned well of the microtiter plate in triplicate. Two hundred microliters of BCA Reagent was added to each well. The plate was incubated at 37°C for 30 min. Using a SpectraMax

Plus Plate Reader (Molecular Devices, San Jose, CA), the plate was read at an absorbance of 562 nm. Total protein was then calculated for each sample from the standard curve. Supernatant samples were stored at −20°C until Wes assay was conducted.

Using an Excel spreadsheet for assay sample plate prep provided in the assay kit, samples were diluted with 0.1X sample buffer (from the 10X sample buffer included in the assay kit) to a concentration of 0.3 mg/mL and 0.1 mg/mL for desmin and troponin-T, respectively. Standard pack reagents (DDT, Fluorescent 5X Master Mix, and Biotinylated Ladder) were prepared following instructions from the Wes assay kit. Primary antibodies (Rabbit Monoclonal Desmin, clone RM234 [Novus Biologicals, Centennial, CO]; Mouse Monoclonal Troponin-T, clone JLT-12 [Sigma-Aldrich, St. Louis, MO]) were diluted to 1:50 and 1:1250 using the Antibody Diluent 2, respectively, and stored on ice. Four microliters of diluted sample extract and 1 µL of 5X fluorescent Master Mix were combined in a 0.5 mL microcentrifuge tube and vortexed. Samples were heated for 5 min at 95°C. Following heating, the samples were vortexed and then centrifuged for 15 s at 16,000 × *g* and then stored on ice until assayed. A peroxide/luminol-s solution was prepared by combining 200 µL of peroxide and 200 µL of luminol-s. Secondary antibodies (Gt Anti-Rabbit Secondary HRP or Anti-Mouse Secondary HRP) were provided in the ProteinSimple detection module and were not diluted. Each Wes assay plate contained 25 lanes. Lane 1 contained the biotinylated ladder. A 0 h postmortem *longissimus lumborum* sample was plated on 3 lanes per plate for desmin degradation plates. The remaining lanes were assigned to specific samples to ensure there was no treatment bias on an individual plate. Once all samples/reagents were plated, the plate was centrifuged (Eppendorf 5804 tabletop centrifuge equipped with a 96-well plate rotor) for 5 min at 1,000 × *g* at room temperature. Five hundred µL of wash buffer was added to the first 3 rows below the samples. The plate was inserted into the Wes Instrument and target proteins were detected with chemiluminescence and quantified based on area under the curve. Desmin and troponin-T were assayed on separate plates from one another. Intact desmin was normalized by calculating percent degradation based on the average intact desmin of the three 0 h samples for each plate. Intact troponin-T and degradation products were normalized by calculating percent degradation as the percentage of troponin-T degradation product area to the total of intact and degraded troponin-T areas.

Slice shear force

Slice shear force (SSF) was conducted using the methods of Shackelford et al. (1999). Steaks were cooked to 71°C using an electric combi-oven (Model SCC WE 62G, Rational USA, Rolling Meadows, IL) set to 204°C and 0% relative humidity. After reaching peak internal temperature, the lateral end of the steak was squared off to expose the muscle fibers, and a 5-cm slice was removed using a sizing box (Tallgrass Solutions, Inc., Manhattan, KS). A 1-cm-thick, 5-cm-wide slice was removed parallel to the muscle fiber orientation using a slice box and double-bladed knife (Tallgrass Solutions, Inc.). Each slice was sheared perpendicular to the muscle fibers using an SSF machine equipped with a flat, blunt-end blade (Tallgrass Solutions, Inc.). Crosshead speed was set to 500 mm/min. Peak force was recorded in kilograms.

Volatile compound analysis

Volatile compounds were determined using the modified methods of Gardner and Legako (2018). Steaks designated for volatile compound analysis were homogenized as previously discussed. Five grams of raw sample were weighed into 20 mL glass vials and 10 µL of an internal standard solution (1,2 dichlorobenzene, 2.5 µg/µL) was added to the vial. Vials were sealed with a 1.3-mm polytetrafluoroethylene septa and metal screw cap (Gerstel, Inc., Linthicum, MD). Samples were loaded into a dry air-cooling block set at −20°C (MéCour Temperature Control, LLC, Groveland, MA). A Gerstel automated sampler (Multipurpose S, Gerstel, Inc.) removed samples from the cooling block and placed them in an agitator for a 5-min incubation period at 65°C. Following incubation, a 25-min extraction period was used to collect volatile compounds from the sample headspace via solid phase microextraction with an 85 µm film thickness carboxen polydimethylsiloxane fiber (Supelco Inc., Bellefonte, PA). After extraction, volatile compounds were injected into the gas chromatograph (7890B series, Agilent) and desorbed onto a VF-5ms capillary column (30 m × 0.25 mm × 1 µm; Agilent J&W GC columns) and separated. Ions were detected by the mass spectrometer (5977A, Agilent) within a range of 45 to 500 m/z using electron impact mode at 70 eV. The internal standard and external authentic standards (Sigma-Aldrich) were used to confirm compound identities through retention time and fragmentation pattern of 3 key ions. Volatile compounds were quantified (nanogram per gram of sample) based on internal standard and a 5-level calibration curve.

Statistical analysis

Data were analyzed as a split-plot design where carcass served as the whole plot and loin portion served as the subplot. Loin portion served as the experimental unit at the interaction level. For all analyses, carcass and collection group trip were included in the model as random effects. Statistical analyses were conducted using the PROC GLIMMIX procedure of SAS (v. 9.4, Cary, NC) where aging temperature, duration, and their interaction served as fixed effects. The Kenward-Roger adjustment was used to estimate denominator degrees of freedom. Least squares means were separated using the PDIFF function. An alpha level of 0.05 was used for all analyses.

Results and Discussion

Spoilage microorganisms

Results of spoilage microorganism growth are presented in Table 2. EB, LAB, M-APC, and P-APC were measured at each aging interval. Two-way interactions were observed for all spoilage microorganisms ($P < 0.001$). Loins aged for 42 d at 4°C possessed the greatest EB counts compared to all other treatments ($P < 0.001$). Loins aged for 28 or 56 d at 4°C possessed similar EB counts ($P > 0.05$). In the 4°C environment, loins aged for 14 d had the lowest EB counts ($P < 0.05$). Regardless of aging duration, loins aged at 4°C had increased EB growth compared to loins aged at 0°C or 2°C ($P < 0.001$). No differences were observed between loins aged at 0°C and −2°C regardless of duration ($P > 0.05$). M-APC were the lowest in loins aged for 14 d at −2°C compared to all other treatments ($P < 0.001$). Aging for 28, 42, or 56 d at 4°C and for 28 or 42 d at 0°C produced similar M-APC ($P > 0.05$). M-APC were similar when loins were aged for 14 d at 4°C or for 42 or 56 d at −2°C ($P > 0.05$). Moreover, loins aged for 14 d at 4°C had more M-APC growth than loins aged for 28 d at −2°C ($P < 0.05$). Loins aged 28 d or longer at 4°C and for 42 d at 0°C had the greatest LAB counts compared to all other treatments ($P < 0.05$). When aged in the 0°C environment, loins aged for 56 d produced greater LAB counts compared to loins aged for 28 d ($P < 0.05$). Aging for 14 d at either −2°C or 0°C produced similar LAB counts ($P > 0.05$). P-APC were similar in loins aged for 14 d in either −2°C or 0°C environments ($P > 0.05$) and produced the lowest counts compared to all other treatments ($P < 0.05$). Loins aged for 28, 42, or 56 d at 4°C and for 56 d at 0°C had similar P-APC ($P > 0.05$) and produced the greatest P-APC ($P < 0.05$). Loins aged for 28 d at 0°C produced greater P-APC ($P < 0.05$) than loins aged for either 14 d at 4°C or 42 d at −2°C, which were similar ($P > 0.05$).

These data indicate a clear relationship between aging temperature and duration where the growth rates of spoilage organisms were influenced by aging temperature during extended aging. It is well established that microbial counts increase during storage (Hodges et al., 1974; Newsome et al., 1984; Wicklund et al., 2005; Colle et al., 2015). Newsome et al. (1984) reported an increase in aerobic, anaerobic, and *Lactobacillus* counts in vacuum-packaged beef stored for 1, 3, or 5 wk at 4°C. Colle et al. (2015) reported an

Table 2. Interaction of spoilage microorganism growth[1] from beef strip loins wet aged in 3 aging temperatures[2] and 4 aging durations[3]

	−2°C				0°C				4°C					
Spoilage Organism	14 d	28 d	42 d	56 d	14 d	28 d	42 d	56 d	14 d	28 d	42 d	56 d	SEM[4]	*P* Value[5]
EB	<0.24^{d}	<0.29^{d}	<0.33^{d}	<0.27^{d}	<0.57^{d}	<0.58^{d}	<0.44^{d}	<0.47^{d}	<1.64^{c}	<2.65^{b}	3.74^{a}	2.70^{b}	0.277	<0.001
M-APC	<0.67^{g}	2.27^{e}	3.50^{cd}	3.42^{d}	<1.67^{f}	4.43^{ab}	4.65^{a}	4.07^{bc}	2.94^{d}	4.84^{a}	4.61^{a}	4.43^{ab}	0.319	<0.001
LAB	<0.49^{f}	<1.19^{de}	<1.64^{d}	<1.72^{d}	<0.77^{ef}	2.64^{c}	4.19^{a}	3.25^{b}	2.93^{bc}	4.14^{a}	3.96^{a}	4.18^{a}	0.402	<0.001
P-APC	1.64^{f}	2.80^{e}	3.70^{d}	3.83^{cd}	1.91^{f}	4.09^{c}	4.79^{b}	5.08^{ab}	3.60^{d}	5.23^{a}	5.31^{a}	5.22^{a}	1.570	<0.001

[1]*Enterobacteriaceae* (EB), mesophilic aerobic plate count (M-APC), lactic acid bacteria (LAB), psychrotrophic aerobic plate count (P-APC). Reported as $\log_{10}$ colony-forming units (CFU)/50 cm^2.

[2]−2°C, 0°C, 4°C.

[3]14, 28, 42, 56 d.

[4]Largest standard error of the least squares means. SEM = standard error of the mean.

[5]Observed significance level.

[a–g]Means in the same row without a common superscript differ ($P < 0.05$). Means with a less than symbol (<) indicate that at least one sample had a count that was below the detectable limit (0.02 CFU/cm^2).

increase in M-APCs when aging up to 63 d. The present study is in agreement with these works. However, in regard to EB, counts peaked at 42 d then decreased at 56 d. This decrease in EB counts could be explained by the increase of LAB counts, subsequently increasing lactic acid. Signorini et al. (2006) suggested LAB could reduce the presence of EB through lactic acid production and direct competition. Grau (1983) reported reduced EB growth in beef stored at 1°C compared to 5°C. Our results are congruent with the abovementioned study where 4°C promoted the greatest EB growth and lower temperatures inhibited growth. Developed growth curves of spoilage organisms in beef at different temperatures showed an increase in time in the lag phase, increased specific growth rate, and decreased maximum populations at lower temperatures (Chai et al., 2017). Moreover, Small et al. (2012) reported that storing beef subprimals at −0.5°C greatly extended the shelf life of vacuum-packaged beef subprimals. The interactive effects of storage temperature and duration were observed by Martin et al. (2013). These investigators reported an increase in APCs in overwrap packaged ground beef stored for 14, 21, and 28 d at 2.3°C compared to those stored at −1.7°C.

Protein degradation

Aging temperature × aging duration interactions were observed for both desmin and troponin-T degradation ($P \leq 0.002$; Figures 1 and 2). Aging for 14 d at either −2°C or 0°C ($P > 0.05$) resulted in the least desmin degradation compared to all treatments ($P < 0.05$). Moreover, aging loins for 14 d at 4°C produced similar desmin degradation as for loins aged 28 d at −2°C and

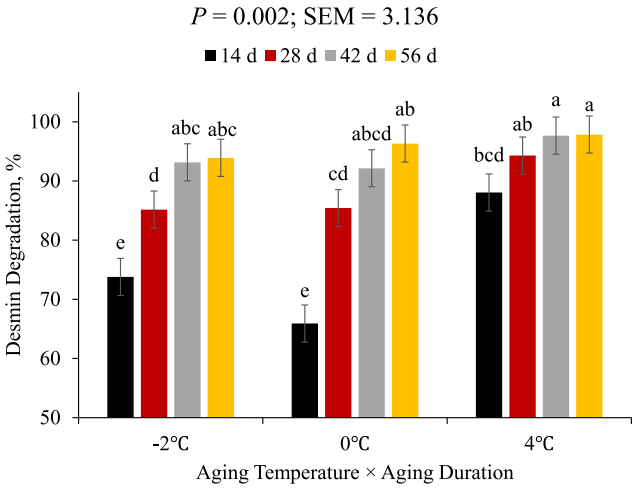

Figure 1. Interaction of percent desmin degradation in beef strip loins aged in 3 temperature environments and 4 aging durations. SEM = standard error of the mean. [a–e]Means without a common superscript differ ($P < 0.05$).

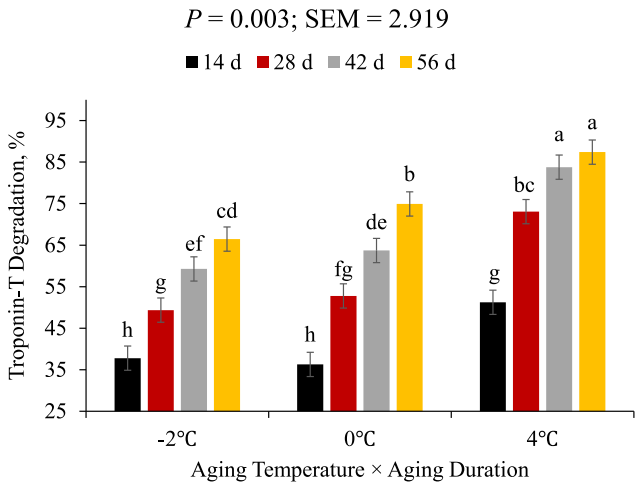

Figure 2. Interaction of percent troponin-T degradation in beef strip loins aged in 3 temperature environments and 4 aging durations. SEM = standard error of the mean. [a–h]Means without a common superscript differ ($P < 0.05$).

for 28 and 42 d at 0°C ($P > 0.05$). Loins aged for 42 or 56 d had similar percentage of degraded desmin, regardless of aging temperature ($P > 0.05$). Similar to desmin, aging for 14 d at 4°C produced a greater percentage of troponin-T degradation ($P < 0.05$) compared to loins aged for 14 d at either −2°C or 0°C, which were similar ($P > 0.05$). Loins aged for 14 d at 4°C were similar to loins aged for 28 d at either −2°C and 0°C ($P > 0.05$). Loins aged for 56 d at 0°C had greater percentage of troponin-T degradation compared to loins aged for 56 d at −2°C ($P < 0.05$). Loins aged at 4°C for either 42 or 56 d were similar ($P > 0.05$) and produced the greatest percentage of troponin-T degradation compared to all other treatments ($P < 0.05$).

Desmin and troponin-T degradation were readily influenced by aging temperature and duration. The rate of proteolysis was clearly driven by aging temperature. Koohmaraie et al. (1986) reported decreased calpain 1 activity at colder temperatures. Moreover, colder temperatures decreased the rate of tenderization (Davey and Gilbert, 1976; Whipple et al., 1990). These concepts were evident in the present study because aging in either −2°C or 0°C resulted in less desmin degradation at 14 d compared to aging at 4°C. Furthermore, aging 14 d at 4°C achieved similar troponin-T degradation as aging for 28 d at colder temperatures. Our desmin degradation results are in agreement with King et al. (2009), who reported increased desmin degradation in *longissimus lumborum* steaks aged at 3.3°C compared to −0.5°C and during intermediate and extended aging. In the present study, desmin degradation was more extensive at earlier aging times and plateaued at 42 d. This is supported by Gruber et al. (2006),

who reported the rate of tenderization decreases as aging time increases. Conversely, troponin-T degradation continually increased approximately 10% at each aging interval beyond 28 d in a more linear response across all aging temperatures. Calpain-2 has been suggested to not be active early postmortem compared to its counterpart, calpain-1. Colle and Doumit (2017) reported increased autolyzed calpain-2 activity beginning at 14 d and increasing up to 42 d. It could be speculated that calpain-2 may be responsible for this continued degradation of troponin-T during extended aging.

Slice shear force

No interactions were observed for SSF ($P = 0.670$). Storage temperature impacted SSF values ($P = 0.014$; Figure 3). Aging loins in 4°C produced more tender steaks compared to −2°C ($P < 0.05$). Steaks from loins aged at −2°C and 0°C produced steaks of similar tenderness ($P > 0.05$). However, 0 and −2°C environments still produced steaks that would classify as "Very Tender" and "Tender," respectively (ASTM, 2011). Aging duration also influenced SSF values ($P < 0.001$; Figure 4). Extended aging (42 and 56 d) produced the most tender steaks compared to shorter aged product ($P < 0.05$). Steaks aged for 14 d produced the greatest SSF value ($P < 0.05$). Like storage temperature, each aging duration produced steaks that would classify as "Very Tender" or "Tender."

Regarding storage temperature, the present study's results agree with King et al. (2009), who reported elevated aging temperatures (−0.5°C vs. 3.3°C) improved SSF values. Furthermore, this was supported

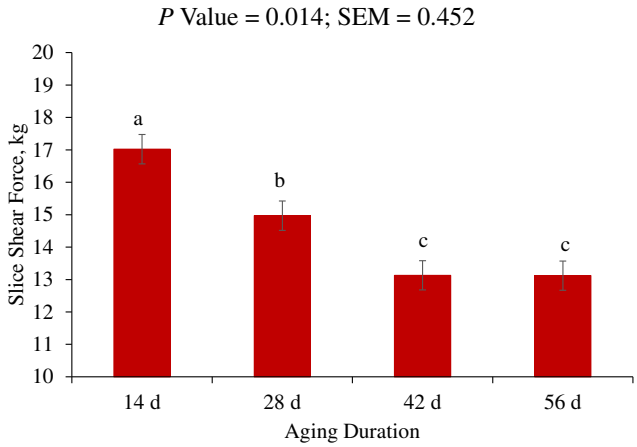

Figure 4. Least squares means of slice shear force values from beef strip loin steaks aged for 4 aging durations. SEM = standard error of the mean. [a–c]Means without a common superscript differ ($P < 0.05$).

by desmin and troponin-T degradation data previously discussed. When comparing the protein degradation data (Figures 1 and 2) and the temperature × duration SSF data (Figure 5), it is clear that as myofibrillar protein degradation increases, SSF values decrease. Although the contribution to tenderness of individual myofibrillar proteins has not been discerned, the desmin degradation treatment means more closely mirror the decline in SSF than does the degradation of troponin-T. However, across all samples the correlation of desmin degradation to SSF was −0.45 compared to −0.50 for troponin-T (data not shown).

Cassens et al. (2018) reported no difference in Warner-Bratzler shear force (WBSF) values in beef subprimals aged at 0°C to 1.1°C and 3.3°C to 4.4°C; however, subprimals were aged in their respective environment for 7 d only. This may explain this

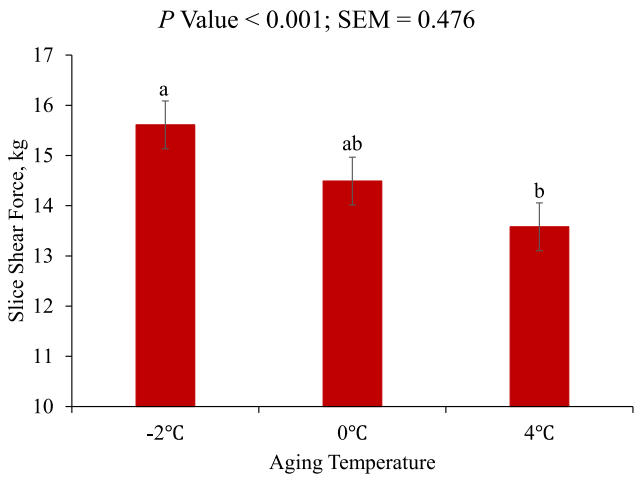

Figure 3. Least squares means of slice shear force values from beef strip loin steaks aged in 3 temperature environments. SEM = standard error of the mean. [a,b]Means without a common superscript differ ($P < 0.05$).

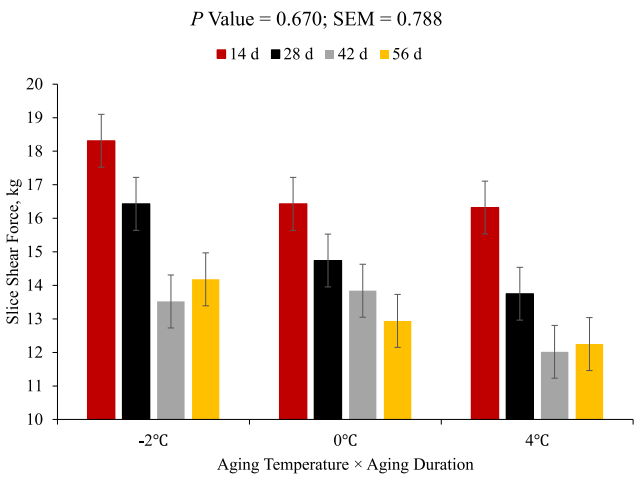

Figure 5. Interaction means of slice shear force values from beef strip loin steaks aged in 3 temperature environments and 4 aging durations. SEM = standard error of the mean.

discrepancy with the present study. Furthermore, Juárez et al. (2010) reported no difference in SSF values between steaks aged in different temperature environments (1°C and 5°C). It should be noted that individual steaks were aged, whereas in the current study, loin portions were aged, although this difference has been shown not to impact tenderness (Wheeler et al., 1996). Historically, it has been generally accepted that ultimate tenderness was realized at 28 d postmortem (Gruber et al., 2006); however, data are accumulating that indicate this is not true. In the present study, ultimate tenderness was realized at 42 d. Our results are congruent with Lepper-Blilie et al. (2016), who reported the lowest WBSF values in beef aged for 42 d. However, the investigators concluded the improvements in WBSF values were minimal in terms of economics and maintaining flavor integrity. Juárez et al. (2010) reported similar results as Lepper-Blilie et al. (2016). In contrast with the previous 2 studies, we observed a 1.49 kg decrease in SSF values from 28 to 42 d of age, whereas the previous studies observed much smaller differences. Consumers can detect differences in beef steaks determined to be tough, intermediate, and tender (Huffman et al., 1996; Boleman et al., 1997). The 2015 National Beef Tenderness Survey reported approximately 96% of top loin steaks were "very tender" and 4% of top loin steaks were "tender" (Martinez et al., 2017). As a majority of beef steaks are considered tender, it is probable that consumers are now able to distinguish between "tender" and "very tender" steaks. Corbin et al. (2015) reported differences in consumer tenderness liking and acceptability in steaks considered "tender" (WBSF $\leq$ 3.4 kg). The authors were not able to conclude whether consumers are able categorize "very tender" steaks based on tenderness or if juiciness and flavor influenced consumer perception of tenderness.

Free amino acids

Two-way interactions were observed for asparagine, aspartic acid, cysteine, cystine, glutamic acid, glycine, isoleucine, leucine, methionine, ornithine, phenylalanine, proline, threonine, valine, and total amino acids ($P \leq 0.007$; Table 3). Loins aged for 56 d at 4°C produced the greatest concentration of asparagine, aspartic acid, glutamic acid, isoleucine, leucine, and valine ($P < 0.05$) compared to all other treatments followed by loins aged for 42 d at 4°C ($P < 0.05$). Asparagine, aspartic acid, glutamic acid, isoleucine, leucine, and valine concentrations were greater in loins aged for 28 d at 4°C compared to loins aged for 28 d at −2°C ($P < 0.05$). Storage temperature had no influence on asparagine, aspartic acid, glutamic acid, isoleucine, leucine, or valine content in loins aged for 14 d ($P > 0.05$) and were comparable to loins aged for 28 d at −2°C ($P > 0.05$). Cysteine, glycine, methionine, phenylalanine, proline, and threonine concentrations were the greatest in loins aged for 42 or 56 d at 4°C compared to all other treatments ($P < 0.05$). Loins aged for 14 d at either 0°C or 4°C and 28 d at −2°C or 0°C have similar cysteine, glycine, methionine, and threonine concentrations ($P > 0.05$) and had lower concentrations compared to loins aged for 42 d at 0°C ($P < 0.05$). Loins aged for 28 d at 4°C, 42 d at 0°C, and 56 d at either −2°C or 0°C had similar phenylalanine and proline concentrations ($P > 0.05$). Loins aged for 56 d at 4°C produced the greatest concentration of cystine compared to all other treatments ($P < 0.05$). Loins aged for 42 d at 4°C and 56 d at 0°C had similar cystine concentrations ($P > 0.05$) and had greater concentrations than loins aged for 14 d at −2°C ($P < 0.05$). Aging loins for 56 d at 4°C produced the greatest levels of ornithine compared to all other treatments ($P < 0.05$). Furthermore, loins aged for 42 d at 4°C and 56 d at either −2°C or 0°C possessed similar ornithine concentrations ($P > 0.05$). Total free amino acid content was the greatest in loins aged for 56 d at 4°C ($P < 0.05$) followed by loins aged for 42 d at 4°C ($P < 0.05$).

β-Alanine, glutamine, and hydroxyproline were not influenced by either aging temperature or duration ($P > 0.05$). Aging duration readily impacted alanine, histidine, lysine, serine, tryptophan, and tyrosine ($P < 0.05$; Table 4). Loins aged for 42 and 56 d had the greatest concentration of alanine, tryptophan, and tyrosine compared to loins aged for 14 or 28 d ($P < 0.05$). Histidine and serine concentrations were greatest in loins aged for 56 d compared to all other duration treatments ($P < 0.05$). Aging for 28 d produced a greater amount of serine, tryptophan, and tyrosine than loins aged for 14 d ($P < 0.05$). Lysine concentration was greater in loins aged for 56 d compared to 14 d ($P < 0.05$). Aging temperature influenced serine and tryptophan ($P < 0.05$). Aging beef subprimals at 4°C produced the greatest concentration of serine and tryptophan ($P < 0.05$) compared to −2°C or 0°C, which were similar ($P > 0.05$).

The present study indicated aging temperature and duration readily influenced the release of free amino acids. Multiple studies have reported an increase in free amino acids in aged product (Nishimura et al., 1988; Koutsidis et al., 2008; Foraker et al., 2020). The present study is in agreement with these works and has

Table 3. Interaction of free amino acid content (mmol/kg) in beef strip loins aged in 3 temperature environments and 4 aging durations

	−2°C				0°C				4°C					
Amino Acid (mmol/kg)	14 d	28 d	42 d	56 d	14 d	28 d	42 d	56 d	14 d	28 d	42 d	56 d	SEM[3]	*P* Value[4]
Asparagine	0.246^{gh}	0.246^{gh}	0.362^{efg}	0.513^{cd}	0.185^{h}	0.325^{fg}	0.474^{cde}	0.570^{c}	0.282^{gh}	0.434^{def}	0.739^{b}	0.861^{a}	0.065	0.002
Aspartic Acid	0.039^{f}	0.038^{f}	0.121^{ef}	0.260^{cd}	0.032^{f}	0.0877^{ef}	0.179^{de}	0.356^{c}	0.056^{f}	0.170^{de}	0.483^{b}	0.823^{a}	0.070	<0.001
Cysteine	0.448^{bcde}	0.332^{de}	0.669^{bcde}	0.720^{bcde}	0.142^{e}	0.396^{cde}	0.962^{b}	0.746^{bcd}	0.277^{de}	0.961^{bc}	1.865^{a}	2.252^{a}	0.242	<0.001
Cystine	0.004^{c}	0.009^{bc}	0.012^{bc}	0.012^{bc}	0.005^{bc}	0.010^{bc}	0.015^{bc}	0.022^{b}	0.005^{bc}	0.007^{bc}	0.023^{b}	0.056^{a}	0.010	0.007
Glutamic Acid	1.366^{ef}	1.351^{ef}	1.900^{de}	2.891^{c}	1.108^{f}	1.852^{de}	2.793^{c}	3.100^{c}	1.406^{ef}	2.439^{cd}	3.946^{b}	4.799^{a}	0.332	<0.001
Glycine	0.837^{ef}	0.780^{f}	1.058^{de}	1.347^{bc}	0.833^{ef}	0.993^{def}	1.548^{b}	1.382^{bc}	0.886^{ef}	1.176^{cd}	1.909^{a}	1.950^{a}	0.148	<0.001
Isoleucine	0.553^{gh}	0.632^{gh}	0.977^{ef}	1.357^{c}	0.444^{h}	0.772^{fg}	1.287^{cd}	1.406^{c}	0.608^{gh}	1.072^{de}	1.782^{b}	2.206^{a}	0.151	<0.001
Leucine	0.913^{gh}	1.015^{gh}	1.527^{ef}	2.039^{cd}	0.716^{h}	1.220^{fg}	1.961^{cd}	2.064^{c}	1.039^{gh}	1.662^{de}	2.607^{b}	3.029^{a}	0.220	0.006
Methionine	0.230^{fg}	0.288^{fg}	0.477^{de}	0.688^{bc}	0.178^{g}	0.382^{ef}	0.712^{bc}	0.734^{b}	0.289^{fg}	0.554^{cd}	1.010^{a}	1.147^{a}	0.095	<0.001
Ornithine	0.113^{c}	0.089^{c}	0.096^{c}	0.306^{b}	0.073^{c}	0.115^{c}	0.153^{c}	0.442^{b}	0.083^{c}	0.152^{c}	0.335^{b}	0.687^{a}	0.069	<0.001
Phenylalanine	0.515^{ef}	0.551^{ef}	0.796^{cd}	1.107^{b}	0.407^{f}	0.667^{de}	1.139^{b}	1.131^{b}	0.608^{def}	0.954^{bc}	1.469^{a}	1.673^{a}	0.148	0.004
Proline	0.323^{cd}	0.306^{d}	0.381^{cd}	0.529^{b}	0.302^{d}	0.349^{cd}	0.537^{b}	0.525^{b}	0.315^{d}	0.429^{bc}	0.701^{a}	0.779^{a}	0.060	<0.001
Threonine	0.817^{de}	0.871^{de}	1.238^{cd}	1.868^{b}	0.548^{e}	1.087^{cde}	1.619^{bc}	1.915^{b}	0.783^{de}	1.586^{bc}	2.866^{a}	3.191^{a}	0.225	0.002
Valine	0.821^{gh}	0.916^{gh}	1.394^{ef}	1.932^{cd}	0.673^{h}	1.137^{fg}	1.835^{cd}	2.041^{c}	0.898^{gh}	1.586^{de}	2.582^{b}	3.157^{a}	0.195	<0.001
Total Amino Acids	16.434^{fgh}	16.285^{gh}	21.346^{efg}	30.155^{cd}	12.996^{h}	20.261^{efg}	26.020^{cde}	30.974^{c}	16.030^{gh}	23.434^{def}	38.791^{b}	46.332^{a}	3.673	0.001

[1]−2°C, 0°C, 4°C.

[2]14, 28, 42, 56 d.

[3]Largest standard error of the least squares means. SEM = standard error of the mean.

[4]Observed significance level.

[a–h]Means in the same row without a common superscript differ ($P < 0.05$).

Table 4. Least squares means of free amino acid content (mmol/kg) from beef strip loins wet aged in 3 temperature environments[1] and 4 durations[2]

Amino Acid (mmol/kg)	Alanine	Beta-Alanine	Glutamine	Histidine	Hydroxyproline	Lysine	Serine	Tryptophan	Tyrosine
Temperature									
−2°C	2.563	0.088	0.008	5.616	0.054	0.524	1.720^{b}	0.056^{b}	0.768
0°C	2.738	0.089	0.007	5.399	0.056	0.587	1.932^{b}	0.056^{b}	0.756
4°C	3.009	0.092	0.006	7.192	0.061	0.704	2.565^{a}	0.087^{a}	0.920
SEM[3]	0.150	0.011	0.001	1.059	0.006	0.076	0.180	0.012	0.110
***P* Value**[4]	0.116	0.945	0.421	0.147	0.610	0.238	0.003	<0.001	0.221
Duration									
14 d	2.168^{b}	0.093	0.006	4.478^{b}	0.052	0.351^{c}	1.068^{d}	0.034^{c}	0.537^{c}
28 d	2.463^{b}	0.077	0.007	5.564^{b}	0.057	0.538^{bc}	1.633^{c}	0.057^{b}	0.721^{b}
42 d	3.237^{a}	0.103	0.007	5.601^{b}	0.068	0.674^{ab}	2.527^{b}	0.079^{a}	0.966^{a}
56 d	3.212^{a}	0.086	0.007	8.632^{a}	0.051	0.860^{a}	3.062^{a}	0.096^{a}	1.034^{a}
SEM	0.151	0.103	0.001	1.084	0.006	0.075	0.178	0.019	0.106
***P* Value**	<0.001	0.202	0.644	<0.001	0.073	<0.001	<0.001	<0.001	<0.001
Temperature* × *Duration									
***P* Value**	0.301	0.420	0.688	0.376	0.087	0.782	0.165	0.078	0.551

[1]−2°C, 0°C, 4°C.

[2]14, 28, 42, 56 d.

[3]Largest standard error of the least squares means. SEM = standard error of the mean.

[4]Observed significance level.

[a–c]Means in the same row without a common superscript differ ($P < 0.05$).

elucidated the influence of aging temperature as well. The release of free amino acids has been attributed to proteolytic activity. Zhao et al. (2020) reported an increase in free amino acid concentration after incubating beef *semimembranosus* with exogenous enzymes, i.e., papain, bromelain. As previously discussed, extended aging at 4°C resulted in increased proteolytic activity in the present study. This increase in proteolytic activity is consistent with the increase of free amino acid concentration. Moreover, sarcoplasmic protein degradation has been implicated in the production of free amino acids (Nishimura et al., 1988). Some literature suggests microbial growth and metabolism are linked to the production of free amino acids (Toldra, 1998; Christensen et al., 1999; Ercolini et al., 2011). Previous literature has reported no increase in β-alanine and glutamine during postmortem aging (Locker, 1960; Koutsidis et al., 2008). The present study is in agreement with these works as β-alanine and glutamine were unchanged. The stability of these amino acids during aging is relatively unknown. Amino acids are a primary substrate in the Maillard reaction; therefore, it could be speculated that the increased free amino acid content would result in increased Maillard reaction products (Mottram, 1998; Khan et al., 2015). The increase in sour and bitter tastes associated with extended aged product could be the result of the overaccumulation of amino acids associated with sour and bitter tastes as well as by-products of microbial metabolism. Foraker et al. (2020) reported strong correlations between amino acids and sourness determined by a trained panel.

Raw volatile compound analysis

Forty-nine volatile compounds were detected in raw beef steaks. Aging temperature × aging duration interactions were observed for ethanol, acetic acid, butanoic acid, hexanoic acid, 2,3-butanedione, and dimethyl sulfide ($P < 0.05$; Table 5). A dramatic increase in ethanol concentration was observed when loins were aged for 56 d at 4°C ($P < 0.05$). Likewise, aging 28 d and beyond at 4°C produced the greatest concentration of acetic acid compared to all other treatments ($P < 0.05$). Moreover, aging for 14 and 56 d at −2°C or 0°C and for 14 d at 4°C were similar ($P > 0.05$) and produced the lowest concentrations of acetic acid ($P < 0.05$). Extended aging at 4°C produced the greatest concentration of butanoic acid ($P < 0.05$). However, aging for 42 d at 4°C produced similar butanoic acid concentration compared to aging for 42 d at −2°C and 0°C ($P > 0.05$).

Aging duration readily influenced the production of alcohols, aldehydes, alkenes, carboxylic acids, esters, furans, hydrocarbons, ketones, and sulfur-containing compounds ($P < 0.05$; Table 6). Aging for 42 d produced the greatest concentration of 1-pentanol and 2,3-butanediol compared to all other treatments ($P < 0.05$). The 1-hexanol and 1-octen-3-ol concentrations were the greatest in loins aged for 42 and 56 d

Table 5. Interaction of volatile compound concentration from beef strip loins aged in 3 temperature environments[1] and 4 aging durations[2]

	−2°C				0°C				4°C					
Volatile Compound (ng/g)	14 d	28 d	42 d	56 d	14 d	28 d	42 d	56 d	14 d	28 d	42 d	56 d	SEM[3]	*P* Value[4]
Alcohols														
Ethanol	10.54^{d}	16.06^{d}	27.83^{cd}	13.11^{d}	12.34^{d}	24.83^{cd}	23.66^{cd}	46.77^{bc}	10.88^{d}	26.61^{cd}	54.22^{b}	99.45^{a}	9.780	<0.001
Carboxylic Acids														
Acetic Acid	31.38^{f}	85.83^{cd}	78.81^{cd}	33.76^{ef}	28.89^{f}	68.93^{de}	105.67^{bc}	55.65^{def}	40.51^{ef}	117.35^{ab}	143.00^{a}	143.90^{a}	13.670	0.003
Butanoic Acid	1.87^{e}	3.00^{de}	4.50^{bc}	1.94^{e}	1.64^{e}	2.76^{de}	4.94^{bc}	2.74^{de}	2.62^{de}	3.65^{cd}	5.48^{ab}	6.62^{a}	0.727	<0.001
Hexanoic Acid	1.45^{g}	2.18^{def}	3.08^{abc}	1.34^{g}	1.43^{g}	2.54^{bcd}	3.00^{abc}	1.81^{efg}	1.72^{fg}	2.39^{cde}	3.67^{a}	3.12^{ab}	0.341	0.005
Ketones														
2,3-Butanedione	8.34^{a}	4.81^{bcd}	8.31^{ab}	9.18^{a}	7.98^{ab}	6.49^{abc}	6.04^{abc}	3.19^{cd}	8.76^{a}	4.34^{cd}	4.00^{cd}	1.99^{d}	1.338	0.018
Sulfur Containing														
Dimethyl Sulfide	3.22^{abcde}	3.48^{abcd}	3.32^{abcde}	2.61^{ef}	2.88^{def}	3.66^{abc}	3.77^{ab}	2.36^{f}	3.89^{a}	2.94^{cdef}	3.03^{bcdef}	2.75^{def}	0.286	0.020

[1]−2°C, 0°C, 4°C.

[2]14, 28, 42, 56 d.

[3]Largest standard error of the least squares means. SEM = standard error of the mean.

[4]Observed significance level.

[a–f]Means in the same row without a common superscript differ ($P < 0.05$).

($P < 0.05$). Aging for 56 d resulted in the greatest 1-penten-3-ol concentration ($P < 0.05$). Moreover, aging for 28 and 42 d produced the most 1-octanol ($P < 0.05$). Likewise, decanal concentration was the greatest in loins aged for 28 and 42 d ($P < 0.05$). Dodecanal and nonanal concentrations were the greatest in loins aged for 28 d ($P < 0.05$). Aging for 14 and 56 d produced similar concentrations of dodecanal and nonanal

Table 6. Least squares means of volatile compound content (ng/g) of raw beef strip loins aged in 3 temperature environments[1] and 4 aging durations[2]

	Aging Duration						Aging Temperature				
Volatile Compound	14 d	28 d	42 d	56 d	SEM[3]	*P* Value[4]	−2°C	0°C	4°C	SEM	*P* Value
Alcohols											
1-Hexanol	0.31^{b}	0.28^{b}	0.47^{a}	0.40^{a}	0.067	<0.001	0.33	0.35	0.41	0.067	0.126
1-Octanol	0.96^{b}	1.27^{a}	1.21^{a}	0.94^{b}	0.060	<0.001	1.06	1.10	1.12	0.052	0.733
1-Octen-3-ol	0.98^{a}	0.72^{b}	1.05^{a}	1.13^{a}	0.243	<0.001	0.98	0.98	0.95	0.245	0.959
1-Pentanol	0.48^{b}	0.45^{b}	0.83^{a}	0.38^{b}	0.137	<0.001	0.56	0.54	0.50	0.137	0.751
1-Penten-3-ol	0.12^{c}	0.14^{b}	0.13^{bc}	0.17^{a}	0.008	<0.001	0.13^{b}	0.13^{b}	0.16^{a}	0.007	0.015
2,3-Butanediol	1.58^{b}	2.09^{b}	4.26^{a}	1.32^{b}	0.569	<0.001	1.81^{b}	1.89^{b}	3.24^{a}	0.553	0.011
Aldehydes											
Acetaldehyde	39.86^{b}	74.71^{a}	44.83^{b}	13.14^{c}	6.599	<0.001	46.09	44.15	39.16	10.330	0.454
AQ1 **Benzaldehyde**	10.66^{c}	15.21^{a}	13.70^{b}	7.61^{d}	1.040	<0.001	11.08^{b}	11.81^{ab}	12.50^{a}	1.016	0.034
Dodecanal	5.51^{bc}	17.16^{a}	9.31^{b}	1.76^{c}	3.850	<0.001	9.09	8.37	7.85	3.770	0.814
Heptanal	3.45	3.29	3.94	3.57	0.733	0.357	3.55	3.35	3.79	0.733	0.545
Hexanal	9.73^{b}	7.86^{bc}	12.11^{a}	7.61^{c}	2.708	<0.001	9.68	9.44	8.87	2.733	0.796
Nonanal	9.19^{bc}	11.54^{a}	9.46^{b}	7.97^{c}	0.669	<0.001	9.60	9.48	9.53	0.624	0.981
Octanal	3.42	3.75	3.66	3.65	0.317	0.756	3.73	3.42	3.71	0.302	0.474
Pentanal	1.24^{ab}	1.20^{bc}	1.48^{a}	0.82^{c}	0.356	<0.001	1.21	1.18	1.07	1.640	0.709
2-Methylbutanal	0.10^{b}	0.17^{b}	0.27^{a}	0.12^{b}	0.260	<0.001	0.14^{b}	0.14^{b}	0.22^{a}	0.023	0.022
3-Methylbutanal	1.88	2.77	2.22	1.25	0.572	0.089	1.94	1.66	2.49	0.512	0.363
Alkenes											
Toluene	0.31^{ab}	0.25^{bc}	0.36^{a}	0.18^{c}	0.041	<0.001	0.25	0.26	0.30	0.040	0.342
p-Xylene	0.03^{b}	0.02^{c}	0.02^{c}	0.04^{a}	0.002	<0.001	0.03	0.03	0.03	0.002	0.839
Carboxylic Acids											
Heptanoic Acid	7.56^{b}	7.85^{a}	7.86^{a}	7.53^{b}	0.032	<0.001	7.72	7.70	7.69	0.032	0.209
Nonanoic Acid	0.47^{b}	0.51^{b}	0.52^{b}	0.71^{a}	0.086	<0.001	0.56	0.53	0.57	0.085	0.535
Octanoic Acid	0.39^{b}	0.90^{a}	0.92^{a}	0.42^{b}	0.063	<0.001	0.56^{b}	0.65^{ab}	0.77^{a}	0.064	0.038
Esters											
Butanoic Acid, Methyl Ester	2.19^{b}	2.96^{b}	5.93^{a}	3.17^{b}	0.770	<0.001	3.01^{b}	3.07^{b}	4.60^{a}	0.772	0.010
Hexanoic Acid, Methyl Ester	3.16^{b}	3.31^{b}	5.25^{a}	4.85^{a}	0.620	<0.001	3.75	3.88	4.80	0.648	0.083
Octanoic Acid, Methyl Ester	1.00^{c}	1.28^{ab}	1.39^{a}	1.12^{bc}	0.126	<0.001	1.16	1.18	1.26	0.138	0.714
Furans											
2-Pentyl Furan	0.41^{a}	0.31^{bc}	0.34^{b}	0.26^{c}	0.101	<0.001	0.34	0.35	0.30	0.103	0.483
Hydrocarbons											
Decane	0.90	0.80	0.91	0.95	0.073	0.431	0.98	0.78	0.92	0.064	0.053
Ethylbenzene	0.06^{a}	0.05^{ab}	0.40^{bc}	0.03^{c}	0.009	0.002	0.04	0.05	0.05	0.009	0.497
Nonane	0.50^{b}	0.49^{b}	0.63^{a}	0.57^{ab}	0.102	0.032	0.55	0.52	0.58	0.102	0.552
Octane	0.70^{b}	0.25^{c}	1.07^{a}	0.94^{ab}	0.168	<0.001	0.77	0.70	0.75	0.160	0.852
Tetradecane	0.51	0.65	0.63	0.74	0.121	0.186	0.63	0.65	0.62	0.117	0.965
Ketones											
Butyrolactone	0.89^{c}	1.19^{b}	1.69^{a}	0.78^{c}	0.094	<0.001	1.10	1.08	1.23	0.091	0.382
2-Butanone	4.71^{a}	5.17^{b}	7.65^{a}	3.81^{c}	0.325	<0.001	5.08	5.35	5.57	0.305	0.526
2-Heptanone	0.15^{b}	0.12^{c}	0.17^{a}	0.14^{bc}	0.015	<0.001	0.15	0.15	0.13	0.015	0.181
2-Pentanone	0.20^{b}	0.16^{bc}	0.28^{a}	0.15^{c}	0.018	<0.001	0.19	0.19	0.21	0.018	0.721
2-Propanone	11.20^{b}	18.47^{a}	18.97^{a}	12.96^{b}	0.986	<0.001	14.97	15.02	16.21	0.968	0.580
3-Hydroxy-2-Butanone	12.86^{a}	10.06^{a}	12.92^{a}	4.98^{b}	1.340	<0.001	11.87	9.87	8.88	1.179	0.180

Table 6. (*Continued*)

	Aging Duration						Aging Temperature				
Volatile Compound	14 d	28 d	42 d	56 d	SEM[3]	*P* Value[4]	−2°C	0°C	4°C	SEM	*P* Value
Sulfur Containing											
Carbon Disulfide	6.77[a]	7.16[a]	5.15[b]	5.44[b]	2.432	0.003	5.94	5.97	6.50	2.421	0.521
Dimethyl-Disulfide	0.01	0.01	0.04	0.01	0.023	0.521	0.01	0.01	0.03	0.019	0.449
Methanethiol	26.80[b]	36.19[a]	17.05[c]	29.59[ab]	2.961	<0.001	25.73	25.97	30.52	2.610	0.276

[1]−2°C, 0°C, 4°C.

[2]14, 28, 42, 56 d.

[3]Largest standard error of the least squares means. SEM = standard error of the mean.

[4]Observed significance level.

Aging duration × aging temperature interaction not significant ($P > 0.05$).

[a–c]Means in the same main effect and row without a common superscript differ ($P < 0.05$).

($P > 0.05$). Loins aged for 42 d had the highest hexanal concentration compared to all other treatments ($P < 0.05$). Aging for 56 d resulted in the lowest pentanal concentration compared to all other treatments ($P < 0.05$). Toluene concentrations were the lowest in loins aged for 56 d ($P < 0.05$). In contrast, p-xylene concentration was the greatest in loins aged for 56 d ($P < 0.05$). Heptanoic acid and octanoic acid concentrations were the greatest in loins aged for 28 and 42 d ($P < 0.05$). Aging for 56 d produced the greatest concentration of nonanoic acid compared to all other treatments ($P < 0.05$). Loins aged for 42 d produced the greatest amounts of butanoic acid, methyl ester and octanoic acid, methyl ester ($P < 0.05$). Hexanoic acid, methyl ester concentration was the greatest in loins aged for 42 and 56 d ($P < 0.05$). Aging for 14 d produced the highest concentration of 2-pentyl furan compared to all other treatments ($P < 0.05$). Octane and nonane production was the greatest in loins aged for 42 and 56 d compared to the remaining treatments ($P < 0.05$). Loins aged for 42 d produced the greatest concentrations of butyrolactone, 2-heptanone, and 2-pentanone compared to all other treatments ($P < 0.001$). Aging for 56 d produced the lowest concentration of 3-hydroxy-2-butanone compared to all other treatments ($P < 0.05$). Acetaldehyde and benzaldehyde concentrations were the greatest in loins aged for 28 d ($P < 0.05$). Aging for 42 d produced the greatest concentration of 2-methylbutanal ($P < 0.05$). Aging for 42 and 56 d were similar ($P > 0.05$) and produced the lowest concentration of carbon disulfide compared to aging for 14 or 28 d ($P < 0.05$). Moreover, aging for 42 d produced the lowest concentration of methanethiol compared to all other treatments ($P < 0.05$).

Of the volatile compound classifications, only alcohols, aldehydes, carboxylic acids, and esters were influenced by aging temperature ($P < 0.05$; Table 6). Aging subprimals at 4°C produced the greatest concentration of 1-penten-3-ol and 2,3-butanediol compared to 0°C and −2°C ($P < 0.05$). Loins aged at −2°C produced lower benzaldehyde concentration compared to 4°C ($P < 0.05$). The 2-methylbutanal concentration was the greatest in loins aged at 4°C compared to all other treatments ($P < 0.05$). Octanoic acid concentration was greater in loins aged at 4°C compared to −2°C ($P < 0.05$). Aging loins at 4°C produced the greatest concentration of butanoic acid, methyl ester compared to all other treatments ($P < 0.05$).

These data indicate aging temperature and duration influenced the production of volatile compounds. The purpose of measuring the raw volatile profile was to track lipid oxidation via secondary products, i.e., alcohols and aldehydes (Amaral et al., 2018). However, our method of measuring volatiles compounds (gas-chromatography-mass spectrometry) was able to detect a variety of other volatile compounds. Aging duration readily influenced a majority of volatile compounds. In contrast with our original hypothesis, aging for 56 d did not always produce the greatest concentration of volatiles, especially known products of lipid oxidation. Often, volatile concentration peaked at 42 or even 28 d. Foraker et al. (2020) reported peak aldehyde concentration at 35 d of age. Shahidi and Pegg (1994) reported a peak in hexanal concentration in ground pork at 5 d of storage followed by a decline. The investigators suggested hexanal degraded into other flavor-active compounds i.e., hexanoic acid (Palamand and Dieckmann, 1974). It was also speculated that hexanal participated in cross-linking reactions with other components in the meat matrix (Palamand and Dieckmann, 1974; Shahidi and Pegg, 1994).

As previously discussed, some spoilage microorganism counts peaked at 42 d then decreased at 56 d.

A relationship between microbial counts and volatile production may be present. EB and *Pseudomonas* spp. utilize acetic acid as a substrate for metabolism following the depletion of glucose and glucose-6-phosphate (Casaburi et al., 2015). This may explain the decrease in acetic acid production at 56 d in −2°C and 0°C environments. Ercolini et al. (2011) reported relationships between LAB and butanoic acid production. Furthermore, EB, *Pseudomonas* spp., and *Brochothrix thermosphacta* were associated with 2,3-butanediol, hexanoic acid, octanoic acid, methyl ester, and acetoin (Ercolini et al., 2011). The 2-methylbutanal in cooked beef is a product of Strecker degradation. However, in raw meat, it is product of spoilage bacteria, namely *Enterococcus* spp. (Casaburi et al., 2015).

Conclusions

This work clearly indicates aging temperature and duration readily influence spoilage organism growth, proteolytic activity, and the subsequent release of free amino acids and volatile compound production. Microbial growth increased during extended aging but was dependent on aging temperature. Aging temperature readily influenced the rate of proteolysis and subsequent tenderness development and free amino acid accumulation. Proteolytic activity continued through 56 d of aging, but desmin degradation and tenderness plateaued at 42 d. Volatile production seemed to be driven by spoilage organisms. Retailers and purveyors who intend to hold product beyond 28 d should store product at −2°C to slow the growth of spoilage organisms. In addendum, aging at −2°C will not negatively affect tenderness development as aging beyond 28 d will allow for continued proteolytic activity and tenderness development.

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