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# Flavor, Tenderness, and Related Chemical Changes of Aged Beef Strip Loins

Blake A. Foraker[1], Devin A. Gredell[1], Jerrad F. Legako[2], Richard D. Stevens[3], J. Daryl Tatum[1], Keith E. Belk[1], and Dale R. Woerner[2]*

[1]Department of Animal Sciences, Colorado State University, Fort Collins, TX 80523, USA
[2]Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79409, USA
[3]Department of Natural Resources Management, Texas Tech University, Lubbock, TX 79409, USA
*Corresponding author. Email: Dale.Woerner@TTU.edu (Dale R. Woerner)

**Abstract:** Varying aging times and methods were evaluated for their effect on flavor, tenderness, and related changes in volatile compounds and flavor precursors. Strip loin sections from USDA Choice beef carcasses ($n = 38$) were randomly assigned to treatments: (1) 3 d wet-aged, (2) 14 d wet-aged, (3) 28 d wet-aged, (4) 35 d wet-aged, (5) 49 d wet-aged, (6) 63 d wet-aged, (7) 21 d dry-aged, and (8) 14 d wet-aged followed by 21 d dry-aged. Samples were analyzed for trained sensory attributes, shear force, volatile compounds, and flavor precursors (fatty acids, free amino acids, and sugars). Discriminant function analysis was used to identify sensory attributes contributing the greatest to treatment differences. Flavor notes were not differentiated in beef aged up to 35 d, regardless of aging method. A shift in flavor occurred between 35 d and 49 d of wet-aging time that was characterized by more intense sour and musty/earthy notes. Both shear force assessment and trained panelists agreed that tenderness was not affected ($P > 0.05$) by additional aging beyond 28 d. Volatile compound production and liberation of amino acids and sugars increased ($P < 0.01$) during the progression of aging time, with no change ($P > 0.05$) in fatty acid composition, which may be a result of metabolic processes like microbial metabolism. Chemical properties shared strong positive relationships ($r > 0.50$, $P < 0.001$) with sour, musty/earthy, and overall tenderness. These results substantiate the deteriorative effect of extended aging times of 49 d or greater on flavor of beef strip loins without tenderness improvement.

**Key words:** beef, aging, flavor, tenderness, flavor precursors, volatile compounds
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## Introduction

Postmortem aging of beef is universally accepted to enhance eating quality and is commonly accomplished by (1) wet aging, in which meat is stored in a vacuum package, and/or (2) dry aging, in which beef is held in a controlled, open-air environment. Beef is most commonly vacuum packaged for efficient distribution; even so, dry aging has found a popular presence in niche markets, despite increased costs resulting from yield loss. Consumers have been willing to pay for eating experiences with altered flavor profiles imparted by a particular aging method (Sitz et al., 2006). Yet an acceptable level of tenderness affects the perception of flavor and its contribution to overall eating satisfaction (Feuz et al., 2004). Management of postmortem aging time can overcome tenderness challenges in muscles from varying quality grades (Gruber et al., 2006). Still, disagreement exists about the effect of aging time and method on beef flavor (Warren and Kastner, 1992; Idolo Imafidon and Spanier, 1994; Campbell et al., 2001; Sitz et al., 2006; Laster et al., 2008; Smith et al., 2008; O'Quinn et al., 2016). Aging method can affect volatile compounds known to impact flavor (King et al., 1995). Evaluation of the precursors to these flavor compounds, including fatty acids, reducing sugars, and free amino acids,

provides insight to changes in eating quality resulting from different aging parameters. Fatty acid profiles in wet- and dry-aged beef have been previously related to intensity of individual flavor attributes (Gredell et al., 2018). These compounds produce a complex matrix of odors and flavors in cooked meat, and the indirect effect of certain attributes on other individual eating quality descriptors has been difficult to parse (Brewer, 2007; King et al., 2009a). This study aimed to characterize the eating quality of aged beef using a discriminant function analysis (DFA) and identify associated volatile compounds and flavor precursors.

## Materials and Methods

Institutional Animal Care and Use Committee approval was not required for this study as samples were obtained postmortem from a federally inspected harvest facility.

### *Sample collection, treatment designation, and fabrication*

Matched pairs of boneless strip loins (Institutional Meat Purchase Specifications #180; NAMP, 2010) were collected from "A" maturity, commodity USDA Choice (Small$^{00}$–Small$^{99}$) beef carcasses ($n = 38$) of cattle harvested on the same day. Carcasses were chilled 36 h postmortem and selected 1 to 2 h post-ribbing. Visual marbling score (USDA, 2017) within the *longissimus* muscle between the 12th and 13th ribs was determined by a trained evaluator using official US Department of Agriculture marbling photographs (National Cattlemen's Beef Association, Centennial, CO). On the same day as collection, strip loins were transported under refrigeration (2°C) to the Colorado State University Meat Laboratory, and each was fabricated into four 9-cm sections, starting from the anterior end, producing 8 sections per carcass. Sections within each carcass were randomly assigned to 1 of 8 post-collection aging treatments: (1) 3 d wet-aged, (2) 14 d wet-aged, (3) 28 d wet-aged, (4) 35 d wet-aged, (5) 49 d wet-aged, (6) 63 d wet-aged, (7) 21 d dry-aged, and (8) 14 d wet-aged followed by 21 d dry-aged (combination). Sections assigned to a wet-aging treatment were vacuum packaged and stored in the absence of light at 2°C for their respective aging period. A dry-aging cabinet was created using a commercial grade refrigerator (Model CFD-2RR; Avantco Refrigeration, Lititz, PA) set to maintain a temperature of 3.1°C ± 1.2°C with 70%–90% relative humidity and continuous air flow using a household humidifier. Commercial dry-aging facilities are often inhabited by naturally occurring molds. Because the dry-aging cabinet in this study was purchased new and exhibited a nearly sterile environment, sections assigned to a dry-aging treatment (21 d dry-aged and combination) were inoculated with Bactoferm 600 Mould (*Penicillium nalgiovense*; The SausageMaker Inc., Buffalo, NY) according to supplier instructions. Sections were fully submersed in inoculant and placed subcutaneous fat side up on racks in the dry-aging cabinet. Sections were randomly relocated within the dry-aging cabinet after 14 d. Initial (before inoculation) and final weights were recorded for dry-aged sections to capture yield loss. After aging, all sections were vacuum packaged (if not already) and placed in −20°C frozen storage. To obtain steaks for analysis, frozen sections were faced on the anterior and posterior ends. Two 2.54-cm-thick steaks were cut from each section, and each steak was identified for either sensory or shear force analysis. Remaining steak(s) from each section were identified for chemical analysis. Steaks were trimmed free of fat, connective tissue, secondary muscles, and external crust from dry-aged samples, such that only *longissimus* muscle remained. Steaks were vacuum packaged and stored at −20°C until analysis.

### *Cooking procedures*

Steaks for trained sensory analysis and shear force analysis were tempered for 24 to 48 h at 2°C to an internal temperature of 0°C to 4°C at time of cooking. Groups of steaks (4 at a time for sensory analysis; 6 at a time for shear force analysis) were singly layered and cooked on a centrally located grill grate (Model SCC WE 61 E; Rational, Landberg am Lech, Germany) in a combi-oven (Model SCC WE 61 E; Rational, Landberg am Lech, Germany) at 204°C, 0% relative humidity, and default fan speed. Steak temperature was monitored in the cooking process using an oven core temperature probe (Model SCC WE 61 E; Rational, Landberg am Lech, Germany) placed in the geometric center of one centrally located steak. Steaks were removed from the oven to target a peak internal temperature of 71°C. Individual peak temperatures were recorded using a calibrated type K thermocouple thermometer (AccuTuff 340, model 34040, Cooper-Atkins Corporation, Middlefield, CT) placed in the geometric center of each steak. To appropriately account for potential differences in cooking rates, groups of steaks from wet-aging treatments were cooked separately from those including a dry-aging treatment.

### *Trained sensory analysis*

Trained sensory analysis was conducted at Colorado State University. Flavor attributes associated with aged beef were evaluated using the lexicon developed by Adhikari et al. (2011). Panelists were trained to identify and quantify the attributes listed in Table 1 according to the "Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat" (AMSA, 2016). A 15-point scale was used to objectively quantify the presence or absence of each flavor note (0 = none/not present, 2 = barely detectable, 4 = identifiable but not very intense, 6 = slightly intense, 8 = moderately intense, 10 = intense, 12 = very intense, 15 = extremely intense). Upon training, discriminative triangle tests were used to qualify a pool of 14 trained panelists for sensory evaluation.

Steaks were cooked and temperatures measured according to aforementioned procedures. Steaks were removed from the oven and served to panelists within 1 h. Immediately after cooking, each steak was vacuum packaged and held in a combi-oven (Model SCC WE 61 E; Rational, Landberg am Lech, Germany) set at 57°C, 100% humidity, and default fan speed until all steaks were cooked. At the designated panel time, samples were transferred within proximity of trained panel location. For the duration of the panel until served, sealed samples were held in a 55°C circulating water bath (Fisher Scientific Isotemp Heated Immersion Circulators: Model 6200 H24; Thermo Fisher Scientific, Waltham, MA). Steaks were randomly assigned to 1 of 19 independent 1-h panel sessions, such that 16 steaks (2 from each treatment) were fed in random order during each panel. Samples were evaluated every 3.5–4 min by 5–7 panelists per session. No panelist served on more than 2 panels per day, with a minimum of 1 h between sessions. Panelists were seated in individual cubicles in a dark room under red incandescent lighting. Distilled water and unsalted saltine crackers were supplied as palate cleansers. Immediately before serving, cooked steaks were trimmed of any remaining external fat and connective tissue and cut into cubes (1 cm × 1 cm × steak thickness). Each panelist received 2 to 3 cubes for sensory attribute evaluation. Panelist responses were recorded on an electronic ballot generated by an online survey software (Qualtrics,

**Table 1.** Sensory attributes, descriptors, and anchors on a 15-point scale used for trained sensory analysis adapted from Adhikari et al. (2011)

| Attribute | Description | Anchor |
|---|---|---|
| **Beef Flavor ID** | The flavor associated with cooked beef; basic meaty flavor of unseasoned beef broth | Swanson's (Camden, NJ) beef broth = 5.0<br>80% lean ground beef = 7.0<br>Beef brisket cooked to 71°C = 11.0 |
| **Browned** | The flavor associated with grilled beef; caramelized | Beef suet (broiled) = 8.5 |
| **Roasted** | The flavor associated with roasted beef | 80% lean ground chuck = 10.0 |
| **Metallic** | The impression of slightly oxidized metal, such as iron, copper, and silver spoons | 0.10% potassium chloride solution = 1.5<br>Select strip steak = 4.0<br>Dole (Westlake Village, CA) canned pineapple juice = 6.0 |
| **Fat-Like** | The aromatics associated with cooked animal fat | Hillshire Farms (Chicago, IL) Lit'l beef smokies = 7.0<br>Beef suet = 12.0 |
| **Sour** | The fundamental taste factor associated with citric acid | 0.015% citric acid solution = 1.5<br>0.050% citric acid solution = 3.5 |
| **Oxidized** | The aromatics commonly associated with oxidized fat and oils; cardboard, painty, varnish, and fishy | Microwaved Wesson (Memphis, TN) vegetable oil (3 min at high) = 7.0<br>Microwaved Wesson vegetable oil (5 min at high) = 9.0 |
| **Nutty** | A combination of slightly sweet, brown, woody, oily, musty, astringent, and bitter aromatics commonly associated with nuts, seeds, beans, and grains | Mixture of Diamond (Stockton, CA) sliced almonds and Diamond shelled walnuts = 7.5 |
| **Musty/Earthy** | Musty, sweet, decaying vegetation | Raw mushroom = 12.0 |
| **Liver-Like** | Aromatics associated with cooked organ meat/liver | Beef liver (broiled) = 7.5 |
| **Overall Tenderness** | The amount of force required to masticate a piece of meat | Beef shank cooked to 71°C = 7.0<br>Select strip steak cooked to 71°C = 9.0<br>Tenderloin steak cooked to 71°C = 14.0 |
| **Initial Juiciness** | The amount of perceived juice initially released from the product during mastication (within the first 5 chews) | Select strip steak cooked to 58°C = 11.0<br>Select strip steak cooked to 82°C = 9.0 |
| **Sustained Juiciness** | The amount of perceived juice released from delayed mastication (after 5 chews) | Select strip steak cooked to 71°C = 5.0<br>Upper 2/3 Choice strip steak cooked to 71°C = 8.0 |

Provo, UT). Intensity ratings for each attribute were averaged among panelists for each sample.

### Shear force

Shear force steaks were randomly assigned to 1 of 3 shear force days, and treatments were equally represented within each day. Warner-Bratzler shear force (WBSF) and slice shear force (SSF) measurements were obtained from every steak using procedures described by Lorenzen et al. (2010). Steaks were grouped, according to similar weight, size, and shape, and cooked using aforementioned procedures. Pre-cook and post-cook temperatures and weights were recorded on each steak. Within 1 to 2 min of recording peak internal temperature, the lateral end of each cooked steak was squared, and a 1-cm-thick × 5-cm-long slice was removed parallel to muscle fibers. This slice was sheared perpendicular to muscle fibers, using a SSF machine (Tallgrass Solutions, Inc., Manhattan, KS) equipped with a flat, blunt-end blade (crosshead speed: 500 mm/min, load capacity: 50 kg), resulting in a single peak SSF measurement for each steak. Remaining steak portions were equilibrated to room temperature (22°C) or below, and 4 to 6 cores (1.2 cm diameter) were removed parallel to muscle fibers. Each core was sheared perpendicular to muscle fibers using a WBSF machine (Tallgrass Solutions, Inc., Manhattan, KS) fitted with a Warner-Bratzler shear head (crosshead speed: 225 mm/min, load cell capacity: 50 kg). Peak shear force of each core was recorded, and resulting values were averaged to obtain a single WBSF measurement for each steak.

### Sample homogenization

Frozen steaks designated for chemical analysis were individually thawed in ice water for approximately 1 min, or enough to be hand cut into small pieces. Steak pieces were frozen using liquid nitrogen, transferred to a blender (NutriBullet LEAN, Pacoima, CA), ground into a fine powder, and stored in an individual bag at −20°C. Blender cups, blades, and other utensils were rinsed, dried, and chilled between samples. Individual homogenized samples ($n = 38$ per treatment) were randomly assigned to 1 of 8 composites per treatment, producing a total of 64 composites (8 composites × 8 treatments). Of the 8 composites per treatment, 7 consisted of 5 individual homogenized samples, and 1 consisted of 3 individual homogenized samples. On the same day as sample homogenization, Equal proportions of individual homogenized sample were weighed to generate a 100 g composited sample. Composites were vacuum packaged and stored at −80°C.

### Fatty acids

Fatty acid composition was determined from raw composites ($n = 8$ per treatment). Lipid constituents were extracted using a modified Folch method (Folch et al., 1957). Extracted lipid were fractionated using a Sep-Pak silica gel cartridge (Waters Corporation, Milford, MA). Fatty acids in polar lipid (phospholipid) were saponified and derivatized to fatty acid methyl esters (FAME) using sodium methoxide in methanol (Li and Watkins, 2001), whereas saponification and derivatization for those in neutral lipid was performed using methanolic potassium hydroxide (Maxwell and Marmer, 1983). FAME were analyzed on an Agilent Technologies (Santa Clara, CA) 7890B series gas chromatograph (GC) equipped with an HP-88 capillary column (100 m × 0.25 mm internal diameter; Agilent Technologies, Santa Clara, CA) and a flame-ionization detector. Identification and quantification of FAME was carried out by an internal standard calibration, comparing with FAME authentic standard (Nu-Check Prep, Inc., Standard Group 610). After analysis of fatty acids at Colorado State University, samples were shipped on dry ice to Texas Tech University for analysis of free amino acids, sugars, and volatile compounds.

### Free amino acids and sugars

Extraction of water-soluble compounds was conducted from raw composites ($n = 8$ per treatment) using the procedures of Koutsidis et al. (2008a). Two grams of frozen homogenate was added to 10 mL of cold water with 50 µL of internal standards sarcosine, norvaline, rhamnose, and purine. Compounds were extracted using a Burell wrist-action shaker for 30 min, followed by centrifugation at 3000 × $g$ at 4°C for 15 min. A 0.2-µm nylon membrane filter was used to screen remaining supernatant into a 3-kDa cutoff membrane for centrifugal filtration for 4 h, and remaining solution was stored in a 1.5-mL polypropylene vial at −80°C. This aqueous beef extract was used for both free amino acid and sugar analyses.

Free amino acids were derivatized using an EZ-Fast amino acid derivatization kit (Phenomenex, Torrance, CA). Aqueous extract was filtered using a sorbent tip, and a chloroform and propyl chloroformate mixture was added to emulsify the solution. The organic layer was decanted into an autosampler vial and evaporated with nitrogen. Remaining derivatives were re-dissolved into a mixture of 80% iso-octane and 20% chloroform and transferred to an insert placed

in an autosampler vial, and vials were stored at −80°C until analysis. Derivatized amino acids were quantified by GC-mass spectrometry (MS) (Agilent 7890B-5977A). A volume of 1.5 µL of sample was injected into the instrument at a 1:15 split ratio at 250°C with helium as a carrier gas at 1.1 mL/min constant flow and a 30°C/min increase in oven temperature from 110°C to 320°C. Amino acids were separated in a Zebron EZ-AAA Amino Acid GC Column (10 m × 0.25 mm × 0.15 mm; a 5890 Agilent capillary column; Phenomenex, Torrance, CA). Authentic standards (Phenomenex, Torrance, CA) were used to identify peaks. Internal standard norvaline was used to calculate quantities by relative response.

Silylation and GC-MS were used to determine sugar content in each sample, similar to methods by Leblanc and Ball (1978) and Koutsidis et al. (2008a). Freeze-dried pellets produced from 250 µL of aqueous extract were added to a solution of anhydrous dimethyl sulfoxide, hexamethyl disilazane, trimethylchlorosilane, and cyclohexane. The resulting solution was sonicated and stored overnight at room temperature before removing the organic layer, which was analyzed on a GC-MS (Agilent 7890B-5977A, Palo Alto, CA) in electron impact mode (70 eV). Injection temperature was set at 250°C, and oven temperature was programmed at 60°C for an initial minute, followed by a 30°C/min increase to 130°C for 2 min, a 2°C/min increase to 170°C/min, and a final 4°C/min increase to 300°C/min. A DB-17ms capillary column (30 m × 0.25 mm; 0.25-µm film thickness), coupled with 1.5 m of deactivated methylsilicone fused silica capillary retention gap, was used for compound retention and separation. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Authentic standards (Sigma-Aldrich, Bellefonte, PA) were used to identify peaks. Internal standard Rhamnose was used to calculate quantities by relative response.

## Volatile compounds

An Agilent 7890B series GC (Agilent Technologies, Santa Clara, CA) in combination with a 5977A mass selection detector (Agilent Technologies) was used to collect volatile compounds. Five grams of raw composite ($n = 8$ per treatment) was weighed into a 20-mL glass GC vial (Art #093640-036-00, Gerstel Inc., Linthicum, MD), and 10 µL of an internal standard solution (1,2 dichlorobenzene, 2.5 µg/µL) was added to the vial. Each vial was capped with a 1.3-mm polytetrafluoroethylene septa and metal screw cap (Art #093640-040-00, Gerstel Inc.). Prepared vials were loaded onto a Gerstel automated sampler (MPS, Gerstel Inc.) for a 5-min incubation period at 30°C in a Gerstel agitator (500 rotations/min). Incubation was followed by a 20-min extraction period during which volatile compounds were collected from the headspace of the vial by solid-phase microextraction, utilizing an 85-µm film thickness carboxen polydimethylsiloxane fiber (Stableflex 24 Ga, Supelco, Bellefonte, PA). After extraction, volatile compounds were injected into a VF-5 ms capillary column (30 m × 0.25 mm × 1.00 µm; Agilent J&W GC Columns, Netherlands) and separated. Ions were detected within the range of 33–500 m/z by the mass spectrometer with an electron impact mode at 70 eV. Validation of volatile compound identities was completed using comparison to external authentic standards. Samples that failed to reach the minimum threshold detection level were recorded as zeros.

## Statistical analysis

Data were analyzed using R statistical software, version 3.6.0 (R Core Team, 2019). Univariate analysis of sensory data was only performed because sensory studies of beef eating quality are traditionally analyzed using univariate methods. Boxplots and histograms were used to visually assess outliers and univariate normality of each variable before analysis. Sensory and shear force data from each carcass (experimental unit; $n = 38$ per treatment) were analyzed as a randomized complete block design. The lmer() function from the lme4 (Bates et al., 2015) package was used to fit a restricted maximum likelihood (REML)-based, mixed model with Kenward-Roger approximation of denominator degrees of freedom for each eating quality attribute, SSF, WBSF, and cook loss. Treatment was included as a fixed effect, and carcass served as a random effect to account for the blocking structure. Final cooking temperature was included in shear force analysis as a covariate. Volatile compound and flavor precursor data from each composite ($n = 8$ per treatment) were analyzed as a completely randomized design because block effect of carcass was lost when samples were composited. The lm() function from base R was used to fit a linear model for each chemical compound with treatment as the fixed effect. Treatment effect on each response variable was tested with an analysis of variance (ANOVA) using the anova() function from the lmerTest package (Kuznetsova et al., 2017). When effect of treatment from ANOVA was significant, treatment means were separated with Tukey adjusted pairwise comparisons using the emmeans package

(Lenth, 2018). Significance of treatment effect and pairwise comparisons was considered at $P < 0.05$. To identify relationships of sensory data to volatile compounds and flavor precursors, sensory data for each sample represented in a composite were averaged for each attribute. Pearson correlation coefficients were calculated and tested for significance between sensory attributes and chemical compounds using the rcorr() function from the Hmisc package (Harrell, 2019). The primary objective of this analysis was to explore and generalize these relationships; thus, only significant ($P < 0.05$) and strong ($r > |0.50|$) Pearson correlations were interpreted.

Sensory attributes were analyzed in a multivariate space using a DFA to assess differentiation between treatments. Before analysis, data were evaluated for test assumptions. Samples with missing values were identified and removed, if present. Highly correlated variables were assessed for redundancy at $r > 0.70$. Mahalanobis' distances were calculated to assess multivariate normality for the set of sensory attributes on each sample at a $\chi^2$ critical value of 32.91 (df = 12, $P = 0.001$). Homogeneity of variance-covariance matrices was evaluated using Box's M test from the heplots package (Fox et al., 2018), where $\alpha < 0.001$ indicated heterogeneity (Tabachnick and Fidell, 2013). Multicollinearity was formally assessed using the olsrr package (Hebbali, 2018). A linear model was estimated between sensory attributes and sample sequence numbers, and condition indices greater than 30 at each eigen value were considered a violation. After evaluation of assumptions, a linear model of sensory attributes was used to predict treatment membership, and the candisc package (Friendly and Fox, 2017) was used to evaluate statistical significance of discriminant functions built using the model. Loadings and standardized coefficients for each sensory attribute were assessed on each function to determine the discriminating ability of each attribute to aging treatment differentiation. On each function, composite sensory scores were generated using standardized coefficients for each sensory attribute and fit to a mixed linear model with treatment as a fixed effect and carcass as a random effect. The model was tested for treatment effect using ANOVA, and treatments were compared using Tukey adjusted pairwise comparisons, with significance at $P < 0.05$.

## Results and Discussion

All aging treatments were blocked within each beef carcass, which removed variation between carcasses and increased the power of the study to identify meaningful aging differences. Strip loins were collected from carcasses with a mean and standard error hot carcass weight of 450 ± 10 kg, fat thickness of 1.2 ± 0.1 cm, ribeye area (*longissimus* muscle) of 100 ± 2 $cm^2$, and marbling score of 430 ± 10 (Small). Beef exposed to 21 d dry aging and combination aging experienced shrinks of 25.7% ± 0.3% and 24.2% ± 0.5%, respectively, in the dry-aging cabinet.

### Trained sensory performance

Intercorrelations of sensory ratings for 13 attributes evaluated by trained panelists for each sample ($N = 304$) are shown in Table 2. Data for sour, oxidized, nutty, musty/earthy, and liver-like notes were square root transformed to a normal distribution before statistical testing, including correlations. Trained sensory analysis aims for objective ratings of each attribute. However, the "halo effect," dependency of ratings for some attributes on the ratings of others, has been reported in trained sensory studies (King et al., 2009a) and was shown in the present study. Ratings for beef flavor identity (ID) showed strong negative associations with sour ($r = -0.56$, $P < 0.001$) and musty/earthy ($r = -0.45$, $P < 0.001$). Furthermore, sour, oxidized, nutty, musty/earthy, and liver-like flavor notes were all positively related to each other ($r = 0.34$–$0.69$, $P < 0.001$). These data demonstrate the multidimensionality of sensory ratings, especially related to flavor, in the present study. Thus, an analysis with the ability to distinguish sensory attributes based on unique and correlated contribution to differences between treatments was warranted.

Estimated marginal means and treatment comparisons for each sensory attribute are presented in Table 3. Univariate ANOVA results of sensory data showed a treatment effect ($P < 0.05$) for beef flavor ID, browned, roasted, sour, oxidized, nutty, musty/earthy, liver-like, and overall tenderness. However, intercorrelations of sensory ratings for many of these attributes made it difficult to decipher whether differences were unique to the effect of treatment or the attribute's dependence on one or more other attribute(s). Moreover, the number of treatment comparisons in the present study created complexity. Thus, intercorrelations increased the opportunity to encounter Type I error in the univariate ANOVA of individual attributes, and interpretation of these treatment comparisons was only used to supplement multivariate results.

Principal component analysis (PCA) is a relatively common statistical approach in meat science studies

**Table 2.** Pearson intercorrelation coefficients[1] between sensory attributes evaluated by trained panelists in beef strip loin steaks (N = 304) representing 8 aging treatments[2]

| Sensory Attribute | 1. | 2. | 3. | 4. | 5. | 6. | 7. | 8. | 9. | 10. | 11. | 12. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1. Beef Flavor ID** | | | | | | | | | | | | |
| **2. Browned** | 0.34*** | | | | | | | | | | | |
| **3. Roasted** | 0.34*** | 0.44*** | | | | | | | | | | |
| **4. Metallic** | −0.23*** | −0.01 | −0.02 | | | | | | | | | |
| **5. Fat-Like** | 0.12* | 0.14* | 0.15** | 0.29*** | | | | | | | | |
| **6. Sour** | −0.56*** | −0.11* | −0.07 | 0.36*** | 0.02 | | | | | | | |
| **7. Oxidized** | −0.34*** | −0.18** | −0.23*** | 0.20** | −0.09 | 0.40*** | | | | | | |
| **8. Nutty** | −0.05 | 0.07 | −0.07 | 0.00 | 0.02 | 0.37*** | 0.24*** | | | | | |
| **9. Musty/Earthy** | −0.41*** | −0.11 | −0.17** | 0.22*** | 0.02 | 0.67*** | 0.36*** | 0.56*** | | | | |
| **10. Liver-Like** | −0.24*** | −0.06 | 0.00 | 0.14* | −0.07 | 0.40*** | 0.40*** | 0.27*** | 0.38*** | | | |
| **11. Overall Tenderness** | −0.10 | −0.13* | −0.03 | 0.19** | 0.24*** | 0.17** | 0.17** | 0.07 | 0.16** | 0.05 | | |
| **12. Initial Juiciness** | 0.22*** | −0.14* | −0.18** | −0.04 | 0.17** | −0.10 | −0.03 | 0.06 | −0.05 | −0.18** | 0.34*** | |
| **13. Sustained Juiciness** | 0.15** | −0.18** | −0.23*** | 0.02 | 0.16** | −0.06 | 0.01 | 0.08 | 0.02 | −0.15** | 0.33*** | 0.84*** |

[1]Correlation coefficients differ from 0 at: $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

[2]Aging treatments included: (1) 3 d wet-aged, (2) 14 d wet-aged, (3) 28 d wet-aged, (4) 35 d wet-aged, (5) 49 d wet-aged, (6) 63 d wet-aged, (7) 21 d dry-aged, and (8) 14 d wet-aged followed by 21 d dry-aged.

**Table 3.** Trained sensory ratings[1], SSF, WBSF, and cook loss values, and composite sensory scores from discriminant function analysis of beef strip loin steaks (n = 38 per treatment) representing 8 aging treatments

| | Wet-Aged (d) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | 3 | 14 | 28 | 35 | 49 | 63 | 21 d Dry-Aged | Comb.[2] | SEM[3] | *P* Value |
| ***Sensory Analysis*** | | | | | | | | | | |
| Beef flavor ID | 7.4$^{a}$ | 7.4$^{a}$ | 7.5$^{a}$ | 7.3$^{a}$ | 6.7$^{b}$ | 6.5$^{b}$ | 7.5$^{a}$ | 7.3$^{a}$ | 0.1 | <0.01 |
| Browned | 4.6$^{a}$ | 4.5$^{ab}$ | 4.5$^{ab}$ | 4.4$^{ab}$ | 4.2$^{ab}$ | 4.2$^{b}$ | 4.6$^{a}$ | 4.6$^{a}$ | 0.1 | <0.01 |
| Roasted | 5.0$^{abc}$ | 5.1$^{abc}$ | 5.2$^{abc}$ | 5.1$^{abc}$ | 4.8$^{c}$ | 4.9$^{bc}$ | 5.5$^{a}$ | 5.3$^{ab}$ | 0.1 | <0.01 |
| Metallic | 1.8 | 1.7 | 1.7 | 1.8 | 1.9 | 2.0 | 1.8 | 1.8 | 0.1 | 0.11 |
| Fat-like | 1.6 | 1.7 | 1.6 | 1.6 | 1.5 | 1.5 | 1.6 | 1.6 | 0.1 | 0.62 |
| Sour | 1.5$^{c}$ | 1.4$^{c}$ | 1.6$^{c}$ | 1.7$^{bc}$ | 2.6$^{a}$ | 2.8$^{a}$ | 1.7$^{bc}$ | 2.0$^{b}$ | 0.1 | <0.01 |
| Oxidized | 0.3$^{cd}$ | 0.2$^{d}$ | 0.3$^{cd}$ | 0.4$^{abc}$ | 0.6$^{ab}$ | 0.7$^{a}$ | 0.3$^{cd}$ | 0.3$^{bcd}$ | 0.1 | <0.01 |
| Nutty | 0.5$^{b}$ | 0.6$^{b}$ | 0.5$^{b}$ | 0.6$^{b}$ | 1.2$^{a}$ | 1.1$^{a}$ | 0.5$^{b}$ | 0.8$^{ab}$ | 0.1 | <0.01 |
| Musty/earthy | 0.6$^{c}$ | 0.6$^{c}$ | 0.7$^{bc}$ | 1.0$^{bc}$ | 2.1$^{a}$ | 2.4$^{a}$ | 0.7$^{c}$ | 1.3$^{b}$ | 0.1 | <0.01 |
| Liver-like | 0.2$^{bc}$ | 0.2$^{c}$ | 0.3$^{bc}$ | 0.3$^{bc}$ | 0.6$^{a}$ | 0.6$^{a}$ | 0.3$^{abc}$ | 0.4$^{ab}$ | 0.1 | <0.01 |
| Overall tenderness | 8.0$^{c}$ | 8.8$^{ab}$ | 9.1$^{ab}$ | 9.2$^{a}$ | 9.3$^{a}$ | 9.2$^{a}$ | 8.6$^{b}$ | 8.9$^{ab}$ | 0.1 | <0.01 |
| Initial juiciness | 5.5 | 5.6 | 5.6 | 5.6 | 5.7 | 5.3 | 5.4 | 5.5 | 0.1 | 0.46 |
| Sustained juiciness | 5.5 | 5.6 | 5.6 | 5.6 | 5.7 | 5.3 | 5.4 | 5.4 | 0.1 | 0.36 |
| ***Shear Force Analysis*** | | | | | | | | | | |
| SSF (kg) | 14.6$^{a}$ | 13.1$^{ab}$ | 12.1$^{bc}$ | 11.5$^{bc}$ | 11.5$^{bc}$ | 11.0$^{c}$ | 11.4$^{bc}$ | 11.4$^{c}$ | 0.4 | <0.01 |
| WBSF (kg) | 3.6$^{a}$ | 3.3$^{ab}$ | 2.8$^{cd}$ | 2.7$^{cd}$ | 2.6$^{d}$ | 2.6$^{d}$ | 3.1$^{bc}$ | 2.9$^{bcd}$ | 0.1 | <0.01 |
| Cook loss (%) | 25.0$^{a}$ | 24.3$^{ab}$ | 24.4$^{ab}$ | 22.4$^{b}$ | 22.4$^{b}$ | 24.4$^{ab}$ | 18.5$^{c}$ | 17.1$^{c}$ | 0.5 | <0.01 |
| ***Composite Sensory Scores***[4] | | | | | | | | | | |
| Function 1 | 1.3$^{a}$ | 1.0$^{ab}$ | 0.5$^{bcd}$ | 0.1$^{cd}$ | −1.6$^{e}$ | −1.9$^{e}$ | 0.8$^{abc}$ | −0.2$^{d}$ | 0.2 | <0.01 |
| Function 2 | 0.8$^{a}$ | −0.2$^{bcd}$ | −0.6$^{d}$ | −0.5$^{cd}$ | 0.1$^{bc}$ | 0.2$^{b}$ | 0.1$^{bc}$ | 0.1$^{bc}$ | 0.2 | <0.01 |

[1]Attributes were scored using a 15-point scale: 0 = very tough, very dry, and not present; 15 = very tender, very juicy, and very intense.

[2]Combination aging treatment with wet-age period of 14 d followed by dry-age period of 21 d.

[3]Standard error (largest) of the estimated marginal means.

[4]Composite sensory scores were generated using standardized coefficients for each sensory attribute from each discriminant function. Function 1 represented flavor, and function 2 represented tenderness.

[a–e]Estimated marginal means in the same row lacking a common superscript differ ($P < 0.05$).

SSF, slice shear force; WBSF, Warner-Bratzler shear force.

used to graphically display variation in a dataset. However, PCA does not statistically test for differences between treatments, which is often of primary interest in experimental studies. Both PCA and DFA calculate linear combinations of original variables (known as components or functions) that maximize variation. Each function/component is orthogonal to previous ones, such that all cumulative variation (100%) is explained by addition of the last function/component. However, DFA calculates these functions to maximize variation between groups and minimize variation within groups. Thus, DFA predicts group membership (dependent variable) from a matrix of independent variables, whereas PCA only identifies variation within one matrix of independent variables.

Total number of discriminant functions calculated in a DFA is equal to the lesser of either the number of variables or one less than the number of groups. Added variation accounted by each function is then tested for significance. Canonical correlations are used to assess the relationship between groups and the combination of variables on each function. Loadings indicate the overall relationship between variables and a function. Standardized coefficients show the unique contribution of variables to a function, which account for differences in magnitudes between variables. An understanding of both loadings and standardized coefficients provides insight into the most discriminating variables (Enders, 2003). Loadings may be partially inflated by a variable's interdependency on other variables, yet standardized coefficients are derived simultaneously for all variables, so highly intercorrelated variables compete for weighted values. Comrey and Lee (1992) identified that loading values of |0.32| represent 10% of overlapping variance between variables and the discriminant function; thus, a variable with a high loading and a high standardized coefficient is most discriminatory. Standardized coefficients for each variable are used to derive a composite score for each individual case on each function. Thus, the direction of these composite scores is dependent on the uniquely positive or negative contribution of a variable's standardized coefficient to a function. Means of these scores can be plotted for visual evaluation of group differences and/or formally tested for group differences.

Tests of assumptions for the use of DFA in the present study were validated. Given the strong correlation between initial and sustained juiciness ($r = 0.84$), only initial juiciness was retained so its unique contribution was assessed. Box's M test was significant at $P < 0.001$, indicating heterogeneity among variance-covariance matrices. However, because sample sizes were large and equal, this violation was not a concern for inference of results (Tabachnick and Fidell, 2013). Even though individual attributes were intercorrelated, formal testing showed that overall multicollinearity was not violated.

Seven discriminant functions were calculated to predict the membership of cases to 1 of 8 aging treatments using 12 sensory attributes. The first discriminant function (DF1) accounted ($P < 0.001$) for 76.9% of variation between treatments. The second discriminant function (DF2) explained ($P = 0.003$) 11.1% of variation between treatments. Subsequent functions were not significant ($P > 0.05$). Thus, the majority (88%) of variation between treatments was explained on DF1 and DF2. As assessed from canonical correlations, most of the variance (55%) in sensory attributes on DF1 was explained by treatment, whereas only 15% of the variance was explained on DF2. Eigen values explain the discriminating ability of the functions relative to each other, and DF1 was 6.9 times more effective at explaining treatment differences than DF2. As shown in Figure 1a, DF1 maximally separated beef wet-aged 49 d and 63 d from beef aged 35 d and less, regardless of aging method. Although it was less effective at explaining treatment differences, DF2 separated beef wet-aged 3 d from all other treatments. These conclusions were formally supported by univariate tests of the composite sensory scores derived from the combination of all attributes on each function (Table 3). Composite sensory scores on DF1 for beef wet-aged 49 d and 63 d were different ($P < 0.01$) from all other treatments, and composite scores on DF2 for beef wet-aged 3 d were different ($P < 0.01$) from all other treatments. Nevertheless, DF2 explained a marginal amount of variation between treatments compared to DF1, which indicated the greatest difference in eating quality between treatments existed at extended aging times of 49 d and 63 d.

Loadings on DF1 suggested this function represented flavor, as beef flavor ID ($r = 0.57$), sour ($r = -0.81$), nutty ($r = -0.56$), and musty/earthy ($r = -0.84$) all strongly loaded onto this function (Table 4; Figure 1b). Standardized coefficients for sour ($b = -0.42$) and musty/earthy ($b = -0.41$) were weighted greatest on DF1, while beef flavor ID ($b = 0.09$) and nutty ($b = -0.25$) contributed less to treatment separation. Thus, the contribution of beef flavor ID and nutty to DF1 was likely because of their dependency on ratings for sour and musty/earthy. Although the contribution of overall tenderness was much more distinct ($r = -0.79$, $b = -0.94$) on DF2, its contribution to DF1 was moderate ($r = -0.43$; $b = -0.46$).

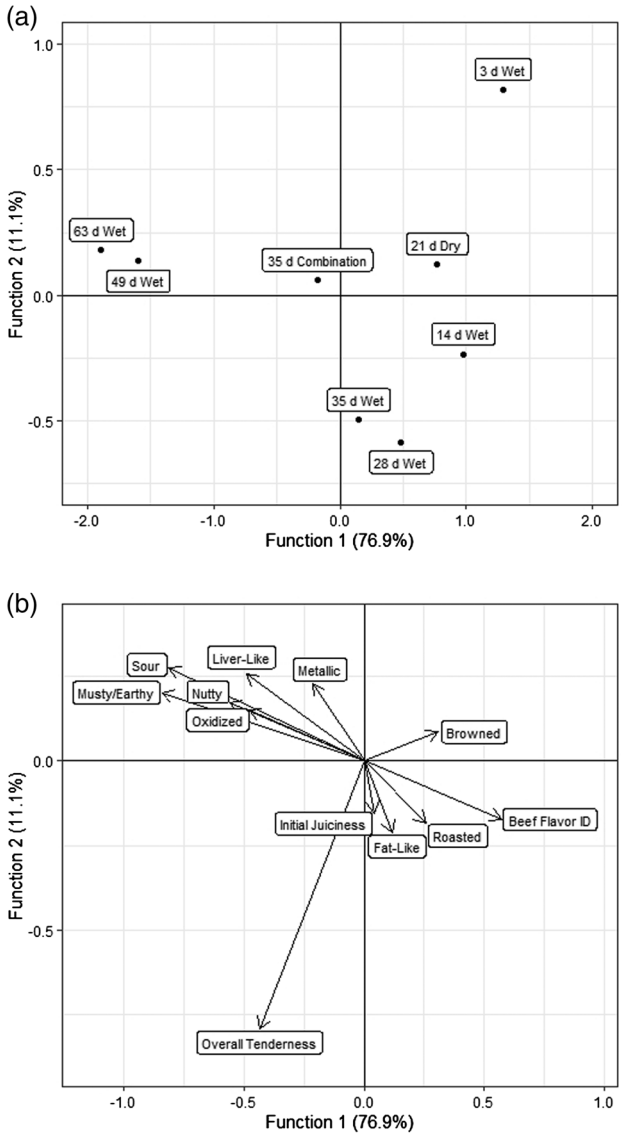


**Figure 1.** Discriminant function analysis of trained sensory evaluation of aged beef strip loin steaks (N = 304), where (a) illustrates aging treatment centroids and (b) illustrates sensory attribute loadings.

This explains some of the treatment differences ($P < 0.01$) in composite sensory scores (and centroids) on DF1 at aging times of 35 d or less, despite these differences being of less numerical magnitude than those at extended aging times of 49 d and 63 d. This was supported by the univariate ANOVA of individual flavor attributes, where no meaningful or statistical difference ($P > 0.05$) was noted for any attribute at aging times of 35 d or less, regardless of aging method.

Together, treatment separation, loadings, and standardized coefficients on DF1 suggested a shift in flavor of beef strip loins between 35 d and 49 d of aging time that was characterized by more intense sour and musty/earthy notes (Figure 2). Univariate ANOVA

**Table 4.** Loadings and standardized coefficients[1] for each sensory attribute on significant ($P < 0.05$) discriminant functions used in prediction of aging treatments[2]

| | Discriminant Function 1 | | Discriminant Function 2 | |
|---|---|---|---|---|
| Item | Loadings | Standardized Coefficients | Loadings | Standardized Coefficients |
| ***Sensory Attribute*** | | | | |
| Beef flavor ID | 0.57 | 0.09 | −0.17 | −0.05 |
| Brown | 0.31 | 0.19 | 0.09 | 0.15 |
| Roasted | 0.26 | 0.10 | −0.18 | −0.16 |
| Metallic | −0.21 | 0.07 | 0.23 | 0.34 |
| Fat-like | 0.12 | 0.22 | −0.21 | −0.11 |
| Sour | −0.81 | −0.42 | 0.28 | 0.19 |
| Oxidized | −0.48 | 0.01 | 0.15 | 0.05 |
| Nutty | −0.56 | −0.25 | 0.17 | 0.07 |
| Musty/earthy | −0.84 | −0.41 | 0.20 | 0.00 |
| Liver-like | −0.49 | −0.14 | 0.26 | 0.16 |
| Overall tenderness | −0.43 | −0.46 | −0.79 | −0.94 |
| Initial juiciness | 0.04 | 0.14 | −0.16 | 0.27 |
| ***Canonical $R^2$*** | 0.55 | | 0.15 | |
| ***Eigen Value*** | 1.24 | | 0.18 | |
| ***P Value*** | <0.001 | | 0.003 | |

[1]Loadings indicate the overall relationship between variables and discriminant functions, and standardized coefficients show the unique contribution of each variable to a function.

[2]Aging treatments included: (1) 3 d wet-aged, (2) 14 d wet-aged, (3) 28 d wet-aged, (4) 35 d wet-aged, (5) 49 d wet-aged, (6) 63 d wet-aged, (7) 21 d dry-aged, and (8) 14 d wet-aged followed by 21 d dry-aged.

results of individual attributes shared a similar conclusion, but intercorrelations made the results more difficult to parse. Similar to our study, a change in flavor has been previously noted in beef aged greater than 35 d (Lepper-Blilie et al., 2016), and many studies have shown no flavor change at aging times less than 35 d (Minks and Stringer, 1972; Jeremiah and Gibson, 2003; Bruce et al., 2005; Laster et al., 2008; Lepper-Blilie et al., 2016). Acid flavor (sour) has also been previously associated with extended wet-aging time (Campo et al., 1999). Although the objective nature of trained panels limits the ability to infer consumer acceptability, advanced aging time has been previously described as detrimental to desirable meat flavor (Van Ba et al., 2012; O'Quinn et al., 2016).

Overall tenderness had the greatest impact ($r = -0.79$, $b = -0.94$) on DF2, where the contribution of flavor notes was minimal (Table 4). This suggests that DF2 represented tenderness, despite its marginal contribution to treatment separation compared to DF1. Multivariate composite sensory scores on DF2 showed that beef wet-aged 3 d was rated toughest ($P < 0.01$) of all treatments, which was reflected ($P < 0.01$) by the

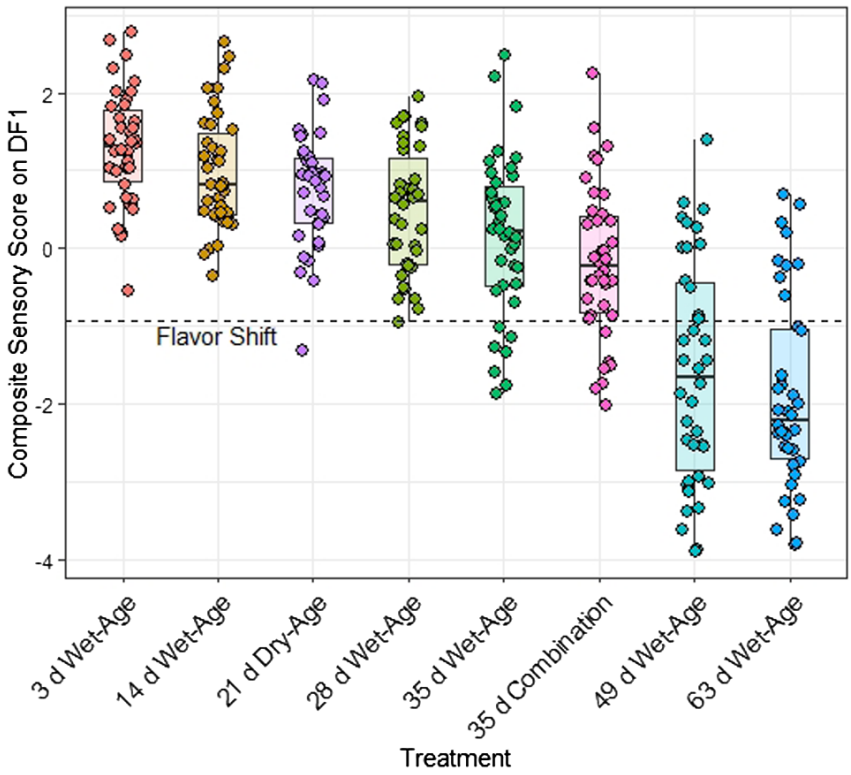


**Figure 2.** Composite sensory scores on discriminant function 1 (DF1) derived from 12 attributes of beef strip loin steaks (n = 38 per treatment) assessed by trained panelists. Notes of sour and musty/earthy loaded most strongly onto DF1, which indicated a flavor shift between 35 d and 49 d of aging time.

univariate ANOVA of overall tenderness. Previous studies have also shown improved sensory ratings for tenderness with increased aging time (Warren and Kastner, 1992; Campo et al., 1999). Numerically more subtle treatment differences ($P < 0.01$) in composite sensory scores (and centroids) on DF2 between aging times of 14 d and 63 d were likely a result of drastic differences in flavor during these aging times, more so than tenderness. Composite scores were derived from all attributes; thus, while the contribution of flavor to DF2 was minimal compared to tenderness, the drastic flavor difference between aging times of 14 d and 63 d was enough to drive significant differences between these treatments on this function. This was supported by the univariate ANOVA of overall tenderness, which was not different ($P > 0.05$) between beef wet-aged 14 d up to 63 d. This substantiates the influence of flavor, and not tenderness, on sensory performance at extended wet-aging times of 49 d and 63 d.

Although only 2 dry-aging treatments were represented, neither discriminant function showed trained panelists differentiated dry-aged beef from wet-aged beef (Table 3; Figure 1a). Composite sensory scores on both discriminant functions were not different ($P > 0.05$) between wet-aged beef and dry-aged beef (either independent from or in combination with wet aging) for similar aging times. Multiple previous studies also showed no flavor differences between wet and dry aging (Parrish et al., 1991; Jeremiah and Gibson, 2003; Sitz et al., 2006; Laster et al., 2008; Smith et al., 2008). Campbell et al. (2001) reported that vacuum aging for 7 d or 14 d before dry aging, similar to our combination aging treatment, had no effect on flavor. Mold and bacterial growth—or lack thereof—and/or inconsistent trimming of external dry-aged crust may partially explain discrepancies among studies. A lack of flavor differences between aging methods in this study may be attributed to the absence of visible mold growth during the dry-aging process, despite confirmed viability of the culture at the time of inoculation on tryptic-soy agar plates (Accumedia-Neogen, Lansing, MI). Visual mold growth has been speculated to contribute to unique flavors in dry-aged beef, and variation in microbial communities are expected to affect its sensory properties (Clark et al., 2020). Excessive air circulation leading to premature external dehydration may have inhibited mold growth in our study. Without visible mold growth on the beef, we suspect the dry aging achieved in this study was simply

dehydration of the product. Furthermore, low levels of marbling (less than Small[50]), such as those represented in our sample, have been shown to limit discernible flavor differences between wet- and dry-aging methods (Lepper-Blilie et al., 2016). Because of a lack of meaningful flavor differences resulting from dry aging in this study, dry-aging treatments were not extensively discussed in chemical analysis.

## Shear force assessment

Instrumental tenderness did not improve after 28 d of aging time, as shown by shear force assessment (Table 3). Beef wet-aged 3 d exhibited greater ($P < 0.05$) SSF and WBSF values than beef wet-aged 28 d. Beef wet-aged 14 d exhibited intermediate SSF values not different ($P > 0.05$) from beef wet-aged 3 d or 28 d. Moreover, WBSF values for beef wet-aged 14 d were not different ($P > 0.05$) from beef wet-aged 3 d but were greater ($P < 0.05$) than beef wet-aged 28 d. Subsequent wet aging at 35, 49, and 63 d resulted in SSF and WBSF values not different ($P > 0.05$) from beef wet-aged 28 d, which agreed with trained panelist ratings. Gruber et al. (2006) reported improvements in WBSF values for wet-aged Select strip steaks up to 28 d postmortem, with 96% of the aging response occurring by 26 d. Moreover, King et al. (2009b) suggested that tenderness improvements in *longissimus lumborum* were more noticeable up to 28 d than after 28 d. Freezing and thawing steaks before shear force analysis has been shown to affect instrumental tenderness (Grayson et al., 2014); thus, this may have diminished the realization of tenderness improvements in our study after 28 d of aging time. Beef dry-aged 21 d or aged using a combination of methods had SSF and WBSF values not different ($P > 0.05$) from wet-age treatments comparable in aging time. However, beef dry-aged 21 d or using a combination of methods resulted in less ($P < 0.01$) percent cook loss than beef from all wet-aging treatments, which is likely a factor of dehydration in the dry-aging cabinet before cooking.

## Volatile compounds

Estimated marginal means and treatment comparisons of volatile compound concentrations are presented in Table 5. Data for all volatile compounds were logarithmically transformed using $\log_{10}(x + 1)$,

**Table 5.** Concentrations (ng/g) of volatile compounds of raw beef strip loin steak composites (n = 8 per treatment) representing 8 aging treatments[1]

| | Wet-Aged (d) | | | | | | Dry-Aged (d) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Volatile (ng/g) | 3 | 14 | 28 | 35 | 49 | 63 | 21 | Comb.[2] | SEM[3] | *P* Value |
| ***Alcohols*** | | | | | | | | | | |
| 1-hexanol | 0.62 | 0.76 | 0.94 | 1.19 | 0.94 | 0.76 | 1.15 | 0.80 | 0.23 | 0.45 |
| 1-octanol | 1.29 | 1.36 | 1.54 | 1.82 | 1.47 | 0.93 | 1.48 | 0.92 | 0.26 | 0.22 |
| 1-octen-3-ol | 2.26 | 2.41 | 2.55 | 3.29 | 2.41 | 1.69 | 3.16 | 1.62 | 0.59 | 0.32 |
| 1-pentanol | $2.73^{b}$ | $3.13^{b}$ | $3.23^{b}$ | $3.90^{ab}$ | $2.58^{b}$ | $8.54^{a}$ | $4.41^{ab}$ | $1.90^{b}$ | 0.96 | <0.01 |
| 1-penten-3-ol | 0.05 | 0.06 | 0.06 | 0.09 | 0.08 | 0.10 | 0.13 | 0.07 | 0.02 | 0.07 |
| 2,3-butanediol | $0.04^{d}$ | $0.20^{d}$ | $0.28^{d}$ | $1.08^{cd}$ | $2.25^{c}$ | $8.58^{b}$ | $0.18^{d}$ | $29.13^{a}$ | 3.60 | <0.01 |
| Ethanol | $0.51^{e}$ | $3.85^{d}$ | $24.41^{c}$ | $77.91^{b}$ | $311.46^{a}$ | $569.05^{a}$ | $5.54^{d}$ | $37.63^{bc}$ | 34.73 | <0.01 |
| ***Carboxylic Acids*** | | | | | | | | | | |
| Acetic acid | $1.50^{d}$ | $2.00^{cd}$ | $2.46^{bcd}$ | $3.29^{abc}$ | $4.46^{ab}$ | $6.29^{a}$ | $2.67^{bcd}$ | $5.26^{a}$ | 0.63 | <0.01 |
| Butanoic acid | $18.35^{d}$ | $25.37^{cd}$ | $28.83^{cd}$ | $49.55^{abc}$ | $39.19^{bc}$ | $70.34^{ab}$ | $67.68^{ab}$ | $92.86^{a}$ | 10.55 | <0.01 |
| Hexanoic acid | 9.71 | 10.78 | 12.96 | 15.17 | 12.74 | 8.78 | 15.69 | 14.27 | 2.58 | 0.28 |
| Nonanoic acid | $50.76^{a}$ | $34.79^{a}$ | $49.84^{a}$ | $45.90^{a}$ | $38.69^{a}$ | $16.98^{a}$ | $36.10^{a}$ | $53.50^{a}$ | 7.74 | 0.04 |
| Octanoic acid | 0.09 | 0.06 | 0.09 | 0.10 | 0.09 | 0.02 | 0.05 | 0.12 | 0.03 | 0.25 |
| ***Esters*** | | | | | | | | | | |
| Butanoic acid, methyl ester | $1.46^{d}$ | $1.92^{cd}$ | $1.90^{bcd}$ | $2.83^{abc}$ | $2.25^{abcd}$ | $3.95^{a}$ | $3.35^{ab}$ | $3.20^{abc}$ | 0.37 | <0.01 |
| Heptanoic acid, methyl ester | $0.71^{a}$ | $0.75^{a}$ | $0.91^{a}$ | $1.01^{a}$ | $1.01^{a}$ | $1.00^{a}$ | $1.05^{a}$ | $0.85^{a}$ | 0.08 | 0.03 |
| Hexanoic acid, methyl ester | $22.97^{b}$ | $22.95^{b}$ | $24.69^{b}$ | $28.34^{ab}$ | $24.77^{b}$ | $30.71^{ab}$ | $38.87^{a}$ | $28.47^{ab}$ | 2.40 | <0.01 |
| Nonanoic acid, methyl ester | $2.38^{b}$ | $3.36^{ab}$ | $2.95^{ab}$ | $3.65^{ab}$ | $2.62^{b}$ | $2.56^{b}$ | $5.41^{a}$ | $3.36^{ab}$ | 0.52 | <0.01 |

**Table 5.** (*Continued*)

| | Wet-Aged (d) | | | | | | Dry-Aged (d) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Volatile (ng/g) | 3 | 14 | 28 | 35 | 49 | 63 | 21 | Comb.[2] | SEM[3] | *P* Value |
| Octanoic acid, methyl ester | 0.52 | 0.65 | 0.59 | 0.53 | 0.54 | 0.54 | 0.60 | 0.42 | 0.10 | 0.80 |
| Propanoic acid, methyl ester | 1.35$^{c}$ | 2.11$^{abc}$ | 2.86$^{ab}$ | 3.12$^{a}$ | 3.13$^{ab}$ | 2.10$^{abc}$ | 2.26$^{abc}$ | 1.71$^{bc}$ | 0.34 | <0.01 |
| ***Furans*** | | | | | | | | | | |
| 2-pentyl furan | 0.14 | 0.16 | 0.22 | 0.23 | 0.16 | 0.07 | 0.22 | 0.10 | 0.05 | 0.26 |
| ***Hydrocarbons*** | | | | | | | | | | |
| 1-octene | 0.65$^{b}$ | 0.65$^{b}$ | 0.77$^{b}$ | 1.09$^{ab}$ | 0.74$^{b}$ | 2.34$^{a}$ | 1.52$^{ab}$ | 1.24$^{ab}$ | 0.39 | <0.01 |
| Decane | 1.34$^{ab}$ | 1.44$^{ab}$ | 1.29$^{b}$ | 1.58$^{ab}$ | 1.32$^{ab}$ | 1.65$^{ab}$ | 1.80$^{a}$ | 1.81$^{a}$ | 0.11 | <0.01 |
| D-limonene | 0.09$^{b}$ | 0.14$^{ab}$ | 0.20$^{a}$ | 0.23$^{a}$ | 0.10$^{b}$ | 0.10$^{b}$ | 0.14$^{ab}$ | 0.14$^{ab}$ | 0.02 | <0.01 |
| Octane | 0.82$^{b}$ | 0.97$^{b}$ | 1.02$^{b}$ | 1.24$^{b}$ | 0.98$^{b}$ | 4.24$^{a}$ | 1.52$^{b}$ | 0.99$^{b}$ | 0.29 | <0.01 |
| Pentane | 1.91$^{b}$ | 2.61$^{b}$ | 2.28$^{b}$ | 3.19$^{b}$ | 2.10$^{b}$ | 21.59$^{a}$ | 3.36$^{b}$ | 1.72$^{b}$ | 2.07 | <0.01 |
| p-xylene | 0.36$^{b}$ | 0.48$^{b}$ | 0.44$^{b}$ | 0.42$^{b}$ | 0.43$^{b}$ | 1.13$^{a}$ | 0.42$^{b}$ | 0.42$^{b}$ | 0.11 | <0.01 |
| Toluene | 24.79$^{a}$ | 20.01$^{a}$ | 21.50$^{a}$ | 30.57$^{a}$ | 24.28$^{a}$ | 4.39$^{b}$ | 41.34$^{a}$ | 27.30$^{a}$ | 6.37 | <0.01 |
| ***Ketones*** | | | | | | | | | | |
| 2,3-butanedione | 21.96$^{abc}$ | 18.40$^{abc}$ | 11.75$^{bcd}$ | 17.43$^{abc}$ | 5.96$^{d}$ | 9.43$^{cd}$ | 28.27$^{ab}$ | 37.83$^{a}$ | 4.17 | <0.01 |
| 2,3-pentanedione | 0.50$^{b}$ | 0.50$^{b}$ | 0.50$^{b}$ | 0.50$^{b}$ | 0.50$^{b}$ | 0.52$^{a}$ | 0.50$^{b}$ | 0.50$^{b}$ | <0.01 | <0.01 |
| 2-butanone | 4.04 | 5.49 | 3.52 | 5.14 | 2.96 | 7.7 | 5.18 | 4.34 | 1.62 | 0.23 |
| 2-heptanone | 0.53$^{b}$ | 0.55$^{b}$ | 0.61$^{b}$ | 0.65$^{b}$ | 0.64$^{b}$ | 0.66$^{b}$ | 1.08$^{a}$ | 1.04$^{a}$ | 0.07 | <0.01 |
| 2-pentanone | 0.06$^{c}$ | 0.07$^{c}$ | 0.06$^{c}$ | 0.08$^{c}$ | 0.05$^{c}$ | 0.25$^{ab}$ | 0.14$^{bc}$ | 0.30$^{a}$ | 0.03 | <0.01 |
| 2-propanone | 13.19$^{c}$ | 23.97$^{bc}$ | 24.85$^{abc}$ | 37.66$^{ab}$ | 18.46$^{bc}$ | 77.86$^{a}$ | 34.70$^{abc}$ | 37.92$^{abc}$ | 9.03 | <0.01 |
| 3-hydroxy-2-butanone | 32.58$^{ab}$ | 36.19$^{ab}$ | 24.13$^{abc}$ | 34.91$^{ab}$ | 10.33$^{c}$ | 14.88$^{bc}$ | 53.93$^{a}$ | 62.46$^{a}$ | 9.58 | <0.01 |
| ***Lactones*** | | | | | | | | | | |
| Butyrolactone | 0.06$^{b}$ | 0.06$^{b}$ | 0.07$^{b}$ | 0.13$^{ab}$ | 0.10$^{ab}$ | 0.11$^{ab}$ | 0.12$^{ab}$ | 0.18$^{a}$ | 0.03 | 0.01 |
| ***n-Aldehydes*** | | | | | | | | | | |
| Acetaldehyde | 0.46$^{ab}$ | 0.65$^{ab}$ | 0.80$^{ab}$ | 0.98$^{ab}$ | 0.00$^{b}$ | 0.00$^{b}$ | 0.96$^{a}$ | 1.76$^{a}$ | 0.32 | <0.01 |
| Heptanal | 0.69$^{a}$ | 1.26$^{a}$ | 1.86$^{a}$ | 2.26$^{a}$ | 1.79$^{a}$ | 1.08$^{a}$ | 0.95$^{a}$ | 0.59$^{a}$ | 0.39 | 0.02 |
| Hexanal | 3.84$^{c}$ | 17.95$^{abc}$ | 32.54$^{ab}$ | 43.03$^{a}$ | 36.67$^{ab}$ | 25.63$^{ab}$ | 13.72$^{bc}$ | 4.42$^{c}$ | 7.14 | <0.01 |
| Nonanal | 0.23$^{b}$ | 0.41$^{ab}$ | 0.69$^{ab}$ | 0.92$^{a}$ | 0.63$^{ab}$ | 0.31$^{ab}$ | 0.39$^{ab}$ | 0.29$^{ab}$ | 0.14 | 0.02 |
| Octanal | 1.74$^{b}$ | 2.90$^{ab}$ | 4.37$^{ab}$ | 5.11$^{a}$ | 4.25$^{ab}$ | 3.34$^{ab}$ | 1.87$^{b}$ | 2.51$^{ab}$ | 0.73 | 0.01 |
| Pentanal | 0.02$^{bc}$ | 0.07$^{abc}$ | 0.11$^{abc}$ | 0.16$^{a}$ | 0.13$^{abc}$ | 0.14$^{ab}$ | 0.04$^{abc}$ | 0.01$^{c}$ | 0.03 | <0.01 |
| ***Pyrazines*** | | | | | | | | | | |
| 2,5-dimethyl-pyrazine | 0.00$^{b}$ | 0.02$^{b}$ | 0.04$^{ab}$ | 0.11$^{ab}$ | 0.07$^{ab}$ | 0.01$^{b}$ | 0.07$^{ab}$ | 0.56$^{a}$ | 0.13 | 0.03 |
| Methyl-pyrazine | 0.00$^{b}$ | 0.01$^{b}$ | 0.02$^{b}$ | 0.06$^{ab}$ | 0.03$^{ab}$ | 0.01$^{b}$ | 0.04$^{ab}$ | 0.33$^{a}$ | 0.07 | 0.02 |
| ***StreckerAldehydes*** | | | | | | | | | | |
| 2-methylbutanal | 0.03$^{c}$ | 0.04$^{c}$ | 0.09$^{c}$ | 0.26$^{bc}$ | 0.77$^{ab}$ | 1.70$^{a}$ | 0.04$^{c}$ | 0.67$^{bc}$ | 0.24 | <0.01 |
| 3-methylbutanal | 0.05$^{d}$ | 0.06$^{d}$ | 0.67$^{cd}$ | 2.74$^{bc}$ | 7.27$^{b}$ | 21.54$^{a}$ | 0.13$^{d}$ | 5.65$^{b}$ | 2.78 | <0.01 |
| Benzaldehyde | 0.76 | 0.91 | 1.11 | 1.10 | 1.12 | 0.92 | 0.71 | 0.96 | 0.18 | 0.66 |
| Phenylacetaldehyde | 0.51$^{b}$ | 0.55$^{b}$ | 0.68$^{b}$ | 1.11$^{b}$ | 3.82$^{a}$ | 3.65$^{a}$ | 0.54$^{b}$ | 1.32$^{b}$ | 0.46 | <0.01 |
| ***Sulfides*** | | | | | | | | | | |
| Carbon disulfide | 1,027.16$^{b}$ | 2,875.02$^{ab}$ | 3,817.50$^{a}$ | 2,919.12$^{a}$ | 2,388.87$^{ab}$ | 1,365.46$^{ab}$ | 3,965.28$^{a}$ | 3,386.91$^{ab}$ | 642.27 | <0.01 |
| Dimethyl-disulphide | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | <0.01 | 0.24 |
| Dimethyl sulfide | 1.78$^{ab}$ | 2.44$^{a}$ | 2.12$^{ab}$ | 2.96$^{a}$ | 1.19$^{ab}$ | 1.33$^{ab}$ | 1.41$^{ab}$ | 0.93$^{b}$ | 0.43 | <0.01 |
| ***Thiols*** | | | | | | | | | | |
| Methanethiol | 0.26 | 0.25 | 0.31 | 0.40 | 0.45 | 0.38 | 0.47 | 0.67 | 0.13 | 0.42 |

[1]Treatments: (1) 3 d wet-aged; (2) 14 d wet-aged; (3) 28 d wet-aged; (4) 35 d wet-aged; (5) 49 d wet-aged; (6) 63 d wet-aged; (7) 21 d dry-aged; (8) 14 d wet-aged followed by a 21 d dry-aged (combination).

[2]Combination: Wet-age period of 14 d followed by dry-age period of 21 d.

[3]Standard error (largest) of the estimated marginal means.

[a–d]Estimated marginal means in the same row lacking a common superscript differ ($P < 0.05$).

where $x$ was a compound's measured value, in order to meet test assumptions of normality for treatment comparisons. Treatment influenced ($P < 0.05$) concentrations of 38 compounds, and 27 of these were different ($P < 0.05$) between 3, 14, 28, 35, 49, and/or 63 d of wet-aging time. Seventeen compounds increased ($P < 0.01$) from 3 to 63 d of wet aging. Only toluene decreased ($P < 0.01$) from 3 to 63 d of wet aging.

Effective sample size of sensory scores was reduced by aggregating to match composites for chemical analysis (n = 8 per treatment), which exaggerated intercorrelations between sensory attributes even more than discussed in sensory analysis. Thus, a conservative approach to the correlation analysis of sensory attributes and chemical compounds was warranted, and these relationships are discussed within the context of interrelatedness between sensory attributes. Only strong correlation coefficients ($r \geq |0.50|$) were extracted and interpreted because of the exploratory objective of identifying general associations between sensory attributes and chemical compounds, despite the likely existence of direct associations. These strong relationships indicate the similar influence of treatment on eating quality and chemical constituents of aged beef.

Seven sensory attributes showed a strong relationship ($r \geq |0.50|$) with at least 1 of 7 volatile compounds (Table 6). Sour and musty/earthy flavor notes, which were most discriminatory in treatment separation on

**Table 6.** Strong Pearson correlation coefficients[1] (greater than or equal to |0.50|) between sensory attributes and volatile compounds and flavor precursors

| Component | Beef Flavor ID | Sour | Oxidized | Nutty | Musty/Earthy | Liver-Like | Overall Tenderness |
|---|---|---|---|---|---|---|---|
| ***Volatile Compounds*** | | | | | | | |
| 2,3-butanediol | | 0.54 | | | 0.59 | 0.52 | |
| 2-methylbutanal | | 0.67 | 0.53 | 0.59 | 0.71 | 0.55 | |
| 3-methylbutanal | −0.54 | 0.73 | 0.61 | 0.64 | 0.82 | 0.63 | 0.51 |
| Acetic acid | | 0.58 | | | 0.59 | 0.50 | |
| Ethanol | −0.59 | 0.72 | 0.60 | 0.61 | 0.79 | 0.61 | 0.68 |
| Phenylacetaldehyde | −0.57 | 0.72 | 0.52 | 0.66 | 0.77 | 0.54 | |
| Toluene | 0.51 | | | | | | |
| ***Free Amino Acids*** | | | | | | | |
| Alanine | | 0.53 | | | 0.51 | | |
| Alpha-aminoadipic acid | | 0.70 | 0.52 | 0.59 | 0.76 | 0.60 | 0.66 |
| Asparagine | −0.57 | 0.78 | 0.54 | 0.61 | 0.79 | 0.66 | |
| Aspartic acid | −0.60 | 0.78 | 0.58 | 0.63 | 0.83 | 0.61 | 0.55 |
| Cysteine | −0.54 | 0.67 | 0.56 | 0.57 | 0.73 | | 0.62 |
| Cystine | −0.53 | 0.56 | | | 0.55 | | |
| Glutamic acid | −0.57 | 0.77 | 0.60 | 0.59 | 0.77 | 0.61 | 0.66 |
| Histidine | | | | | | | 0.61 |
| Hydroxyproline | | | | | | | 0.56 |
| Isoleucine | | 0.55 | | | 0.60 | | 0.50 |
| Leucine | | 0.63 | | | 0.57 | 0.53 | |
| Lysine | −0.52 | 0.74 | 0.55 | 0.58 | 0.76 | 0.59 | 0.65 |
| Methionine | | 0.58 | | 0.54 | 0.68 | | 0.61 |
| Ornithine | −0.58 | 0.75 | 0.62 | 0.58 | 0.76 | 0.57 | 0.51 |
| Phenylalanine | −0.55 | 0.75 | 0.54 | 0.60 | 0.78 | 0.62 | 0.62 |
| Proline | | | | | | | 0.50 |
| Serine | | 0.59 | | | 0.59 | 0.50 | 0.61 |
| Threonine | | 0.60 | | | 0.60 | 0.51 | 0.61 |
| Tryptophan | | 0.67 | | 0.58 | 0.71 | 0.56 | 0.52 |
| Tyrosine | | 0.70 | | 0.55 | 0.73 | 0.57 | 0.70 |
| Total amino acids | −0.52 | 0.72 | 0.53 | 0.55 | 0.73 | 0.58 | 0.68 |
| ***Sugars*** | | | | | | | |
| Fructose | | | | | | 0.50 | |
| Mannose | | | | | | 0.51 | |

[1]All coefficients shown were statistically significant at $P < 0.001$.

DF1, both showed strong positive associations ($r =$ 0.54 to 0.82, $P < 0.001$) with each of 6 compounds: ethanol, acetic acid, 3-methylbutanal, 2-methylbutanal, 2,3-butanediol, and phenylacetaldehyde. Treatment means also indicated a greater presence of these compounds at extended aging times, where concentrations of ethanol, acetic acid, 3-methylbutanal, 2-methylbutanal, 2,3-butanediol, and phenylacetaldehyde each increased (Table 5; $P < 0.01$) from 3 d to 63 d in nearly linear fashion. Oxidized, nutty, and liver-like were strongly related to fewer volatile compounds but at a lower magnitude ($r = 0.50$ to 0.66, $P < 0.001$) than sour and musty/earthy, but again, their relationship was likely because of an intercorrelation with sour and musty/earthy rather than a truly unique contribution. The same holds true for beef flavor ID, which was negatively associated with ethanol, 3-methyl-butanal, and phenylacetaldehyde ($r = -0.54$ to $-0.59$, $P < 0.001$). The large effect of aging time on the production of ethanol and 3-methylbutanal likely produced the strong relationship of overall tenderness to these compounds ($r = 0.68$ and 0.51, respectively, $P < 0.001$).

While associations of volatile compounds and flavor notes are highly variable and complex in the literature, previous beef flavor research has shown results similar to our study. Gredell et al. (2018) reported that sour/acidic and earthy/mushroom were strongly correlated with 2- and 3-methylbutanal in aged beef trimmings. Moreover, acetic acid has been previously associated with a sour aroma and 2-methylbutanal with a musty aroma (Kerth and Miller, 2015). Previous work has shown aging time to affect volatile compounds derived from lipid oxidation (Stetzer et al., 2008). Moreover, the Maillard reaction has been identified as primary contributor to volatile compounds when meat is cooked (Mottram, 1998). However, fatty acid analysis (data not reported) in our study suggested limited lipid oxidation, and volatile compounds were extracted from raw samples at 30°C. This leads us to believe that metabolic processes in aged beef may contribute to volatile compound production and consequent flavor changes. Ismail et al. (2008) showed drastic increases of ethanol during aging of irradiated meat, and lactic acid–producing bacteria have been shown to generate ethanol in an anaerobic environment, such as vacuum-packaged meat (Mayr et al., 2003). Various groups of lactic acid bacteria have also been shown to generate 3-methylbutanal in vacuum-packaged meat (Hernández-Macedo et al., 2012), and Nychas et al. (2008) reported 2-methylbutanal as a product of gram-negative bacteria metabolism. Right-skewed distributions of these compounds during aging time progression also indicated the exponential growth of microbial communities. Microbiological analysis is needed to confirm or refute this speculation.

## Flavor precursors

Aging time progression generally resulted in increased concentrations of free amino acids (Table 7) and sugars (Table 8), while fatty acid composition was minimally affected (data not reported). Concentrations of 22 free amino acids and 6 individual sugars increased ($P < 0.05$) from 3 to 63 d. Previous studies have also found free amino acid and sugar content to increase during postmortem aging time (Ginger et al., 1954; Nishimura et al., 1988; Koutsidis et al., 2008b). Twenty individual free amino acids showed at least one strong association ($r \geq |0.50|$, $P < 0.001$) with a sensory attribute (Table 6). The strongest and greatest number of these individual associations were with sour ($r = 0.53$–$0.78$, $P < 0.001$), musty/earthy ($r = 0.51$–$0.79$, $P < 0.001$), and overall tenderness ($r =$ 0.50–0.70, $P < 0.001$). Total free amino acid content was positively associated ($r \geq 0.50$, $P < 0.001$) with sour, oxidized, nutty, musty, liver-like, and overall tenderness and negatively associated ($r = -0.52$, $P <$ 0.001) with beef flavor ID. The redundancy of these relationships is likely the large effect of aging time on both sensory attributes and free amino acid content and not necessarily a direct relationship between the two. However, Brewer (2007) reported that aspartic acid and asparagine were associated with sour flavor, and both amino acids were strongly positively correlated ($r = 0.78$) with sour in the present study. The liberation of free amino acids and sugars at extended aging times may be the result of microbial metabolism, proteolytic breakdown, or a relationship between both factors. Microbial growth and exogenous enzymatic activity have been linked to free amino acid production (Toldrá, 1998), and conversion of peptides to amino acids is a primary metabolic function of lactic acid bacteria (Christensen et al., 1999). Further, free amino acids and sugars were measured in raw sample that did not undergo the Maillard reaction, at least not to the extent seen in cooking. Given that free amino acids and sugars are substrates in the Maillard reaction, it would be reasonable to hypothesize that their increased bioavailability at extended aging times would increase intensities of flavors associated with the Maillard reaction upon cooking, such as browned and roasted. However, browned and roasted were not discriminating attributes between aging treatments. This suggests

**Table 7.** Concentrations (mmol/kg) of free amino acids for raw beef strip loin steak composites (n = 8 per treatment) representing 8 aging treatments

| | Wet-Aged (d) | | | | | | Dry-Aged (d) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Amino Acid (mmol/kg) | 3 | 14 | 28 | 35 | 49 | 63 | 21 | Combination[1] | SEM[2] | *P* Value |
| **Alanine** | 0.563$^{c}$ | 0.736$^{bc}$ | 0.683$^{c}$ | 0.676$^{c}$ | 0.892$^{bc}$ | 1.331$^{a}$ | 0.777$^{bc}$ | 1.052$^{ab}$ | 0.077 | <0.001 |
| **Beta-Alanine** | 0.154$^{b}$ | 0.156$^{ab}$ | 0.158$^{a}$ | 0.156$^{ab}$ | 0.156$^{ab}$ | 0.157$^{ab}$ | 0.157$^{ab}$ | 0.156$^{ab}$ | 0.001 | 0.033 |
| **Alpha-Aminoadipic acid** | 0.001$^{f}$ | 0.004$^{e}$ | 0.007$^{c}$ | 0.007$^{c}$ | 0.011$^{b}$ | 0.014$^{a}$ | 0.005$^{d}$ | 0.010$^{b}$ | 0.001 | <0.001 |
| **Asparagine** | 0.109$^{c}$ | 0.111$^{c}$ | 0.138$^{c}$ | 0.156$^{c}$ | 0.436$^{ab}$ | 0.518$^{a}$ | 0.187$^{c}$ | 0.350$^{b}$ | 0.024 | <0.001 |
| **Aspartic Acid** | 0.088$^{e}$ | 0.094$^{de}$ | 0.115$^{cd}$ | 0.133$^{c}$ | 0.174$^{b}$ | 0.218$^{a}$ | 0.109$^{de}$ | 0.137$^{c}$ | 0.005 | <0.001 |
| **Cysteine** | 0.114$^{e}$ | 0.150$^{d}$ | 0.199$^{c}$ | 0.215$^{c}$ | 0.252$^{b}$ | 0.309$^{a}$ | 0.140$^{d}$ | 0.148$^{d}$ | 0.008 | <0.001 |
| **Cystine** | 0.002$^{d}$ | 0.003$^{cd}$ | 0.005$^{bcd}$ | 0.005$^{bcd}$ | 0.009$^{a}$ | 0.007$^{ab}$ | 0.005$^{bcd}$ | 0.006$^{abc}$ | 0.001 | <0.001 |
| **Glutamic Acid** | 0.138$^{f}$ | 0.256$^{e}$ | 0.427$^{cd}$ | 0.489$^{c}$ | 0.641$^{b}$ | 0.776$^{a}$ | 0.353$^{de}$ | 0.468$^{c}$ | 0.025 | <0.001 |
| **Glutamine** | 0.138$^{f}$ | 0.256$^{e}$ | 0.427$^{c}$ | 0.489$^{c}$ | 0.641$^{b}$ | 0.776$^{a}$ | 0.353$^{d}$ | 0.468$^{c}$ | 0.025 | <0.001 |
| **Glycine** | 0.146$^{d}$ | 0.452$^{c}$ | 0.768$^{ab}$ | 0.799$^{a}$ | 0.629$^{abc}$ | 0.488$^{bc}$ | 0.638$^{abc}$ | 0.598$^{abc}$ | 0.068 | <0.001 |
| **Histidine** | 0.246$^{d}$ | 0.336$^{bcd}$ | 0.388$^{ab}$ | 0.379$^{abc}$ | 0.350$^{abc}$ | 0.445$^{a}$ | 0.282$^{cd}$ | 0.321$^{bcd}$ | 0.022 | <0.001 |
| **Hydroxyproline** | 0.007$^{b}$ | 0.011$^{ab}$ | 0.013$^{a}$ | 0.012$^{ab}$ | 0.012$^{ab}$ | 0.013$^{a}$ | 0.012$^{a}$ | 0.014$^{a}$ | 0.001 | 0.005 |
| **Isoleucine** | 0.098$^{d}$ | 0.162$^{bc}$ | 0.160$^{bc}$ | 0.163$^{bc}$ | 0.178$^{bc}$ | 0.320$^{a}$ | 0.128$^{cd}$ | 0.188$^{b}$ | 0.013 | <0.001 |
| **Leucine** | 0.307$^{d}$ | 0.304$^{d}$ | 0.469$^{cd}$ | 0.521$^{c}$ | 0.953$^{a}$ | 0.632$^{bc}$ | 0.552$^{c}$ | 0.810$^{ab}$ | 0.048 | <0.001 |
| **Lysine** | 0.159$^{f}$ | 0.225$^{ef}$ | 0.304$^{d}$ | 0.337$^{cd}$ | 0.424$^{b}$ | 0.517$^{a}$ | 0.264$^{de}$ | 0.391$^{bc}$ | 0.017 | <0.001 |
| **Methionine** | 0.095$^{d}$ | 0.126$^{cd}$ | 0.166$^{bcd}$ | 0.208$^{bc}$ | 0.228$^{ab}$ | 0.296$^{a}$ | 0.140$^{cd}$ | 0.207$^{bc}$ | 0.019 | <0.001 |
| **Ornithine** | 0.130$^{e}$ | 0.138$^{d}$ | 0.142$^{cd}$ | 0.143$^{cd}$ | 0.156$^{b}$ | 0.188$^{a}$ | 0.140$^{cd}$ | 0.146$^{c}$ | 0.002 | <0.001 |
| **Phenylalanine** | 0.138$^{e}$ | 0.217$^{d}$ | 0.289$^{c}$ | 0.315$^{c}$ | 0.427$^{b}$ | 0.541$^{a}$ | 0.265$^{cd}$ | 0.398$^{b}$ | 0.015 | <0.001 |
| **Proline** | 0.192$^{d}$ | 0.239$^{bc}$ | 0.244$^{bc}$ | 0.250$^{b}$ | 0.218$^{cd}$ | 0.297$^{a}$ | 0.212$^{cd}$ | 0.231$^{bc}$ | 0.007 | <0.001 |
| **Serine** | 0.237$^{d}$ | 0.364$^{cd}$ | 0.565$^{b}$ | 0.569$^{b}$ | 0.550$^{b}$ | 0.800$^{a}$ | 0.489$^{bc}$ | 0.595$^{b}$ | 0.035 | <0.001 |
| **Threonine** | 0.112$^{c}$ | 0.201$^{c}$ | 0.409$^{b}$ | 0.463$^{ab}$ | 0.490$^{ab}$ | 0.567$^{a}$ | 0.359$^{b}$ | 0.469$^{ab}$ | 0.032 | <0.001 |
| **Tryptophan** | 0.005$^{d}$ | 0.012$^{cd}$ | 0.016$^{cd}$ | 0.031$^{bc}$ | 0.042$^{b}$ | 0.066$^{a}$ | 0.019$^{cd}$ | 0.029$^{bc}$ | 0.004 | <0.001 |
| **Tyrosine** | 0.151$^{g}$ | 0.216$^{f}$ | 0.288$^{de}$ | 0.309$^{cd}$ | 0.365$^{ab}$ | 0.410$^{a}$ | 0.251$^{ef}$ | 0.342$^{bc}$ | 0.012 | <0.001 |
| **Valine** | 0.182$^{c}$ | 0.203$^{bc}$ | 0.374$^{abc}$ | 0.408$^{abc}$ | 0.531$^{a}$ | 0.243$^{abc}$ | 0.382$^{abc}$ | 0.491$^{ab}$ | 0.068 | 0.002 |
| **Total Amino Acids** | 3.373$^{f}$ | 4.713$^{e}$ | 6.326$^{d}$ | 6.744$^{cd}$ | 8.123$^{ab}$ | 9.151$^{a}$ | 5.864$^{d}$ | 7.558$^{bc}$ | 0.253 | <0.001 |

[1]Wet-age period of 14 d followed by dry-age period of 21 d.

[2]Standard error (largest) of the estimated marginal means.

[a–g]Estimated marginal means in the same row lacking a common superscript differ ($P < 0.05$).

**Table 8.** Concentrations (mmol/kg) of sugars for raw beef strip loin steak composites (n = 8 per treatment) representing 8 aging treatments

| | Wet-Aged (d) | | | | | | Dry-Aged (d) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sugar (mmol/kg) | 3 | 14 | 28 | 35 | 49 | 63 | 21 | Combination[1] | SEM[2] | *P* Value |
| **Ribose** | 0.18$^{d}$ | 0.37$^{cd}$ | 0.61$^{cd}$ | 0.51$^{cd}$ | 2.14$^{a}$ | 1.21$^{bc}$ | 2.01$^{ab}$ | 2.44$^{a}$ | 0.19 | <0.01 |
| **Fructose** | 0.64$^{c}$ | 1.27$^{c}$ | 2.54$^{c}$ | 2.02$^{c}$ | 5.47$^{b}$ | 5.15$^{b}$ | 5.19$^{b}$ | 8.15$^{a}$ | 0.52 | <0.01 |
| **Mannose** | 0.40$^{e}$ | 0.88$^{de}$ | 1.43$^{cd}$ | 1.31$^{d}$ | 2.50$^{ab}$ | 2.12$^{bc}$ | 2.36$^{ab}$ | 3.03$^{a}$ | 0.17 | <0.01 |
| **Glucose** | 3.41$^{d}$ | 5.35$^{d}$ | 7.36$^{cd}$ | 6.54$^{cd}$ | 13.83$^{ab}$ | 10.00$^{bc}$ | 14.84$^{a}$ | 15.41$^{a}$ | 0.96 | <0.01 |
| **Myo-Inositol** | 0.05$^{c}$ | 0.06$^{c}$ | 0.08$^{c}$ | 0.08$^{c}$ | 0.23$^{ab}$ | 0.16$^{b}$ | 0.28$^{a}$ | 0.24$^{ab}$ | 0.02 | <0.01 |
| **Maltose** | 0.09 | 0.11 | 0.11 | 0.11 | 0.10 | 0.10 | 0.10 | 0.11 | <0.01 | 0.06 |

[1]Wet-age period of 14 d followed by dry-age period of 21 d.

[2]Standard error (largest) of the estimated marginal means.

[a–e]Estimated marginal means in the same row lacking a common superscript differ ($P < 0.05$).

the overwhelming impact that some flavor notes may have in masking browned and roasted notes.

Fatty acids (both polar and neutral fractions) were not meaningfully different between treatments nor strongly related to any sensory attribute. Anaerobic conditions of vacuum packaging and intact meat have been shown to prevent lipid oxidation (King et al., 1995; Spanier et al., 1997), which supports the limited differences in fatty acid profiles of our study and suggests that flavor differences in our study were not the

result of lipid oxidation. Only C14:1 in the polar fraction generated a treatment effect, where combination aging produced a greater ($P = 0.02$) percent (0.90%) C14:1 than 35 d wet aging (1.17%). Work by Gredell et al. (2018) conflicts with this finding, as levels of C14:1 *cis*-9 were greater in fresh beef than dry-aged beef. The reason for this discrepancy is unknown, but the small magnitude of difference reported in our study may not have been biologically meaningful. Limited research has been conducted on fatty acid profiles of wet- and dry-aged beef. O'Quinn et al. (2016) and Gredell et al. (2018) found different fatty acid profiles for wet and dry aging, suggesting differences in oxidative stability between the 2 methods. However, processing differences (such as trimming of crust on dry-aged samples), muscle form (whole vs. ground), and the simple dehydrative nature of our dry-aging process may have contributed to these discrepancies.

## Conclusions

This study indicates the deteriorative effect of extended aging time on the flavor of beef strip loins. This conclusion was derived by accounting for interrelatedness between eating quality attributes using a DFA. Flavor notes were not differentiated by trained panelists in beef aged up to 35 d, regardless of aging method (wet vs. dry); however, beef wet-aged for 49 d or 63 d was characterized as uniquely sour and musty/earthy. Further research is needed to evaluate dry-aging parameters, including storage conditions and the use of inoculums. Still, tenderness improved to a point with increased aging time, but both shear force assessment and trained panelists agreed that additional aging beyond 28 d did not meaningfully improve tenderness. Volatile compounds, many of which have been previously described as products of microbial metabolism, including ethanol, were especially prevalent at extended aging times. Intrinsic metabolic processes were also evident by the liberation of amino acids and sugars, with no change in fatty acid composition, during the progression of aging time. Therefore, a sacrifice in flavor, with no realization of tenderness improvement, can be expected when beef strip loins are wet-aged to 49 d or greater.

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