Flavor Characterization of Grass- and Grain-Fed Australian Beef *Longissimus Lumborum* Wet-Aged 45 to 135 Days

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Abstract: The study objective was to evaluate the effects of extended postmortem wet aging of Australian beef, cattle diet, and their interaction on objective and subjective measures of beef flavor of the *longissimus lumborum*. Beef strip loins were collected from grass- and grain-fed cattle ($n = 50$ total) at a commercial abattoir near Brisbane, Australia. Beef *longissimus lumborum* were portioned and assigned randomly to one of 3 postmortem aging periods (45, 70, or 135 d). As each section reached its respective postmortem aging designation, that section was fabricated into 2.5-cm steaks, individually vacuum packaged, and frozen ($-21°C$). Trained panelists evaluated cooked steaks for numerous flavor attributes, tenderness, and juiciness; volatile compounds were also evaluated. Sensory scores differed ($P < 0.05$) due to diet, whereby grain-fed samples were rated more intense ($P < 0.05$) for beef flavor identity, fat-like, liver-like, and sweet but grass-fed samples were more intense ($P < 0.05$) for green-hay and bitter. Juiciness, tenderness, and 4 flavor attributes (bloody/serumy, metallic, umami, and rancid) were similar ($P > 0.05$) between diets. Extending postmortem aging from 45 to 135 d resulted in decreased beef and umami flavors ($P < 0.05$), along with concurrent increased detection of off-flavors, such as liver-like, rancid, bitter, and sour ($P < 0.05$). Volatile flavor compounds were more influenced by postmortem aging than diet. Increased postmortem aging time increased concentration for both lipid oxidation and Maillard-reaction–derived volatiles, resulting in the concentration of negative flavor volatile compounds rather than the absence of positive flavor compounds. Based on these results, differences existed in the flavor profile of *longissimus lumborum* from grass- and grain-fed beef, regardless of postmortem aging. However, aging beef strip loins 135 d is not recommended based on reduced beef flavor and increased off-flavor detection compared to samples aged 45 or 70 d postmortem.

Introduction

The characteristic flavors of grass- versus grain-fed beef are highly dependent on different volatile compounds derived from the lipid source, particularly intramuscular fat (Mottram, 1998). Steaks from grass-fed beef have been described as having greater barny, bitter, gamey, and grassy flavor notes—all of which have been classified as "negative" attributes—than grain-fed beef while also being less juicy and having less umami recognition by panelists (Maughan et al., 2012). Fruet et al. (2018) found that forage-fed beef had improved fatty acid profiles, decreased concentrations of volatile compounds associated with lipid oxidation, and less off-flavor compared to beef finished entirely or partially with concentrates. These conflicting results highlight the potential regional differences in comparisons of grass- versus grain-fed beef and the dependency on diet quality. Even so, concentrations of certain aroma volatiles differ between concentrate- and forage-based cattle diets. According to Elmore et al. (2004), the concentration of 1-octen-3-ol, hexanal, 2-pentyl-furna, trimethylamine, *cis*- and *trans*-2-octene, and 4,5-dimethyl-2-pentyl-3-oxazoline were over 3 times

greater in beef from concentrate-fed steers compared to beef from steers fed grass silage. Meanwhile, 1-phytene was found at higher levels in the grass silage-fed beef (Elmore et al., 2004).

Historically, postmortem aging has been used to improve quality characteristics, such as tenderness, juiciness, and flavor (Mottram, 1998), as it has a well-established positive influence on meat tenderness (Gruber et al., 2006; Brewer and Novakofski, 2008; Juárez et al., 2010; Hughes et al., 2015). However, its impacts on beef flavor are inconsistent and less well defined. The biochemical changes that occur during postmortem aging have drastic effects on tenderness (Wicklund et al., 2005) but can also result in flavor changes (Kato and Nishimura, 1987). Research has shown that meat flavor improves with aging to a certain point, but rancid or other off-flavors may develop after long storage periods (Killinger et al., 2004). The characteristic beef flavor originates from heat-induced reactions, specifically the Maillard reaction and lipid degradation, which produce a wide variety of volatile flavor compounds (Mottram, 1998). During postmortem aging, enzymes cause an alteration of compounds such as peptides, free amino acids, and fatty acids in the meat, which leads to an increase in concentration of flavor precursors (MacLeod and Seyyedain-Ardebili, 1981).

Until recently, 100 d was the commonly accepted shelf life of Australian vacuum-packaged beef stored at −1°C during shipping and storage (Small et al., 2012), but more conservative limits (77–84 d) are in place for some Australian exports to account for potential deficiencies in the supply chain (MLA, 2016). Recent studies have suggested that an extended shelf life of up to 20–30 wk (140–210 d) for vacuum-packaged beef is feasible if temperature is well controlled and maintained from −2°C to −1°C (Small et al., 2012; Hughes et al., 2015). Although shelf life of Australian chilled beef can be extended to 20 or more weeks, off-odors and off-flavors can still be detected (Rodas-González et al., 2011; Small et al., 2012).

Given these findings, there is a need to characterize the flavor of Australian vacuum-packaged beef that has been exposed to extended periods of postmortem aging, especially in the export market where the product is destined to be consumed. Therefore, this study was conducted to evaluate the effects of extended postmortem wet aging of Australian beef up to 135 d postmortem, along with cattle diet and their interaction, on the traits associated with beef flavor of the *longissimus lumborum*.

Materials and Methods

Carcass selection and subprimal fabrication

Animals were selected randomly at a commercial abattoir near Brisbane, Australia, to equally represent grain- and grass-fed diets ($n = 50$; 25 per diet). Live animal information pertaining to diet (grass vs. grain) was made available through the animal identification system in Australia, but no data were collected on farm. Animals were harvested over a period of 10 days. On the first, fourth, fifth, eighth, and ninth days, grain-fed animals were slaughtered. On the second, third, sixth, seventh, and tenth days, grass-fed animals were slaughtered. Carcasses were fabricated 24 h postmortem, and strip loins (Institutional Meat Purchase Specifications #180) were collected from the selected carcasses each day. Subprimals were portioned into sections measuring approximately 10 to 13 cm and assigned randomly to one of three postmortem aging periods (45, 70, or 135 d). Sections were individually vacuum packaged and shipped refrigerated (0°C–4°C), with temperature monitored during shipping, to Texas Tech University in Lubbock, Texas. Upon arrival, the strip loin sections were sorted into their respective preassigned aging groups of 45 d, 70 d, and 135 d.

Steak fabrication, pH, and instrumental color

As each section reached its respective postmortem aging designation, that section was fabricated into three to four 2.54-cm steaks. Steaks were assigned randomly to proximate analysis, sensory analysis, or volatile flavor compound analysis. Steaks were individually vacuum packaged and frozen (−21°C).

Prior to steak fabrication, pH of the strip loin sections was taken using a pH meter (TPS WP-80M, TPS, QLD, Australia) with a pH probe and temperature sensor. Both probes were inserted in the *longissimus lumborum* 3 times; pH values were averaged prior to statistical analysis.

After steaks were fabricated, the second steak from the anterior-most end of each section was allowed to oxygenate for 20 min prior to color measurement. Color values were determined by a colorimeter with an observer angle of 2°, a pulsed xenon lamp light source, and an 8-mm aperture opening (Minolta CR-400 Chroma Meter, Konica Minolta Sensing Americas, Ramsey, NJ). The surface of the exposed *longissimus lumborum* was scanned 3 times to obtain L^*, a^*, and b^* values, which were averaged prior to statistical analysis. Hue angle was calculated as $\tan^{-1}(b^* \div a^*)$ and chroma as $\mathrm{sqrt}(a^{*2} + b^{*2})$.

Cooked sample preparation

Before cooking, steaks were thawed at 2°C to 4°C for 24 h. Electric clamshell grills (model GR150; Cuisinart Griddler Deluxe, East Windsor, NJ) were used to cook steaks to a medium degree of doneness (71°C). Steak temperatures were monitored using digital thermometers (Thermapen, Classic Super-Fast, Thermoworks, American Fork, UT) and were recorded for steaks designated for trained sensory panels and cooked analyses.

Proximate analysis

Steaks for compositional analysis were obtained solely from sections aged 45 d. Steaks were thawed for 24 h at 2°C to 4°C. After thawing, steaks were trimmed of subcutaneous fat and perimysial connective tissue and ground 3 times through a 4-mm plate of an electric meat grinder (IK-541091, Cabela's Inc., Sidney, NE). An AOAC International-approved method (Anderson, 2007) was used to conduct proximate analysis through a near infrared spectrophotometer (FoodScan, FOSS NIR systems, Inc., Laurel, MD). Percentages of protein, fat, and moisture were determined for each sample.

Trained descriptive sensory panels

Panelists ($n = 14$) consisted of staff and graduate students from Texas Tech University Department of Animal and Food Sciences and were trained for the evaluation of several sensory attributes utilizing the American Meat Science Association (AMSA, 2016) sensory guidelines. Panelists were exposed to the anchors for flavor intensities shown in Table 1 and were evaluated for 3 wk to be able to objectively evaluate intensity of beef flavor attributes similar to attributes included and described in the beef flavor lexicon (Adhikari et al., 2011). Flavors included the following: beef flavor identity (amount of beef flavor identity in the sample), bloody/serumy (aromatics associated with blood on cooked meat products; closely related to metallic aromatic), fat-like (aromatics associated with cooked animal fat), liver-like (aromatics associated with cooked organ meat/liver), metallic (impression of slightly oxidized metal, such as iron,

Table 1. Definitions and references used for beef flavor attributes, as well as overall juiciness and tenderness, as adapted from the AMSA sensory guidelines (2016)

Sensory Attribute	Definition	Reference (Score)
Beef Flavor Identity	The amount of beef identity in a sample	Swanson's beef broth diluted with water 50:50 (30) 80% lean ground chuck—pan fried until brown (50) Beef brisket (75)
Bloody/Serumy	An aromatic associated with blood in cooked meat. Closely related to metallic.	USDA Choice strip steak cooked to 60°C (40)
Fat-Like	Aromatic associated with cooked animal fat	Beef brisket cooked to 71°C (10) Ground beef 90/10—pan fried until brown (30) Ground beef 70/30—pan fried until brown (60)
Liver-Like	Aromatics associated with cooked organ meat/liver	Flat iron steak cooked to 71°C (20)
Sour	Fundamental taste factor associated with a citric acid solution	0.015% citric acid solution (10) 0.050% citric acid solution (25)
Bitter	The fundamental taste associated with caffeine solution	0.01% caffeine solution (15) 0.02% caffeine solution (25)
Sweet	A fundamental taste associated with sucrose	0.50% sucrose solution (25)
Metallic	Impression of slightly oxidized metal	Dole canned pineapple juice (40)
Rancid	Aromatics commonly associated with oxidized fat and oils; may include cardboard, painty, varnish, and fishy	Microwaved vegetable oil—3 min (45) Microwaved vegetable oil—5 min (55)
Umami	Flat, salty, and somewhat brothy with taste similar to glutamate, salts of amino acids and other molecules called nucleotides	Swanson's 99% fat- and sodium-free beef broth (30)
Green-hay	Brown/green dusty aromatics associated with dry grasses, hay, dry parsley, and tea leaves	Dry parsley (40)
Juiciness		Strip steak cooked to 85°C (20) Strip steak cooked to 71°C (50) Strip steak cooked to 60°C (75)
Tenderness		Eye of round steak cooked to 85°C (20) Strip steak cooked to 71°C (55) Tenderloin steak cooked to 65°C (90)

USDA, US Department of Agriculture.

copper, and silver spoons), rancid (aromatics commonly associated with oxidized fat and oils; may include cardboard, painty, varnish, and fishy), green-hay (brown/green dusty aromatics associated with dry grasses, hay, dry parsley, and tea leaves), umami (flat, salty, somewhat brothy; taste of glutamate, salts of amino acids, and other molecules called nucleotides), sour (fundamental taste factor associated with citric acid), bitter (fundamental taste factor associated with a caffeine solution), and sweet (fundamental taste factor associated with sucrose).

Twenty-five steaks from each diet × aging period treatment combination ($n = 150$) were randomly assigned to one of 25 panel sessions. Panel sessions were conducted over 15 d with no more than 2 panel sessions per day. On days with multiple sessions, a minimum 90-min rest period was observed between panel sessions. Six samples were served in each panel session, representing the 6 diet × aging treatment combinations. Six to eight panelists participated in every session.

Prior to panels, steaks were thawed at 2°C to 4°C for 24 h and cooked as previously described. Steaks were removed from the grill when they reached 71°C, at which time peak temperature was recorded. Steaks were then sliced into 1.27 × 1.27 cm pieces (½" sensory box, Tallgrass Solutions Inc., Manhattan, KS), and a minimum of 2 pieces were placed in 2-oz. plastic portion cups and covered with plastic lids. While remaining steaks were being cooked, samples were placed into a warmer (Cambro Ultra Heated Holding Pan Carrier, 214UPCH400, Webstaurant Store, Lititz, PA) and held at 55°C for no more than 30 min before serving to panelists.

Panelists evaluated all samples individually in a private booth under red incandescent lights to mask color differences. In each booth, panelists were given a napkin, plastic fork, toothpick, and an expectorant cup, along with palate cleansers consisting of unsalted crackers and distilled water to use between each sample. In addition, panelists were provided with the anchors for each flavor attribute as described in Table 1. Traits—including 11 flavor attributes, tenderness, and juiciness—were scored on 100-point line scales, in which 0 = slight, 50 = moderate, and 100 = strong. All ballots were completed using iPads (Foxconn Technology Group, Tucheng, New Taipei, Taiwan) equipped with online software (Qualtrics, Provo, UT).

Volatile compound analysis

Volatile flavor compounds were collected with a combination of an Agilent 7890B series gas chromatograph (Agilent Technologies, Santa Clara, CA) and a 5977A mass selection detector (Agilent Technologies, Santa Clara, CA). A protocol method adapted to include an auto-sampler from Legako et al. (2015) was used to collect compounds on cooked ($n = 150$) samples representing all diet × aging combinations. Cooked volatile samples were cooked as outlined previously. For each sample, a coring device was used to collect six 1.27-cm-diameter cores perpendicular to the cut surface of the steak; 2 each from the medial, middle, and lateral sections of the sample. The cores were minced in a coffee grinder (Coffee Grinder, Mr. Coffee, Cleveland, OH) for 2–3 quick pulses to mimic a chewed-like texture. After the sample was minced, 5.0 g was weighed into a 20-mL glass gas chromatograph vial (Art #093640-036-00, Gerstel, Linthicum, MD), and 10 μL of the internal standard solution (1,2 dichlorobenzene, 2.5 μg/μl) was added. The vials were capped with a 1.3-mm polytetrafluoroethylene septa and metal screw cap (Art #093640-040-00, Gerstel, Linthicum MD). Once the samples were prepared, they were loaded by a Gerstel automated sampler (MPS, Gerstel Inc., Linthicum, MD) for an incubation period at 65°C for 5 min in the Gerstel agitator (500 rotations/min). After incubation, a 20-min extraction period occurred. During this time, the volatile compounds were collected by solid phase microextraction from the vial headspace while in the agitator, using a carboxen polydimethylsiloxane fiber (Stableflex 24 Ga, Supelco, Bellefonte, PA) of 85-μm film thickness. The compounds were then injected in a VF-5ms capillary column (30 m × 0.25 mm × 1.00 μm; Agilent J&W GC Columns, the Netherlands) and separated. The mass spectrometer detected the ions in electron impact mode at 70 eV within the range of 33–500 m/z.

Volatile compound identity was initially determined from a mass spectral library (NIST MS Search 2.0, National Institute of Standards and Technology, Gaithersburg, MD). Compound identity was validated by comparison with retention times and ion fragmentation patterns of external standards (Sigma-Aldrich, Saint Louis, MO). Compounds consistently identified in each chromatogram were retained for treatment comparison. A 5-point external standard method was used for quantification. Standard reference compounds (Sigma-Aldrich, Saint Louis, MO) were injected (0.1 μL) in solutions of pentane (late eluting compounds) or toluene (early eluting compounds) in splitless mode. Calibration curve slopes and intercepts were produced for each compound based on target area ratios and concentration ratios between individual compounds and the internal standard. Unknown

concentrations were determined in sample runs relative to the calibration curve slope, intercept, internal standard concentration, and internal standard ion area. Final values were calculated as nanograms of volatile compound extracted divided by sample weight in grams.

Statistical analysis

Data generated for pH, color, proximate analysis (total fat, moisture, and protein), trained descriptive sensory panels, and volatile compounds were analyzed using statistical procedures in SAS version 9.4 (SAS Institute Inc., Cary, NC). Data were analyzed as a split plot design when applicable, with diet as the whole plot factor and postmortem aging as the subplot factor. Portioned pieces of the strip loins served as the experimental unit. Diet and postmortem aging and their interaction were the fixed effects. For compositional data, postmortem aging was not a factor as analysis was conducted only on samples aged 45 d postmortem. For trained descriptive sensory data, carcass, harvest day, panel, and panelist were included as random effects. For volatile flavor compounds, carcass and harvest day were included as random effects. Least-squares means were generated for all analyses utilizing generalized linear mixed models (PROC GLIMMIX) and separated with the PDIFF function, with significance defined as $\alpha = 0.05$.

Results and Discussion

Proximate analysis

Proximate analysis values for raw samples are presented in Table 2. Diet influenced ($P = 0.01$) fat and moisture percentages but had no effect ($P = 0.54$) on protein percentage. Grain-fed samples had greater ($P < 0.01$) fat percentage, whereas grass-fed samples were

Table 2. Proximate composition of raw Australian *longissimus lumborum* ($n = 50$) from samples aged 45 days postmortem

| | Diet | | | |
	Grass	Grain	SEM[1]	P value
Fat, %	1.84[b]	3.76[a]	0.44	0.01
Moisture, %	74.13[a]	71.99[b]	0.23	0.01
Protein, %	23.54	23.43	0.12	0.54

[1]SEM (largest) of the least-squares (LS) means.

[ab]LS means within a row lacking a common superscript differ ($P < 0.05$).

greater ($P < 0.01$) in moisture percentage. Previous studies have shown an inverse relationship between fat and moisture content, in alignment with these results (O'Quinn et al., 2016; Bueso et al., 2018; Garmyn et al., 2019). The fat content from the grass-fed steaks is comparable to the expected fat content from a US Department of Agriculture (USDA) Standard strip steak, whereas the fat content from the grain-fed treatment is similar to the expected fat percentage of a USDA Select strip steak (Corbin et al., 2015; Gredell et al., 2018). Generally, grain-fed cattle in Australia are fed a concentrated diet for approximately 70–90 d (ALFA, 2019). According to the national Meat Standards Australia grading reports (MLA, 2019), grass-fed carcasses are leaner with slightly less 12th rib fat (7 mm vs. 9 mm, respectively) and lower marbling scores (330 vs. 370, respectively) compared to carcasses from grain-fed cattle, suggesting that grass-fed beef has less intramuscular fat. French et al. (2001) found similar results, in which concentrate-finished cattle had greater fat and lower moisture; they saw no differences in protein between grass- and grain-finished beef. However, Bueso et al. (2018) found a difference in percent protein between Honduran grain-fed, Honduran grass-fed, USDA Select, and USDA Top Choice beef with the Honduran grain-fed having the highest percent protein. This increase in protein was attributed to the age of the cattle at processing; the Honduran grain-finished cattle were older animals.

Color and pH

Diet and postmortem aging interacted ($P < 0.01$) to influence $a*$, $b*$, and chroma, which are presented in Table 3. The main effects of diet and postmortem aging on the remaining instrumental color traits and pH are listed in Table 4. Grain-fed samples aged for 70 d postmortem had greater ($P < 0.05$) $a*$ values than samples from grain-fed aged 45 d, grain-fed aged 135 d, and grass-fed aged 45 d, but they did not differ ($P > 0.05$) from grass-fed samples aged 70 d or grass-fed samples aged 135 d. Grain-fed samples aged 70 d had greater ($P < 0.05$) $b*$ values than all other treatments, whereas grain-fed samples aged 45 d had greater ($P < 0.05$) $b*$ values than grain-fed samples aged 135 d, but all grass-fed treatments did not differ ($P > 0.05$) from each other and were similar ($P > 0.05$) to grain-fed samples aged 45 d and 135 d. Grain-fed samples aged 70 d had greater ($P < 0.05$) chroma values than all other treatments, while chroma values of grain-fed samples aged 45 d were greater ($P < 0.05$) than grain-fed samples aged 135 d and grass-fed samples aged 70 d but did

Table 3. Least-squares means of color of Australian *longissimus lumborum* samples based on diet × aging interactions (n = 150)

Attribute	Grass, d			Grain, d			SEM[1]	Diet × Age P value
	45	70	135	45	70	135		
*a**	20.59[bc]	20.79[ab]	20.80[ab]	20.59[bc]	21.74[a]	19.68[c]	0.37	0.01
*b**	12.90[bc]	13.05[bc]	13.16[bc]	13.52[b]	14.51[a]	12.67[c]	0.34	0.01
Chroma	24.31[bc]	24.55[bc]	24.61[bc]	24.67[b]	26.14[a]	23.41[c]	0.49	0.01

[1]SEM largest of the least-squares (LS) means.

[abc]LS means within a row, lacking a common superscript differ ($P < 0.05$).

*a**: (−) = green, (+) = red.

*b**: (−) = blue, (+) = yellow.

Chroma = a*2+b*2

Chroma: 0 = dull/least intense color; 20+ = full chroma/color.

Table 4. Least-squares means of color and pH based on diet and aging times of Australian *longissimus lumborum* samples (n = 150)

Attribute	Diet				Aging, d				
	Grass	Grain	SEM[1]	P value	45	70	135	SEM	P value
*L**	42.24[b]	45.14[a]	0.33	0.01	43.96[x]	42.79[y]	44.32[x]	0.28	0.01
Hue	0.56[b]	0.58[a]	0.01	0.01	0.57	0.57	0.56	0.01	0.29
pH	5.72	5.71	0.01	0.42	5.74[x]	5.72[x]	5.68[y]	0.02	0.01

[1]SEM largest of the least-squares (LS) means.

[ab]LS means within a row, specific to diet, lacking a common superscript differ ($P < 0.05$).

[xyz]LS means within a row, specific to age, lacking a common superscript differ ($P < 0.05$).

*L**: 0 = black, 100 = white.

Hue: $\tan^{-1}(b*/a*)$.

Hue: 0° = red, 275° = violet.

not differ ($P > 0.05$) from grass-fed samples aged 45 d and 135 d. In the grain-fed samples specifically, the *a** and *b** values increased until 70 d and then decreased, which was similar to the results found by Colle et al. (2015), in which the *a** values increased until 21 d of aging and then declined until 63 d of aging. The *b** values followed suit as they increased until 21 d of aging and decreased for the remaining aging treatments (Colle et al., 2015).

As seen in Table 4, diet influenced *L** ($P < 0.01$) and hue ($P < 0.01$), but pH was similar ($P = 0.42$) between diets. Lightness (*L**) values were greater ($P < 0.05$) for grain-fed treatments than grass-fed, which concurs with previous research (Baublits et al., 2004; Bruce et al., 2004) that show that forage-fed cattle produce darker colored lean. Vestergaard et al. (2000) attributed the darker color lean of beef from forage-fed cattle to the amount of glycogen in the muscle; less glycogen and higher pH leads to darker lean color scores. In the current study, hue values were greater ($P < 0.05$) for grain-fed samples than grass-fed samples. Although carcass traits were not assessed in

the current study and age was not documented, it is possible that age of the animals also influenced lean color between grass- and grain-fed carcasses. Some previous work would suggest that grass-fed cattle could be older than grain-fed counterparts as it may take longer to reach similar end points, either measured by final weight or external fat estimates (Mandell et al., 1997; Garmyn et al., 2010). While it is possible the animals in the current study varied in age, we believe the animals were likely similar in physiological age based on national average ossification scores for Meat Standards Australia graded carcasses designated as grass-fed or grain-fed. Ossification score is assessed much like skeletal maturity in the USDA quality grading system and serves as a measure of physiological maturity. According to the Australian Beef Eating Quality Insights (MLA, 2019), grass-fed and grain-fed carcasses have similar average ossifications scores (170 vs. 160, respectively). In fact, grass-fed cattle had a larger proportion (56%) of cattle with ossification scores of 150 or less compared to 46% of grain-fed carcasses. As a result of probable similarity in cattle

age between the 2 diets, future discussion will focus more on diet rather than the possible age difference between cattle finished using the 2 diets.

Aging influenced $L*$ ($P < 0.01$) and pH ($P < 0.01$). $L*$ values were similar ($P > 0.05$) and greater ($P < 0.05$) for samples aged 45 d and 135 d compared to samples aged 70 d. Wicklund et al. (2005) found that $L*$ values declined as aging periods lengthened out to 28 d; the current study shows that $L*$ values decreased up to 70 d. Colle et al. (2015), however, found that $L*$ values of *longissimus lumborum* increased from 14 to 21 d of postmortem aging and remained elevated until 63 d. Rodas-González et al. (2011) found that $L*$ values increased while color deteriorated (as assessed by trained panelists) as vacuum storage time of Australian strip loins increased from 10 to 16 wk (70–112 d). It has been previously suggested by Seideman et al. (1976) that production of lactic acid by lactobacilli could result in the brighter color. Even so, we did not expect the nonlinear response of $L*$ with aging time. However, little is known about color at aging times that have been extended to 135 d. Aging also influenced pH ($P < 0.01$); pH values decreased slightly in samples aged 135 d compared to samples aged 45 d or 70 d, which did not differ ($P > 0.05$) from each other. Colle et al. (2015) found that pH increased up to 63 d postmortem. Conversely, English et al. (2016) observed a decrease in pH of loin sections as aging time increased to 62 d postmortem, which aligns more with the current findings. The relationship between color and pH observed in the current study up to 70 d is widely accepted and has been found in previous research (Abril et al., 2001; Brewer et al., 2001; Viljoen et al., 2002). As pH increases, lean color darkens, which is supported by the negative relationship between $L*$ values and pH documented by Ijaz et al. (2020). According to Offer and Trinick (1983), myofibrillar shrinkage is less pronounced with less sarcoplasmic denaturation in meat with higher pH values. This results in meat seeming translucent and appearing dark (Offer and Trinick, 1983). However, in the current study, pH declined after 70 d, and $L*$ values increased at 135 d.

Descriptive sensory panels

An interaction between diet and postmortem aging was detected ($P < 0.01$) for the sour flavor attribute as shown in Table 5. Grass-fed samples aged 135 d had the strongest ($P < 0.05$) sour flavor according to trained panelists; grain-fed samples aged 135 d were intermediate, while grass- and grain-fed samples aged 45 d and 70 d were all similar ($P > 0.05$) with the least intense sour flavor. These results were similar to past research, in which increased wet aging time consistently increased the sour off-flavor (Campo et al., 1999; Gorraiz et al., 2002; Dikeman et al., 2013). Sour is a basic taste with negative associations in beef flavor (Kerth and Miller, 2015). Bruce et al. (2005) reported similar results, in which grass-fed beef samples were associated with sour attributes more than grain-fed. However, not all results support grass-fed beef having greater off-flavor, as some researchers have shown that grain-fed beef is more prone to sour off-flavors (Larick and Turner, 1990; Maruri and Larick, 1992). Sour flavor has been attributed to the increased amount of glycogen in grain-finished beef and anaerobic glycolysis creating lactic acid, thus leading to the sour flavor (Dube et al., 1971).

As seen in Table 6, diet and aging had no effect ($P > 0.05$) on bloody/serumy in descriptive sensory evaluation. However, diet, independent from aging, impacted ($P < 0.05$) the following flavor intensities: beef flavor identity, fat-like, liver-like, green-hay, bitter, and sweet. Beef flavor identity, fat-like, liver-like, and sweet were stronger ($P < 0.05$) in grain-fed samples than grass-fed samples. The increased beef flavor identity in grain-fed beef was also reported by Duckett et al. (2013) and Garmyn et al. (2010). The increased fat-like flavor in the grain-fed samples aligned with the greater fat percentage of grain-fed samples compared to grass-fed samples (3.76% vs. 1.84%, respectively). The greater intensity of the liver-like in grain-fed

Table 5. Trained descriptive sensory least-squares means[1] for sour attribute of Australian *longissimus lumborum* samples based on diet × aging type interaction ($n = 150$)

Attribute	Grass, d			Grain, d			SEM[2]	Diet × Age P value
	45	70	135	45	70	135		
Sour	6.12[c]	7.22[c]	11.28[a]	6.75[c]	7.04[c]	9.37[b]	0.51	0.01

[1]Sensory scores: 0 = slight, 50 = moderate, and 100 = strong.

[2]SEM largest of the least-squares (LS) means.

[a,b]LS means within a row lacking a common superscript differ ($P < 0.05$).

Table 6. Least-squares means[1] of trained descriptive sensory flavor attributes of Australian *longissimus lumborum* samples based on diet and aging time ($n = 150$)

	Diet				Aging, d				
Attribute	Grass-Fed	Grain-Fed	SEM[2]	P Value	45	70	135	SEM	P value
Beef Flavor	46.19[b]	47.85[a]	0.84	0.03	51.21[x]	47.94[y]	41.91[z]	0.94	0.01
Bloody/Serumy	9.05	9.95	0.46	0.08	8.94	9.49	10.06	0.52	0.19
Fat-Like	11.43[b]	12.00[a]	0.32	0.05	11.54	11.95	11.65	0.35	0.53
Liver-Like	6.86[b]	9.00[a]	0.66	0.01	4.95[z]	7.39[y]	11.46[x]	0.65	0.01
Metallic	7.87	7.37	0.41	0.19	6.12[z]	7.39[y]	9.33[x]	0.46	0.01
Rancid	10.16	9.22	0.82	0.26	6.00[y]	7.52[y]	15.54[x]	0.88	0.01
Green-hay	13.31[a]	9.94[b]	0.74	0.01	8.38[z]	10.96[y]	15.52[x]	0.82	0.01
Umami	10.82	10.99	0.52	0.62	11.78[x]	11.11[x]	9.81[y]	0.55	0.01
Bitter	3.97[a]	3.33[b]	0.25	0.01	2.73[z]	3.52[y]	4.73[x]	0.28	0.01
Sweet	0.47[b]	0.73[a]	0.14	0.02	0.66	0.51	0.63	0.16	0.92
Juiciness	43.99	43.50	1.14	0.72	41.00[y]	44.46[x]	45.78[x]	1.20	0.01
Tenderness	54.36	57.01	1.05	0.07	52.79[y]	54.49[y]	59.77[x]	1.01	0.01

[1]Sensory scores: 0 = slight, 50 = moderate, and 100 = strong.

[2]SEM largest of the least-squares (LS) means.

[a,b]LS means within a row, specific to diet, lacking a common superscript differ ($P < 0.05$).

[x,y,z]LS means within a row, specific to age, lacking a common superscript differ ($P < 0.05$).

samples was unexpected, as the majority of previous research has shown that grass-fed beef has had greater off-flavor intensities (Calkins and Hodgen, 2007; Garmyn et al., 2010; Duckett et al., 2013). However, Melton et al. (1982) found that liver-flavor intensity increased up to 86 d on corn-based diets compared to 0 d on a corn-based diet. As expected, the grass-fed samples had greater green-hay and bitter flavor intensities compared to grain-fed samples ($P < 0.01$); the green-hay flavor is likely due to the decrease in unsaturated fats and conjugated linoleic acids in grass-finished beef (French et al., 2001).

Aging influenced ($P < 0.01$) beef flavor identity, liver-like, metallic, rancid, green-hay, umami, and bitter flavor attributes (Table 6). Beef flavor identity ($P < 0.01$) was stronger in samples aged 45 d and decreased as aging time increased, with samples aged 135 d having the least intense flavor. Samples aged 45 and 70 d similarly had stronger ($P < 0.05$) umami flavor than samples aged 135 d. Beefy, brothy, browned/caramel, and sweet flavors were shown to decrease with aging time in ground beef and Brangus beef (Spanier et al., 1997; Jiang et al., 2010). Aging had the opposite effect on the remaining flavors and attributes tested, as each attribute increased in intensity as aging time was extended. For liver-like, metallic, green-hay, and bitter, panelists detected the strongest ($P < 0.05$) flavor in samples aged 135 d. Samples aged 70 d were intermediate, and samples aged 45 d had the mildest flavor detection ($P < 0.05$). For rancid,

samples aged 135 d had greater ($P < 0.05$) intensity than samples aged 45 d and 70 d, but rancid was similar between the shorter aging periods ($P > 0.05$). Campo et al. (1999) found similar results in which livery flavor increased with extended postmortem aging. However, Colle al. (2015) found no differences in flavor of beef aged up to 63 d postmortem.

Juiciness and tenderness were influenced ($P < 0.01$) by aging time (Table 6). Juiciness was the lowest ($P < 0.05$) at 45 d compared to 70 d and 135 d, which did not differ ($P > 0.05$). Tenderness was greater ($P < 0.05$) at 135 d compared to 45 d and 70 d, which did not differ ($P > 0.05$). Previous research has shown that tenderness improves with postmortem aging (Campo et al., 1999; Brewer and Novakofski, 2008; Colle et al., 2015; Lepper-Blilie et al., 2016). However, Hughes et al. (2015) found that consumer eating quality of *longissimus lumborum*, including tenderness, improved between 2 and 12 wk of vacuum-packaged storage, but no further improvement was observed at 20 wk. In the current study, tenderness was similar at 45 and 70 d, but improved at 135 d. We would like to point out that the first time point in the Hughes study was 14 d, whereas the first time point for assessment in the current study was 45 d. Hughes et al. (2015) also relied on consumer responses for eating quality assessment, whereas trained panelists were used in the current study. This difference alone could explain the variation between the 2 studies. Even so, we would expect to see an improvement in

tenderness from 14 to 84 d, as Hughes did, but by 45 d, it is possible and probable that the samples in the current study were already quite tender. So although tenderness did not improve between 84 and 140 d, according to consumers, it was not unexpected for tenderness to continue to improve during the final 65 d of aging between 70 and 135 d in the current study, despite the lack of difference in tenderness between the first 25 d of aging between 45 and 70 d. Additionally, Campo et al. (1999) showed no effect of aging on juiciness.

Volatile compound analysis

Lipid-derived volatile quantities of cooked beef are shown by diet in Tables 7–8 for diet and aging, respectively. Hundreds of volatile compounds have been found in cooked meat and are derived from lipid degradation, including aldehydes, ketones, hydrocarbons, alcohols, carboxylic acids, and esters. These compounds are secondary products from the oxidation of fatty acids in lipids, and this oxidation can lead to rancid off-flavors during long-term storage; however, in cooked beef the reaction occurs quicker and can lead to more desirable flavors (Mottram, 1998). The lipid-derived volatiles are known to be a major contributor to beef flavor (Brewer and Novakofski, 2008). Differences of lipid-derived compounds between grass- and grain-fed beef were observed in compounds belonging to alkenes, n-aldehydes, alkenes, hydrocarbons, and ketones. In particular, grass-fed samples had greater ($P < 0.05$) heptanal, alpha-pinene, toluene, p-xylene, octane, and 2-propanone compared to grain-fed samples. Vasta and Priolo (2006) found that forage-finished beef had greater concentrations of the phenolic compounds which is related to the fact that phenolic compounds are secondary metabolites of plants. Raes et al. (2003) also found an increase in aldehyde concentration in beef from cattle fed in confinement versus cattle on pasture. Hydrocarbons are generally linked with undesirable and rancid off-flavors (Mottram, 1998).

The quantities of lipid-derived volatile compounds of cooked beef are shown by aging in Table 8. The alcohols affected ($P \le 0.02$) by aging treatments were ethanol, 1-hexanol, and 1-octen-3-ol; concentrations for all 3 compounds were greater ($P < 0.05$) in samples aged 135 d than in samples aged 45 d and 70 d, which were similar ($P > 0.05$). Only one alkene was affected by aging treatments: p-xylene. Samples aged 135 d had a greater ($P < 0.05$) concentration of p-xylene than samples aged 45 or 70 d, which did not differ

Table 7. Least-squares means of lipid-derived volatile flavor compounds from cooked samples of Australian *longissimus lumborum* from two diets[1] ($n = 150$)

Volatile Compound (ng/g)	Diet		SEM[2]	P value
	Grass	Grain		
Alcohols				
Ethanol	342.94	227.11	57.03	0.12
1-hexanol	10.93	7.82	1.34	0.11
1-octanol	7.23	7.27	0.66	0.97
1-octen-3-ol	8.57	7.12	1.32	0.44
1-pentanol	13.61	12.51	2.48	0.75
n-Aldehydes				
Heptanal	83.15[a]	48.71[b]	10.47	0.02
Hexanal	190.75	181.00	35.71	0.79
Pentanal	12.14	9.13	1.68	0.21
Butanal	9.78	6.41	1.41	0.09
Octanal	13.70	9.75	1.79	0.12
Decanal	20.02	17.19	5.38	0.71
Dodecanal	48.46	55.81	16.78	0.76
Alkenes				
Alpha-pinene	6.85[a]	0.16[b]	1.46	0.01
Toluene	133.88[a]	85.95[b]	16.86	0.04
p-xylene	148.98[a]	97.97[b]	17.81	0.04
1-octene	18.17	14.19	1.97	0.15
Carboxylic Acids				
Nonanoic acid	159.78	150.65	18.12	0.72
Benzoic acid	0.40	0.45	0.11	0.79
Esters				
Butanoic acid, methyl ester	0.46	0.62	0.16	0.47
Heptanoic acid, methyl ester	0.41	0.43	0.06	0.87
Hexanoic acid, methyl ester	0.82	2.53	0.64	0.06
Nonanoic acid, methyl ester	0.69	0.79	0.08	0.33
Octanoic acid, methyl ester	2.46	2.15	0.36	0.56
Methyl propionate	2.06	1.84	0.24	0.35
Furan				
2-pentyl furan	2.49	2.51	0.43	0.98
Hydrocarbons				
Decane	9.44	8.21	0.79	0.27
Octane	40.12[a]	27.74[b]	3.61	0.02
Pentane	33.99	21.02	5.04	0.07
Tetradecane	11.85	11.14	3.47	0.88
Ketones				
2-butanone	208.72	136.98	26.79	0.06
2-heptanone	3.56	3.08	0.43	0.43
2-pentanone	2.48	2.09	0.29	0.35
2-propanone	264.34[a]	182.62[b]	27.94	0.04

[1]Diets include grass- and grain-fed treatments.

[2]SEM (largest) of the least-squares (LS) means.

[a,b]LS means within a row lacking a common superscript differ ($P < 0.05$).

($P > 0.05$). Octanoic acid, methyl ester (an ester), decane (a hydrocarbon), and 2-heptanone (a ketone) were all affected ($P < 0.04$) by aging, with greater concentrations in samples aged 135 d compared to samples aged

Table 8. Least-squares means of lipid-derived volatile flavor compounds from cooked samples of Australian *longissimus lumborum* from three aging periods[1] (*n* = 150)

Volatile Compound (ng/g)	Aging, d			SEM[2]	*P* value
	45	70	135		
Alcohols					
Ethanol	83.28[y]	99.45[y]	657.34[x]	98.32	0.01
1-hexanol	7.55[y]	7.75[y]	12.82[x]	1.59	0.02
1-octanol	6.81	6.74	8.20	0.79	0.31
1-octen-3-ol	5.53[y]	6.31[y]	11.68[x]	1.64	0.01
1-pentanol	12.47	9.78	16.91	3.08	0.24
n-Aldehydes					
Heptanal	61.412	59.91	76.47	12.41	0.54
Hexanal	189.40	142.00	226.23	44.31	0.15
Pentanal	11.47	8.08	12.35	2.09	0.30
Butanal	9.30	5.38	9.60	2.46	0.16
Octanal	11.95	11.01	12.21	2.04	0.89
Decanal	18.61	21.41	15.80	6.79	0.83
Dodecanal	55.14	44.23	57.04	20.82	0.89
Alkenes					
Alpha-pinene	3.23	3.16	4.11	1.51	0.84
Toluene	116.77	74.77	138.22	20.91	0.08
p-xylene	103.78[y]	82.05[y]	184.86[x]	22.09	0.01
1-octene	16.91	13.55	18.07	2.42	0.39
Carboxylic Acids					
Nonanoic acid	152.97	139.61	173.07	22.47	0.55
Benzoic acid	0.39	0.24	0.64	0.14	0.10
Esters					
Butanoic acid, methyl ester	0.47	0.38	0.75	0.19	0.36
Heptanoic acid, methyl ester	0.37	0.33	0.53	0.07	0.07
Hexanoic acid, methyl ester	0.86	1.24	2.93	0.78	0.13
Nonanoic acid, methyl ester	0.68	0.77	0.78	0.13	0.69
Octanoic acid, methyl ester	1.91[y]	1.64[y]	3.37[x]	0.44	0.01
Methyl propionate	2.26	1.93	1.67	0.29	0.13
Furan					
2-Pentyl furan	2.38	1.77	3.35	0.53	0.10
Hydrocarbons					
Decane	8.09[y]	6.98[y]	11.41[x]	0.99	0.01
Octane	33.89	28.87	39.03	5.85	0.21
Pentane	28.12	22.45	31.96	8.93	0.54
Tetradecane	11.92	9.14	13.43	4.47	0.77
Ketones					
2-butanone	182.91	119.96	215.68	46.61	0.11
2-heptanone	2.57[y]	2.54[y]	4.85[x]	0.52	0.01
2-pentanone	2.64	1.93	2.29	0.51	0.39
2-propanone	226.69	165.79	277.95	94.13	0.07

[1]Aging treatments include 45 d, 70 d, and 135 d.

[2]SEM (largest) of the least-squares (LS) means.

[xyz]LS means within a row lacking a common superscript differ (*P* < 0.05).

45 or 70 d, which were similar (*P* > 0.05). Results were similar to those of Gorraiz et al. (2002), who found an increase in hydrocarbons with aging time along with an increase in aftertaste intensity. The increase in hydrocarbons throughout aging can be explained by the increase in free fatty acids levels that is caused by lipolytic enzyme activity (Hood and Allen, 1971).

The least-squares means of Maillard-reaction–derived volatile compounds of cooked beef are shown by diet in Table 9. The Maillard reaction occurs when amino compounds react with sugars; this reaction can be the most influential in cooked meat flavors by producing a wide variety of volatile compounds (Mottram, 1998). Diet caused differences in concentrations of one Strecker aldehyde and one sulfur-containing compound. Grass-fed samples had greater (*P* < 0.05) concentrations of acetaldehyde and 2-methyl thiophene. Although sulfur-containing volatile compounds are in very low concentrations in meat, they still play a large role in flavor simply due to the low sensory detection threshold (Drumm and

Table 9. Least-squares mean of Maillard-reaction–derived volatile flavor compounds of cooked samples of Australian *longissimus lumborum* from two diet treatments (*n* = 150)

Volatile Compound (ng/g)	Diet		SEM[1]	*P* value
	Grass	Grain		
Ketones				
2,3-butanedione	146.75	186.15	19.39	0.15
3-hydroxy-2-butanone	222.70	298.61	30.68	0.08
Pyrazines				
Methyl-pyrazine	24.02	25.27	5.49	0.82
Trimethylpyrazine	2.69	2.52	0.34	0.73
2,5-dimethyl-pyrazine	37.02	70.83	6.18	0.66
2-ethyl-3,5-dimethyl pyrazine	13.07	13.41	2.13	0.91
Strecker Aldehydes				
Acetaldehyde	36.04[a]	17.55[b]	6.17	0.03
Benzaldehyde	242.96	167.80	32.95	0.11
Methional	14.46	10.61	1.49	0.07
Phenylacetaldehyde	12.14	9.81	1.50	0.27
2-methyl butanal	89.39	69.57	11.95	0.24
3-methyl butanal	63.50	47.02	9.12	0.20
Isobutyraldehyde	190.29	124.77	27.47	0.09
Sulfur Containing				
Carbon disulfide	20.75	15.49	1.96	0.06
Dimethyl disulfide	1.05	0.69	0.21	0.25
Dimethyl sulfide	14.46	10.59	1.74	0.12
Methanethiol	43.79	35.11	5.31	0.24
2-Methyl thiophene	1.03[a]	0.53[b]	0.14	0.01

[1]SEM (largest) of the least-squares (LS) means.

[ab]LS means within a row lacking a common superscript differ (*P* < 0.05).

Spainer, 1991). Raes et al. (2003) found double the concentration of sulfur-containing volatile compounds in grass-fed grilled beef than grain-fed beef. Results from the current study did not show double the concentrations, but an increase was observed. Despite greater acetaldehyde concentration for grass-fed samples compared to grain-fed samples in the current study, Descalzo et al. (2005) and Maruri and Larick (1992) found that grain-fed steers had greater total aldehyde concentration.

The quantities of Maillard-reaction–derived volatile flavor compounds of cooked beef are shown by aging in Table 10. Aging treatments primarily affected Strecker aldehydes and sulfur-containing compounds. Methional and phenylacetaldehyde were the 2 Strecker aldehydes impacted ($P < 0.01$) by aging. Those compounds were greater ($P < 0.05$) in samples aged 135 d than samples aged 45 d and 70 d, which were similar ($P > 0.05$). Ba et al. (2014) found an increase in Strecker aldehydes in beef as postmortem aging time increased and believed that the increase in concentration was due to the increase in free fatty acids released over time. Lipid oxidation and the release of free fatty acids that occurs during extended storage time can certainly contribute to undesirable flavors in beef. In the current study, samples aged 135 d had greater ($P < 0.05$) concentrations of carbon disulfide and methanethiol compared to samples aged 45 or 70 d, which had similar ($P > 0.05$) concentrations of those compounds. Braggins (1996) found that dimethyl disulfide and dimethyl trisulfide concentrations decreased as the pH of the meat increased; Vasta and Priolo (2006) stated that lower pH values allow for greater breakdown of cysteine, which creates more sulfur-containing volatiles in meat. Thus, lower pH from beef aged for an extended amount of time in the current study possibly created a significant increase in sulfur-containing volatile compounds.

Table 10. Least-squares mean of Maillard-reaction–derived volatile flavor compounds of cooked samples of Australian *longissimus lumborum* from three aging treatments ($n = 150$)

Volatile Compound (ng/g)	Aging, d			SEM[1]	P value
	45	70	135		
Ketones					
2,3-butanedione	193.93	137.29	168.14	32.90	0.23
3-hydroxy-2-butanone	288.11	214.92	278.93	37.79	0.32
Pyrazines					
Methyl-pyrazine	27.77	17.19	28.97	4.77	0.16
Trimethylpyrazine	2.58	2.34	2.88	0.39	0.57
2,5-dimethyl-pyrazine	46.86	28.92	40.99	7.50	0.22
2-ethyl-3,5-dimethyl pyrazine	14.14	9.71	15.88	2.59	0.20
Strecker Aldehydes					
Acetaldehyde	27.17	17.03	36.22	7.77	0.20
Benzaldehyde	207.42	147.96	260.76	40.86	0.14
Methional	12.27[y]	8.30[y]	17.01[x]	1.74	0.01
Phenylacetaldehyde	8.01[y]	8.54[y]	16.37[x]	1.86	0.01
2-methyl butanal	88.65	53.75	96.03	20.79	0.09
3-methyl butanal	61.43	37.78	66.56	15.88	0.14
Isobutyraldehyde	181.06	104.73	186.80	47.81	0.16
Sulfur Containing					
Carbon disulfide	11.96[y]	12.81[y]	29.61[x]	2.44	0.01
Dimethyl disulfide	1.06	0.52	1.05	0.26	0.26
Dimethyl sulfide	12.76	8.81	16.03	3.04	0.06
Methanethiol	31.98[y]	30.87[y]	55.49[x]	9.12	0.01
2-methyl thiophene	0.84	0.53	0.97	0.17	0.14

[1]SEM (largest) of the least-squares (LS) means.

[xyz]LS means within a row lacking a common superscript differ ($P < 0.05$).

Conclusions

The results of this study indicate that postmortem aging, diet, and diet × postmortem aging interactions impacted beef flavor. Extended postmortem wet aging has a detrimental effect on beef color and has the ability to increase negative descriptive sensory attributes while reducing palatability. Grass-fed samples tended to magnify this adverse effect on descriptive sensory attributes. Additionally, this study suggests that a certain amount of aging can be favorable to an eating experience, particularly with increased tenderness and juiciness. Volatile flavor compounds were more influenced by postmortem aging than diet. Increased postmortem aging time increased concentration for both lipid-oxidation–derived and Maillard-reaction–derived volatiles. The increased lipid-oxidation–derived volatile compounds is related to the amount of fatty acid degradation that occurs with increased storage time. Therefore, the undesirable flavor formation was related to the concentration of negative flavor volatile compounds instead of the absence of positive flavor compounds. Based on these results, differences existed in the flavor profile of *longissimus lumborum* from grass- and grain-fed beef, regardless of postmortem aging. However, aging beef strip loins 135 d is not recommended based on reduced beef flavor and increased off-flavor detection compared to samples aged 45 or 70 d postmortem, which is supported by increased concentrations of volatile compounds associated with off-flavors in samples aged 135 d.

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