Meat and Muscle Biology™

Determination of Package and Muscle-Type Influence on Proteolysis, Beef-Flavor-Contributing Free Amino Acids, Final Beef Flavor, and Tenderness

K. R. Vierck[1], J. F. Legako[1]*, J. K. Kim[2], B. J. Johnson[1], and J. C. Brooks[1]

[1]Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79409, USA
[2]Department of Animal and Food Sciences, Department of Human Nutrition, Michigan State University, East Lansing, MI, 48824
*Corresponding author. Email: jerrad.legako@ttu.edu (J. F. Legako)

Abstract: The objectives of this study were to determine the influence of package and muscle type on postmortem proteolysis and subsequent release of flavor-contributing free amino acids during storage. Beef strip loins and top sirloin butts ($n = 20$/subprimal) from USDA Low Choice carcasses were fabricated into 2.54-cm steaks (*M. longissimus lumborum* and *M. gluteus medius*) at 7 d postmortem. Steaks were randomly assigned to packaging treatments (carbon monoxide mother-bag [CO], high-oxygen modified atmosphere packaging [HIOX], polyvinyl overwrap [OW], or rollstock [ROLL]) and aged for an additional 14 d in dark storage. Steaks intended for OW were initially vacuum packaged during dark storage, then overwrapped just prior to display. Steaks were placed in coffin-style retail cases for 48 h under fluorescent lighting to simulate retail display. HIOX steaks exhibited the highest Warner-Bratzler shear force values ($P < 0.05$); the lowest desmin degradation rate ($P < 0.05$); the highest ratings for fishy, bitter, sour, and oxidized flavors; and the lowest overall tenderness scores ($P < 0.05$) and, in general, produced the lowest amount of free amino acids ($P < 0.05$) compared with all other treatments. Contrastingly, ROLL packaging produced the highest ratings for beef flavor identity, brown/roasted, bloody/serumy, and umami flavors ($P < 0.05$). Additionally, ROLL packaging exhibited ($P < 0.05$) greater desmin degradation in comparison with HIOX steaks. These data indicate that the optimum package for storage and aging is an anaerobic environment to maintain optimum flavor, tenderness, and postmortem proteolysis.

Key words: aging, beef, packaging type, postmortem proteolysis, tenderness, volatile flavor compounds
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Introduction

The packaging method of meat products is an important factor in the meat industry, as it serves to protect the product and improve shelf life and quality as well as factors into the consumer's purchasing decision (McMillin, 2017; Polkinghorne et al., 2018). Different packaging types can result in different eating experiences. Multiple consumer studies in both the United States and Australia have consistently shown beef packaged in high-oxygen (80% O_2) modified atmosphere packaging (HIOX) to be lower than both polyvinyl overwrap and vacuum packaging for tenderness, juiciness, flavor liking, and overall liking when fed to consumers (Polkinghorne et al., 2018; Ponce et al., 2019). Additionally, HIOX has been implicated with increased toughness when compared to vacuum-packaged or polyvinyl chloride overwrapped steaks (Geesink et al., 2015; Moczkowska et al., 2017). Currently, toughening of beef steaks after exposure to high-oxygen environments is not fully understood. Conflicting results are reported, as Geesink et al. (2015) did not observe any differences in desmin degradation in HIOX. However, Moczkowska et al. (2017) and Fu et al. (2017) both observed increased desmin degradation in vacuum packaging in comparison to HIOX. Increased desmin degradation would

indicate a greater amount of postmortem proteolysis occurring, therefore resulting in a more tender product. In addition to tenderness, one of the primary precursors to beef flavor development are free amino acids. Presently, it is unclear whether any alterations to protein degradation, as described earlier, would influence content of free amino acids and beef flavor.

From a flavor standpoint, HIOX has been shown to produce lower beef flavor identity and umami ratings, as well as increased oxidized, cardboardy, and sour flavors when analyzed by trained descriptive panels (Ponce et al., 2019). This is likely due to induced lipid oxidation from the HIOX oxidative environment, which contributes greatly to production of off-flavors (Min and Ahn, 2005; Bekhit et al., 2013).

Previous research indicates that packaging type has an impact on tenderization and flavor development of meat products. With regard to tenderization, Fu et al. (2017) reported increased desmin degradation in vacuum packaging in comparison to both polyvinyl overwrap and modified atmosphere packaging (MAP). Similarly, Moczkowska et al. (2017) reported that vacuum packaging had increased degradation of both desmin and troponin-T (TnT) in both the *M. longissimus lumborum* (LL) and *M. biceps femoris*. Moreover, Moczkowska et al. (2017) reported reduced Warner-Bratzler shear force (WBSF) values in vacuum-packaged steaks for both muscles, which indicates that an increased rate of proteolysis occurred in vacuum packages compared with MAP steaks. Additionally, MAP and polyvinyl overwrap produced a greater amount of carbonyl products from oxidation in both the *M. psoas major* and *M. semimembranosus* in comparison to vacuum packaging (Fu et al., 2017). The impact of many postmortem production practices on beef flavor is unknown, or the scope has not been fully investigated. Therefore, the objective of this study was to determine the influence of package and 2 diverging muscle types in oxidative stability on postmortem proteolysis and subsequent release of flavor-contributing free amino acids during storage and distribution and to determine their influence on final beef flavor and tenderness through volatile flavor compounds, descriptive sensory analysis, and WBSF.

Materials and Methods

Product selection and subprimal fabrication

Beef strip loins (Institutional Meat Purchase Specification #180; NAMP, 2010) and top sirloin butts (Institutional Meat Purchase Specification #184; NAMP, 2010) were selected from USDA Low Choice A maturity carcasses ($n = 20$) free of quality defects for this study. Trained Texas Tech University personnel collected data for yield and quality grading, including preliminary yield grade; ribeye area, kidney, pelvic, and heart fat; skeletal and lean maturity; and marbling score. Subprimals were collected in 3 separate collections 48 h postmortem. Collections were performed within the same week, with 2 collections for 7 subprimals and the final collection for 6 subprimals. Following each collection, subprimals were transported under refrigeration (0°C–4°C) to the Gordon W. Davis Meat Laboratory in Lubbock, Texas. Subprimals were wet aged in the dark for 7 d postmortem, then fabricated into representative steaks of the *M. gluteus medius* (GM) and the LL. A steak from the most anterior portion of each subprimal was saved (not subjected to retail display) and immediately frozen at −20°C at 7 d postmortem to be used as a negative control for raw steak analyses. Steaks were then randomly assigned into one of 4 packaging schemes: carbon monoxide motherbag (CO; 0.4% CO/30% CO_2/69.6% N_2; Certified Molar/Volume Concentrations 4,080 ppm CO/30.3% CO_2/Balance N_2; Praxair, Lubbock, TX), HIOX lidded trays (80% O_2/20% CO_2; Certified Volume Concentrations 19.9% CO_2/Balance O_2; Praxair, Lubbock, TX), polyvinyl overwrap (OW), and rollstock (ROLL; forming and nonforming films [T6035B and T6235B, Sealed Air, Cryovac, Charlotte, NC]). Steaks designated for CO were placed on black expanded polystyrene trays overwrapped with polyvinyl chloride film and placed into a high-barrier master bag (Product No. PM9120B; Sealed Air, Charlotte, NC) flushed with a mixture of 0.4% CO, 30% CO_2, and 69.6% N_2 gas using a CVP A600 FRESH VAC (CVP Systems, Downers Grove, IL) packaging machine. HIOX packages were created using a Mondini Tray Sealer, CV/VG-S (Cologne, Italy). The trays used for MAP packages had an oxygen transmission rate (OTR) of 0.1 cc/d at 73°C at 0% relative humidity (RH) and a moisture vapor transmission rate (MVTR) of 2 g/d. The tray film used for the MAP packages had an OTR of 7 cc/m^2/d at 40°C at 0% RH and an MVTR of 9 g/m^2/d at 38°C at 100% RH. Steaks placed in ROLL packaging were produced using a Multivac Baseline F100 (Kansas City, MO) using a forming film (OTR: 2 cc/m^2/d at 23°C at 0% RH; MVTR of 7 g/m^2/d at 38°C at 100% RH) and nonforming film (OTR of 3 cc/m^2/d at 23°C at 0% RH, and a MVTR of 9 g/m^2/d at 38°C at 100% RH). A Minipack-torre, Minispenser (Dalmine, Italy) was used to produce the OW packaging. Prior to retail display, OW steaks were stored in

ROLL packaging. Steaks were held in their respective packaging type for an additional 14 d of aging in the absence of light. Following the aging period, steaks were subjected to a 48-h retail display under continuous fluorescent lighting in 3 coffin-style cases with a temperature range of 2°C–4°C. Packages were rotated every 12 h with light intensity measurements taken concurrently. Immediately following retail display, steaks were removed from their respective packaging and vacuum packaged, then frozen at −20°C until further analysis.

Trained descriptive panel analysis

Trained descriptive panel analysis was conducted according to the American Meat Science Association Sensory Guidelines (AMSA, 2015). Seven panelists were trained according to the American Meat Science Association Sensory Guidelines for 13 traits: beef flavor identity, brown/roasted, bloody/serumy, fat-like, liver-like, oxidized, fishy, buttery, umami, bitter, sour, overall juiciness, and overall tenderness, described in Table 1. Panelists evaluated 2 cubed samples on continuous 100-point line scales using digital surveys on tablets (Qualtrics Surveys, Provo, UT; iPad, Apple, Inc., Cupertino, CA). Each scale was anchored at each endpoint and had a neutral midpoint (0 = extremely bland/dry/tough; 50 = neither tough/dry nor tender/juicy; 100 = extremely tender/juicy/intense). Panels consisted one steak of each treatment ($n = 8$) in a randomly assigned order. Prior to panel analysis, steaks were thawed for 24 h at 2°C–4°C. Using clamshell grills (Cuisinart Griddler Deluxe GR-250, Cuisinart, Stamford, CT), steaks were cooked to a medium degree of doneness (71°C). After cooking, steaks were cut into cubes 1 × 1 cm thick, and 2 cubes were served to each panelist.

Western blot analysis

Western blot analysis was conducted using the methods of Knobel (2014) and Phelps et al. (2015). Samples for both western blot and free amino acid analysis were prepared for analysis through liquid nitrogen homogenization. Accessory muscles, external fat, and connective tissue were removed, and then steaks were diced. The cubes were placed into liquid nitrogen and frozen, then homogenized using a food processor (Robot Coupe Blixer 3, Robot Coupe USA, Jackson, MS). Following homogenization, samples were stored at −80°C for approximately 1 mo until further analysis.

Table 1. Descriptive attributes and references

Flavor Attribute	Anchor	Location on Scale (0–100)
Beef Flavor Identity	Beef broth (heated to 74°C, served warm)	30
	80% ground chuck (71°C)	50
	Brisket (71°C)	75
Bloody/ Serumy	USDA Choice strip steak (60°C)	40
Brown/ Roasted	80% ground chuck (71°C)	40
	Well-done strip steak (77°C)	65
Fat-Like	90/10 ground beef (71°C)	30
	70/30 ground beef (71°C)	60
Liver-Like	Flat iron steak (71°C)	20
	Calf liver	90
Oxidized	Microwaved vegetable oil	30
	Cooked, stored (12 h at 4°C) and microwaved ground beef (71°C)	60
Buttery	Unsalted butter, 0.1-cm-thick slice	65
Fishy	Cod liver oil	30
	Canned tuna	60
Umami	Beef broth, sodium free (heated to 74°C, served warm)	30
Sour	0.015% citric acid	10
	0.050% citric acid	25
Salty	0.15% NaCl	10
	0.25% NaCl	45
Bitter	0.01% caffeine	15
	0.02% caffeine	25
Overall Tenderness	Eye of round (77°C)	30
	Strip steak (71°C)	55
	Tenderloin (65°C)	90
Overall Juiciness	Strip steak (85°C)	25
	Strip steak (71°C)	50
	Strip steak (60°C)	75

Desmin and TnT were the proteins of interest. Proteins were isolated from muscles using whole muscle extraction buffer (2% sodium dodecyl sulfate, 10 mM phosphate, pH 7.0). Following the addition of the buffer, samples were mixed on a vortex mixer at 2,000 RPM for 2 min, then centrifuged for 15 min at 15,000 × g. Protein concentration was determined using the Pierce BCA protein assay (Thermo Fisher Scientific, Fairlawn, NJ). To confirm protein concentration, a NanoDrop 1000 spectrophotometer was used to analyze protein concentration at 562 nm. Following concentration analysis, all samples were diluted to a similar concentration using phosphate buffered saline, and Modified Wang's tracking solution was added with β-mercaptoethanol and then incubated for 10 min at 100°C. Proteins were loaded on to a Novex 4 to 12%

Bis Tris Gel (Invitrogen, Grand Island, NY) and were separated via electrophoresis. Gels ran for 35 min at 165 V and 30 mA. Following running, proteins were transferred to nitrocellulose membranes for 7 min. Membranes were then incubated with nonfat dry milk (Bio-Rad, Hercules, CA) and 1 × tris buffered saline (TBS) for 1 h at 25°C to block for nonspecific antibody binding. Primary antibody solution consisting of antibodies for desmin (1:10,000 dilution, ab6322, Anti-desmin cytoskeleton marker, Abcam, Cambridge, UK) and TnT (1: 10,000 dilution, ab83907, Anti-Troponin/TNT antibody, Abcam); 1 × TBS-1% Tween was then added, and samples were incubated and gently rocked overnight at 4°C. Membranes were then rinsed with 1 × TBS-1% Tween solution 3 times each for 5 min, and then secondary antibodies were added to the membranes and allowed to incubate in the absence of light on a rocker for 1 h at 25°C. Secondary antibody solution consisted of 1 × TBS-1% Tween solution and 1:2 dilution of antibodies (desmin: A21126, AlexaFluor 633 Goat Anti-Mouse; TnT: A21070, AlexaFluor 633 Goat Anti-Rabbit; Thermo Fisher Scientific). Following secondary antibody incubation, membranes were again rinsed 3 times for 5 min with 1 × TBS-1% Tween solution. Following incubation, membranes were dried and imaged using a VersaDoc Imaging System (Bio-Rad); intact and degraded bands were detected and measured using the Quantity One Band Analysis software (Bio-Rad). Degraded and intact forms of desmin were measured with bands located at approximately 55 kDA, and TnT was measured at approximately 30 kDA. Band intensity was equalized to a pooled sample on each blot. Average intensity of each band was measured in relation to the internal standard (pooled composite from each sample of the gel) and reported as measurements of relative degradation.

Free amino acid analysis

Free amino acid analysis was conducted using the modified methods of Koutsidis et al. (2008). For analysis, 3 g of sample was weighed into a 50-mL conical tube. Ten mL. of autoclaved, cold, double-distilled water was added to each sample, then shaken for 10 min. Following shaking, samples were centrifuged at 29,900 × *g* for 33 min. All supernatant was decanted, and then an additional 5 mL of water was added. Samples were re-extracted as described previously, and then the 2 extracts were combined together. The combined supernatant was filtered through a 0.2-µm disc filter. Free amino acids ($n = 23$) were derivatized using 100 µL of the aqueous extract from the combined supernatant and an EZ-Faast amino acids kit (Phenomenex, Torrance, CA). Free amino acid content was determined using a gas chromatography-mass spectrometer in electron impact mode with a 3:1 split ratio (6890A; 5975B, Agilent, Santa, Clara, CA). Derivatives were separated using a Zebron ZB-AAA capillary column (10 m × 0.25 mm; 0.25-µm film thickness, Phenomenex). A 3-level calibration curve based on response and concentration ratios between an internal standard (norvaline) and authentic standards for each amino acid was used for quantitation (millimoles per kilogram of initial wet sample).

WBSF

WBSF was conducted according to the Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat (AMSA, 2015). Steaks were cooked as previously described for both trained panel and volatile compound analysis. Following the removal of volatile compound analysis cores, steaks were chilled for 12 h at 2°C–4°C. Six 1.27-cm cores were removed parallel to the muscle fiber orientation. Cores were then sheared perpendicular to the muscle fiber using a WBSF instrument equipped with a v-shaped blade with a 200 mm/min crosshead speed (GR-151, Tall Grass Solutions, Manhattan, KS). Measurements were recorded as peak force (kilograms) and averaged across the 6 cores for each steak.

Volatile compound analysis

The methods of Gardner and Legako (2018) were used to determine volatile compound composition of steaks. Steaks designated for volatile compound analysis were prepared as previously described for trained descriptive panel analysis. Immediately following cooking, six 1.27-cm cores were removed from the center of the steak perpendicular to the steak cut surface. The cores were then minced for 10 s using a coffee grinder (4- to 12-cup Mr. Coffee grinder; Sunbeam Corporation, Boca Raton, FL). Five grams of sample was weighed into 20-mL glass vials (Gerstel Inc, Linthicum, MD). Ten microliters of internal standard (1,2-dichlorobenzene, 2.5 mg/µL) was pipetted into the vial and then sealed using a polytetrafluoroethylene septa screw cap (#093640-040-00, 1.3 mm polytetrafluoroethylene septa and metal screw cap; Gerstel Inc., Linthicum, MD). The samples were then loaded using a Gerstel automatic sampler (MPS, Gerstel, Inc.) for a 5-min incubation time at 65°C in the Gerstel

agitator prior to a 25-min extraction time. Solid phase microextraction was used to collect the volatile compounds from the headspace of the sample with an 85-µm film thickness carboxen polydimethylsiloxane fiber (Supelco Inc., Bellefonte, PA). Volatile compounds extracted from the headspace were placed onto a VF-5 MS capillary column (30 m × 0.25 mm × 1.0 µm; Agilent J&W GC Column; Agilent Technologies, Inc., Santa Clara, CA). Authentic standards (Sigma-Aldrich, St. Louis, MO) were used to confirm compound identities through retention time. Furthermore, authentic standards were utilized to quantitate individual volatile compounds relative to sample weight (nanograms per gram of cooked samples).

Statistical analysis

Data were analyzed as a 2×4 factorial design with muscle, packaging type, and their interaction serving as fixed effects. Individual package served as the experimental unit. Collection and carcass ID were incorporated into the model as a random effect for all analyses. For cooked analyses, peak temperature was included as a covariate. Probability values (P values) less than or equal to $\alpha = 0.05$ were considered significant. The Kenward-Rogers adjustment was also used to estimate denominator degrees of freedom.

Results and Discussion

Carcass characteristics

Carcass characteristics are presented in Table 2. Carcasses used in this study were from the USDA Choice quality grade (Small^{00}–Small^{100} marbling score) and A maturity. Additionally, carcasses possessed approximately 1.0 cm preliminary and 1.2 cm adjusted fat thickness with 100.6 cm^2 ribeye area. Moreover, carcasses also possessed approximately 3.5% kidney, pelvic, and heart fat, with an average final yield grade of 2.9.

Trained descriptive panels

No interactions ($P \geq 0.233$) between packaging scheme and muscle type were observed for trained panel attributes (Table 3). Additionally, no differences ($P \geq 0.056$) in any sensory traits were observed between the GM and the LL. However, packaging type had a substantial impact on flavor and tenderness when evaluated by the trained panel. No differences were observed ($P \geq 0.357$) between packaging schemes for fat-like, liver-like, buttery, or salty flavors. When evaluating positive flavor traits, steaks in OW and ROLL packaging produced greater beef flavor identity ($P < 0.05$) in comparison with CO and HIOX packaging. Moreover, HIOX steaks were the lowest for beef flavor identity ($P < 0.05$) compared with all other treatments. OW and ROLL steaks produced more brown roasted flavors ($P < 0.05$) in comparison to HIOX steaks; however, CO steaks were similar to both treatment groups ($P > 0.05$). For bloody/serumy, HIOX steaks produced ($P < 0.05$) the least bloody/serumy flavor compared with all other treatments. However, ROLL steaks produced ($P < 0.05$) the highest ratings for bloody/serumy compared with OW steaks but were similar ($P > 0.05$) to CO steaks. When evaluating negative flavor traits, HIOX packaging produced the most intense oxidized and fishy flavors ($P < 0.05$) in comparison with all other treatments, followed by CO packaging ($P < 0.05$). For the basic tastes, ROLL and OW produced the most intense umami flavor ($P < 0.05$) compared with all other treatments. For bitter, HIOX steaks were most intense ($P < 0.05$) compared with all other treatments, followed by CO and ROLL packaging ($P < 0.05$), with OW steaks producing the least intense bitter flavor ($P < 0.05$). Additionally, HIOX packaging produced the most intense sour flavor ($P < 0.05$) compared with all other treatments. Furthermore, CO packaging produced juicier steaks ($P < 0.05$) than HIOX steaks, but ROLL and OW were similar to both CO and HIOX ($P > 0.05$). HIOX also produced the least tender steaks ($P < 0.05$) in comparison with all other treatments.

Table 2. Least-squares means (±SEM[1]) of beef carcass ($n = 20$) measurements

Carcass Characteristics	
Quality Attributes	
Lean maturity[2]	139 ± 21
Skeletal maturity[2]	124 ± 28
Overall maturity[2]	130 ± 21
Marbling score[3]	443 ± 24
Yield Attributes	
Preliminary fat thickness, cm	1.0 ± 0.4
Adjusted fat thickness, cm	1.2 ± 0.4
Ribeye area, cm^2	100.6 ± 10.6
Hot carcass weight, kg	412.4 ± 39.4
Kidney, pelvic, and heart fat, %	3.5 ± 0.5
Final yield grade	2.9 ± 0.4

[1]Standard error of the mean.

[2]100 = A; 200 = B.

[3]200 = Traces; 300 = Slight; 400 = Small.

Table 3. Least-squares means of trained descriptive panel evaluation[1] of beef steaks ($n = 160$) from two different muscles[2] and four different packaging schemes

	Beef Flavor Identity	Brown/ Roasted	Bloody/ Serumy	Fat-Like	Liver-Like	Oxidized	Fishy	Buttery	Umami	Salty	Bitter	Sour	Overall Juiciness	Overall Tenderness
Packaging Type														
Carbon monoxide[3]	34.9^{b}	31.2^{a}	14.4^{ab}	14.2	8.2	29.3^{b}	23.6^{b}	13.5	22.6^{b}	7.1	10.4^{b}	11.7^{b}	47.1^{a}	52.3^{a}
High oxygen[4]	28.6^{c}	28.1^{b}	10.5^{c}	13.6	8.4	43.0^{a}	35.4^{a}	12.7	19.5^{c}	6.4	12.8^{a}	14.5^{a}	42.6^{b}	47.9^{b}
PVC overwrap[5]	39.3^{a}	33.8^{a}	13.6^{b}	13.5	8.6	23.8^{c}	17.9^{c}	13.0	25.2^{a}	6.8	8.8^{c}	10.1^{b}	44.6^{ab}	52.3^{a}
Rollstock[6]	41.7^{a}	32.6^{a}	17.3^{a}	15.1	8.4	18.8^{d}	14.3^{c}	14.9	26.2^{a}	7.3	10.2^{b}	11.1^{b}	44.7^{ab}	53.8^{a}
SEM[7]	1.5	1.5	1.4	0.9	0.9	2.0	2.2	1.3	0.9	0.4	0.7	0.9	1.3	1.6
P value	< 0.001	0.003	< 0.001	0.390	0.971	< 0.001	< 0.001	0.497	< 0.001	0.357	< 0.001	< 0.001	0.048	0.011
Muscle														
GM	35.5	31.1	13.8	14.0	8.7	30.2	24.0	13.8	22.8	6.6	11.0	12.5	44.8	51.7
LL	36.7	31.8	14.0	14.2	8.1	27.2	21.5	13.3	23.9	7.2	10.1	11.2	44.7	51.3
SEM	1.3	1.3	1.2	0.8	0.8	1.7	1.7	1.1	0.8	0.3	0.6	0.8	1.0	1.3
P value	0.250	0.536	0.834	0.787	0.389	0.058	0.174	0.639	0.143	0.063	0.065	0.056	0.995	0.747
Packaging × Muscle														
P value	0.812	0.103	0.901	0.551	0.564	0.310	0.920	0.546	0.606	0.619	0.361	0.368	0.233	0.918

[1]Sensory scores: 0 = absence of specific flavor/extremely tough/dry; 50 = neither tough nor tender/neither dry nor juicy; 100 = extremely intense specific flavor/extremely tender/juicy.

[2]Muscles included the *gluteus medius* (GM) and *longissimus lumborum* (LL).

[3]Carbon monoxide motherbag (0.4% CO/30% CO_2/69.6% N_2).

[4]High-oxygen modified atmosphere packaging (80% O_2/20% CO_2).

[5]Polyvinyl chloride (PVC) overwrap; prior to retail display, polyvinyl overwrap (OW) steaks were stored in rollstock (ROLL) packaging.

[6]Rollstock.

[7]SE (largest) of the least-squares means in the same main effect (packaging type or muscle).

[a–c]Least-squares means in the same main effect (packaging type or muscle) without a common superscript differ ($P < 0.05$).

From a flavor standpoint, HIOX has been shown to produce lower beef flavor identity, umami, and tenderness ratings, as well as increased oxidized, cardboardy, and sour flavors when analyzed by trained descriptive panels (Ponce et al., 2019). This is likely due to induced lipid oxidation from the oxidative environment of HIOX, which contributes greatly to production of off-flavors (Min and Ahn, 2005; Bekhit et al., 2013). Additionally, this is likely due to the increased lipid oxidation products observed in the volatile compound analysis.

ROLL and OW packages produced a greater umami flavor compared with all other treatments. This is likely due to the increased concentration of aspartic and glutamic acid (Tables 4–5), 2 free amino acids linked to increased umami flavors (Dashdorj et al., 2015).

Western blot analysis

Results from western blot analysis are presented in Table 6. No differences were observed in TnT degradation for packaging type ($P = 0.442$), muscle ($P = 0.074$), or their interaction ($P = 0.093$). However, desmin was readily impacted by both packaging type ($P < 0.001$) and muscle ($P < 0.001$), with no interactive effects ($P = 0.263$). Initial samples pulled 7 d postmortem, and HIOX samples possessed ($P < 0.05$) the greatest relative intensity of degraded desmin compared with all other packaging types, which indicates a higher concentration of desmin and less degradation during postmortem proteolysis. Additionally, GM samples exhibited ($P < 0.05$) greater relative intensity of degraded desmin compared with LL samples, which indicates that LL steaks had a greater amount of desmin degradation.

These results are partially in agreement with previous work. Moczkowska et al. (2017) and Fu et al. (2017) both reported increased desmin degradation in LL steaks stored in vacuum packaging compared with those stored in HIOX, as observed in the current study. However, in the current study, TnT degradation was similar across treatments, whereas Moczkowska et al. (2017) observed increased TnT degradation in

Table 4. Interaction of packaging type and muscle on free amino acid content of beef steaks ($n = 160$) from two different muscles and four different packaging schemes

Free Amino Aacid, mmol/kg	Aspartic Acid	Cysteine	Orthinine
Gluteus Medius			
No packaging[1]	0.019^{d}	0.389^{c}	0.068^{e}
Carbon monoxide[2]	0.048^{bcd}	0.186^{d}	0.101^{bcd}
High oxygen[3]	0.027^{cd}	0.130^{d}	0.085^{cde}
Overwrap[4]	0.051^{bc}	0.542^{bc}	0.106^{bc}
Rollstock[5]	0.043^{bcd}	0.682^{b}	0.117^{b}
Longissimus Lumborum			
No packaging	0.022^{cd}	0.459^{c}	0.077^{e}
Carbon monoxide	0.066^{b}	0.420^{c}	0.087^{cde}
High oxygen	0.032^{cd}	0.158^{d}	0.077^{de}
Overwrap	0.099^{a}	0.942^{a}	0.112^{bc}
Rollstock	0.112^{a}	1.110^{a}	0.171^{a}
SEM[6]	0.011	0.081	0.012
P value	0.011	0.004	0.009

[1]Initial sample taken at the beginning of the aging period; no packaging treatment or aging applied.

[2]Carbon monoxide motherbag (0.4% CO/30% CO_2/69.6% N_2).

[3]High-oxygen modified atmosphere packaging (80% O_2/20% CO_2).

[4]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[5]Rollstock.

[6]SE (largest) of the least-squares means in the same main effect (packaging type or muscle).

[a–e]Least-squares means in the same main effect (packaging type or muscle) without a common superscript differ ($P < 0.05$).

Table 5. Least-squares means of free amino acids from beef steaks ($n = 160$) from two different muscles

	Muscle Type			
	Gluteus medius	*Longissimus lumborum*	SEM[6]	*P* value
Free amino acid, mmol/kg				
Alanine	4.825^{b}	5.150^{a}	0.263	<0.001
Cystine	4.825^{b}	5.150^{a}	0.265	0.008
Glutamine	0.014^{b}	0.017^{a}	0.003	0.001
Glycine	1.387^{b}	1.498^{a}	0.084	<0.001
Proline	0.408^{b}	0.446^{a}	0.022	<0.001
Tyrosine	0.431^{b}	0.474^{a}	0.039	0.050
Valine	1.410^{b}	1.564^{a}	0.071	<0.001

[1]SE (largest) of the least-squares means in the same main effect (muscle type).

[a,b]Least-squares means in the same main effect (packaging type) without a common superscript differ ($P < 0.05$).

vacuum-packaged LL steaks compared with those stored in HIOX. Additional research with oxidative environments has also indicated similar rates of TnT degradation throughout packaging types (Kim et al., 2010; Xue et al., 2012). However, both of these studies have reduced aging periods (14 d and 9 d) compared with the current study (21 d).

Table 6. Least-squares means of relative intensity of degraded desmin and troponin-T from beef steaks ($n = 160$) from two muscles and four packaging schemes

	Desmin	Troponin-T
Treatment		
Packaging Type		
No packaging[1]	1.28^{a}	0.99
Carbon monoxide[2]	0.97^{b}	0.95
High oxygen[3]	1.03^{a}	0.98
Overwrap[4]	0.98^{b}	0.95
Rollstock[5]	0.97^{b}	0.98
SEM[6]	0.06	0.03
P value	< 0.001	0.442
Muscle		
Gluteus medius	1.09^{a}	0.98
Longissimus lumborum	1.00^{b}	0.96
SEM	0.05	0.01
P value	< 0.001	0.074
Packaging × Muscle		
P value	0.263	0.093

[1]Initial sample taken at the beginning of the aging period; no packaging treatment or aging applied.

[2]Carbon monoxide motherbag (0.4% CO/30% CO_2/69.6% N_2).

[3]High-oxygen modified atmosphere packaging (80% O_2/20% CO_2).

[4]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[5]Rollstock.

[6]SE (largest) of the least-squares means in the same main effect (packaging type or muscle).

[a,b]Least-squares means in the same main effect (packaging type or muscle) without a common superscript differ ($P < 0.05$).

Free amino acid analysis

Only one amino acid, beta-alanine, ($P \geq 0.629$) was not significant for muscle, packaging type, or their interaction. Three amino acids—aspartic acid, cysteine, and ornithine—were impacted by the interaction of packaging type and muscle ($P < 0.011$; Table 4). For aspartic acid and cysteine, LL ROLL and OW steaks produced ($P < 0.05$) the greatest amount of each respective amino acid. Aspartic acid and cysteine contribute umami, savory, meat-like, and sulfurous flavors to meat products (Dashdorj et al., 2015). These flavors were present in increased intensity in ROLL and OW steaks, according to the trained panelists in the current study (Table 3). The increased concentration of aspartic acid and cysteine as free amino acids in LL ROLL and OW steaks indicate an increased reservoir of positive

amino acids to contribute to beefy, savory flavors in steaks through the Maillard reaction. In comparison, LL and GM HIOX steaks produced ($P < 0.05$) the lowest concentration of aspartic acid and cysteine, as LL ROLL steaks exhibited almost 7 times more cysteine than LL HIOX steaks. Similarly, GM ROLL steaks possessed 5.25 times more cysteine than GM HIOX steaks. For ornithine, LL ROLL steaks possessed ($P < 0.05$) the most ornithine compared with all other treatments, possessing 1.5 times more ornithine than GM ROLL steaks.

Six amino acids (alanine, cystine, glycine, proline, tyrosine, and valine) were impacted ($P \leq 0.01$) by both the main effects of packaging (Table 7) and muscle (Table 5). For all 6 amino acids, LL steaks possessed a greater ($P < 0.05$) concentration of each respective amino acid in comparison with GM steaks. Additionally, initial, day-0, unaged samples possessed the lowest ($P < 0.05$) concentration of all free amino acids, as they were not able to be freed through the postmortem aging process. Valine and glycine were present in the greatest ($P < 0.05$) concentration in OW and ROLL steaks, followed by CO, which was greater than HIOX ($P < 0.05$). HIOX steaks only contained greater ($P < 0.05$) concentrations of valine and glycine than the initial unaged subprimal samples. Similarly, ROLL and OW steaks possessed ($P < 0.05$) a greater concentration of proline than HIOX steaks; however, CO steaks were intermediate and similar ($P > 0.05$) to both treatment groups in proline concentration. Cystine and alanine were present ($P < 0.05$) in greater concentrations in OW steaks compared with HIOX steaks. Both ROLL and CO steaks were intermediate ($P > 0.05$) and similar to both treatments for cystine and alanine concentration. Cystine is known for contributing meat-like, sweet, and sulfurous flavor to meat products due to its sulfurous side chain (Dashdorj et al., 2015). Alanine also contributes both sweet and sour flavors to meat products (Dashdorj et al., 2015). These free amino acids may have contributed to

Table 7. Least-squares means of free amino acid content of beef steaks ($n = 160$) from four different packaging schemes

	Packaging Type						
	No Packaging[1]	Carbon Monoxide[2]	High Oxygen[3]	Overwrap[4]	Rollstock[5]	SEM[6]	*P* Value
Free Amino Acid, mmol/kg							
Alanine	3.797^{c}	5.240^{ab}	5.058^{b}	5.510^{a}	5.332^{ab}	0.284	0.008
Asparagine	0.167^{d}	0.298^{b}	0.257^{c}	0.360^{a}	0.342^{a}	0.014	< 0.001
Cystine	3.797^{c}	5.240^{ab}	5.058^{b}	5.511^{a}	5.332^{ab}	0.284	< 0.001
Glycine	1.094^{d}	1.478^{b}	1.356^{c}	1.673^{a}	1.610^{a}	0.090	0.003
Glutamic acid	0.720^{c}	1.520^{b}	1.293^{b}	1.781^{a}	1.863^{a}	0.091	< 0.001
Histidine	4.791^{b}	5.232^{b}	6.664^{a}	5.628^{ab}	5.395^{b}	0.431	0.036
Hydroxyproline	0.027^{c}	0.042^{ab}	0.038^{b}	0.047^{a}	0.041^{ab}	0.004	< 0.001
Isoleucine	0.428^{d}	0.928^{b}	0.792^{c}	1.047^{a}	1.055^{a}	0.471	< 0.001
Leucine	0.662^{c}	1.490^{b}	1.336^{b}	1.662^{a}	1.664^{a}	0.081	< 0.001
Lysine	0.309^{c}	0.650^{b}	0.650^{b}	0.762^{a}	0.708^{ab}	0.038	< 0.001
Methionine	0.128^{d}	0.362^{b}	0.309^{c}	0.430^{a}	0.420^{a}	0.020	< 0.001
Phenylalanine	0.255^{c}	0.626^{b}	0.562^{b}	0.700^{a}	0.704^{a}	0.035	< 0.001
Proline	0.332^{c}	0.437^{ab}	0.427^{b}	0.470^{a}	0.470^{a}	0.025	0.006
Serine	0.579^{b}	1.255^{a}	1.182^{a}	1.374^{a}	1.281^{a}	0.095	< 0.001
Threonine	0.394^{c}	0.731^{ab}	0.633^{b}	0.808^{a}	0.831^{a}	0.056	< 0.001
Tryptophan	0.028^{c}	0.060^{ab}	0.055^{b}	0.067^{a}	0.057^{ab}	0.005	< 0.001
Tyrosine	0.245^{c}	0.508^{ab}	0.533^{a}	0.514^{ab}	0.461^{b}	0.043	< 0.001
Valine	0.786^{d}	1.613^{b}	1.412^{c}	1.809^{a}	1.816^{a}	0.088	0.010
Total free amino acids	15.227^{c}	22.735^{b}	22.773^{b}	25.334^{a}	24.987^{ab}	1.043	< 0.001

[1]Initial sample taken at the beginning of the aging period; no packaging treatment or aging applied.

[2]Carbon monoxide motherbag (0.4% CO/30% CO_2/69.6% N_2).

[3]High-oxygen modified atmosphere packaging (80% O_2/20% CO_2).

[4]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[5]Rollstock.

[6]SE (largest) of the least-squares means in the same main effect (packaging type).

[a–d]Least-squares means in the same main effect (packaging type) without a common superscript differ ($P < 0.05$).

the increase in beef flavor identity ratings for OW steaks compared with HIOX steaks when fed to trained panelists in the current study. Previously, no work has evaluated the impact of packaging types on flavor precursors, such as free amino acids.

Contrastingly, tyrosine was present ($P < 0.05$) in the highest concentration in HIOX steaks compared with ROLL steaks. The increase in tyrosine in HIOX steaks likely influenced the bitterness ratings observed by trained panelists, as tyrosine is a water-soluble taste-active compound that contributes to bitter flavors (Dashdorj et al., 2015). Additionally, proteins with large amounts of tyrosine residues are more susceptible to oxidation via singlet oxygen (Papuc et al., 2017). Moreover, tyrosine also has a hydroxyl group present on the aromatic ring of its side chain which renders it especially labile to oxidation (Papuc et al., 2017). By forcing tyrosine's abstraction from peptide chains through protein oxidation, it would be present in greater amounts in HIOX environments compared with the anaerobic ROLL environment.

The majority of free amino acids ($n = 12$; Table 7) were impacted solely by the packaging main effect ($P < 0.04$). Initial samples from the beginning of the aging period exhibited the lowest concentration of amino acids compared with all other treatments, with the exception of histidine ($P < 0.05$). With the exception of histidine, ROLL and OW steaks possessed ($P < 0.05$) the greatest concentration of the remaining free amino acids, followed by CO steaks ($P < 0.05$) and then HIOX steaks ($P < 0.05$). Histidine was present ($P < 0.05$) in greater concentrations in HIOX steaks in comparison with ROLL, CO, and initial steaks. Overwrap steaks were similar to all other treatments ($P > 0.05$). Proteins with amino acid residues with high electron density, such as histidine or tyrosine, are very labile to oxidation by singlet oxygen (Papuc et al., 2017). Examples of these proteins would be myoglobin, which uses histidine to play key structural roles in maintaining myoglobin structure and function (Lee et al., 2003; Mancini and Hunt, 2005). In an oxidative environment, it could contribute to increased release of histidine from HIOX steaks. This increase in histidine concentration, similar to tyrosine, likely contributed to the increased bitter intensity of HIOX steaks reported by the trained panelists in the current study, as it has been linked to bitter flavors (Dashdorj et al., 2015).

Glutamine was the lone amino acid impacted solely by a muscle main effect ($P = 0.001$; Table 5). Similar to other amino acids, LL steaks possessed ($P < 0.05$) a greater concentration of glutamine compared with GM steaks. Glutamine has been observed to be a precursor to α-ketoglutarate, an important component to the Krebs cycle (Tapiero et al., 2002). The LL has consistently been rated higher by trained panelists than the GM for beef flavor, and it is likely that glutamine's contribution to those beefy flavors has aided that advantage (Calkins and Hodgen, 2007).

WBSF

No interactions or muscle effects were observed ($P > 0.05$) for WBSF (Table 8). However, HIOX packaging produced the greatest ($P < 0.05$) WBSF values compared with all other treatments, with OW producing the lowest WBSF values ($P < 0.05$). These packaging results are in agreement with the previous literature. Moczkowska et al. (2017), Zakrys-Waliwander et al. (2012), and Lagerstedt et al. (2011) observed substantial differentiation between HIOX and vacuum-packaged LL steaks, as HIOX steaks were substantially higher for WBSF. Additionally, Kim et al. (2010) observed increased star probe values (another instrumental determination for tenderness) for HIOX steaks

Table 8. Least-squares means of Warner-Bratzler shear force values of beef steaks ($n = 160$) from two muscles and four packaging schemes

	Warner-Bratzler shear force, kgf
Packaging Type	
Carbon monoxide[1]	2.5[b]
High oxygen[2]	3.1[a]
Overwrap[3]	2.2[c]
Rollstock[4]	2.4[bc]
SEM[5]	0.1
P value	< 0.001
Muscle	
Gluteus medius	2.5
Longissimus lumborum	2.6
SEM	0.1
P value	0.355
Packaging Type × Muscle	
P value	0.275

[1]Carbon monoxide motherbag (0.4% CO/30% CO_2/69.6% N_2).

[2]High-oxygen modified atmosphere packaging (80% O_2/20% CO_2).

[3]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[4]Rollstock.

[5]SE (largest) of the least-squares means in the same main effect (packaging type or muscle).

[a–c]Least-squares means in the same main effect (packaging type or muscle) without a common superscript differ ($P < 0.05$).

Table 9. Interaction of packaging type and muscle on the production of volatile flavor compounds from beef steaks ($n = 160$)

Volatile Compound, ng/g	Benzaldehyde	2,3-Butanediol	Hexanal	Hexanoic Acid	2-Pentylfuran	Nonanal	Ethanol
Gluteus Medius							
Carbon monoxide[1]	15.54[b]	189.06[a]	76.87[b]	119.59[bc]	4.72[b]	2.37[b]	90.46[a]
High oxygen[2]	29.74[a]	62.59[b]	667.76[a]	479.12[a]	31.94[a]	8.18[a]	19.65[b]
Overwrap[3]	14.64[b]	119.43[b]	156.46[b]	157.73[bc]	4.03[b]	3.28[b]	19.82[b]
Rollstock[4]	14.06[b]	89.79[b]	109.39[b]	116.66[bc]	5.18[b]	2.18[b]	21.10[b]
Longissimus Lumborum							
Carbon monoxide[1]	12.35[b]	77.49[b]	86.98[b]	113.79[bc]	4.99[b]	2.14[b]	18.29[b]
High oxygen[2]	16.19[b]	76.43[b]	254.40[b]	220.08[a]	7.11[b]	3.75[b]	10.69[b]
Overwrap[3]	14.41[b]	77.40[b]	136.20[b]	99.14[c]	7.27[b]	2.95[b]	26.64[b]
Rollstock[4]	13.33[b]	81.74[b]	118.23[b]	108.12[bc]	5.19[b]	1.85[b]	32.67[b]
SEM[5]	2.61	28.83	74.00	42.02	4.19	0.84	13.68
P value	0.034	0.019	0.008	0.003	0.002	0.020	0.005

[1]Carbon monoxide motherbag (0.4% CO/30% CO_2/69.6% N_2).

[2]High-oxygen modified atmosphere packaging (80% O_2/20% CO_2).

[3]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[4]Rollstock.

[5]SE (largest) of the least-squares means in the same main effect (packaging type or muscle).

[a,b]Least-squares means in the same main effect (packaging type or muscle) without a common superscript differ ($P < 0.05$).

compared with vacuum-packaged steaks. Although an instrumental measurement of tenderness, the packaging differences also translated to a difference observed by the trained panelists in the current study (Table 3), as they rated HIOX steaks lower for tenderness compared with all other treatments. This is likely due to reduced protein degradation occurring postmortem, as observed by the western blot results of this study. Previous literature indicates that oxidative environments can arrest the aging process through the inactivation of calpains (Rowe et al., 2004; Kemp et al., 2010; Lonergan et al., 2010; Xue et al., 2012). If calpains are being inactivated during the aging period because of the HIOX environment, it would explain the increased WBSF, reduced desmin degradation, and reduced tenderness scores observed by trained panelists in HIOX steaks.

Volatile compound analysis

Seven compounds—benzaldehyde, 2,3-butanediol, hexanal, hexanoic acid, 2-pentylfuran, nonanal, and ethanol—elicited a packaging type × muscle interaction ($P \leq 0.034$; Table 9). For all interactions except 2,3-butanediol and ethanol, HIOX GM steaks produced the greatest concentration ($P < 0.05$) compared with all other treatments. HIOX GM steaks produced ($P < 0.05$) 2.6 times the amount of hexanal than the next closest mean. This trend was apparent throughout these compounds; however, for hexanoic acid, HIOX LL steaks were similar ($P > 0.05$) to HIOX GM steaks, indicating that, regardless of muscle, hexanoic acid was produced in exorbitant amounts in HIOX packaging. These lipid-derived compounds are primarily products

Table 10. Least-squares means of volatile compounds produced from beef steaks ($n = 160$) of four packaging schemes and two muscles

	2-Heptanone	2-Propanone	Octanoic Acid
Packaging Type			
Carbon monoxide[1]	2.86[b]	105.52[b]	26.55[b]
High oxygen[2]	5.68[a]	150.66[a]	32.95[a]
Overwrap[3]	2.06[b]	112.67[b]	25.72[b]
Rollstock[4]	2.04[b]	82.38[b]	24.80[b]
SEM[5]	0.53	12.27	2.37
P value	< 0.001	0.001	0.039
Muscle			
Gluteus medius	3.79[a]	130.53[a]	31.68[a]
Longissimus lumborum	2.53[b]	95.09[b]	23.33[b]
SEM	0.38	8.55	1.72
P value	0.014	0.003	< 0.001
Muscle × Packaging			
P value	0.129	0.561	0.944

[1]Carbon monoxide motherbag (0.4% CO/30% CO_2/69.6% N_2).

[2]High-oxygen modified atmosphere packaging (80% O_2/20% CO_2).

[3]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[4]Rollstock.

[5]SE (largest) of the least-squares means in the same main effect (packaging type or muscle).

[a,b]Least-squares means in the same main effect (packaging type or muscle) without a common superscript differ ($P < 0.05$).

of lipid oxidation (Min and Ahn, 2005). The combination of the HIOX packaging with the oxidation labile GM escalated the lipid oxidation process and forced oxidation products to be produced in inflated concentrations. For 2,3-butanediol and ethanol, CO GM steaks produced these intermediate compounds in the highest ($P < 0.05$) concentrations compared with all other treatments. 2,3-butanediol is a C4 sugar fragment Maillard reaction intermediate that originates from the retro-aldol reactions of reducing sugars and is a metabolite of acetaldehyde (Yaylayan and Keyhani, 1999; Martins et al., 2000). Additionally, Enterobacteriaceae have been implicated with the production of 2,3-butanediol through fermentation and the metabolism of acetaldehyde. Previous work has indicated that the GM possesses increased concentrations of these compounds (Legako et al., 2015; Hunt et al., 2016); however, this effect was limited to only CO GM steaks. This indicates that CO may have interfered with the cooking process, thus increasing the concentration of 2,3-butanedione. Since 2,3-butanedione is a Maillard intermediate; these results imply that CO is halting the Maillard reaction prior to the retro-aldol reaction, Strecker degradation, and production of sulfur-containing compounds, resulting in a buildup of 2,3-butanedione during cooking (Mottram et al., 1982; Mottram, 1993, 1998).

Three compounds—2-heptanone, 2-propanone, and octanoic acid—were impacted by both a packaging main effect ($P \leq 0.002$) and muscle main effect ($P \leq$

Table 11. Least-squares means of volatile compounds produced from beef steaks ($n = 160$) of four packaging types

	Packaging Type					
Volatile Compound, ng/g	Carbon Monoxide[1]	High Oxygen[2]	Overwrap[3]	Rollstock[4]	SEM[5]	*P* Value
Maillard Reaction Products						
Strecker aldehydes						
Isobutyraldehyde	7.94^{ab}	7.11^{b}	10.38^{a}	7.19^{b}	0.96	0.049
Methional	12.29^{a}	9.68^{b}	10.41^{b}	10.18^{b}	0.71	0.004
Ketone						
2,3-pentanedione	0.21^{b}	0.82^{a}	0.23^{b}	0.19^{b}	0.11	< 0.001
Sulfur-containing compound						
Methanethiol	3.37^{ab}	4.10^{a}	2.87^{b}	2.63^{b}	0.36	0.020
Lipid Degradation Products						
Alcohols						
1-hexanol	11.42^{b}	70.18^{a}	10.56^{b}	12.68^{b}	16.88	0.001
1-octanol	6.02^{b}	9.97^{a}	4.95^{b}	4.22^{b}	0.84	< 0.001
1-octen-3-ol	12.33^{b}	29.89^{a}	12.30^{b}	11.67^{b}	3.36	< 0.001
1-pentanol	11.66^{b}	27.11^{a}	12.44^{b}	15.05^{b}	2.78	< 0.001
Aldehydes						
Decanal	10.49^{a}	8.37^{ab}	6.79^{b}	6.25^{b}	0.99	0.008
Heptanal	9.58^{b}	30.23^{a}	13.32^{b}	8.91^{b}	3.11	< 0.001
Octanal	0.99^{b}	2.18^{a}	1.22^{b}	0.85^{b}	0.19	< 0.001
Pentanal	1.65^{b}	4.80^{a}	2.66^{b}	2.06^{b}	0.47	< 0.001
Alkanes						
Pentane	11.66^{b}	17.74^{a}	8.97^{b}	7.43^{b}	1.59	< 0.001
Tetradecane	0.71^{a}	1.27^{b}	0.83^{b}	0.52^{ab}	0.22	0.035
Carboxylic acid						
Benzoic acid	0.31^{ab}	0.37^{a}	0.40^{ab}	0.25^{b}	0.03	0.014
Esters						
Butanoic acid, methyl ester	0.82^{b}	0.67^{b}	1.33^{a}	1.12^{ab}	0.21	0.022
Hexanoic acid, methyl ester	9.97	24.34	9.28	13.13	4.94	0.038

[1]Carbon monoxide motherbag (0.4% CO/30% CO_2/69.6% N_2).

[2]High-oxygen modified atmosphere packaging (80% O_2/20% CO_2).

[3]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[4]Rollstock.

[5]SE (largest) of the least-squares means in the same main effect (packaging type).

[a,b]Least-squares means in the same main effect (packaging type) without a common superscript differ ($P < 0.05$).

0.013; Table 10). For all 3 compounds, HIOX steaks produced ($P < 0.05$) a greater concentration than all other treatments, which were not different ($P > 0.05$). Additionally, GM steaks produced ($P < 0.05$) a greater concentration of all 3 compounds compared with LL steaks. Lipid-derived ketones are primarily produced via lipid oxidation and have negative impacts on flavor (Min and Ahn, 2005). The GM is more labile to oxidation than the LL, which is more stable, which likely contributed to the increased concentration of 2-heptanone and 2-propanone produced (Lanari and Cassens, 1991; Colle et al., 2015). Similarly, octanoic acid, a straight chain saturated fatty acid, has an unpleasant, rancid odor and taste (Bekhit et al., 2013). It is developed through lipid oxidation, so it is logical for it to be present in the greatest amount in HIOX GM steaks (Bekhit et al., 2013)

Seventeen compounds, primarily lipid derived, were impacted by a packaging main effect ($P \leq 0.049$; Table 11). Not surprisingly, for all of the lipid-derived alcohols and aldehydes and the lone carboxylic acid, HIOX steaks produced ($P < 0.05$) the greatest concentration compared with all other treatments. However, HIOX steaks also produced ($P < 0.05$) the highest concentration of 2,3-pentanedione (a Maillard reaction intermediate) and methanethiol (a sulfur-containing compound developed during the Maillard reaction) through cysteine, methionine, and methional degradation, compared with all other treatments (Resconi et al., 2013). These results indicate that oxidation can arrest the Maillard reaction, as these compounds typically undergo further reactions, such as the retro-aldol reaction and heterocyclization (Bekhit et al., 2013). Moreover, previous work has illustrated the antagonistic effect of lipid-derived reactive carbonyls and phenolic compounds on production of Strecker aldehydes (Delgado et al., 2016). When added together with phenylalanine, phenylacetaldehyde production was substantially reduced (Delgado et al., 2016). This indicates that oxidation products halt the further production of different compounds.

Two compounds were impacted by the muscle main effect ($P \leq 0.003$; Table 12). The GM steaks produced a greater concentration of 2,3-butanedione ($P = 0.003$) and 3-hydroxy-2-butanone ($P = 0.002$) than the LL steaks. In previous studies, the GM has produced greater concentrations of 2,3-butanedione compared with the LL (Legako et al., 2015). Additionally, 3-hydroxy-2-butanone is a Maillard-reaction–produced ketone, which is associated with buttery flavors, that has previously been observed in increased levels in GM steaks over LL steaks (Legako et al., 2015)

Table 12. Least-squares means of volatile compounds produced from beef steaks ($n = 160$) of two muscles[1]

	Muscle type			
	GM	LL	SEM[2]	*P* value
2,3-butanedione	154.83[a]	113.16[b]	12.23	0.003
3-hydroxy-2-butanone	184.41[a]	125.33[b]	17.00	0.002

[1]Muscles in the study included the *gluteus medius* (GM) and *longissimus lumborum* (LL).

[2]SE (largest) of the least-squares means in the same main effect.

[a,b]Least-squares means in the same main effect without a common superscript differ ($P < 0.05$).

Conclusions

This work clearly indicates that environment and muscle type influence beef flavor and tenderness. Results from this study contribute to the growing understanding of beef flavor development and help to validate the impediment of proteolysis and tenderness development by high-oxygen environments. These results distinctly illustrate that HIOX is detrimental to quality, especially flavor and tenderness.

Acknowledgments

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