Meat and Muscle Biology™

Chemical Characterization and Sensory Relationships of Beef *M. longissimus lumborum* and *M. gluteus medius* Steaks After Retail Display in Various Packaging Environments

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Abstract: Volatile compounds, carbonyls, non-heme iron, and thiobarbituric acid reactive substances (TBARS) were measured in both raw and cooked beef samples to determine the effects of muscle and packaging type on beef flavor development. All paired strip loins and top sirloin butts were packaged under vacuum and aged for 14 d postmortem. After initial aging, all subprimals were fabricated to produce *M. gluteus medius* (GM) or *M. longissimus lumborum* (LL) steaks. At 14 d postmortem, steaks were randomly assigned to 1 of 5 package types: high-oxygen modified atmosphere lidded trays (80% O_2/20% CO_2 ["HIOX"]), carbon monoxide modified atmosphere lidded trays (0.4% CO/30% CO_2/69.6% N_2 ["CO"]), rollstock (forming and non-forming films ["ROLL"]), vacuum packaging without retail display ("VAC"), and traditional overwrap ("OW") remained under vacuum prior to retail display. Steaks were stored in darkness an additional 7 d prior to display. At 21 d postmortem, HIOX, OW, CO, and ROLL packages were displayed for 48 h under continuous fluorescent lighting, while VAC steaks remained in dark storage. Packaging and muscle type impacted ($P < 0.05$) quantities of multiple volatile flavor compounds, including alcohols, n-aldehydes, esters, furans, hydrocarbons, sulfur-containing compounds, and ketones in both raw and cooked samples. Volatile compounds related to lipid oxidation were more ($P < 0.05$) prominent in HIOX packaging. Package type ($P < 0.05$) and muscle ($P < 0.05$) had an impact on raw-steak TBARS, although package type did not influence ($P > 0.05$) cooked-steak TBARS. The GM possessed greater ($P < 0.05$) TBARS values than the LL in both raw and cooked samples. Package type had no effect ($P > 0.05$) on carbonyl and non-heme iron content although these analyses differed among muscles ($P < 0.05$), with the GM being greater ($P < 0.05$) than the LL. These results indicate that the development of lipid oxidation that occurs during storage and display was muscle and packaging specific. Therefore, to maintain flavor, quality packaging systems should be selected on a muscle-specific basis.

Key words: Beef, flavor, lipid oxidation, volatile compounds
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Introduction

The most critical characteristic of case-ready product is an appealing visual appearance as consumers largely depend on color to make purchasing decisions. However, packaging systems can promote or deter degradative processes that affect shelf life. Many factors can impact oxidation, such as amount of prooxidants in the product, enzymatic activity, fatty acid composition, processing, packaging, and storage conditions (Faustman et al., 2010). Lipid oxidation is capable of masking other acceptable quality traits, such as flavor, and potentially causing interactions between lipid oxidation products and proteins (amino acids, etc.), which induce additional oxidative reactions (Resconi et al., 2013).

Beef aroma volatiles are developed in the presence of heat via the Maillard reaction and thermal lipid degradation, generating numerous flavor compounds, including pyrazines, ketones, acids, alcohols,

esters, ethers, aldehydes, furans, hydrocarbons, pyrroles, sulfides, and thiophenes (Donald, 1998). However, volatile products formed during lipid oxidation prior to cooking are undesirable and impart rancid off-flavors (Drumm and Spanier, 1991). Although protein oxidation can take place without the presence of lipids, protein and lipid oxidation systems can interact and exchange radicals such as peroxyl radicals to continue the process of protein oxidation (Park et al., 2006). Furthermore, the formation of carbonyls causes detrimental effects on fresh meat quality, including degradation of amino acids, increase of protein cross-links, and loss of proteolytic enzyme activity (Estévez, 2011).

There are many packaging applications available; however, more research needs to be conducted pertaining to beef flavor chemistry relative to packaging types. Therefore, this study aimed to investigate the effects of package type on oxidation and volatile flavor compounds of beef *gluteus medius* (GM) and *longissimus lumborum* (LL) muscles. Additionally, chemical components were evaluated in relation to previously described sensory results (Ponce et al., 2019).

Materials and Methods

Product selection and fabrication

A factorial arrangement of 2 muscles and 5 package types was utilized to determine the effects of muscle and package type on beef flavor. Paired strip loins (Institutional Meat Purchasing Specifications [IMPS] 180; NAMP, 2010) and top sirloin butts (IMPS 184; NAMP, 2010) were collected from USDA Choice "A" maturity beef carcasses ($n = 10$) at a commercial processing facility in the Texas panhandle. Subprimals were packaged under vacuum, stored in dark storage at 0°C–4°C, and aged until 14 d postmortem. After initial aging, all top butts and strip loins were fabricated and sliced to produce 2.54-cm thick GM and LL steaks ($n = 400$), respectively. Steaks from each muscle were randomly assigned to 1 of 5 package types: high-oxygen modified atmosphere lidded trays (80% O_2/20% CO_2 ["HIOX"]), carbon monoxide modified atmosphere lidded trays (0.4% CO/30% CO_2/69.6% N_2 ["CO"]), rollstock (forming and non-forming films (T6035B and T6235B, Sealed Air, Cryovac, Charlotte, NC ["ROLL"]), vacuum packaging without retail display ("VAC"), and traditional overwrap ("OW"). Modified atmosphere packages (MAP) were produced using a Mondini Tray Sealer, CV/VG-S (Cologne, Italy). The trays used for MAP had an oxygen transmission rate (OTR) of 0.1 cc/day at 73°C at 0% relative humidity (RH) and a moisture vapor transmission rate (MVTR) of 2 g/day. The tray film used for the MAP had an OTR of 7 cc/m^2/day at 40°C at 0% RH and an MVTR of 9 g/m^2/day at 38°C at 100% RH. Rollstock and VAC packages were produced using a Multivac Baseline F100 (Kansas City, MO). The forming film had an OTR of 2 cc/m^2/day at 23°C at 0% RH and an MVTR of 7 g/m^2/day at 38°C at 100% RH. The non-forming film had an OTR of 3 cc/m^2/day at 23°C at 0% RH and an MVTR of 9 g/m^2/day at 38°C at 100% RH. The OW packages remained under vacuum prior to being placed on foam trays and sealed with polyvinyl chloride film and displayed in retail cases on day 21 postmortem. The polyvinyl chloride film had an OTR of 150 cc/m^2/day at 90°C at 90% RH. Overwrap packages were produced using a Minipacktorre, Minispenser (Dalmine, Italy). All package types were held in dark storage at 0°C–4°C for an additional 7 d prior to display. At 21 d postmortem, HIOX, OW, CO, and ROLL packages were removed from dark storage and displayed in coffin-style retail cases (Hussmann, BEXD-8, Bridgeton, MO) (0°C–2°C) for 48 h under continuous fluorescent lighting. However, VAC steaks remained in dark storage. All steaks were rotated every 12 h during display to ensure all packages were held at similar temperatures and lighting throughout the case. Temperature fluctuations and retail case temperatures were monitored continuously with remote temperature recorders (Multitrip temperature recorders, Temprecord, Auckland, New Zealand). After 48 h of retail display, all steaks were individually vacuum packaged and frozen (−20°C) until subsequent analyses.

Cooking procedures

Before cooking, steaks were tempered at 2°C to 4°C for 24 h to thaw. Electric clamshell grills (Cuisinart Griddler Deluxe, model GR150, East Windsor, NJ) were used to cook all designated cooked samples. Both heating plates were in contact with steaks during the cooking process, and steaks were not flipped. Steaks were pulled from grills accordingly to reach a peak temperature of 71°C, a medium degree of doneness. Cooked temperatures (Thermapen, Classic Super-Fast, Thermoworks, American Fork, UT) were collected for steaks designated for cooked analyses.

Raw and cooked steak homogenization

After tempering at 2°C to 4°C for 24 h to thaw, raw steaks were trimmed, cubed, and flash frozen in liquid

nitrogen. Cooked steaks were flash frozen immediately after cooking. For both, all heavy connective tissue, external fat, and exterior muscles of each steak were trimmed prior to homogenization. Frozen samples were homogenized to a fine powder with a commercial blender (Robot Coupe, Blixer 3 Food Processor, Robot Coupe, Jackson, MS). Frozen homogenates were stored in labelled bags (Whirl-Pak Standard 13-oz bag, Whirl-Pak, Madison, Wisconsin) at −80°C until subsequent analyses.

Volatile compound analysis

An Agilent 7890B series gas chromatograph (Agilent Technologies, Santa Clara, CA) in combination with a 5977A mass selection detector (Agilent Technologies, Santa Clara, CA) was used to collect volatile flavor compounds. Compound collection was completed on cooked and raw ($n = 200$) samples representing all muscle × packaging combinations similar to Legako et al. (2015). Samples intended for cooked volatiles were cooked as previously described. For all samples, six 1.27-cm-diameter cores were taken, perpendicular to the cut surface of the steak, and minced in a coffee bean grinder (Coffee grinder, Mr. Coffee, Cleveland, OH) to produce a texture similar to a chewed sample. Once minced, 5.0 g of sample was weighed out into a 20-mL glass GC vial (Art #093640-036-00, Gerstel, Linthicum, MD), and 10 µL of an internal standard solution (1,2 dichlorobenzene, 2.5 µg/µL) was added to the vial. Each vial was capped with a 1.3-mm polytetrafluoroethylene septa and metal screw cap (Art #093640-040-00, Gerstel, Linthicum, MD). Vials were loaded by a Gerstel automated sampler (MPS, Gerstel Inc., Linthicum, MD) for a 5-min incubation period at 30°C for raw samples and 65°C for cooked samples in the Gerstel agitator (500 rotations/min). Incubation was followed by a 20-min extraction period during which volatile compounds were collected from the headspace of the vial while in the agitator by solid phase microextraction, utilizing an 85-µm film thickness carboxen polydimethylsiloxane fiber (Stableflex 24 Ga, Supelco, Bellefonte, PA). After extraction, volatile compounds were desorbed onto a VF-5ms capillary column (30 m × 0.25 mm × 1.00 µm; Agilent J&W GC Columns, the Netherlands) and separated. Ions were detected within the range of 45–500 m/z by the mass spectrometer with an electron impact mode at 70 eV. Validation of volatile compound identities was completed by comparing ion fragmentation patterns to external standards.

Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) were measured as an indication of lipid oxidation using the protocol of Buege and Aust (1978) as described by Luqué et al. (2011). Ten grams of powdered sample were homogenized with 30 mL of ice-cold deionized water for 30 s and centrifuged for 10 min at 1,850*g*. Once centrifuged, 2 mL of supernatant, 4 mL of a 15% trichloroacetic acid (TCA), and 20 mM thiobarbituric acid solution, and 1 mL of 10% butylated hydroxyanisole was added, and the sample was vortexed. Samples were placed in a boiling water bath (100°C) for 15 min, followed by an ice bath for 10 min, and were centrifuged for 10 min at 1,850*g*. Absorbances were read at 531 nm and are presented as milligrams of malondialdehyde per kilogram of sample. A standard curve was generated from multiple 1,1,3,3-tetraethoxypropane solutions.

Protein oxidation byproducts

Carbonyl content of raw samples was evaluated by derivatization with 2,4 dinitrophenylhydrazine (DNPH), as described by Vossen and De Smet (2015) with modifications. Three grams of frozen powdered homogenate was mixed with 30 mL of phosphate buffer (20 mM, pH 6.5, containing 0.6 M NaCl). Three aliquots of 0.2 mL were taken from each sample. To precipitate the proteins, 1 mL of ice-cold 10% TCA was added to all aliquots. Samples were then placed in a cooler (2°C–4°C) for 15 min and centrifuged for 30 min at 2,500*g*, followed by discarding of the supernatant. Precipitation of the proteins was repeated, beginning with the addition of 1 mL of ice-cold 10% TCA and finishing with discarding the supernatant. Two aliquots were treated with 0.5 mL of 10 mM DNPH dissolved in 2.0 M HCl, and one aliquot was treated with 0.5 mL of 2.0 M HCl to serve as a blank. All microtubes were vortexed to ensure thorough mixing and were placed on a shaker stored in the dark overnight (>8 h). Following overnight shaking, 0.5 mL of ice-cold 20% TCA was added, and tubes were vortexed and placed in a cooler (2°C–4°C) for 15 min. Samples were centrifuged for 20 min at 2,500*g*, and the supernatant was discarded. To wash samples, 1 mL of (1:1) ethanol/ethyl acetate was added, the sample was vortexed and centrifuged for 20 min at 2,500*g*, and the supernatant was discarded. This washing process works to remove excess DNPH and was completed an additional 2 times. Subsequent to the final discarding of supernatant, microtubes were placed under a fume hood for 20 min to allow for the evaporation of excess solvent. After 20 min, 1 mL of 6 M guanidine-HCl in

20 mM phosphate buffer (pH 6.5) was added to dissolve the remaining pellet, and tubes were placed on a shaker for 30 min covered from light. Finally, samples were then centrifuged at 4,500*g* for 10 min to remove the insoluble portion. Samples were analyzed in duplicate, and those with a coefficient of variation (CV) greater than 15% were reanalyzed. Values with less than 15% CV were averaged prior to statistical analysis. Absorbance was read at 280 and 370 nm, and carbonyl concentrations were calculated as nanomole per milligram. The calculated concentration of the samples designated as blanks were subtracted from the concentration of the samples treated with DNPH. Carbonyl concentration was calculated utilizing the following equation:

$$\frac{C_{\text{hydrazone}}}{C_{\text{protein}}} = \frac{A_{370}}{\varepsilon_{\text{hydrazone},370} \times (A_{280} - A_{370} \times 0.43)} \times 10^6$$

Non-heme iron content

Non-heme iron (NHI) concentration was measured on raw samples using a ferrozine method as described by Li et al. (2012) with some modifications. Five grams of powdered sample was homogenized with 15 mL of citrate-phosphate buffer (0.1 M, pH 5.5) for 15 s. A volume of 1.5 mL of homogenate was mixed with 0.5 mL of 1% ascorbic acid in 0.2 N hydrochloric acid (HCl). All tubes were held at room temperature for 15 min following the addition of 0.5 mL of ascorbic acid. After 15 min, 1 mL of 11.3% TCA was added and held for an additional 5 min at room temperature. Tubes were then centrifuged for 15 min at 3,000*g*. Immediately after centrifugation, 2 mL of clear supernatant was removed and added to 0.8 mL of 10% ammonium acetate. Then, 0.2 mL of the ferrozine color reagent was added. Tubes were then vortexed and held at room temperature for 10 min. Absorbances were read at 562 nm, and NHI concentration was presented as microgram of NHI per gram of sample. A standard curve was prepared utilizing an iron standard solution to determine concentration (micrograms per gram) of NHI in each sample. Samples were analyzed in duplicate, and those with a CV greater than 10% were reanalyzed. Values with less than 10% CV were averaged prior to statistical analysis.

Sensory evaluation, proximate analysis, and pH

Consumer and trained sensory evaluation methodologies and results were described in detail in Ponce et al. (2019). Likewise, proximate composition and pH were previously determined (Ponce et al., 2019). The same data are retained here to evaluate relationships with chemical components. In brief, consumer panel sessions ($n = 5$) were completed using methods similar to Corbin et al. (2015) and Legako et al. (2015), approved by the Texas Tech University Institutional Review Board. Each panel session consisted of 20 untrained, paid panelists ($n = 100$). Panelists were given a plastic fork, toothpick, napkin, and an expectorant cup, along with a cup of water, cup of diluted apple juice, and unsalted crackers to serve as palate cleansers between samples. Steaks were thawed at 2°C–4°C for 24 h prior to consumer panels. Steaks were cooked as discussed earlier. Ten samples were derived from each steak and served to ten pre-assigned panelists immediately following plating. Each panelist was served one 1.5-cm-×-1.5-cm piece per panel round. Ten panel rounds were conducted representing all possible muscle × packaging combinations. Panelists evaluated all samples for overall liking, liking of flavor, tenderness, and juiciness. Attributes were measured on a 100-mm continuous line scale with "Dislike Extremely, Not Tender, or Not Juicy" representing 0 and "Like Extremely, Very Tender, or Very Juicy" representing 100.

Trained descriptive attribute panels were conducted utilizing the Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat (AMSA, 2015). Panelists were trained and tested for 4 wk to objectively evaluate intensity of beef flavor attributes similar to attributes included and described in a published beef flavor lexicon (Adhikari et al., 2011): beef flavor identity (amount of beef flavor identity in the sample), brown/roasted (round, full aromatic generally associated with beef suet that has been broiled), 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), oxidized (stale, aromatics associated with old oil), cardboardy (aromatic associated with slightly oxidized fats and oils, reminiscent of wet cardboard packaging), umami (flat, salty, somewhat brothy; taste of glutamate, salts of amino acids, and other molecules called nucleotides), sweet (fundamental taste factor associated with sucrose), salty (fundamental taste factor of which sodium chloride is typical), bitter (fundamental taste factor associated with a caffeine solution), and sour (fundamental taste factor associated with citric acid). Additional palatability characteristic evaluation was conducted by asking "Overall Juiciness" and "Overall Tenderness" following flavor

attributes for each sample. Anchors that panelists were trained to reference for each flavor attribute were made available to each panelist at all panels. Sample steaks were thawed at 2°C–4°C for 24 h prior to panels and cooked as previously described. Once a steak reached peak temperature, it was immediately weighed, sliced into 1.27-×-1.27 cm pieces, and placed in 2-oz plastic portion cups and covered with corresponding plastic lids. Sensory attributes were quantified on an unstructured line scale with "0" representing absence of specific flavor, extreme toughness, or extreme dryness and "100" representing extreme intensity of specific flavor attribute, extreme tenderness, or extreme juiciness.

In brief, total fat percent, moisture content, ash, protein, and pH analyses were conducted according to the following cited methodologies. An AOAC 983.23 approved chloroform: methanol extraction method was used to determine fat percent, as described by Folch et al. (1957). Percent moisture of raw and cooked samples was measured utilizing the AOAC 950.46 oven drying method. Percent ash content of raw and cooked was measured using samples produced immediately following the completion of moisture analysis with the AOAC 923.03 protocol. Crude protein was measured using an AOAC 992.15 approved method on raw and cooked samples, utilizing a LECO TruMacN (Leco Corporation, St. Joseph, MI). Analysis of pH was completed on raw samples using the method as described in Luqué et al. (2011).

Statistical analysis

All data were analyzed utilizing statistical procedures in SAS version 9.4 (SAS Institute Inc., Cary, NC). Individual steak served as the experimental unit, and package type and muscle type were the fixed effects in a 2 × 5 factorial arrangement. Retail case, carcass, and replication were used as random effects for all lab analyses. Least-squares means were generated for all analyses utilizing generalized linear mixed models (PROC GLIMMIX) and separated with the PDIFF function, with significance determined at $\alpha = 0.05$. Principal component analysis (PCA) was performed on all raw analyses and cooked analyses separately, which included consumer and descriptive sensory data. Data were analyzed using PROC FACTOR in SAS version 9.4. Two principal components (PC1 and PC2) were established for both raw and cooked analyses, separately. Prior to PCA, all data were mean centered and standardized.

Results and Discussion

Raw volatile compounds

A muscle × package type interaction occurred for isobutyraldehyde ($P < 0.02$) and pentane ($P = 0.01$) in raw samples as shown in Table 1. For both compounds, LL HIOX possessed a greater ($P < 0.05$) amount than all other muscle × package type combinations. Raw volatile mean quantities by package type are shown in Table 2 and by muscle in Table 3. The secondary products of lipid oxidation that contribute to off-flavors include n-aldehydes, ketones, alcohols, and hydrocarbons. The HIOX packaging treatment possessed the greatest ($P < 0.05$) quantity of all n-aldehydes including heptanal, hexanal, nonanal, and pentanal. Those secondary products of lipid oxidation contribute to off-flavors; hexanal is considered to be the most prominent n-aldehyde in cooked beef and is proportional to TBARS, therefore it is associated with lipid oxidation–derived off-flavors (Ullrich and Grosch, 1987).

The HIOX treatment possessed the greatest ($P < 0.05$) amount of 2-heptanone, 1-octen-3-ol, and 2-pentyl-furan. A study conducted by Jääskeläinen et al. (2016) reported that the volatiles found in beef samples

Table 1. Least-squares means of volatile flavor compounds of raw samples based on package type[1] × muscle[2] interaction

		Volatile Compound (ng/g)	
Package Type	Muscle	Isobutyraldehyde	Pentane
CO	GM	1.28[b]	4.54[b]
HIOX	GM	0.89[b]	8.49[b]
ROLL	GM	1.42[b]	2.52[b]
OW	GM	1.52[b]	6.55[b]
VAC	GM	1.25[b]	1.43[b]
CO	LL	0.97[b]	3.35[b]
HIOX	LL	2.98[a]	29.86[a]
ROLL	LL	1.65[b]	1.30[b]
OW	LL	1.44[b]	5.66[b]
VAC	LL	1.31[b]	1.17[b]
SEM[3]		0.60	3.77
P value		0.029	0.010

[1]Package types included carbon monoxide modified atmosphere lidded trays (0.4% CO/30% CO_2/69.6% N_2 ["CO"]), high-oxygen modified atmosphere lidded trays (80% O_2/20% CO_2 ["HIOX"]), traditional overwrap ("OW"), rollstock (forming and non-forming films ["ROLL"]), and vacuum packaging without retail display ("VAC").

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

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

[a,b]Means within a column lacking a common superscript differ ($P < 0.05$).

Table 2. Least-squares means of volatile flavor compounds from raw samples from five package types[1]

	Package Type						
Volatile Compound (ng/g)	CO	HIOX	ROLL	OW	VAC	SEM[2]	*P* Value
Alcohols							
Ethanol	7.01	5.08	8.53	4.20	8.22	5.84	0.763
1-Octanol	0.28	0.46	0.28	0.29	0.24	0.06	0.073
1-Octen-3-ol	0.48^{b}	2.12^{a}	0.41^{b}	0.45^{b}	0.38^{b}	0.22	<0.001
1-Pentanol	0.82	0.80	0.93	0.84	0.83	0.12	0.811
1-Penten-3-ol	0.07	0.18	0.06	0.09	0.06	0.05	0.176
n-Aldehydes							
Heptanal	0.10^{b}	0.74^{a}	0.09^{b}	0.06^{b}	0.01^{b}	0.16	0.027
Hexanal	8.46^{b}	30.06^{a}	5.69^{b}	5.97^{b}	2.91^{b}	8.20	0.015
Nonanal	0.07^{b}	0.25^{a}	0.07^{b}	0.08^{b}	0.05^{b}	0.04	0.015
Pentanal	0.10^{b}	0.21^{a}	0.04^{b}	0.07^{b}	0.03^{b}	0.05	0.021
Alkenes							
Toluene	1.57^{b}	1.59^{b}	2.28^{a}	2.20^{a}	1.64^{b}	0.26	0.002
Xylene	99.45	111.94	139.75	107.74	83.89	17.36	0.081
1-Octene	0.08^{c}	0.19^{ab}	0.27^{a}	0.20^{ab}	0.12^{bc}	0.02	<0.001
2,4-Dimethyl-1-heptene	126.78	417.46	202.98	146.30	112.60	112.8	0.225
Carboxylic Acids							
Acetic acid	1.84	2.17	2.50	2.07	1.91	0.56	0.742
Butanoic acid	66.76	62.82	104.70	79.80	79.36	21.57	0.238
Nonanoic acid	3.73	4.55	2.81	2.76	2.82	0.72	0.313
Octanoic acid	0.02	0.03	0.02	0.02	0.02	0.005	0.925
Hexanoic acid	4.29^{b}	19.85^{a}	4.13^{b}	4.54^{b}	3.32^{b}	1.61	<0.001
Esters							
Butanoic acid, methyl ester	4.50^{a}	3.42^{b}	5.27^{a}	4.57^{a}	4.93^{a}	0.80	0.004
Heptanoic acid, methyl ester	0.57^{b}	0.86^{a}	0.37^{c}	0.44^{bc}	0.36^{c}	0.06	<0.001
Hexanoic acid, methyl ester	32.42^{b}	88.35^{a}	24.47^{b}	27.36^{b}	21.17^{b}	4.99	<0.001
Nonanoic acid, methyl ester	0.20^{b}	0.30^{a}	0.19^{b}	0.20^{b}	0.19^{b}	0.01	<0.001
Octanoic acid, methyl ester	1.83	1.67	1.72	1.71	1.75	0.29	0.990
Methyl propionate	3.86	4.48	4.40	4.19	4.53	0.59	0.735
Furan							
2-Pentyl furan	0.10^{b}	1.44^{a}	0.01^{b}	0.06^{b}	0.00^{b}	0.30	0.002
Hydrocarbons							
Decane	1.35	1.45	1.45	1.29	1.32	0.14	0.889
Nonane	0.15^{b}	0.35^{a}	0.13^{b}	0.14^{b}	0.12^{b}	0.06	0.035
Octane	1.05^{b}	1.64^{a}	0.94^{b}	1.05^{b}	0.63^{b}	0.15	0.009
Tetradecane	0.05^{b}	0.09^{a}	0.05^{b}	0.04^{b}	0.04^{b}	0.01	0.039
4-Methyl-heptane	0.37	0.35	0.37	0.37	0.37	0.01	0.184
Ketones							
Butyrolactone	0.03	0.05	0.04	0.04	0.04	0.009	0.606
2-Butanone	3.33	3.78	3.80	4.26	3.48	0.52	0.641
2-Heptanone	0.94^{b}	2.12^{a}	0.55^{b}	0.70^{b}	0.49^{b}	0.18	0.004
2-Pentanone	0.06	0.10	0.07	0.07	0.06	0.01	0.255
2-Propanone	38.95	80.27	43.31	61.88	35.55	18.12	0.159
3-Hydroxy-2-butanone	34.01	38.27	42.60	53.30	38.82	18.16	0.374
2,3-Butanedione	33.89	50.97	39.00	48.60	34.37	13.73	0.112
2,3-Pentanedione	0.49^{b}	0.51^{a}	0.49^{b}	0.49^{b}	0.49^{b}	0.001	<0.001
Strecker Aldehydes							
Acetaldehyde	0.22	0.58	0.23	0.41	0.23	0.17	0.349
Benzaldehyde	0.15	0.25	0.17	0.18	0.14	0.05	0.398

Table 2. (*Continued*)

Volatile Compound (ng/g)	Package Type					SEM[2]	*P* Value
	CO	HIOX	ROLL	OW	VAC		
Phenylacetaldehyde	0.40	0.41	0.40	0.40	0.39	0.01	0.744
2-methyl-Butanal	0.30	0.02	0.18	0.17	0.15	0.17	0.077
3-methylbutanal	6.01	0.55	4.26	3.01	3.75	3.58	0.114
Sulfur Containing							
Carbon disulfide	0.97[b]	0.45[c]	1.59[a]	0.93[bc]	0.97[b]	0.26	0.009
Dimethyl-Disulfide	0.002	0.002	0.001	0.002	0.002	0.004	0.500
Dimethyl sulfide	4.18	3.38	7.05	5.08	5.95	2.18	0.298
Dimethyl sulfone	0.16	0.18	0.21	0.20	0.18	0.04	0.638
Methanethiol	0.09	0.11	0.12	0.11	0.13	0.02	0.687

[1]Package types include carbon monoxide modified atmosphere lidded trays (0.4% CO/30% CO_2/69.6% N_2 ["CO"]), high-oxygen modified atmosphere lidded trays (80% O_2/20% CO_2 ["HIOX"]), traditional overwrap ("OW"), rollstock (forming and non-forming films ["ROLL"]), and vacuum packaging without retail display ("VAC").

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

[a–c]Means within a row lacking a common superscript differ ($P < 0.05$).

stored in high-oxygen MAP at 6°C were greater in hexanoic acid, nonanal, and 1-octen-3-ol than vacuum-packaged samples. Hexanal, an indicator of lipid oxidation, was similar in concentration between vacuum packaging and high-oxygen MAP at 2 d of storage; however, it was present in higher concentrations in high-oxygen MAP compared with vacuum packaging from 4 to 14 d. The results of the current study suggest that anaerobic packaging system prevents the generation of volatile flavor compounds that are detrimental to beef flavor. Spanier et al. (1992) reported that vacuum packaging ground beef patties prevented the production of volatile compounds associated with lipid oxidation, including pentanal, heptanal, hexanal, and nonanal. Similar findings were reported by Argyri et al. (2015), including an increase in aldehydes (pentanal, hexanal, nonanal, heptanal) and alcohols (1-octen-3-ol) in minced beef stored in high-oxygen MAP compared with air-permeable packaging. Samples were stored for 27, 20, 16, and 9 d, and ketones (including 2-pentanone and 2-heptanone) increased whereas 2-butanone decreased. Nonetheless, lipid oxidation that occurs during storage and display can produce nonvolatile products that ultimately result in the development of volatile flavor compounds in cooked product that contribute to undesirable flavors and aromas.

Muscles did not differ ($P > 0.05$) in quantities of alkenes, furans, and hydrocarbons in raw samples, as shown in Table 3. Hexanal was greater ($P < 0.05$) in GM samples than LL samples, indicating that the extent of oxidation was greater in the GM. Additionally, GM possessed a greater ($P < 0.05$) quantity of compounds that are also associated with lipid oxidation, including esters and ketones. Overall, the results of this study indicate that oxidative atmospheres can cause an increase in the development of volatile compounds that promote the generation of undesirable flavors and aromas in the cooked product. However, muscles also are differentiated based on differences in lipid stability.

Cooked volatile compounds

A variety of volatile flavor compounds were generated through multiple pathways, including thermal lipid degradation and the Maillard reaction. Many volatile compounds were measured and categorized as Strecker aldehydes, n-aldehydes, sulfur-containing compounds, ketones, pyrazines, alkanes, alcohols, alkenes, carboxylic acids, esters, furans, or hydrocarbons from cooked samples. Cooked mean quantities of lipid-derived volatiles detected from package type are shown in Table 4 and displayed by muscle type in Table 5.

Alcohols are associated with products of oxidative degradation of lipids and are known to impact flavor (Garcia et al., 1991). Half of all alcohols quantified differed ($P < 0.05$) among package types including 1-octanol, 1-octen-3-ol, 1-penten-3-ol, and 2,3 butanediol. The greatest ($P < 0.05$) quantities of 1-octanol and 1-octen-3-ol were detected in HIOX, and CO and OW possessed greater ($P < 0.05$) quantities of 1-octen-3-ol than ROLL and VAC. The HIOX, CO, and OW package types had similar ($P > 0.05$) quantities of 1-penten-3-ol, but HIOX and CO exhibited greater ($P < 0.05$) quantities than VAC and ROLL. However, CO

Table 3. Least-squares means of volatile flavor compounds of raw *gluteus medius* and *longissimus lumborum* muscles

	Muscle			
Volatile Compound (ng/g)	GM	LL	SEM[1]	*P* Value
Alcohols				
Ethanol	3.63[b]	9.58[a]	4.81	0.005
1-Octanol	0.32	0.30	0.03	0.502
1-Octen-3-ol	0.81	0.72	0.20	0.463
1-Pentanol	1.01[a]	0.68[b]	0.10	<0.001
1-Penten-3-ol	0.08	0.10	0.03	0.338
n-Aldehydes				
Heptanal	0.29	0.11	0.12	0.142
Hexanal	15.19[a]	6.05[b]	7.22	0.023
Nonanal	0.13	0.08	0.03	0.095
Pentanal	0.12[a]	0.07[b]	0.05	0.043
Alkenes				
Toluene	1.83	1.88	0.22	0.759
Xylene	112.71	104.40	13.30	0.512
1-Octene	0.17	0.17	0.01	0.956
2,4-Dimethyl-1-heptene	158.47	243.97	80.09	0.260
Carboxylic Acids				
Acetic acid	2.28	1.92	0.35	0.195
Butanoic acid	106.34[a]	51.04[b]	18.51	<0.001
Nonanoic acid	3.27	3.40	0.45	0.849
Octanoic acid	0.03	0.02	0.003	0.191
Hexanoic acid	8.27	6.18	1.02	0.143
Esters				
Butanoic acid, methyl ester	5.34[a]	3.73[b]	0.75	<0.001
Heptanoic acid, methyl ester	0.64[a]	0.40[b]	0.05	<0.001
Hexanoic acid, methyl ester	43.60[a]	33.91[b]	3.71	0.004
Nonanoic acid, methyl ester	0.22	0.22	0.01	0.790
Octanoic acid, methyl ester	2.51[a]	0.96[b]	0.24	<0.001
Methyl propionate	3.64[b]	4.94[a]	0.51	0.003
Furan				
2-Pentyl furan	0.49	0.15	0.23	0.125
Hydrocarbons				
Decane	1.41	1.33	0.09	0.459
Nonane	0.21	0.15	0.04	0.094
Octane	0.98	1.15	0.10	0.158
Tetradecane	0.05	0.06	0.009	0.302
4-Methyl-heptane	0.37	0.36	0.007	0.218
Ketones				
Butyrolactone	0.03[b]	0.05[a]	0.006	0.005
2-Butanone	3.83	3.64	0.42	0.560
2-Heptanone	1.01	0.91	0.11	0.449
2-Pentanone	0.07	0.07	0.01	0.993
2-Propanone	67.19[a]	36.07[b]	14.69	0.001
3-Hydroxy-2-butanone	55.50[a]	27.31[b]	17.28	<0.001
2,3-Butanedione	50.49[a]	32.24[b]	12.98	0.006
2,3-Pentanedione	0.50	0.50	0.50	0.270
Strecker Aldehydes				
Acetaldehyde	0.31	0.36	0.12	0.612
Benzaldehyde	0.18	0.17	0.03	0.893
Phenylacetaldehyde	0.40	0.40	0.007	0.329
2-methyl-Butanal	0.22	0.11	0.16	0.064
3-methylbutanal	4.32	2.71	3.44	0.115
Sulfur Containing				
Carbon disulfide	0.92	1.05	0.22	0.404
Dimethyl-disulfide	0.002[a]	0.001[b]	0.0002	0.011
Dimethyl sulfide	8.17[a]	2.08[b]	1.80	<0.001
Dimethyl sulfone	0.18	0.19	0.03	0.356
Methanethiol	0.10	0.12	0.01	0.123

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

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

GM = *gluteus medius*; LL = *longissimus lumborum*.

possessed the greatest ($P < 0.05$) amount of 2,3 butanediol compared with all other package types. A study conducted by Ercolini et al. (2011) reported that 2,3 butanediol and 1-octen-3-ol were correlated with aerobic packaging versus vacuum packaging. These results indicate there is a difference in the development of lipid oxidation that occurs in vacuum-packaged product, displayed or stored in darkness, versus product packaged in certain MAP atmospheres, and the accumulation of these lipid oxidation volatile compounds can collectively impact flavor.

A majority of alkenes were influenced by package type, and in 2 instances, CO was similar ($P > 0.05$) to all package types although HIOX and OW had greater ($P < 0.05$) amounts of D-limonene and xylene than ROLL and VAC. Additionally, HIOX contained the greatest ($P < 0.05$) quantity of 1-octene. Finally, ROLL possessed the greatest ($P < 0.05$) amount of 2,4-dimethyl-1-heptene, although OW and VAC had greater ($P < 0.05$) quantities than CO and HIOX. Alkenes are generated by lipid oxidation, and small amounts are capable of impacting flavor. MacLeod and Coppock (1976) found that alkenes were associated with off-odors in beef, such as cardboardy, sour, and pungent. Overall, alkenes were more abundant in aerobic packaging and can negatively influence flavor if present over a certain threshold.

The greatest ($P < 0.05$) concentration of 2-heptanone was found in HIOX, and similar to a pattern seen in other compounds, the CO and OW treatments possessed greater ($P < 0.05$) concentrations than ROLL and VAC. The difference in 2-heptanone concentration is likely due to the increased amount of oxidation that occurs in high-oxygen environments; however, Legako et al. (2016) reported a greater

Table 4. Least-squares means of lipid-derived volatile flavor compounds of cooked samples from five package types[1]

	Package Type						
Volatile Compound (ng/g)	CO	HIOX	ROLL	OW	VAC	SEM[2]	*P* Value
Alcohols							
Ethanol	4.52	1.65	3.49	2.98	4.46	3.20	0.592
1-Hexanol	0.97	0.92	0.08	0.29	0.05	0.47	0.363
1-Octanol	0.74^{b}	1.30^{a}	0.45^{b}	0.55^{b}	0.42^{b}	0.20	<0.001
1-Octen-3-ol	3.10^{b}	7.11^{a}	0.60^{c}	2.64^{b}	0.74^{c}	1.39	<0.001
1-Pentanol	1.92	3.51	0.75	2.82	1.70	1.41	0.527
1-Penten-3-ol	0.07^{a}	0.06^{a}	0.01^{b}	0.05^{ab}	0.02^{b}	0.01	0.017
2-Phenyl isopropanol	5.83	7.47	9.17	5.95	6.77	2.73	0.810
2,3-Butanediol	3.05^{a}	1.65^{b}	1.68^{b}	1.43^{b}	1.77^{b}	0.73	0.038
n-Aldehydes							
Heptanal	1.57	3.55	1.46	2.15	1.86	0.90	0.206
Hexanal	27.68	84.46	19.90	45.45	31.62	25.86	0.092
Nonanal	0.58	1.08	0.73	0.56	0.79	0.22	0.251
Pentanal	0.31	0.44	0.23	0.93	0.43	0.43	0.662
Alkenes							
Alpha-pinene	0.05	0.03	0.04	0.05	0.03	0.01	0.507
D-limonene	23.66^{ab}	30.92^{a}	18.03^{b}	29.79^{a}	17.29^{b}	3.95	0.031
Toluene	2.90	2.34	3.59	3.29	3.01	0.45	0.158
Xylene	87.68^{ab}	109.55^{a}	66.65^{b}	97.88^{a}	65.63^{b}	8.63	0.007
1-Octene	0.09^{d}	0.98^{a}	0.53^{b}	0.47^{bc}	0.16^{cd}	0.17	<0.001
2,4-Dimethyl-1-heptene	48.26^{c}	52.84^{c}	420.79^{a}	188.67^{b}	189.04^{b}	74.12	<0.001
Carboxylic Acids							
Acetic acid	3.69	2.80	2.96	4.32	2.93	0.84	0.410
Butanoic acid	293.30	218.12	235.53	276.36	231.63	34.79	0.461
Nonanoic acid	14.18	17.09	16.19	12.59	14.52	2.78	0.695
Octanoic acid	0.18	0.19	0.17	0.17	0.17	0.03	0.981
Hexanoic acid	42.44^{b}	105.24^{a}	26.68^{b}	42.70^{b}	25.47^{b}	12.46	<0.001
Esters							
Butanoic acid, methyl ester	0.18	0.30	0.08	0.23	0.12	0.14	0.636
Heptanoic acid, methyl ester	0.03	0.08	0.03	0.05	0.03	0.01	0.125
Hexanoic acid, methyl ester	1.81	6.46	0.98	2.63	1.31	1.68	0.096
Nonanoic acid, methyl ester	0.15^{b}	0.16^{b}	0.15^{b}	0.22^{a}	0.15^{b}	0.009	<0.001
Octanoic acid, methyl ester	0.11	0.27	0.05	0.11	0.08	0.08	0.417
Methyl propionate	0.77	0.71	0.67	0.70	0.69	0.05	0.580
Furan							
2-Pentyl furan	0.76^{b}	4.57^{a}	0.18^{b}	0.77^{b}	0.10^{b}	0.82	<0.001
Hydrocarbons							
Decane	0.90^{b}	1.98^{a}	1.07^{b}	0.94^{b}	0.90^{b}	0.25	0.007
Nonane	0.61^{b}	1.30^{a}	0.61^{b}	0.69^{b}	0.57^{b}	0.24	0.040
Octane	2.04^{b}	3.45^{a}	1.58^{b}	2.84^{a}	1.36^{b}	0.60	<0.001
Pentane	2.21^{bc}	5.22^{a}	1.24^{c}	3.14^{b}	1.24^{c}	1.01	<0.001
Tetradecane	0.24^{b}	0.47^{a}	0.20^{b}	0.25^{b}	0.16^{b}	0.07	0.004
Ketones							
Butyrolactone	0.13	0.10	0.13	0.13	0.11	0.02	0.494
2-Butanone	6.16	4.21	5.79	5.90	5.35	0.91	0.396
2-Heptanone	1.50^{b}	3.17^{a}	0.64^{c}	1.31^{b}	0.63^{c}	0.35	<0.001
2-Pentanone	0.06	0.05	0.05	0.05	0.05	0.007	0.781
2-Propanone	15.49	22.54	12.09	16.13	11.01	3.44	0.073

[1]Package types include carbon monoxide modified atmosphere lidded trays (0.4% CO/30% CO_2/69.6% N_2 ["CO"]), high-oxygen modified atmosphere lidded trays (80% O_2/20% CO_2 ["HIOX"]), traditional overwrap ("OW"), rollstock (forming and non-forming films ["ROLL"]), and vacuum packaging without retail display ("VAC").

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

[a–d]Means within a row lacking a common superscript differ ($P < 0.05$).

Table 5. Least-squares means of lipid-derived volatile flavor compounds from cooked *gluteus medius* and *longissimus lumborum* muscles

	Muscle			
Volatile Compound (ng/g)	GM	LL	SEM[2]	*P* Value
Alcohols				
Ethanol	1.96[b]	4.88[a]	2.99	0.023
1-Hexanol	0.51	0.41	0.31	0.788
1-Octanol	0.71	0.68	0.17	0.815
1-Octen-3-ol	2.90	2.78	1.27	0.852
1-Pentanol	1.92	2.36	1.03	0.678
1-Penten-3-ol	0.03	0.05	0.01	0.151
2-Phenyl isopropanol	5.84	8.24	2.05	0.229
2,3-Butanediol	2.23	1.60	0.65	0.078
n-Aldehydes				
Heptanal	1.87	2.37	0.71	0.411
Hexanal	34.48	49.17	21.53	0.351
Nonanal	0.70	0.79	0.16	0.571
Pentanal	0.21	0.73	0.31	0.115
Alkenes				
Alpha-pinene	0.04	0.04	0.006	0.421
D-limonene	22.86	25.02	2.41	0.532
Toluene	3.00	3.04	0.34	0.903
Xylene	83.82	87.13	5.27	0.659
1-Octene	0.43	0.46	0.14	0.781
2,4-Dimethyl-1-heptene	185.93	173.92	63.80	0.771
Carboxylic acids				
Acetic acid	3.36	3.31	0.65	0.923
Butanoic acid	294.20[a]	207.77[b]	21.25	0.005
Nonanoic acid	14.35	15.48	1.99	0.597
Octanoic acid	0.24[a]	0.11[b]	0.02	<0.001
Hexanoic acid	59.94[a]	37.07[b]	9.82	0.007
Esters				
Butanoic acid, methyl ester	0.23	0.13	0.10	0.314
Heptanoic acid, methyl ester	0.05	0.04	0.009	0.488
Hexanoic acid, methyl ester	3.46	1.82	1.12	0.235
Nonanoic acid, methyl ester	0.17	0.16	0.007	0.765
Octanoic acid, methyl ester	0.17	0.08	0.05	0.237
Methyl propionate	0.71	0.71	0.03	0.934
Furan				
2-Pentyl furan	1.54	1.01	0.69	0.279
Hydrocarbons				
Decane	1.23	1.08	0.20	0.386
Nonane	0.71	0.81	0.19	0.563
Octane	2.33	2.18	0.56	0.522
Pentane	2.35	2.87	0.88	0.342
Tetradecane	0.28	0.25	0.06	0.407
Ketones				
Butyrolactone	0.08[b]	0.16[a]	0.02	<0.001
2-Butanone	5.10	5.86	0.68	0.258
2-Heptanone	1.52	1.38	0.32	0.345
2-Pentanone	0.05	0.06	0.06	0.429
2-Propanone	18.71[a]	12.20[b]	2.42	0.017

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

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

GM = *gluteus medius*; LL = *longissimus lumborum*.

occurrence of differences in ketones in cooked *longissimus dorsi* (LD) steaks of varying quality grades. Product in this study were of the same quality grade, which can potentially explain similarities among most ketones and all n-aldehydes. The proportion of phospholipids and available prooxidants determine the amount of lipid oxidation products formed that generate undesirable off-flavors and odors in cooked product.

All hydrocarbons were influenced ($P < 0.05$) by package type. The HIOX treatment exhibited the greatest ($P < 0.05$) quantity of decane, nonane, and tetradecane. The HIOX and OW package treatments possessed greater ($P < 0.05$) amounts of octane than ROLL, VAC, and CO. Similarly, the greatest ($P < 0.05$) amount of pentane was detected in HIOX; however, OW had a greater ($P < 0.05$) amount than ROLL and VAC, although CO was similar ($P > 0.05$) to all package treatments other than HIOX. Hur et al. (2004) reported an increase in hydrocarbons, in addition to alcohols, esters, ketones, and aldehydes, attributed to an increase in lipid oxidation. An aerobic environment clearly influences the quantity of hydrocarbons present in cooked samples that likely impact flavor. The HIOX treatment contained greater ($P < 0.05$) amounts of 2-pentyl furan compared with all package types.

Muscle type had no impact ($P > 0.05$) on n-aldehydes, alkenes, esters, furans, or hydrocarbons found in cooked samples. The amount of butyrolactone was greater ($P < 0.05$) in LL than GM, whereas 2-propanone was greater ($P < 0.05$) in GM, which may be attributed to differences in muscle lipid stability. However, Legako et al. (2015) reported that 2-propanone was similar between the LL and GM. The only alcohol that differed between the 2 muscles was ethanol ($P = 0.03$), which was greater in the LL. Ethanol has been reported as a product of metabolism from gram-negative bacteria (Argyri et al., 2015). More than half of the carboxylic acids detected were found in greater quantities in the GM than the LL, including butanoic acid ($P < 0.01$), octanoic acid ($P < 0.01$), and hexanoic acid ($P < 0.01$). Carboxylic acids are a product of lipid degradation, and the higher occurrence found in GM is likely due to its having a lower chemical stability than LL (Jeremiah et al., 2003; Chail et al., 2016).

Maillard-reaction–derived volatiles in cooked samples are presented by package type in Table 6 and according to muscle type in Table 7. Similar to a pattern seen in the effect of package type on lipid-derived compounds, 2,3-pentanedione was the highest ($P < 0.05$) in HIOX, whereas OW and CO had a greater ($P < 0.05$) amount than VAC and ROLL. These results

Table 6. Least-squares means of Maillard-reaction–derived volatile flavor compounds of cooked samples from five package types[1]

	Package Type						
Volatile Compound (ng/g)	CO	HIOX	ROLL	OW	VAC	SEM[2]	*P* Value
Ketones							
2,3-Butanedione	70.66	55.83	55.90	66.53	55.87	10.21	0.604
3-Hydroxy-2-butanone	82.23	64.52	70.80	86.62	70.21	13.84	0.699
2,3-Pentanedione	0.502[b]	0.508[a]	0.498[c]	0.503[b]	0.498[c]	0.002	<0.001
Pyrazines							
Methyl-pyrazine	0.04	0.04	0.02	0.05	0.02	0.01	0.317
Trimethylpyrazine	0.02	0.03	0.01	0.04	0.02	0.009	0.352
2,5-Dimethyl-pyrazine	0.08	0.09	0.05	0.09	0.05	0.01	0.416
2-Ethyl-3,5-dimethyl pyrazine	0.05	0.04	0.03	0.04	0.03	0.01	0.658
3-Ethyl-2,5-dimethyl pyrazine	0.06	0.06	0.04	0.07	0.04	0.02	0.794
Strecker Aldehydes							
Acetaldehyde	0.82	0.68	0.78	0.83	0.71	0.14	0.770
Benzaldehyde	4.87	4.22	4.89	4.82	4.63	0.76	0.906
Methional	0.07	0.06	0.05	0.06	0.05	0.01	0.845
Phenylacetaldehyde	0.54	0.59	0.51	0.54	0.50	0.02	0.121
2-Methyl butanal	0.13[a]	0.09[ab]	0.08[b]	0.11[ab]	0.06[b]	0.01	0.040
3-Methyl butanal	0.54[ab]	0.36[b]	0.36[b]	0.60[a]	0.40[b]	0.07	0.026
Isobutyraldehyde	3.04[a]	2.06[b]	3.42[a]	3.28[a]	2.65[ab]	0.45	0.047
Sulfur containing							
Carbon disulfide	4.01	3.24	4.93	3.41	3.25	1.01	0.485
Dimethyl disulfide	0.009	0.006	0.009	0.009	0.012	0.002	0.631
Dimethyl sulfide	0.73[ab]	0.45[c]	0.83[a]	0.51[bc]	0.78[a]	0.08	0.004
Dimethyl sulfone	0.58	0.39	0.52	0.53	0.44	0.13	0.464
Methanethiol	0.73	0.72	0.56	0.76	0.64	0.12	0.742
2-Methyl thiophene	0.025	0.041	0.025	0.058	0.024	0.017	0.488

[1]Package types include carbon monoxide modified atmosphere lidded trays (0.4% CO/30% CO_2/69.6% N_2 ["CO"]), high-oxygen modified atmosphere lidded trays (80% O_2/20% CO_2 ["HIOX"]), traditional overwrap ("OW"), rollstock (forming and non-forming films ["ROLL"]), and vacuum packaging without retail display ("VAC").

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

[a–c]Means within a row lacking a common superscript differ ($P < 0.05$).

indicate that there are differences in oxidation products that develop in the raw product due to the oxidative potential typical of each packaging environment. Sulfur-containing compounds are critical contributors to the development of desirable beef flavor (Drumm and Spanier, 1991). Dimethyl sulfide was impacted by package type as HIOX and OW treatments possessed lesser ($P < 0.05$) amounts than ROLL and VAC; however, CO was similar ($P > 0.05$) to ROLL, VAC, and OW. Aerobic packaging environments seem to impact the presence of volatile flavor compounds that contribute to desirable flavor development. Package type had no effect ($P > 0.05$) on pyrazines, which provide desirable nutty, roasted, and caramelized flavors (Mottram, 1998; Moon et al., 2006). This suggests that undesirable flavor development caused by packaging environment is not caused by an absence of desirable flavor-promoting compounds but by an increase in the presence of compounds that promote off-flavors and aromas. Dimethyl sulfide was greater ($P < 0.05$) in the GM. Previously, it was shown that aldehydes formed from polyunsaturated fatty acids aid in the development of these sulfur-containing compounds (Drumm and Spanier, 1991). It may be speculated that an overall increase in aldehydes in the GM contributed to accumulation of dimethyl sulfide.

TBARS

Lipid oxidation was further measured utilizing the TBARS procedure. Package type ($P < 0.01$) and muscle ($P < 0.04$) had an impact on raw TBARS as shown in Table 8. The HIOX treatment had the greatest ($P < 0.05$) TBARS value compared with all other package types. Additionally, raw samples packaged in CO MAP had a greater ($P < 0.05$) TBARS value than VAC

Table 7. Least-squares means of Maillard-reaction–derived volatile flavor compounds from cooked *gluteus medius* and *longissimus lumborum* muscles

	Muscle			
Volatile Compound (ng/g)	GM	LL	SEM[1]	*P* Value
Ketones				
2,3-Butanedione	70.55[a]	51.37[b]	10.21	0.016
3-Hydroxy-2-butanone	92.44[a]	57.32[b]	9.31	0.002
2,3-Pentanedione	0.50	0.50	0.002	0.319
Pyrazines				
Methyl-pyrazine	0.02[b]	0.04[a]	0.006	0.029
Trimethylpyrazine	0.02	0.03	0.007	0.081
2,5-Dimethyl-pyrazine	0.06	0.09	0.01	0.052
2-Ethyl-3,5-dimethyl pyrazine	0.03	0.04	0.009	0.384
3-Ethyl-2,5-dimethyl pyrazine	0.05	0.06	0.01	0.348
Strecker Aldehydes				
Acetaldehyde	0.70	0.83	0.11	0.167
Benzaldehyde	4.93	4.45	0.61	0.336
Methional	0.06	0.06	0.009	0.840
Phenylacetaldehyde	0.53	0.54	0.01	0.641
2-Methyl butanal	0.08[b]	0.11[a]	0.01	0.024
3-Methyl butanal	0.48	0.42	0.04	0.308
Isobutyraldehyde	2.67	3.11	0.36	0.152
Sulfur Containing				
Carbon disulfide	3.53	4.00	0.78	0.503
Dimethyl disulfide	0.010	0.009	0.001	0.816
Dimethyl sulfide	0.93[a]	0.39[b]	0.05	<0.001
Dimethyl sulfone	0.42	0.56	0.12	0.052
Methanethiol	0.69	0.67	0.08	0.869
2-Methyl thiophene	0.03	0.03	0.01	0.767

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

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

GM = *gluteus medius*; LL = *longissimus lumborum*.

Table 8. Least-squares means of cooked and raw TBARS[1] (mg MDA/kg meat) of five package types[2] and two muscles[3]

		TBARS (mg MDA/kg meat)	
Package Type	Muscle	Raw	Cooked
CO		0.64[b]	1.31
HIOX		1.08[a]	1.59
OW		0.55[bc]	1.16
ROLL		0.48[bc]	1.15
VAC		0.36[c]	1.35
SEM[4]		0.12	0.29
P value		<0.001	0.320
	GM	0.73[a]	1.45[a]
	LL	0.51[b]	1.17[b]
	SEM	0.10	0.26
	P value	0.004	0.020

[1]Thiobarbituric acid reactive substances (TBARS).

[2]Package types include carbon monoxide modified atmosphere lidded trays (0.4% CO/30% CO_2/69.6% N_2 ["CO"]), high-oxygen modified atmosphere lidded trays (80% O_2/20% CO_2 ["HIOX"]), traditional overwrap ("OW"), rollstock (forming and non-forming films ["ROLL"]), and vacuum packaging without retail display ("VAC").

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

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

[a–c]Means within a column, specific to package type or muscle, lacking a common superscript differ ($P < 0.05$).

MDA = malondialdehyde.

samples. Multiple studies have reported that packaging type can impact TBARS (Luño et al., 2000; John et al., 2005; Clausen et al., 2009; Kim et al., 2010). A study conducted by Kim et al. (2010) reported an increase in lipid oxidation in high-oxygen MAP LL steaks over 9 d of display under fluorescent lighting, while TBARS in vacuum-packaged steaks exposed to similar display conditions remained the same throughout display. Luño et al. (2000) proposed that including at least 0.25% CO within a MAP gas mixture inhibits lipid oxidation as LL steaks packaged in a 70% O_2/20% CO_2/10% N_2 MAP possessed higher TBARS values. Similar to this study, CO did not display lipid oxidation to the same extent as HIOX although it displayed greater lipid oxidation than VAC, suggesting that the difference in lipid oxidation is potentially due to exposure to light. Clausen et al. (2009) reported that TBARS were similar between vacuum-packaged LD steaks and overwrapped LD steaks aged for 23 d postmortem and then placed in display conditions for 2 d. This finding agrees with the current study as ROLL and OW treatments did not differ.

Package type did not influence ($P > 0.05$) TBARS values in cooked samples. However, in both raw and cooked samples, the GM possessed a greater ($P < 0.05$) TBARS value than the LL; similar results were reported by Łopacka et al. (2017). The GM is a more unstable muscle and is therefore more susceptible to lipid oxidation than the LL (O'Keeffe and Hood, 1982). The GM contains less intramuscular fat than the LL; consequently, a higher proportion of the total fat content is composed of phospholipids that largely contribute to the increase in lipid oxidation between the two muscles (Donald, 1998; Enser et al., 1998; Faustman et al., 2010). Nonetheless, it is apparent that high-oxygen packaging environments are detrimental to the lipid stability of raw beef, affecting muscles of lower stability at a faster rate.

Protein oxidation byproducts

Raw sample carbonyl content was influenced by muscle type ($P < 0.01$) as shown in Table 9. The

Table 9. Least-squares means of carbonyl content (nmol/mg meat) and non-heme iron content (μg/g meat) from raw samples of five package types[1] and two muscles[2]

Package Type	Muscle	Carbonyl Content (nmol/mg meat)	Non-heme Iron (μg/g meat)
CO		1.39	0.73
HIOX		1.43	0.83
OW		1.39	0.70
ROLL		1.28	0.77
VAC		1.46	0.80
SEM[3]		0.13	0.06
P value		0.820	0.25
	GM	1.56[a]	0.89[a]
	LL	1.22[b]	0.64[b]
	SEM	0.08	0.05
	P value	0.001	<0.001
Muscle × Package Type			
P value		0.900	0.590

[1]Package types include carbon monoxide modified atmosphere lidded trays (0.4% CO/30% CO_2/69.6% N_2 ["CO"]), high-oxygen modified atmosphere lidded trays (80% O_2/20% CO_2 ["HIOX"]), traditional overwrap ("OW"), rollstock (forming and non-forming films ["ROLL"]), and vacuum packaging without retail display ("VAC").

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

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

[a,b]Means within a column, specific to package type or muscle, lacking a common superscript differ ($P < 0.05$).

GM possessed greater ($P < 0.05$) carbonyl content than the LL. Protein-oxidation–derived carbonyls are generated in meat products by direct oxidation of amino acid side chains via lipid-, myoglobin-, or metal-catalyzed oxidation (Stadtman and Levine, 2003; Estévez, 2011). The difference in carbonyl content within muscles corresponds to the difference in lipid oxidation found between the GM and LL as protein and lipid oxidation systems are capable of interacting and exchanging radicals to continue the degradative processes (Park et al., 2006). The larger proportion of unsaturated fatty acids make the GM more susceptible to lipid oxidation, potentially initiating and supporting an increased rate of protein oxidation.

There was no package type effect on carbonyl content ($P = 0.82$) as shown in Table 9. Similar to the findings in this study, Lund et al. (2007) found no differences in carbonyl content at 4, 8, and 14 d in pork LD samples stored in 70% O_2/30% CO_2 MAP and vacuum skin packaging displayed under fluorescent lighting. Contrastingly, a study conducted by Zakrys-Waliwander et al. (2012) reported higher carbonyl content in beef LD steaks packaged in high-oxygen MAP compared with vacuum packaging after 8 and 14 d stored at 4°C under fluorescent lighting. Fu et al. (2014) determined that beef LD steaks stored in darkness at 4°C packaged in vacuum packaging and high-oxygen MAP possessed a higher carbonyl content than OW after 4 d. However, after 10 d of storage, OW displayed a greater concentration of carbonyls than vacuum packaging and high-oxygen MAP. The differences in carbonyl content found in other studies due to package type may have been attributed to duration of storage and display, as samples in this study were stored in their respective package types for 7 d in darkness followed by 2 d in retail display conditions. Although there were no differences in protein oxidation as indicated by carbonyls, protein oxidation has been linked to a decrease in tenderness due to the formation of protein cross-links (Lund et al., 2007).

NHI content

NHI is the main promoter of oxidation found in meat systems being more relevant to oxidative potential than total iron content (Rhee et al., 1987; Min and Ahn, 2005). Package type had no effect ($P = 0.25$) on NHI content; however, NHI was higher ($P < 0.01$) in the GM than in the LL as shown in Table 9. Kanner (1994) reported NHI was higher in raw, dark muscles than white muscles in turkey and chicken. The same study found that NHI increased throughout 7 d of storage at 4°C in raw—dark and white—turkey and chicken samples. The results from a study completed on cooked and stored liver pâté by Estévez and Cava (2004) suggest a potential relationship between the release of NHI and protein oxidation. The increase in protein oxidation during storage in combination with varying chemical stability may explain the differences found in carbonyl and NHI content between LL and GM. However, Li et al. (2012) determined slices of cooked, cured ham packaged with films of various OTR and multiple intensities of lighting had no effect on NHI content.

PCA

PCA was completed for raw and cooked data separately. For the raw-data PCA, PC1 explained 46.0% and PC2 explained 31.0% of the variation associated with proximates, pH, carbonyls, NHI, volatiles, and TBARS of all muscle × package type combinations, as shown in Figure 1. PC1 separated HIOX packaging treatments from all other package types, while GM HIOX clustered with a majority of lipid-derived

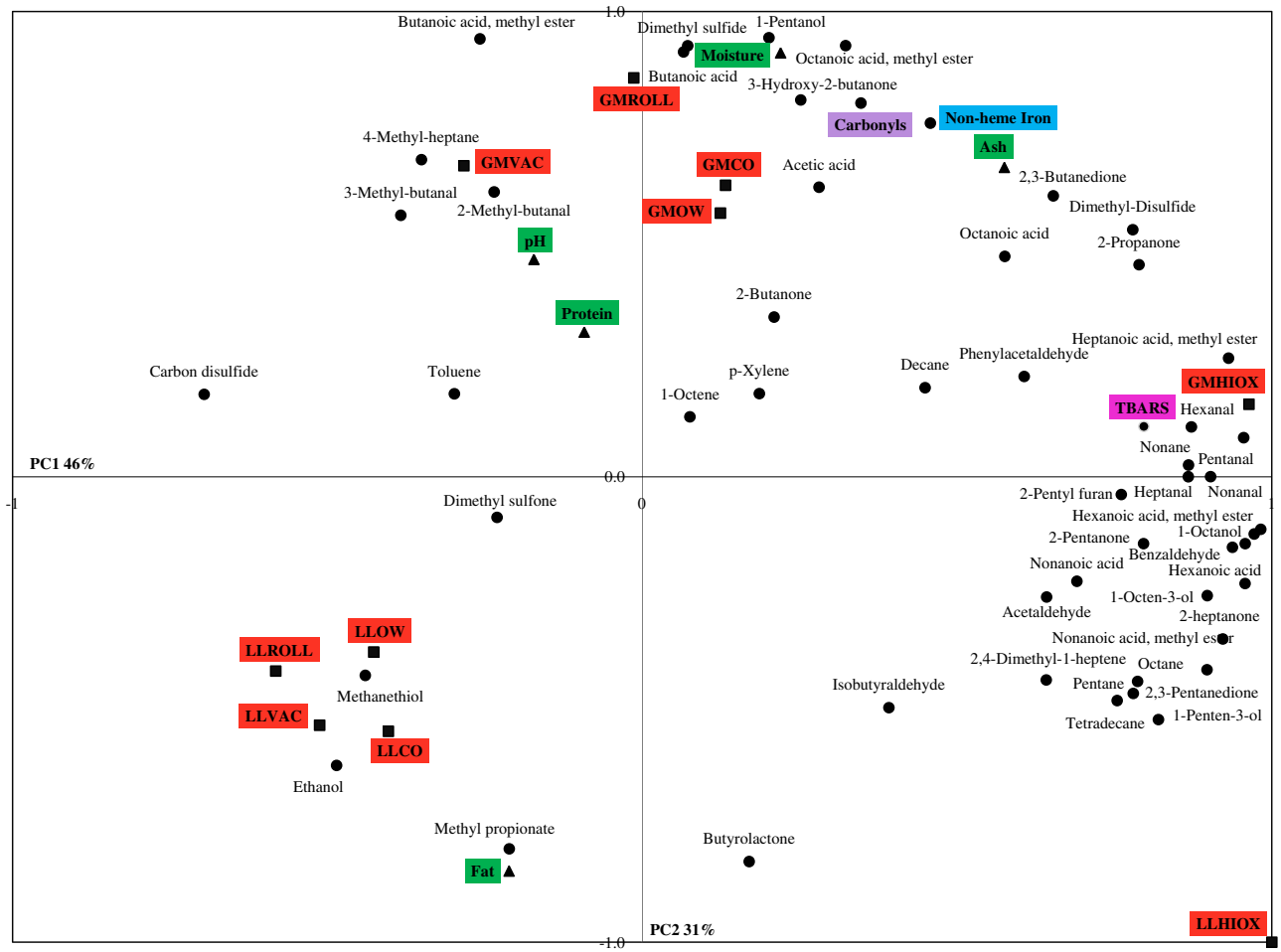

Figure 1. Principle component (PC) analysis for carbonyls, non-heme iron, pH, proximates, thiobarbituric acid reactive substances (TBARS), and volatile compounds of all muscle (*gluteus medius* [GM] and *longissimus lumborum* [LL]) × package type combinations of raw samples. Package types: carbon monoxide modified atmosphere lidded trays (0.4% CO/30% CO_2/69.6% N_2 ["CO"]), high-oxygen modified atmosphere lidded trays (80% O_2/20% CO_2 ["HIOX"]), traditional overwrap ("OW"), rollstock (forming and non-forming films ["ROLL"]), and vacuum packaging without retail display ("VAC").

volatile flavor compounds, including hydrocarbons, ketones, n-aldehydes, and alcohols. PC2 separated LL from GM, and corresponding proximate composition, moisture, ash, and protein were more associated with the GM. Similar to the findings of Legako et al. (2015), there were few volatiles surrounding the LL treatments, while fat content was more associated with the LL than the GM. Fat acts as a reservoir for soluble compounds, and products with less fat release a greater amount of volatile compounds than products that contain more fat (Chevance et al., 2000). The GM is clustered with a greater number of volatiles than the LL, which further supports this finding. Carbonyls and NHI clustered closer to the GM than the LL. An increase in oxidative products and prooxidants present in the GM is due to a lower chemical stability compared with the LL. Although TBARS are clustered in the same area as GM HIOX and other lipid oxidation compounds, it is more associated with the GM than the LL which corresponds to all other oxidation measurements done in the current study. Overall, the raw data PCA depicts a clear relationship between packaging environment and how the chemical stability of muscles can be influenced. These data imply that the production of undesirable volatile flavor compounds can be mitigated by selecting a packaging application that is effective at minimizing oxidation.

For the cooked-data PCA, PC1 explained 46.0% and PC2 explained 27.0% of the variation associated with consumer sensory scores, descriptive attribute scores, proximate composition, TBARS, and volatiles of all muscle × package type combinations, as shown in Figure 2. PC2 established a clear separation between muscles and corresponding chemical composition measurements; moisture, protein, and ash were more associated with the GM, whereas fat content was more associated with the LL. PC1 depicts GM HIOX as the treatment least associated with consumer sensory

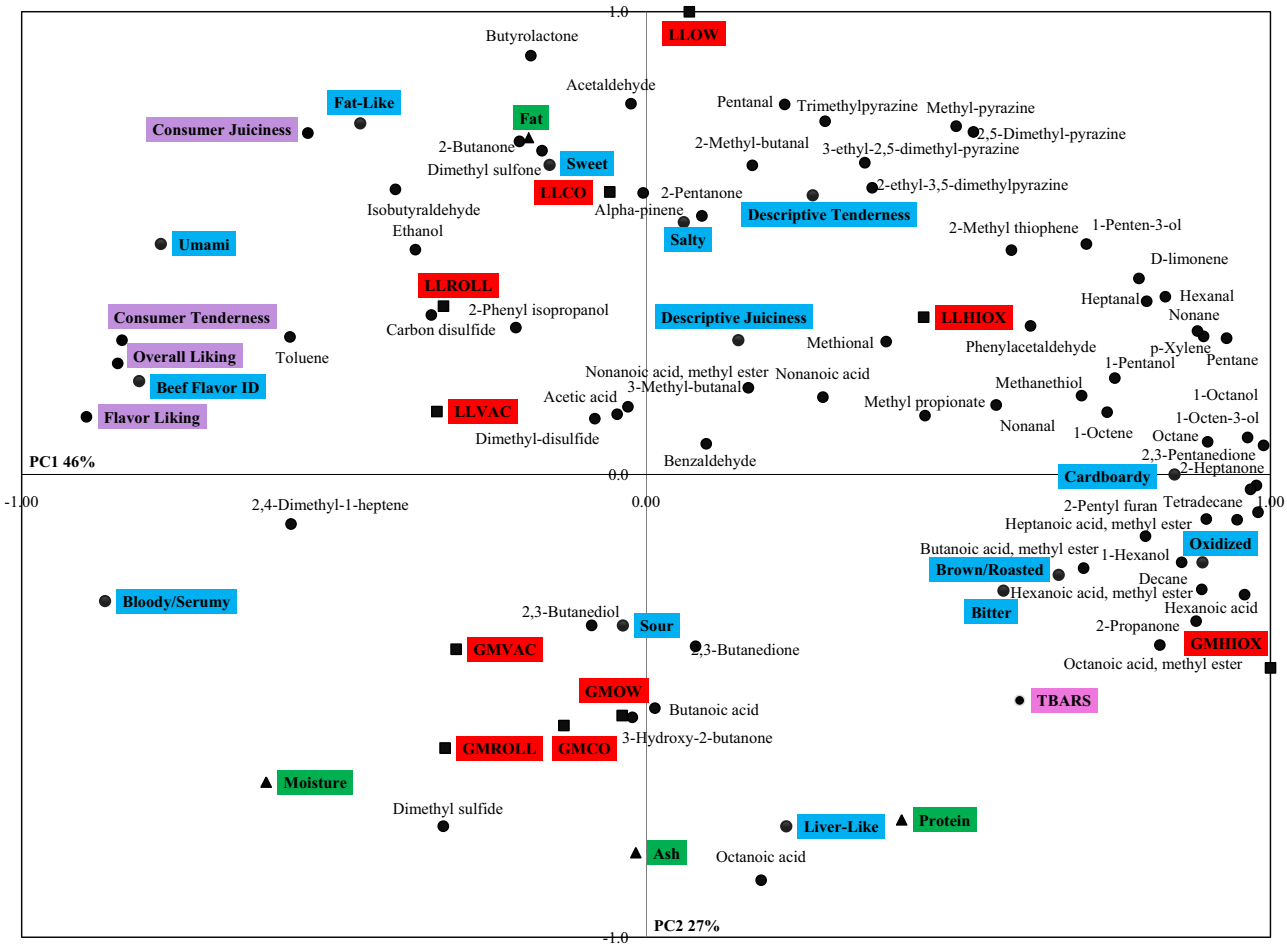

Figure 2. Principle component (PC) analysis for consumer sensory scores, descriptive attributes, proximates, thiobarbituric acid reactive substances (TBARS), and volatile compounds of all muscle (*gluteus medius* [GM] and *longissimus lumborum* [LL]) × package type combinations of cooked samples. Package types: carbon monoxide modified atmosphere lidded trays (0.4% CO/30% CO_2/69.6% N_2 ["CO"]), high-oxygen modified atmosphere lidded trays (80% O_2/20% CO_2 ["HIOX"]), traditional overwrap ("OW"), rollstock (forming and non-forming films ["ROLL"]), and vacuum packaging without retail display ("VAC").

scores including tenderness, juiciness, overall liking, and flavor liking. However, PC2 shows that consumer sensory scores are all clustered together and closer to the LL than the GM. The GM treatments were more associated with negative flavor attributes such as sour and liver-like, while PC2 depicts positive flavor attributes including sweet, fat-like, beef flavor identity, umami, and descriptive juiciness and tenderness grouped with the LL. The GM HIOX is surrounded by undesirable flavor attributes such as cardboardy, oxidized, and bitter. However, despite increased susceptibility to oxidative processes of less stable muscles, the LL HIOX was more associated with cardboardy, oxidized, and bitter than all other GM treatments in relation to PC1. This finding suggests that high-oxygen MAP systems are detrimental to flavor development no matter the stability of a muscle. The GM HIOX is grouped with numerous lipid-oxidation–derived volatile flavor compounds, including alcohols, ketones, and n-aldehydes. The LL HIOX placement in comparison to all other muscle × package type combinations suggests that LL is more stable than GM; however, the high-oxygen environment had a greater impact on the extent of oxidation compared with all other package types. Additionally, TBARS were grouped with GM HIOX and the surrounding negative flavor attributes. This further supports the correlation of increased oxidation in HIOX treatments and the influence it can have on the presence of undesirable flavor attributes. In relation to PC2, pyrazines were more associated with LL, suggesting that a greater presence of positive volatile compounds and the lack of negative lipid oxidation compounds explains overall acceptable palatability of LL compared with GM. Nonetheless, the cooked PCA presents a relationship between desirable palatability and package system.

Conclusions

The results of this study indicate that high-oxygen packaging environments are detrimental to beef flavor. Chemical stability of a particular muscle can influence the impact of various packaging systems on flavor development. Maillard reaction volatile flavor compounds such as pyrazines were similar between the 2 muscles, whereas lipid-oxidation–derived volatiles were more associated with the GM. This suggests that undesirable flavor formation is caused by an increase in the presence of negative volatile flavor compounds rather than an absence of positive flavor attributes. Nonetheless, there is a relationship between palatability and packaging application that can be better understood that might improve industry packaging decisions based on the chemical nature of a muscle.

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