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The Impact of Enhancement, Degree of Doneness, and USDA Quality Grade on Beef Flavor Development

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Abstract: The objective of this study was to determine the impact of enhancement, degree of doneness (DOD), and USDA quality grade on beef volatile flavor compounds from cooked strip loin steaks. This study also aimed to evaluate relationships between volatile compounds and consumer sensory responses. Beef strip loins (n = 72; 24/grade) of 3 quality grades (USDA Prime, Low Choice, and Low Select) were enhanced (8% of green weight with brine containing 0.35% salt and 0.4% sodium phosphate) or not enhanced, and cooked to 3 DOD (Rare: 60°C; Medium: 71°C; Very Well Done: 83°C) before volatile analysis. Volatile compounds were evaluated through a split-plot design where enhancement level and quality grade were used as the whole plot factors and DOD served as the subplot factor. Principal component analysis (PCA) was utilized to explore relationships between volatile compounds, consumer response, and treatments. The majority of compounds (n = 32) were impacted ($P < 0.05$) by the interaction between DOD × enhancement × quality grade. Four compounds- methanethiol, dimethyl-disulfide, methyl-benzene, and acetic acid were affected ($P < 0.05$) by enhancement × DOD. Two compounds- dimethyl sulfone and d-limonene, were impacted ($P < 0.05$) by quality grade × enhancement. Phenylacetaldehyde and hexanoic acid, methyl ester was affected ($P < 0.05$) by the interaction of USDA quality grade × DOD. The DOD main effect was only implicated in Maillard products ($P < 0.05$), including 2-methylbutanal, a Strecker aldehyde, and 2 pyrazines (methyl-pyrazine and trimethylpyrazine). Nonanal was the only compound impacted ($P < 0.05$) by a quality grade effect. No enhancement main effects were observed for any compounds ($P > 0.05$). In agreement, PCA indicated volatile compound production was primarily driven by degree of doneness and quality grade. There was no strong link between enhancement and beef volatile flavor compound development, despite the dramatically improved flavor liking scores from consumers.

Keywords: beef, degree of doneness, enhancement, flavor, volatile compounds
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Introduction

Flavor has been well established as a main contributor to beef palatability. Recent studies revolving around beef quality factors have indicated an increased focus on flavor by consumers (Corbin et al., 2015; Lucherk et al., 2016; Wilfong et al., 2016; Nyquist et al., 2018; Vierck et al., 2018). These studies indicated that over 50% of the respondents selected flavor as the most important beef attribute when consuming beef. This has drastically increased from approximately 30% in older studies (Huffman et al., 1996). Additionally, flavor has been strongly correlated (r = 0.88, 0.85) to consumer overall liking (O'Quinn et al., 2012; Legako et al., 2015a). Beef flavor can be evaluated in several different ways, including consumer panel evaluation and volatile flavor compound production. Volatile flavor compounds contribute substantially to flavor (Mottram, 1998; Legako et al., 2015a). These compounds are formed through various pathways of flavor development, including the Maillard reaction and thermal lipid degradation (Mottram, 1993, 1998).

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Enhancement is a processing strategy used by the beef industry to provide a more consistent eating experience of beef products (Vote et al., 2000; Baublits et al., 2006). Typically, an enhancement solution consists of water, salt, and phosphates to improve flavor and juiciness (Vote et al., 2000). However, when evaluating the effects of enhancement on beef volatile flavor compounds, there has been minimal research previously conducted. When investigating the effects of enhancement and different aging times on 10 different muscles, Stetzer et al. (2008) evaluated 10 compounds from various flavor development pathways. No differences were observed for all compounds between enhanced and non-enhanced *Longissimus dorsi* steaks, with the exception of butanoic acid, a lipid derived carboxylic acid (Elmore et al., 1999; Stetzer et al., 2008). Butanoic acid and other lipid derived carboxylic acids are typically associated with off-flavors produced during of lipid oxidation (Ercolini et al., 2011; Gardner and Legako, 2018).

In addition to providing a consistent eating experience, enhancement is also used to ensure that palatability traits are maintained at higher degrees of doneness (Drey et al., 2018). Previous studies which asked consumers their preferred DOD had approximately 41 to 52% of consumer panel participants who responded with medium or medium-rare (McKillip et al., 2017; Vierck et al., 2018). However, little research has evaluated the effects of degree of doneness (DOD) on volatile flavor compound production. Gardner and Legako (2018) reported that as DOD increased, there was a relative increase in concentration in Maillard products. Yet, this trend was not as well defined in lipid degradation products (Gardner and Legako, 2018).

Quality grade has long been a primary indicator of the eating experience of beef **(**Legako et al., 2016**;** O'Quinn et al., 2018**)**. The influence of USDA quality grade on beef flavor volatile compound production is not well defined and inconsistent in the literature. Previous studies indicate that increasing intramuscular lipid content does not necessarily lead to an increase in volatile compounds (Legako et al., 2015b; Hunt et al., 2016; O'Quinn et al., 2016). However, Gardner and Legako (2018) reported substantially greater concentrations of several volatile compounds from both the Maillard reaction and lipid degradation in USDA Prime steaks in comparison to Low Choice and Standard steaks. This indicates that increased concentration of lipids may actually contribute to flavor changes through both reactions. Furthermore, with the addition of enhancement, lower quality grades, such as USDA Select and USDA Low Choice have shown drastic improvements in consumer flavor liking scores (Lucherk et al., 2016). However, minimal research has been conducted to investigate the chemical factors that may impact flavor enhancement. Additionally, the interaction between marbling and enhancement has not been well investigated, especially at higher quality grades.

Therefore, the objective of this study was to determine the impact of enhancement, DOD, and USDA quality grade on beef volatile flavor compounds from cooked strip loin steaks. Second, this study aimed to evaluate relationships between volatile compounds and consumer sensory responses.

Materials and Methods

Product selection and fabrication

Product selection and fabrication was previously described in detail by McKillip et al. (2017). Beef strip loins (Institutional Meat Purchasing Specifications [IMPS] #180; NAMP, 2010; N = 72; 24/grade) were procured from beef carcasses of 3 quality grades: USDA Prime, Low Choice, and Low Select. At 2 d post-mortem, selected carcasses were fabricated into wholesale subprimals. Beef loins selected for this study were immediately collected, vacuum packaged, and transported to the Kansas State Meat Laboratory (Manhattan, KS) under refrigerated conditions (2°C) for fabrication. In total, strip loins were aged for 21 d post-mortem at 2 to 4°C. At 14 d of aging, one-half of each quality grade (n = 12) were injected with an enhancement solution consisting of 0.35% salt and 0.40% sodium phosphate (Brifisol 512, ICL Food Specialties, Saint Louis, MO) to reach a target pump of 8% of the final product weight. Following enhancement, strip loins were vacuum packaged and continued to age at 2 to 4°C for an additional 7 d.

Following aging, all strip loins were fabricated into 2.54-cm thick steaks from anterior to posterior using a slicer (Berkel X13A-Plus, Berkel, Inc, Houston, TX). After removal of the most anterior face steak, the following 12 steaks (anterior to posterior) were grouped into sets of 4 steaks that were randomly assigned to 1 of 3 DOD: rare (60°C; RARE), medium (70°C; MED) and very well done (82°C; VWD). Within each DOD section, 1 steak was designated for volatile flavor analysis and consumer sensory evaluation. Following fabrication, all steaks were vacuum packaged and frozen at -20°C until further analysis.

Cooking procedures

Prior to cooking, all steaks were thawed at 2 to 4°C for 24 h. Following thawing, remaining external fat and accessory muscles (*Gluteus medius* and *Multifidus dorsi*) were removed prior to cooking. Steaks were then cooked on a clamshell grill (Cuisinart Griddler Deluxe, Cuisinart, East Windsor, NJ) to the previously assigned DOD. During cooking, thermocouples (30-gauge copper and constant; Omega Engineering, Stamford, CT) were used to monitor internal temperature. Peak temperature was confirmed using a hand-held thermocouple (Model HH509, Omega Engineering, Stamford, CT).

Volatile compound analysis

Immediately following cooking, steaks (n = 214; 12/treatment) were subjected to volatile compound analysis, performed using the protocol of Gardner and Legako (2018). Five 1.27-cm cores were removed from each steak, immediately perpendicular to the steak surface. After removal, the cores were ground for 10 s using a coffee grinder (KRUPS, Medford, MA; Type #F203). Five grams of the ground sample were weighed into 20-mL glass vials (093640–036–00; Gerstel, Linthicum, MD) and sealed using a polytetrafluoroethylene septa and screw cap (093640–092–00; Gerstel). After sealing, 10 microliters of internal standard (1,2-dichlorobenzene; 0.801 mg/ml) was added to the ground sample. The vials were then loaded using a Gerstel automated sampler (MPS) for a 5 min incubation period at 65°C in the Gerstel agitator. Following incubation, a 20 min extraction phase was used to collect volatile compounds from the headspace of the cooked samples, using solid phase microextraction (SPME) with an 85 mm film thickness carboxen polydimethylsiloxane fiber (Supelco, Bellfonte, PA). After extraction, volatile compounds were desorbed and separated with a VF-5 MS capillary column (30 m × 0.25 mm × 1.00 mm; Agilent J&W GC Columns, Santa Clara, CA). Authentic standards (Sigma-Aldrich, St. Louis, MO) were used to validate compound identities by matching ion fragmentation patterns and retention times. Quantitation was achieved by internal standard calibration with the same authentic standards.

Consumer panel evaluation

The Kansas State University Institutional Review Board approved the procedures used in this study (IRB 7440, 21 Nov. 2014). Consumer panel evaluation is described in detail by McKillip et al. (2017). Briefly, untrained consumer panelists (n = 252) were recruited from the Manhattan, KS area. Panelists were rewarded monetarily for their time. Thirty-six panels, consisting of 7 consumers, were held at the KSU Sensory Laboratory, where panelists were placed in individual sensory booths and fed all samples under red incandescent lighting to mask DOD differences present within the samples.

Panelists evaluated each sample for tenderness, juiciness, flavor, and overall liking using continuous 100-point line scales. Verbal anchors were located at endpoints and the midpoints of the scale, where 0 = extremely dislike/extremely tough/extremely dry; 50 = neither like or dislike/neither tough nor tender/neither dry or juicy; 100 = extremely like/extremely tender/extremely juicy.

Statistical analysis

Statistical analysis was completed using the GLIMMIX procedure of SAS (Version 9.4; SAS Inst. Inc., Cary, NC). For volatile compound analysis, a split-plot design was used. To aid in the analysis of this study, quality treatment was used to define the combination of USDA quality grade and enhancement level to accomplish the objectives of the study. In the model, the quality treatment was used as the whole plot and DOD served as the subplot. For all three-way interactions, the SLICE function of SAS was used with DOD serving as the SLICE option to restrict comparisons between quality treatments within the same degree of doneness. The Kenward-Roger approximation was used on all analyses to estimate the denominator degrees of freedom and for means separation, a protected t-test was used with the PDIFF option. Statistical significance was determined at $\alpha \leq 0.05$. Principal component analysis (PCA) was conducted on volatile compounds and consumer sensory results using the FACTOR procedure of SAS. Data were pre-processed to reflect a normal distribution using meat centering and standardization techniques. Two principal components, principal component 1 and principal component 2, were used to evaluate volatile compound and consumer sensory results relationships with treatment groups (x = principal component 1, y = principal component 2).

Results and Discussion

Enhancement × degree of doneness × quality grade interactions

Fifty-three compounds were evaluated during this study. The compounds of interest were derived from

Table 1. Interaction between degree of doneness (DOD), enhancement[1] (EN), and USDA quality grade[2] (QG) for volatile compounds produced from Strecker degradation through the Maillard reaction from grilled beef strip steaks (n = 214). Three-way interaction was sliced by DOD using the SLICE function of SAS[3]

	Volatile compound, ng/g of cooked sample			
Treatment	Acetaldehyde	3-Methylbutanal	Methional	Benzaldehyde
Rare, 60°C				
Non-enhanced				
Prime	43.64	6.30	0.37	1.74^{b}
Low Choice	32.99	5.00	0.34	1.99^{b}
Low Select	38.30	5.75	0.46	2.68^{b}
Enhanced				
Prime	35.68	8.73	0.64	2.24^{b}
Low Choice	43.32	11.76	1.07	4.52^{a}
Low Select	37.28	10.57	0.67	3.04^{ab}
SEM[4]	5.04	3.55	0.28	0.81
P-value	0.20	0.29	0.08	< 0.01
Medium, 71°C				
Non-enhanced				
Prime	30.88	12.78	0.98	2.86^{b}
Low Choice	37.95	12.32	0.64	3.84^{ab}
Low Select	28.88	11.80	0.82	4.03^{ab}
Enhanced				
Prime	29.80	14.98	1.13	2.79^{b}
Low Choice	31.95	10.29	0.93	2.89^{b}
Low Select	36.33	15.47	1.31	5.06^{a}
SEM[4]	5.01	3.54	0.27	0.79
P– value	0.34	0.65	0.17	0.02
Very Well Done, 82°C				
Non-enhanced				
Prime	43.50^{a}	28.28^{a}	1.92^{b}	5.30^{b}
Low Choice	45.16^{a}	22.88^{abc}	1.46^{bc}	7.33^{a}
Low Select	36.31^{a}	16.50^{c}	1.00^{c}	6.88^{a}
Enhanced				
Prime	31.88^{b}	18.08^{bc}	1.32^{c}	2.91^{c}
Low Choice	42.68^{a}	23.92^{ab}	2.46^{a}	6.17^{ab}
Low Select	44.40^{ab}	26.00^{a}	1.91^{b}	6.36^{ab}
SEM[4]	5.03	3.54	0.27	0.79
P-value	0.04	0.01	< 0.01	< 0.01
DOD × QG × EN				
P-value	0.03	0.03	< 0.01	0.04

$^{a–c}$Least squares means within the same column within a degree of doneness without a common superscript are different ($P < 0.05$).

[1]Enhanced (8% of green weight with brine containing 0.35% salt and 0.4% sodium phosphate, or not enhanced).

[2]USDA Prime, Low Choice, and Low Select.

[3]Version 9.4, SAS Inst. Inc., Cary, NC.

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

Table 2. Interaction between degree of doneness (DOD), enhancement[1] (EN), and USDA quality grade[2] (QG) for volatile compounds produced from the Maillard reaction from grilled beef strip steaks (n = 214). Three-way interaction was sliced by DOD using the SLICE function of SAS[3]

	Volatile compound, ng/g cooked sample			
Treatment	Dimethyl sulfide	Carbon disulfide	2,5-Dimethylpyrazine	2,3-Butanedione
Rare, 60°C				
Non-enhanced				
Prime	15.73	15.08^{c}	0.09	65.24
Low Choice	12.81	17.25^{bc}	0.03	49.98
Low Select	10.81	26.65^{ab}	0.08	51.98
Enhanced				
Prime	14.84	14.81^{c}	0.07	67.71
Low Choice	15.92	29.32^{a}	0.11	79.00
Low Select	17.53	21.21^{abc}	0.12	59.94
SEM[4]	2.81	5.89	0.23	10.26
P-value	0.17	0.04	0.99	0.08
Medium, 71°C				
Non-enhanced				
Prime	9.81^{b}	7.65^{c}	0.25	45.19^{bc}
Low Choice	17.17^{a}	31.04^{a}	0.12	67.02^{a}
Low Select	8.16^{b}	25.13^{ab}	0.31	38.79^{c}
Enhanced				
Prime	11.29^{b}	10.54^{c}	0.27	59.75^{ab}
Low Choice	8.97^{b}	17.03^{bc}	0.06	46.44^{bc}
Low Select	17.47^{a}	28.68^{a}	0.24	59.67^{ab}
SEM[4]	2.74	5.74	0.18	10.01
P-value	< 0.01	< 0.01	0.71	0.04
Very Well Done, 82°C				
Non-enhanced				
Prime	14.36^{a}	11.89^{cd}	1.07^{a}	59.35
Low Choice	16.77^{a}	21.05^{abc}	0.55^{b}	60.59
Low Select	11.66^{ab}	30.81^{a}	0.34^{b}	44.91
Enhanced				
Prime	8.95^{b}	7.84^{d}	0.27^{b}	43.53
Low Choice	12.50^{ab}	19.47^{bc}	0.31^{b}	52.58
Low Select	16.52^{a}	25.77^{ab}	0.59^{b}	50.81
SEM[4]	2.75	5.74	0.18	10.02
P-value	0.03	< 0.01	< 0.01	0.39
DOD × QG × EN				
P-value	0.03	0.01	0.01	< 0.01

$^{a–d}$Least squares means within the same column within a degree of doneness without a common superscript are different ($P < 0.05$).

[1]Enhanced (8% of green weight with brine containing 0.35% salt and 0.4% sodium phosphate or not enhanced).

[2]USDA Prime, Low Choice, and Low Select.

[3]Version 9.4, SAS Inst. Inc., Cary, NC.

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

major beef flavor development pathways, such as the Maillard reaction and thermal lipid degradation. No compound was impacted by enhancement alone ($P >$ 0.05). The majority of compounds (n = 32) evaluated were impacted by the three-way interaction of quality grade, degree of doneness, and enhancement. Due to

Table 3. Interaction between degree of doneness (DOD), enhancement[1] (EN), and USDA quality grade[2] (QG) for volatile compounds produced from lipid degradation from grilled beef strip steaks (n = 214). Three-way interaction was sliced by DOD using the SLICE function of SAS[3]

	Volatile compound, ng/g cooked sample					
Treatment	1-Hexanol	1-Penten-3-ol	1-Octen-3-ol	1-Octanol	2-Pentyl furan	Octane
Rare, 60°C						
Non-enhanced						
Prime	0.74^{ab}	0.69^{b}	1.06^{b}	8.95	1.37^{cd}	1.02^{c}
Low Choice	0.52^{bc}	0.34^{b}	0.78^{b}	8.60	1.29^{cd}	0.97^{c}
Low Select	0.89^{a}	0.55^{b}	1.45^{ab}	14.20	2.41^{a}	2.31^{ab}
Enhanced						
Prime	0.38^{c}	0.41^{b}	0.79^{b}	7.08	1.02^{d}	1.21^{bc}
Low Choice	0.92^{a}	1.27^{a}	2.10^{a}	13.07	2.10^{ab}	2.71^{a}
Low Select	0.48^{bc}	0.49^{b}	1.15^{b}	10.18	1.61^{bc}	2.19^{abc}
SEM[4]	0.16	0.22	0.37	2.97	0.29	0.65
P-value	< 0.01	< 0.01	< 0.01	0.12	< 0.01	0.02
Medium, 71°C						
Non-enhanced						
Prime	0.35^{c}	0.52	0.58^{b}	4.02^{c}	1.08^{c}	0.85^{b}
Low Choice	0.69^{ab}	0.59	0.94^{b}	10.11^{ab}	1.53^{bc}	1.94^{b}
Low Select	0.48^{bc}	0.48	0.80^{b}	8.01^{bc}	1.87^{b}	1.27^{b}
Enhanced						
Prime	0.28^{c}	0.50	0.65^{b}	5.46^{bc}	1.03^{c}	0.77^{b}
Low Choice	0.37^{c}	0.43	1.07^{b}	6.42^{bc}	1.40^{bc}	1.29^{b}
Low Select	0.78^{a}	0.78	2.06^{a}	13.96^{a}	2.71^{a}	3.75^{a}
SEM[4]	0.16	0.21	0.36	2.97	0.28	0.64
P-value	< 0.01	0.56	< 0.01	< 0.01	< 0.01	< 0.01
Very Well Done, 82°C						
Non-enhanced						
Prime	0.20	0.81	0.37	4.53	0.95^{bc}	0.49^{c}
Low Choice	0.36	0.66	0.65	10.95	1.42^{ab}	1.39^{bc}
Low Select	0.30	0.51	0.68	6.54	1.69^{a}	1.85^{ab}
Enhanced						
Prime	0.16	0.39	0.42	4.09	0.83^{c}	0.60^{c}
Low Choice	0.32	0.47	0.69	6.55	1.23^{abc}	1.63^{bc}
Low Select	0.41	0.73	0.92	6.54	0.51^{a}	3.03^{a}
SEM[4]	0.16	0.22	0.36	7.39	0.29	0.64
P-value	0.59	0.29	0.69	0.90	0.02	< 0.01
DOD × QG × EN						
P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

$^{a–d}$Least squares means within the same column within a degree of doneness without a common superscript are different ($P < 0.05$).

[1]Enhanced (8% of green weight with brine containing 0.35% salt and 0.4% sodium phosphate or not enhanced).

[2]USDA Prime, Low Choice, and Low Select.

[3]Version 9.4, SAS Inst. Inc., Cary, NC.

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

the overwhelming amount of three-way interactions, 6 compounds of interest will be discussed in depth: carbon disulfide, butanal, 3-methylbutanal, hexanal, methional, and 2,5-dimethylpyrazine. These compounds are representative markers of both the Maillard reaction and lipid degradation. All compounds impacted by the three-way interactions are present in Tables 1 through 6.

Methional and 3-methylbutanal are representatives of Strecker degradation during the Maillard reaction. Strecker aldehydes are produced through the reactions of individual amino acids with reductones or dehydroreductones, which are compounds formed during the dehydration step and Amadori or Heynes rearrangement of the Maillard reaction (Mottram, 1993; Resconi et al., 2013). Methional is produced using me-

Table 4. Interaction between degree of doneness (DOD), enhancement[1] (EN), and USDA quality grade[2] (QG) for lipid-derived aldehydes produced from lipid degradation of grilled beef strip steaks (n = 214). Three-way interaction was sliced by DOD using the SLICE function of SAS[3]

	Volatile compound, ng/g cooked sample				
Treatment	Butanal	Hexanal	Heptanal	Octanal	Pentanal
Rare, 60°C					
Non-enhanced					
Prime	2.16	116.57^{b}	3.84^{bc}	2.99	4.99^{b}
Low Choice	2.00	81.77^{b}	3.76^{bc}	3.29	3.21^{b}
Low Select	2.55	129.55^{ab}	6.43^{ab}	6.58	6.03^{b}
Enhanced					
Prime	3.27	89.76^{b}	3.26^{c}	2.54	3.89^{b}
Low Choice	4.82	183.34^{a}	8.45^{a}	6.13	10.72^{a}
Low Select	4.02	111.38^{b}	4.80^{bc}	4.10	4.90^{b}
SEM[4]	1.65	29.41	1.54	1.79	2.03
P-value	0.43	< 0.01	< 0.01	0.09	< 0.01
Medium, 71°C					
Non-enhanced					
Prime	4.57	100.64^{b}	2.63^{c}	1.84^{c}	4.76
Low Choice	5.83	127.48^{ab}	6.31^{ab}	5.89^{ab}	6.43
Low Select	4.60	111.17^{b}	5.34^{bc}	4.75^{abc}	5.53
Enhanced					
Prime	5.14	84.95^{b}	2.78^{c}	2.25^{c}	3.96
Low Choice	3.68	109.92^{b}	3.60^{bc}	2.73^{bc}	4.80
Low Select	6.95	175.53^{a}	8.25^{a}	7.82^{a}	9.46
SEM[4]	1.61	28.67	1.51	1.75	1.29
P-value	0.35	0.02	< 0.01	< 0.01	0.05
Very Well Done, 82°C					
Non-enhanced					
Prime	11.99^{a}	53.91	2.55	2.54	3.41
Low Choice	10.64^{ab}	91.73	4.17	3.66	5.36
Low Select	8.32^{bc}	106.84	4.69	4.37	6.76
Enhanced					
Prime	6.45^{c}	47.73	2.01	1.80	2.45
Low Choice	9.80^{ab}	72.16	3.99	3.69	4.29
Low Select	11.26^{ab}	100.00	4.98	4.55	6.11
SEM[4]	1.62	28.78	1.51	1.75	1.99
P-value	< 0.01	0.18	0.26	0.57	0.21
DOD × QG × EN					
P-value	0.02	< 0.01	< 0.01	0.02	< 0.01

$^{a–c}$Least squares means within the same column within a degree of doneness without a common superscript are different ($P < 0.05$).

[1]Enhanced (8% of green weight with brine containing 0.35% salt and 0.4% sodium phosphate or not enhanced).

[2]USDA Prime, Low Choice, and Low Select.

[3]Version 9.4, SAS Inst. Inc., Cary, NC.

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

Table 5. Interaction between degree of doneness (DOD), enhancement[1] (EN), and USDA quality grade[2] (QG) for lipid-derived carboxylic acids produced from lipid degradation of grilled beef strip steaks (n = 214). Three-way interaction was sliced by DOD using the SLICE function of SAS[3]

	Volatile compound, ng/g cooked sample			
Treatment	Heptanoic acid	Hexanoic acid	Nonanoic acid	Octanoic acid
Rare, 60°C				
Non-enhanced				
Prime	0.27	2.32^{ab}	3.92	0.20
Low Choice	0.32	1.47^{c}	26.06	0.18
Low Select	0.33	1.90^{bc}	26.45	0.21
Enhanced				
Prime	0.33	1.94^{bc}	12.35	0.22
Low Choice	0.34	3.01^{a}	14.52	0.25
Low Select	0.31	2.07^{bc}	8.35	0.24
SEM[4]	0.08	0.45	15.33	0.07
P-value	0.96	0.02	0.54	0.96
Medium, 71°C				
Non-enhanced				
Prime	0.30	1.79^{bc}	27.97^{bc}	0.16^{b}
Low Choice	0.39	2.37^{ab}	58.41^{a}	0.36^{a}
Low Select	0.29	1.59^{bc}	20.33^{c}	0.18^{b}
Enhanced				
Prime	0.30	1.46^{c}	4.40^{c}	0.13^{b}
Low Choice	0.27	1.55^{bc}	20.33^{c}	0.18^{b}
Low Select	0.35	2.83^{a}	52.38^{ab}	0.40^{a}
SEM[4]	0.08	0.45	14.50	0.07
P-value	0.60	< 0.01	< 0.01	< 0.01
Very Well Done, 82°C				
Non-enhanced				
Prime	0.32^{b}	1.96	12.55	0.23
Low Choice	0.31^{b}	2.13	27.78	0.24
Low Select	0.29^{b}	1.72	19.34	0.23
Enhanced				
Prime	0.27^{b}	1.05	22.35	0.15
Low Choice	0.52^{a}	1.53	38.71	0.21
Low Select	0.31^{b}	2.16	3.62	0.23
SEM[4]	0.23	0.45	14.31	0.07
P-value	0.02	0.10	0.11	0.70
DOD × QG × EN				
P-value	0.04	< 0.01	< 0.01	< 0.01

$^{a–c}$Least squares means within the same column within a degree of doneness without a common superscript are different ($P < 0.05$).

[1]Enhanced (8% of green weight with brine containing 0.35% salt and 0.4% sodium phosphate or not enhanced).

[2]USDA Prime, Low Choice, and Low Select.

[3]Version 9.4, SAS Inst. Inc., Cary, NC.

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

thionine, whereas 3-methylbutanal is produced from leucine (Resconi et al., 2013; Dashdorj et al., 2015). Once sliced by DOD, both compounds exhibited no differences within RARE or MED steaks ($P > 0.05$). However, within VWD, methional was produced in the greatest amount by enhanced Low Choice steaks ($P <$

Table 6. Interaction between degree of doneness (DOD), enhancement[1] (EN), and USDA quality grade[2] (QG) for ketones produced from lipid degradation of grilled beef strip steaks (n = 214). Three-way interaction was sliced by DOD using the SLICE function of SAS[3]

	Volatile compound, ng/g of cooked sample				
Treatment	2-Propanone	2-Butanone	2-Pentanone	2-Heptanone	2,3-Pentanedione
Rare, 60°C					
Non-enhanced					
Prime	38.00^{ab}	15.15	1.19^{ab}	0.160^{b}	1.51
Low Choice	20.02^{c}	7.27	0.74^{c}	0.124^{b}	1.50
Low Select	32.59^{abc}	13.30	0.94^{bc}	0.231^{b}	1.52
Enhanced					
Prime	44.92^{a}	10.15	1.35^{a}	0.203^{b}	1.52
Low Choice	27.88^{bc}	12.36	0.99^{bc}	0.366^{a}	1.55
Low Select	33.39^{abc}	11.67	1.12^{ab}	0.255^{ab}	1.52
SEM[4]	6.98	3.29	0.15	0.07	0.02
P-value	0.01	0.20	< 0.01	< 0.01	0.05
Medium, 71°C					
Non-enhanced					
Prime	29.00	13.20	0.81^{bc}	0.125^{b}	1.52^{b}
Low Choice	29.77	13.94	0.99^{ab}	0.183^{b}	1.52^{b}
Low Select	24.23	12.44	0.74^{bc}	0.178^{b}	1.52^{b}
Enhanced					
Prime	30.54	11.34	1.19^{a}	0.156^{b}	1.52^{b}
Low Choice	19.18	8.98	0.69^{c}	0.182^{b}	1.52^{b}
Low Select	35.81	14.76	1.13^{a}	0.471^{a}	1.56^{a}
SEM[4]	6.87	3.22	0.15	0.07	0.02
P-value	0.21	0.51	< 0.01	< 0.01	0.04
Very Well Done, 82°C					
Non-enhanced					
Prime	38.15	23.93^{a}	1.05	0.152^{b}	1.56
Low Choice	33.32	21.55^{ab}	0.92	0.216^{b}	1.53
Low Select	35.73	22.59^{ab}	0.80	0.216^{b}	1.54
Enhanced					
Prime	28.54	11.84^{c}	0.85	0.131^{b}	1.54
Low Choice	27.77	17.55^{bc}	0.82	0.194^{b}	1.57
Low Select	33.24	19.41^{ab}	1.12	0.365^{a}	1.56
SEM[4]	6.92	3.25	0.15	0.07	0.02
P-value	0.63	< 0.01	0.15	0.01	0.08
DOD × QG × EN					
P-value	0.03	0.02	< 0.01	< 0.01	0.02

$^{a–c}$Least squares means within the same column within a degree of doneness without a common superscript are different ($P < 0.05$).

[1]Enhanced (8% of green weight with brine containing 0.35% salt and 0.4% sodium phosphate or not enhanced).

[2]USDA Prime, Low Choice, and Low Select.

[3]Version 9.4, SAS Inst. Inc., Cary, NC.

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

0.05), but all other treatments were similar ($P > 0.05$). Previous research indicates that methional is produced in higher concentrations in lower, more acidic pH products, which is not indicated in the current study, as enhanced Low Choice VWD steaks produced the greatest concentration (Meynier and Mottram, 1995; Dashdorj et al., 2015). Enhanced steaks in the current study had higher pH values in comparison to non-enhanced steaks (McKillip et al., 2017).

In comparison, 3-methylbutanal was produced in the lowest quantities ($P < 0.05$) by VWD non-enhanced Low Select steaks and VWD enhanced Prime steaks. All other treatments produced a greater concentration ($P < 0.05$) of 3-methylbutanal. As a Strecker aldehyde, 3-methylbutanal is produced as an intermediate of the

Maillard reaction (Resconi et al., 2013; Dashdorj et al., 2015). As an intermediate of the reaction, 3-methylbutanal could further interact with other compounds such as hydrogen sulfide or ammonia to form other flavor products during the cooking process, such as thiazoles, as well as undergo heterocyclization and thus appear in the lowest concentration in VWD steaks.

Similar trends were observed for 2,5-dimethylpyrazine, a pyrazine produced during the Maillard reaction. Once again, no differences ($P > 0.05$) were observed between treatments for RARE or MED steaks. However, 2,5-dimethylpyrazine was present in the greatest concentration ($P < 0.05$) in VWD non-enhanced Prime steaks. In comparison, all other treatments had a similar concentration ($P > 0.05$) that was lower ($P < 0.05$) than VWD non-enhanced Prime steaks. Pyrazines are a final product of the Maillard reaction (Resconi et al., 2013; Dashdorj et al., 2015). Prime steaks have the greatest concentration of intramuscular fat, which may influence the rate at which heterocyclic compounds such as pyrazines are formed (Gardner and Legako, 2018). These results contrast those observed by O'Quinn et al. (2016), who observed Low Choice steaks produced a greater amount of 2,5-dimethylpyrazine in comparison to other steaks in a wide range of marbling scores and antemortem production characteristics. However, a different methodology was used between studies. Namely, evaluation of patties by O'Quinn et al. (2016) and evaluation of steaks in this study. Previous work has indicated that steaks will produce a greater concentration of volatile compounds than patties and Prime steaks will produce a greater amount of volatiles than Standard steaks (Gardner and Legako, 2018).

For carbon disulfide, a sulfur-containing compound produced during the Maillard reaction, different concentrations were present within each DOD ($P < 0.01$; Table 2). Within RARE, enhanced Low Choice steaks produced a greater concentration of carbon disulfide ($P < 0.05$) than enhanced and non-enhanced Prime steaks and non-enhanced Low Choice steaks, but was similar to non-enhanced and enhanced Low Select steaks ($P > 0.05$). Within MED, enhanced Low Select and non-enhanced Low Choice exhibited the greatest concentration of carbon disulfide ($P < 0.05$) in comparison to both enhanced and non-enhanced Prime as well as enhanced Low Choice but was similar to non-enhanced Low Select. Lower quality grades once again produced a greater concentration ($P < 0.01$) of carbon disulfide within VWD. Non-enhanced Low Select, Low Choice and non-enhanced Low Select steaks produced a greater concentration of carbon disulfide than all other treatments ($P < 0.05$). Additionally, enhanced Low Choice exhibited a greater concentration than enhanced Prime ($P < 0.05$), which produced the lowest concentration of carbon disulfide ($P < 0.05$). Previous research has indicated no differences between quality grades within the *Longissimus lumborum* for carbon disulfide concentration (Hunt et al., 2016). This may contribute to the conflicted effects of quality grade within each DOD.

Butanal and hexanal, 2 aldehydes produced through lipid oxidation were selected due to their negative influence on flavor (Shahidi and Pegg, 1994; Legako et al., 2015b). Butanal was not different ($P > 0.05$) between treatments for RARE or MED, however, was influenced in the VWD group ($P < 0.05$; Table 4). Within VWD, enhanced Prime and non-enhanced Low Select steaks produced the lowest concentration of butanal in comparison to all other treatments ($P < 0.05$). In direct contrast, hexanal was influenced within the RARE and MED groups ($P < 0.05$; Table 4). In the RARE group, hexanal was produced in the greatest concentration in the enhanced Low Choice and non-enhanced Low Select ($P < 0.05$), in comparison to all other treatments. However, in the MED group, it was the non-enhanced Low Choice and the enhanced Low Select that exhibited hexanal in the greatest amounts ($P < 0.05$). These variations in hexanal and butanal may be explained by increased lipid oxidation that occurs in beef with lower intramuscular fat content (Wood et al., 2003, 2008; Legako et al., 2015a). As lipids are deposited intramuscularly, a concurrent increase in the neutral lipid fraction results, as the neutral lipid fraction is primarily composed of triglycerides, which are more stable due to a greater concentration of saturated fatty acids. (De Smet et al., 2004; Wood et al., 2008). In contrast, the polar lipid fraction is primarily composed of phospholipids, which are more susceptible to lipid oxidation, due to their increased levels of monounsaturated and polyunsaturated fatty acids (De Smet et al., 2004; Wood et al., 2008). As intramuscular fat content is reduced, and thus resulting in a reduced quality grade, there is a subsequent increase in the proportion of the polar fraction in total lipids (Legako et al., 2015a). Therefore, the lower quality grades (Low Choice and Select) would be more susceptible to lipid oxidation and more likely to produce a greater amount of hexanal and other lipid oxidation products.

Enhancement × degree of doneness interactions

Methanethiol and dimethyl-sulfide, acetic acid, and methylbenzene were impacted by the interaction of enhancement and DOD ($P \leq 0.005$; Table 7). For all 3 compounds, non-enhanced VWD steaks pos-

Table 7. Interaction between enhancement[1] (EN) and degree of doneness (DOD) treatment for volatile compounds produced from grilled beef strip steaks ($n = 214$)

	Volatile compound, ng/g of cooked sample			
Treatment	Methanethiol	Dimethyl-disulfide	Methyl-benzene	Acetic acid
Enhanced[1]				
Rare, 60°C	0.62^{c}	0.14^{bc}	0.46^{d}	57.91^{a}
Medium, 71°C	0.91^{c}	0.11^{c}	0.41^{cd}	45.62^{b}
Very Well Done, 83°C	2.14^{b}	0.23^{b}	0.72^{b}	32.09^{c}
Non-enhanced				
Rare, 60°C	0.63^{c}	0.25^{b}	0.41^{d}	47.25^{ab}
Medium, 71°C	1.09^{bc}	0.15^{bc}	0.61^{bc}	45.79^{b}
Very Well Done, 83°C	5.28^{a}	0.52^{a}	0.98^{a}	52.20^{ab}
SEM[2]	0.41	0.43	0.05	4.40
EN × DOD				
P-value	< 0.01	< 0.01	< 0.01	< 0.01

$^{a–d}$Least squares means within the same column without a common superscript are different ($P < 0.05$).

[1]Enhanced (8% of green weight with brine containing 0.35% salt and 0.4% sodium phosphate or not enhanced).

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

sessed the greatest concentration ($P < 0.05$), followed by enhanced VWD steaks and non-enhanced MED steaks ($P < 0.05$). However, for methanethiol concentration, non-enhanced MED steaks were similar ($P > 0.05$) to non-enhanced RARE as well as enhanced MED and RARE steaks. For dimethyl-disulfide concentration, non-enhanced MED and enhanced RARE were similar ($P > 0.05$) to both enhanced MED and non-enhanced RARE. However, non-enhanced RARE steaks possessed a greater concentration of dimethyl-disulfide ($P < 0.05$) than enhanced MED steaks.

Sulfur-containing compounds, such as methanethiol and dimethyl-sulfide, are primary contributors to meat flavor, largely due to their low detection thresholds (Resconi et al., 2013). Additionally, these compounds are present in low concentrations and are very reactive with other flavor compounds within beef products (Resconi et al., 2013). These compounds have been negatively correlated with bloody/serumy and brown/roasted flavors (r = -0.42; Legako et al., 2016). Due to the reactivity of sulfur-containing products, this could have contributed to the differences observed between concentrations of dimethyl-disulfide and methanethiol for MED and RARE treatments.

In comparison, methylbenzene, more commonly known as toluene, was present in the greatest concentration in non-enhanced VWD steaks ($P < 0.05$), followed by enhanced VWD steaks, which were similar to non-enhanced MED steaks ($P > 0.05$). Non-enhanced MED steaks were also similar to enhanced MED steaks for methylbenzene concentration ($P > 0.05$). Both enhanced and non-enhanced RARE steaks possessed the lowest concentration of methylbenzene ($P < 0.05$). Toluene is a homocyclic hydrocarbon which is typically developed during lipid oxidation and has been associated with grass-fed beef flavors (Tansawat et al., 2013; Watanabe et al., 2015). Since toluene is produced during degradation of fatty acids, it could have been produced during thermal degradation of lipids during cooking, which explains its increased concentration in steaks cooked to increased DOD, such as VWD or MED (Min et al., 1977; Dashdorj et al., 2015; Watanabe et al., 2015). The results in the current study are similar to that of other DOD studies (Ji et al., 2010). When cooking steaks from the *Longissimus lumborum* to 50, 70, or 90°C, Ji et al. (2010) observed a concurrent increase in toluene concentration with an increase in DOD, which indicates that toluene could be produced through thermal degradation of fatty acids.

Within enhanced steaks, acetic acid concentration was inversely related with DOD. As DOD increased, there was a reduction in acetic acid concentration ($P < 0.05$). However, within non-enhanced steaks, all DOD were similar ($P > 0.05$) for acetic acid concentration. Acetic acid can be formed from the Maillard reaction, specifically Strecker degradation from alanine, as well as being applied as an organic acid as a microbial hurdle (Mikel et al., 1996; Resconi et al., 2013). Strecker degradation acid products, such as acetic acid, are more readily formed at an increased pH in comparison to lower pH, which explains why significant results were observed with in enhanced steaks (Hofmann et al., 2000). This data indicates that acetic acid, if being formed by the Maillard reaction, is occurring in the beginning of the reaction as an early intermediate rather than an end product, as it is present in greater concentration in lower DOD, such as RARE.

Enhancement × quality grade interactions

Two compounds, dimethyl sulfone and d-limonene were affected by the interaction of quality grade and enhancement ($P \leq 0.04$; Table 8). Enhanced Low Choice steaks possessed a greater amount ($P < 0.05$) of dimethyl sulfone than enhanced Prime steaks, as well as non-enhanced Low Choice and Select steaks. However, it was similar to enhanced Low Select and non-enhanced Prime steaks ($P > 0.05$). Enhancement increased the production of dimethyl sulfone for lower quality grades. A similar trend was observed for d-limonene, a lipid-derived monoterpene that is associated with fruity and sweet odors (Shi et al., 2013).

Table 8. Interaction between enhancement[1] (EN) and USDA quality grade[2] (QG) treatment for volatile compounds from produced grilled beef strip steaks ($n = 214$)

	Volatile compound, ng/g of cooked sample	
Treatment	Dimethyl sulfone	D-limonene
Enhanced		
Prime	22.64^{bc}	347.34^{b}
Low Choice	48.18^{a}	700.46^{a}
Low Select	27.07^{abc}	306.60^{b}
Non-enhanced		
Prime	38.83^{ab}	304.03^{b}
Low Choice	13.63^{c}	399.80^{b}
Low Select	8.79^{c}	509.09^{ab}
SEM[3]	8.31	143.14
QG × EN		
P-value	0.01	0.04

$^{a–c}$Least squares means within the same column without a common superscript are different ($P < 0.05$).

[1]Enhanced (8% of green weight with brine containing 0.35% salt and 0.4% sodium phosphate or not enhanced).

[2]USDA Prime, Low Choice, and Low Select.

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

Enhanced Low Choice steaks and non-enhanced Low Select steaks produced a greater amount of d-limonene ($P < 0.05$) than all other treatments.

Typically, sulfur-containing compounds are produced at a greater concentration in meat products with a lower, more acidic pH (Meynier and Mottram, 1995; Dashdorj et al., 2015). Additionally, dimethyl sulfone has been associated with pasture-fed beef flavor and has been positively correlated with negative flavors such as barny ($r = 0.97$), bitter ($r = 0.90$), gamey ($r = 0.43$), and grassy ($r = 0.75$; Tansawat et al., 2013). It has also been negatively related with juiciness ($r = -0.55$) and umami ($r = -0.81$; Tansawat et al., 2013). Contrasting results were observed in the current study, as non-enhanced steaks produced a lower concentration of dimethyl sulfone. This indicates that the enhancement may mask the negative impacts of dimethyl sulfone on flavor, as when fed to consumers, enhanced steaks outperformed non-enhanced steaks substantially, with the exception of Prime (McKillip et al., 2017).

Degree of doneness × quality grade interactions

An interaction between quality grade and DOD ($P \leq 0.01$) was observed for 3 compounds: phenylacetaldehyde; butanoic acid, methyl ester; and hexanoic acid, methyl ester (Table 9). Phenylacetaldehyde, a Strecker aldehyde produced during the Maillard reaction from the amino acid phenylalanine, generally

Table 9. Interaction between degree of doneness (DOD) and USDA quality grade[1] (QG) treatment for volatile compounds produced from grilled beef strip steaks ($n = 214$)

	Volatile compound, ng/g of cooked sample		
Treatment	Phenylacetaldehyde	Butanoic acid, methyl ester	Hexanoic acid, methyl ester
Rare, 60°C			
Prime	0.22^{d}	19.55^{a}	76.96^{a}
Low Choice	0.29^{cd}	13.61^{b}	52.06^{b}
Low Select	0.20^{d}	10.50^{b}	41.36^{b}
Medium, 71°C			
Prime	0.49^{b}	4.43^{c}	14.05^{c}
Low Choice	0.32^{cd}	3.63^{cd}	12.34^{c}
Low Select	0.20^{bc}	2.72^{cd}	10.94^{c}
Very Well Done, 83°C			
Prime	0.69^{a}	0.50^{d}	1.47^{c}
Low Choice	0.76^{a}	0.77^{cd}	2.37^{c}
Low Select	0.53^{b}	0.55^{d}	1.69^{c}
SEM[2]	0.57	1.41	5.99
QG × DOD			
P-value	< 0.01	< 0.01	0.01

$^{a–d}$Least squares means within the same column without a common superscript are different ($P < 0.05$).

[1]USDA Prime, Low Choice, and Low Select.

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

increased in concentration with a concurrent increase in DOD (Dashdorj et al., 2015). Phenylacetaldehyde was present in the greatest amounts in Prime and Low Choice steaks cooked to VWD ($P < 0.05$). Low Select VWD steaks were similar ($P > 0.05$) to Prime and Low Select MED steaks for phenylacetaldehyde concentration. Low Choice MED steaks were similar to all RARE treatments for phenylacetaldehyde ($P > 0.05$).

Phenylacetaldehyde has generally been regarded as a positive flavor compound in beef products. When comparing carcasses of 3 different USDA quality grades, Legako et al. (2016) observed a positive correlation ($r = 0.43$) with consumer overall liking and tenderness scores. Additionally, the results of the current study are in agreement with Gardner and Legako (2018), which observed a significant increase in phenylacetaldehyde with a concurrent increase in USDA quality grade and DOD, as the greatest concentration observed in that study were Prime steaks cooked to 77°C.

An opposing trend was observed for the interaction between quality grade and DOD for both butanoic and hexanoic acid, methyl ester, 2 fatty acids created through lipid degradation during cooking (Kerth and Miller, 2015). As DOD increased from RARE to VWD, both compounds were present in the greatest amount ($P < 0.05$) in Prime RARE steaks, then Low Choice

and Select RARE steaks ($P < 0.05$). All RARE treatments were greater than MED or VWD ($P < 0.05$). For hexanoic acid, methyl ester, no differences were observed between MED or VWD for any of the 3 quality grades ($P > 0.05$), which indicates that once cooked to a MED DOD, there is no differentiation in hexanoic acid, methyl ester concentration. In comparison, a similar trend for RARE was observed for butanoic acid, methyl ester; however, MED Prime steaks possessed a higher concentration ($P < 0.05$) of butanoic acid, methyl ester than Prime or Low Select VWD steaks. All other MED and VWD treatments were similar ($P > 0.05$) in butanoic acid, methyl ester concentration. Stetzer et al. (2008) investigated the impact of enhancement and aging on 10 different muscles and 10 different volatile compounds, with a specific focus on compounds associated with lipid oxidation. Within this study, the concentration of butanoic acid was 5 times higher in enhanced steaks in comparison to non-enhanced steaks (Stetzer et al., 2008). This may be due to butanoic acid's role as a derivative of the lipid oxidation reaction (Stetzer et al., 2008; Ercolini et al., 2011; Gardner and Legako, 2018). Butanoic acid and other carboxylic acids, such as hexanoic acid, have been positively associated with livery off-flavors ($r = 0.22$; Stetzer et al., 2008). However, this relationship with liver-like off flavors was not established in the current study.

Main effects

Three compounds, 2-methylbutanal, methylpyrazine, and trimethylpyrazine, were impacted by the DOD ($P \leq 0.04$; Table 10). Methylpyrazine, trimethylpyrazine, and 2-methylbutanal are pyrazines and a Strecker aldehyde produced by the Maillard reaction. The greatest concentration of these 3 compounds was observed in VWD steaks ($P < 0.05$). Additionally, 2-methylbutanal concentration increased with a concurrent increase in DOD (RARE < MED < VWD; $P < 0.05$). However, no differences were observed for concentration of both pyrazines in MED and RARE steaks ($P > 0.05$).

Degree of doneness has been associated with increased production of Maillard derived compounds (Mottram, 1993; Resconi et al., 2013). In the current study, 2-methylbutanal, a Strecker aldehyde, methylpyrazine, and trimethylpyrazine, were present in the greatest concentration in VWD. This is in agreement with previous research, as Gardner and Legako (2018) reported an increasing concentration of trimethylpyrazine in steaks as DOD simultaneously increased. This was echoed throughout the study for both methylpyrazine and 2-methylbutanal which were present in the greatest concentration at VWD in the present study and well done (77°C) in the previous study (Gardner and Legako, 2018).

Table 10. Least squares means for volatile compounds derived from beef *longissimus lumborum* steaks ($n = 214$)

	Volatile compound, ng/g of cooked sample			
Treatment	2-Methyl-butanal	Methyl-pyrazine	Trimethyl-pyrazine	Nonanal
Degree of doneness				
Rare, 60°C	4.49[c]	0.059[b]	0.076[b]	
Medium, 71°C	11.57[b]	0.111[b]	0.112[b]	
Very Well Done, 83°C	25.52[a]	0.270[a]	0.207[a]	
SEM[1]	1.54	0.036	0.042	
P-value	< 0.01	< 0.01	< 0.01	
USDA quality grade[2]				
Prime				2.94[c]
Low Choice				6.22[b]
Low Select				7.41[a]
SEM				0.92
P-value				< 0.01

[a–c]Least squares means within the same column without a common superscript are different ($P < 0.05$).

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

[2]USDA Prime, Low Choice, and Low Select.

One compound, nonanal, was impacted by the quality grade main effect ($P < 0.05$). Nonanal concentration was inversely related with USDA quality grade, as Prime steaks produced the lowest concentration of nonanal ($P < 0.05$), followed by Low Choice, and Low Select having the greatest concentration of nonanal ($P < 0.05$). Previous research on the impact of USDA quality grade on volatile compound production has been varied. Legako et al. (2015b) and O'Quinn et al. (2016) reported no differences due to quality grade within the *Longissimus lumborum*. However, these studies were conducted using whole muscle samples or ground patties in comparison to more recent research, which used a minced sample. In comparison, Gardner and Legako (2018) reported substantial differentiation between quality grades for both Maillard derived and lipid degradation compounds within steaks. Prime steaks generally produced the greatest concentration of various volatile compounds in comparison to Low Choice or Standard steaks (Gardner and Legako, 2018). Similar to O'Quinn et al. (2016), there was less differentiation between quality grades for volatile compound production within ground patties (Gardner and Legako, 2018). For nonanal specifically, the lone compound impacted by the quality grade main effect in the present study, Gardner and Legako (2018) reported a concurrent increase in nonanal concentration with an increase in USDA quality grade, however, non-

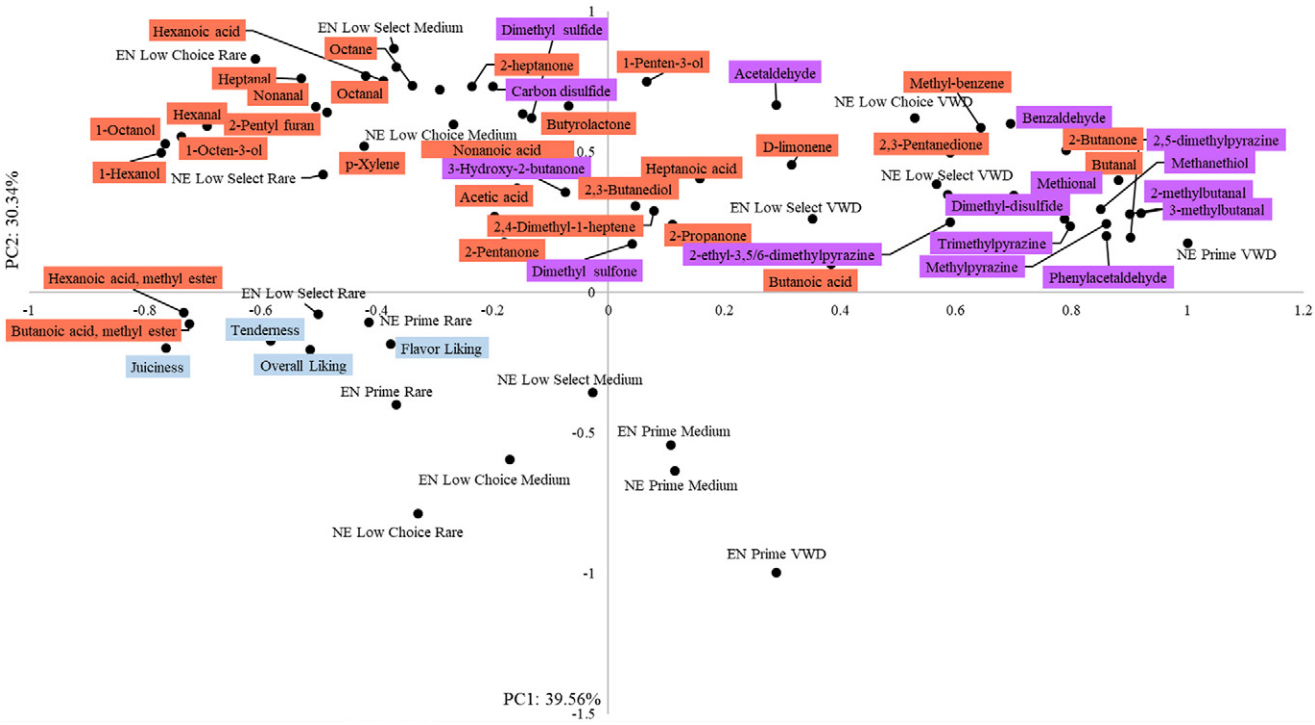

Figure 1. Principal component analysis relating volatile compound analysis to consumer ratings of grilled beef strip loin steaks of 3 USDA quality grades (USDA Prime, Low Choice, and Low Select) cooked to 3° of doneness (Rare, 60°C; Medium, 71°C; Very Well Done [VWD], 83°C) and either enhanced (8% of green weight with brine containing 0.35% salt and 0.4% sodium phosphate; EN) or non-enhanced (NE). Products derived from the Maillard reaction are highlighted in purple and lipid derived compounds are highlighted in red. Consumer liking scores are highlighted in light blue.

anal concentration also was reduced with an increase in DOD. In comparison, the present study exhibited the direct opposite trend, where nonanal concentration was reduced as USDA quality grade increased, despite using the same sampling methodology. Nonanal, as an aldehyde produced during lipid oxidation, contributes to warmed over flavors and negatively impacts flavor perception (Kerth and Miller, 2015).

Within the current study, no compound was solely impacted by enhancement ($P < 0.05$). In agreement with this finding, Stetzer et al. (2008) observed no differences between enhanced and non-enhanced steaks for all compounds within the *Longissimus dorsi*, with the exception of butanoic acid, as previously discussed.

Principal component analysis

Principal component analysis (PCA) was conducted to observe the relationships between DOD, enhancement, and quality grade (Fig. 1). Consumer ratings and volatile compounds were used to determine the principal components (PC). When PCA was conducted, PC1 represented 39.56% and PC2 represented 30.34% of the variation. Principal component 1 segregated volatile compounds by DOD. Rare steaks were associated with lipid degradation products, such as lipid derived alcohols, aldehydes, and esters. Medium steaks were associated with a combination of Maillard products and lipid degradation products. This resulted in MED steaks being most closely associated with total volatile products, which may be due to MED steaks garnering both Maillard and lipid degradation products, instead of primarily one or the other. Additionally, MED steaks were most closely associated with sulfur-containing compounds. Very Well Done steaks were most closely associated with Maillard products, such as Strecker aldehydes and pyrazines. However, VWD steaks were negatively related with consumer tenderness, juiciness, flavor and overall liking, whereas rare steaks were positively and more closely related with those traits.

However, when comparing the volatile compound analysis to results produced from the consumer sensory analysis, it is interesting to note that the consumers resoundingly preferred enhanced product, regardless of quality grade, for flavor liking (McKillip et al., 2017). In addition to the additional moisture, the addition of salt with the sodium phosphate may be the contributing factor. Consumer panelists may have preferred enhanced beef with addition of salt, as it is a contributing flavor enhancer, rather than

non-enhanced steaks without the contribution of salt. Previous research with soups have indicated that salt is a stronger influence on flavor, rather than lipids with consumer panelists (Bolhuis et al., 2018). Within the chemical analysis, however, there would not be bias toward the saltier sample, rather a more quantitative, objective look at the compounds produced.

Conclusions

Volatile compound production in beef is primarily driven by degree of doneness and quality grade. There is no strong link between enhancement and beef flavor development from a chemical standpoint, but it has a dramatic impact on consumer beef flavor liking scores.

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