

Volatile flavor compounds vary by beef product type and degree of doneness¹

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ABSTRACT: This study aimed to determine how quality grade and degree of doneness (DOD) influence the development of volatile compounds among beef whole muscle and ground patties. Volatile compounds were quantified via head space solid phase microextraction from samples tempered in refrigerated temperatures (3 to 5 °C), room temperature (24 to 26 °C), or cooked on an electric clamshell-style grill to an endpoint temperature of 55, 60, 71, or 77 °C. Collected samples were subsequently determined by gas chromatography mass spectrometry. Prominent compounds known to be the result of the Maillard reaction or lipid degradation were retained for comparison. Four Strecker aldehydes, 4 pyrazines, and one ester had a 3-way interaction between quality grade, DOD, and product type (each $P < 0.001$). Pyrazine concentrations did not differ ($P > 0.05$) in ground patties and was comparably greater ($P < 0.05$) in steaks; in Prime and Low Choice steaks, pyrazine concentration increased ($P < 0.05$) as DOD increased. A 2-way interaction between quality

grade and product type was observed for acetaldehyde, dimethyl disulfide, 1-penten-3-ol, butanoic acid, hexanal, octanal, nonanal, and 2-heptanone. Among which, octanal and nonanal were greater ($P < 0.05$) in Prime steaks compared with ground patties. Another 2-way interaction, quality grade and DOD, was observed in 2 ketones, an alcohol, 2 esters, and 2 aldehydes. For example, 2,3-butanedione was greater ($P < 0.05$) in concentration in Prime 4 °C samples compared with Low Choice and Standard. The final 2-way interaction of DOD and product type was observed in 3 ketones, 2 sulfur compounds, 2 esters, 5 aldehydes, 2 carboxylic acids, and a ketone. For example, 2-heptanone was greater ($P < 0.05$) in concentration in ground patties compared to steaks in all degrees of doneness except 4 °C. Overall, these results indicate that the volatile flavor profile of beef is greatly influenced by product type and DOD. Generally, consumers select beef based on product type and determine their cookery approach. Therefore, consumers may greatly influence final beef flavor profile.

Key words: beef flavor, degree of doneness, lipid degradation, Maillard reaction, quality grade

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INTRODUCTION

Flavor is a combination of taste and odor and requires a combination of olfactory and gustatory senses. Volatile compounds contribute to the aroma portion of flavor and thus play a large role in flavor perception (Legako et al., 2015).

Intramuscular lipids are a source of many volatiles that are present in high concentrations even in lean muscle (Bailey and Einig, 1989; Buckholz, 1989). Some studies have found, however, that increased intramuscular fat (i.e., higher quality grades) has rarely produced increases in volatile flavor compounds (Cross et al., 1980; Mottram et al., 1982; Mottram and Edwards, 1983). Legako et al. (2015) found that among 26 quantified compounds, none differed due to quality grade alone. Other studies suggest that fat acts as a solvent and retains volatile compounds, thus delaying flavor

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release (Chevance and Farmer, 1999; Chevance et al., 2000; Farmer et al., 2013). Volatile compounds are generated from nonvolatile water-soluble precursors and lipids via multiple reactions resulting from lipid oxidation and thermal degradation. The reaction between lipids or lipid degradation products and Maillard intermediates creates reactions that compete with the lipid oxidation reaction; these competing reactions may affect the amount and type of volatile compounds formed (Mottram, 1994). Compared to cooked beef, raw beef has not received much attention by way of volatile compound research (King et al., 1993; Insausti et al., 2002). The effect of heat on sugars and amino acids directly relates to Strecker degradations and Maillard reactions, which are important contributors to volatile compound formation (MacLeod, 1994). It is possible that during cooking, some volatile compounds are degraded as fast as they are formed because of their participation in further reactions, resulting in what seems to be little or no change in the levels of the compounds toward the end of the Maillard reaction (Balagiannis et al., 2010). The objective of this study was to evaluate volatile compound development in response to varied degree of doneness (DOD) across multiple product types.

MATERIALS AND METHODS

Product Selection

Paired beef strip loins [IMPS 180 (NAMP, 2010)] were collected from 24 carcasses across 3 USDA quality grades (Prime, Low Choice, and Standard, $n = 8$ per quality grade; USDA, 2017) of "A" maturity animals. Carcasses were selected at a commercial beef processing plant in the Intermountain West after approximately 24 h postmortem chilling. Carcass measures included hot carcass weights (kg), external fat thickness (mm), ribeye area (cm^2), skeletal maturity, lean maturity, marbling scores, and percentages of kidney, pelvic, and heart fat. Yield grade was calculated as $\{2.50 + [0.0984252 \times \text{fat thickness (mm)}] - [0.0496 \times \text{REA (cm}^2)] + [0.20 \times \text{KPH}\%] + [0.008378 \times \text{HCW (kg)}]\}$. Carcasses representing USDA Prime had a minimum marbling score of Slightly Abundant⁰⁰ (700) or greater, USDA Low Choice carcasses were within Small⁰⁰ (400) to Small⁹⁹ (499), and USDA Standard carcasses had Traces⁹⁹ (299) or lower marbling score based on comparison with standard photographs (National Cattlemen's Beef

Association, Centennial, CO). Paired strip loins from each selected carcass were collected following fabrication by plant personnel and transported under vacuum and refrigeration (4 °C) to the Utah State University Meat laboratory. Intact strip loins were stored under vacuum, in darkness, and under refrigeration (4 °C) until 21 d postmortem.

Processing

At day 21 of postmortem aging, loins were removed from packaging to produce steaks and ground patties. Strip loins were cut into 2.54-cm thick steaks progressing anterior to posterior using a meat slicer (Globe Food Equipment Co., Model 3600N, Dayton, OH). All external fat and minor muscles were removed. Additionally, more posterior steaks containing the Gluteus medius were excluded leaving only the Longissimus lumborum muscle within sample steaks. Steaks were randomly assigned to a raw or cooked DOD, as determined by internal temperature [4 (raw), 25 (raw), 55 (rare), 60 (medium), 71 (medium well), or 77 °C (well done)]. Steaks were then individually vacuum sealed and stored at -20 °C until analysis. Steaks throughout the paired loins were also randomly designated for grinding. Grinding was carried out on fully-denuded and heavy connective tissue-free remaining Longissimus lumborum muscle combined from previously cut steaks designated for grinding. Grinding was achieved by using a grinder (Hobart, Model 4i52, Troy, OH) equipped with a 0.64-cm plate. Following grinding, ground material was stuffed into approximately 50-mm diameter, plastic perforated casings (Package Concepts and Materials, Inc., Item A712X42HP100, Greenville, SC) and frozen at -20 °C. Resulting in a single frozen chub per loin which were subsequently sliced on a band saw (American Meat Equipment, LLC, Butcher Boy, Model SA-16, Selmer, TN) into 1.9-cm patties and assigned to various degrees of doneness for cooking and subsequent chemical analysis.

Cooking Procedure

Before cooking, steak and patty samples were allowed to thaw under refrigeration (4 °C) for at least 12 h but no more than 24 h to a temperature range of 3 to 5 °C. The samples designated to represent 4 °C were taken directly from refrigeration; their raw temperatures were recorded and any remaining subcutaneous fat was removed from the steak samples, leaving only the intramuscular fat. The steak and patty samples designated to

represent 25 °C were tempered in an incubator (140 Series, Model 12-140E, Quincy Lab, Inc., Chicago, IL) for approximately 2 h after first being thawed to 3 to 5 °C. The remaining steak and patty samples were cooked on an electrical clamshell-style grill (Cuisinart Griddler Deluxe, Model GR-150, Cuisinart, East Windsor, NJ) to an internal temperature of 55 (rare), 60 (medium), 71 (medium well), or 77 °C (well done) after being thawed to a temperature of 3 to 5 °C. Before cooking or tempering, the raw temperature of each sample was recorded, and the steak samples were removed of any subcutaneous fat, identical to the procedure for samples designated as 4 °C. The average grill plate surface temperature was 245 °C. Internal temperature of the steaks and patties was monitored via an Omega Engineering MDSSi8 series benchtop 10-channel thermometer (Omega Engineering Inc., Stamford, CT) with a 5TC series thermocouple wire (Omega Engineering Inc., Stamford, CT). The final temperature reached, grill temperature, and cook time was recorded for each cooked sample.

Volatile Compound Analysis

Volatile analysis was carried out similar to the method described by Legako et al. (2015). After the steak samples were tempered or cooked to the required temperature, five 1.27-cm diameter cores cut perpendicular to the steak cut surface were extracted and minced in a coffee bean grinder (KRUPS, Medford, MA; Type #F203). After the ground patties were tempered or cooked to the required temperature, each patty was cut into quarters and minced in the same coffee bean grinder. Five grams of the resulting minced sample were weighed into 20 mL glass GC vials, 10- μ L of an internal standard (1,2-dichlorobenzene, 0.801 mg/mL) were added to each vial, and the vials were capped with polytetrafluoroethylene septa and screw caps (Gerstel, Linthicum, MD). The vials were loaded by a Gerstel automated sampler (MPS, Linthicum, MD) into the Gerstel agitator for a 5-min incubation period at 65 °C. The vials were then subjected to 20 min of extraction, during which volatile compounds were extracted via headspace solid phase microextraction (SPME) using a polydimethylsiloxane fiber (Supelco, Bellefonte, PA). The extracted volatile compounds were injected onto a capillary column (30 m \times 0.25 mm \times 1.00 μ m; Agilent J&W GC Columns, Santa Clara, CA). Selective ion monitoring in the scan mode was used to collect the data. Volatile compound identity was confirmed by comparing the data to external standards. An

internal standard calibration was used to quantitate the data. Volatile concentrations were calculated as amount extracted (ng) per sample weight.

Statistical Analysis

Statistical analysis was performed by SAS version 9.4 (SAS Institute, Cary, NC) using the GLIMMIX procedure. A 3-way analysis of variance was utilized to determine the influence of the fixed effects (quality grade, whole muscle vs. ground, and DOD). Means were separated by protected *t*-test using the LSMEANS/PDIFF option. The statistical significance was determined at $P \leq 0.05$. The experimental design included a whole plot, sub-plot, and sub-sub-plot. The whole plot was quality grade (Prime, Low Choice, and Standard). The sub-plot was the sample type (whole steaks vs. ground patties). The sub-sub-plot was the thermal processing temperature (4, 25, 55, 60, 71, and 77 °C).

RESULTS

Carcass Characteristics

The data collected during carcass selection can be found in Table 1. Quality grade affected ($P \leq 0.009$) hot carcass weight, marbling scores, percentage of kidney, pelvic, and heart fat, ribeye area, and calculated yield grade. The HCW of Low Choice and Standard animals were similar ($P > 0.05$), while the HCW of Prime animals were comparably lower ($P < 0.05$). The REA of the Standard animals were larger ($P < 0.05$) than other quality grades. As anticipated, the marbling scores for each quality grade were different ($P < 0.001$), indicating that the carcasses obtained for this study achieved differing levels of intramuscular fat. The kidney, pelvic, and heart fat (KPH) percentages of Standard carcass were greater ($P < 0.05$) than Prime and Low Choice. The calculated yield grade is dependent upon the fat thickness (mm), ribeye area (cm²), KPH percent, and HCW (kg) measurements, and differed by quality grade ($P < 0.001$). Lean maturity and skeletal maturity did not differ ($P > 0.05$) between quality grades. Carcasses of similar lean and skeletal maturity, independent of quality grade, were purposefully selected to minimize the effect of animal maturity. Factors such as diet, breed, and preharvest handling were not confirmed. However, per requirements of the beef processor, these carcasses would be in line with

Table 1. LS means of carcass characteristics from USDA Prime, Low Choice, and Standard carcasses

Item	Quality Grade			SEM	P-value
	Prime	Low Choice	Standard		
HCW ¹ , kg	394.9 ^b	424.2 ^a	419.2 ^a	6.4	0.009
Marbling ²	803.8 ^a	446.3 ^b	265.0 ^c	9.2	<0.001
KPH ³ , %	2.8 ^b	3.1 ^b	4.0 ^a	0.2	0.001
REA ⁴ , cm ²	86.8 ^b	86.6 ^b	101.2 ^a	2.1	<0.001
Fat thickness, mm	19.4 ^a	11.7 ^b	5.5 ^c	2.0	<0.001
Calculated YG ⁵	3.9 ^a	3.4 ^b	2.6 ^c	0.2	<0.001
Lean Maturity ⁶	36.3	43.8	41.3	3.9	0.414
Skeletal Maturity ⁶	63.8	53.8	60.0	8.6	0.711

^{a,b,c} LS means within a row lacking common superscript differ ($P < 0.05$).

¹HCW = hot carcass weight.

²Marbling assessed at Longissimus dorsi surface between the 12th and 13th ribs by comparison with official USDA marbling photographs (National Cattlemen's Beef Association, Centennial, CO). Marbling score units: 200 = Traces⁰⁰; 300 = Slight⁰⁰; 400 = Small⁰⁰; 500 = Modest⁰⁰; 600 = Moderate⁰⁰; 700 = Slightly Abundant⁰⁰; and 800 = Moderately Abundant⁰⁰.

³KPH = kidney, pelvic, and heart fat; KPH is measured subjectively as an approximation of 2 to 4 percent of carcass weight

⁴REA = ribeye area.

⁵YG = yield grade. Calculated yield grade = $2.50 + (0.0984252 \times \text{mm fat thickness}) - (0.0496 \times \text{cm}^2 \text{ REA}) + (0.20 \times \text{KPH, \%}) + (0.008378 \times \text{kg HCW})$

⁶Lean and skeletal maturity scale: 100 to 599: 100 = A⁰⁰; 200 = B⁰⁰; 300 = C⁰⁰; 400 = D⁰⁰; and 500 = E⁰⁰.

common commercial North American genotypes and feedlot production practices.

Volatile Compounds

A total of 27 different volatile compounds were evaluated in this study: 6 Strecker aldehydes, 4 ketones, 2 sulfur containing compounds, 2 esters, 2 alcohols, 2 carboxylic acids, 5 aldehydes, and 4 pyrazines. Each volatile compound resulted from either the Maillard reaction or lipid degradation. Maillard reaction compounds included: acetaldehyde, 2-methyl-propanal, 3-methyl-butanal, 2-methyl-butanal, benzaldehyde, benzeneacetaldehyde, carbon disulfide, dimethyl disulfide, 2,3-butanedione, 3-hydroxy-2-butanone, methyl-pyrazine, 2,5-dimethyl-pyrazine, trimethyl-pyrazine, and 2-ethyl-3,5-dimethyl-pyrazine. Lipid degradation compounds included: 2-propanone, 2-heptanone, methyl ester acetic acid, methyl ester butanoic acid, 1-penten-3-ol, 1-octen-3-ol, hexanal, heptanal, octanal, nonanal, decanal, butanoic acid, and octanoic acid. Compounds representing important trends will be described in the text below. Additionally, example compounds will be represented in figures in order to visualize trends.

Four Strecker aldehydes, 4 pyrazines, and 1 ester had 3-way interactions between quality grade, DOD, and product type (each $P < 0.001$). The concentration of pyrazines, such as trimethyl pyrazine, did not differ ($P > 0.05$) among ground patties, regardless of quality grade or DOD (Fig. 1). Meanwhile, concentrations were comparably

greater in steaks ($P < 0.05$). Standard steak samples did not differ ($P > 0.05$), regardless of quality grade or DOD, in 3 out of the 4 pyrazines measured: methyl-pyrazine, 2,5-dimethyl-pyrazine, and trimethyl-pyrazine. Standard 77 °C steak samples differed ($P < 0.05$) from 4 °C and 25 °C samples for 2-ethyl-3,5-dimethyl-pyrazine (Fig. 2). In Prime and Low Choice steak samples, the concentration of pyrazines increased ($P < 0.05$) as DOD increased. The most dramatic effect on pyrazine concentration was seen in the Prime steak samples. The concentration in Prime 4 °C and 25 °C samples differed ($P < 0.05$) from Prime cooked samples (55, 60, 71, and 77 °C) in all 4 pyrazines. In all pyrazine compounds except 2-ethyl-3,5-dimethyl-pyrazine, 71 °C and 77 °C Prime steak samples were different ($P < 0.05$) from all DOD and quality grades.

A 3-way interaction was observed for 4 Strecker aldehydes: 2-methyl-propanal ($P = 0.013$), 2-methyl-butanal ($P < 0.001$), 3-methyl-butanal ($P = 0.001$; Fig. 3), and benzeneacetaldehyde ($P = 0.004$). Overall, the concentration of each of these compounds was greatest ($P < 0.05$) in Prime 77 °C steak samples. In all 4 Strecker aldehydes, the concentration was greater ($P < 0.05$) in steaks compared with ground patties in Prime 55, 60, 71, and 77 °C samples, Low Choice 77 °C samples, and Standard 77 °C samples. Among steak samples, the concentration of these Strecker aldehydes increased ($P < 0.05$) in Prime and Low Choice samples as DOD increased. In Prime steak samples, concentrations were greatest ($P < 0.05$) in cooked samples (55, 60, 71, and 77 °C) compared with raw (4 and

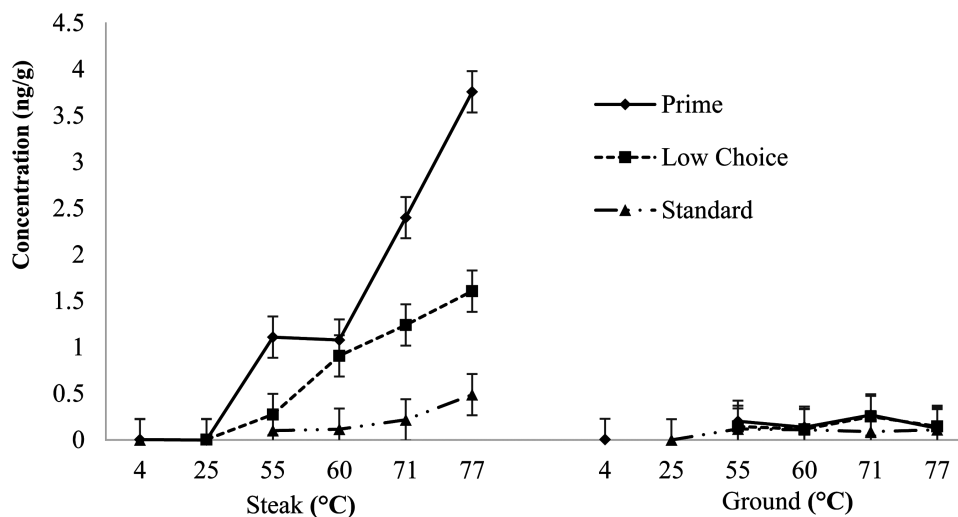


Figure 1. Concentration (ng/g) of trimethyl pyrazine from USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) of 6 degrees of doneness. A 3-way interaction (quality grade \times degree of doneness \times product type) was observed ($P < 0.001$).

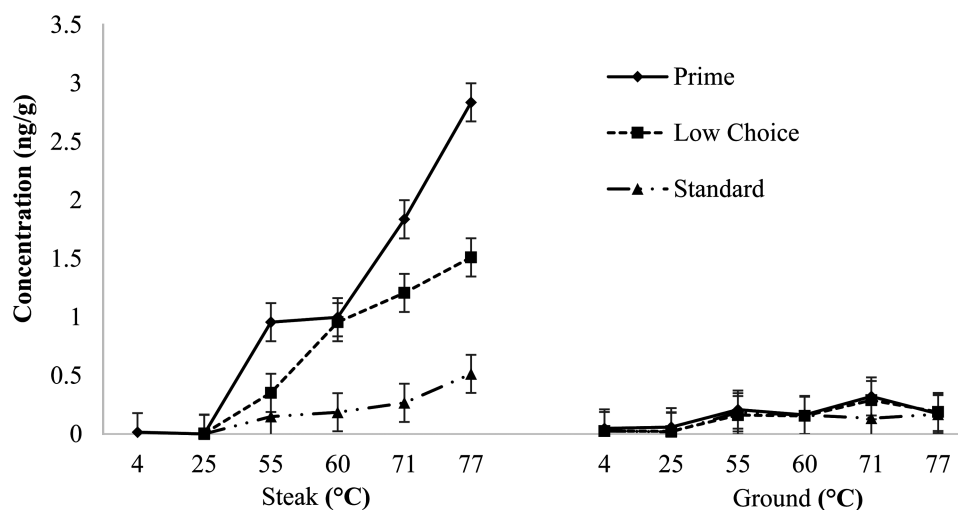


Figure 2. Concentration (ng/g) of 2-ethyl-3,5-dimethyl-pyrazine from USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) of 6 degrees of doneness. A 3-way interaction (quality grade \times degree of doneness \times product type) was observed ($P < 0.001$).

25 °C). Among cooked steak samples, the concentration of each Strecker aldehyde was greatest ($P < 0.05$) in Prime compared with Low Choice and Standard. The concentration of each compound in raw steak samples (4 and 25 °C) did not differ ($P > 0.05$) for benzeneacetaldehyde, 2-methyl-butanal, and 2-methyl-propanal. Similarly, among ground patties, the concentrations of all 4 Strecker aldehydes within the raw DOD did not differ ($P > 0.05$); furthermore, raw concentrations were similar ($P > 0.05$) with cooked ground patties in some cases (55 °C and 60 °C).

A 2-way interaction between quality grade and product type was observed for 2 Maillard reaction compounds and 6 lipid degradation compounds. The 2 Maillard compounds were a Strecker aldehyde (acetaldehyde; $P = 0.015$) and a sulfur compound (dimethyl disulfide; $P = 0.003$; Fig. 4). Both

compounds were present in the greatest ($P < 0.05$) concentration in Prime steak samples. In ground patties, the concentration for each of these two compounds was lowest ($P < 0.05$) in Standard samples. The concentration of dimethyl disulfide decreased ($P < 0.05$) in steak samples with a decrease in quality grade. The 4 lipid degradation compounds affected were an alcohol (1-penten-3-ol; $P < 0.001$; Fig. 5), a carboxylic acid (butanoic acid; $P < 0.001$; Fig. 6), 3 aldehydes (hexanal: $P = 0.029$; octanal: $P = 0.001$; and nonanal: $P = 0.005$), and a ketone (2-heptanone; $P = 0.035$). 2-Heptanone (Fig. 7) and hexanal (Fig. 8) were greatest ($P < 0.05$) in concentration in ground patties. Both compounds were lowest ($P < 0.05$) in Standard ground patties compared with the other quality grades. The concentration of hexanal in steaks did not differ ($P > 0.05$) by quality grades. Meanwhile, the concentration

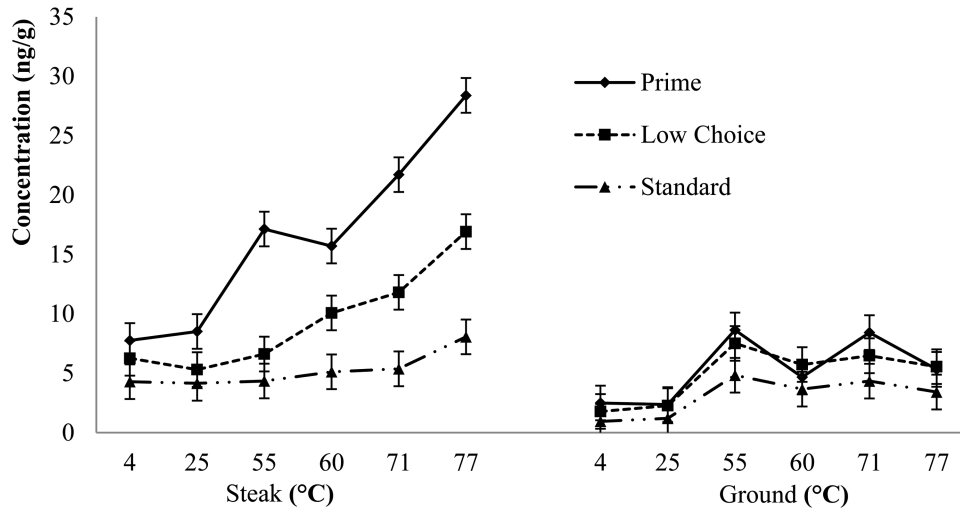


Figure 3. Concentration (ng/g) of 3-methyl-butanal in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at 6 degrees of doneness. A 3-way interaction (quality grade \times degree of doneness \times product type) was observed ($P < 0.001$).

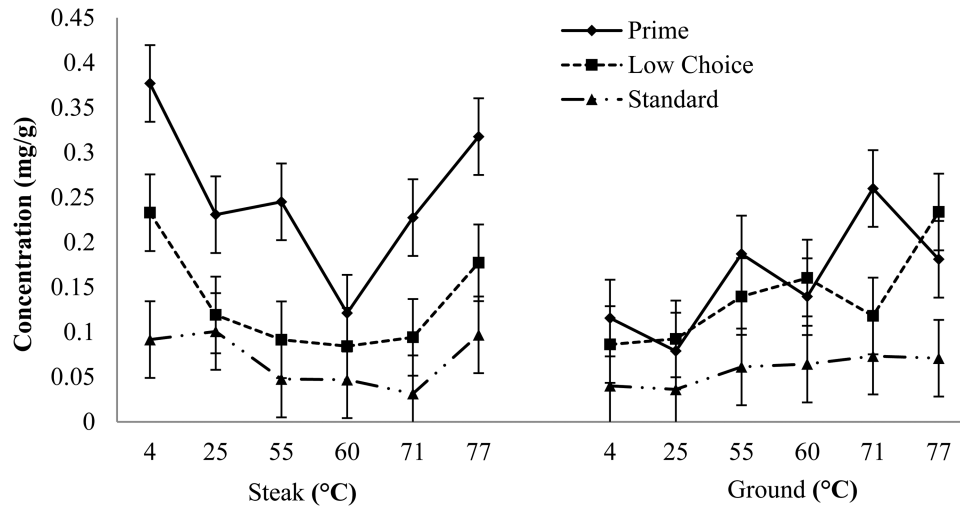


Figure 4. Concentration (ng/g) of dimethyl disulfide in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at 6 degrees of doneness. Two-way interactions were observed: quality grade \times product type ($P < 0.001$) and product type \times degree of doneness ($P < 0.001$).

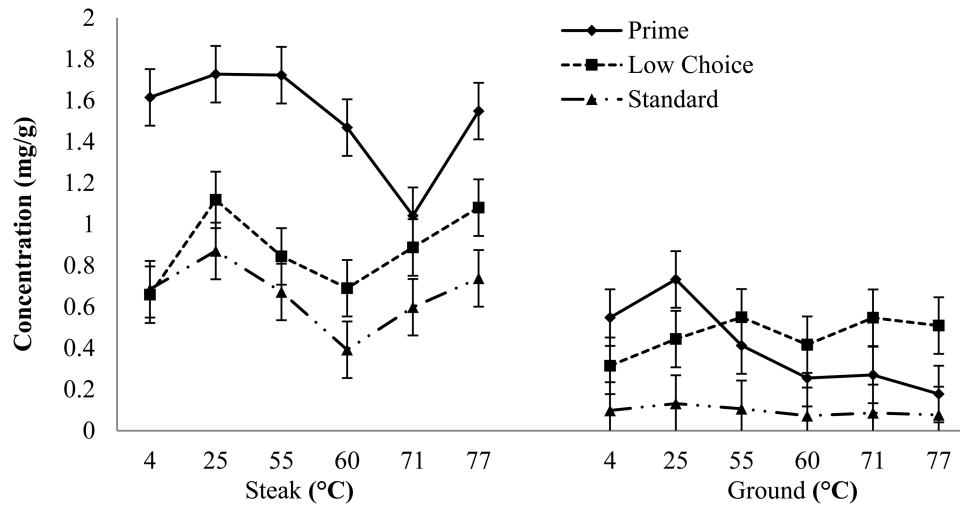


Figure 5. Concentration (ng/g) of 1-penten-3-ol in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at 6 degrees of doneness. Two-way interactions were observed: quality grade \times product type ($P < 0.001$) and quality grade \times degree of doneness ($P = 0.016$).

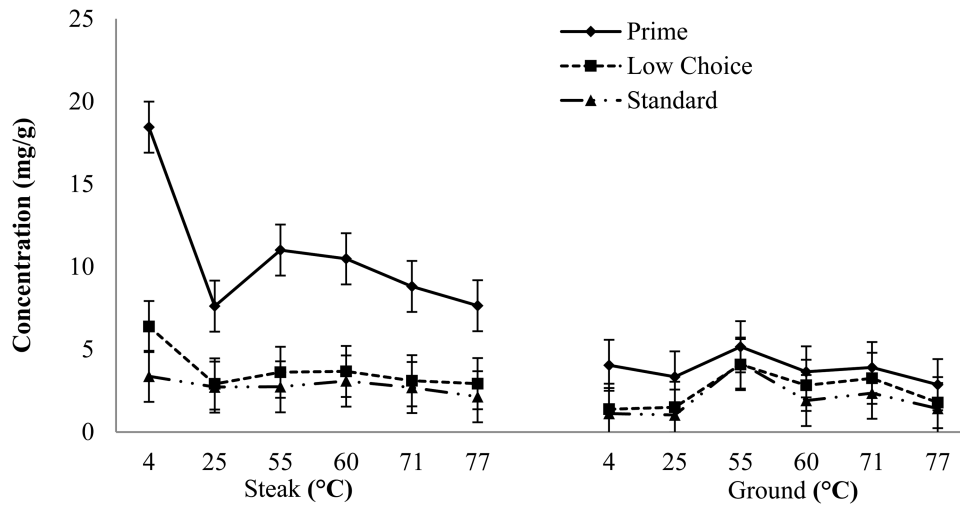


Figure 6. Concentration (ng/g) of butanoic acid in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at 6 degrees of doneness. Two-way interactions were observed: quality grade \times product type ($P < 0.001$) and product type \times degree of doneness ($P = 0.002$).

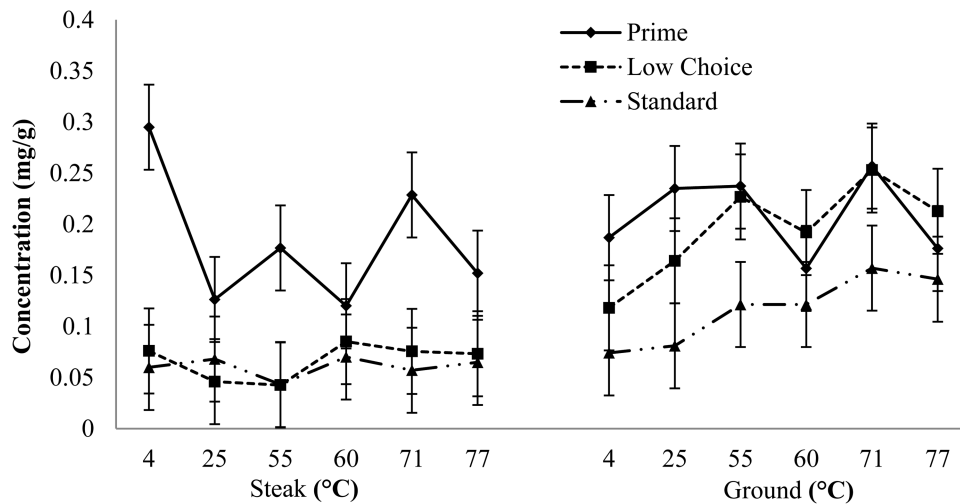


Figure 7. Concentration (ng/g) of 2-heptanone in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at 6 degrees of doneness. Two-way interactions were observed: quality grade \times product type ($P = 0.035$) and product type \times degree of doneness ($P = 0.028$).

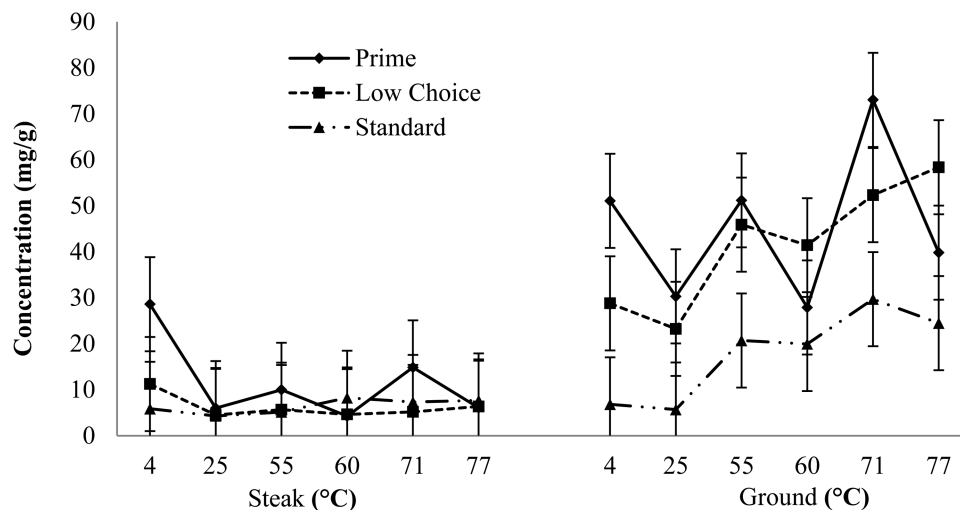


Figure 8. Concentration (ng/g) of hexanal in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at 6 degrees of doneness. Two-way interactions were observed: quality grade \times product type ($P = 0.029$) and product type \times degree of doneness ($P = 0.019$).

of 2-heptanone, octanal, and nonanal was greatest ($P < 0.05$) in Prime steaks. Octanal and nonanal in ground patties did not differ ($P > 0.05$) by quality grade. The concentration of 1-penten-3-ol was greatest ($P < 0.05$) in steak samples compared with ground patties. Within these steak samples, the concentration of the compound was greatest ($P < 0.05$) in Prime samples followed by Low Choice and Standard. Butanoic acid was greatest ($P < 0.05$) in Prime steak samples and did not differ by any other quality grades or degrees of doneness.

Another 2-way interaction, quality grade \times DOD, was observed in 2 Maillard reaction compounds and 5 lipid degradation compounds. The 2 Maillard compounds were both ketones (2,3-butanedione: $P = 0.002$; and 3-hydroxy-2-butanone: $P = 0.005$). These two compounds (Figs. 10 and 11) were each greatest ($P < 0.05$) in concentration in Prime 4 °C samples. Neither compound differed ($P > 0.05$) by quality grades in 60, 71, or 77 °C samples. The lipid degradation compounds affected by this interaction were an alcohol (1-penten-3-ol; $P = 0.016$; Fig. 5), 2 esters (butanoic acid, methyl ester: $P < 0.001$; and acetic acid, methyl ester: $P < 0.001$), and 2 aldehydes (octanal: $P = 0.038$; and nonanal: $P = 0.022$). The concentration of 1-penten-3-ol was greatest ($P < 0.05$) in Prime samples cooked or tempered to 4, 25, 55, and 60 °C, followed by Low Choice and Standard. At 71 and 77 °C, the concentration of the compound did not differ ($P > 0.05$) in Prime and Low Choice samples and was lower ($P < 0.05$) in Standard samples. The concentration of butanoic acid, methyl ester was greatest ($P < 0.05$) in Prime samples tempered or cooked to 4, 25, and 55 °C. Meanwhile, the concentration in 60, 71, and 77 °C samples did not differ

($P > 0.05$) by quality grade. Quantity of acetic acid, methyl ester (Fig. 11) was greatest ($P < 0.05$) in Prime samples cooked or tempered to 4, 25, and 71 °C, while 60 °C and 77 °C samples did not differ ($P > 0.05$) by quality grade. Octanal and nonanal (Fig. 12) concentrations did not differ ($P > 0.05$) by quality grades in 60, 71, and 77 °C samples. In 4, 25, and 55 °C samples, octanal and nonanal were greatest ($P < 0.05$) in Prime samples, and Low Choice and Standard samples did not differ ($P > 0.05$).

The final 2-way interaction that was observed for volatile compounds was DOD and product type. This interaction was significant for 5 Maillard reaction compounds and 11 lipid degradation products. The Maillard compounds affected included 3 ketones (2,3-butanedione: $P < 0.001$; 3-hydroxy-2-butanone: $P = 0.002$; and 2-heptanone: $P = 0.028$) and 2 sulfur compounds (dimethyl disulfide: $P < 0.001$; and carbon disulfide: $P < 0.001$). 2,3-butanedione (Fig. 9) and 3-hydroxy-2-butanone (Fig. 10), did not differ ($P > 0.05$) by product type in the cooked samples, but were greater ($P < 0.05$) in concentration in steaks compared to ground patties in raw samples. The third ketone, 2-heptanone, was greatest ($P < 0.05$; Fig. 7) in concentration in ground patties compared to steaks in all DOD, except 4 °C. Dimethyl disulfide (Fig. 4) and carbon disulfide were both greatest ($P < 0.05$) in 4 °C steak samples, while their concentration in cooked samples did not differ ($P > 0.05$) by product type.

The lipid degradation compounds affected by this 2-way interaction were 2 esters (butanoic acid, methyl ester: $P < 0.001$; and acetic acid, methyl ester: $P < 0.001$), 5 aldehydes (hexanal: $P = 0.019$; heptanal: $P = 0.006$; octanal: $P = 0.001$; nonanal: $P = 0.002$; and decanal: $P = 0.001$), 2 carboxylic

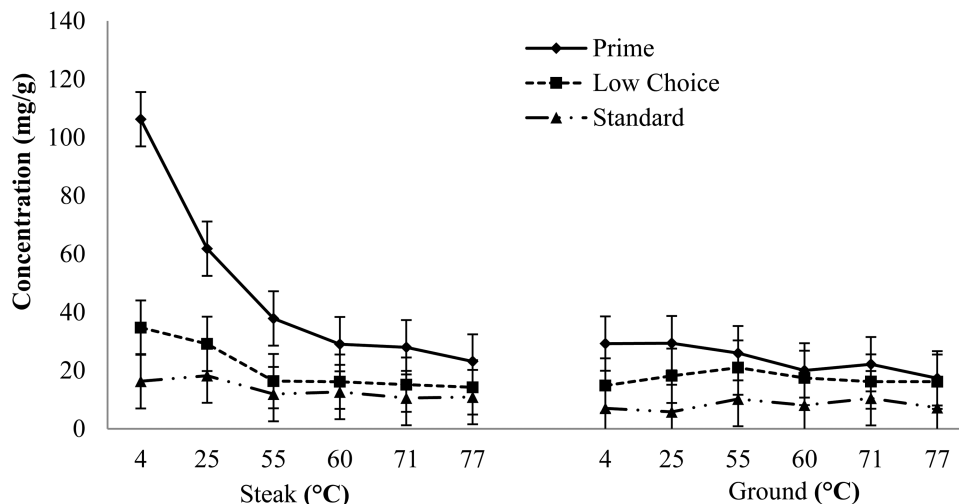


Figure 9. Concentration (ng/g) of 2,3-butanedione in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at 6 degrees of doneness. Two-way interactions were observed: quality grade \times degree of doneness ($P = 0.002$) and product type \times degree of doneness ($P < 0.001$).

acids (butanoic acid: $P = 0.002$; and octanoic acid: $P = 0.006$), a ketone (2-heptanone; $P = 0.028$), and an alcohol (1-octen-3-ol; $P = 0.029$). Butanoic acid, methyl ester and acetic acid, methyl ester (Fig. 11) concentrations were greatest ($P < 0.05$) in raw steak samples. Their concentrations in cooked samples did not differ ($P > 0.05$) by product type; however, butanoic acid, methyl ester was an exception in that the concentration in 60 °C samples was greater ($P < 0.05$) in ground patties compared to steaks. All 5 aldehyde compounds were greatest ($P < 0.05$) in concentration in ground patties compared to steaks and were greatest ($P < 0.05$) in one of the higher DOD (71 °C or 77 °C) compared to raw samples, although the concentration of 71 and 77 °C samples were often similar ($P > 0.05$). The only exception to this observation was octanal, where

the concentration in 55 °C samples was greater ($P < 0.05$) in steaks compared to ground patties. Among steaks, hexanal (Fig. 8) and octanal were greatest ($P < 0.05$) in concentration in cooked samples compared with raw, while nonanal (Fig. 12) was found in the greatest ($P < 0.05$) amount in 4 °C samples. Butanoic acid (Fig. 6) and octanoic acid were greatest ($P < 0.05$) in 4 °C steaks. The concentration of octanoic acid in ground patties was greater ($P < 0.05$) in 25 °C samples compared to 60 °C but did not differ ($P > 0.05$) by any other DOD. 2-heptanone (Fig. 7) and 1-octen-3-ol (Fig. 13) were greatest ($P < 0.05$) in ground patties compared to steaks. The concentration of 1-octen-3-ol did not differ ($P > 0.05$) among steaks, but among ground patties, it was greatest ($P < 0.05$) in 25 °C samples.

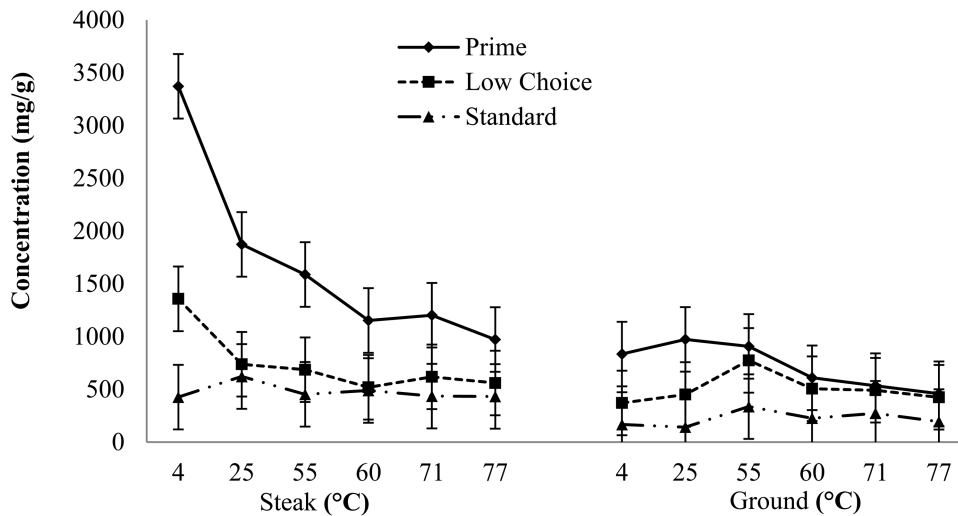


Figure 10. Concentration (ng/g) of 3-hydroxy-2-butanone in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at 6 degrees of doneness. Two-way interactions were observed: quality grade \times degree of doneness ($P = 0.005$) and product type \times degree of doneness ($P = 0.002$).

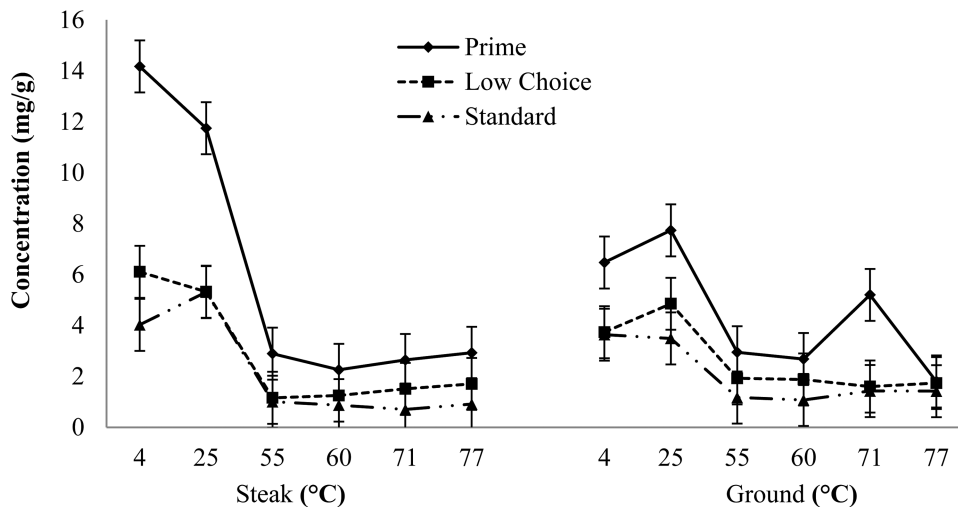


Figure 11. Concentration (ng/g) of acetic acid, methyl ester in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at 6 degrees of doneness. A 3-way interaction (quality grade \times degree of doneness \times product type) was observed ($P = 0.029$).

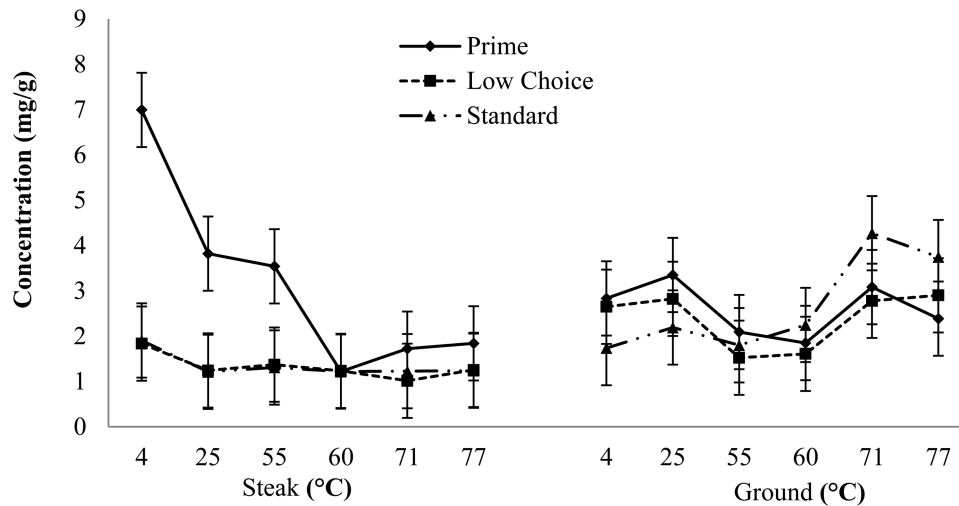


Figure 12. Concentration (ng/g) of nonanal in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at 6 degrees of doneness. Two-way interactions were observed: quality grade \times product type ($P = 0.005$); quality grade \times degree of doneness ($P = 0.022$); and product type \times degree of doneness ($P = 0.002$).

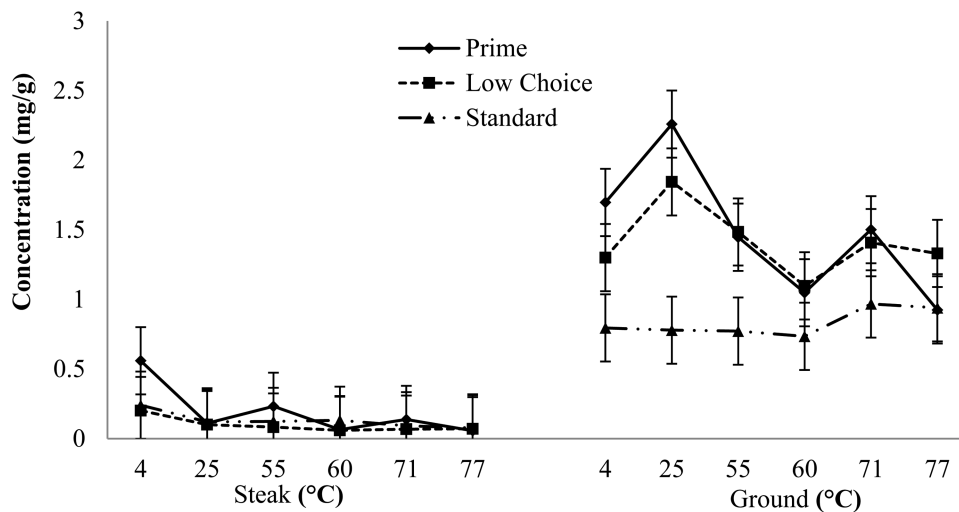


Figure 13. Concentration (ng/g) of 1-octen-3-ol in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at 6 degrees of doneness. A 2-way interaction (degree of doneness \times product type) was observed ($P = 0.029$).

DISCUSSION

It is important to note that this study utilized mincing of the meat samples to measure volatile compounds. Previous studies carried out in our lab have measured volatile compounds from intact meat samples (Legako et al., 2015; Legako et al., 2016). Preliminary results (unpublished) indicated that mincing of the meat provided improved volatile extraction and quantitation. Furthermore, mincing may more closely simulate a chewed product and thus provide a volatile profile more similar to what is perceived during consumption, in comparison to an intact sample. According to previous research, we would expect an increase in quality grade, i.e., an increase in intramuscular fat to be associated with the output of fewer volatile compounds (Cross et al., 1980; Mottram et al., 1982; Mottram

and Edwards, 1983). The results of this study, however, are not fully in agreement with this, as there were many compounds that were present in greater concentrations in samples with a greater amount of intramuscular fat. Previous research suggests that lipids stifle the formation of some volatile compounds (Chevance and Farmer, 1999; Chevance et al., 2000; Farmer et al., 2013). However, increased intramuscular (IM) fat did not seem to be associated with lower abundance of volatile compounds in this study. Recent research has demonstrated that certain volatile compounds are lipophilic in nature and greater quantities of these volatile compounds are retained in high fat beef up until consumption (Frank et al., 2017). This phenomenon is supported by our data where specific volatile compounds were expressed in greater quantities from Prime

steaks after mincing. Interestingly many of these compounds that appear to be retained by fat are not lipid derived but are products of the Maillard reaction. This indicates that fat content itself may indirectly influence consumer perception of flavor through enhanced delivery of flavor compounds during consumption. These results support the U.S. beef industries efforts to increase palatability through enhancement of marbling content in beef carcasses.

There are many factors that can contribute to the formation of volatile compounds in meat, with one factor being cooking duration. Cooking times (not presented) of products in this study indicated that steaks took significantly longer to cook than ground patties. Additionally, there were cooking duration differences due to grade for steaks, where Prime generally required a longer time to reach designated DOD in comparison with Low Choice and Standard. Volatile compound formation, like any chemical reaction, is greatly influenced by time and temperature; therefore, volatile compound profiles are likely different in steaks at each designated DOD due to variation in time spent on the heating surface. For patties, the production of Maillard reaction compounds were limited due to the short cooking time that was characteristic of the clamshell cook method used in this study. In practice patties are typically cooked on open flat top or char broiler grills. We would expect less efficient heat transfer and lengthier cook times to reach DOD with more practical cookery methods. Therefore, interpretation of this data must be done from a scientific viewpoint as our intent was to cook products by a similar mechanism to isolate differences between intact and nonintact products. Subsequent work should be done to evaluate volatile flavor compounds as they relate to common beef cookery methods.

Steaks showed clear differentiation between quality grades for certain classes of compounds. Namely, pyrazines and Strecker aldehydes from the Maillard reaction pathway were increased in Prime, followed by Low Choice being greater than Standard. This trend is of note since these components are a direct result of cooking. Presently, it is unclear what the exact mechanism is for this differentiation. However, as previously described, duration differences may provide longer heating times for additional Maillard reaction product formation. Additionally, moisture content differences between grades may influence Maillard reaction rate. It is well established that multiple dehydrations occur during the initial stages of the Maillard

reaction and Maillard browning is slowed in foods with greater amounts of free water (Huber and BeMiller, 2017). Therefore, it may be that moisture content also influences volatile compound development. Alternatively, variation in flavor precursor compounds, free-amino acids and reducing sugars, between grades may impact availability of starting ingredients that lead to pyrazines and Strecker aldehydes. This concept will be explored in a subsequent paper.

Several compounds, such as dimethyl disulfide, had nonlinear responses to DOD or decreased as DOD increased. This may be due to interactions with other compounds or components of other compounds to create new volatile compounds. Some compounds may be degraded as fast as they were formed, resulting in little or no change in concentration throughout cooking (Balagiannis et al., 2010). Furthermore, Parker et al. (2010) found that in a meat-based pet food, the formation of trimethyl pyrazine involves the incorporation of 2,3-butanedione. The reduction of compound abundance with increase DOD was also found for 3-hydroxy-2-butanone. Indeed, the results of this study indicate that as 2,3-butanedione and 3-hydroxy-2-butanone decreased, the concentration of trimethyl pyrazine and other pyrazines increased. Interestingly, it was recently determined that 2,3-butanedione and 3-hydroxy-2-butanone, typically thought of as Maillard reaction components, may be present in raw beef (Legako et al., 2018). Prior work has indicated that these compounds may result from microbial catabolism of sugars (Joffraud et al., 2001). The results of this study clearly indicate that these compounds are present in raw beef. Furthermore, it is evident that the presence of these compounds in raw beef is influenced by quality grade and product type. Presently, it is unclear why these compounds may be varied due to these factors in raw beef. Further work exploring microbial populations among various beef products is required to understand a more direct link for these compounds in raw beef. Additionally, decreases of acetaldehyde were observed as DOD increased. This result is in agreement with Bailey (1994) who determined that during cooking acetaldehyde interacts with hydrogen sulfide compounds to form other volatile compounds.

Overall, there were higher proportions of lipid-derived volatiles produced from ground patties compared with steaks. Similar to the results of Ahn and Nam (2004), the volatile compounds produced in ground patties of this study were lipid oxidation products, such as 2-propanone

and hexanal. The process of grinding is known to increase the surface area of meat products and allow for greater exposure of lipids to oxygen. This likely increased lipid degradation products in the patties of this study. However, this lipid degradation may have occurred at different points. For hexanal and 1-octen-3-ol, initial raw ground patties had greater content of these compounds compared with raw steaks. This indicates that lipid degradation occurred to a greater degree for patties compared with steaks, as we would expect after grinding. After cooking hexanal and 1-octen-3-ol continued to be elevated in cooked patties compared to cooked steaks. However, other lipid degradation products, butanoic acid, 2-heptanone, acetic acid-methyl ester, 1-penten-3-ol, and nonanal, were comparable between patties and steaks, or were in some cases greater in steaks. These results clearly indicate that the lipid-derived volatile profile is different between beef patties and steaks. These differences are likely due to differences in the status of lipid oxidation of patties compared to steaks, where products express specific lipid-derived compounds depending on oxidative status. Generally, greater lipid oxidation during processing and storage of beef leads to negative off-flavors. However, it has been stated that thermal lipid degradation during cooking may promote characteristic beef flavors (Kerth and Miller, 2015). These results indicate that the final volatile profile is influenced by product handling prior to cooking. Therefore, beef processors, retailers, and consumers may influence the final flavor profile of beef. As such, these groups may control the quantity of lipid oxidation products and limit the likelihood of off-odors and off-flavors through avoidance of pro-oxidants.

In conclusion, this study indicates that volatile beef flavor compounds are greatly influenced by quality grade, product type, and DOD. Previous sensory research has indicated that these factors influence flavor perception. However, this study reveals that specific characteristics, such as varied cooking duration or lipid oxidative status, of these factors may influence chemical flavor development. Overall, it may be concluded that postharvest processing and cookery greatly impact the final flavor profile of beef. Ultimately consumers select beef based on quality grade and product type, then determine the cookery approach. Therefore, in consideration of beef from farm-to-fork a large proportion of influence on beef flavor is in the hands of consumers. Therefore, additional research must be conducted with common consumer beef handling

and cookery methods to further understand how flavor is impacted under more practical conditions.

Conflict of interest statement. None declared.

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