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COMPARISON OF BEEF FLAVOR COMPOUNDS FROM STEAKS AND GROUND
PATTIES OF THREE USDA QUALITY GRADES AND VARIED DEGREES OF
DONENESS

by

Kourtney Gardner

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Science

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Logan, Utah

2017

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ABSTRACT

Comparison of Beef Flavor Compounds from Steaks and Ground Patties of Three USDA
Quality Grades and Varied Degrees of Doneness

by

Kourtney T. Gardner, Master of Science

Utah State University, 2017

Major Professor: Dr. Jerrad F. Legako
Department: Nutrition, Dietetics, and Food Science

This study determined how quality grade and degree of doneness influence the development of beef flavor compounds among whole muscle and ground patties. Proximate composition, pH, cooking duration, neutral and polar lipid fatty acids, free and total amino acids, total reducing sugars, and volatile compounds were evaluated in beef strip steaks and ground patties of *Longissimus lumborum* from three USDA quality grades (Prime, Low Choice, and Standard; n=8 per quality grade) and six degrees of doneness (4, 25, 55, 60, 71, and 77°C). In the split-plot experiment, quality grade was the whole-plot, product-type was a sub-plot, and degree of doneness was the sub-sub-plot. The 3-way interaction of quality grade, degree of doneness, and product type impacted moisture ($P = 0.004$) and protein content ($P = 0.006$); pH ($P < 0.001$); neutral and polar lipid fatty acids ($P \leq 0.048$); free and total amino acids ($P \leq 0.044$); total reducing sugars ($P < 0.001$); and volatile compounds ($P \leq 0.029$). The 2-way interaction of quality grade

and degree of doneness impacted free amino acids ($P \leq 0.036$); PUFA within the neutral lipid fraction ($P \leq 0.033$); fatty acids within the polar lipid fraction ($P \leq 0.043$); volatile compounds ($P \leq 0.038$); and the total fat percentage ($P = 0.046$). The 2-way interaction of quality grade and product type impacted fatty acids within the neutral lipid fraction ($P \leq 0.042$); fatty acids within the polar lipid fraction ($P \leq 0.015$); and volatile compounds ($P \leq 0.047$). The 2-way interaction of product type and degree of doneness affected fatty acids within the neutral lipid fraction ($P \leq 0.046$); fatty acids within the polar lipid fraction ($P \leq 0.035$); free amino acids ($P \leq 0.005$) and total amino acids ($P \leq 0.004$); volatile compounds ($P \leq 0.029$); and cooking duration ($P < 0.001$). Overall the results of this study indicated that quality grade, grinding, and cooking have interacting effects on flavor related compounds. Thus, each factor must be considered during any model development which aims to predict beef flavor.

(186 pages)

PUBLIC ABSTRACT

Comparison of Beef Flavor Compounds from Steaks and Ground Patties of Three USDA Quality Grades and Varied Degrees of Doneness

Kourtney T. Gardner

The objective of this study was to determine how quality grade (Prime, Low Choice, and Standard) and degree of doneness (4, 25, 55, 60, 71, and 77°C) influence the development of the flavor-producing compounds in beef whole muscle and ground patties. The content and type of many compounds influence beef flavor, including: proximate composition, pH, neutral and polar lipid fatty acids, free and total amino acids, reducing sugars, and volatile compounds, in addition to cooking duration. The important proximate components include fat, moisture, and protein content. Amino acids and reducing sugars alone contribute to the five basic tastes, but they can also react to create volatile compounds that contribute to a diverse flavor profile. Degradation of fatty acids also creates a diverse flavor profile that contributes to beef eating quality. Thus, varied interactions between quality grade, degree of doneness, and product type were determined for each of these measured compounds. In general, there were less of these compounds in ground patties compared with steaks. Total fat percentage and volatile compounds resulting from lipid degradation, however, were greater in ground patties compared with steaks. Furthermore, grinding reduced the amount of water soluble compounds (amino acids and reducing sugars). This implies that grinding significantly impacts beef flavor precursor compounds and may alter the perception of beef flavor.

Generally, volatile compounds increased with cooking and showed little to no response upon further cooking. Fatty acids also increased with cooking in most cases. The water-soluble compounds decreased with cooking. The effect of quality grade on these measurements varied by degree of doneness, product type, and individual compound.

DEDICATION

I would like to dedicate this thesis to my parents, Don and Becky Keck. While it has been difficult to take on a new chapter in life that includes marriage and graduate school from so far away, your love, support, encouragement, and advice never waned, and I could not have done it without you. Thank you for instilling in me an impeccable work ethic and the value of hard work and perseverance by setting an example through the way that you live. Thank you for also reminding me of the importance to have a little fun and to take time for myself. Thank you for living out a great example of what a loving marriage should look like. I am grateful for our time spent physically apart, because it has allowed us to grow closer in ways that would not have been possible if we had not moved so far from home. I am forever grateful for your love and guidance and forever proud to be your daughter.

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CHAPTER I

INTRODUCTION AND OBJECTIVES

Introduction

Tenderness, juiciness, and flavor all play an important role in the palatability of beef. Tenderness has most often been considered the defining trait of consumer acceptance of beef (Huffman et al., 1996; Miller et al., 2001; Platter et al., 2003). The 2010 National Beef Tenderness Survey revealed that between 85 and 95% of steaks coming from the rib and loin were within the “very tender” category. This increase in the availability of very tender beef may have now shifted consumers’ thoughts about which sensory attribute contributes most to a satisfactory beef eating experience. Recent consumer studies reveal that consumers may now consider beef flavor more important than tenderness (Corbin et al., 2015; O’Quinn 2015; Tatum 2015.) When asked whether tenderness, flavor, or juiciness was most important when eating beef steaks, 50.8% of consumers said they considered flavor most important, followed by tenderness (30.8%) and juiciness (18.4%) (Corbin et al., 2015). O’Quinn (2015) and Chail (2016) found similar results. Thus, beef flavor has arguably become the most important factor in consumers’ assessments of eating quality and acceptability (Meinert et al., 2007; Dashdorj et al., 2015).

Flavor itself is a combination of taste and odor, requiring gustatory, olfactory, and trigeminal senses (Dashdorj et al., 2015). The five basic tastes, sweet, sour, salty, bitter,

and umami are produced by non-volatile, water-soluble compounds. Sour and bitter sensations can stem from amino acids, while sweetness can stem from both amino acids and sugars (MacLeod 1994). Salty taste characteristics come primarily from inorganic salts and sodium salts of glutamate and aspartate, while the umami sensation stems mostly from glutamic acid and monosodium glutamate (MacLeod 1994). Many researchers categorize flavor precursors into water-soluble components and non-water-soluble components, such as lipids (Mottram 1998; Shahidi 1994; Dashdorj et al., 2015). These flavor precursors react to form volatile compounds that contribute to the characteristic meaty flavor. Important key reactions include lipid oxidation and thermal degradation (Mottram 1998; Shahidi 1994; Dashdorj et al., 2015).

Most flavor research in meat has been conducted using model systems containing a mixture of some reactants, such as one sugar and one amino acid, or use an extract instead of the meat itself (Balagiannis et al., 2010; Balagiannis et al., 2009; Fagerson 1969; Heyns et al., 1966; Sugisawa & Edo, 1966; Tai & Ho, 1997). These systems have advantages, such as their ability to be heated more uniformly; extraction of volatiles is simpler; and interferences from interactions not of interest can be avoided (Balagiannis et al., 2009). However, these simple aqueous systems also have limitations. For example, when trying to study the Maillard reaction in meat, a model system that contains one amino acid and one reducing sugar may create a clear dependence on the amino acid concentration because it is the only source of amino groups in the system (Balagiannis et al., 2009). In a more complex meat system, many more reactive amino groups, such as peptides and protein-bound amino groups exist (Balagiannis et al., 2009). In real foods,

other meat components, such as multiple amino acids, multiple fatty acids, and multiple fat types contribute to flavor generation during the Maillard reaction (Mottram & Elmore, 2010). Furthermore, model systems do not offer all the sensory background effects associated with cooked foods, making them more susceptible to small variation in reaction conditions (Mottram & Elmore, 2010). This study eliminated all subcutaneous fat from the samples used and only considered intramuscular fat content, so it can still be considered an in-situ model system; however, the study analyzed the chemical composition of meat in actual steak or ground patty meat sample rather than in a simple mixture of reactants, making it different from a traditional model system.

The chemical composition, including reducing sugars, free and total amino acids, neutral and polar lipid fatty acids, and volatile compounds; proximate composition; and pH values were evaluated in beef from three different quality grades: Prime, Low Choice, and Standard. Marbling scores for this study were as follows: Slightly Abundant⁰⁰ or greater (Prime); Small⁰⁰ to Small¹⁰⁰ (Low Choice); and Traces¹⁰⁰ or lower (Standard). Of the four quality grades that represent A maturity animals, these three quality grades were used for this study because they each have marbling scores different enough to adequately represent differing fat levels. The same chemical measurements were analyzed in beef across six different degrees of doneness (4, 25, 55, 60, 71, and 77°C), in both whole muscle steaks and in ground beef patties with no added fat. Previous studies have been conducted in which some of these measurements have been observed in cooked products. Most of these studies, however, only use one or two cooked

temperatures, for example medium and well-done temperatures (approximately 60°C and 77°C) (Spanier et al., 1997).

It is important to note that, generally, flavor studies include qualitative data such as sensory data or a combination of both qualitative and quantitative measures. This study, however, determined only the quantitative measurements of the chemical compounds associated with beef flavor. Once it is known how the development of beef flavor precursor compounds is affected by these parameters, future research may unveil whether there is a means to manage flavor development in order to provide consumers with a consistent eating experience. For example, certain pathways leading to the development of desirable or undesirable flavor compounds can be promoted or inhibited by parameters such as feeding and post-mortem processing (van Boekel 2006). Better understanding of the chemical composition of beef will allow for the development of technologies that can deliver enhanced palatability of beef muscles (Jeremiah et al., 2003). New technologies may be able to reduce the variation of chemical properties in order to provide consumers with a consistent, flavorful eating experience. Providing a “Prime” beef flavor in lower quality products may have important implications for those consumers who prefer the flavor of Prime beef but can only afford Low Choice or Standard beef products.

Hypothesis

The composition and content of flavor related compounds in whole muscle and ground beef strip steaks of multiple quality grades and degrees of doneness differ.

Objectives

Determine how quality grade (Prime, Low Choice, and Standard) and degree of doneness (4°C, tempered to 25°C, or cooked to 55°, 60°, 71°, or 77°C) influences beef proximate composition, pH, volatile compounds, reducing sugars, amino acids, and fatty acids among whole muscle and ground patties.

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CHAPTER II

REVIEW OF LITERATURE

Overview

Tenderness, juiciness, and flavor are three attributes that, together, play a role in determining palatability of beef products. While tenderness is usually referred to as the most important contributor to eating quality, recent consumer research shows that once tenderness is within an acceptable range, flavor often becomes the predominant factor in consumers' assessment of eating quality (Huffman et al., 1996; Goodson et al., 2002; Killinger et al., 2004; Behrends et al., 2005a; Behrends et al., 2005b). Kerth and Miller (2015) suggest that consumer liking is more dependent on flavor than either juiciness or tenderness. While it is known that applying heat to beef alters its chemical components in a way that changes flavor, it is not as well understood how the flavor-related compounds of beef respond to specific degrees of doneness. Another area of interest related to these flavor related compounds is how differing levels of intramuscular fat interact with the different degrees of doneness to alter these compounds and influence their development. Finally, although there is evidence to show that the physical process of grinding meat changes the particle size of its components, it is unknown how the development of flavor-related compounds is affected by this process. Once this knowledge gap is filled, potential will exist for further understanding of flavor compound development and cookery considerations among steaks and ground patties from different quality grades.

Meat Flavor

Flavor is a combination of taste and odor. Figure 2-1 highlights the specific flavor active components that develop during heating and contribute to meat flavor. Taste is detected on the tongue as a sweet, sour, salty, bitter, or umami sensation. These sweet, sour, salty, and bitter flavors typical of meat are the result of sugars, amino acids, and organic and inorganic salts. Sweetness is associated with glucose, fructose, ribose, and many L-amino acids (MacLeod & Seyyedain-Ardebili, 1981; MacLeod, 1986; Kuninaka, 1981; Haefeli & Glaser, 1990). The sour sensation often results from aspartic acid, glutamic acid, histidine, and asparagine, while bitterness is derived from peptides and several L-amino acids (MacLeod 1994). Amino acids, peptides, nucleotides, acids, salts, and minerals all contribute to the basic tastes including sour, bitter, umami, and salty via a series of chemical reactions during heating. Odor, on the other hand, is an aroma resulting from volatile compounds, and this aroma is detected by the nose and plays a large role in flavor perception (Legako et al., 2015a). The formation of these volatile compounds via thermally induced reactions of non-volatile components results in meat flavor (Khan et al., 2015).

The aroma component of flavor results from the presence of volatile compounds. Lipids, reducing sugars, and free amino acids are important contributors to the formation of volatile compounds via oxidation, degradation, dehydration, and the Maillard reaction. In beef, volatile compounds can be formed via thermal oxidation of lipids leading to a free radical chain reaction (Shahidi & Zhong, 2005). Amino acids and reducing sugars participate in the Maillard reaction, which provides savory, meaty, roast, and boiled

flavors (Mottram, 1998). This reaction is considered the primary pathway contributing to the formation of cooked meat volatile compounds (Khan et al., 2015; Farmer and Mottram, 1990; Mottram and Nobrega, 2002). Thermal oxidation and the Maillard reaction together are considered most important in forming the characteristic flavor of cooked meat (Warriss, 2000).

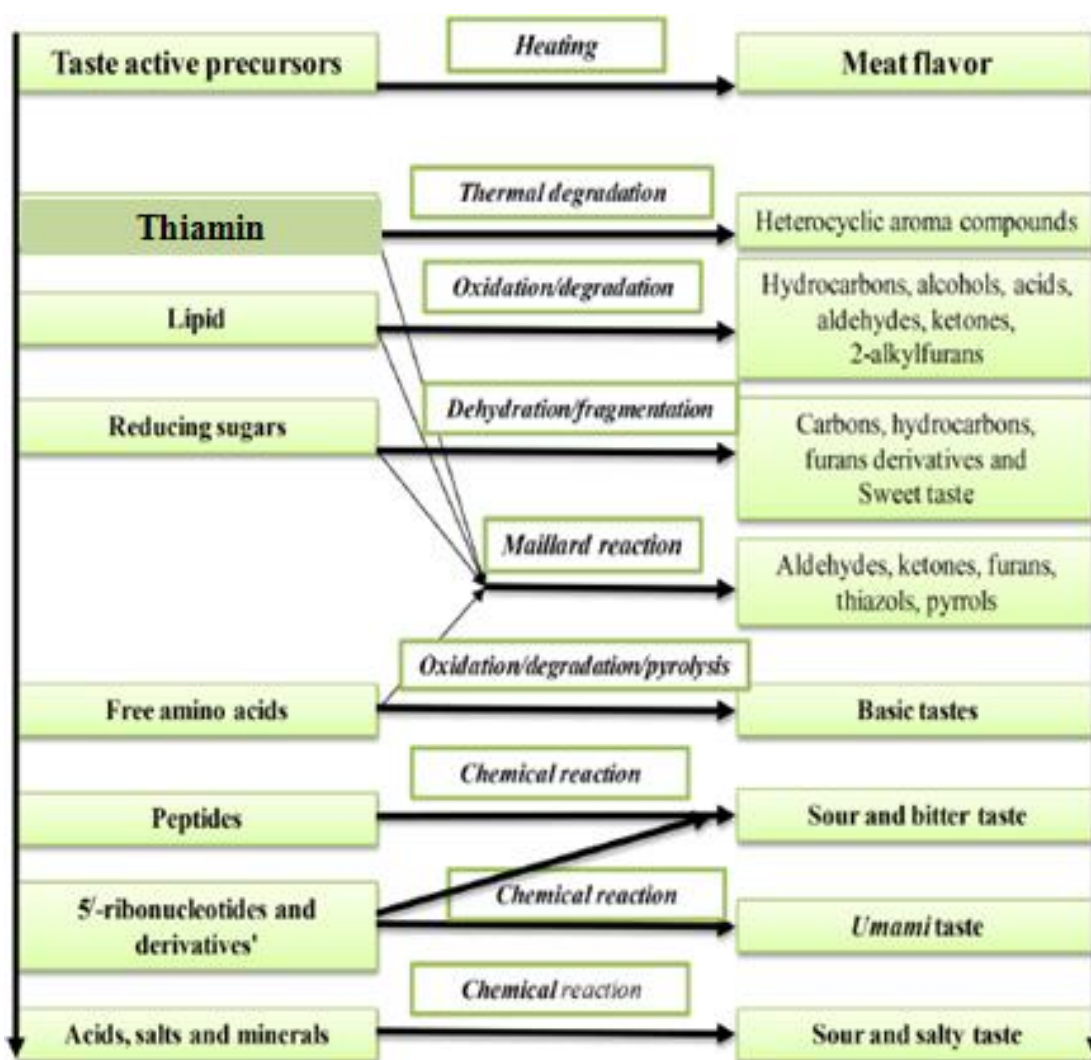


Figure 2-1. Schematic representation of meat flavor developing reactions from taste-active water-soluble precursors (Adapted from Dashdorj et al. 2015).

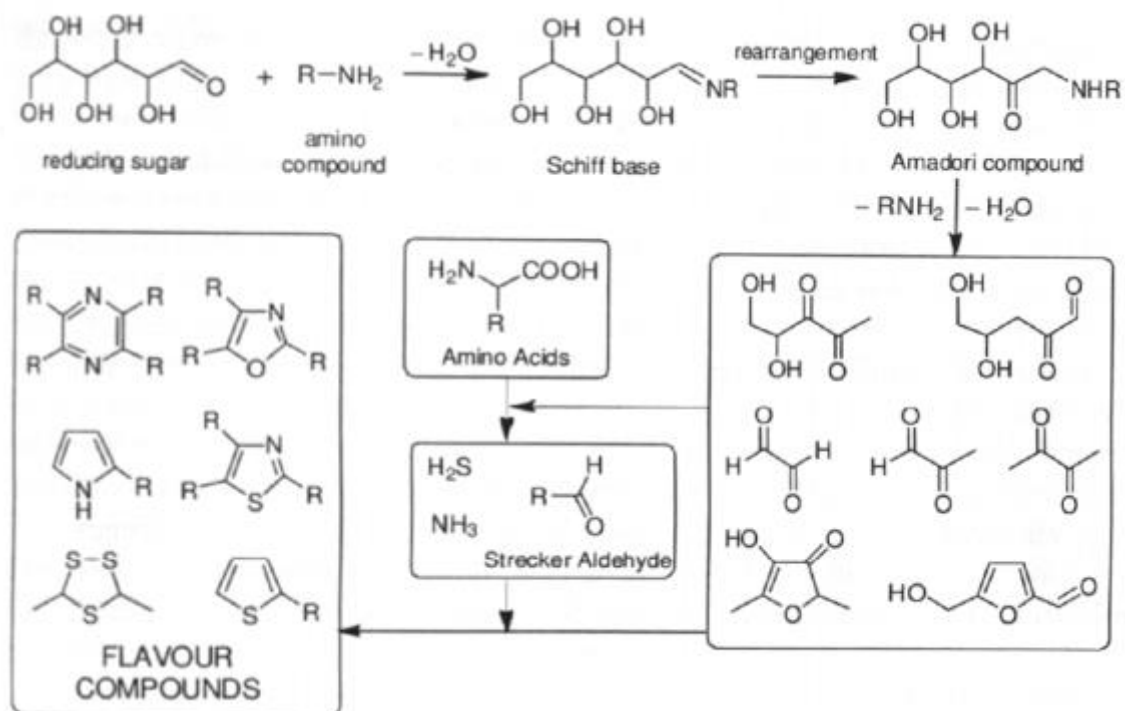


Figure 2-2. The essential steps of the Maillard reaction leading to the formation of aroma compounds (Mottram & Elmore 2010).

The Maillard reaction and Strecker degradation are important pathways contributing to the formation of volatile compounds via reactions involving free amino acids and sugars (Mottram 1994). Figure 2-2 shows the basic steps of the Maillard reaction that generate flavor compounds. The reaction involves the interaction of a free amino group with an aldehyde, ketone, or reducing sugar to create an Amadori and/or Heyns compound, which decomposes thermally and is rearranged into other reaction products including sugar dehydration and fragmentation products with one or more carbonyl components (Mottram & Elmore 2010). These reaction products become reactants for later interactions, creating a complex network of reactions (MacLeod 1994). Namely, these carbonyl groups interact with available amino acids and undergo Strecker

degradation, yielding a Strecker aldehyde that not only contributes to flavor characteristics on its own but is also an intermediate for the formation of additional volatile compounds (Mottram & Elmore 2010). An example of a Strecker degradation reaction is shown in figure 2-3. When these carbonyl compounds interact with each other and amino compounds, flavor compounds such as heterocyclics form (Mottram & Elmore 2010). Because of the role free amino acids play in the Maillard reaction, they are important water-soluble meat flavor precursors (Mottram, 1998). Van Boekel (2001) suggests that changes in amino acid concentrations can result from reacting with a sugar during the initial stage of the Maillard reaction; from amino acid regeneration from Amadori products; and from reactions with later-stage Maillard products. Products of the Maillard reaction, as well as resulting intermediates for other flavor-forming reactions, are particularly important for the characteristic aroma of meat, namely heterocyclic compounds and sulfur compounds (Mottram, 1998).

Strecker degradation, a reaction associated with the Maillard reaction, can yield hydrogen sulfide, ammonia, and acetaldehyde (Mottram 1998; Mottram & Elmore, 2010). These compounds are important, because along with carbonyl compounds formed during the Maillard reaction, they lead to the formation of other flavor compound classes such as pyrazines, furans, thiazoles, and other heterocyclic compounds (Mottram 1998). Sulfur compounds derived from ribose and cysteine are particularly important to characteristic meat aroma (Mottram 1998). These sulfur compounds can also originate from the transformation of alanine to acetaldehyde via Strecker degradation (Bailey 1994). Besides Maillard-derived compounds, there are also lipid-derived volatile

compounds. These compounds, especially aldehydes as they are major products of lipid degradation, are believed to contribute to the fatty flavors of cooked meats (Mottram, 1998).

Both the Maillard reaction and lipid degradation pathways require heat, so cooking affects these reactions and the proportion of compounds that participate in them (Mottram 1994). Beef with a higher fat content will contain more lipids to participate in lipid degradation, a reaction which produces fatty aroma compounds and compounds that readily interact with Maillard intermediates (Mottram 1994). The likelihood of interaction between Maillard products and lipid-derived products makes cooked beef a complex reaction medium with several possible reaction products. Often the interaction of compounds from the Maillard reaction and lipid degradation are so complex that the existence of products from one reaction completely block or partially inhibit the formation of other products (Kerth & Miller, 2015). Figure 2-4 shows an example of this, in which lipid-derived products and Maillard-derived products compete for hydrogen sulfide (derived from ribose and cysteine) to generate an aroma.

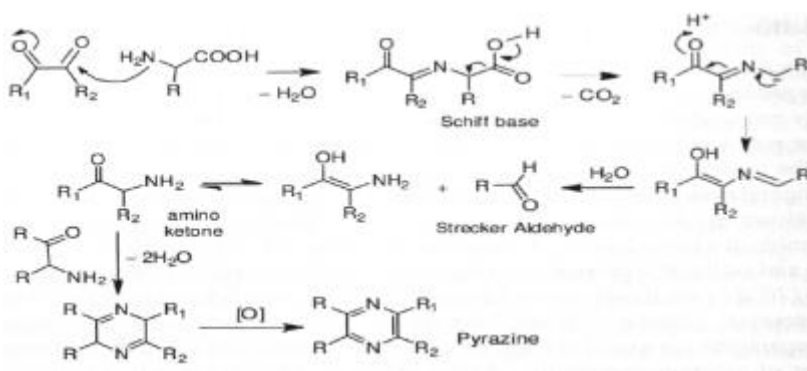


Figure 2-3. Strecker degradation of amino acids and formation of alkyldiazepines (Mottram & Elmore 2010).

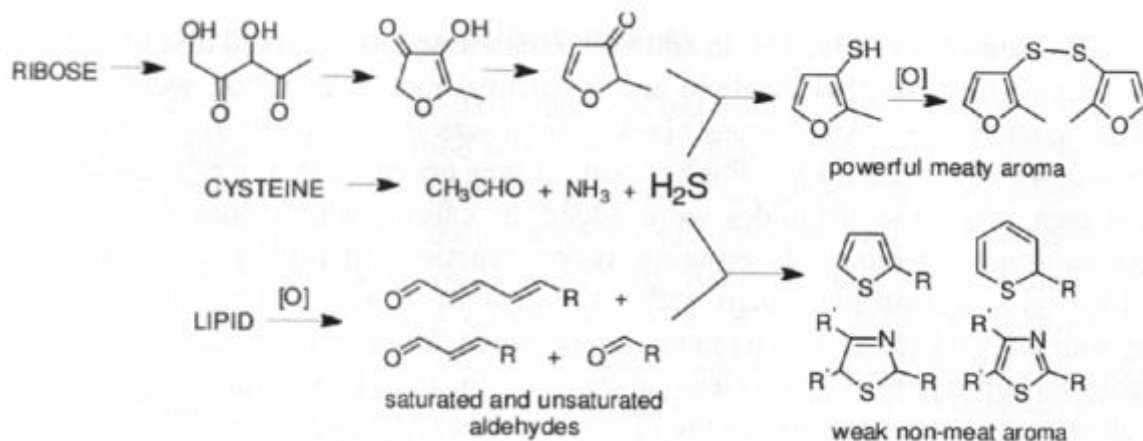


Figure 2-4. Competition between lipid derived aldehydes and Maillard-derived furanones for available hydrogen sulfide in thermal generation of meat flavor (Mottram & Elmore 2010).

Quality Grade

The quality grade (Prime, Choice, Select, Standard, etc.) (USDA 1997) is assigned to a beef carcass dependent on carcass marbling score and carcass maturity. According to Smith et al. (1983), marbling score indirectly assesses concentrations of aroma compounds in beef because it reflects intramuscular fat content, and carcasses with higher marbling scores are more likely to produce “beefy” tasting meat. Consumers in the U.S. prefer the fatty flavor of beef that is achieved by an increase in intramuscular fat content (Khan et al., 2015; Miller et al., 2000). The minimum intramuscular fat level preferred by these consumers is approximately 3% (Khan et al., 2015; Miller, 2001). This intramuscular fat, or marbling, contains lipids that are oxidized during cooking to create important flavor compounds. Beef contains higher levels of C16:0 (palmitic acid), C18:0 (stearic acid), and C18:1cis 9 (oleic acid) than other fatty acids (Smith et al., 2004). The most abundant fatty acid of intramuscular fat (IMF) is C18:1 cis 9, although it contains

less C18:1 cis 9 and more C18:0 acid than subcutaneous fat, otherwise known as backfat (Smith et al., 2004). Smith and Johnson (2014) found that an increase in monounsaturated fatty acids, such as C18:1 cis 9, was associated with an increase in overall IMF content (Sturdivant et al., 1992; May et al., 1993; Archibeque et al., 2005). Increasing C18:1 cis 9 content in beef has been shown to allow consumers to differentiate between different levels of marbling (Killinger et al., 2004; O'Quinn et al., 2012; Hunt et al., 2014; Smith & Johnson 2014). Furthermore, monounsaturated fatty acids are positively correlated with flavor intensity (Garmyn et al., 2011). The higher the C18:1 cis 9 content in beef, the greater its overall palatability (Waldman et al., 1968; Westerling & Hedrick, 1979). Smith and Johnson (2014) explain that this may be due to the fat softness associated with C18:1 cis 9, which gives beef a juicier mouthfeel (Smith et al., 1998; Wood et al., 2004; Chung et al., 2006). Additionally, monounsaturated fatty acids have a lower melting point than saturated fatty acids (Smith & Johnson, 2014). The lower melting point could contribute an increased juiciness and thus enhance overall palatability. Thus, quality grade is an important factor to consider when studying effects on flavor development in beef.

Influence of Quality Grade on Flavor-Contributing Compounds

Corbin et al. (2015) found that fat percentage plays an important role in all three palatability factors of beef (juiciness, tenderness, and flavor) and that consumer flavor liking scores increased with increased fat percentage. An increase in quality grade implies an increased level of intramuscular fat. Fat content of closely trimmed whole meats is directly proportional to marbling and inversely proportional to moisture content

(Hedrick et al., 1981; Brackebusch et al., 1991). Therefore, fat content tends to increase with an increase in marbling, while the moisture content tends to decrease with an increase in marbling (Seggern et al., 2005). Troutt et al. (1992) found that in ground patties, protein percentage was higher in low-fat samples.

Fatty acid composition plays an important role in consumers' perception of the sensory quality attributes associated with beef (Wood et al., 2004). Fatty acids participate in the oxidation of lipids, which can occur during storage and cooking (Legako et al., 2015b). This lipid oxidation, while considered undesirable during storage of meats, is essential for the development of typical meaty aroma (Khan et al., 2015; Shahidi et al., 1986). The proportion of fatty acids that become oxidized is small but significant enough to alter flavor (Khan et al., 2015; Belitz et al., 2009). Fatty acids may be separated into neutral (NL) and polar (PL) lipid fractions, and these lipid fractions are affected by quality grade (Legako et al., 2015b). As the amount of NL stored in the adipose tissues increases, this deposition of the NL is associated with increased intramuscular fat content (Legako et al., 2015b). As intramuscular fat content increases, the NL makes up most of the overall fatty acid composition (Wood et al., 2008). Because the PL is a structural component of cell membranes, it remains fairly constant in concentration (Legako et al., 2015b). In the Legako et al. (2015b) study, it was observed that in raw steaks, Primes had the most NL, and in cooked steaks, Prime and Low Choice steaks had proportionally more NL and less PL than Standard steaks. A dramatic change in intramuscular fat content could possibly present enough of a tissue structure difference to alter the relative composition of structural components, i.e. phospholipids in the PL (Rule, Macneil, &

Short, 1997). For example, in lean beef with a low amount of intramuscular fat, the amount of polar lipids present is markedly lower than in beef with a greater amount of intramuscular fat (Larick and Turner, 1989; Warren et al., 2008).

Several researchers have revealed that as fat content increases, so do the concentrations of all fatty acids (Wood et al., 2008; Scollan et al., 2006). In the Legako et al. (2015b) study, clear differences in concentration of the NL mono-unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were observed between Prime, Low Choice, and Standard raw steaks. Concentrations of PL MUFA and PUFA were lower in Standard steaks than in Prime and Low Choice. An increase in marbling score had a positive correlation with the percent of MUFA, while it had a negative correlation with the percent of PUFA. Several studies have found that C18:0 has a negative correlation with beef flavor desirability (Westerling & Hendrick, 1979; Melton et al., 1982). Legako et al. (2015b) found that the concentration of this fatty acid increased with increased intramuscular fat content, most likely because thermal effects cause beef to lose moisture, which results in a proportional increase in the fat percent. These results reveal that quality grade impacts the composition and amounts of fatty acids present in beef, and the fatty acid composition and amount affect consumer flavor preferences.

Influence of Quality Grade on Volatile Compounds

More than 1000 volatile compounds have been identified in meat (Mottram, 1998), including sulfur-containing compounds, furanathiols, disulfides, aldehydes, ketones, and other heterocyclic compounds (Cerny & Grosch, 1992; Farmer & Patterson, 1991; Gasser & Grosch, 1988; Mottram, 1991). As previously described, the bulk of

volatile compounds can be placed into two categories: (1) those compounds resulting from the Maillard reaction and (2) those compounds resulting from lipid degradation via thermal oxidation. Maillard reaction compounds include Strecker aldehydes, pyrazines, some ketones, and sulfur compounds. Lipid degradation products include alcohols, n-aldehydes, alkanes, carboxylic acids, furans, and some ketones. Since these pathways are catalyzed by heat, cooking will most likely cause more of an effect on the volatile compounds resulting from these reactions, but the amount of intramuscular lipid present is not to be ignored.

Intramuscular lipids are the major source of volatile components (Mottram et al., 1982) and consist of marbling fat and structural or membrane lipids. The marbling fat contains mostly triglycerides, while the structural lipids contain mostly phospholipids (MacLeod 1994). These intramuscular triglycerides and structural phospholipids are the main components of lean tissue that react with products of thermal lipid oxidation to create distinct flavors (Khan et al., 2015; Mottram and Edwards, 1983). Intramuscular lipids are a source of many volatiles that are present in high concentrations even in lean muscle (Bailey & Einig, 1989; Buckholz, 1989). Thus, lipid oxidation products should still be prevalent even in leaner beef. Other studies, however, have found that increased intramuscular fat (i.e. higher quality grades) has rarely produced increases in volatile flavor compounds (Cross, Berry, & Wells, 1980; Mottram & Edwards, 1983; Mottram, Edwards, & MacFie, 1982). Legako et al. (2015a) found that among 26 quantified compounds, none differed due to quality grade alone. Additionally, long chain n-aldehydes showed negative correlations with percent fat (Legako et al., 2015a). This may

be due to the evidence from other studies that suggests that fat acts as a solvent and retains volatile compounds, thus delaying flavor release (Farmer et al., 2013; Chevance et al. 2000; Chevance & Farmer, 1999). Farmer et al., (2013) found that lower fat content correlated with greater amounts of volatile compounds, since there were lower amounts of lipid in which volatile aroma compounds could be solubilized. Mottram and Edwards found that lipids in beef may inhibit the formation of some heterocyclic compounds that are a product of Maillard reactions (1983). 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).

Sensory Relationships with Quality Grade

Consumers often associate an increase in flavor desirability with an increase in quality grade, or intramuscular fat (O'Quinn et al., 2012; Smith et al., 1983; Legako et al., 2015a). Flavor desirability ratings for the beef longissimus muscle increase as marbling score increases from Practically Devoid to Moderately Abundant (McBee and Wiles, 1967; Smith et al. 1980). Francis (1977) found that consumers preferred steaks with higher amounts of marbling for flavor, juiciness, tenderness, and overall acceptability (Kerth & Miller 2015). On the other hand, Legako et al. (2015a) found that increasing quality grade did not show consistent increases in consumer palatability scores for juiciness, flavor liking, and overall liking among different beef muscles. Thus, consumers often prefer the flavor in meats from a higher quality grade, dependent on muscle.

Degree of Doneness

Uncooked meat has almost no aroma and merely a serummy flavor, implying that meat flavor is thermally derived (Mottram 1994). Formation of volatile flavor compounds via the interactions between flavor precursor compounds are time and temperature dependent. These thermal treatment conditions lead to controlled oxidation of lipids, which as previously discussed is essential to the development of meaty aroma (Khan et al., 2015; Byrne et al., 2002). During cooking, the compounds are constantly changing, thus changing the way flavor is perceived. Cooking can include a wide range of temperature conditions; meat can be grilled, roasted, boiled, or stewed for example, creating a wide range of flavor sensations perceived (Mottram 1994). With higher degrees of doneness, flavor may play a stronger role in determining customer satisfaction than other parameters, such as tenderness (Lorenzen et al., 1999). This implies that the degree of doneness is likely to affect flavor development, and thus, factors such as tenderness and juiciness may become less important. The formation of Maillard reaction products is enhanced at higher cooking temperatures with a dry-heat cooking method (Imafidon and Spanier, 1994). Therefore, differing degrees of doneness may impact the intensity of meat flavor (Myers et al., 2009). The association between flavor development and specific degrees of doneness is not well understood. The following three subsections highlight the effect of heating on the flavor-related compounds and sensory perception.

Influence of Degree of Doneness on Flavor-Contributing Compounds

In a study conducted by Smith et al. (1989), the proximate composition of cooked retail cuts of beef was measured. The study found that the percentage of total fat increased

with cooking due to a decrease in moisture; and the percentage of total protein increased with cooking. Spanier et al. (1990) found that as cooking temperature increased, so did the type and amounts of peptides.

Sugars are a key component in the Maillard reaction, and heating no doubt affects their concentrations. Sugars are subject to other reactions as well, such as isomerization and degradation. However, in a model system, these reactions occur mostly at temperatures above 80°C (van Boekel 2001), which are not typical internal temperatures of most meats; therefore, these reactions will not be discussed in detail. Mottram (1994) found that upon heating of a meat model system, quantities of carbohydrates decreased, with ribose experiencing the most significant loss. In raw meat, glucose has a higher concentration than ribose (Balagiannis et al., 2010 & Balagiannis et al., 2009). Despite its smaller concentration, ribose is much more reactive than glucose (Balagiannis et al., 2010; Laroque et al., 2008; Macey et al., 1964). These findings imply that ribose should decrease with cooking more than glucose. Balagiannis et al. (2010) also found that adding ribose to a meat system increased the amount of Maillard-derived volatiles present, but it did not affect the amount of lipid-derived compounds.

Sugars are much more reactive to heat and are present in smaller quantities compared to amino acids; thus, the amount of sugars present rather than the amount of amino acids is the limiting factor for the Maillard reaction (Balagiannis et al., 2010). For this reaction, the concentration of the active form of the sugar, the open chain, increases with temperature (van Boekel 2001). Normally a reducing sugar in this open chain form is required to begin the first stage of the Maillard reaction (van Boekel 2001).

Degradation of Amadori products, created during the Maillard reaction, is prevalent during the latter stages of the reaction (i.e. at higher temperatures), and this degradation results in the regeneration of some sugar fragments (van Boekel 2001). Balagiannis et al. (2009) found that in raw meat, mannose levels were very small and fructose was not detected. As temperature increased and the Maillard reaction progressed, however, the levels of both sugars increased, reached a maximum, and began to decrease through involvement in other interactions. Glucose concentration has a positive correlation with Strecker aldehydes, implying that Strecker degradation may be limited by glucose availability (Balagiannis et al., 2009).

Furthermore, Mottram and Elmore (2010) claim that in foods high in protein, such as meat, amino acids are present in excess over sugars, and because of this there is competition between the amino acids for available sugars. Since sugars are the limiting factor in the Maillard reaction, excess amino acids are present in comparison with sugars after heating (Balagiannis et al., 2010). In a study conducted by Balagiannis et al. (2009), amino acids were rapidly consumed during initial heating, and then concentrations leveled off. Like some volatile compounds, however, it is possible for some free amino acids to be regenerated, possibly from Amadori product-breakdown, after being consumed, resulting in no net loss or gain toward the end of the Maillard reaction (Balagiannis et al., 2009; Labuza & Baisier, 1992; Baisier & Labuza, 1992; van Boekel 2001). At higher temperatures, however, a continuous loss of free amino groups occurs through rapid advanced stages of the Maillard reaction, meanwhile relatively less regeneration occurs (van Boekel 2001).

As mentioned previously, fatty acids are important contributors to flavor because of their participation in lipid oxidation. Thermal oxidation involving fatty acids produces desirable volatile compounds, like saturated and unsaturated aldehydes and ketones, and other important components of beef flavor (Nawar, 1984; Mottram, 1998; Selke, Rohwedder, & Dutton, 1977, 1980). Legako et al. (2015b) revealed that there is evidence to show that fatty acids are affected differently by cooking based on their lipid fractions. Regarding the entire lipid fractions, cooking increased the NL and decreased the PL across all quality grades (Legako et al., 2015b). In this same study, the PL concentration decreased and the NL concentration increased with cooking, and cooking affected PL more than the NL. This could possibly be explained by a fatty acid influx into the NL after PLs are broken down and migrate to other areas of the meat (Legako et al., 2015b).

The PL with a greater proportion of unsaturated fatty acids is very susceptible to thermal degradation (Igene & Pearson, 1979; Min & Ahn, 2005). The greater amount of conjugated double bonds within polyunsaturated fatty acids make them more susceptible to oxidation than MUFA and SFA. Polar lipids, also known to be more susceptible to oxidation (Mottram 1998), contain a greater proportion of PUFA. Due to this susceptibility, PUFAs in the PL are affected by thermal oxidation more than those in the NL (Terrell et al., 1968). Meat with higher PUFA levels has been shown to result in higher concentrations of lipid-derived aldehydes during cooking (Elmore et al., 1997; Elmore et al., 1999; Mottram & Elmore 2010). Legako et al. (2015b) found that with cooking, percentages of MUFA in the PL decreased, while percentages of PUFA in the PL increased due to the disappearance of a large amount of PL C18:1 cis 9. C18:1 cis 9

readily participates in the development of volatile compounds in meat, giving aldehydes (Cerny 2007). When these unsaturated fatty acids are introduced to a mixture of Maillard intermediates, they provide competing reactions which help build other volatile compounds (Mottram 1994).

With regard to cooking, pH is important to the Maillard reaction, because it affects the state of the reactants required to start the reaction. The reactive form of a sugar, its open chain form, depends on pH. The equilibrium between the open chain form and the ring form of the sugar shifts to more open chain and less ring form as pH and/or temperature increases (van Boekel 2001). The active form of the amino group is also pH dependent, as it must be unprotonated in order to provide a free electron pair to react with the carbonyl group of the sugar (van Boekel 2001). Van Boekel (2001) states that the pH will decrease with temperature, as there is an increased disassociation of water. Furthermore, at a lower pH fewer unprotonated amino groups will be present. Namiki (1988) claimed that the maximal rate of reaction between amino acids and sugars occurs at weakly acidic pH. Carboxylic acids formed during cooking cause the pH to decrease, which slows down the Maillard reaction (van Boekel 2001). This effect, however, is more noticeable at higher temperatures, i.e. above 80°C (Berg & van Boekel, 1994; van Boekel & Brands, 1998). When the pH of beef increases (for example, in high pH meat such as dark, firm, and dry meat), so do the water-holding capacity and heat transfer (Meynier & Mottram, 1995). As pH increases, the proteins have increased water-binding properties (Calkins & Hodgen, 2007). In meat with a low water- holding capacity, free water travels to the heating surface

and cools it, resulting in the formation of more lipid degradation products and less Maillard reaction products (Kerth & Miller 2015).

Influence of Degree of Doneness on Volatile Compounds

As mentioned previously, volatile compounds are generated from non-volatile water-soluble precursors and lipids via multiple reactions resulting from lipid oxidation and degradation and thermal degradation. The main reactions during cooking are the Maillard reaction between amino acids and reducing sugars and the thermal degradation of lipids (Mottram 1994). 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). The amount of reactions that occur involving the products of the aforementioned reactions increases the variety of compounds created (MacLeod 1994).

Compared to cooked beef, raw beef has not received much attention by way of volatile compound research (Insausti et al., 2002; King et al., 1993). Insausti et al. (2002) identified 53 volatile compounds in raw beef from Spanish cattle breeds: 19 aliphatic hydrocarbons, 11 aliphatic ketones, five aromatic hydrocarbons, six aliphatic aldehydes, four aliphatic alcohols, 2 alicyclic hydrocarbons, one sulfur compound, one furan, one terpenoid, and three esters. 2-propanone was the predominant compound. Of these 53 compounds, 23 were also previously reported in raw beef in other studies (Chung et al., 1994; King et al., 1993; Jackson et al., 1992; Dainty et al., 1989; Spanier et al., 1988; St. Angelo et al., 1987). The study done by Insausti et al. (2002) identified these compounds in raw beef that had been stored for a various number of days at a refrigerated

temperature, similar to the number of days that beef may be displayed in a retail setting. Upon storage, 13 of the 53 compounds identified had also been identified in cooked beef, implying that some of the chemical changes that take place in aging meat may contribute to formation of some volatile compounds that were previously considered to be formed via thermal degradation (Insausti et al., 2002).

The Maillard reaction is one of the most important pathways associated with cooked foods, because it does not require very high temperatures and easily produces volatile compounds at common cooked food temperatures (Mottram 1994). In an early stage of the Maillard reaction, Amadori products rearrange to create many compounds, including dicarbonyl compounds (Mottram 1994). Mottram (1994) suggests that these dicarbonyl compounds (i.e. acetone, propanal, isobutanal) are most important as contributing reactants for the formation of other volatiles more closely associated with contributing to meat flavor. Thus, these dicarbonyl compounds may show an increase in concentration followed by a decrease as they participate in other reactions. Amadori and Heyns rearrangement products, which are also formed during the Maillard reaction, are unstable above ambient temperature and readily react with other compounds (Mottram & Elmore 2010).

One class of compounds resulting from the Maillard reaction that many researchers consider the most important volatiles formed during meat cookery are sulfur compounds, and large amounts of hydrogen sulfide are produced during heating (Bailey 1994). Acetaldehyde, formed via Strecker degradation, can react with these hydrogen sulfide compounds to produce other volatile compounds (Bailey 1994). Thus, like the

dicarbonyl compounds, the concentration of acetaldehyde may show an initial increase with temperature as it is formed but may then decrease with higher temperatures as it participates in other reactions to become a different product. 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). Balagiannis et al. (2010) and Mottram and Elmore (2010) reveal that aldehydes, such as the methyl butanals, can behave in this manner. Pyrazines are a class of volatile compounds characteristic of cooked beef, and Parker et al. (2010) found that in a meat-based pet food, the formation of trimethyl pyrazine involves the incorporation of another volatile compound, 2,3-butanedione. So, the concentration of 2,3-butanedione may decrease with temperature as trimethylpyrazine increases.

Sensory Relationships with Degree of Doneness

Cooking imparts a wide range of temperature conditions; therefore, a variety of flavor profiles are possible (Mottram 1994). The 1999 Beef Customer Satisfaction Study (Lorenzen et al.) determined that consumer ratings tended to be higher for steaks cooked to lower degrees of doneness, meanwhile steaks cooked “well done or more” were more closely related to those cooked “medium” than those cooked to “medium well.” Glascock (2014) and Miller et al. (1995) found that as degree of doneness increases, consumer liking overall and liking for flavor, beef flavor, juiciness, and tenderness decreases across various cuts and cooking methods.

Grinding

Ground beef is easy to prepare, relatively inexpensive, and versatile in preparation (Troutt et al., 1992). It is one of the most popular meat products and represents a multi-billion dollar asset to the meat industry (Glover, 1968; Cross et al., 1980; Parizek et al., 1981; Miller et al., 1987, Troutt et al., 1992). However, relatively few previous works have documented the inherent influence of grinding on flavor compounds. Thus, knowing how grinding of a meat product affects flavor development will contribute valuable information to the realm of meat flavor research.

Influence of Grinding on Flavor-Contributing Compounds

Troutt et al. (1992) found that the results of proximate analysis of ground beef patties varying in fat percent from five to 30 percent were similar to results found in whole muscle beef steaks. Moisture and protein content had a negative correlation with fat percent. With cooking, moisture was highest in the lower cooked temperature, while fat and protein were highest in the higher cooked temperature. It is not known whether these proximate parameters affect the flavor development of ground beef in the same way as they do in whole muscle steaks. In a study conducted by O'Quinn (2012), increased intramuscular fat in ground strip steaks was associated with increased percentages of C14:1, C16:1 c9, and C18:1 c9.

Influence on Volatile Compounds

Processing of beef causes particle size reduction and increased surface area (Lee et al. 2005). These traits make ground beef more susceptible to oxidation. Therefore, it is

necessary to employ methods to decrease some off-flavors associated with oxidation, such as the addition of antioxidants or application of irradiation to reduce growth of spoilage organisms. The major volatile compounds responsible for off-odor in irradiated meats are sulfur compounds (Ahn et al., 1999; Ahn et al., 2000; Ahn et al., 2001; Ahn & Nam 2004). Ahn and Nam (2004) found that unlike most whole muscle beef, almost all volatiles produced in ground beef were lipid oxidation products with the predominant compounds being 2-propanone, 2-butanone, and 2,3-butanedione; hexanal was the predominant aldehyde compound. Other studies have also identified 2-propanone as the main compound in cooked ground beef (MacLeod & Ames 1986; Gorraiz 1999). O'Quinn (2012) discovered that as intramuscular fat of ground strip steaks increased, so did the amount of 2,3-butanedione.

Sensory Relationships

While the differences between flavor development of ground beef versus whole muscle beef are not well known, the sensory characteristics of ground beef have been extensively studied. It is important to note, however, that the following studies prepared ground beef in a traditional way by adding fat instead of maintaining the fat content of the original cut of meat. Melton et al. (1982a) found that the intensity of beef-fat flavor in ground beef from cows fed a corn diet increased the longer the cows were fed, while the intensities of milky-oily, sour, liver, fishy, and metallic flavors decreased. Melton et al. (1982b) found that flavor scores of ground beef from grass-fed steers were lower than those from grain-fed steers. Troutt et al. (1992) and Berry and Leddy (1984) found that ground beef patties with higher fat content caused an increase in moisture release and juiciness.

Troutt et al. (1992) also found that beef flavor intensity was higher in ground beef with more fat.

O'Quinn (2016) found that beef with higher percentages of intramuscular fat containing more MUFAs and less SFAs and PUFAs experiences stronger preference from sensory panelists. In a study conducted by McHenry (2013), sensory attributes of ground beef from different muscles were evaluated. Monounsaturated fatty acids were related to positive beef flavor characteristics, while an increased percent of SFA was related to negative off-flavors. Meanwhile, MUFA were positively related to beefy/brothy and browned/grilled flavors and negatively associated with sour/acidic off-flavors. Saturated fatty acids, however, particularly C18:0, were associated with negative off-flavors. Similarly, O'Quinn (2016) discovered that the concentrations of MUFAs including C12:1, C14:1, C16:1 c9, and C18:1 cis 9 were positively correlated with overall flavor desirability scores, while stearic acid concentration was negatively correlated with overall flavor desirability. O'Quinn (2016) also discovered that overall flavor desirability was negatively correlated with concentrations of PUFA including C18:2, C18:3n3, and C22:5n3. This same study showed that 2,3-butanedione, 3-hydroxy-2-butanone, 3-methyl butanal, and pentanal concentrations were positively correlated with overall flavor desirability scores, while high concentrations of dimethyl sulfide were not as desired.

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CHAPTER III

EFFECTS OF QUALITY GRADE, DEGREE OF DONENESS, AND GRINDING ON
PROXIMATE COMPOSITION AND FATTY ACIDS OF BEEF STRIP STEAKS

Abstract

Percent moisture, protein, total fat, fatty acid content, and cooking duration was determined for beef strip steaks and ground patties of three USDA quality grades (Prime, Low Choice, and Standard) tempered in refrigerated temperatures (3-5°C), room temperature (24-26°C), or cooked on an electric clamshell-style grill to an endpoint temperature of 55, 60, 71, or 77°C. Steaks took longer to cook compared with ground patties ($P < 0.05$), and Standard samples took longer to cook compared with Prime and Low Choice ($P < 0.05$). Protein content decreased ($P < 0.05$) as fat level increased, and Prime 25°C samples had the lowest ($P < 0.05$) protein percentages in both steaks and ground patties. Fatty acids were impacted by the 3-way interaction of quality grade, degree of doneness, and product type; the 2-way interaction of degree of doneness by product type; the 2-way interaction of quality grade by degree of doneness; and the 2-way interaction of quality grade by product type. These effects varied per individual fatty acid. Generally, an increase in quality grade was associated with an increase in the amount of fatty acid deposition. There were some instances, however, where Standard samples, containing the least amount of intramuscular fat, had the greatest ($P < 0.05$) amount of fatty acids compared with Prime and Low Choice samples. Ground patties contained a greater ($P < 0.05$) amount of fatty acids compared with steaks. The 3-way

interaction of quality grade, degree of doneness, and product type impacted the amount of fatty acids in the neutral lipid fraction to a greater extent than the polar lipid fraction.

Introduction

The objective of this study was to determine how cooking duration, proximate composition, and fatty acids of differing quality grades respond to grinding and differing degrees of doneness. Corbin et al. (2015) determined that consumer flavor liking scores increased with increased fat percentage. Fat content of closely trimmed whole meats is directly proportional to marbling and inversely proportional to moisture content (Hedrick et al., 1981; Brackebusch et al., 1991). Smith et al. (1989) found that the percentage of total fat increased with cooking due to a decrease in moisture; and the percentage of total protein increased with cooking. Troutt et al. (1992) found that the results of proximate analysis of ground beef patties varying in fat percent from five to 30 percent were similar to results found in whole muscle beef steaks.

Moisture is a factor that effects the cook time of steaks and ground patties in that increased moisture promotes increased heat transfer (Ngadi et al., 2001). A difference in time that the sample is exposed to heat may create a decreased or increased amount of time available for lipid degradation to occur and thus the creation of more or less fatty acid products. This fatty acid composition plays an important role in consumers' perception of the sensory quality attributes associated with beef (Wood et al., 2004). Fatty acids may be separated into neutral (NL) and polar (PL) lipid fractions, and these lipid fractions are affected by quality grade (Legako et al., 2015b). Accumulation of fatty acids in the NL increases intramuscular fat content (Wood et al., 2008). Since the PL is a

structural component, it remains fairly constant in concentration (Legako et al., 2015b). Legako et al. (2015b) revealed that there is evidence to show that fatty acids are affected differently by cooking based on their lipid fractions. Regarding the entire lipid fractions, cooking increased the NL and decreased the PL across all quality grades (Legako et al., 2015b). Several researchers have revealed that as fat content increases, so do the concentrations of all fatty acids (Wood et al., 2008; Scollan et al., 2006). In a study conducted by O'Quinn (2012), increased intramuscular fat in ground strip steaks was associated with increased percentages of C14:1, C16:1 n9, and C18:1 n9. Knowing how these compounds develop in response to quality grade, degree of doneness, and grinding and how they relate with the formation of volatile compounds via lipid degradation will allow us to better utilize sensory data associated with these fatty acids. This could then create the potential for purposeful development of those volatile compounds involving fatty acid degradation that consumers find most enjoyable when eating beef.

Materials and Methods

Product Selection

Paired beef strip loins [IMPS 180, (NAMP, 2010)] were collected from 24 carcasses across three USDA quality grades (Prime, Low Choice, and Standard, n = 8 per quality grade; USDA 1977) of "A" maturity animals. Carcasses were selected at a commercial beef processing plant in Hyrum, UT after approximately 24 hours post-mortem chilling. Carcass measures included hot carcass weights (kg), external fat thickness (mm), ribeye area (cm²), 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 marble score of Slightly abundant⁰⁰ (700) or greater, USDA Low Choice carcasses were within Small⁰⁰ (400) to Small¹⁰⁰ (499), and USDA Standard carcasses had Traces¹⁰⁰ (200) 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 days post-mortem.

Processing

At day 21 of post-mortem aging, loins were removed from packaging to produce steaks and ground patties. Strip loins were cut into 2.54 cm steaks progressing anterior to posterior using a 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 degree of doneness, 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 *Longissimus lumborum* muscle. 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 frozen chubs were then 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 hours but no more than 24 hours to a temperature range of $3 - 5^{\circ}\text{C}$. 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 two hours after first being thawed to $3 - 5^{\circ}\text{C}$. 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 (well done) $^{\circ}\text{C}$ after being thawed to a temperature of $3 - 5^{\circ}\text{C}$. 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 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.

Cooking Duration

Cooking times for steaks and patties were measured in minutes by a timer that started when the sample was placed on the grill and ended when the sample reached the appropriate internal temperature (55, 60, 71, or 77°C).

Sample Preparation for Chemical Analysis

Following tempering or cooking, samples were frozen in liquid nitrogen and pulverized in a blender (Nutri Ninja: Model BL642, Euro-Pro Operating LLC, Newton, MA). The resulting homogeneous samples were stored in 4.5 x 9 inch VWR sterile sampling bags (VWR International, Cat. No. 82007-706, Radnor, PA) at -80°C until later analyses.

Proximate Analysis

Moisture Analysis

An AOAC official oven-drying method (950.46 and 934.01; AOAC 1995) was used to determine moisture percentages. One gram of sample was weighed into a 57 mm VWR aluminum pan (VWR International, Cat. No. 25433-008, Radnor, PA) and placed in an oven (National Appliance Company, Model 430, Portland, OR) at 100°C for 16 hours. The pans were then placed in desiccators to cool to room temperature and be weighed. The moisture percentage was calculated as, $\text{Moisture \%} = (\text{initial weight of sample, g} - \text{weight of dried sample, g}) / (\text{initial weight of sample, g}) \times 100$.

Protein Analysis

The percent protein measurement was performed by combustion with a LECO (Model FP-528) using the AOAC method 992.15 (AOAC, 2006). Percent protein was generated by multiplying nitrogen content by 6.25.

Fat Analysis

A chloroform-methanol procedure was used to extract fat from the meat samples, similar to Folch et al. (1957), so that the fat percentage could be calculated. One gram of homogenized sample was weighed into a 50 mL conical tube and vortexed. The sample then underwent additions of methanol (8 mL) and chloroform (8 mL) and additional vortexing. Following centrifugation, 4 mL of the chloroform extract were pipetted into culture tubes and evaporated to dryness. The dry fat residue and tube were then weighed and the final weights recorded. The fat percentage was calculated as (g residue / g wet sample) x 2 x 100. The multiplication factor of two was used because only half of the extract was evaporated.

Fatty Acids

Total lipid was extracted from 0.5 g of homogenized samples via a chloroform-methanol method (Folch et al. 1957). Briefly, chloroform and methanol were added to the samples and mixed via a Polytron-PT 2100 (Kinematica, Inc., Bohemia, NY). The contents were filtered through Whatman no. 40 filter paper into another 30 mL glass screw-cap tube, KCl was added, and the mixture vortexed for 10 minutes. Samples were stored at refrigeration (4°C) overnight to allow for separation of aqueous (discarded) and organic phases. Phospholipid separation was carried out per the method described by

Juaneda and Rocquelin (1985). The organic phase was evaporated to dryness, and the test tubes were washed with 2 mL of chloroform twice. The extracted lipids were then loaded onto a pre-rinsed (via 10 mL of methanol followed by 10 mL of chloroform) Resprep SPE EPH silica gel cartridge (Restek Corporation, Bellefonte, PA) for separation of neutral and polar lipids. The NL eluted first by 10 mL of chloroform, followed by the PL with 15 mL of methanol. The lipid fractions were transferred to a 15 mL glass screw-cap tube and evaporated to dryness. Methylation of lipid fractions to produce fatty acid methyl esters (FAME) was carried out per the method described by O'Fallon (2007). An internal standard (1 mL of 0.5 mg of C13:0/mL of methanol), KOH, and methanol were added, and the tubes were placed in a 55°C water bath for 1.5 hours before sulfuric acid was added, and the tubes were again placed in the water bath. Hexane was added to extract FAME, the contents were vortexed for 5 minutes, centrifuged for 10 minutes, and the hexane layer containing the FAME was transferred to a GC vial. Separation of FAME was carried out by a GC equipped with an HP-88 capillary column (30m × 0.25 mm × 0.20 µm; Agilent Technologies, Palo Alto, CA, USA) and a flame ionization detector (FID). One microliter of sample was injected with a split ratio of 50:1. The oven method was as follows: 120°C held for 1 min, increased to a temperature of 170 °C at the rate of 15°C/min, held for 2 min, then increased to a temperature of 200°C at the rate of 3°C/min, held for 1 min, and finally increased to a temperature of 235°C at a rate of 20°C/min and held for 1 min. Hydrogen was used as the carrier gas. The FID will be operated at 300°C. Fatty acids were identified based on the similarity of retention times

with GC reference standards. Concentrations of fatty acids were calculated on a dry-weight basis.

Statistical Analysis

All statistical analyses were performed by SAS® version 9.4 (SAS Institute, Cary, NC) using the GLIMMIX procedure. One-way analysis of variance was used to determine the effect of quality grade on carcass characteristics: hot carcass weight (HCW); marbling; kidney, pelvic, and heart fat (KPH); ribeye area (REA); calculated yield grade (YG); ribeye color; lean maturity; and skeletal maturity. The statistical significance was determined at $P \leq 0.05$. A 3-way analysis of variance was utilized to determine the influence of the fixed effects (quality grade, whole muscle vs. ground, and degree of doneness) on each compound measured. 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), in which $n=8$. The sub-plot was the sample type (whole steaks vs. ground patties), in which $n=24$. The sub-sub-plot was the thermal processing temperature (4, 25, 55, 60, 71, and 77°C), in which $n=48$.

Results

Carcass Data

The data collected during carcass selection can be found in Table 3-1 in the appendix. 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 ($P < 0.05$). 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 (cm^2), KPH percent, and HCW (kg) measurements, and differed by quality grade ($P < 0.001$). Measurements of ribeye color, 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 pre-harvest handling were not confirmed. However, per requirements of the beef processor, these carcasses would be in line with common commercial North American genotypes and feedlot production practices.

Cooking Duration and Proximate Analysis

Cooking Duration

The LS means for cooking duration of each treatment can be found in Table 3-2. The main effects and interactions impacting cooking duration can be found in Table 3-3. The interaction of product (steak versus ground patty) and degree of doneness (DOD) impacted cooking duration ($P < 0.001$). Cooking duration increased ($P < 0.05$) with DOD in both steaks and ground patties but was longer ($P < 0.05$) in steaks. This interaction is depicted in Figure 3-1. Prime and Low Choice sample cook times were similar ($P >$

0.05), but longer ($P < 0.05$) than Standard samples, i.e. the Standard samples cooked faster than the other quality grades.

Moisture

The LS means of percent moisture can be found in Table 3-2. The main effects and interactions impacting the moisture percentage can be found in Table 3-3. A 3-way interaction between quality grade, DOD, and steak versus ground patty on percent moisture was observed ($P = 0.004$; Figure 3-2). While steaks and patties followed a similar trend, there were some differences in the percent moisture of the cooked samples (55, 60, 71, 77°C) depending on quality grade between the two product types. For both steaks and patties: Standard samples had the greatest percent moisture ($P < 0.05$), followed by Low Choice and Prime, meanwhile the overall percent moisture decreased as DOD increased ($P < 0.05$). The percent moisture was higher in steaks than in patties ($P < 0.05$). Percentages of moisture of Standard 4°C and 25°C samples were similar ($P > 0.05$) in both steak and ground samples, but at cooked degrees of doneness (55, 60, 71, 77°C) moisture differed ($P < 0.05$) in Standard steaks and patties. In Low Choice samples, the percentages of moisture at 25, 55, 71, and 77°C differed ($P < 0.05$) between steaks and ground patties. In Prime samples, percent moisture was greater ($P < 0.05$) in steaks compared with ground patties at 25°C and 55°C.

Protein

A 3-way interaction between quality grade, DOD, and steak versus ground patty on percent protein was observed ($P = 0.006$; Figure 3-3).

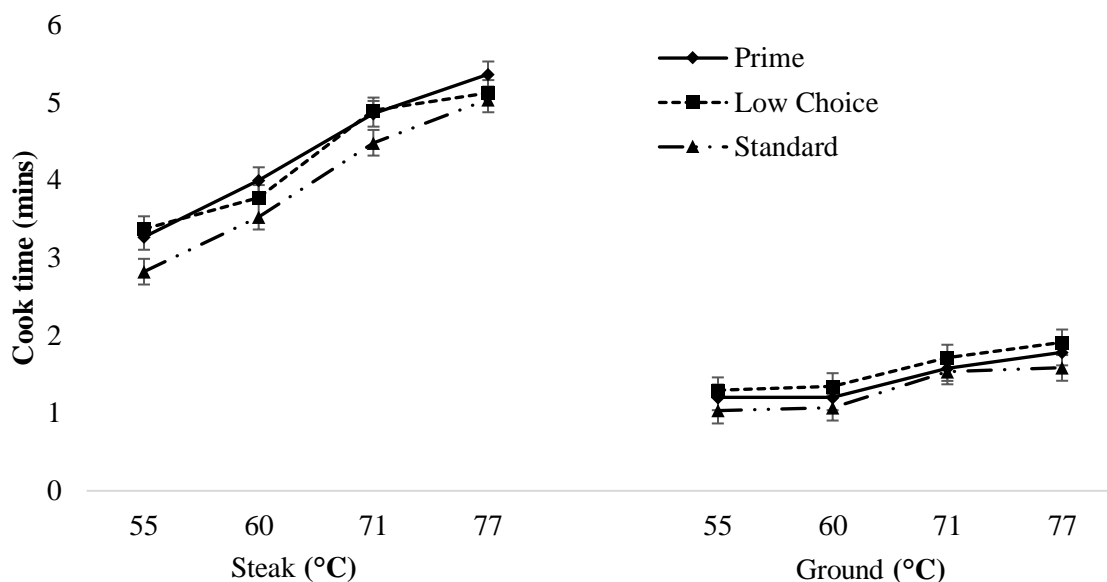


Figure 3-1. Cooking duration of USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A two-way interaction (DOD \times product type) was observed ($P < 0.001$). Quality grade also influenced cooking duration ($P \leq 0.004$).

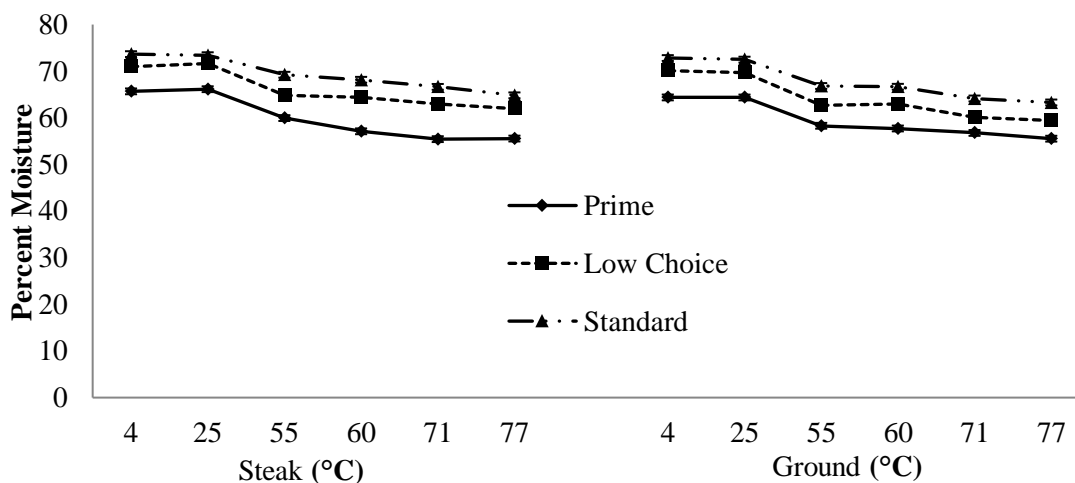


Figure 3-2. Percent of moisture in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P < 0.001$).

The LS means of these protein percentages can be found in Table 3-2. The main effects and interactions impacting protein content can be found in Table 3-3. For both steak and ground patties, 25°C Prime samples had the lowest ($P < 0.05$) protein percentages of all treatments. Both steaks and ground patties showed an increase ($P < 0.05$) in the percentage of protein from 25°C to 55°C and then tended to level off ($P > 0.05$) as DOD increased, until 77°C, where protein percentage increased again ($P < 0.05$). Standard 4, 25, and 71°C samples differed between steaks and ground patties ($P < 0.05$). Low Choice samples followed this same trend ($P = 0.0059$). In Prime samples, the 4, 25, 60, and 77°C samples differed ($P < 0.05$) across the two product types ($P < 0.05$). The percent of protein was greatest ($P < 0.05$) in steaks for the following samples: Standard, Low Choice, and Prime 4°C; Standard, Low Choice, and Prime 25°C; and Prime 77°C. Meanwhile, Standard 71°C and Prime 60°C samples had protein percentages greater ($P < 0.05$) in ground patties.

Fat

Quality grade, DOD, and product each individually influenced fat content ($P < 0.001$). The LS means for fat percent can be found in Table 3-2. The main effects and interactions impacting the percentage of fat can be found in Table 3-3. Fat percent was greatest ($P < 0.05$; Figure 3-4) in Prime samples, followed by Low Choice and Standard. The percentages of fat in raw samples (4°C and 25°C) were similar ($P > 0.05$) to each other but lower ($P < 0.05$) than the cooked samples (55, 60, 71, and 77°C). Among cooked samples, fat percentages differed ($P < 0.05$) between 60 and 77°C, being greater ($P < 0.05$) in 77°C beef. Fat percent of ground patties was greater ($P < 0.05$) than steaks.

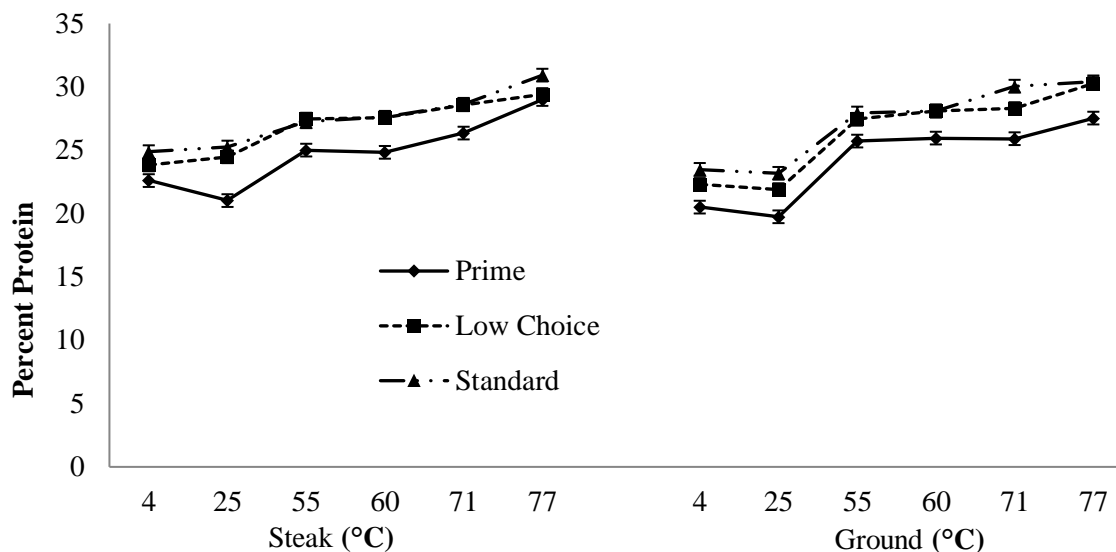


Figure 3-3. Percent of protein in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P = 0.005$).

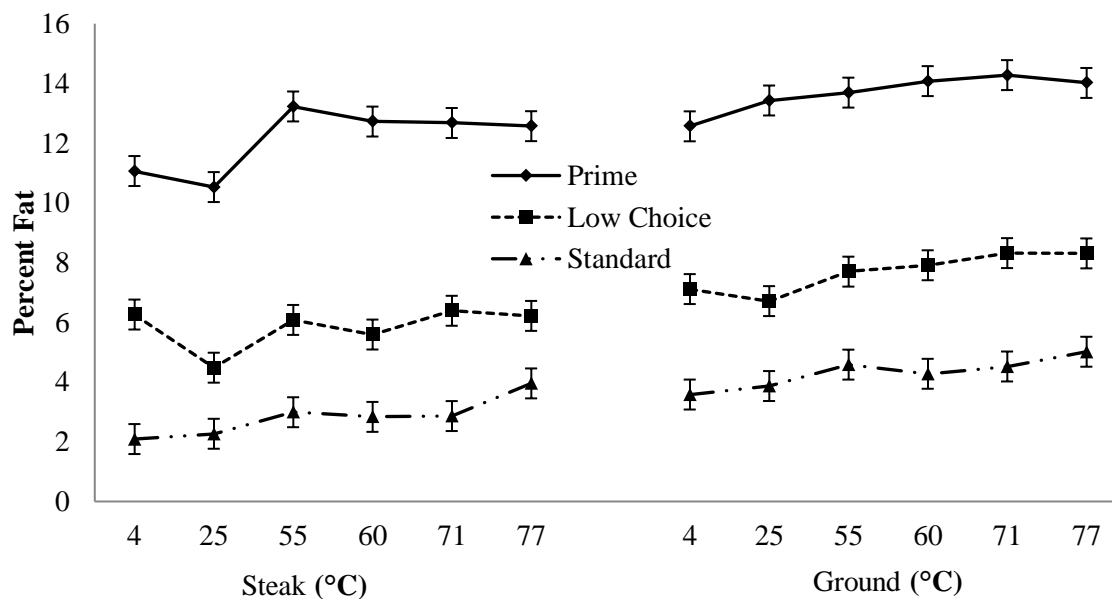


Figure 3-4. Percent of total intramuscular fat in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. The two-way interaction (quality grade \times DOD) was observed ($P = 0.0465$). Product type also influenced intramuscular fat ($P < 0.001$).

Fatty Acids

Neutral Lipids

A total of ten saturated fatty acids (SFA), eight monounsaturated fatty acids (MUFA), and eight polyunsaturated fatty acids (PUFA) were identified in steaks and ground patties. The common name for each fatty acid identified in this study is defined in Table 3-4. These fatty acids were separated into their neutral and polar lipid fractions and were measured on a concentration and a percentage basis. The main effects and interactions impacting the concentrations and percentages of neutral lipids can be found in Tables 3-5 and 3-6, respectively. Within the neutral lipid fraction, 15 individual fatty acids and the total SFA, MUFA, and PUFA were affected by the 3-way interaction of quality grade, degree of doneness, and product type. The SFA affected were: C14:0 ($P < 0.001$), C15:0 ($P < 0.001$), C16:0 ($P < 0.001$), C17:0 ($P < 0.001$), C18:0 ($P = 0.007$), and C22:0 ($P = 0.005$). C14:0 (Figure 3-5), C14:1 n5, and C15:0. The LS means of neutral lipid SFA concentrations can be found in Table 3-5a in the appendix. Among Prime samples these fatty acids were greater ($P < 0.05$) in ground patties compared with steaks of 4, 25, and 55°C, while their concentration in 60, 71, and 77° patties were lower ($P < 0.05$) or did not differ ($P > 0.05$) compared with steaks at the same degrees of doneness. This was also true for the other SFA affected (C16:0, C17:0, and C18:0), but they were greater ($P < 0.05$) in Prime ground patties compared with steaks only in 4 and 25°C samples (Figure 3-6). This means that these SFA increased ($P < 0.05$) with degree of doneness in Prime steaks but decreased ($P < 0.05$) with degree of doneness in Prime ground patties. SFA in Low Choice and Standard samples tended to not differ between

steaks and ground patties, but there were some instances where the concentration in ground patties was greater ($P < 0.05$), but this varied by degree of doneness and individual SFA. Within product type, these SFAs tended to be greatest in Prime samples, however the concentration in raw Prime samples was not always significantly different from Low Choice. The concentration in Low Choice samples also tended to be greater than Standard, however, this difference was not always significant. The final SFA affected by this 3-way interaction was C22:0 (Figure 3-7), and among Prime samples, it was greater ($P < 0.05$) in 4° and 77°C samples. Low Choice and Standard samples did not differ by product type.

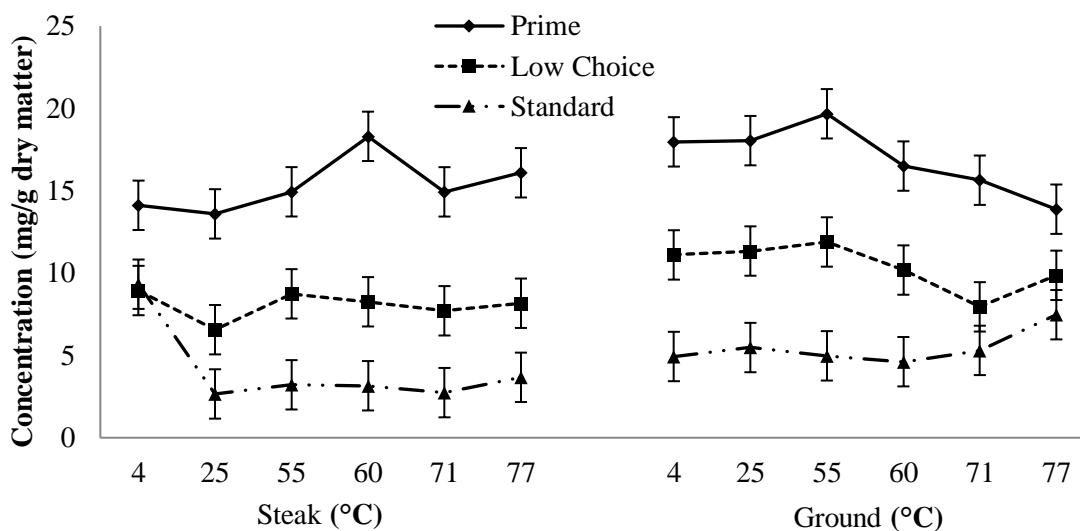


Figure 3-5. Concentration of C14:0 from the neutral lipid fraction of USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P < 0.001$).

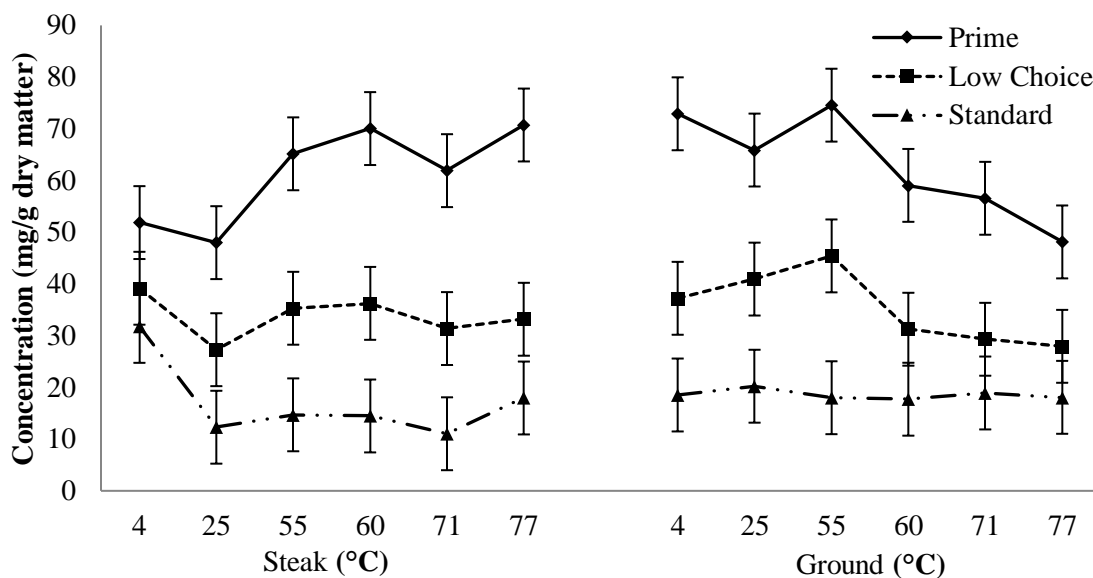


Figure 3-6. Concentration of C18:0 from the neutral lipid fraction of USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. A three-way interaction (quality grade × DOD × product type) was observed ($P < 0.001$).

MUFA affected by the 3-way interaction included: C14:1 n5 ($P = 0.015$), C16:1 n7 ($P = 0.005$; Figure 3-8, C17:1 n8 ($P = 0.008$), C18:1 trans ($P = 0.013$), C18:1 n9 cis ($P = 0.010$), and C20:1 n9 ($P = 0.007$; Figure 3-9). The LS means for neutral lipid MUFA concentrations can be found in Table 3-5b in the appendix. MUFA behaved similarly to SFA in that their concentration in Prime ground patties was greater ($P < 0.05$) compared with steaks until 60°C at which point the concentration became similar ($P > 0.05$) to that in steaks with an increase in degree of doneness. MUFA in Low Choice samples were greater ($P < 0.05$) in 25°C ground patties compared with 25°C steaks, and the concentration in ground patties became similar ($P > 0.05$) to that in steaks at cooked degrees of doneness. However, there were some cases where the concentration in Low

Choice ground patties was greater ($P < 0.05$) compared with steaks, but this varied by degree of doneness and individual MUFA. Concentrations of C16:1 n7, C14:1 n5, C20:1 n9, C18:1 trans, and C18:1 n9 cis in Standard ground patties differed ($P < 0.05$) from Standard steaks at 4° and 77°C; ground patties were greater ($P < 0.05$) in concentration compared with steaks at 77°C, while steaks were greater ($P < 0.05$) at 4°C.

PUFA affected by the 3-way interaction included: C18:2 n6 ($P = 0.012$), C18:2 trans ($P = 0.001$), and C20:4 n6 ($P = 0.014$). The LS means for neutral lipid PUFA concentrations can be found in Table 3-5c in the appendix. The former two PUFA listed behaved similarly to the aforementioned MUFA. C20:4 n6 (Figure 3-10) on the other hand, behaved differently. This PUFA in Standard 77°C ground patties was greater ($P < 0.05$) than all steak samples, regardless of quality grade or degree of doneness.

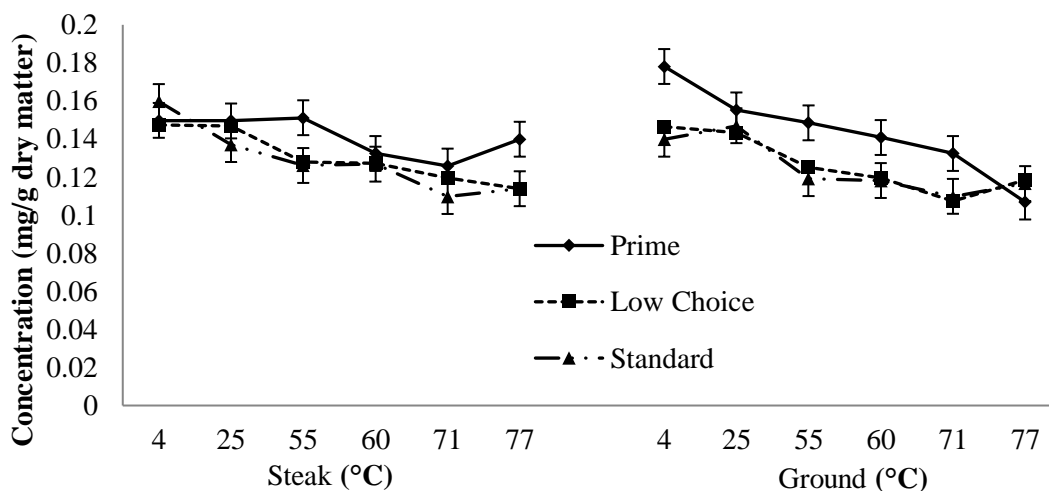


Figure 3-7. Concentration of C22:0 from the neutral lipid fraction of USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P < 0.001$).

Meanwhile, its concentration within the other degrees of doneness in Standard ground patties did not differ ($P > 0.05$) from those in steaks. The concentration in Prime and Low Choice samples also did not differ ($P > 0.05$) by product type.

Total SFA (Figure 3-11) in Prime ground patties did not differ ($P > 0.05$) between 4, 25, and 55°C, but decreased ($P < 0.05$) in 60°C samples and then did not differ ($P > 0.05$) in cooked degrees of doneness. Similarly, total SFA in Prime steaks, were similar ($P > 0.05$) in raw degrees of doneness, increased ($P < 0.05$) at 55°C, and then did not differ ($P > 0.05$) among cooked samples. However, total SFA increased ($P < 0.05$) from raw to cooked samples in steaks, while they decreased ($P < 0.05$) from raw to cooked samples in ground patties. Total MUFA (Figure 3-12) in Prime ground patties was greater ($P < 0.05$) compared with steaks in 4, 25, 55, and 71°C samples; Low Choice ground

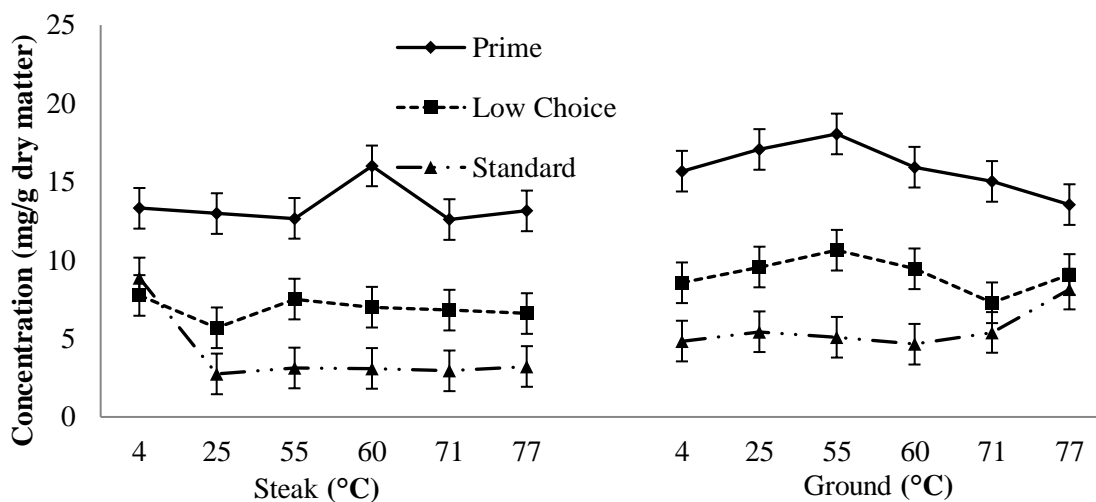


Figure 3-8. Concentration of C16:1 n7 from the neutral lipid fraction of USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P < 0.001$).

patties were greater ($P < 0.05$) than steaks in 25° and 60°C samples; and Standard ground patties were greater ($P < 0.05$) than steaks in only 25°C samples. Total MUFA in Standard steaks was greater ($P < 0.05$) compared with ground patties in 4°C samples. Otherwise, there were no differences ($P > 0.05$) within the quality grades based on degree of doneness. Total PUFA (Figure 3-13) was greater ($P < 0.05$) in ground patties in more instances than MUFA. Total PUFA in Prime ground patties was greater ($P < 0.05$) compared with steaks in 4, 25, 55, and 71°C samples; Low Choice was greater ($P < 0.05$) in 25, 55, 60, and 77°C samples; and Standard was greater ($P < 0.05$) in 25, 55, 71, and 77°C samples. Overall, there was a greater amount of SFA and MUFA than PUFA.

The 2-way interaction of product type and degree of doneness affected the following compounds: C18:3 n3 ($P = 0.002$), C20:2 ($P = 0.004$), C20:3 n6 ($P = 0.011$), C22:1 n9 ($P = 0.037$), and C20:5 n3 ($P = 0.047$). The LS means for the concentrations of these fatty acids can be found in Tables 3-5b and 3-5c in the appendix. C18:3 n3 was greater ($P < 0.05$) in ground patties compared with steaks in all degrees of doneness. C20:2 was greater ($P < 0.05$) in ground patties in 25, 55, 60, and 77°C samples. C20:3 n6 was greater ($P < 0.05$) in ground patties in 25, 55, 71, and 77°C samples. C22:1 n9 differed by product type only in steak 71°C samples, and these samples were greater ($P < 0.05$) compared with ground patties. C20:5 n3 (Figure 3-14) was greater ($P < 0.05$) in 4°C steaks compared with 4°C ground patties; meanwhile it was greater ($P < 0.05$) in 77°C ground patties compared with 77°C steaks.

The 2-way interaction of quality grade and degree of doneness impacted only two compounds: C18:3 n3 ($P = 0.018$) and C20:2 ($P = 0.033$). The LS means for the

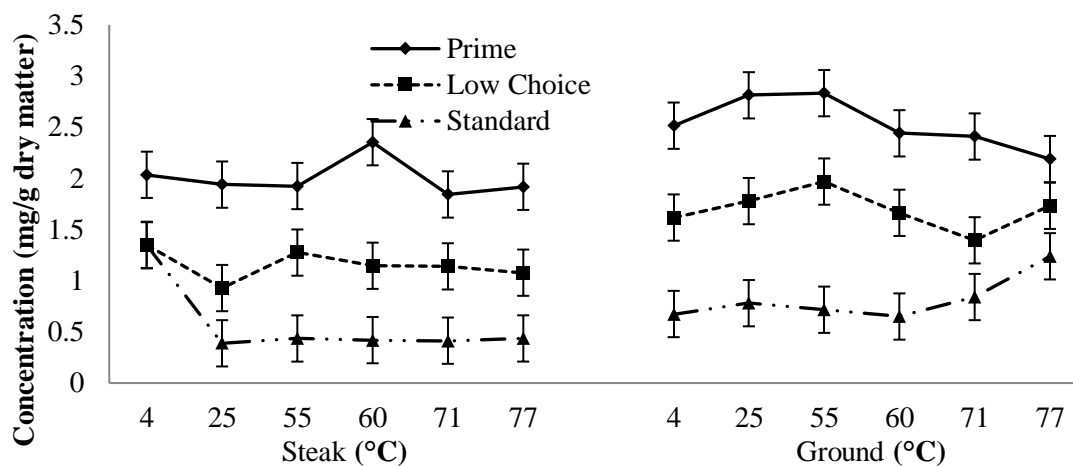


Figure 3-9. Concentration of C20:1 n9 from the neutral lipid fraction of USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. A three-way interaction (quality grade × DOD × product type) was observed ($P < 0.001$).

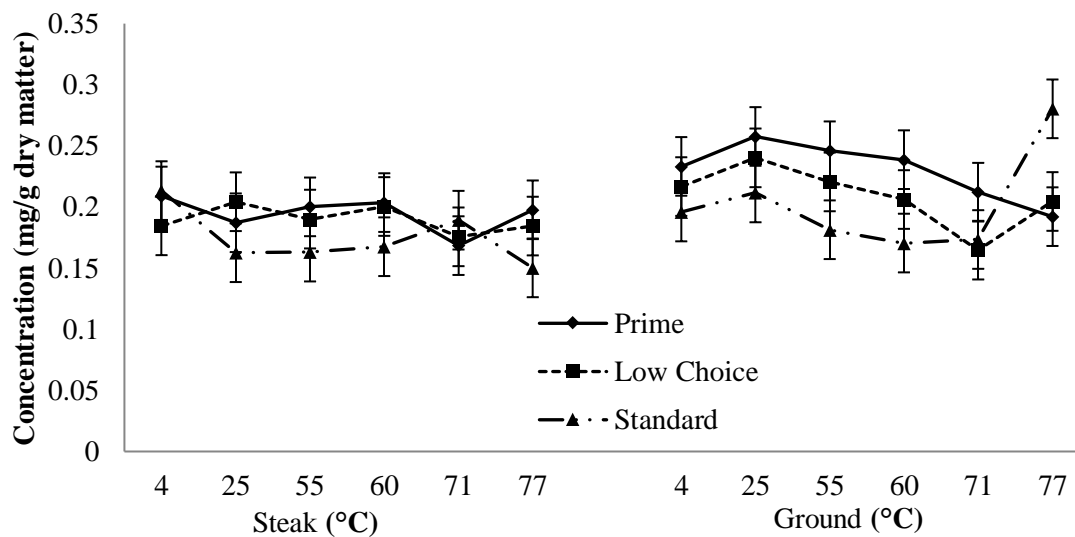


Figure 3-10. Concentration of C20:4 n6 from the neutral lipid fraction of USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. A three-way interaction (quality grade × DOD × product type) was observed ($P = 0.014$).

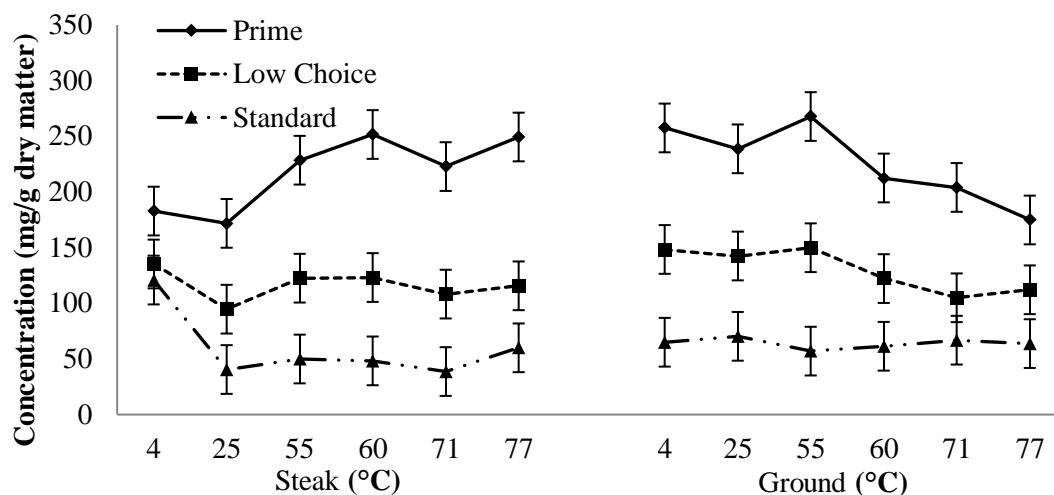


Figure 3-11. Concentration of total SFA from the neutral lipid fraction of USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P < 0.001$).

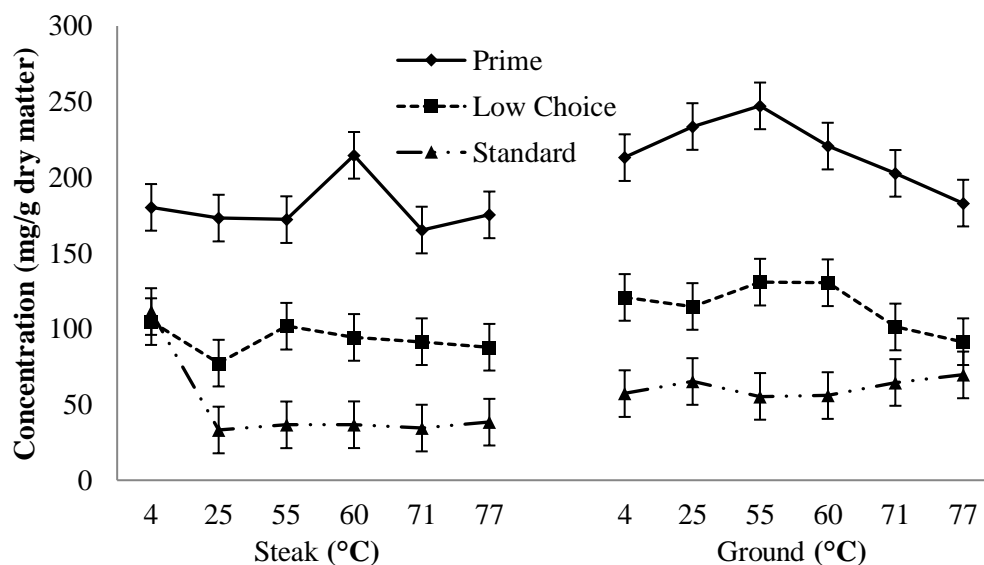


Figure 3-12. Concentration of total MUFA from the neutral lipid fraction of USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P = 0.012$).

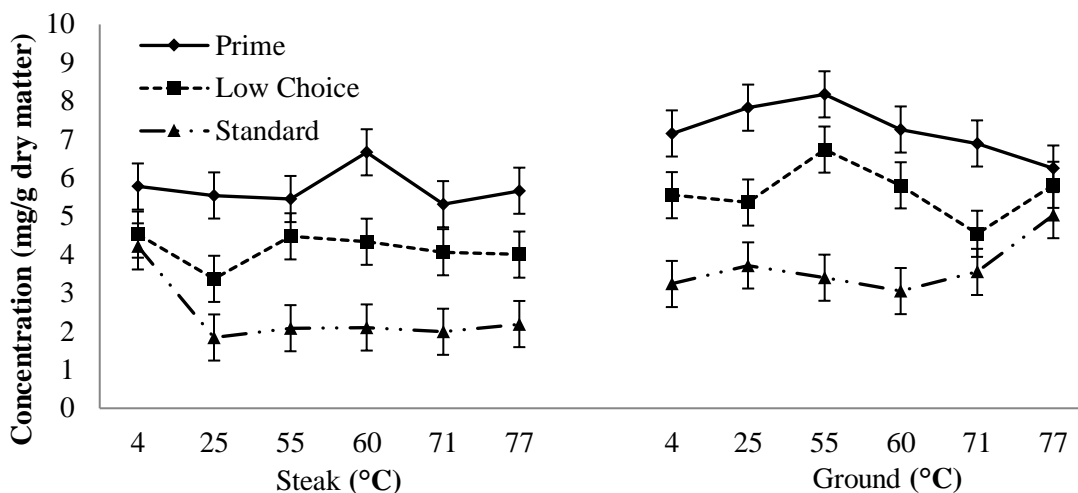


Figure 3-13. Concentration of total PUFA from the neutral lipid fraction of USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P = 0.016$).

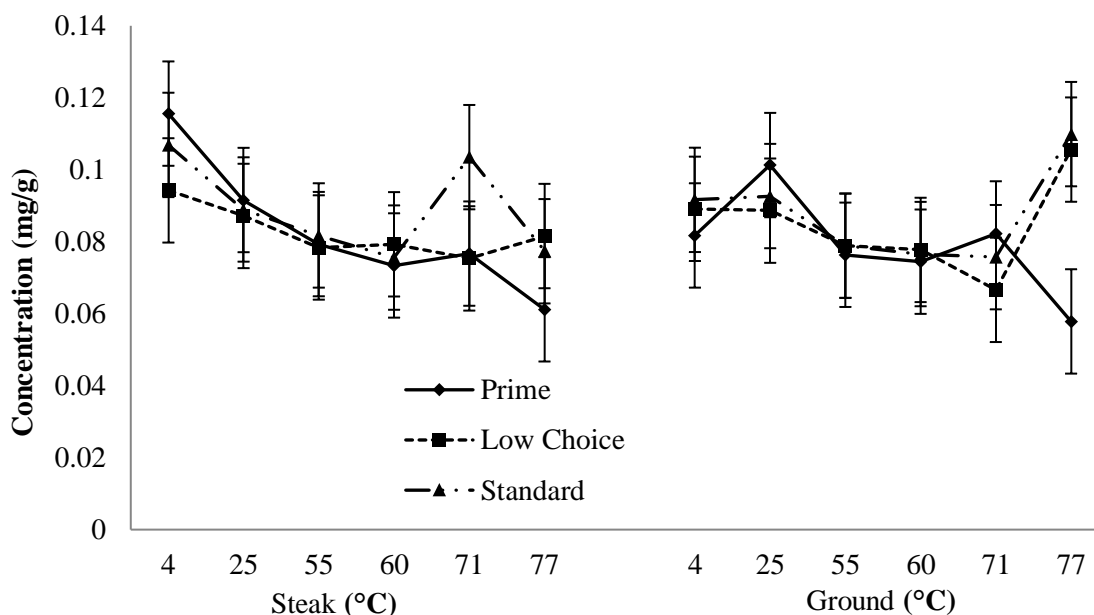


Figure 3-14. Concentration of C20:5 n3 from the neutral lipid fraction in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A two-way interaction (DOD \times product type) was observed ($P = 0.047$).

concentrations of these two fatty acids can be found in Table 3-5c in the appendix. C18:3 n3 was greatest ($P < 0.05$) in Prime samples compared to Low Choice and Standard in 25, 60, and 71°C samples. Low Choice samples were greater ($P < 0.05$) than Standard at all degrees of doneness. C20:2 was greatest ($P < 0.05$) in Prime samples compared with the other quality grades at all degrees of doneness except 77°C. Low Choice samples were greater ($P < 0.05$) than Standard samples at all degrees of doneness except 71°C. Three fatty acids were influenced by the interaction of quality grade and product type: C20:0 ($P = 0.043$), C21:0 ($P = 0.031$), and C20:2 ($P = 0.001$). C20:0 was greater ($P < 0.05$) in Prime steak samples compared with Prime ground patties, and Low Choice and Standard samples did not differ ($P > 0.05$) by product type. C21:0 was greater ($P < 0.05$) in ground patties compared with steaks in Prime and Low Choice samples. C20:2 was greatest ($P < 0.05$) in ground patties compared with steaks in all quality grades.

The interaction of product type and degree of doneness affected the percentage of the following fatty acids from the neutral lipid fraction: C14:1 n5 ($P = 0.042$), C16:1 n7 ($P = 0.033$), C18:0 ($P = 0.006$), C18:1 n9 cis ($P < 0.001$), C18:2 trans ($P = 0.023$), C18:3 n3 ($P = 0.032$), C20:1 n9 ($P = 0.009$), C20:3 n6 ($P = 0.013$), total SFA ($P = 0.007$), and total MUFA ($P = 0.014$). C14:1 n5, C16:1 n7, C18:2 trans, C18:3 n3 (Figure 3-15), and C20:1 n9 were greater ($P < 0.05$) in concentration in ground patties compared with steaks in 55° and 77°C samples. C20:3 n6 differed by product type in only 77°C samples, in which it was greater ($P < 0.05$) in ground patties. Percentages of C18:1 n9 cis were greater in ground patties compared with steaks at 55, 60, 71, and 77°C, while total MUFA percent was greater in ground patties at 60, 71, and 77°C. C18:0 was greatest ($P <$

0.05) in steaks compared with ground patties at 55, 60, and 77°C, while total SFA percent was greater in steaks at 55, 60, 71, and 77°C. The percentages of two fatty acids in the neutral lipid fraction, C21:0 ($P = 0.001$; Figure 3-16) and C22:0 ($P = 0.001$), were influenced by the interaction of quality grade and product type. Both fatty acids were greater ($P < 0.05$) in steaks compared with ground patties in Standard samples.

The following fatty acid percentages were affected by product type: C14:0 ($P = 0.041$), C15:0 ($P = 0.049$), C17:1 n8 ($P < 0.001$), C18:1 trans ($P = 0.025$), C18:2 n6 ($P = 0.003$), C20:0 ($P = 0.009$), C20:5 n3 ($P = 0.008$), and total PUFA ($P = 0.001$). The LS means of neutral lipid SFA, MUFA, and PUFA percentages can be found in Tables 3-6a, 3-6b, and 3-6c in the appendix.

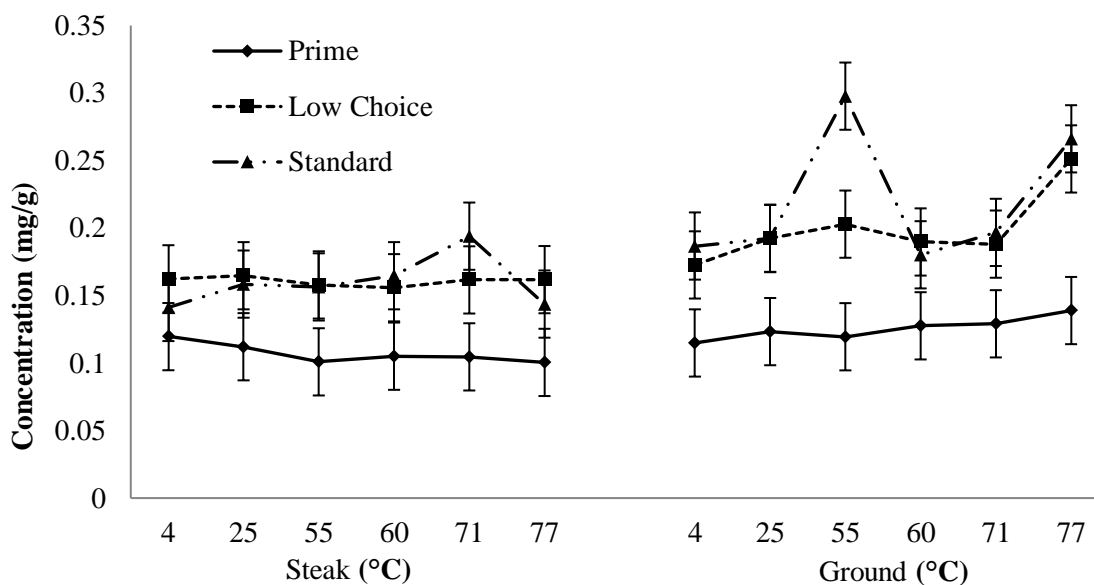


Figure 3-15. Percent of C18:3 n3 in the neutral lipid fraction in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A two-way interaction (DOD \times product type) was observed ($P = 0.032$).

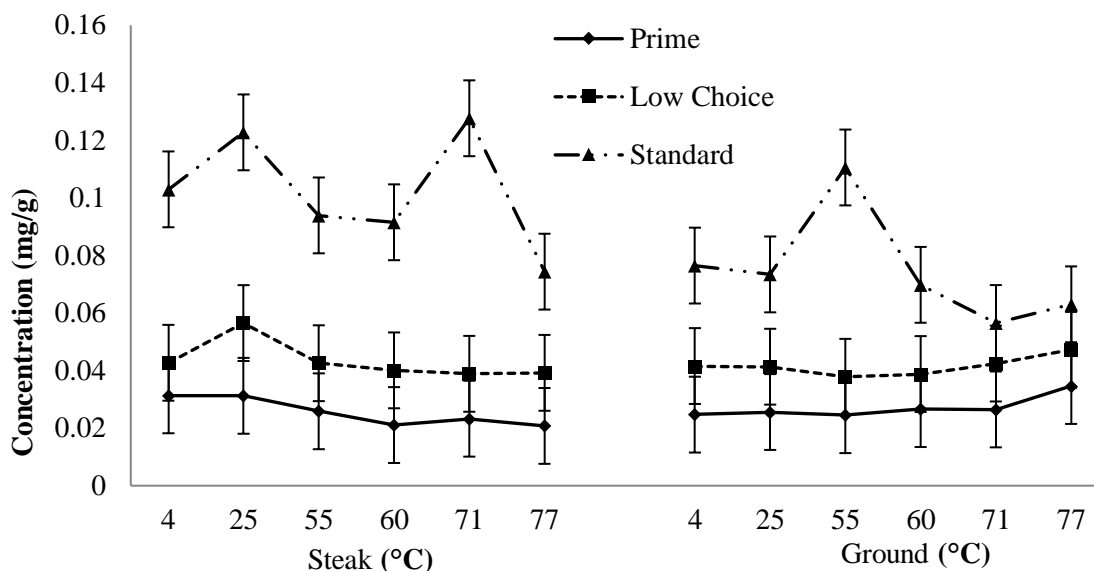


Figure 3-16. Percent of C21:0 in the neutral lipid fraction in USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. A two-way interaction (QG × product type) was observed (P = 0.001).

The LS means of neutral lipid SFA, MUFA, and PUFA percentages can be found in Tables 3-6a, 3-6b, and 3-6c in the appendix. C20:0 and C20:5 n3 were greater (P < 0.05) in steaks compared with ground patties, while the other fatty acids listed were all greater (P < 0.05) in ground patties. The following fatty acid percentages were influenced by quality grade: C15:0 (P < 0.001), C15:1 (P = 0.001), C17:0 (P = 0.029), C17:1 n8 (P = 0.004), C18:2 n6 (P < 0.001), C18:3 n6 (P = 0.014), C20:0 (P = 0.017), C18:3 n3 (P < 0.001), C20:2 (P = 0.001), C20:4 n6 (P < 0.001), C23:0 (P = 0.016), C20:5 n3 (P < 0.001), and total PUFA (P < 0.001). All of these fatty acid percentages were lowest (P < 0.05) in Prime samples, and percentages in Standard samples either did not differ (P >

0.05) compared with Low Choice or were greatest ($P < 0.05$) compared with the other quality grades.

Polar Lipids

The main effects and interactions impacting the concentrations and percentages of polar lipids can be found in Tables 3-7 and 3-8, respectively. The LS means of polar lipid SFA, MUFA, and PUFA concentrations can be found in Tables 3-7a, 3-7b, and 3-7c in the appendix. The LS means of the polar lipid SFA, MUFA, and PUFA percentages are found in Tables 3-8a, 3-8b, and 3-8c. Within the polar lipid fraction, four fatty acids were influenced by the 3-way interaction of quality grade, degree of doneness, and product type: C14:0 ($P = 0.048$), C18:0 ($P = 0.021$), C18:1 n9 cis ($P = 0.029$; Figure 3-17), and C20:4 n6 ($P = 0.029$; Figure 3-18). The LS means for the concentrations of these fatty acids can be found in Tables 3-7a (polar lipid SFA), 3-7b (polar lipid MUFA), and 3-7c (polar lipid PUFA) in the appendix. The concentration of C18:0 in Low Choice samples was greater ($P < 0.05$) in ground patties compared with steaks at 77°C; meanwhile, its concentration did not differ ($P > 0.05$) by quality grade at any other degree of doneness. The concentrations of both C18:1 n9 cis and C14:0 in Prime samples were greater ($P < 0.05$) in ground patties compared with steaks at 4, 25, and 77°C; in Low Choice samples, each of these fatty acids were greater ($P < 0.05$) in patties at 25, 71, and 77°C, although C14:0 was also greater in these samples at 60°C; and in Standard samples, they were both greater in patties at 55, 60, 71, and 77°C. Of the fatty acids from the polar lipid fraction that exhibited a 3-way interaction, C20:4 n6 was the only fatty

acid whose concentration was greater ($P < 0.05$) in steaks; the concentration in Prime steak samples was greater ($P < 0.05$) compared with steaks at 4°C; Low Choice steaks were greater in 60°C; and Standard steaks were greater ($P < 0.05$) in 55°C. However, the concentration of C20:4 n6 in Low Choice samples was greater ($P < 0.05$) in ground patties compared with steaks at 77°C.

The 2-way interaction of product type and degree of doneness influenced 20 of the fatty acids in the polar lipid fraction. The LS means for the concentrations of all the following fatty acids discussed can be found in Tables 3-7a (polar lipid SFA), 3-7b (polar lipid MUFA), and 3-7c (polar lipid PUFA) in the appendix. The concentrations of MUFA and C16:1 n7 were greater ($P < 0.05$) in ground patties compared to steaks at all degrees of doneness. C20:1 n9 was greater ($P < 0.05$) in ground patties compared with steaks at all degrees of doneness except 55°C. Aside from the three previously listed, the fatty acids whose concentrations were greater ($P < 0.05$) in ground patties compared to steaks at 77°C included: total PUFA, C17:1 n8, C15:1, C16:0, C14:1 n5, C18:3 n6, C18:1 trans, C18:2 trans, total SFA, C20:3 n6, C22:0, C20:0, and C18:3 n3. The fatty acids whose concentrations were greater ($P < 0.05$) in ground patties compared with steaks at 71°C included: C16:0, C14:1 n5, C18:1 trans, C18:2 trans, and total SFA. Two fatty acids were also greater ($P < 0.05$) in ground patties at 60°C: C18:1 trans and C18:2 trans. C14:1 n5 and C18:1 trans were both greater ($P < 0.05$) in patties compared with steaks at 25°C, while C18:1 trans was also greater ($P < 0.05$) in patties at 4°C. Two fatty acids were greater ($P < 0.05$) in steaks at all degrees of doneness except 77°C: C22:0 and C24:0. C21:0 was greater ($P < 0.05$) in steaks at all degrees of doneness except 60° and 77°C.

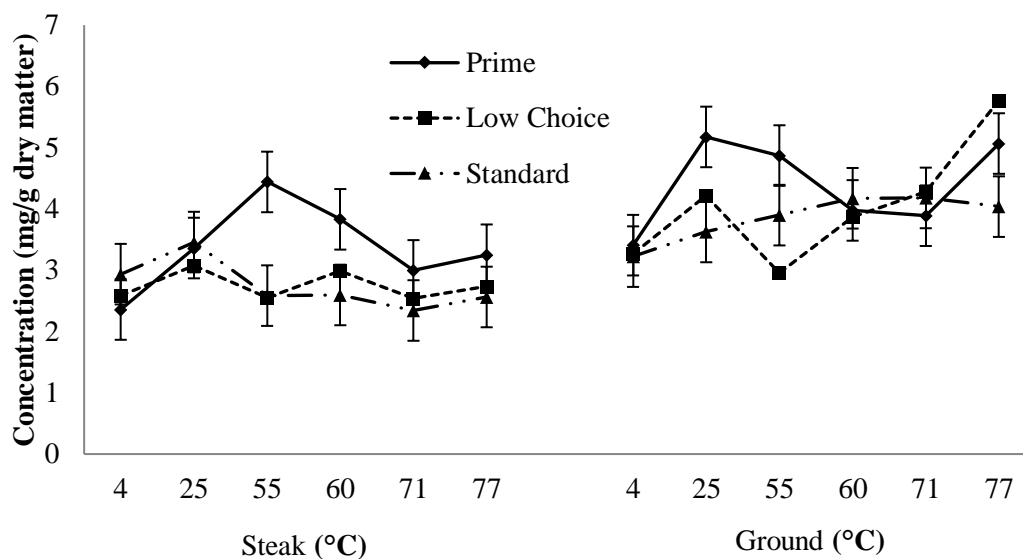


Figure 3-17. Concentration of C18:1 n9 cis from the polar lipid fraction of USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. A three-way interaction (quality grade × DOD × product type) was observed (P = 0.029).

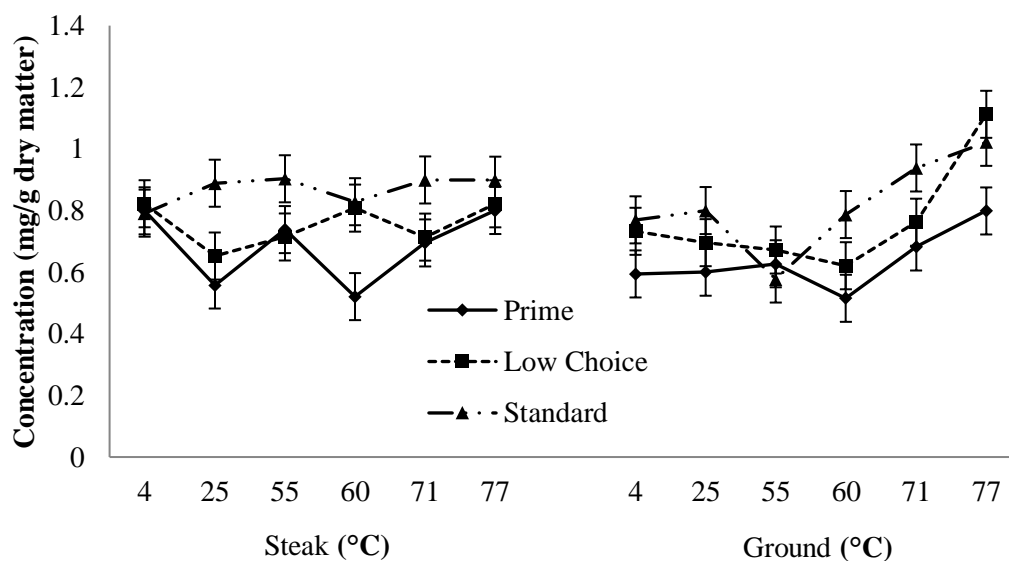


Figure 3-18. Concentration of C20:4 n6 from the polar lipid fraction of USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. A three-way interaction (quality grade × DOD × product type) was observed (P = 0.029).

Fatty acids that were greater ($P < 0.05$) in steaks at 55°C were: C18:3 n3, C18:2 n6, total PUFA, C20:5 n3, and C20:3 n6. Fatty acids that were greater in steaks compared with ground patties at 4°C included: C20:0, total PUFA, C20:5 n3, and C20:3 n6. C20:5 n3 was also greater in steaks at 60°C.

The 2-way interaction of quality grade and degree of doneness affected 13 fatty acids of the polar lipid fraction. The LS means for the concentrations of polar lipid SFA can be found in Table 3-7a in the appendix. Generally, the SFAs affected by this interaction (C21:0, C22:0, and C24:0) were greatest ($P < 0.05$) in concentration in Standard samples, followed by Low Choice and Prime. This was true at 4, 25, and 60°C for each of these three fatty acids. This trend was also seen in C21:0 at 55 and 71°C, in C22:0 at 71°C, and in C24:0 at 55°C. The only degrees of doneness impacted by quality grade for total SFA, however, were 55° and 77°C. At 55°C, total SFA concentration in Prime and Standard samples did not differ ($P > 0.05$), but Prime was greater ($P < 0.05$) than Low Choice. At 77°C, total SFA concentration was greatest ($P < 0.05$) in Low Choice samples. The LS means for the concentrations of polar lipid MUFA can be found in Table 3-5b in the appendix. The MUFAs influenced by this interaction (C16:1 n7, C18:1 trans, and C20:1 n9) were all greatest ($P < 0.05$) in Prime samples compared to Low Choice and Standard at 55°C. At 77°C, C18:1 trans and C20:1 n9 were lowest ($P < 0.05$) in Standard samples compared to Prime and Low Choice. Total MUFA concentration was greatest ($P < 0.05$) in Prime samples compared to Low Choice and Standard at 25° and 55°C. PUFAs influenced by the interaction of quality grade and degree of doneness included: C18:2 trans, C18:3 n6, C20:5 n3, and C20:3 n6. The LS

means for the concentrations of polar lipid PUFA can be found in Table 3-7c in the appendix. The concentrations of the latter three fatty acids were all lowest ($P < 0.05$) in Prime samples compared with Low Choice and Standard at 60°C. The concentration of C18:3 n6 was greatest ($P < 0.05$) in Standard samples compared with the other quality grades at 4, 25, 55, and 71°C in addition to 60°C as mentioned previously. C18:2 trans was different in that it was instead greater ($P < 0.05$) in Prime samples compared with Standard at 25, 55, and 77°C. Total PUFA concentration was lowest ($P < 0.05$) in Prime samples compared with Low Choice and Standard at 4, 25, 60, and 77°C. At 55° and 71°C, total PUFAs were greatest ($P < 0.05$) in Standard samples compared with Prime and Low Choice.

Only two fatty acids in the polar lipid fraction were influenced by the 2-way interaction of quality grade and product type: C20:1 n9 ($P = 0.015$) and C21:0 ($P = 0.002$). The LS means for the concentrations of these two polar lipid fatty acids can be found in Tables 3-7b and 3-7a in the appendix, respectively. C20:1 n9 was greater ($P < 0.05$) in ground patties compared with steaks in all quality grades. Within each quality grade, C21:0 was greater ($P < 0.05$) in steaks compared with ground patties. One fatty acid was influenced by degree of doneness; C22:1 n9 was greatest ($P < 0.05$; Table 3-5b) in 25°C samples compared with the rest of the degrees of doneness. C17:0 (Table 3-5a) was affected by only product type and was greater ($P < 0.05$) in ground patties compared with steaks. Three fatty acids were influenced by quality grade: both C18:2 n6 and C18:3 n3 (Table 3-5c) were lowest ($P < 0.05$) in Prime samples compared with Low Choice and

Standard, while C14:1 n5 was greatest ($P < 0.05$; Table 3-5b) in Prime samples compared with the other quality grades.

A total of 15 fatty acids from the polar lipid fraction calculated on a percentage basis were influenced by the 3-way interaction of quality grade, degree of doneness, and product type. Three of these were SFA: C14:0, C18:0, and C21:0. The LS means for the percentages of polar lipid SFA can be found in Table 3-8a in the appendix. There was no common trend between the percentages of these SFA; the interaction varied between them. C21:0 percentages were greater ($P < 0.05$) in steaks compared with ground patties in Prime 4, 25, 55, and 71°C samples; in Low Choice 4, 25, 71, and 77°C samples; and in Standard 55, 60, 71, and 77°C. C14:0 was greater ($P < 0.05$) in ground patties compared with steaks in Prime 4, 25, 55, and 77°C samples; in Low Choice 4, 25, 60, 71, and 77°C samples; and in Standard 55, 60, 71, and 77°C. MUFA percentages affected by this 3-way interaction included C14:1 n5, C16:1 n7, and C18:1 trans. The LS means for the percentages of polar lipid MUFA can be found in Table 3-8b in the appendix. Percentages of these three MUFA were greater ($P < 0.05$) in ground patties compared with steaks in all quality grades, but the degree of doneness at which they were greater was varied. The total MUFA percentage was greater ($P < 0.05$) in ground patties compared with steaks in Prime 4, 25, 55, and 77°C samples; in Low Choice 25, 60, and 71°C samples; and in Standard 55, 60, and 71°C samples. Seven PUFA percentages were influenced by this interaction: C18:2 trans, C18:2 n6, C18:3 n6, C18:3 n3, C20:3 n6, C20:4 n6, and C20:5 n3. The LS means for the percentages of polar lipid PUFA can be found in Table 3-8c in the appendix. C18:2 trans was the only PUFA percentage that was

greater ($P < 0.05$) in ground patties compared with steaks and this was true in Prime 60° and 77°C samples; in Low Choice 77°C samples; and in Standard 55, 60, 71, and 77°C samples. The other PUFA were greater ($P < 0.05$) in steaks compared with ground patties in all quality grades, but the degree of doneness at which they were greater varied between them. Total PUFA percentage was greater ($P < 0.05$) in steaks compared with ground patties in Prime 4, 25, and 77°C samples; in Low Choice 4, 25, 60, 71, and 77°C samples; and in Standard 55, 60, 71, and 77°C samples.

Discussion

Carcass Data

Animals graded Prime have a greater amount of marbling within the lean muscle, and generally mature earlier than animals graded Low Choice or Standard (Camfield et al., 1997; Camfield et al., 1994). Once intramuscular fat begins to deposit within the lean, the growth of that lean muscle has normally slowed. Low Choice and Standard animals have a smaller deposition of marbling throughout their lean, and generally are larger framed animals that do not mature as quickly as Prime animals (Camfield et al., 1997; Camfield et al., 1994). This difference in frame size allows animals graded Prime to reach a mature level of muscle growth and to begin deposition of intramuscular fat more quickly than the larger framed Low Choice and Standard animals that must accumulate more muscle mass (Camfield et al., 1997; Camfield et al., 1994; Lewis et al., 1993; Galloway et al., 1993). Thus, the Low Choice and Standard animals had greater HCW. Furthermore, this explains why REA of the Standard animals were larger than other quality grades.

Cooking Duration

Organized tissue structure of whole muscle is damaged during grinding, which creates a more porous structure in ground beef (Tuntivanich et al., 2008). This porous structure influences heat transfer; it results in increased water availability between muscle cells for heat transfer (Ngadi et al., 2001). This change in heat transfer may explain why ground patties cooked more quickly compared with steaks. Additionally, ground patties were thinner and smaller in overall size compared with steaks, and this size difference may have also contributed to a shorter cooking duration of ground patties. By opening the meat tissue particles, the grinding process allows for the migration of liquid fat to the center of the product, and this fat carries heat faster than the remaining moisture. Standard samples contained the most moisture and cooked the quickest. If increased moisture translates to increased water availability for heat transfer, as previously described, this may explain why Standard samples cooked more quickly.

Proximate Composition

The moisture content results are in agreement with Seggern et al. (2005), who found that moisture content tends to decrease with an increase in marbling. For both steak and ground patties, 25°C Prime samples had the lowest protein percentages of all treatments. These results are contrary to the findings of Troutt et al. (1992), in which ground patties that were low in fat had the highest protein percentages. However, Corbin et al. (2015) found similar results, where protein content of beef strip loin steaks decreased as fat level increased. Serrano et al. (2007) also found similar results in restructured beef steaks. The low protein percentage in 25°C Prime samples could be

explained by purge loss that occurred as these samples were tempered. Some proteins may have been lost in the purge. Moisture loss during cooking causes a proportional change in the other cellular components that are not lost during cooking. Thus, as moisture was lost with increasing DOD, the protein percentage increased. Increased fat percent upon cooking was in agreement with the findings of Juarez et al. (2010) and Garcia-Arias et al. (2003) in buffalo meat and fish, respectively. Fat percent increases in cooked samples was likely due to moisture loss during cooking causing a proportional increase in the percentage of fat present (Garcia-Arias et al., 2003; Juarez et al., 2010).

Fatty Acids

The difference in fat content between the three quality grades as evidenced by the fat proximate data proves that carcasses chosen for this study and samples resulting from those carcasses were successful in representing differing levels of intramuscular fat. These differing levels of intramuscular fat allowed for a diverse fatty acid profile. Results from this study are in agreement with those found by Legako et al. (2015) in that generally, an increase in quality grade was associated with an increase in the amount of fatty acid deposition. There were some instances, however, where Standard samples, containing the least amount of intramuscular fat, had the greatest amount of fatty acids compared with Prime and Low Choice samples. Both neutral lipids and polar lipids are found within the intramuscular fat, but neutral lipids consist mostly of triglycerides that are stored inside of adipocytes within a matrix of collagen. Collagen within meat is relatively heat stable (thus the need to cook collagen-rich roasts for long periods of time) and consequently protects these stored triglycerides from immediate effects of heating.

Polar lipids, on the other hand, consist mostly of phospholipids that contribute to cell structure. These phospholipids are contained within the sarcolemma that surrounds muscle cells and are anchored by other lipids and lipoproteins. Igene et al. (1980) found that both triglycerides and phospholipids are susceptible to oxidation, but phospholipids oxidize first, especially the PUFA of phospholipids. These phospholipids are thought to contribute more to volatile compound production (Farmer and Mottram, 1990).

Furthermore, unsaturated fatty acids are more susceptible to degradation because of their double bonds. Free radicals are formed by removing a hydrogen atom from a carbon atom adjacent to a double bond, which triggers a continuation of the oxidation reaction. PUFA contain the most double bonds, and the polar lipid fraction contains a larger proportion of PUFA; thus, polar lipid PUFA should be the fatty acid type that is most susceptible to this process. Legako et al. (2015) found that cooking and quality grade impacted the fatty acids of the polar lipid fraction to a greater extent than the neutral lipid fraction. On the contrary, the results of this study show that overall, there was less variation in the way the fatty acids of the polar lipid fraction were impacted by these factors and product type, versus the neutral lipid fraction.

The NL SFA, MUFA, and PUFA were consistent with the total fat data, where their concentrations were greatest in Prime samples, followed by Low Choice and Standard. All three fatty acid types within the neutral lipid did not differ within Low Choice or Standard samples, regardless of product type, but Prime samples did have a dependence on product type. SFA increased in steaks when exposed to initial cooking. This could be attributed to the longer cooking time needed to get Prime steaks to the

appropriate temperature. The longer cooking time needed for these samples may have provided more time for proteins to leach out, which may have concentrated the existing SFA content. The SFA, MUFA, and PUFA within Low Choice and Standard steaks showing no response upon further cooking is in agreement with the results found by Legako et al. (2015). SFA and MUFA in ground product decreased upon cooking. Grinding alters the structure of these lipids and they melt out more easily. In addition, SFA are shorter chain fatty acids and have a lower melting point, which also makes them more prone to melt out. Within ground patties, PUFA content became similar at 77°C, regardless of quality grade. In Prime samples, perhaps the degree of saturation gives these PUFA a lower melting point, so they melt out of the product, translating to a more marked decrease in PUFA content at 77°C. In Low Choice and Standard samples, perhaps the loss of soluble proteins at this point could lead to these PUFA becoming more concentrated, which translates to an increase in their content.

Polar lipid SFA and MUFA within the ground product were elevated at 77°C, regardless of quality grade. This could be related to the very short cooking time associated with the ground patties. Perhaps the time it took the patty to reach the desired temperature was enough time for protein loss to have occurred, but not enough time for most of the SFA to have melted out of the product yet. Thus, they may have become more concentrated, translating to an increase in content. On the other hand, PUFA in ground patties eventually decreased in content. PUFA content was greater in steaks compared with ground patties at 4° and 55°C, implying that the act of grinding led to greater oxidation of PUFA and an increase in fat content. Regardless of degree of

doneness, PUFA in the polar lipid fraction were greater in concentration in Standard samples compared with Prime. Standard samples cooked more quickly, so PUFA in these samples may not have had time to melt out yet.

Conclusion

These data reveal that quality grade, grinding, and degree of doneness impact proximate composition and fatty acids in both the neutral and polar lipid fraction. Within the neutral lipid fraction, SFA, MUFA, and PUFA showed similar trends in that their content was greatest in Prime samples, followed by Low Choice and Standard; they showed no response with cooking within Low Choice and Standard samples, regardless of product type, but Prime samples were dependent on product type. Within the polar lipid fraction, SFA, MUFA, and PUFA showed little to no response with cooking within steaks; SFA and MUFA in ground patties increased at the highest endpoint temperature, while PUFA content in ground patties decreased with cooking. These data have potential for management of important flavor contributing compounds as a function of cooking, grinding, and quality grade. By manipulating the proximate composition and the fatty acid profile of beef, consumers may be able to receive the same flavorful eating experience from lower quality grades as Prime beef. Previous work has primarily utilized model systems to study beef compounds known to influence its flavor. This data set reveals that basic understanding of flavor development may be attained from beef steaks. Furthermore, future research could explore the development of predictive models for chemical changes that occur with cooking. One overall model, especially one that

includes chemical changes involving fatty acids, is not sufficient; the model must be dependent on fat content (quality grade) and product type.

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CHAPTER IV

EFFECTS OF QUALITY GRADE, DEGREE OF DONENESS, AND GRINDING ON
AMINO ACIDS AND REDUCING SUGARS OF BEEF STRIP STEAKS

Abstract

A total of 23 free amino acids were identified. Total amino acids were determined from hydrolysates of homogenized samples upon derivatization, and 23 were identified. The concentration of total reducing sugars was determined using the dinitrosalicylic acid method per Sadasivam and Manickam (1996). The pH of all samples was also measured. A 3-way interaction effect of quality grade, DOD, and steaks versus ground patties was observed for pH ($P < 0.001$). The pH of the raw samples was lower ($P < 0.05$) than the pH of all cooked samples ($P < 0.05$), and both steaks and ground patties showed a steep decline ($P < 0.05$) in pH at 25°C. Other pH differences between steaks and ground patties were dependent on quality grade and degree of doneness and varied. A 3-way interaction effect of quality grade, DOD, and steaks versus ground patties was observed for 18 free amino acids. Most these free amino acids were greater ($P < 0.05$) in concentration in steaks compared with ground patties and in Standard or Low Choice samples compared with Prime. Amino acid concentrations in both steaks and ground patties spiked ($P < 0.05$) in concentration at 25°C, where the pH showed a steep decline ($P < 0.05$). Fifteen total amino acids were affected by the interaction of quality grade, degree of doneness, and product type. Within ground patties, none of these amino acids differed ($P > 0.05$) by quality grade or degree of doneness, and none differed ($P > 0.05$) from the lowest

concentration found in streaks. This indicates that these factors affected total amino acid concentrations in steaks to a greater extent, and the interaction effect varied per individual amino acid. A 3-way interaction between quality grade, degree of doneness, and product type was observed for the concentration of total reducing sugars ($P < 0.001$). Overall, the sugar concentration in Standard 4°C steak samples was greater ($P < 0.05$) than all other quality grades and temperatures.

Introduction

Amino acids and reducing sugars are important water-soluble compounds that contribute to the formation of flavor-related compounds in beef. The behavior of these compounds is dependent on each other and pH of the system. Amino acids and sugars interact upon heating during the Maillard reaction, arguably one of the most important reactions associated with the development of beef flavor, to create a variety of compounds such as aldehydes, ketones, pyrazines, and sulfur compounds (Dashdorj et al. 2015). Amino acids compete for interaction with sugars, which are the limiting factor in the Maillard reaction, and can be consumed upon initial interaction with sugars, regenerated during the middle of the Maillard reaction, and consumed again at latter stages of the reaction (van Boekel 2001). Free amino acids can also undergo oxidation and degradation to create the basic tastes (Dashdorj et al., 2015). Meanwhile, reducing sugars undergo dehydration and fragmentation that produce carbons, hydrocarbons, and furans that contribute a sweet taste (Dashdorj et al., 2015). The goal of this study was to determine how pH, free and total amino acids, and reducing sugars of multiple quality grades respond to grinding and cooking to different degrees of doneness. Depending on

how these compounds react to these parameters, new information about the development of beef flavor may be revealed and create the potential for management of flavor precursor compounds.

Materials and Methods

Product Selection

Details regarding carcass characteristics were previously described in Chapter 3. Briefly, paired beef strip loins [IMPS 180, (NAMP, 2010)] were collected from 24 carcasses across three USDA quality grades (Prime, Low Choice, and Standard, $n = 8$ per quality grade; USDA 1977) of “A” maturity animals. 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. Intact strip loins were stored under vacuum, in darkness, and under refrigeration (4°C) until 21 days post-mortem.

Processing

Details regarding fabrication of loins into steaks and patties were previously discussed in Chapter 3. Briefly, strip loins were cut into 2.54 cm steaks progressing anterior to posterior using a 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 degree of doneness, 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 *Longissimus lumborum* muscle. Following grinding, ground material was stuffed into approximately 50-mm diameter, plastic perforated casings and frozen at -20°C. Resulting frozen chubs were then sliced 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 hours but no more than 24 hours to a temperature range of 3 – 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 two hours after first being thawed to 3 – 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 (well done) °C after being thawed to a temperature of 3 – 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 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.

Sample Preparation for Chemical Analysis

Following tempering or cooking, samples were frozen in liquid nitrogen and pulverized in a blender (Nutri Ninja: Model BL642, Euro-Pro Operating LLC, Newton, MA). The resulting homogeneous samples were stored in 4.5 x 9 inch VWR sterile sampling bags (VWR International, Cat. No. 82007-706, Radnor, PA) at -80°C until later analyses.

pH

A Thermo Fisher Orion Star A111 benchtop pH meter (Thermo Fisher Scientific, Beverly, MA) and a Sure-Flow refillable Ag/AgCl combination pH electrode (Thermo Fisher Scientific, Beverly, MA) were used to determine the pH of homogenized samples. Nine mL of distilled water were added to one gram of sample in a VWR 15 mL polypropylene test tube. The samples were then vortexed for 30 seconds or until the meat was dispersed, the pH electrode was placed directly into the sample, and the pH recorded (John et al., 2004).

Free Amino Acids

Following the methods of Koutsidis, Elmore, Oruna-Concha, Campo, Wood, and Mottram (2008), water soluble compounds were extracted from the homogenized samples, with an adaptation to the centrifugation step. Two grams of frozen sample

homogenates were weighed into 15 mL polypropylene vials, to which 10 mL of cold water were added. The tubes were shaken for 30 minutes and centrifuged at 3000 x g for 15 minutes. The resulting material was filtered through a 0.2 μm , 30 mm nylon membrane syringe filter (MicroLiter, Millville, NJ) into a 3 kDa cutoff membrane for centrifugal filtration for 1.5 hours. 100 μL of the resulting aqueous extract from each sample was introduced to the EZ-Faast amino acid kit (Phenomenex, Torrance, CA), by which free amino acids were derivatized. Derivatized amino acids were determined by GC-MS in electron impact mode with a 3:1 split ratio. The initial injection temperature was 280°C, while the oven was 110°C for one minute, with a 30°C per minute increase until 320°C was reached. Free amino acid derivatives were separated using a Zebron ZB-AAA capillary column (10 m x 0.25 mm; 0.25 μm film thickness, Agilent J&W GC Columns, Santa Clara, CA) with helium as the carrier gas. Amino acid identity and quantity were confirmed by comparing the data to external standards. Quantities of the amino acids were determined by relative responses to Norvaline, an internal standard. Concentrations of free amino acids were calculated on a dry-weight basis.

Total Amino Acids

Total amino acids were determined from hydrolysates of the homogenized samples. Five mL of 6 N HCl followed by 0.7 mL of 12 N HCl were added to one gram of sample. Ultra-high-purity nitrogen gas was added to the headspace of each tube before being capped and placed in an oven at 120°C for 22 hours. After removal from the oven, the hydrolysate solutions were diluted 50 times in water, and 100 μL were introduced to the EZ-Faast amino acid kit (Phenomenex, Torrance, CA), by which total amino acids

were derivatized. Derivatized amino acids were determined by GC-MS in electron impact mode with a 15:1 split ratio. The initial injection temperature was 280°C, while the oven was 110°C for one minute, with a 30°C per minute increase until 320°C was reached. Total amino acid derivatives were separated using a Zebron ZB-AAA capillary column (10 m x 0.25 mm; 0.25 µm film thickness, Agilent J&W GC Columns, Santa Clara, CA) with helium as the carrier gas. Amino acid identity and quantity were confirmed by comparing the data to external standards. Quantities of the amino acids were determined by relative responses to Norvaline, an internal standard. Concentrations of total amino acids were calculated on a dry-weight basis.

Reducing Sugars

The concentration of total reducing sugars was determined using the dinitrosalicylic acid method per Sadasivam and Manickam (1996). Sugars were extracted from the homogenized samples by weighing 0.5 g of the sample into a VWR 15 mL polypropylene, conical tube and adding 5 mL of 80% ethanol. The tubes were then centrifuged for 10 minutes. After repeating this step for a total of 10 mL of ethanol added, the supernatant was poured off into glass culture tubes and evaporated to dryness via nitrogen gas in an 80°C water bath. Next, 10 mL of water were added to dissolve the remaining sugars, and the tubes were shaken by hand for 5 seconds. After shaking, 3 mL of the extract were pipetted into different glass culture tubes, to which 3 mL of DNS reagent were added. The contents were then heated in a 90°C water bath for 5 minutes. After removal from the water bath, but while the contents were still warm, 1 mL of 40% Rochelle salt solution was added. The tubes were then set in a rack on the benchtop and

allowed to cool to room temperature before 2 mL of each sample were transferred to cuvettes, and their absorbance values were read using a spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan) set at 510 nm. A 7-point standard curve was constructed using glucose as the standard ($R^2 = 0.989$) in concentrations of 0.5, 0.4, 0.3, 0.2, 0.1, 0.05, and 0.025 mg/mL. The concentrations of total reducing sugars for each sample were calculated based on the equation of this curve. The concentrations of sugars were calculated on a dry-weight basis.

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 degree of doneness). 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), in which $n=8$. The sub-plot was the sample type (whole steaks vs. ground patties), in which $n=24$. The sub-sub-plot was the thermal processing temperature (4, 25, 55, 60, 71, and 77°C), in which $n=48$.

Results

pH

A 3-way interaction effect of quality grade, DOD, and steaks versus ground patties was observed for pH ($P < 0.001$) and can be found in Figure 4-1. The LS means of

pH values can be found in Table 4-1. The main effects and interactions impacting pH can be found in Table 3-3. The pH of the raw samples was lower ($P < 0.05$) than the pH of all cooked samples ($P < 0.05$). In Prime samples, pH values for the two product types did not differ ($P > 0.05$), except pH was greater ($P < 0.05$) in ground patties at 71°C. In Low Choice samples, the pH values of steaks at each DOD were similar ($P > 0.05$) to those of ground patties. The pH of Standard 60°C and 71°C samples differed ($P < 0.05$) between steaks and ground patties, where 60°C ground samples had a greater ($P < 0.05$) pH than steaks. Meanwhile, 71°C steak samples had a higher ($P < 0.05$) pH compared with ground patties.

Free Amino Acids

A total of 23 free amino acids were quantified in this study. The main effects and interactions impacting free amino acid concentrations can be found in Table 4-3. The LS means of all free amino acids can be found in Tables 4-3a and 4-3b in the appendix. The common names for the abbreviated amino acids in these tables can be found in Table 4-2. A 3-way interaction was observed for 18 free amino acids ($P \leq 0.0181$). The concentration of 10 of these 18 amino acids, alanine, glycine, valine, beta-alanine, leucine, isoleucine, asparagine, hydroxyproline, glutamine, and tyrosine, was greater ($P < 0.05$; Figure 4-2) in steaks compared with ground patties. The only free amino acid whose concentration was greater ($P < 0.5$) in ground patties versus steaks was aspartate. Ten of these 18 amino acids had greater ($P < 0.05$; Figure 4-2) concentrations in Standard or Low Choice steaks versus Prime: glutamate, phenylalanine, asparagine, aspartate, beta-alanine, leucine, isoleucine, glycine, valine, and alanine. The concentration of the

other amino acids affected by quality grade, degree of doneness, and product type, in no particular pattern, varied between being greatest ($P < 0.05$) in Prime and Low Choice or Standard depending on the temperature and product type. The concentration of each amino acid at 77°C was either lower than ($P < 0.05$; Figure 4-3) or equal to ($P > 0.05$) its concentration at 4°C. Most free amino acid concentrations showed an increase ($P < 0.05$; Figure 4-3) between 4°C and 55°C and then decreased ($P < 0.05$). The exceptions to this observation were asparagine, aspartate in steak samples, hydroxyproline in ground samples, and phenylalanine in steak samples.

Total Amino Acids

A total of 23 amino acids were quantified among steaks and ground patties. The main effects and interactions impacting total amino acid concentrations can be found in Table 4-4. The LS means of all total amino acids can be found in Tables 4-4a and 4-4b in the appendix. Of these 23 amino acids, 15 were affected by the interaction of quality

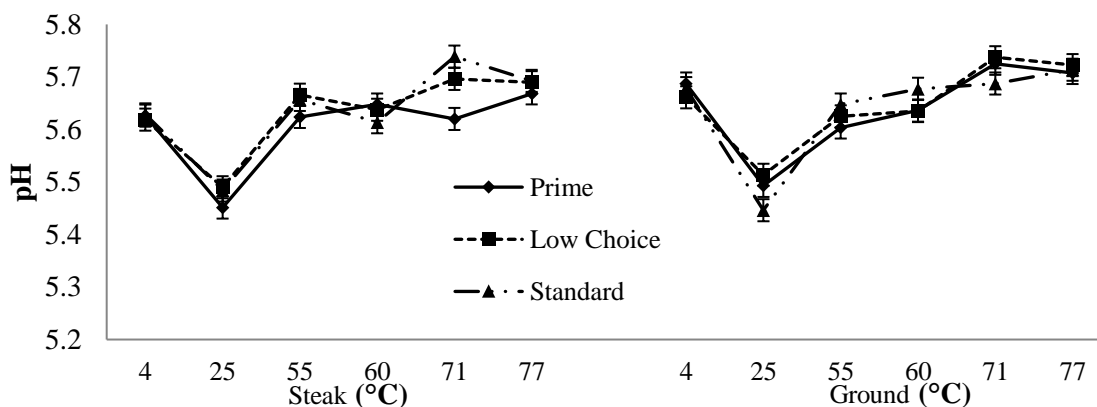


Figure 4-1. pH of USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (QG \times DOD \times product type) was observed ($P < 0.001$).

grade, degree of doneness, and product type: alanine ($P < 0.001$), valine ($P = 0.036$), beta-alanine ($P < 0.001$), threonine ($P = 0.001$), serine ($P = 0.010$), proline ($P = 0.001$), aspartate ($P = 0.003$), methionine ($P < 0.001$), hydroxyproline ($P < 0.001$), glutamate ($P = 0.020$), phenylalanine ($P = 0.002$), cysteine ($P < 0.001$), lysine ($P = 0.004$), histidine ($P < 0.001$), and tyrosine ($P = 0.024$). Within ground patties, none of these amino acids differed ($P > 0.05$) by quality grade or degree of doneness, and none differed ($P > 0.05$) from the lowest concentration found in streaks. This indicates that these factors affected total amino acid concentrations in steaks to a greater extent. Therefore, the remainder of this 3-way interaction discussion will address total amino acid concentrations within only steak samples. Each amino acid exhibited a steep increase ($P < 0.05$) that resulted in its greatest ($P < 0.05$) concentration at 71°C , dependent on quality grade. For beta-alanine, aspartate, methionine, hydroxyproline, phenylalanine, cysteine, lysine, and histidine, this increase ($P < 0.05$) occurred in Low Choice samples (Figure 4-4). For threonine, glutamate, and tyrosine, this increase ($P < 0.05$) occurred in Standard samples (Figure 4-5). For serine and proline, this increase ($P < 0.05$) occurred in Prime samples (Figure 4-6). For valine, this increase ($P < 0.05$) occurred in both Low Choice and Standard samples (Figure 4-7), while in alanine, it occurred in both Prime and Low Choice samples (Figure 4-8). Tyrosine also had a steep increase ($P < 0.05$) in concentration in Prime 25°C samples in addition to the increase ($P < 0.05$) in Standard 71°C steaks. The amino acid concentrations tended to not differ ($P > 0.05$) between 4, 25, 55, and 60°C samples, then sharply increased ($P < 0.05$) at 71°C in one or two quality grades as

previously described, and then decreased ($P < 0.05$) to an amount that did not differ ($P > 0.05$) from the concentration at 4, 25, 55, and 60°C.

Amino acids that were affected by the interaction of product type and degree of doneness included: glycine ($P = 0.002$), leucine ($P = 0.005$), isoleucine ($P = 0.004$), and glutamine ($P = 0.006$). Concentrations of glycine (Figure 4-9), isoleucine, and glutamine (Figure 4-10) did not differ ($P > 0.05$) within ground patties, and the concentration in ground patties were lower ($P < 0.05$) compared with steaks in all degrees of doneness except 60° (for glycine and isoleucine) and 77°C (for glutamine). The concentration of leucine (Figure 4-11) was greater ($P < 0.05$) in ground patties compared with steaks in all degrees of doneness except 60°C. Cysteine was affected only by product type, and its concentration was greater ($P < 0.05$) in steaks compared with ground patties.

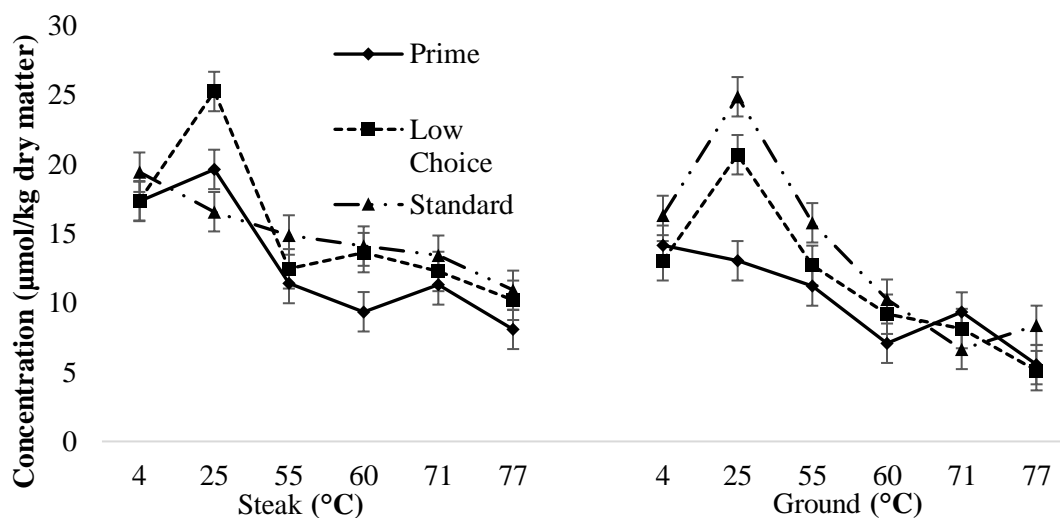


Figure 4-2. Concentration of free valine in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P < 0.001$).

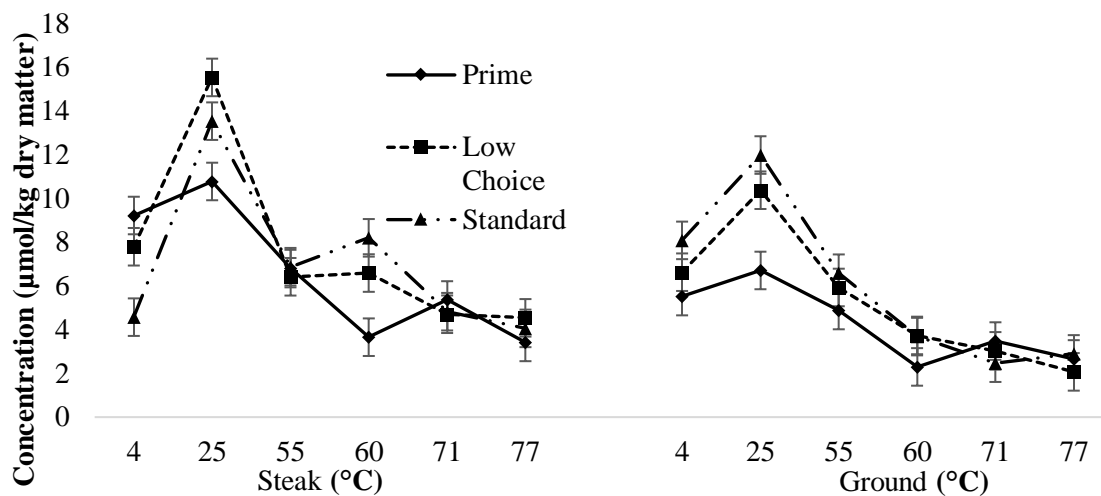


Figure 4-3. Concentration of free proline in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P < 0.001$).

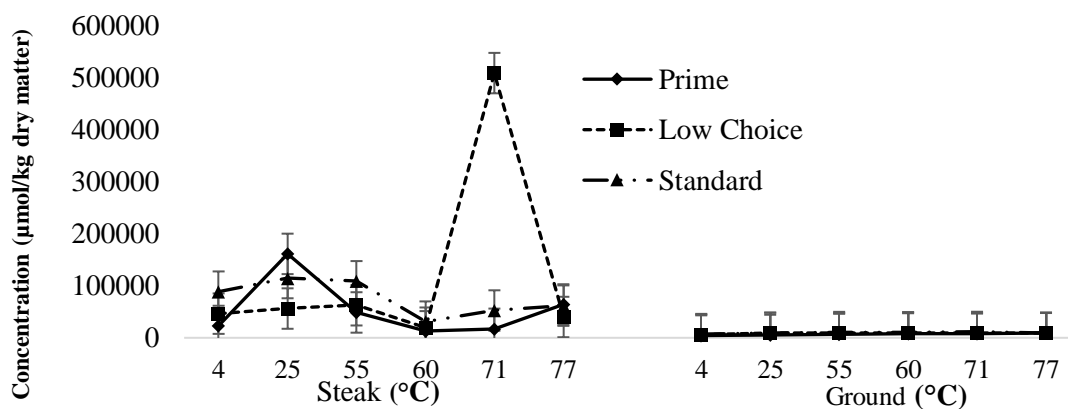


Figure 4-4. Concentration of total methionine in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P < 0.001$).

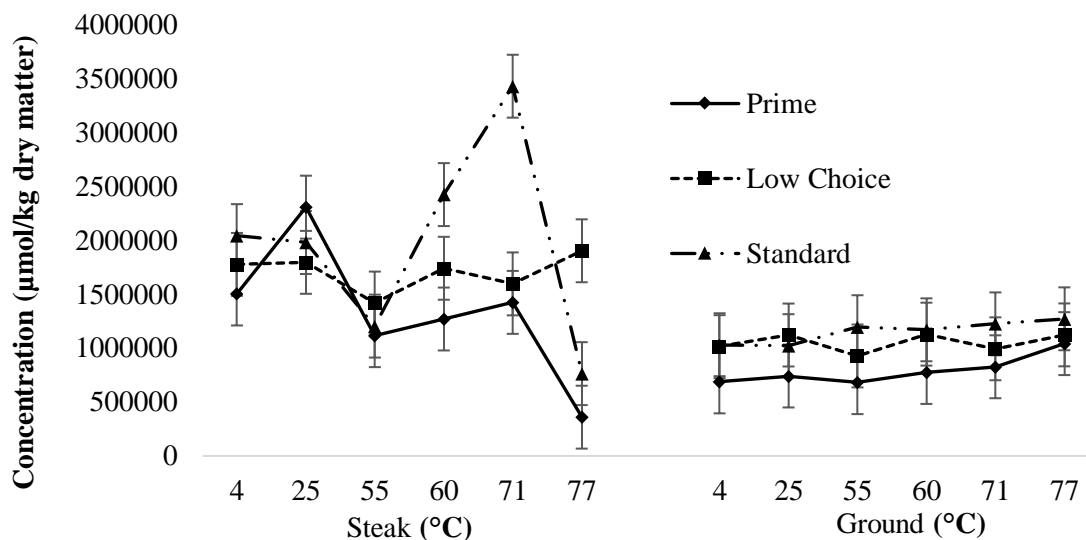


Figure 4-5. Concentration of total threonine in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P < 0.001$).

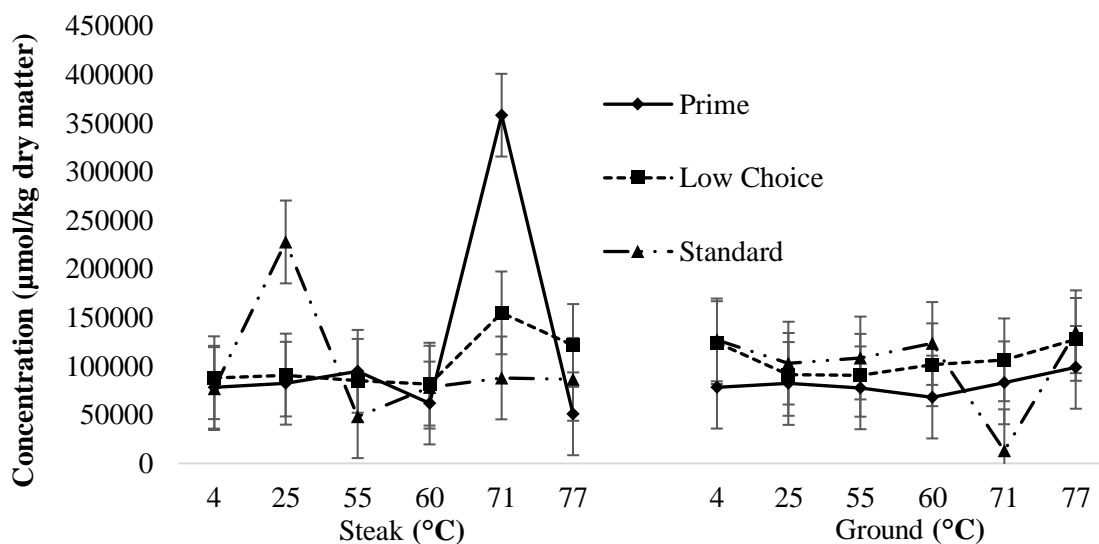


Figure 4-6. Concentration of total serine in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (QG \times DOD \times product type) was observed ($P = 0.01$).

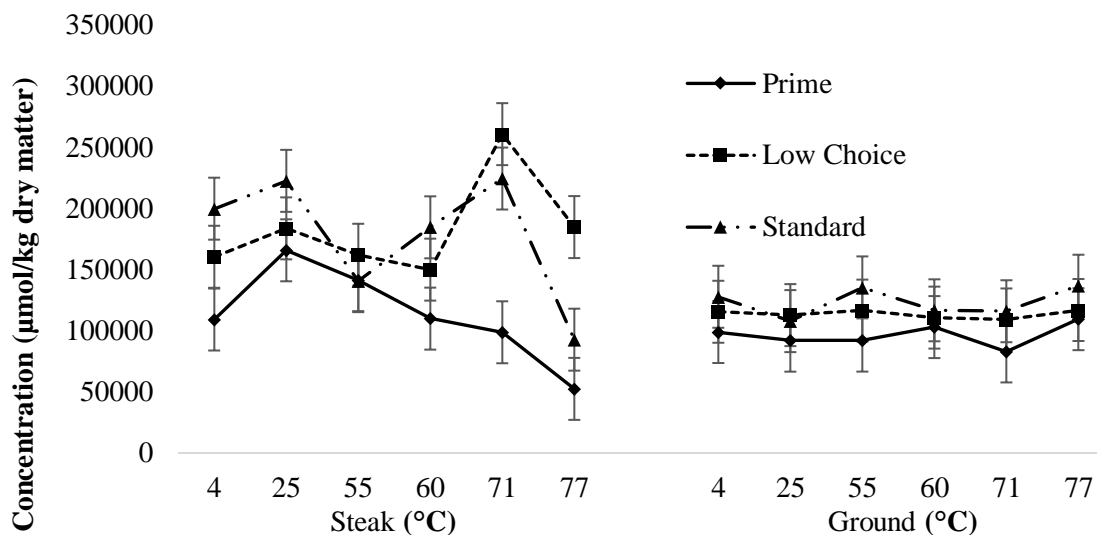


Figure 4-7. Concentration of total valine in USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. A three-way interaction (quality grade × DOD × product type) was observed (P = 0.036).

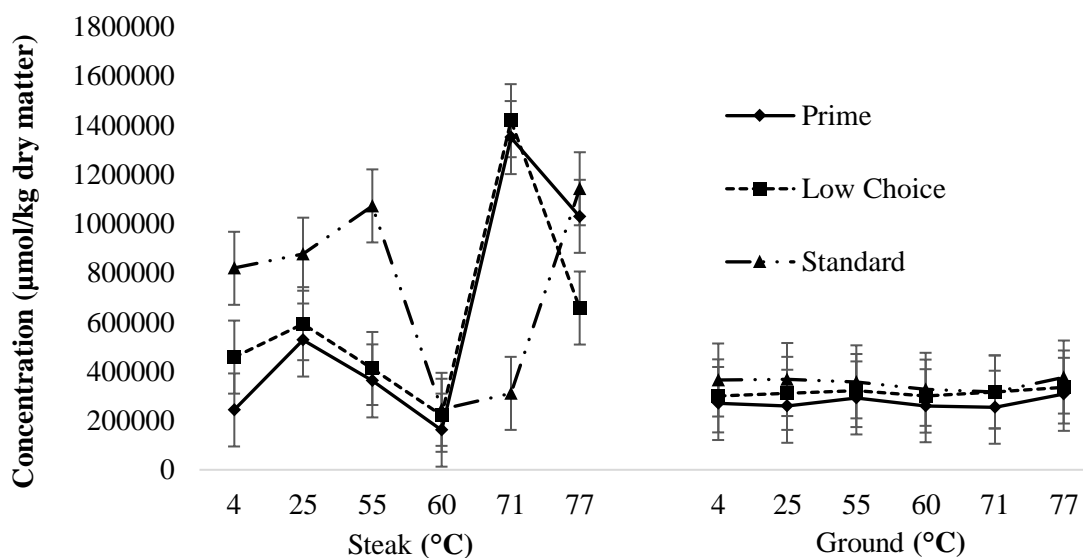


Figure 4-8. Concentration of total alanine in USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. A three-way interaction (quality grade × DOD × product type) was observed (P < 0.001).

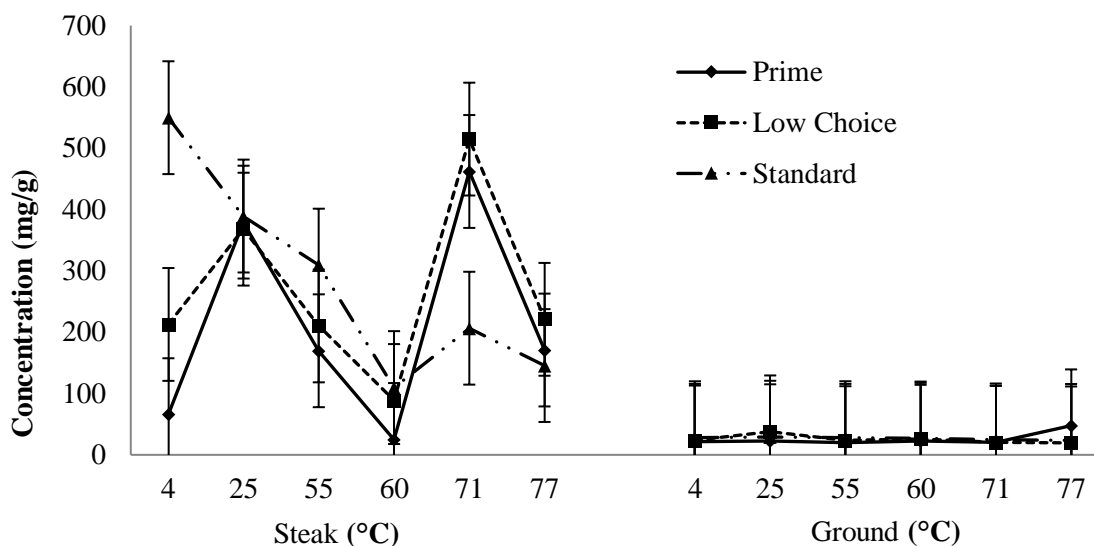


Figure 4-9. Concentration of total glycine in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A two-way interaction (DOD \times product type) was observed ($P = 0.002$).

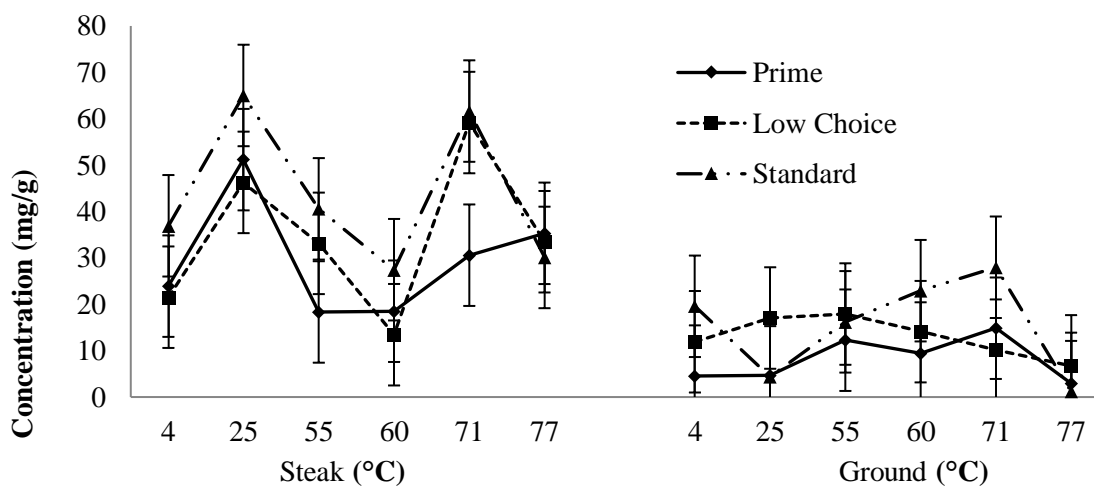


Figure 4-10. Concentration of total glutamine in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A two-way interaction (DOD \times product type) was observed ($P = 0.002$). Quality grade also influenced glutamine ($P < 0.05$).

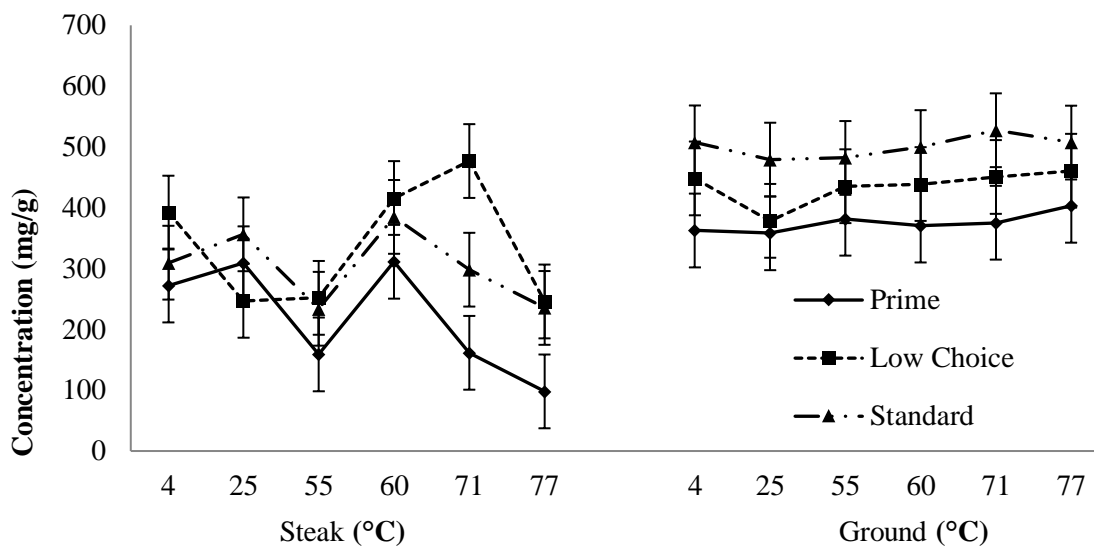


Figure 4-11. Concentration of total leucine in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A two-way interaction (DOD \times product type) was observed ($P = 0.005$). Quality grade also influenced leucine ($P < 0.05$).

Reducing Sugars

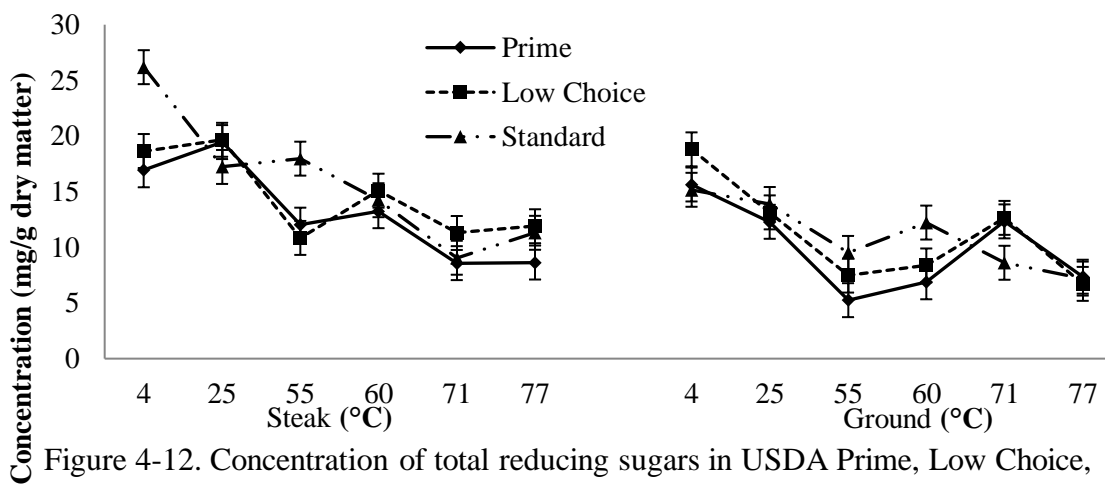


Figure 4-12. Concentration of total reducing sugars in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P < 0.001$).

A 3-way interaction between quality grade, degree of doneness, and product type was observed for the concentration of total reducing sugars ($P < 0.001$; Figure 4-12). The LS means of total reducing sugar concentrations can be found in Table 4-5 in the appendix. The main effects and interactions impacting these sugars can be found in Table 3-3. Overall, the concentration in Standard 4°C steak samples was greater ($P < 0.05$) than all other quality grades and temperatures. The concentration of sugars in steaks was greater ($P < 0.05$) than ground patties in Prime 25, 55, and 60° samples; in Low Choice 25, 60, and 77°C samples; and in Standard 4, 55, and 77°C samples. Among steaks, Standard samples were greater ($P < 0.05$) than the other quality grades in 4, 25, and 55°C samples. The 60, 71, and 77°C steak samples each did not differ ($P > 0.05$) by quality grade. Among ground patties, 4, 25, 55, and 77°C samples did not differ ($P > 0.05$) by quality grade. The Standard ground patties were greater ($P < 0.05$) than Prime in 60°C samples. In 71°C ground patties, the concentrations in Standard samples were lower ($P < 0.05$) than Low Choice.

Discussion

The increase in pH that was observed after samples reached 25°C could be due to proteolysis and the formation of peptides and free amino acids (Dierick et al., 1974; Garcia de Fernando & Fox, 1991; Incze, 2004; Irscher et al., 2013). The drop in pH at 25°C matches the spike in amino acid concentration at this same temperature. There are less unprotonated amino groups at a lower pH, and an unprotonated amino group is needed to react with a reducing sugar. The larger proportion of protonated amino acids at

this lower pH made them predominantly unreactive at this point; thus, they showed an increase in concentration, because they were not being consumed. It is important to note that this was not a continuous system, meaning that each sample was cooked to one, individual endpoint temperature rather than being cooked in a progression from 4 to 77°C; the development of pH in the system may have been different in a continuous system.

Sugars and amino acids interact during the Maillard reaction to form volatile compounds, and the activity of both of these water-soluble compounds is dependent on pH and the abundance of each other. Balagiannis et al. (2010) claims that because sugars are the limiting factor in the reaction, amino acids are present in excess over sugars. Furthermore, they found that amino acids were rapidly consumed during initial heating, and then concentrations leveled off. While these results show that free amino acids were indeed rapidly consumed during initial heating, as degree of doneness increased, the concentrations of free amino acids did not always level off. In fact, they fluctuated between increased and decreased concentrations until a final decrease at 77°C. Total amino acids showed a spike in concentration at 71°C, but at 77°C decreased to a concentration lower or equal to the concentration seen in raw samples. The decrease in free amino acid concentration with cooking indicates their participation in the Maillard reaction. The behavior of free amino acids between steaks and ground patties was usually similar, but total amino acids in ground patties did not fluctuate much. Some amino acids could be regenerated from Amadori product breakdown after being consumed, resulting in no net loss or gain (Balagiannis et al., 2009; Labuza & Baisier, 1992; Baisier &

Labuza, 1992; van Boekel 2001). At higher temperatures, however, a continuous loss of free amino groups occurs with less regeneration, which explains why there was always a decreased concentration of amino acids at 77°C than was observed at initial heating (van Boekel 2001).

The concentration of reducing sugars was lower in ground patties compared with steaks, due to loss of water soluble compounds during grinding. The effect of quality grade on reducing sugars is unclear, however they were consumed rapidly upon cooking. The active form of sugar is its open chain form. This active form is dependent on pH and is present in greater amounts with an increase in pH and temperature (van Boekel 2001). This active form is what reacts with an amino group, so the sugars are consumed quickly once they become active, which may make it difficult to notice an increase in the active form of the sugars with an increase in degree of doneness. The active form of amino groups is also dependent on pH. The amino group must be unprotonated to provide a free electron pair to react with the carbonyl group of the sugar (van Boekel 2001). As mentioned previously, there are less unprotonated amino groups at a lower pH, and this may explain why there was a spike in amino acid concentration at 25°C, the degree of doneness at which the pH was lowest. The larger proportion of protonated amino acids at this pH around 5.4 made the amino acids predominantly unreactive at this point. Alternatively, this accumulation of free amino acids could cause the pH to drop. The implication of the relationship between free amino acids and pH is that it could be possible to thaw meat to enhance flavor. As more water-soluble compounds, like these free amino acids, are consumed in the Maillard reaction, more volatile compounds are

produced. The spike in free amino acid concentration at 25°C, a common thawed temperature, means there are more amino acids available to participate in the volatile-producing reaction. Thus, there may be increased volatile production in meat that is thawed to room temperature and subsequently cooked.

Conclusion

This data revealed that pH, free and total amino acids, and reducing sugars are impacted by quality grade, degree of doneness, and grinding. Furthermore, the behavior of amino acids and reducing sugars is dependent on each other and pH. The free amino acid behavior matches the pH of the system, providing implications for increased volatile production upon thawing of beef to room temperature before cooking. These water-soluble compounds were initially greater in concentration in steaks compared with ground patties, due to the loss of these compounds during the grinding process. These amino acids and sugars were also rapidly consumed upon cooking. These data have potential for management of important flavor contributing compounds as a function of cooking, grinding, and quality grade. Knowing how these data respond to these factors may allow us to couple it with sensory data related to these compounds in order to discover management possibilities of compounds that consumers find desirable. By manipulating the water-soluble compounds in beef, consumers may be able to receive the same flavorful eating experience from lower quality grades as Prime beef. Previous work has primarily utilized model systems to study beef compounds known to influence its flavor. This data set reveals that basic understanding of flavor development may be

attained from beef steaks. Furthermore, future research could explore the development of predictive models for chemical changes that occur with cooking. One overall model, especially one that includes chemical changes involving sugars and amino acids, is not sufficient; the model must be dependent on fat content (quality grade) and product type.

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CHAPTER V

EFFECTS OF QUALITY GRADE, DEGREE OF DONENESS, AND GRINDING ON
VOLATILE COMPOUNDS OF BEEF STRIP STEAKS

Abstract

A total of 27 volatile compounds (nanograms per gram) were quantified via head space solid phase microextraction from samples tempered in refrigerated temperatures (3-5°C), room temperature (24-26°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, four pyrazines, and one ester had a 3-way interaction between quality grade, degree of doneness, and product type (each $P < 0.001$). For example, pyrazine concentration 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 degree of doneness 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 two ketones, an alcohol, two esters, and two 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 degree of doneness and product type was observed in three ketones, two sulfur compounds, two esters, five aldehydes, two 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. How each volatile compound was affected by each of the interactions previously described varied greatly, thus the effects on each individual compound are omitted in this abstract.

Introduction

The objective of this study was to determine how volatile compounds in beef strip steaks of differing quality grades respond to grinding and differing degrees of doneness. Flavor is a combination of taste and odor and requires a combination of olfactory and gustatory senses, and 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 & 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, Berry, & Wells, 1980; Mottram & Edwards, 1983; Mottram, Edwards, & MacFie, 1982). 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 release (Farmer et al., 2013; Chevance et al. 2000; Chevance & Farmer, 1999). Volatile compounds are generated from non-volatile water-soluble precursors and lipids via

multiple reactions resulting from lipid oxidation and degradation 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 (Insausti et al., 2002; King et al., 1993). 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). Knowing how these compounds develop in response to these factors will allow us to determine whether management of these compounds is possible.

Materials and Methods

Product Selection

Details regarding carcass characteristics were previously described in Chapter 3. Briefly, paired beef strip loins [IMPS 180, (NAMP, 2010)] were collected from 24 carcasses across three USDA quality grades (Prime, Low Choice, and Standard, $n = 8$ per quality grade; USDA 1977) of “A” maturity animals. 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. Intact

strip loins were stored under vacuum, in darkness, and under refrigeration (4°C) until 21 days post-mortem.

Processing

Details regarding fabrication of loins into steaks and patties were previously discussed in Chapter 3. Briefly, strip loins were cut into 2.54 cm steaks progressing anterior to posterior using a 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 degree of doneness, 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 *Longissimus lumborum* muscle. Following grinding, ground material was stuffed into approximately 50-mm diameter, plastic perforated casings and frozen at -20°C. Resulting frozen chubs were then sliced 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 hours but no more than 24 hours to a temperature range of 3 – 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 two hours after first being thawed to 3 – 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 (well done) °C after being thawed to a temperature of 3 – 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 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.

Sample Preparation for Chemical Analysis

Following tempering or cooking, samples were frozen in liquid nitrogen and pulverized in a blender (Nutri Ninja: Model BL642, Euro-Pro Operating LLC, Newton, MA). The resulting homogeneous samples were stored in 4.5 x 9 inch VWR sterile sampling bags (VWR International, Cat. No. 82007-706, Radnor, PA) at -80°C until later analyses.

Volatile Compounds

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, 5

cores cut parallel with the muscle fiber 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 microliters 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 five-minute incubation period at 65°C. The vials were then subjected to 20 minutes 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 (30m × 0.25mm × 1.00µm; 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 cooked 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 degree of doneness). 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), in which $n=8$. The sub-plot was the sample type (whole steaks vs. ground patties), in which $n=24$. The sub-sub-plot was the thermal processing temperature (4, 25, 55, 60, 71, and 77°C), in which $n=48$.

Results

A total of 27 different volatile compounds were identified in the samples in this study: six Strecker aldehydes, four ketones, two sulfur compounds, two esters, two alcohols, two carboxylic acids, five aldehydes, and four 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. The main effects and interactions impacting these compounds can be found in Table 5-1. The LS means of all volatile compounds can be found in Tables 5-1a and 5-1b.

Four Strecker aldehydes, four pyrazines, and one ester had 3-way interactions between quality grade, degree of doneness, and product type (each $P < 0.001$). The

concentration of pyrazines, such as trimethylpyrazine, did not differ ($P > 0.05$) among ground patties, regardless of quality grade or degree of doneness (Figure 5-1).

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

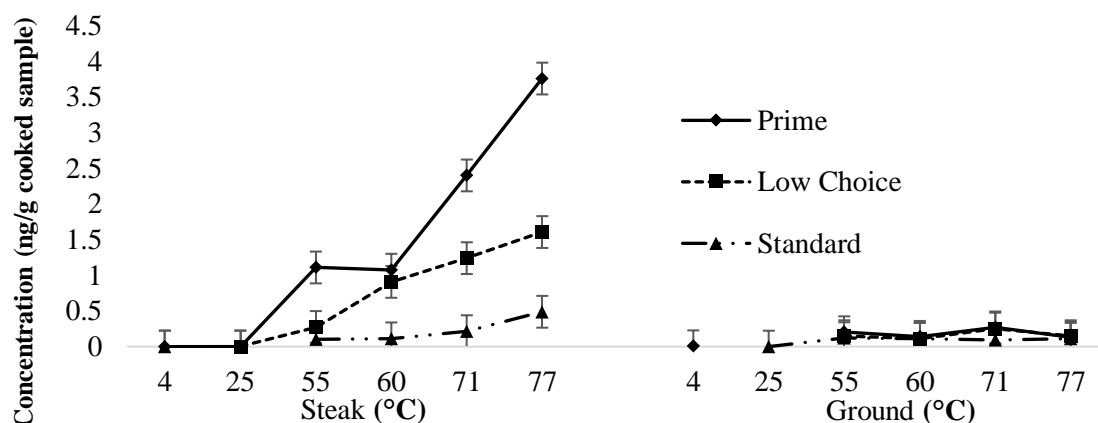


Figure 5-1. Concentration (ng/g cooked sample) of trimethyl pyrazine from USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) of six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P < 0.001$).

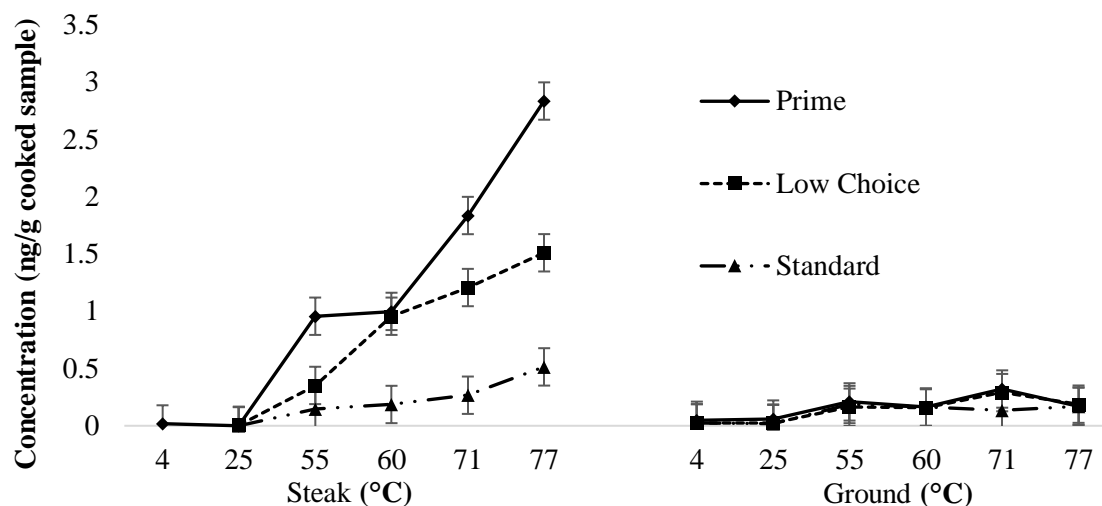


Figure 5-2. Concentration (ng/g cooked sample) of 2-ethyl-3,5-dimethyl-pyrazine from USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) of six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P < 0.001$).

A 3-way interaction was observed for four Strecker aldehydes: 2-methyl-propanal ($P = 0.013$; Figure 5-3), 2-methyl-butanal ($P < 0.001$), 3-methyl-butanal ($P = 0.001$; Figure 5-4), 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 four 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 degree of doneness 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 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-methylbutanal, and 2-methyl-propanal. Similarly, among ground patties, the concentrations of all four Strecker aldehydes within the raw degrees of doneness did not differ ($P > 0.05$); furthermore, raw concentrations were similar ($P > 0.05$) with cooked ground patties in some cases (55° and 60°C).

A 2-way interaction between quality grade and product type was observed for two Maillard reaction compounds and six lipid degradation compounds. The two Maillard compounds were a Strecker aldehyde (acetaldehyde; $P = 0.015$) and a sulfur compound (dimethyl disulfide; $P = 0.003$; Figure 5-5). 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.

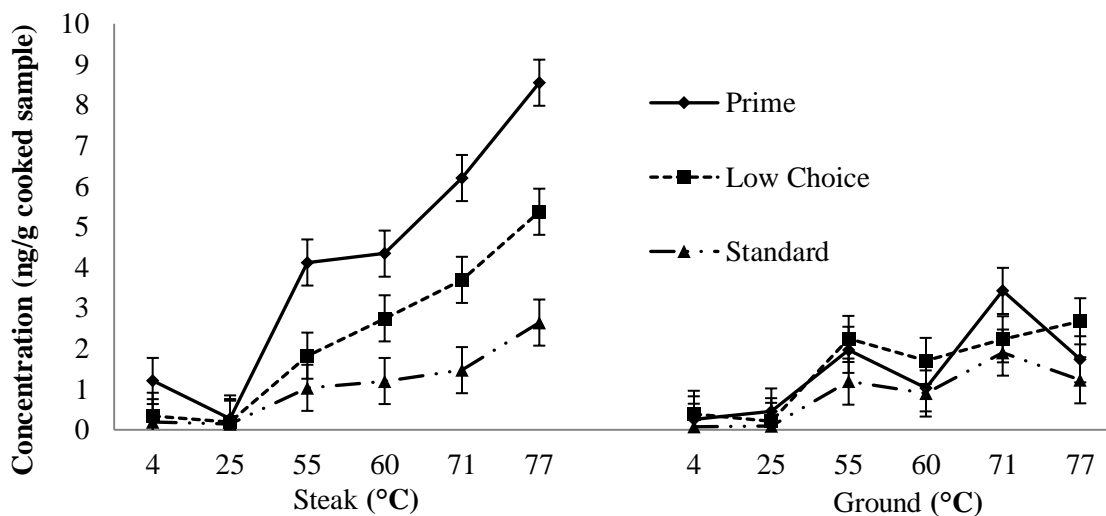


Figure 5-3. Concentration (ng/g cooked sample) of 2-methyl-Propanal in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A three-way interaction (quality grade \times DOD \times product type) was observed ($P = 0.013$).

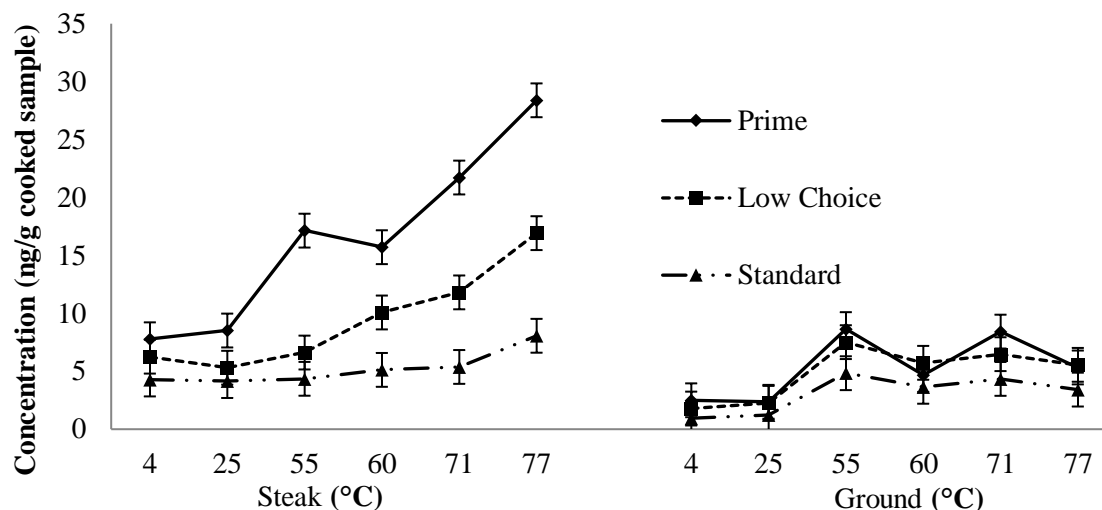


Figure 5-4. Concentration (ng/g cooked sample) of 3-methyl-Butanal in USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. A three-way interaction (quality grade × DOD × product type) was observed ($P < 0.001$).

The concentration of dimethyl disulfide decreased ($P < 0.05$) in steak samples with a decrease in quality grade. The four lipid degradation compounds affected were an alcohol (1-penten-3-ol; $P < 0.001$; Figure 5-6), a carboxylic acid (butanoic acid; $P < 0.001$; Figure 5-7), three aldehydes (hexanal: $P = 0.029$; octanal: $P = 0.001$; and nonanal: $P = 0.005$), and a ketone (2-heptanone; $P = 0.035$; Figure 5-8). 2-heptanone and hexanal (Figure 5-9) 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 of 2-heptanone, octanal, and nonanal was greatest ($P < 0.05$) in Prime steaks. Octanal (Figure 5-10) 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 two Maillard reaction compounds and five lipid degradation compounds. The two Maillard compounds were both ketones (2,3-butanedione: $P = 0.002$; and 3-hydroxy-2-butanone: $P = 0.005$). These two compounds were both 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 (Figure 5-11). The lipid degradation compounds affected by this interaction were an alcohol (1-penten-3-ol; $P = 0.016$; Figure 5-6), two esters (methyl ester butanoic acid:

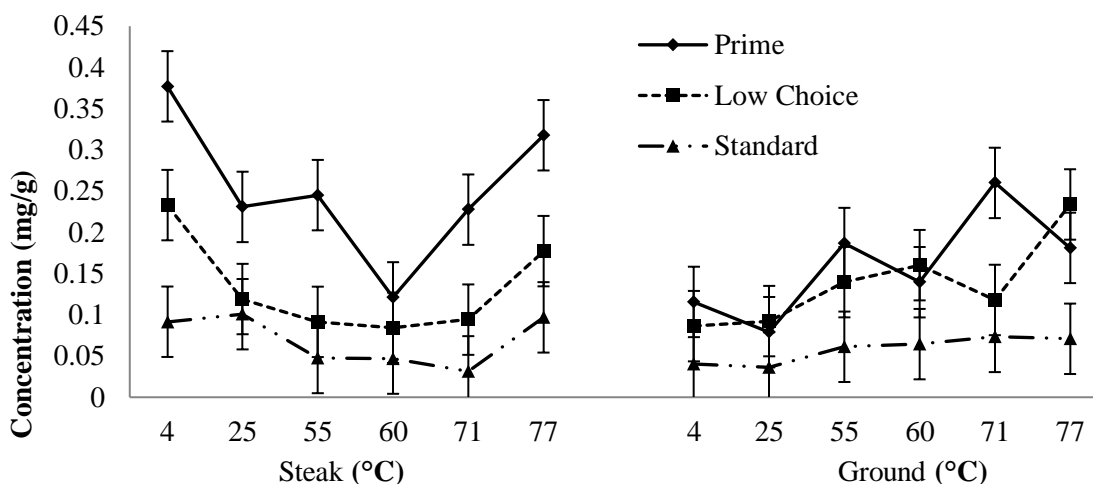


Figure 5-5. Concentration (ng/g cooked sample) of dimethyl disulfide in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. Two-way interactions were observed: QG \times product type ($P < 0.001$) and product type \times DOD ($P < 0.001$).

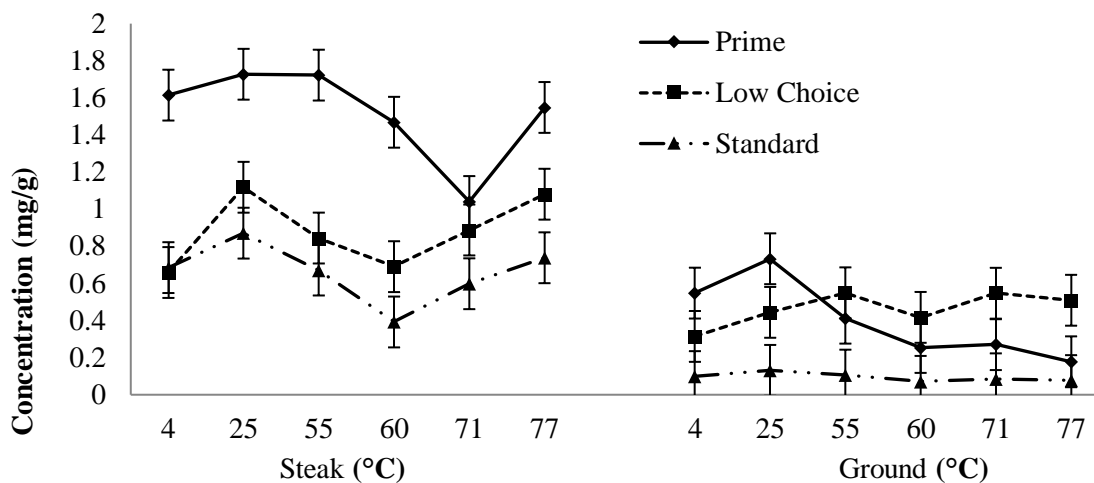


Figure 5-6. Concentration (ng/g cooked sample) of 1-penten-3-ol in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. Two-way interactions were observed: QG \times product type ($P < 0.001$) and QG \times DOD ($P = 0.016$).

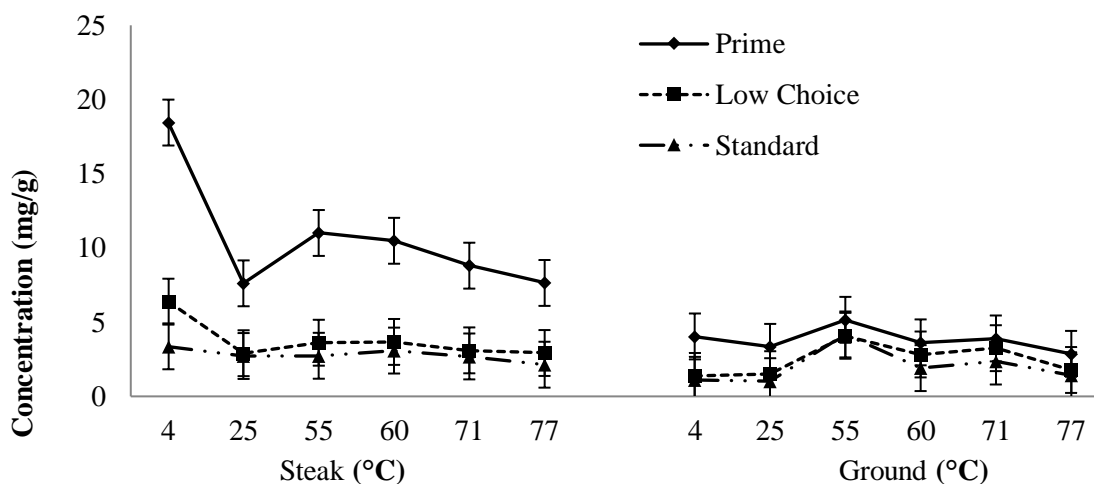


Figure 5-7. Concentration (ng/g cooked sample) of butanoic acid in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. Two-way interactions were observed: QG \times product type ($P < 0.001$) and product type \times DOD ($P = 0.002$).

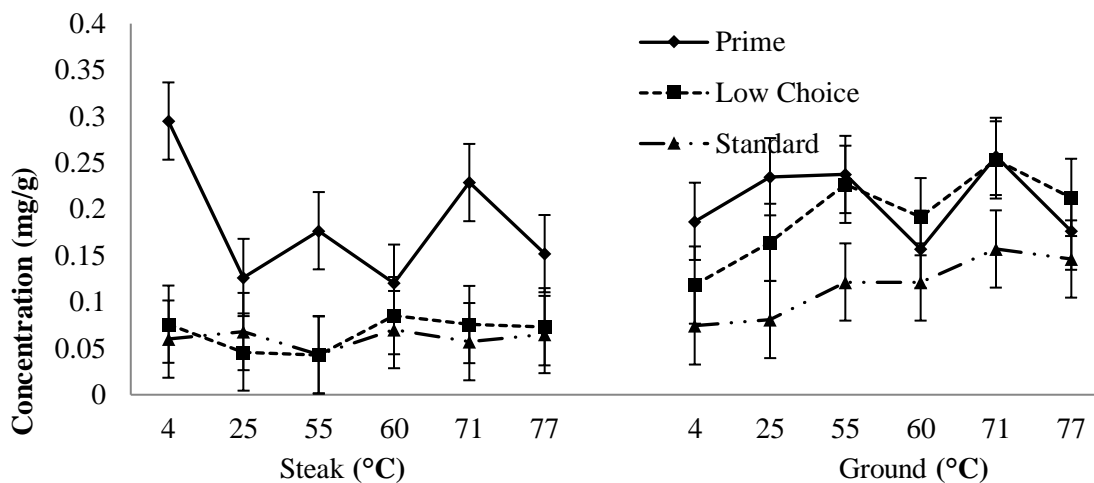


Figure 5-8. Concentration (ng/g cooked sample) of 2-heptanone in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. Two-way interactions were observed: QG \times product type ($P = 0.035$) and product type \times DOD ($P = 0.028$).

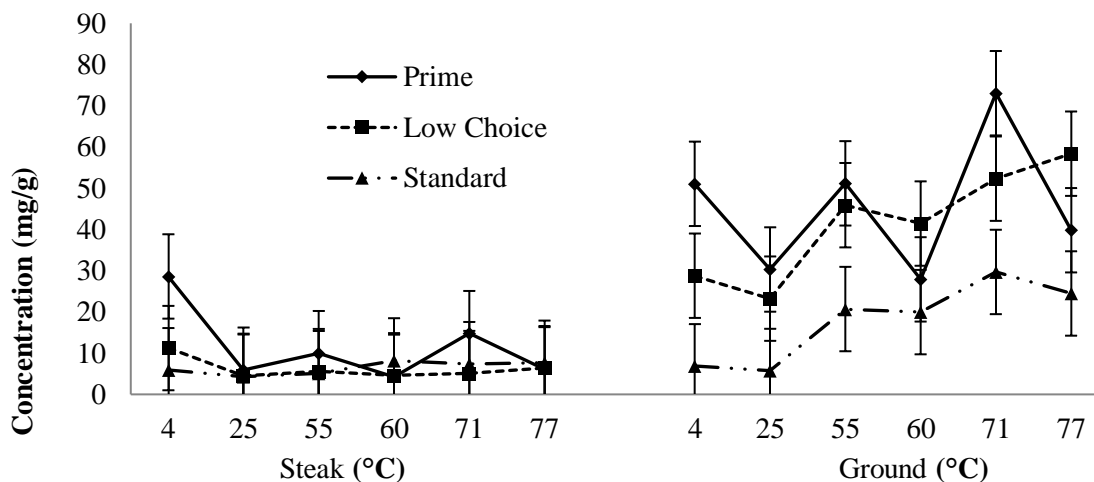


Figure 5-9. Concentration (ng/g cooked sample) of hexanal in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. Two-way interactions were observed: QG \times product type ($P = 0.029$) and product type \times DOD ($P = 0.019$).

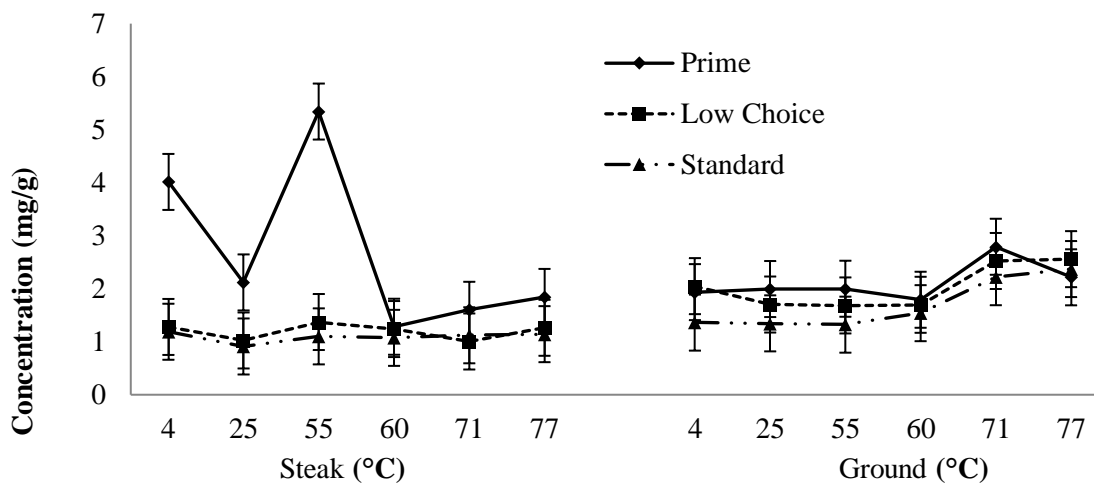


Figure 5-10. Concentration (ng/g cooked sample) of octanal in USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. Two-way interactions were observed: QG × product type (P = 0.001); QG × DOD (P = 0.038); and product type × DOD (P = 0.001).

P < 0.001; and methyl ester acetic acid: P < 0.001), and two 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 (Figure 5-12) 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 (Figure 5-13) was greatest (P < 0.05) in Prime samples cooked or tempered to 4, 25, and 71°C, while 60° and 77°C samples did not differ (P > 0.05) by quality grade. Octanal and nonanal (Figure 5-14) concentrations did not differ (P > 0.05) by quality grades in 60, 71, and 77°C samples (P ≤ 0.0385). In 4, 25,

and 55°C samples, their concentrations 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 degree of doneness and product type. This interaction was significant for five Maillard reaction compounds and 11 lipid degradation products. The Maillard compounds affected included three ketones (2,3-butanedione: $P < 0.001$; 3-hydroxy-2-butanone: $P = 0.002$; and 2-heptanone: $P = 0.028$) and two sulfur compounds (dimethyl disulfide: $P < 0.001$; and carbon disulfide: $P < 0.001$). 2,3-butanedione and 3-hydroxy-2-butanone (Figure 5-15), 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 ($P < 0.05$; Figure 5-8) in concentration in ground

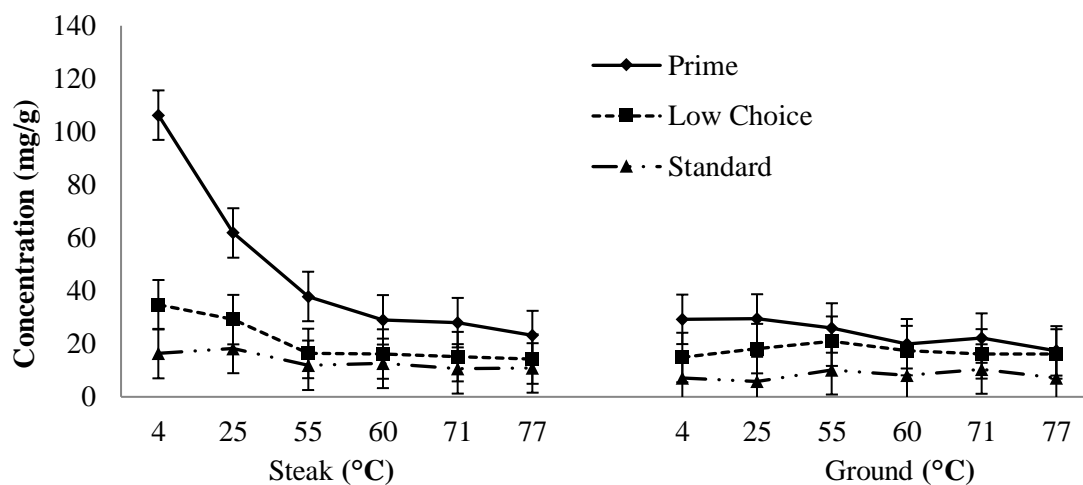


Figure 5-11. Concentration (ng/g cooked sample) of 2,3-butanedione in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. Two-way interactions were observed: QG \times DOD ($P = 0.002$) and product type \times DOD ($P < 0.001$).

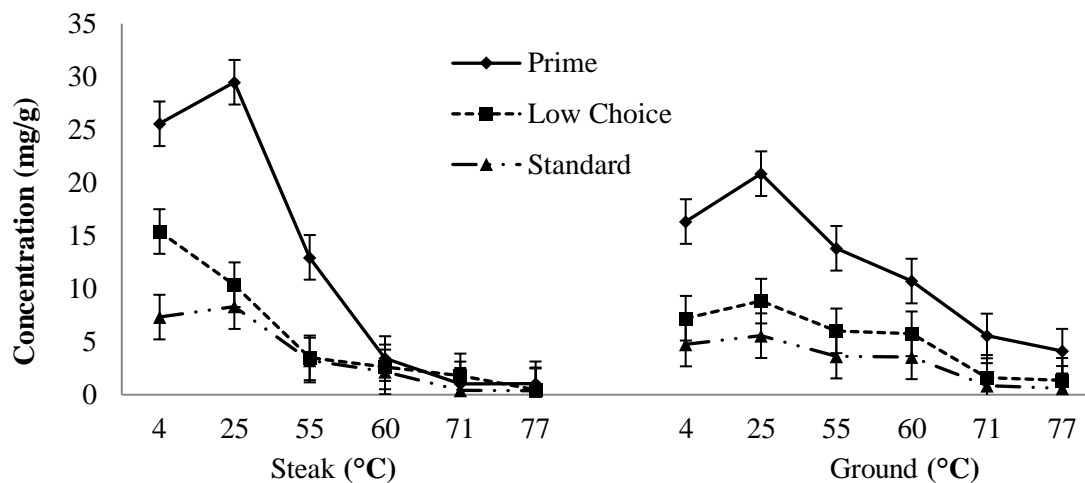


Figure 5-12. Concentration (ng/g cooked sample) of butanoic acid, methyl ester in USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. Two-way interactions were observed: quality grade \times DOD ($P < 0.001$) and product type \times DOD ($P < 0.001$).

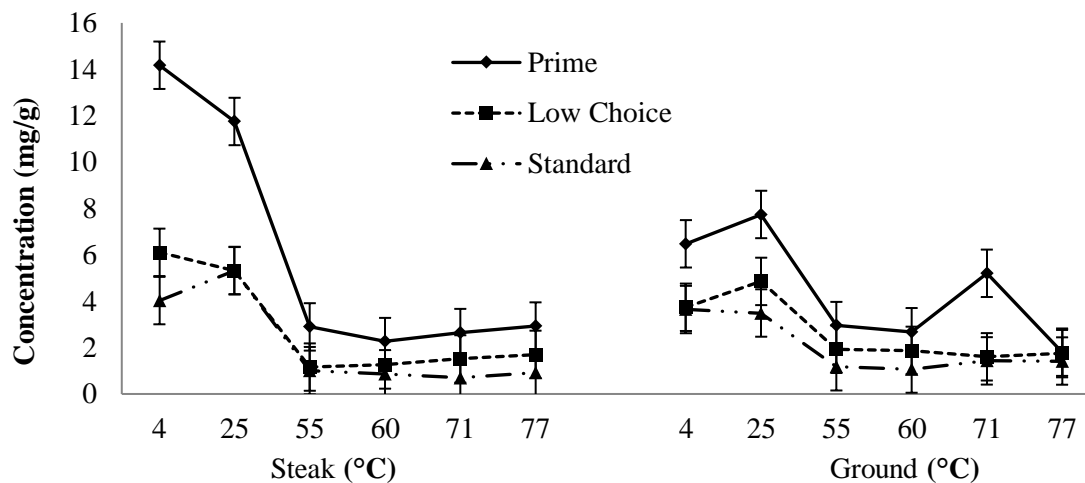


Figure 5-13. Concentration (ng/g cooked sample) of acetic acid, methyl ester in USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. A three-way interaction (QG \times DOD \times product type) was observed ($P = 0.029$).

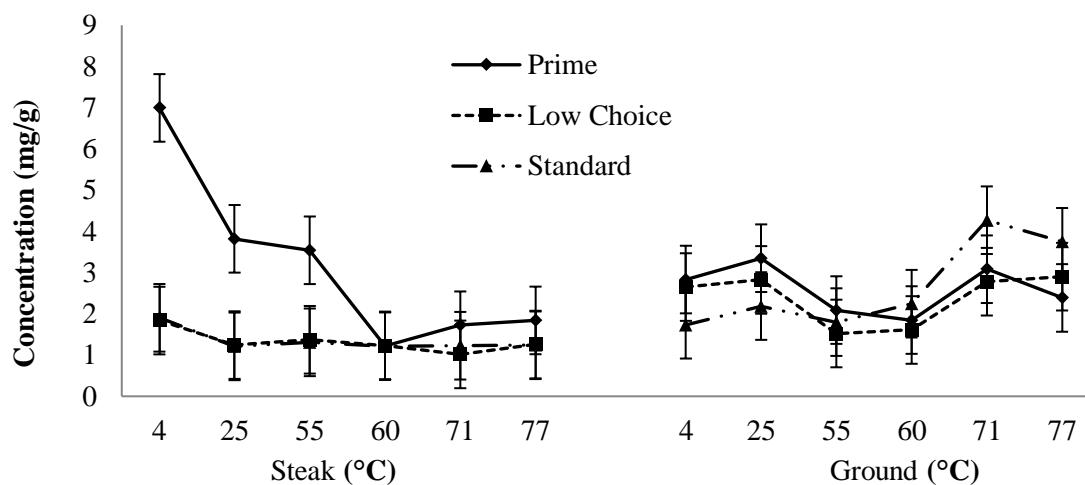


Figure 5-14. Concentration (ng/g cooked sample) of nonanal in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. Two-way interactions were observed: QG \times product type ($P = 0.005$); QG \times DOD ($P = 0.022$); and product type \times DOD ($P = 0.002$).

patties compared to steaks in all degrees of doneness except 4°C. Dimethyl disulfide (Figure 5-5) and carbon disulfide were both greatest ($P < 0.05$) in 4° 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 two esters (butanoic acid, methyl ester: $P < 0.001$; and acetic acid, methyl ester: $P < 0.001$), five aldehydes (hexanal: $P = 0.019$; heptanal: $P = 0.006$; octanal: $P = 0.001$; nonanal: $P = 0.002$; and decanal: $P = 0.001$), two carboxylic 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 (Figure 5-12) and acetic acid, methyl ester (Figure 5-13) concentrations were greatest ($P < 0.05$) in raw steak samples.

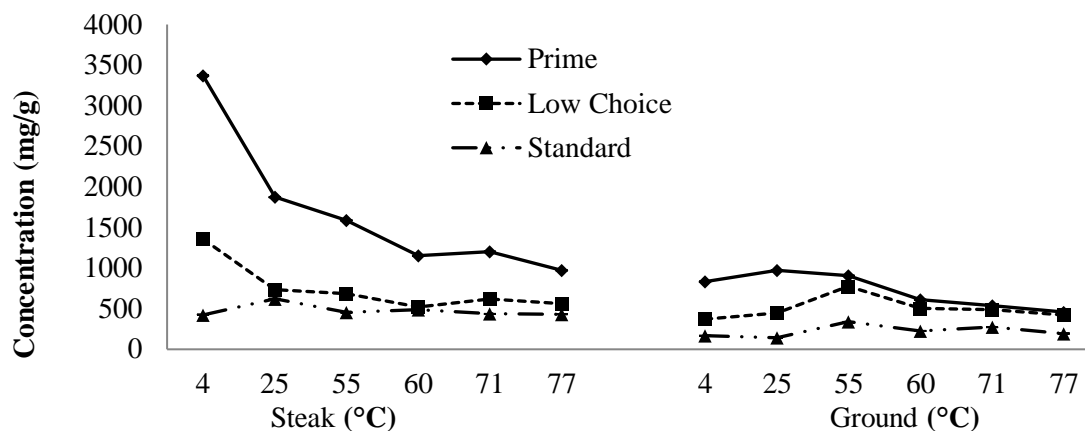


Figure 5-15. Concentration (ng/g cooked sample) of 3-hydroxy-2-butanone in USDA Prime, Low Choice, and Standard steaks and ground patties (n = 8) at six degrees of doneness. Two-way interactions were observed: QG × DOD (P = 0.005) and product type × DOD (P = 0.002).

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 five 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 degrees of doneness (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 (Figure 5-10), where the concentration in 55°C samples was greater ($P < 0.05$) in steaks compared to ground patties. Among steaks, hexanal (Figure 5-9) and octanal were greatest ($P < 0.05$) in concentration in cooked samples compared with raw, while nonanal (Figure 5-14) was found in the greatest ($P < 0.05$) amount in 4°C samples. Butanoic acid (Figure 5-7) and octanoic acid (Figure 5-16) 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 degrees of doneness. 2-heptanone (Figure 5-8) and 1-octen-3-ol (Figure 5-17) 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.

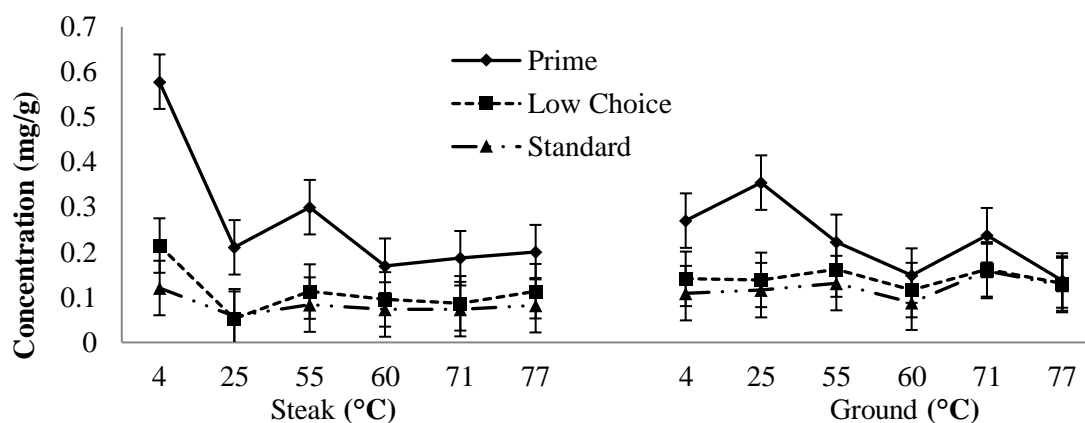


Figure 5-16. Concentration (ng/g cooked sample) of octanoic acid in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A two-way interaction (DOD \times product type) was observed ($P = 0.007$).

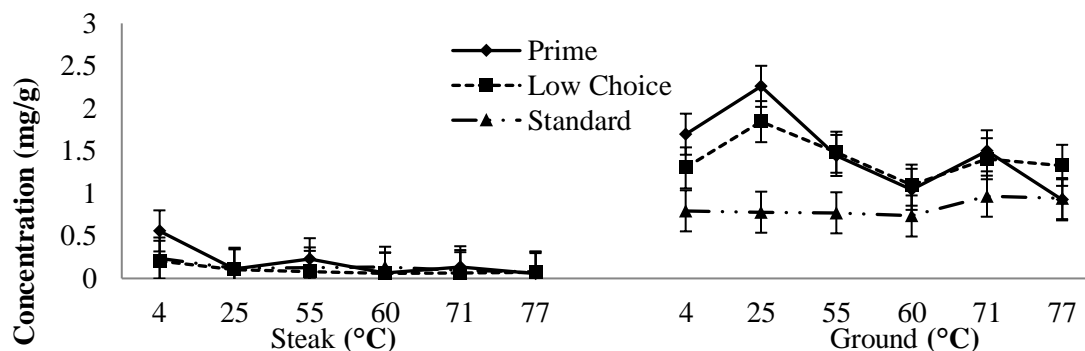


Figure 5-17. Concentration (ng/g cooked sample) of 1-octen-3-ol in USDA Prime, Low Choice, and Standard steaks and ground patties ($n = 8$) at six degrees of doneness. A two-way interaction (DOD \times product type) was observed ($P = 0.029$).

Discussion

It is important to note that a unique attribute of the method used to measure volatile compounds in this study was mincing of the meat samples. Other studies that have measured volatile compounds have utilized an intact meat sample. 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 accumulation of less volatile compounds (Cross, Berry, & Wells, 1980; Mottram & Edwards, 1983; Mottram, Edwards, & MacFie, 1982). 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 (Farmer et al., 2013; Chevance et al. 2000; Chevance & Farmer, 1999). However, increased intramuscular (IM) fat did not seem to be associated with a lesser appearance of volatiles in this study.

There are many factors that can contribute to the formation of volatile compounds in meat, but one factor not often discussed is cooking duration. Previous data (Figure 3-2) indicated that Standard samples generally took longer to cook, and steaks took longer to cook than ground patties. These samples may have a different volatile compound profile than other samples that reached the same degree of doneness due to an increased amount

of time spent on the heating surface. The prolonged heating time that was necessary to heat the Standard samples and the steak samples to the same endpoint temperature as Low Choice and Prime samples and ground patties may have allowed for the formation of volatile compounds that did not have the chance to form yet in other samples.

Likewise, this prolonged heating could have also led to some compounds that were observed in other samples to be driven off and not observed in the samples that required longer cooking times. The production of Maillard reaction compounds in general were limited due to the relatively short cooking time that was characteristic of the cooking method used in this study compared to others.

There was a higher proportion of lipid-derived volatiles produced in ground patties compared with steaks. The process of grinding increased the surface area of the lipids in the patties and made them more susceptible to degradation and more available for participation in volatile-producing reactions. Similar to the results found by Ahn and Nam (2004), the volatile compounds produced in ground patties were primarily lipid oxidation products, with 2-propanone, 2,3-butanedione, and hexanal being the predominant compounds formed. Furthermore, Parker et al. (2010) found that in a meat-based pet food, the formation of trimethylpyrazine involves the incorporation of 2,3-butanedione. Indeed, these results show that as 2,3-butanedione decreased, the concentration of trimethylpyrazine increased. In steaks, acetaldehyde and carbon disulfide decreased with cooking, which supports Bailey's claim (1994) that acetaldehyde interacts with hydrogen sulfide compounds to form other volatiles. Many compounds increased upon cooking and eventually decreased at higher degrees of doneness. This

may be due to interactions with other compounds or components of other compounds to create new volatile compounds. Some compounds could have been degraded as fast as they were formed, resulting in little or no change in concentration throughout cooking (Balagiannis et al., 2010).

Conclusion

These data reveal that quality grade, grinding, and degree of doneness impact important volatile compounds. Contrary to previous research, increased fat content was associated with increased volatile production. A higher proportion of lipid-derived volatiles were produced in ground patties compared with steaks. The shorter cooking duration characteristic of the cooking method used in this study limited the production of Maillard-derived volatiles. These data have potential for management of important flavor contributing compounds as a function of cooking, grinding, and quality grade. By manipulating the rate and pathways of volatile compound production in beef, consumers may be able to receive the same flavorful eating experience from lower quality grades as Prime beef. Previous work has primarily utilized model systems to study the development of volatile flavor compounds from beef. This data set reveals that basic understanding of flavor development may be attained from beef steaks. Furthermore, future research could explore the development of predictive models for chemical changes that occur with cooking of beef. One overall model, especially one that includes chemical changes involving volatile compounds, is not sufficient; the model must be dependent on fat content (quality grade) and product type.

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CHAPTER VI

CONCLUSION

This data revealed that quality grade, grinding, and degree of doneness affect the proximate composition, pH, cooking duration, lipids and water-soluble compounds known to influence beef flavor. Unique findings of this study include that the act of grinding impacted flavor related compounds, and cooking duration had more of an effect on the flavor-producing compounds than was originally expected. Therefore, these data have potential for management of important flavor contributing compounds as a function of cooking, grinding, and quality grade. By manipulating the proximate composition, the fatty acid profile, the water-soluble compounds, and the rate and pathways of volatile compound production in beef, consumers may be able to receive the same flavorful eating experience from lower quality grades as Prime beef. Previous work has primarily utilized model systems to study the development of volatile flavor compounds from beef. This data set reveals that basic understanding of flavor development may be attained from beef steaks. Furthermore, future research could explore the development of predictive models for chemical changes that occur with cooking of beef. This model must also incorporate physical and thermal properties with this data to be complete. One overall model is not sufficient; the model must be dependent on fat content (quality grade) and product type.

The 3-way interaction of quality grade, degree of doneness, and product type (steaks vs. ground patties) impacted moisture and protein content, pH, neutral and polar lipid fatty acids, free and total amino acids, reducing sugars, and volatile compounds. The

2-way interaction of quality grade and degree of doneness affected few free amino acids, few neutral and polar lipid fatty acids, and few volatiles. The 2-way interaction of quality grade and product type impacted fatty acids, amino acids, and volatiles, while the 2-way interaction of product type and degree of doneness affected these same compounds in addition to cook times. The way these interactions affected each measurement varied per the individual measurement or compound. In general, however, there were less ($P < 0.05$) of these compounds known to influence beef flavor in ground patties compared with steaks. The exceptions were fat content and volatile compounds that result from lipid degradation, which were greater ($P < 0.05$) in ground patties compared with steaks. This implies that beef flavor originating from compounds other than lipids, such as water-soluble compounds, is most likely diminished during the process of grinding. The lipid profile and compounds resulting from it, however, increased ($P < 0.05$) in ground patties, and this may alter the perceived flavor of beef.

Generally, volatile compounds increased with cooking or showed little to no change among cooked temperatures, because they are constantly degraded and regenerated. Fatty acids also increased with cooking in most cases. The water-soluble compounds, such as amino acids and sugars, which react to form flavor compounds decreased with degree of doneness as they were being consumed to form other products. The effect of quality grade on these measurements varied according to degree of doneness, product type, and individual compound, but it was usually Prime or Standard samples that had a greater ($P < 0.05$) amount of flavor compounds. Knowing how these compounds develop in response to quality grade, degree of doneness, and grinding will

allow us to better utilize sensory data associated with these compounds. This could then create the potential for purposeful development of those volatile compounds involving the integration of flavor precursor compounds that consumers find most enjoyable when eating beef. Overall, this study reveals that there are different flavor profiles between whole muscle steaks and ground beef products. Ground beef containing only intramuscular fat generally has a less complex flavor profile than whole muscle steaks containing the same fat. The grinding process leads to an increase in fat content and lipid-derived volatile compounds but a decrease in water-soluble compounds. The compounds known to impact flavor were generally more abundant in quality grades of higher fat content and in lower cooked temperatures, such as 55 or 60°C before they became less abundant and less reactive.

APPENDIX
LS MEANS TABLES

Table 3-1. 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	394.85 ^b	424.17 ^a	419.18 ^a	6.417	0.009
Marbling ²	803.75 ^a	446.25 ^b	265.00 ^c	9.186	<.001
KPH ³ , %	2.75 ^b	3.12 ^b	4.00 ^a	0.195	0.001
REA ⁴ , cm ²	86.77 ^b	86.58 ^b	101.23 ^a	2.071	<.001
Calculated YG ⁵	3.99 ^a	3.36 ^b	2.57 ^c	0.154	<.001
Ribeye Color	4.75	5.12	5.12	0.208	0.356
Lean Maturity ⁶	36.25	43.75	41.25	3.981	0.414
Skeletal Maturity ⁶	63.75	53.75	60.00	8.583	0.711

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 x mm fat thickness) - (0.0496 x cm² REA) + (0.20 x KPH%) + (0.008378 x kg HCW)

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

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

Table 3-2. The LS means for the percent of moisture, protein, and fat; and cooking duration (minutes) in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

QG	DOD	Product	Moisture	Protein	Fat	Cooking Duration ¹
Prime	4	Steak	65.67	22.60	11.07	NA
Prime	25	Steak	66.14	21.02	10.53	NA
Prime	55	Steak	59.95	25.01	13.23	3.27
Prime	60	Steak	57.11	24.83	12.72	4.00
Prime	71	Steak	55.43	26.35	12.68	4.86
Prime	77	Steak	55.55	29.00	12.57	5.36
Low Choice	4	Steak	71.00	23.84	6.26	NA
Low Choice	25	Steak	71.64	24.48	4.48	NA
Low Choice	55	Steak	64.76	27.45	6.08	3.37
Low Choice	60	Steak	64.36	27.58	5.59	3.77
Low Choice	71	Steak	62.98	28.59	6.39	4.90
Low Choice	77	Steak	62.01	29.40	6.21	5.13
Standard	4	Steak	73.66	24.88	2.09	NA
Standard	25	Steak	73.43	25.25	2.27	NA
Standard	55	Steak	69.24	27.24	2.99	2.82
Standard	60	Steak	68.16	27.60	2.83	3.53
Standard	71	Steak	66.63	28.63	2.86	4.48
Standard	77	Steak	64.82	30.93	3.95	5.04
Prime	4	Ground	64.37	20.51	12.56	NA
Prime	25	Ground	64.36	19.74	13.43	NA
Prime	55	Ground	58.29	25.72	13.69	1.20
Prime	60	Ground	57.71	25.96	14.08	1.20
Prime	71	Ground	56.76	25.91	14.28	1.58
Prime	77	Ground	55.52	27.54	14.02	1.78
Low Choice	4	Ground	70.09	22.30	7.11	NA
Low Choice	25	Ground	69.70	21.89	6.71	NA
Low Choice	55	Ground	62.69	27.44	7.70	1.30
Low Choice	60	Ground	62.92	28.08	7.91	1.35
Low Choice	71	Ground	60.08	28.29	8.32	1.72
Low Choice	77	Ground	59.42	30.26	8.31	1.91
Standard	4	Ground	72.82	23.49	3.58	NA
Standard	25	Ground	72.48	23.18	3.87	NA
Standard	55	Ground	66.82	27.94	4.58	1.03
Standard	60	Ground	66.66	28.11	4.28	1.07
Standard	71	Ground	64.15	30.06	4.52	1.54
Standard	77	Ground	63.29	30.40	5.01	1.58
SEM			0.62	0.50	0.57	0.16
	QG		<0.001	<0.001	<0.001	0.004
	Product		<0.001	0.016	<0.001	<0.001
	QG × Product		0.035	0.674	0.639	0.320
P-values	DOD		<0.001	<0.001	<0.001	<0.001
	QG × DOD		0.006	0.013	0.046	0.973
	Product × DOD		0.200	<0.001	0.196	<0.001
	QG × Product × DOD		0.004	0.006	0.465	0.901

¹ NA = these samples were not cooked, no data available

Table 3-3. Table of main effects, two-way interaction, and three-way interaction effect on proximate composition, pH, and total reducing sugars. Significance level was determined at $P \leq 0.05$

Measurement	Effect/Interaction						
	QG ¹	Product Type	QG × Product Type	DOD ²	QG × DOD	Product Type × DOD	QG × Product Type × DOD
Moisture %							X
Protein %							X
Fat %	X	X		X	X		
Cooking Duration	X	X		X		X	
pH							X
Total Reducing Sugars							X

¹QG = quality grade

²DOD = degree of doneness

Table 3-4. Common names of fatty acid abbreviations

Abbreviation	Fatty Acid	
	Type	Common Name
C14:0	SFA ¹	Myristic acid
C14:1 n5	MUFA ²	Myristoleic
C15:0	SFA	Pentadecylic acid
C15:1	MUFA	Pentadecenoic acid
C16:0	SFA	Palmitic acid
C16:1 n7	MUFA	Palmitoleic acid
C17:0	SFA	Margaric acid
C17:1 n8	MUFA	Heptadecenoic acid
C18:0	SFA	Stearic acid
C18:1 trans	MUFA	Elaidic acid
C18:1 n9 cis	MUFA	Oleic acid
C18:2 trans	PUFA ³	Linolelaidic acid
C18:2 n6	PUFA	Linoleic acid
C18:3 n6	PUFA	Γ -Linolenic acid
C20:0	SFA	Arachidic acid
C18:3 n3	PUFA	α -Linolenic acid
C20:1 n9	MUFA	Gondoic acid
C21:0	SFA	Heneicosylic acid
C20:2	PUFA	Eicosadienoic acid
C20:3 n6	PUFA	Eicosatrienoic acid
C22:0	SFA	Behenic acid
C20:4 n6	PUFA	Arachidonic acid
C22:1 n9	MUFA	Erucic acid
C23:0	SFA	Tricosylic acid
		Eicosapentaenoic acid
C20:5 n3	PUFA	acid
C24:0	SFA	Lignoceric acid

¹SFA = saturated fatty acid

²MUFA = monounsaturated fatty acid

³PUFA = polyunsaturated fatty acid

Table 3-5. Table of main effects, two-way interaction, and three-way interaction effects on neutral lipid fatty acid concentrations. Significance level was determined at $P \leq 0.05$

Fatty Acid	Effect/Interaction						
	QG ¹	Product Type	QG × Product Type	DOD ²	QG × DOD	Product Type × DOD	QG × Product Type × DOD
C14:0							X
C14:1 n5							X
C15:0							X
C15:1							
C16:0							X
C16:1 n7							X
C17:0							X
C17:1 n8							X
C18:0							X
C18:1 trans							X
C18:1 n9 cis							X
C18:2 trans							X
C18:2 n6							X
C18:3 n6	X	X					
C20:0	X		X				
C18:3 n3	X	X			X	X	
C20:1 n9							X
C21:0	X	X	X	X			
C20:2	X	X	X		X	X	
C20:3 n6	X	X				X	
C22:0							X
C20:4 n6							X
C22:1 n9	X	X				X	
C23:0				X			
C20:5 n3				X		X	
C24:0							
SFA							X
MUFA							X
PUFA							X

¹QG = quality grade

²DOD = degree of doneness

Table 3-5a. The LS means of neutral lipid SFA concentrations (mg/g) in beef strip steaks and ground patties from three different USDA quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Fatty Acid										SFA
			C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C21:0	C22:0	C23:0	C24:0	
Prime	4	Steak	14.12	1.95	108.74	5.08	51.87	0.60	0.12	0.15	0.11	0.14	182.84
Prime	25	Steak	13.59	1.82	102.68	4.64	47.99	0.44	0.11	0.15	0.12	0.16	171.77
Prime	55	Steak	14.94	2.23	138.88	6.29	65.17	0.64	0.10	0.15	0.13	0.15	228.52
Prime	60	Steak	18.30	2.61	152.64	7.02	70.05	0.65	0.10	0.13	0.12	0.12	251.63
Prime	71	Steak	14.93	2.19	136.80	6.05	61.91	0.58	0.09	0.13	0.12	0.11	222.80
Prime	77	Steak	16.09	2.40	152.52	6.79	70.74	0.51	0.09	0.14	0.13	0.13	249.39
Low Choice	4	Steak	8.94	1.56	80.66	4.26	39.17	0.30	0.09	0.15	0.10	0.17	135.29
Low Choice	25	Steak	6.56	1.09	56.19	2.80	27.29	0.24	0.09	0.15	0.09	0.16	94.69
Low Choice	55	Steak	8.74	1.45	72.48	3.92	35.30	0.26	0.09	0.13	0.09	0.13	122.54
Low Choice	60	Steak	8.26	1.40	72.69	3.87	36.25	0.29	0.09	0.13	0.09	0.13	123.15
Low Choice	71	Steak	7.71	1.27	63.94	3.37	31.38	0.25	0.08	0.12	0.08	0.12	108.23
Low Choice	77	Steak	8.17	1.32	68.96	3.49	33.18	0.26	0.08	0.11	0.23	0.14	115.69
Standard	4	Steak	9.32	1.57	73.56	3.90	31.80	0.29	0.12	0.16	0.11	0.17	120.87
Standard	25	Steak	2.66	0.57	23.12	1.30	12.30	0.16	0.08	0.14	0.08	0.16	40.48
Standard	55	Steak	3.22	0.67	29.36	1.53	14.70	0.16	0.08	0.13	0.08	0.14	49.94
Standard	60	Steak	3.16	0.64	28.01	1.47	14.48	0.18	0.07	0.13	0.08	0.14	48.25
Standard	71	Steak	2.74	0.58	22.44	1.21	11.04	0.42	0.09	0.11	0.26	0.13	38.66
Standard	77	Steak	3.67	0.78	35.20	1.92	17.95	0.18	0.07	0.11	0.08	0.12	60.00
Prime	4	Ground	17.97	2.68	155.81	7.19	72.91	0.53	0.12	0.18	0.15	0.15	257.53
Prime	25	Ground	18.04	2.71	144.26	6.94	65.90	0.47	0.12	0.16	0.13	0.16	238.77
Prime	55	Ground	19.67	2.81	162.41	7.37	74.57	0.53	0.12	0.15	0.14	0.13	267.77
Prime	60	Ground	16.50	2.35	127.89	5.95	59.06	0.43	0.12	0.14	0.12	0.12	212.55
Prime	71	Ground	15.64	2.21	123.48	5.59	56.57	0.41	0.11	0.13	0.12	0.12	204.08
Prime	77	Ground	13.88	1.95	105.36	4.83	48.13	0.35	0.12	0.11	0.19	0.13	174.85
Low Choice	4	Ground	11.10	1.90	92.44	4.91	37.23	0.35	0.11	0.15	0.09	0.16	148.34
Low Choice	25	Ground	11.34	1.81	83.27	4.40	40.94	0.31	0.11	0.14	0.10	0.15	142.47
Low Choice	55	Ground	11.89	1.94	91.53	5.02	45.43	0.32	0.10	0.13	0.10	0.13	149.91
Low Choice	60	Ground	10.18	1.62	74.53	3.99	31.25	0.27	0.10	0.12	0.09	0.13	122.19
Low Choice	71	Ground	7.95	1.29	59.90	3.22	29.31	0.22	0.09	0.11	0.25	3.79	104.93
Low Choice	77	Ground	9.86	1.66	72.40	3.92	27.94	0.28	0.09	0.12	0.51	0.12	112.12
Standard	4	Ground	4.93	0.98	37.96	2.02	18.53	0.18	0.09	0.14	0.08	0.16	65.00
Standard	25	Ground	5.48	1.06	40.75	2.21	20.23	0.20	0.10	0.15	0.09	0.16	70.32
Standard	55	Ground	4.97	0.96	32.61	1.99	18.00	0.17	0.08	0.12	0.09	0.13	57.00
Standard	60	Ground	4.62	0.91	35.71	1.93	17.72	0.17	0.08	0.12	0.08	0.13	61.39
Standard	71	Ground	5.30	0.99	39.04	2.12	18.92	0.16	0.07	0.11	0.09	0.12	66.84
Standard	77	Ground	7.47	1.40	37.45	3.07	18.08	0.26	0.09	0.12	0.54	0.20	63.78
SEM			1.500	0.244	13.585	0.683	7.048	0.079	0.009	0.009	0.125	0.669	21.882
	QG		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.019	0.892	0.229	<0.001
	Product Type		<0.001	<0.001	0.082	0.009	0.351	0.063	<0.001	0.443	0.142	0.229	0.091
	QG × Product Type		0.473	0.669	0.564	0.694	1.000	0.043	0.031	0.477	0.817	0.235	0.759
P-values	DOD		0.009	0.008	0.006	0.006	0.027	0.263	<0.001	<0.001	0.012	0.289	0.008
	QG × DOD		0.028	0.103	0.173	0.178	0.570	0.177	0.790	0.784	0.832	0.175	0.232
	Product Type × DOD		0.007	0.016	0.002	0.017	0.002	0.069	0.197	0.664	0.126	0.246	0.001
	QG × Product Type × DOD		<0.001	<0.001	<0.001	<0.001	0.007	0.573	0.297	0.005	0.743	0.178	<0.001

Table 3-5b. The LS means of neutral lipid MUFA concentrations (mg/g) in beef strip steaks and ground patties from three different USDA quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Fatty Acid								MUFA
			C14:1 n5	C15:1	C16:1 n7	C17:1 n8	C18:1 trans	C18:1 n9 cis	C20:1 n9	C22:1 n9	
Prime	4	Steak	4.08	0.10	13.31	3.42	15.27	141.32	2.04	0.05	180.22
Prime	25	Steak	3.95	0.08	12.98	3.26	15.07	135.68	1.94	0.09	173.14
Prime	55	Steak	3.86	0.09	12.67	3.32	15.40	134.19	1.93	0.33	172.12
Prime	60	Steak	4.87	0.09	16.02	4.16	18.54	168.27	2.35	0.30	214.60
Prime	71	Steak	3.77	0.09	12.59	3.15	14.64	128.61	1.84	0.30	165.25
Prime	77	Steak	3.96	0.08	13.15	3.34	15.93	136.67	1.92	0.23	175.28
Low Choice	4	Steak	2.45	0.10	7.75	2.50	10.71	79.88	1.35	0.03	104.77
Low Choice	25	Steak	1.76	0.08	5.69	1.71	7.33	58.91	0.93	0.03	77.35
Low Choice	55	Steak	2.42	0.06	7.52	2.48	10.53	77.20	1.27	0.02	101.73
Low Choice	60	Steak	2.22	0.06	7.00	2.16	9.76	71.73	1.15	0.03	94.34
Low Choice	71	Steak	2.13	0.06	6.82	2.18	8.99	70.16	1.14	0.03	91.51
Low Choice	77	Steak	2.10	0.07	6.60	2.18	9.28	66.51	1.08	0.04	87.85
Standard	4	Steak	2.65	0.10	8.87	2.87	11.57	83.99	1.35	0.05	111.42
Standard	25	Steak	0.87	0.07	2.75	0.86	1.83	26.40	0.39	0.02	33.20
Standard	55	Steak	1.04	0.07	3.13	0.93	2.12	28.84	0.44	0.03	36.60
Standard	60	Steak	1.01	0.06	3.10	0.93	2.22	28.86	0.42	0.05	36.65
Standard	71	Steak	1.06	0.18	2.94	0.87	1.93	26.62	0.41	0.43	34.46
Standard	77	Steak	1.05	0.07	3.22	0.98	2.40	30.17	0.44	0.04	38.36
Prime	4	Ground	4.82	0.09	15.68	4.09	19.84	165.80	2.51	0.23	213.07
Prime	25	Ground	5.27	0.10	17.07	4.71	21.92	181.62	2.81	0.05	233.62
Prime	55	Ground	5.47	0.09	18.06	5.01	22.39	193.35	2.83	0.05	247.26
Prime	60	Ground	4.85	0.13	15.94	4.39	19.59	172.96	2.44	0.06	220.69
Prime	71	Ground	4.57	0.12	15.03	4.04	18.82	158.39	2.41	0.04	202.69
Prime	77	Ground	4.14	0.12	13.55	3.64	16.71	142.66	2.19	0.03	183.04
Low Choice	4	Ground	2.79	0.07	8.56	2.94	13.78	90.93	1.62	0.05	120.74
Low Choice	25	Ground	3.16	0.08	9.57	3.28	13.72	98.85	1.78	0.02	114.77
Low Choice	55	Ground	3.44	0.07	10.64	3.71	15.67	113.05	1.97	0.02	130.84
Low Choice	60	Ground	3.02	0.07	9.45	3.22	12.49	100.50	1.66	0.02	130.44
Low Choice	71	Ground	2.34	0.07	7.29	2.46	10.30	77.38	1.39	0.02	101.24
Low Choice	77	Ground	3.00	0.11	9.09	2.98	12.23	92.41	1.73	0.02	91.50
Standard	4	Ground	1.62	0.07	4.84	1.56	3.49	44.93	0.67	0.03	57.21
Standard	25	Ground	1.83	0.07	5.44	1.79	4.18	51.09	0.78	0.03	65.21
Standard	55	Ground	1.68	0.08	5.09	1.75	3.37	47.98	0.72	0.02	55.39
Standard	60	Ground	1.53	0.07	4.64	1.60	3.52	43.90	0.65	0.03	55.93
Standard	71	Ground	1.77	0.06	5.39	1.86	4.25	50.38	0.84	0.02	64.58
Standard	77	Ground	2.56	0.11	8.16	2.61	12.14	48.56	1.24	0.03	69.57
SEM			0.449	0.032	1.296	0.442	2.559	11.296	0.226	0.091	15.403
	QG		<0.001	0.090	<0.001	<0.001	<0.001	<0.001	<.001	0.003	<0.001
	Product Type		<0.001	0.634	<0.001	<0.001	<0.001	<0.001	<.001	0.008	<0.001
	QG × Product Type		0.571	0.250	0.569	0.505	0.236	0.015	0.059	0.132	0.044
P-values	DOD		0.085	0.759	0.084	0.060	0.224	0.001	0.069	0.309	0.002
	QG × DOD		0.013	0.744	0.009	0.008	0.071	0.003	0.014	0.469	0.015
	Product Type × DOD		0.003	0.208	0.003	0.001	0.110	<0.001	0.001	0.037	0.008
	QG × Product Type × DOD		0.015	0.615	0.005	0.008	0.013	0.010	0.007	0.151	0.012

Table 3-5c. The LS means of neutral lipid PUFA concentrations (mg/g) in beef strip steaks and ground patties from three different USDA quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Fatty Acid								PUFA
			C18:2 trans	C18:2 n6	C18:3 n6	C18:3 n3	C20:2	C20:3 n6	C20:4 n6	C20:5	
Prime	4	Steak	0.97	3.75	0.05	0.44	0.14	0.16	0.21	0.12	5.78
Prime	25	Steak	0.95	3.59	0.04	0.40	0.13	0.15	0.19	0.09	5.54
Prime	55	Steak	0.99	3.55	0.05	0.40	0.12	0.12	0.20	0.08	5.45
Prime	60	Steak	1.20	4.37	0.05	0.49	0.15	0.15	0.20	0.07	6.67
Prime	71	Steak	0.92	3.47	0.04	0.40	0.12	0.13	0.17	0.08	5.32
Prime	77	Steak	0.99	3.70	0.06	0.42	0.13	0.14	0.20	0.06	5.67
Low Choice	4	Steak	0.60	3.05	0.04	0.37	0.11	0.09	0.18	0.09	4.52
Low Choice	25	Steak	0.40	2.23	0.03	0.26	0.11	0.07	0.20	0.09	3.37
Low Choice	55	Steak	0.54	3.05	0.04	0.38	0.11	0.09	0.19	0.08	4.48
Low Choice	60	Steak	0.52	2.95	0.04	0.34	0.11	0.09	0.20	0.08	4.34
Low Choice	71	Steak	0.50	2.80	0.03	0.32	0.10	0.07	0.18	0.08	4.07
Low Choice	77	Steak	0.49	2.70	0.04	0.32	0.11	0.09	0.18	0.08	4.00
Standard	4	Steak	0.63	2.85	0.03	0.28	0.11	0.06	0.21	0.11	4.22
Standard	25	Steak	0.15	1.25	0.02	0.12	0.05	0.04	0.16	0.09	1.84
Standard	55	Steak	0.18	1.47	0.02	0.14	0.06	0.03	0.16	0.08	2.09
Standard	60	Steak	0.18	1.47	0.02	0.14	0.05	0.03	0.17	0.08	2.10
Standard	71	Steak	0.16	1.31	0.02	0.14	0.08	0.03	0.19	0.10	1.99
Standard	77	Steak	0.20	1.52	0.02	0.15	0.06	0.03	0.15	0.08	2.19
Prime	4	Ground	1.29	4.63	0.05	0.55	0.19	0.15	0.23	0.08	7.16
Prime	25	Ground	1.40	5.07	0.07	0.59	0.20	0.18	0.26	0.10	7.83
Prime	55	Ground	1.46	5.31	0.07	0.62	0.23	0.18	0.25	0.08	8.18
Prime	60	Ground	1.26	4.71	0.06	0.57	0.19	0.17	0.24	0.07	7.26
Prime	71	Ground	1.19	4.49	0.06	0.53	0.18	0.16	0.21	0.08	6.90
Prime	77	Ground	1.05	4.08	0.05	0.50	0.16	0.15	0.19	0.06	6.24
Low Choice	4	Ground	0.73	3.76	0.05	0.46	0.14	0.10	0.22	0.09	5.55
Low Choice	25	Ground	0.77	4.17	0.05	0.51	0.16	0.12	0.24	0.09	5.36
Low Choice	55	Ground	0.86	4.66	0.05	0.57	0.16	0.13	0.22	0.08	6.74
Low Choice	60	Ground	0.74	3.99	0.05	0.48	0.15	0.11	0.21	0.08	5.81
Low Choice	71	Ground	0.58	3.13	0.04	0.39	0.10	0.08	0.16	0.07	4.55
Low Choice	77	Ground	0.75	3.93	0.06	0.50	0.15	0.13	0.20	0.11	5.82
Standard	4	Ground	0.28	2.28	0.03	0.23	0.08	0.05	0.20	0.09	3.24
Standard	25	Ground	0.33	2.65	0.03	0.27	0.09	0.06	0.21	0.09	3.72
Standard	55	Ground	0.31	2.41	0.05	0.24	0.08	0.05	0.18	0.08	3.40
Standard	60	Ground	0.28	2.16	0.03	0.22	0.07	0.04	0.17	0.08	3.05
Standard	71	Ground	0.32	2.56	0.03	0.27	0.07	0.05	0.17	0.08	3.55
Standard	77	Ground	0.44	3.51	0.10	0.38	0.10	0.11	0.28	0.11	5.03
SEM			0.090	0.422	0.015	0.050	0.017	0.020	0.024	0.013	0.601
	QG		<0.001	<0.001	0.021	<0.001	<0.001	<0.001	0.159	0.255	<0.001
	Product Type		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.730	<0.001
	QG × Product Type		0.003	0.707	0.476	0.299	0.002	0.806	0.606	0.799	0.690
P-values	DOD		0.005	0.073	0.210	0.088	0.054	0.121	0.077	0.005	0.047
	QG × DOD		0.005	0.014	0.597	0.018	0.033	0.142	0.111	0.065	0.009
	Product Type × DOD		0.001	0.001	0.323	0.002	0.004	0.011	0.144	0.047	0.005
	QG × Product Type × DOD		0.001	0.012	0.275	0.063	0.102	0.188	0.014	0.630	0.016

Table 3-6. Table of main effects, two-way interaction, and three-way interaction effects on neutral lipid fatty acid percentages. Significance level was determined at $P \leq 0.05$

Fatty Acid	Effect/Interaction						
	QG ¹	Product Type	QG × Product Type	DOD ²	QG × DOD	Product Type × DOD	QG × Product Type × DOD
C14:0		X					
C14:1 n5		X				X	
C15:0	X	X					
C15:1	X						
C16:0							X
C16:1 n7		X				X	
C17:0	X						
C17:1 n8	X	X					
C18:0		X				X	
C18:1 trans		X					
C18:1 n9 cis		X				X	
C18:2 trans		X				X	
C18:2 n6	X	X					
C18:3 n6	X						
C20:0	X	X					
C18:3 n3	X	X				X	
C20:1 n9		X				X	
C21:0	X	X	X				
C20:2	X						
C20:3 n6		X		X		X	
C22:0	X	X	X				
C20:4 n6	X						
C22:1 n9							
C23:0	X						
C20:5 n3	X	X					
C24:0							
SFA		X				X	
MUFA		X				X	
PUFA	X	X					

¹QG = quality grade

²DOD = degree of doneness

Table 3-6a. The LS means of neutral lipid SFA percentages in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Fatty Acid										SFA
			C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C21:0	C22:0	C23:0	C24:0	
Prime	4	Steak	3.79	0.53	29.67	1.38	14.15	0.16	0.03	0.04	0.03	0.04	49.84
Prime	25	Steak	3.80	0.49	29.33	1.27	14.06	0.12	0.03	0.04	0.03	0.05	49.33
Prime	55	Steak	3.71	0.54	32.81	1.51	15.38	0.16	0.03	0.04	0.03	0.03	54.25
Prime	60	Steak	3.86	0.56	32.17	1.50	14.76	0.14	0.02	0.03	0.03	0.03	53.06
Prime	71	Steak	3.91	0.57	34.01	1.53	15.24	0.15	0.02	0.03	0.03	0.03	55.42
Prime	77	Steak	3.80	0.57	34.45	1.56	15.65	0.11	0.02	0.03	0.03	0.03	56.22
Low Choice	4	Steak	3.78	0.65	32.25	1.68	15.36	0.12	0.04	0.07	0.04	0.08	54.03
Low Choice	25	Steak	3.84	0.62	30.48	1.52	14.29	0.13	0.06	0.09	0.06	0.10	51.14
Low Choice	55	Steak	3.67	0.62	31.75	1.65	15.55	0.12	0.04	0.07	0.05	0.07	53.55
Low Choice	60	Steak	3.69	0.62	32.50	1.68	16.14	0.13	0.04	0.06	0.04	0.06	54.95
Low Choice	71	Steak	3.74	0.62	31.33	1.64	15.31	0.12	0.04	0.06	0.04	0.06	52.93
Low Choice	77	Steak	3.98	0.65	32.62	1.67	15.46	0.12	0.04	0.05	0.16	0.07	54.65
Standard	4	Steak	3.79	0.69	30.06	1.48	14.06	0.22	0.10	0.15	0.10	0.17	50.71
Standard	25	Steak	3.47	0.76	30.18	1.70	16.13	0.23	0.12	0.20	0.12	0.23	53.02
Standard	55	Steak	3.56	0.77	32.64	1.75	16.78	0.20	0.09	0.15	0.10	0.16	56.06
Standard	60	Steak	3.60	0.74	31.28	1.68	16.38	0.21	0.09	0.15	0.09	0.16	54.25
Standard	71	Steak	3.60	0.77	29.03	1.58	14.46	0.54	0.13	0.17	0.33	0.18	50.33
Standard	77	Steak	3.62	0.78	34.74	1.89	17.79	0.18	0.07	0.12	0.08	0.13	59.31
Prime	4	Ground	3.78	0.57	32.60	1.51	15.15	0.11	0.02	0.04	0.03	0.03	53.81
Prime	25	Ground	3.74	0.55	30.01	1.42	13.86	0.10	0.03	0.03	0.03	0.03	49.77
Prime	55	Ground	3.78	0.54	30.36	1.39	13.88	0.10	0.02	0.03	0.03	0.03	50.14
Prime	60	Ground	3.72	0.53	29.05	1.37	13.43	0.10	0.03	0.03	0.03	0.03	48.30
Prime	71	Ground	3.72	0.53	29.13	1.36	13.54	0.10	0.03	0.03	0.03	0.03	48.46
Prime	77	Ground	3.81	0.54	28.84	1.33	13.15	0.10	0.03	0.03	0.06	0.04	47.85
Low Choice	4	Ground	4.02	0.69	33.34	1.78	13.93	0.13	0.04	0.05	0.03	0.06	54.04
Low Choice	25	Ground	4.32	0.69	31.78	1.67	15.61	0.12	0.04	0.05	0.04	0.06	54.35
Low Choice	55	Ground	4.40	0.70	34.08	1.79	14.32	0.12	0.04	0.05	0.04	0.05	53.78
Low Choice	60	Ground	3.93	0.63	28.75	1.55	11.95	0.11	0.04	0.05	0.04	0.05	47.06
Low Choice	71	Ground	3.74	0.61	28.34	1.53	13.86	0.11	0.04	0.05	0.11	1.61	49.48
Low Choice	77	Ground	5.10	0.83	38.03	2.01	11.62	0.15	0.05	0.06	0.23	0.06	56.35
Standard	4	Ground	3.91	0.79	29.97	1.62	14.75	0.15	0.08	0.11	0.07	0.13	51.50
Standard	25	Ground	3.95	0.77	29.37	1.59	14.47	0.14	0.07	0.11	0.07	0.12	50.59
Standard	55	Ground	6.29	1.22	25.16	2.56	14.18	0.22	0.11	0.16	0.11	0.18	48.23
Standard	60	Ground	3.83	0.76	29.51	1.61	14.71	0.14	0.07	0.10	0.07	0.11	50.84
Standard	71	Ground	3.92	0.74	28.83	1.57	14.02	0.11	0.06	0.08	0.07	0.09	49.43
Standard	77	Ground	5.02	0.95	28.37	2.08	13.16	0.19	0.06	0.09	0.51	0.13	46.42
SEM			0.669	0.120	2.482	0.272	1.317	0.072	0.013	0.019	0.110	0.252	2.963
	QG		0.658	<0.001	0.358	0.030	0.658	0.017	<0.001	<0.001	0.016	0.162	0.604
	Product Type		0.041	0.050	0.082	0.424	0.001	0.010	0.008	<0.001	0.700	0.373	0.004
	QG × Product Type		0.182	0.306	0.204	0.448	0.698	0.091	0.001	0.001	0.983	0.197	0.303
P-values	DOD		0.357	0.323	0.091	0.142	0.900	0.627	0.336	0.087	0.074	0.272	0.337
	QG × DOD		0.729	0.627	0.658	0.623	0.757	0.597	0.459	0.493	0.904	0.170	0.856
	Product Type × DOD		0.279	0.494	0.191	0.625	0.006	0.140	0.090	0.230	0.240	0.263	0.007
	QG × Product Type × DOD		0.864	0.790	0.050	0.808	0.584	0.167	0.121	0.400	0.426	0.147	0.068

Table 3-6b. The LS means of neutral lipid MUFA percentages in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Fatty Acid							MUFA	
			C14:1_n5	C15:1	C16:1_n7	C17:1_n8	C18:1trans	C18:1_n9_cis	C20:1_n9		C22:1_n9
Prime	4	Steak	1.09	0.03	3.58	0.92	4.20	38.19	0.55	0.01	48.56
Prime	25	Steak	1.09	0.02	3.61	0.87	4.01	38.99	0.52	0.02	49.11
Prime	55	Steak	0.97	0.02	3.26	0.86	3.99	34.62	0.49	0.09	44.32
Prime	60	Steak	1.03	0.02	3.42	0.89	4.14	35.41	0.51	0.07	45.47
Prime	71	Steak	1.01	0.02	3.39	0.85	4.04	33.26	0.50	0.08	43.15
Prime	77	Steak	0.99	0.02	3.26	0.83	3.86	32.89	0.47	0.06	42.39
Low Choice	4	Steak	1.07	0.04	3.33	1.06	4.55	33.31	0.56	0.01	43.94
Low Choice	25	Steak	1.07	0.05	3.48	1.03	4.57	35.76	0.57	0.02	46.65
Low Choice	55	Steak	1.02	0.03	3.31	1.04	4.30	34.02	0.55	0.01	44.42
Low Choice	60	Steak	1.00	0.03	3.23	0.97	4.29	32.82	0.51	0.01	43.00
Low Choice	71	Steak	1.05	0.03	3.36	1.06	4.43	34.49	0.56	0.01	45.00
Low Choice	77	Steak	1.06	0.04	3.30	1.09	4.58	32.56	0.54	0.02	43.19
Standard	4	Steak	1.29	0.08	4.08	1.18	3.53	36.23	0.55	0.03	46.96
Standard	25	Steak	1.16	0.10	3.68	1.16	2.43	35.28	0.53	0.03	44.37
Standard	55	Steak	1.15	0.08	3.49	1.07	2.43	32.71	0.50	0.03	41.47
Standard	60	Steak	1.17	0.07	3.63	1.11	2.60	34.06	0.50	0.06	43.19
Standard	71	Steak	1.40	0.24	3.98	1.18	2.60	36.13	0.57	0.54	46.63
Standard	77	Steak	1.05	0.07	3.24	0.98	2.37	30.22	0.44	0.04	38.41
Prime	4	Ground	1.02	0.02	3.31	0.86	4.26	34.60	0.53	0.05	44.65
Prime	25	Ground	1.09	0.02	3.55	0.96	4.49	37.91	0.57	0.01	48.58
Prime	55	Ground	1.08	0.02	3.58	0.98	4.46	37.55	0.55	0.01	48.24
Prime	60	Ground	1.09	0.03	3.61	1.01	4.55	39.09	0.56	0.01	50.01
Prime	71	Ground	1.10	0.03	3.67	1.00	4.62	38.84	0.59	0.01	49.84
Prime	77	Ground	1.14	0.03	3.74	0.99	4.64	39.21	0.60	0.01	50.37
Low Choice	4	Ground	1.01	0.03	3.13	1.07	5.05	32.97	0.59	0.02	43.87
Low Choice	25	Ground	1.20	0.03	3.65	1.25	5.06	36.61	0.67	0.01	43.28
Low Choice	55	Ground	1.27	0.03	3.98	1.34	4.84	36.73	0.72	0.01	43.72
Low Choice	60	Ground	1.17	0.03	3.68	1.26	4.91	38.89	0.65	0.01	50.60
Low Choice	71	Ground	1.11	0.03	3.49	1.18	4.89	36.81	0.66	0.01	48.18
Low Choice	77	Ground	1.52	0.05	4.69	1.53	4.98	37.58	0.90	0.01	40.47
Standard	4	Ground	1.28	0.06	3.85	1.25	2.85	35.97	0.55	0.02	45.82
Standard	25	Ground	1.32	0.06	3.91	1.30	2.97	36.54	0.56	0.02	46.66
Standard	55	Ground	2.08	0.12	6.20	2.18	2.61	37.64	0.91	0.03	47.11
Standard	60	Ground	1.26	0.06	3.84	1.34	2.97	36.50	0.54	0.02	46.54
Standard	71	Ground	1.31	0.05	4.00	1.39	3.13	37.36	0.62	0.01	47.87
Standard	77	Ground	1.73	0.09	5.40	1.76	7.57	36.85	0.86	0.02	49.49
SEM			0.22	0.036	0.659	0.22	1.056	1.872	0.104	0.09	2.915
	QG		0.069	<0.001	0.116	0.004	0.069	0.371	0.266	0.252	0.373
	Product Type		0.001	0.191	0.013	<0.001	0.025	<0.001	<0.001	0.096	0.011
	QG × Product Type		0.221	0.191	0.302	0.068	0.65	0.950	0.313	0.371	0.348
P-values	DOD		0.522	0.705	0.534	0.370	0.422	0.157	0.261	0.335	0.181
	QG × DOD		0.745	0.675	0.858	0.806	0.525	0.464	0.776	0.397	0.825
	Product Type × DOD		0.041	0.242	0.032	0.079	0.231	<0.001	0.008	0.249	0.014
	QG × Product Type × DOD		0.689	0.169	0.748	0.62	0.309	0.604	0.708	0.357	0.102

Table 3-6c. The LS means of neutral lipid PUFA percentages in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

			Fatty Acid								
Quality Grade	DOD	Product Type	C18:2 trans	C18:2 n6	C18:3 n3	C18:3 n6	C20:2	C20:3 n6	C20:4 n6	C20:5 n3	PUFA
Prime	4	Steak	0.26	1.02	0.11	0.01	0.03	0.04	0.05	0.03	1.57
Prime	25	Steak	0.25	0.98	0.11	0.01	0.03	0.04	0.05	0.02	1.52
Prime	55	Steak	0.24	0.90	0.10	0.01	0.02	0.02	0.05	0.02	1.38
Prime	60	Steak	0.25	0.94	0.10	0.01	0.03	0.02	0.04	0.01	1.44
Prime	71	Steak	0.24	0.92	0.10	9.28E-3	0.03	0.03	0.04	0.01	1.40
Prime	77	Steak	0.23	0.88	0.10	0.01	0.03	0.03	0.04	0.01	1.35
Low Choice	4	Steak	0.25	1.35	0.16	0.01	0.05	0.03	0.08	0.04	1.98
Low Choice	25	Steak	0.23	1.43	0.16	0.01	0.06	0.04	0.12	0.05	2.13
Low Choice	55	Steak	0.23	1.35	0.15	0.01	0.05	0.04	0.09	0.03	1.97
Low Choice	60	Steak	0.23	1.37	0.15	0.01	0.05	0.04	0.09	0.03	2.00
Low Choice	71	Steak	0.24	1.40	0.16	0.01	0.04	0.03	0.08	0.03	2.03
Low Choice	77	Steak	0.23	1.33	0.16	0.01	0.05	0.04	0.09	0.04	2.00
Standard	4	Steak	0.22	1.54	0.14	0.01	0.05	0.03	0.19	0.05	2.23
Standard	25	Steak	0.19	1.66	0.15	0.02	0.06	0.04	0.23	0.13	2.48
Standard	55	Steak	0.20	1.66	0.15	0.02	0.06	0.03	0.19	0.10	2.37
Standard	60	Steak	0.21	1.71	0.16	0.02	0.06	0.03	0.19	0.09	2.46
Standard	71	Steak	0.22	1.78	0.19	0.02	0.11	0.03	0.26	0.14	2.71
Standard	77	Steak	0.19	1.51	0.14	0.02	0.05	0.03	0.15	0.07	2.19
Prime	4	Ground	0.27	0.97	0.11	0.01	0.04	0.03	0.04	0.01	1.50
Prime	25	Ground	0.28	1.05	0.12	0.01	0.04	0.03	0.05	0.02	1.62
Prime	55	Ground	0.28	1.03	0.11	0.01	0.04	0.03	0.04	0.01	1.59
Prime	60	Ground	0.28	1.07	0.12	0.01	0.04	0.03	0.05	0.01	1.65
Prime	71	Ground	0.29	1.09	0.12	0.01	0.04	0.03	0.05	0.02	1.68
Prime	77	Ground	0.28	1.12	0.13	0.01	0.04	0.04	0.05	0.02	1.72
Low Choice	4	Ground	0.26	1.40	0.17	0.01	0.05	0.03	0.07	0.03	2.06
Low Choice	25	Ground	0.29	1.59	0.19	0.01	0.05	0.04	0.09	0.03	2.33
Low Choice	55	Ground	0.31	1.70	0.20	0.01	0.05	0.04	0.08	0.02	2.45
Low Choice	60	Ground	0.28	1.59	0.18	0.01	0.05	0.04	0.08	0.03	2.30
Low Choice	71	Ground	0.27	1.53	0.18	0.01	0.04	0.04	0.08	0.03	2.22
Low Choice	77	Ground	0.38	1.98	0.25	0.02	0.07	0.06	0.10	0.05	2.94
Standard	4	Ground	0.22	1.83	0.18	0.02	0.06	0.03	0.15	0.07	2.60
Standard	25	Ground	0.23	1.89	0.19	0.02	0.06	0.03	0.15	0.07	2.67
Standard	55	Ground	0.38	3.21	0.29	0.13	0.10	0.06	0.23	0.10	4.54
Standard	60	Ground	0.23	1.80	0.18	0.02	0.06	0.03	0.14	0.06	2.55
Standard	71	Ground	0.23	1.89	0.19	0.02	0.05	0.03	0.12	0.05	2.63
Standard	77	Ground	0.32	2.43	0.26	0.09	0.07	0.08	0.22	0.08	3.58
SEM			0.405	0.343	0.029	0.028	0.014	0.01	0.032	0.019	0.477
	QG		0.218	< 0.001	< 0.001	0.014	0.001	0.100	< 0.001	< 0.001	< 0.001
	Product Type		< 0.001	0.003	< 0.001	0.144	0.175	0.039	0.119	0.008	0.001
	QG × Product Type		0.480	0.169	0.084	0.263	0.631	0.266	0.219	0.098	0.241
P-values	DOD		0.325	0.430	0.233	0.581	0.775	0.020	0.778	0.335	0.431
	QG × DOD		0.597	0.639	0.614	0.778	0.413	0.664	0.973	0.383	0.662
	Product Type × DOD		0.026	0.172	0.032	0.512	0.080	0.013	0.136	0.063	0.117
	QG × Product Type × DOD		0.645	0.707	0.567	0.732	0.197	0.461	0.127	0.055	0.607

Table 3-7. Table of main effects, two-way interaction, and three-way interaction effects on polar lipid fatty acid concentrations. Significance level was determined at $P \leq 0.05$

Fatty Acid	Effect/Interaction						
	QG ¹	Product Type	QG × Product Type	DOD ²	QG × DOD	Product Type × DOD	QG × Product Type × DOD
C14:0							X
C14:1 n5	X	X				X	
C15:0							
C15:1		X		X		X	
C16:0		X		X		X	
C16:1 n7	X	X		X	X	X	
C17:0		X					
C17:1 n8		X		X		X	
C18:0							X
C18:1 trans	X	X		X	X	X	
C18:1 n9 cis							X
C18:2 trans	X	X		X	X	X	
C18:2 n6	X			X		X	
C18:3 n6	X			X	X	X	
C20:0				X		X	
C18:3 n3	X			X		X	
C20:1 n9		X	X	X	X	X	
C21:0	X	X	X	X	X	X	
C20:2							
C20:3 n6				X	X	X	
C22:0	X	X		X	X	X	
C20:4 n6							X
C22:1 n9				X			
C23:0							
C20:5 n3	X	X		X	X	X	
C24:0	X	X		X	X	X	
SFA		X		X	X	X	
MUFA	X	X		X	X	X	
PUFA	X			X	X	X	

¹QG = quality grade

²DOD = degree of doneness

Table 3-7a. The LS means of polar lipid SFA concentration (mg/g) in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Fatty Acid									SFA	
			C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C21:0	C22:0	C23:0		C24:0
Prime	4	Steak	0.14	0.08	2.36	0.08	1.44	0.06	0.03	0.06	0.01	0.07	4.31
Prime	25	Steak	0.27	0.12	3.41	0.15	2.18	0.04	0.03	0.06	0.02	0.07	6.34
Prime	55	Steak	0.39	0.11	4.30	0.17	2.54	0.04	0.03	0.06	0.01	0.06	7.71
Prime	60	Steak	0.33	0.09	3.38	0.13	1.67	0.03	0.03	0.05	0.01	0.05	5.76
Prime	71	Steak	0.21	0.08	2.86	0.11	1.93	0.03	0.03	0.05	0.01	0.05	5.35
Prime	77	Steak	0.19	0.08	2.92	0.10	1.81	0.03	0.03	0.05	0.01	0.05	5.25
Low Choice	4	Steak	0.15	0.09	2.67	0.11	1.81	0.04	0.04	0.07	0.02	0.08	5.05
Low Choice	25	Steak	0.18	0.12	3.17	0.14	2.18	0.05	0.04	0.07	0.02	0.08	6.03
Low Choice	55	Steak	0.14	0.10	2.88	0.12	2.06	0.05	0.03	0.06	0.02	0.07	5.51
Low Choice	60	Steak	0.16	0.09	3.18	0.12	2.05	0.04	0.03	0.06	0.02	0.07	5.78
Low Choice	71	Steak	0.15	0.09	2.55	0.11	1.74	0.04	0.03	0.06	0.02	0.06	4.81
Low Choice	77	Steak	0.16	0.09	2.66	0.11	1.76	0.04	0.03	0.05	0.01	0.06	4.95
Standard	4	Steak	0.21	0.12	2.76	0.12	2.13	0.05	0.05	0.08	0.02	0.09	5.51
Standard	25	Steak	0.25	0.12	3.44	0.14	2.43	0.05	0.05	0.08	0.02	0.09	6.60
Standard	55	Steak	0.30	0.11	2.87	0.11	2.19	0.05	0.04	0.07	0.02	0.08	5.61
Standard	60	Steak	0.28	0.10	2.64	0.11	2.08	0.04	0.04	0.07	0.02	0.07	5.22
Standard	71	Steak	0.31	0.09	2.33	0.10	1.72	0.04	0.04	0.06	0.02	0.07	4.52
Standard	77	Steak	0.30	0.09	2.48	0.09	1.70	0.04	0.03	0.05	0.02	0.06	4.64
Prime	4	Ground	0.29	0.10	3.33	0.13	1.98	0.04	0.03	0.05	0.01	0.06	6.00
Prime	25	Ground	0.44	0.12	4.35	0.18	2.29	0.04	0.03	0.05	0.01	0.06	7.56
Prime	55	Ground	0.40	0.11	4.43	0.18	2.58	0.04	0.03	0.05	0.01	0.05	7.87
Prime	60	Ground	0.32	0.11	3.27	0.14	1.79	0.03	0.03	0.04	0.01	0.05	5.78
Prime	71	Ground	0.29	0.11	4.26	0.18	2.85	0.04	0.03	0.05	0.01	0.05	7.87
Prime	77	Ground	0.41	0.22	5.57	0.33	2.28	0.05	0.03	0.05	0.03	0.06	8.96
Low Choice	4	Ground	0.24	0.10	3.01	0.12	1.91	0.04	0.04	0.06	0.02	0.08	5.59
Low Choice	25	Ground	0.34	0.13	3.80	0.16	2.17	0.05	0.04	0.06	0.02	0.07	6.83
Low Choice	55	Ground	0.20	0.10	2.90	0.12	1.74	0.04	0.03	0.05	0.02	0.06	5.23
Low Choice	60	Ground	0.29	0.12	3.48	0.15	1.98	0.04	0.03	0.05	0.01	0.06	6.19
Low Choice	71	Ground	0.37	0.12	4.05	0.17	2.10	0.04	0.03	0.05	0.02	0.06	6.98
Low Choice	77	Ground	0.47	0.14	7.16	0.31	4.98	0.06	0.03	0.06	0.02	0.06	13.26
Standard	4	Ground	0.16	0.09	3.58	0.12	1.98	0.04	0.04	0.07	0.04	0.08	7.29
Standard	25	Ground	0.20	0.13	3.45	0.14	2.24	0.05	0.04	0.07	0.02	0.08	6.45
Standard	55	Ground	0.12	0.15	4.05	0.19	2.47	0.05	0.03	0.06	0.02	0.07	7.33
Standard	60	Ground	0.11	0.14	3.90	0.17	2.45	0.05	0.04	0.06	0.01	0.07	7.14
Standard	71	Ground	0.10	0.12	3.86	0.17	2.48	0.04	0.03	0.06	0.02	0.06	7.12
Standard	77	Ground	0.11	0.11	4.21	0.24	2.57	0.05	0.03	0.06	0.02	0.06	7.60
SEM			0.041	0.152	0.649	0.119	0.430	0.004	0.002	0.003	0.006	0.003	1.189
	QG		<0.001	0.340	0.1255	0.626	0.730	0.074	<0.001	<0.001	0.131	<0.001	0.688
	Product Type		<0.001	0.106	<0.001	0.003	<0.001	0.368	<0.001	<0.001	0.246	<0.001	<0.001
	QG × Product Type		0.183	0.430	0.863	0.442	0.661	0.879	0.002	0.901	0.678	0.071	0.827
	DOD		0.003	0.606	0.001	0.745	0.013	<0.001	<0.001	<0.001	0.529	<0.001	0.010
	QG × DOD		0.002	0.388	0.074	0.364	0.002	0.250	0.003	0.014	0.625	0.001	0.025
	Product Type × DOD		0.006	0.533	<0.001	0.287	<0.001	<0.001	0.004	<0.001	0.508	<0.001	<0.001
P-values	QG × Product Type × DOD		0.048	0.542	0.239	0.685	0.021	0.227	0.783	0.119	0.491	0.497	0.227

Table 3-7b. The LS means of polar lipid MUFA concentrations (mg/g) in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Fatty Acid								MUFA
			C14:1 n5	C15:1	C16:1 n7	C17:1 n8	C18:1 nans	C18:1 n9 cis	C20:1 n9	C22:1 n9	
Prime	4	Steak	0.07	0.07	0.23	0.21	0.24	2.36	0.06	0.01	3.26
Prime	25	Steak	0.11	0.07	0.35	0.23	0.40	3.36	0.08	0.02	4.62
Prime	55	Steak	0.14	0.07	0.47	0.26	0.48	4.44	0.09	0.01	5.97
Prime	60	Steak	0.13	0.05	0.38	0.21	0.36	3.83	0.08	0.01	5.05
Prime	71	Steak	0.09	0.07	0.29	0.22	0.28	3.00	0.07	0.01	4.02
Prime	77	Steak	0.08	0.07	0.29	0.24	0.28	3.25	0.07	0.01	4.29
Low Choice	4	Steak	0.08	0.09	0.23	0.23	0.30	2.58	0.08	0.01	3.60
Low Choice	25	Steak	0.09	0.08	0.27	0.26	0.34	3.08	0.08	0.02	4.22
Low Choice	55	Steak	0.07	0.08	0.22	0.23	0.26	2.56	0.07	0.01	3.49
Low Choice	60	Steak	0.08	0.08	0.25	0.26	0.29	2.99	0.07	0.01	4.03
Low Choice	71	Steak	0.08	0.07	0.22	0.21	0.26	2.54	0.07	0.01	3.47
Low Choice	77	Steak	0.07	0.08	0.24	0.22	0.29	2.73	0.07	0.01	3.72
Standard	4	Steak	0.09	0.10	0.27	0.21	0.22	2.94	0.09	0.01	3.92
Standard	25	Steak	0.10	0.10	0.31	0.25	0.23	3.46	0.09	0.02	4.53
Standard	55	Steak	0.07	0.09	0.22	0.20	0.16	2.59	0.07	0.01	3.40
Standard	60	Steak	0.07	0.09	0.22	0.22	0.16	2.60	0.08	0.01	3.44
Standard	71	Steak	0.07	0.08	0.20	0.19	0.15	2.34	0.07	0.01	3.11
Standard	77	Steak	0.06	0.09	0.21	0.19	0.16	2.56	0.07	0.01	3.34
Prime	4	Ground	0.11	0.07	0.34	0.20	0.37	3.41	0.09	0.01	4.57
Prime	25	Ground	0.17	0.08	0.51	0.24	0.55	5.18	0.12	0.01	6.86
Prime	55	Ground	0.14	0.07	0.47	0.23	0.46	4.87	0.10	0.02	6.37
Prime	60	Ground	0.12	0.08	0.39	0.21	0.42	3.98	0.09	0.01	5.31
Prime	71	Ground	0.11	0.10	0.36	0.23	0.38	3.89	0.09	0.01	5.17
Prime	77	Ground	0.27	0.47	0.54	0.63	0.75	5.07	0.12	0.01	7.83
Low Choice	4	Ground	0.11	0.09	0.29	0.21	0.36	3.27	0.09	0.02	4.43
Low Choice	25	Ground	0.14	0.09	0.40	0.24	0.50	4.21	0.10	0.02	5.69
Low Choice	55	Ground	0.10	0.10	0.28	0.20	0.31	2.95	0.07	0.01	4.02
Low Choice	60	Ground	0.12	0.09	0.35	0.22	0.44	3.87	0.09	0.01	5.17
Low Choice	71	Ground	0.15	0.08	0.43	0.24	0.53	4.28	0.10	0.01	5.83
Low Choice	77	Ground	0.14	0.12	0.49	0.31	0.64	5.77	0.12	0.01	7.62
Standard	4	Ground	0.09	0.15	0.36	0.27	0.29	3.22	0.08	0.01	4.46
Standard	25	Ground	0.12	0.12	0.35	0.21	0.27	3.62	0.09	0.01	4.80
Standard	55	Ground	0.15	0.13	0.37	0.21	0.33	3.90	0.09	0.01	5.19
Standard	60	Ground	0.13	0.12	0.39	0.24	0.32	4.17	0.09	0.01	5.47
Standard	71	Ground	0.14	0.12	0.42	0.27	0.35	4.18	0.10	0.01	5.59
Standard	77	Ground	0.13	0.29	0.39	0.38	0.44	4.04	0.09	0.01	5.76
SEM			0.033	0.074	0.055	0.062	0.071	0.494	0.008	0.003	0.654
	QG		0.009	0.292	0.002	0.307	0.001	0.045	0.317	0.114	0.004
	Product Type		<0.001	0.005	<0.001	0.009	<0.001	<0.001	<0.001	0.420	<0.001
	QG × Product Type		0.994	0.355	0.355	0.199	0.837	0.575	0.015	0.853	0.847
P-values	DOD		0.154	0.020	0.008	0.001	<0.001	<0.001	0.002	<0.001	<0.001
	QG × DOD		0.290	0.513	0.002	0.094	0.013	0.001	0.001	0.369	0.004
	Product Type × DOD		0.029	0.014	0.008	<0.001	<0.001	0.003	<0.001	0.123	<0.001
	QG × Product Type × DOD		0.103	0.577	0.136	0.366	0.187	0.030	0.086	0.590	0.088

Table 3-7c. The LS means of polar lipid PUFA concentrations (mg/g) in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Fatty Acid								PUFA
			C18:2trans	C18:2 n6	C18:3 n3	C18:3 n6	C20:2	C20:3 n6	C20:4 n6	C20:5 n3	
Prime	4	Steak	0.02	1.88	0.08	0.02	0.04	0.22	0.80	0.12	3.18
Prime	25	Steak	0.03	1.87	0.07	0.02	0.05	0.19	0.56	0.09	2.88
Prime	55	Steak	0.04	2.03	0.09	0.02	0.04	0.23	0.74	0.11	3.29
Prime	60	Steak	0.02	1.49	0.07	0.02	0.04	0.16	0.52	0.08	2.40
Prime	71	Steak	0.02	1.82	0.07	0.02	0.04	0.22	0.69	0.10	2.99
Prime	77	Steak	0.02	2.21	0.09	0.02	0.04	0.25	0.80	0.11	3.55
Low Choice	4	Steak	0.02	2.41	0.10	0.03	0.05	0.24	0.82	0.13	3.80
Low Choice	25	Steak	0.03	2.51	0.09	0.03	0.05	0.22	0.65	0.11	3.68
Low Choice	55	Steak	0.02	2.25	0.09	0.03	0.05	0.22	0.71	0.12	3.49
Low Choice	60	Steak	0.03	2.57	0.10	0.03	0.05	0.25	0.81	0.12	3.95
Low Choice	71	Steak	0.03	2.21	0.09	0.03	0.04	0.22	0.71	0.11	3.43
Low Choice	77	Steak	0.05	2.43	0.10	0.03	0.05	0.24	0.82	0.12	3.80
Standard	4	Steak	0.02	2.46	0.10	0.03	0.05	0.23	0.79	0.13	3.81
Standard	25	Steak	0.02	2.60	0.11	0.04	0.05	0.24	0.89	0.15	4.10
Standard	55	Steak	0.02	2.66	0.10	0.03	0.05	0.24	0.90	0.14	4.15
Standard	60	Steak	0.02	2.64	0.10	0.03	0.05	0.23	0.83	0.13	4.02
Standard	71	Steak	0.01	2.57	0.10	0.03	0.05	0.23	0.90	0.13	4.02
Standard	77	Steak	0.01	2.68	0.10	0.03	0.04	0.24	0.90	0.13	4.14
Prime	4	Ground	0.02	1.70	0.07	0.02	0.04	0.18	0.59	0.09	2.73
Prime	25	Ground	0.04	1.77	0.08	0.02	0.04	0.19	0.60	0.09	2.83
Prime	55	Ground	0.03	1.79	0.07	0.02	0.04	0.20	0.63	0.09	2.87
Prime	60	Ground	0.03	1.57	0.06	0.02	0.04	0.17	0.52	0.08	2.48
Prime	71	Ground	0.03	2.02	0.08	0.02	0.05	0.22	0.68	0.09	3.20
Prime	77	Ground	0.06	2.19	0.10	0.04	0.05	0.28	0.80	0.10	3.61
Low Choice	4	Ground	0.02	2.17	0.09	0.02	0.05	0.21	0.73	0.11	3.41
Low Choice	25	Ground	0.03	2.24	0.09	0.03	0.05	0.21	0.70	0.11	3.43
Low Choice	55	Ground	0.02	2.01	0.09	0.03	0.04	0.19	0.67	0.10	3.16
Low Choice	60	Ground	0.03	2.08	0.08	0.02	0.05	0.20	0.62	0.10	3.18
Low Choice	71	Ground	0.03	2.16	0.10	0.03	0.05	0.22	0.76	0.11	3.47
Low Choice	77	Ground	0.05	3.02	0.13	0.03	0.05	0.32	1.11	0.15	4.86
Standard	4	Ground	0.02	2.36	0.09	0.03	0.07	0.22	0.77	0.11	3.49
Standard	25	Ground	0.02	2.42	0.09	0.03	0.05	0.21	0.80	0.12	3.75
Standard	55	Ground	0.03	2.12	0.08	0.03	0.05	0.18	0.58	0.09	3.13
Standard	60	Ground	0.03	2.60	0.10	0.03	0.05	0.22	0.79	0.12	3.93
Standard	71	Ground	0.03	2.75	0.10	0.03	0.05	0.24	0.94	0.13	4.27
Standard	77	Ground	0.03	2.67	0.11	0.03	0.04	0.27	1.02	0.14	4.31
SEM			0.005	0.244	0.009	0.003	0.008	0.023	0.076	0.013	0.343
	QG		<0.001	0.001	0.002	<0.001	0.172	0.213	<0.001	0.008	<0.001
	Product Type		<0.001	0.212	0.709	0.995	0.149	0.157	0.093	<0.001	0.155
	QG × Product Type		0.840	0.883	0.506	0.257	0.517	0.893	0.284	0.192	0.831
P-values	DOD		<0.001	<0.001	<0.001	0.001	0.149	<0.001	<0.001	<0.001	<0.001
	QG × DOD		0.015	0.068	0.335	0.032	0.100	0.043	0.011	0.035	0.043
	Product Type × DOD		<0.001	0.036	<0.001	<0.001	0.158	<0.001	<0.001	0.001	0.003
	QG × Product Type × DOD		0.089	0.449	0.335	0.162	0.774	0.440	0.030	0.139	0.353

Table 3-8. Table of main effects, two-way interaction, and three-way interaction effects on polar lipid fatty acid percentages. Significance level was determined at $P \leq 0.05$

Fatty Acid	Effect/Interaction						
	QG ¹	Product Type	QG × Product Type	DOD ²	QG × DOD	Product Type × DOD	QG × Product Type × DOD
C14:0							X
C14:1 n5							X
C15:0							
C15:1	X	X		X		X	
C16:0	X	X		X		X	
C16:1 n7							X
C17:0		X					
C17:1 n8		X		X		X	
C18:0							X
C18:1 trans							X
C18:1 n9 cis	X	X		X			
C18:2 trans							X
C18:2 n6							X
C18:3 n6							X
C20:0		X		X			
C18:3 n3							X
C20:1 n9			X	X			
C21:0							X
C20:2		X		X		X	
C20:3 n6							X
C22:0	X	X		X			
C20:4 n6							X
C22:1 n9	X	X		X			
C23:0	X	X		X			
C20:5 n3							X
C24:0	X	X		X			
SFA		X		X		X	
MUFA							X
PUFA							X

¹QG = quality grade

²DOD = degree of doneness

Table 3-8a. The LS means of polar lipid SFA percentages in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Fatty Acid										SFA
			C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C21:0	C22:0	C23:0	C24:0	
Prime	4	Steak	1.28	0.71	21.82	0.71	13.44	0.52	0.32	0.55	0.13	0.64	39.95
Prime	25	Steak	1.96	0.87	24.51	1.06	15.66	0.31	0.25	0.45	0.11	0.50	45.57
Prime	55	Steak	1.93	0.69	23.99	0.96	15.13	0.26	0.20	0.40	0.09	0.43	44.03
Prime	60	Steak	2.46	0.71	25.56	0.94	12.78	0.24	0.19	0.34	0.09	0.38	43.62
Prime	71	Steak	1.67	0.66	22.97	0.88	15.45	0.27	0.22	0.38	0.10	0.43	42.97
Prime	77	Steak	1.42	0.59	22.23	0.76	13.82	0.26	0.20	0.35	0.09	0.39	40.07
Low Choice	4	Steak	1.20	0.75	21.41	0.84	14.36	0.35	0.33	0.56	0.16	0.62	40.34
Low Choice	25	Steak	1.32	0.87	22.76	1.02	15.64	0.33	0.29	0.50	0.12	0.58	43.24
Low Choice	55	Steak	1.29	0.99	23.30	0.99	16.20	0.60	0.23	0.62	0.21	0.73	44.92
Low Choice	60	Steak	1.20	0.68	22.93	0.87	14.63	0.28	0.22	0.42	0.11	0.48	41.54
Low Choice	71	Steak	1.27	0.78	21.79	0.92	14.76	0.32	0.26	0.48	0.13	0.54	41.05
Low Choice	77	Steak	1.26	0.69	21.21	0.85	14.17	0.29	0.26	0.42	0.10	0.48	39.67
Standard	4	Steak	1.20	0.90	20.85	0.93	16.12	0.40	0.34	0.59	0.14	0.70	41.67
Standard	25	Steak	1.29	0.82	22.61	0.92	16.04	0.35	0.30	0.51	0.13	0.60	43.48
Standard	55	Steak	0.92	0.81	21.78	0.83	16.52	0.34	0.30	0.51	0.13	0.61	42.55
Standard	60	Steak	0.87	0.78	20.64	0.83	16.33	0.34	0.28	0.52	0.12	0.58	41.03
Standard	71	Steak	0.82	0.81	19.98	0.81	14.74	0.34	0.30	0.55	0.15	0.63	38.78
Standard	77	Steak	0.89	0.71	20.41	0.71	13.98	0.30	0.26	0.45	0.13	0.52	38.28
Prime	4	Ground	2.15	0.74	25.03	0.95	14.88	0.29	0.24	0.41	0.10	0.46	45.08
Prime	25	Ground	2.57	0.70	25.14	1.02	13.24	0.24	0.20	0.31	0.08	0.35	43.78
Prime	55	Ground	2.35	0.65	25.36	1.02	14.68	0.24	0.16	0.29	0.06	0.32	45.06
Prime	60	Ground	2.21	0.78	23.92	0.98	13.37	0.26	0.21	0.34	0.09	0.40	42.45
Prime	71	Ground	1.85	0.67	24.93	1.03	15.95	0.26	0.17	0.30	0.08	0.33	45.53
Prime	77	Ground	2.06	0.89	26.34	1.37	11.62	0.25	0.16	0.24	0.12	0.28	43.09
Low Choice	4	Ground	1.70	0.79	22.11	0.90	14.21	0.32	0.27	0.49	0.11	0.59	41.35
Low Choice	25	Ground	2.14	0.80	23.78	1.01	13.60	0.30	0.24	0.39	0.11	0.44	42.74
Low Choice	55	Ground	1.63	0.83	23.18	0.98	13.96	0.30	0.23	0.44	0.12	0.50	41.89
Low Choice	60	Ground	2.00	0.82	23.86	1.03	13.55	0.26	0.21	0.36	0.10	0.41	42.48
Low Choice	71	Ground	2.32	0.71	24.88	1.07	12.88	0.22	0.17	0.29	0.10	0.34	42.90
Low Choice	77	Ground	1.76	0.55	25.27	1.09	16.96	0.23	0.13	0.26	0.09	0.28	46.63
Standard	4	Ground	1.30	3.04	22.89	2.52	13.75	0.32	0.29	0.53	0.19	0.60	45.03
Standard	25	Ground	1.65	0.88	22.89	0.95	14.91	0.33	0.28	0.47	0.11	0.55	42.91
Standard	55	Ground	1.93	0.96	25.48	1.16	15.21	0.29	0.22	0.41	0.13	0.44	45.93
Standard	60	Ground	1.69	0.89	23.46	1.02	14.81	0.29	0.21	0.37	0.09	0.41	43.14
Standard	71	Ground	1.76	0.72	22.79	0.97	14.55	0.24	0.18	0.33	0.11	0.36	41.87
Standard	77	Ground	1.65	0.61	23.38	1.33	14.73	0.27	0.19	0.34	0.09	0.36	42.81
SEM			0.185	0.534	1.125	0.402	1.077	0.073	0.019	0.052	0.025	0.065	2.038
		QG	<0.001	0.194	<0.001	0.541	0.009	0.188	<0.001	<0.001	0.007	<0.001	0.144
		Product Type	<0.001	0.351	<0.001	0.012	0.006	0.000	<0.001	<0.001	0.011	<0.001	0.001
		QG × Product Type	0.012	0.380	0.472	0.238	0.691	0.679	0.168	0.688	0.413	0.506	0.468
P-values		DOD	<0.001	0.326	0.015	0.864	0.159	0.006	<0.001	<0.001	0.008	<0.001	0.045
		QG × DOD	0.000	0.349	0.673	0.314	0.012	0.113	0.708	0.240	0.079	0.220	0.349
		Product Type × DOD	0.460	0.436	0.009	0.364	0.183	0.400	0.011	0.244	0.741	0.261	0.011
		QG × Product Type × DOD	<0.001	0.550	0.114	0.759	0.026	0.240	0.007	0.300	0.129	0.299	0.603

Table 3-8b. The LS means of polar lipid MUFA percentages in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Fatty Acid								MUFA
			C14:1 n5	C15:1	C16:1 n7	C17:1 n8	C18:1 trans	C18:1 n9 cis	C20:1 n9	C22:1 n9	
Prime	4	Steak	0.64	0.70	2.09	1.99	2.26	21.73	0.60	0.11	30.12
Prime	25	Steak	0.83	0.54	2.52	1.68	2.87	24.27	0.57	0.13	33.36
Prime	55	Steak	0.78	0.50	2.60	1.66	2.44	25.14	0.55	0.09	33.70
Prime	60	Steak	0.93	0.40	2.84	1.57	2.75	28.75	0.63	0.07	37.93
Prime	71	Steak	0.73	0.57	2.33	1.82	2.32	24.17	0.55	0.08	32.53
Prime	77	Steak	0.59	0.53	2.23	1.81	2.14	24.71	0.55	0.07	32.61
Low Choice	4	Steak	0.64	0.74	1.85	1.83	2.46	20.38	0.62	0.11	28.59
Low Choice	25	Steak	0.63	0.59	1.95	1.87	2.44	22.09	0.57	0.11	30.23
Low Choice	55	Steak	0.71	0.69	1.86	1.75	2.14	21.14	0.63	0.15	29.03
Low Choice	60	Steak	0.58	0.60	1.84	1.92	2.16	21.66	0.53	0.09	29.31
Low Choice	71	Steak	0.66	0.64	1.92	1.83	2.27	21.65	0.59	0.09	29.58
Low Choice	77	Steak	0.58	0.62	1.87	1.75	2.31	21.68	0.59	0.09	29.48
Standard	4	Steak	0.71	0.80	2.01	1.59	1.67	21.86	0.65	0.10	29.34
Standard	25	Steak	0.65	0.65	2.01	1.63	1.48	22.82	0.56	0.10	29.89
Standard	55	Steak	0.54	0.65	1.66	1.55	1.19	19.71	0.56	0.08	25.91
Standard	60	Steak	0.56	0.71	1.73	1.71	1.26	20.38	0.60	0.10	27.00
Standard	71	Steak	0.57	0.71	1.68	1.61	1.32	20.16	0.61	0.12	26.69
Standard	77	Steak	0.47	0.73	1.71	1.56	1.33	20.99	0.57	0.10	27.45
Prime	4	Ground	0.84	0.50	2.53	1.50	2.74	25.62	0.64	0.09	34.43
Prime	25	Ground	0.98	0.48	2.97	1.42	3.20	29.89	0.68	0.07	39.68
Prime	55	Ground	0.85	0.44	2.77	1.39	2.89	28.25	0.57	0.09	37.26
Prime	60	Ground	0.87	0.60	2.80	1.56	3.00	28.72	0.64	0.07	38.27
Prime	71	Ground	0.71	0.63	2.38	1.49	2.45	25.40	0.60	0.05	33.69
Prime	77	Ground	1.14	1.77	2.66	2.69	3.46	25.72	0.62	0.07	38.04
Low Choice	4	Ground	0.78	0.66	2.15	1.64	2.72	23.60	0.68	0.12	32.35
Low Choice	25	Ground	0.88	0.55	2.49	1.48	3.13	26.31	0.65	0.10	35.59
Low Choice	55	Ground	0.81	0.83	2.27	1.61	2.49	23.85	0.60	0.08	32.50
Low Choice	60	Ground	0.83	0.63	2.43	1.52	3.06	26.64	0.65	0.09	35.81
Low Choice	71	Ground	0.96	0.52	2.66	1.46	3.25	26.22	0.63	0.06	35.75
Low Choice	77	Ground	0.60	0.57	1.99	1.54	2.77	23.40	0.54	0.06	31.49
Standard	4	Ground	0.68	0.94	2.22	1.67	1.74	22.05	0.60	0.11	29.83
Standard	25	Ground	0.83	0.77	2.32	1.40	1.79	24.12	0.61	0.09	31.93
Standard	55	Ground	0.96	0.83	2.45	1.37	2.10	25.46	0.57	0.08	33.82
Standard	60	Ground	0.77	0.70	2.36	1.42	1.94	25.16	0.57	0.08	33.01
Standard	71	Ground	0.79	0.76	2.41	1.63	2.07	24.22	0.56	0.08	32.50
Standard	77	Ground	0.74	1.55	2.15	2.13	2.44	22.87	0.50	0.08	32.42
SEM			0.114	0.25	0.185	0.218	0.25	1.761	0.047	0.023	1.874
	QG		0.035	0.022	0.002	0.621	0.001	0.034	0.448	0.031	0.001
	Product Type		<0.001	0.030	<0.001	0.024	<0.001	<0.001	0.057	0.001	<0.001
	QG × Product Type		0.611	0.234	0.014	0.091	0.321	0.491	0.013	0.775	0.508
P-values	DOD		0.182	0.010	0.000	0.001	0.001	0.000	0.009	0.001	<0.001
	QG × DOD		0.130	0.335	0.004	0.065	<0.001	0.207	0.597	0.314	0.042
	Product Type × DOD		0.544	0.014	0.710	0.001	<0.001	0.469	0.172	0.214	0.740
	QG × Product Type × DOD		0.006	0.375	0.009	0.128	<0.001	0.052	0.542	0.077	0.008

Table 3-8c. The LS means of polar lipid PUFA percentages in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Fatty Acid								PUFA
			C18:2trans	C18:2 n6	C18:3 n3	C18:3 n6	C20:2	C20:3 n6	C20:4 n6	C20:5 n3	
Prime	4	Steak	0.18	17.65	0.75	0.22	0.35	2.08	7.47	1.12	29.82
Prime	25	Steak	0.21	13.71	0.53	0.16	0.35	1.36	4.04	0.62	20.98
Prime	55	Steak	0.21	13.52	0.57	0.16	0.27	1.59	5.14	0.73	22.19
Prime	60	Steak	0.19	11.43	0.50	0.14	0.28	1.24	3.97	0.61	18.37
Prime	71	Steak	0.19	14.85	0.61	0.17	0.32	1.78	5.66	0.83	24.40
Prime	77	Steak	0.17	16.92	0.68	0.18	0.33	1.94	6.15	0.84	27.22
Low Choice	4	Steak	0.14	19.72	0.79	0.22	0.41	1.94	6.67	1.07	30.94
Low Choice	25	Steak	0.17	18.07	0.64	0.18	0.38	1.55	4.67	0.77	26.43
Low Choice	55	Steak	0.16	16.51	0.68	0.21	0.40	1.59	5.36	1.00	25.91
Low Choice	60	Steak	0.17	18.99	0.74	0.20	0.34	1.82	5.92	0.89	29.08
Low Choice	71	Steak	0.18	18.84	0.74	0.22	0.37	1.90	6.07	0.93	29.26
Low Choice	77	Steak	0.14	19.64	0.77	0.22	0.39	1.97	6.62	1.00	30.75
Standard	4	Steak	0.16	18.74	0.72	0.23	0.39	1.71	5.97	0.97	28.89
Standard	25	Steak	0.15	16.82	0.69	0.24	0.35	1.53	5.76	0.97	26.52
Standard	55	Steak	0.12	20.15	0.79	0.25	0.36	1.83	6.87	1.09	31.47
Standard	60	Steak	0.12	20.98	0.76	0.24	0.39	1.84	6.53	1.00	31.87
Standard	71	Steak	0.10	21.97	0.83	0.27	0.39	1.99	7.72	1.16	34.48
Standard	77	Steak	0.11	22.24	0.83	0.25	0.35	1.97	7.46	1.07	34.30
Prime	4	Ground	0.18	12.64	0.53	0.14	0.33	1.36	4.45	0.68	20.32
Prime	25	Ground	0.22	10.31	0.45	0.11	0.25	1.10	3.47	0.52	16.44
Prime	55	Ground	0.20	11.15	0.45	0.12	0.27	1.19	3.75	0.54	17.63
Prime	60	Ground	0.24	12.15	0.49	0.15	0.30	1.30	4.01	0.60	19.22
Prime	71	Ground	0.20	13.03	0.51	0.15	0.31	1.44	4.45	0.61	20.70
Prime	77	Ground	0.29	11.33	0.52	0.21	0.28	1.42	4.19	0.56	18.74
Low Choice	4	Ground	0.15	16.70	0.67	0.18	0.38	1.62	5.62	0.88	26.21
Low Choice	25	Ground	0.20	14.06	0.58	0.17	0.32	1.35	4.40	0.69	21.59
Low Choice	55	Ground	0.17	16.37	0.69	0.22	0.29	1.55	5.45	0.83	25.60
Low Choice	60	Ground	0.19	14.20	0.56	0.16	0.32	1.35	4.27	0.65	21.76
Low Choice	71	Ground	0.21	13.15	0.60	0.16	0.30	1.35	4.67	0.69	21.11
Low Choice	77	Ground	0.19	13.68	0.58	0.15	0.24	1.50	5.19	0.71	22.26
Standard	4	Ground	0.15	16.65	0.67	0.22	0.43	1.58	5.73	0.83	24.94
Standard	25	Ground	0.16	16.15	0.63	0.21	0.34	1.39	5.35	0.83	25.05
Standard	55	Ground	0.16	13.64	0.51	0.17	0.30	1.13	3.69	0.58	20.14
Standard	60	Ground	0.17	15.85	0.58	0.20	0.34	1.30	4.66	0.68	23.78
Standard	71	Ground	0.16	16.56	0.62	0.20	0.30	1.45	5.48	0.76	25.53
Standard	77	Ground	0.16	15.31	0.64	0.19	0.25	1.55	5.93	0.83	24.80
SEM			0.018	1.557	0.05	0.217	0.039	0.141	0.483	0.088	2.138
	QG		<0.001	0.002	0.002	<0.001	0.143	0.283	<0.001	0.014	<0.001
	Product Type		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	QG × Product Type		0.352	0.354	0.119	0.081	0.089	0.685	0.079	0.222	0.249
P-values	DOD		0.086	<0.001	<0.001	0.072	<0.001	<0.001	<0.001	<0.001	<0.001
	QG × DOD		0.002	0.048	0.081	0.020	0.548	0.127	0.001	<0.001	0.011
	Product Type × DOD		<0.001	0.021	0.067	0.829	0.030	0.069	0.014	0.015	0.038
	QG × Product Type × DOD		0.038	<0.001	<0.001	0.001	0.213	<0.001	<0.001	0.001	<0.001

Table 4-1. The LS means for pH in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

QG	DOD	pH	
		Steak	Ground
Prime	4	5.63	5.69
Prime	25	5.45	5.49
Prime	55	5.62	5.6
Prime	60	5.65	5.64
Prime	71	5.62	5.73
Prime	77	5.67	5.71
Low Choice	4	5.62	5.66
Low Choice	25	5.49	5.51
Low Choice	55	5.67	5.63
Low Choice	60	5.64	5.64
Low Choice	71	5.7	5.74
Low Choice	77	5.69	5.72
Standard	4	5.63	5.68
Standard	25	5.48	5.45
Standard	55	5.66	5.65
Standard	60	5.61	5.68
Standard	71	5.74	5.69
Standard	77	5.69	5.71
SEM			0.02
	QG		0.734
	Product Type		0.001
	QG × Product Type		0.063
P-values	DOD		<0.001
	QG × DOD		0.003
	Product Type × DOD		<0.001
	QG × Product Type × DOD		<0.001

Table 4-2. Common names of amino acid abbreviations

Abbreviation	Common Name
ALA	Alanine
GLY	Glycine
VAL	Valine
BetaALA	Beta-Alanine
LEU	Leucine
ILE	Isoleucine
THR	Threonine
SER	Serine
PRO	Proline
ASN	Asparagine
ASP	Aspartic acid
MET	Methionine
HYP	Hydroxyproline
GLU	Glutamic acid
PHE	Phenylalanine
CYS	Cysteine
GLN	Glutamine
ORN	Ornithine
LYS	Lysine
HIS	Histidine
TYR	Tyrosine
TRP	Tryptophan
CYS2	Cystine

Table 4-3. Table of main effects, two-way interaction, and three-way interaction effects on free amino acid concentrations. Significance level was determined at $P \leq 0.05$

Amino Acid	Effect/Interaction						
	QG ¹	Product Type	QG × Product Type	DOD ²	QG × DOD	Product Type × DOD	QG × Product Type × DOD
Alanine							X
Glycine							X
Valine							X
Beta-Alanine							X
Leucine							X
Isoleucine							X
Threonine							X
Serine							X
Proline							X
Asparagine							X
Aspartic acid							X
Methionine							X
Hydroxyproline							X
Glutamic acid							X
Phenylalanine							X
Cysteine		X		X			
Glutamine							X
Ornithine				X	X		
Lysine							X
Histidine	X	X		X	X	X	
Tyrosine							X
Tryptophan							
Cystine	X	X		X	X	X	

¹QG = quality grade

²DOD = degree of doneness

Table 4-3a. The LS means of free amino acid concentrations ($\mu\text{mol/kg}$) in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Amino Acid										
			ALA	GLY	VAL	Beta-ALA	LEU	ILE	THR	SER	PRO	ASN	ASP
Prime	4	Steak	24.60	10.20	17.30	33.10	8.23	8.84	11.83	17.49	9.22	0.47	1.91
Prime	25	Steak	30.20	15.30	19.60	112.60	9.22	11.04	4.50	6.68	10.78	0.33	1.67
Prime	55	Steak	19.50	13.30	11.40	84.83	7.37	9.62	5.84	9.96	6.80	0.38	1.17
Prime	60	Steak	14.30	6.02	9.35	26.32	5.00	4.74	5.90	8.87	3.66	0.34	0.11
Prime	71	Steak	18.10	12.40	11.30	60.93	6.28	7.03	4.51	8.00	5.36	0.29	1.37
Prime	77	Steak	12.60	6.20	8.08	17.18	3.93	4.19	4.82	7.46	3.42	0.25	0.71
Low Choice	4	Steak	26.00	15.10	17.40	92.04	6.94	8.20	5.63	7.90	7.79	1.29	1.13
Low Choice	25	Steak	41.40	22.60	25.20	164.80	11.12	14.66	5.19	7.16	15.54	0.46	1.96
Low Choice	55	Steak	21.70	14.40	12.50	75.55	6.58	8.27	5.54	9.40	6.42	0.35	0.98
Low Choice	60	Steak	24.20	15.20	13.60	110.50	7.27	8.60	3.94	5.99	6.59	0.91	0.85
Low Choice	71	Steak	17.50	8.97	12.30	55.01	5.60	5.42	7.82	11.01	4.71	0.33	1.00
Low Choice	77	Steak	16.20	7.36	10.20	26.05	3.88	5.22	5.83	8.04	4.54	0.36	1.07
Standard	4	Steak	29.80	17.20	19.40	130.10	10.13	6.93	7.70	7.75	4.57	2.95	0.79
Standard	25	Steak	34.00	25.30	16.60	146.30	10.38	13.25	9.33	11.75	13.54	0.77	2.67
Standard	55	Steak	24.80	14.70	14.90	69.73	6.46	8.69	6.26	9.29	6.88	0.28	0.93
Standard	60	Steak	27.20	23.30	14.10	212.50	10.70	12.20	4.18	6.02	8.20	0.38	1.58
Standard	71	Steak	20.10	11.60	13.40	81.96	5.97	6.46	8.17	10.40	4.82	0.37	1.50
Standard	77	Steak	17.30	7.35	10.90	30.05	4.47	5.52	6.59	8.22	4.06	0.31	1.42
Prime	4	Ground	20.90	10.40	14.20	36.94	6.28	6.59	6.56	9.19	5.52	0.92	5.06
Prime	25	Ground	19.00	9.35	13.00	30.08	5.55	6.07	6.72	9.56	6.71	0.22	7.56
Prime	55	Ground	15.50	7.13	11.20	42.00	5.77	5.38	3.56	5.73	4.88	0.16	4.52
Prime	60	Ground	10.10	4.45	7.08	31.56	2.80	3.02	3.65	4.24	2.30	0.12	2.86
Prime	71	Ground	13.40	6.69	9.33	26.54	4.85	3.86	4.89	6.28	3.48	0.10	3.35
Prime	77	Ground	8.33	4.72	5.54	17.33	3.31	3.10	2.24	3.67	2.66	0.08	3.93
Low Choice	4	Ground	20.30	11.10	13.00	33.58	5.71	6.41	6.49	8.51	6.63	0.37	3.56
Low Choice	25	Ground	27.50	14.60	20.70	51.33	9.60	8.58	7.40	13.10	10.38	0.28	11.03
Low Choice	55	Ground	18.60	9.93	12.70	48.32	5.75	5.89	5.26	7.48	5.93	0.14	7.73
Low Choice	60	Ground	13.40	7.84	9.18	35.42	4.49	4.24	3.09	5.03	3.74	0.11	4.68
Low Choice	71	Ground	12.30	6.36	8.14	22.77	4.65	3.52	3.77	4.98	3.03	0.11	3.67
Low Choice	77	Ground	7.95	4.09	5.11	16.32	2.64	2.44	1.83	2.74	2.07	0.06	2.01
Standard	4	Ground	23.30	10.60	16.30	37.15	6.60	7.98	11.09	14.60	8.09	0.44	9.70
Standard	25	Ground	30.50	15.20	24.90	79.22	11.19	10.46	12.76	17.03	11.99	0.44	13.79
Standard	55	Ground	22.60	11.00	15.80	72.02	8.24	7.35	7.27	8.90	6.58	0.17	11.22
Standard	60	Ground	14.30	7.39	10.30	33.23	4.41	4.71	4.75	6.62	3.68	0.10	6.31
Standard	71	Ground	9.73	5.28	6.64	23.09	3.87	3.14	2.53	3.26	2.47	0.07	3.97
Standard	77	Ground	12.00	6.01	8.37	22.27	4.31	3.72	3.28	4.10	2.89	0.07	3.55
SEM			1.662	1.514	1.422	12.32	0.882	0.826	1.152	1.705	0.859	0.253	0.762
	QG		1.00E-04	<0.001	0.047	<0.001	0.039	0.05	0.074	0.47	0.041	0.123	2.00E-04
	Product Type		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.03	0.025	<0.001	1.00E-04	<0.001
	QG \times Product Type		0.241	0.004	0.138	<0.001	0.806	0.706	0.293	0.088	0.14	0.034	3.00E-04
P-values	DOD		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	QG \times DOD		<0.001	<0.001	1.00E-04	<0.001	2.00E-04	<0.001	<0.001	0.001	<0.001	0.036	0.001
	Product Type \times DOD		0.001	4.00E-04	0.002	<0.001	0.001	<0.001	<0.001	<0.001	0.004	0.021	<0.001
	QG \times Product Type \times DOD		0.004	0.005	<0.001	<0.001	0.005	2.00E-04	2.00E-04	0.001	0.001	4.00E-04	<0.001

Table 4-3b. The LS means of free amino acid concentrations ($\mu\text{mol/kg}$) in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Amino Acid											
			MET	HYP	GLU	PHE	CYS	GLN	ORN	LYS	HIS	TYR	TRP	CYS2
Prime	4	Steak	5.12	0.99	40.07	4.72	2.45	25.94	1.18	15.87	13.56	7.44	1.21	0.37
Prime	25	Steak	3.16	1.38	46.32	3.94	2.62	24.62	0.79	10.02	13.94	7.66	0.82	0.35
Prime	55	Steak	4.68	1.31	59.41	5.45	0.9	39.22	1.23	12.65	16.73	8.3	0.84	0.33
Prime	60	Steak	3.01	0.11	23.22	4.38	0.84	24.38	0.71	7.94	10.52	3.96	ND	0.3
Prime	71	Steak	3.38	0.85	30.71	2.61	0.6	25.92	1.18	8.77	6.54	5.37	0.85	0.07
Prime	77	Steak	3.51	0.14	20.42	4.28	0.48	17.72	0.59	6.88	6.02	3.4	0.68	0.08
Low Choice	4	Steak	3.62	0.97	32.57	17.66	5.37	25.01	0.99	9.59	12.23	6.52	0.99	0.23
Low Choice	25	Steak	3.07	2.02	64.51	3.88	3.2	33.66	1.32	13.74	19	9.03	1.04	0.3
Low Choice	55	Steak	3.04	1.16	51.79	3.75	0.61	56.01	1.35	9.55	15.43	6.44	0.69	0.28
Low Choice	60	Steak	2.35	1.64	36.63	9	5.24	32.45	1.49	7.35	13.31	5.58	0.63	0.09
Low Choice	71	Steak	3.96	0.25	29.58	5.11	0.71	27.91	1.09	9.48	9.65	4.47	0.74	0.16
Low Choice	77	Steak	4.64	0.18	28.17	5.39	0.58	20.69	1.06	8.54	6.65	4.17	0.73	0.08
Standard	4	Steak	2.81	0.99	23.63	42.03	5.09	32.43	0.6	8.63	12.24	6.43	0.65	0.43
Standard	25	Steak	5.74	2.32	93.22	6.63	2.49	34.44	1.04	19.08	21.37	11.45	1.2	0.66
Standard	55	Steak	2.79	1.21	45.75	2.75	1.04	44.84	1.15	10.49	22.35	6.18	0.79	0.46
Standard	60	Steak	2.29	2.95	58.35	2.5	1.16	54.54	1.86	12.46	19.8	9.53	1.01	0.25
Standard	71	Steak	4.67	0.18	35.48	6.22	0.75	39.9	0.93	10.83	10.95	5.36	0.78	0.16
Standard	77	Steak	4.37	0.24	30.28	6.72	0.73	21.64	0.68	8.44	8.64	4.31	0.68	0.09
Prime	4	Ground	4.1	0.92	25.81	14.84	1.62	19.95	0.86	12.86	11.79	5.12	0.68	0.34
Prime	25	Ground	4.07	0.11	37.98	11.88	0.91	17.07	1.07	12.81	11.78	5.29	0.8	0.28
Prime	55	Ground	4.3	0.25	37.04	9.53	0.17	20.94	0.87	10.56	10.26	4.69	0.85	0.02
Prime	60	Ground	2.57	0.24	18.25	7.74	4.01	5.63	0.84	4.84	6.04	1.98	0.54	0.03
Prime	71	Ground	3.47	0.18	22.36	15.6	0.3	7.92	1.01	6.31	7.55	3.42	0.67	0.14
Prime	77	Ground	2.23	0.42	31.8	4.44	0.26	14.01	0.87	5.89	6.21	2.97	0.67	0.07
Low Choice	4	Ground	3.95	0.16	34.19	8.1	1.09	18.15	1.02	12.52	11.99	5.22	0.62	0.25
Low Choice	25	Ground	5.8	0.6	62.59	11.95	2	41.48	1.55	19.96	17.25	8.52	1.57	0.42
Low Choice	55	Ground	5.41	0.15	38.99	13.22	0.22	21.95	1.3	11.06	11.12	4.7	0.87	0.05
Low Choice	60	Ground	2.7	0.39	36.49	4.9	0.18	19.55	1.04	8.46	11.25	3.82	0.76	0.04
Low Choice	71	Ground	2.7	0.18	24.41	11.71	0.41	14.04	0.92	6.46	6.46	3.21	0.63	0.14
Low Choice	77	Ground	1.62	0.2	22.24	2.35	0.46	13.35	0.75	4.38	4.99	2.25	0.47	0.05
Standard	4	Ground	5.76	0.15	44.93	11.82	1.46	32.19	0.96	16.71	14.33	6.61	1.01	0.28
Standard	25	Ground	8.56	0.21	58.6	20.9	1.9	39.02	1.63	24.6	22.21	9.3	1.58	0.78
Standard	55	Ground	7.04	0.47	64.65	11.81	0.25	38.56	1.87	16.13	19.29	6.74	1.18	0.06
Standard	60	Ground	3.01	0.11	37.21	5.45	0.17	19.68	1.49	9.43	10.74	3.99	0.74	0.05
Standard	71	Ground	1.4	0.54	33.4	2.25	0.49	18.25	0.87	6.08	7.18	3.19	0.57	0.11
Standard	77	Ground	2.61	0.48	36.31	5.19	0.73	14.49	1.13	6.55	7.00	3.28	0.6	0.08
SEM			0.758	0.265	6.978	3.763	1.148	4.868	0.201	1.448	1.588	0.749	0.826	0.088
		QG	0.329	0.098	0.002	0.247	0.622	<0.001	0.057	0.009	1.00E-04	0.023	0.262	0.01
		Product Type	0.34	<0.001	0.104	0.102	0.005	<0.001	0.551	0.596	3.00E-04	<0.001	0.339	0.003
		QG \times Product Type	0.211	0.065	0.675	0.037	0.128	0.967	0.074	0.082	0.984	0.571	0.567	0.415
P-values		DOD	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.133	<0.001
		QG \times DOD	0.001	1.00E-04	0.007	0.001	0.835	0.032	0.001	<0.001	<0.001	<0.001	0.301	0.011
		Product Type \times DOD	<0.001	<0.001	0.081	<0.001	0.251	<0.001	0.066	<0.001	0.005	0.092	0.413	0.001
		QG \times Product Type \times DOD	0.001	<0.001	0.001	2.00E-04	0.205	0.018	0.168	0.002	0.13	0.002	0.341	0.903

Table 4-4. Table of main effects, two-way interaction, and three-way interaction effects on total amino acid concentrations. Significance level was determined at $P \leq 0.05$

Amino Acid	Effect/Interaction						
	QG ¹	Product Type	QG × Product Type	DOD ²	QG × DOD	Product Type × DOD	QG × Product Type × DOD
Alanine							X
Glycine		X		X		X	
Valine							X
Beta-Alanine							X
Leucine	X	X		X		X	
Isoleucine	X	X		X		X	X
Threonine							X
Serine							X
Proline							X
Asparagine							X
Aspartic acid							X
Methionine							X
Hydroxyproline							X
Glutamic acid							X
Phenylalanine							X
Cysteine							X
Glutamine	X	X		X		X	
Ornithine							X
Lysine							X
Histidine							X
Tyrosine							X
Tryptophan							X
Cystine		X					

¹QG = quality grade

²DOD = degree of doneness

Table 4-4a. The LS means of total amino acid concentrations (mmol/kg) in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Amino Acid								
			ALA	GLY	VAL	Beta-ALA	LEU	ILE	PRO	ASN	ASP
Prime	4	Steak	243.18	65.52	109.19	248.86	272.17	106.29	39.05	1.10	121.67
Prime	25	Steak	527.25	379.52	165.82	622.07	308.77	152.17	51.69	0.27	267.98
Prime	55	Steak	361.35	169.74	141.41	362.65	158.89	137.65	98.52	0.19	164.13
Prime	60	Steak	161.20	25.04	109.93	172.56	311.21	99.20	30.22	1.43	74.36
Prime	71	Steak	1349.82	462.15	98.76	343.51	161.50	275.13	635.06	0.25	94.27
Prime	77	Steak	1029.91	170.93	52.45	388.64	98.06	176.49	151.47	0.28	262.09
Low Choice	4	Steak	457.97	212.64	160.53	526.33	392.06	190.83	68.29	1.49	92.35
Low Choice	25	Steak	593.78	368.07	183.78	664.67	246.99	270.59	116.40	0.19	178.46
Low Choice	55	Steak	411.72	210.25	162.17	389.04	251.87	183.02	90.87	0.49	132.01
Low Choice	60	Steak	221.55	88.59	150.01	226.87	416.05	132.82	42.66	1.91	115.02
Low Choice	71	Steak	1418.53	514.96	260.86	1332.10	476.85	437.04	318.05	0.00	731.73
Low Choice	77	Steak	657.28	221.00	184.81	155.20	245.95	107.53	192.39	0.77	166.32
Standard	4	Steak	818.72	549.87	199.87	868.15	309.69	291.45	139.45	ND	181.88
Standard	25	Steak	876.05	389.46	222.64	692.73	356.34	327.23	346.90	1.49	262.22
Standard	55	Steak	1072.31	309.42	140.58	710.00	233.92	352.83	166.39	ND	174.89
Standard	60	Steak	245.71	109.68	184.62	315.47	384.98	165.37	32.50	1.05	206.42
Standard	71	Steak	310.65	206.54	224.53	366.56	298.19	217.35	33.28	ND	324.38
Standard	77	Steak	1141.93	145.62	92.72	454.23	235.30	247.56	183.84	1.43	268.94
Prime	4	Ground	269.46	20.99	98.98	147.30	362.62	101.57	92.19	1.97	30.33
Prime	25	Ground	258.16	23.01	91.90	156.81	358.06	99.34	72.85	1.94	29.90
Prime	55	Ground	292.07	19.87	91.92	140.17	381.97	105.33	86.15	2.40	31.40
Prime	60	Ground	260.42	22.14	102.99	150.90	370.70	107.72	68.17	2.22	34.55
Prime	71	Ground	254.24	20.47	83.07	141.68	375.41	104.33	77.27	2.43	35.90
Prime	77	Ground	306.75	47.31	109.47	141.09	403.26	112.96	84.93	1.92	42.70
Low Choice	4	Ground	300.45	23.68	115.54	167.10	448.27	123.74	120.89	2.29	42.03
Low Choice	25	Ground	310.59	37.44	112.80	216.39	378.49	108.21	66.91	1.46	51.54
Low Choice	55	Ground	322.12	23.65	116.49	164.24	435.45	122.45	90.40	2.51	38.29
Low Choice	60	Ground	299.40	25.33	110.75	174.06	439.03	122.25	78.00	2.44	41.93
Low Choice	71	Ground	316.87	20.76	109.25	147.79	450.64	125.41	105.65	2.74	40.54
Low Choice	77	Ground	336.50	19.48	116.97	142.84	460.92	126.82	116.94	2.62	40.53
Standard	4	Ground	364.89	27.91	127.81	193.23	507.56	138.16	123.42	2.82	43.05
Standard	25	Ground	366.83	28.77	107.89	202.63	479.17	131.09	91.50	2.25	44.31
Standard	55	Ground	357.39	27.99	135.47	194.39	481.94	139.86	93.27	2.98	46.09
Standard	60	Ground	327.11	27.45	116.74	192.86	499.86	137.87	97.12	2.92	46.39
Standard	71	Ground	316.18	24.30	115.97	172.37	527.49	143.45	108.49	3.15	48.56
Standard	77	Ground	376.87	23.52	136.85	165.62	507.19	143.53	110.59	2.45	40.59
SEM			148.380	92.018	25.354	132.910	60.651	50.077	75.451	0.406	77.133
		QG	0.169	0.540	0.001	0.070	0.001	0.004	0.927	0.125	0.224
		Product Type	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.012	<0.001	<0.001
		QG × Product Type	0.766	0.550	0.043	0.213	0.096	0.153	0.642	0.063	0.410
P-values		DOD	<0.001	0.002	0.050	0.001	0.024	0.006	0.001	0.006	0.005
		QG × DOD	<0.001	0.096	0.090	<0.001	0.324	0.060	0.001	0.148	0.004
		Product Type × DOD	<0.001	0.002	<0.001	0.002	0.005	0.004	0.002	<0.001	0.006
		QG × Product Type × DOD	<0.001	0.107	0.036	<0.001	0.645	0.058	0.001	0.044	0.003

Table 4-4b. The LS means of total amino acid concentrations (mmol/kg) in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Amino Acid							
			HYP	GLU	PHE	GLN	ORN	LYS	HIS	TYR
Prime	4	Steak	24.26	1245.45	999.55	23.91	0.31	386.08	56.99	342.37
Prime	25	Steak	36.30	2186.38	12604.00	51.18	0.18	646.89	117.06	595.63
Prime	55	Steak	24.96	390.28	6878.72	18.35	ND	496.80	64.48	71.48
Prime	60	Steak	15.82	991.58	616.63	18.50	0.32	389.93	69.62	253.13
Prime	71	Steak	49.22	618.06	7672.71	30.58	0.26	681.09	94.01	239.17
Prime	77	Steak	49.38	538.25	4652.60	35.30	0.06	685.03	133.90	38.78
Low Choice	4	Steak	43.49	1001.03	5332.82	21.53	0.17	464.21	134.03	334.31
Low Choice	25	Steak	38.65	823.02	10160.00	46.28	0.14	976.92	99.59	195.21
Low Choice	55	Steak	39.96	633.97	5642.19	33.14	0.14	462.02	86.42	126.23
Low Choice	60	Steak	14.85	1428.36	987.80	13.44	0.54	529.21	94.84	325.99
Low Choice	71	Steak	152.56	1801.24	37639.00	59.18	ND	2049.65	410.01	18.68
Low Choice	77	Steak	25.40	1068.44	4870.22	33.48	0.32	365.46	54.94	209.21
Standard	4	Steak	59.19	1250.56	13060.00	36.92	0.96	1290.82	177.63	279.94
Standard	25	Steak	48.79	1582.24	8228.94	65.02	0.08	956.00	150.23	221.51
Standard	55	Steak	66.95	545.47	10168.00	40.57	0.66	849.90	175.09	88.28
Standard	60	Steak	19.85	2339.51	1558.53	27.45	0.97	795.48	86.44	426.81
Standard	71	Steak	26.51	3275.68	2213.21	61.66	1.62	1222.40	56.39	583.71
Standard	77	Steak	50.72	631.01	4565.90	30.10	0.22	839.11	172.05	302.17
Prime	4	Ground	10.71	290.72	590.98	4.52	0.48	189.16	56.47	181.91
Prime	25	Ground	10.84	331.97	563.92	4.70	0.17	193.99	65.40	257.64
Prime	55	Ground	14.49	320.01	677.14	12.25	0.15	158.73	58.21	278.10
Prime	60	Ground	13.21	385.71	670.94	9.49	0.11	174.39	60.77	290.35
Prime	71	Ground	15.26	362.52	723.72	14.84	0.07	183.07	66.90	292.77
Prime	77	Ground	19.30	341.27	675.52	2.94	0.35	225.87	69.95	281.41
Low Choice	4	Ground	17.75	374.75	769.44	11.92	0.75	264.83	86.08	269.60
Low Choice	25	Ground	15.57	593.24	680.22	17.04	0.34	240.84	72.64	320.22
Low Choice	55	Ground	16.94	451.39	724.61	17.90	0.22	196.41	67.50	330.57
Low Choice	60	Ground	18.54	508.52	724.88	14.10	0.33	209.71	77.15	371.70
Low Choice	71	Ground	20.03	375.91	783.85	10.11	0.31	216.56	69.43	336.15
Low Choice	77	Ground	26.32	331.92	734.81	6.72	0.40	236.13	86.21	320.88
Standard	4	Ground	18.92	390.19	863.67	19.56	0.63	273.94	88.69	353.29
Standard	25	Ground	17.42	449.25	801.02	4.35	0.09	239.13	74.58	348.98
Standard	55	Ground	20.79	506.47	869.36	16.21	0.25	230.37	87.00	395.58
Standard	60	Ground	20.64	491.43	974.01	22.93	0.38	245.78	95.96	413.29
Standard	71	Ground	21.15	480.39	1047.64	27.98	0.15	259.36	101.74	447.81
Standard	77	Ground	25.53	349.53	750.61	1.16	0.50	263.88	92.10	355.93
SEM			13.118	399.750	4429.660	10.939	0.217	183.090	33.384	94.527
	QG		0.062	0.032	0.244	0.021	0.016	<0.001	0.016	0.007
	Product Type		<0.001	<0.001	<0.001	<0.001	0.432	<0.001	<0.001	0.013
	QG × Product Type		0.405	0.121	0.245	0.584	0.027	0.012	0.251	0.203
P-values	DOD		<0.001	0.002	0.013	0.004	0.001	<0.001	0.025	0.049
	QG × DOD		<0.001	0.039	0.003	0.853	0.023	0.004	<0.001	0.009
	Product Type × DOD		<0.001	0.006	0.014	0.002	0.001	<0.001	0.044	0.020
	QG × Product Type × DOD		<0.001	0.020	0.002	0.729	0.010	0.004	<0.001	0.024

Table 4-5. The LS means of total reducing sugar concentrations (mg/g) in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Total reducing sugar	
		Steak	Ground
Prime	4	16.92	15.64
Prime	25	19.46	12.29
Prime	55	12.04	5.25
Prime	60	13.25	6.86
Prime	71	8.58	12.33
Prime	77	8.63	7.36
Low Choice	4	18.65	18.80
Low Choice	25	19.66	13.14
Low Choice	55	10.85	7.46
Low Choice	60	15.09	8.37
Low Choice	71	11.29	12.65
Low Choice	77	11.89	6.72
Standard	4	26.18	15.17
Standard	25	17.22	13.89
Standard	55	17.97	9.50
Standard	60	14.23	12.22
Standard	71	9.07	8.61
Standard	77	11.30	7.19
SEM		2.05	
	QG		0.164
	Product Type		<0.001
	QG × Product Type		0.578
P-values	DOD		<0.001
	QG × DOD		<0.001
	Product Type × DOD		<0.001
	QG × Product Type × DOD		<0.001

Table 5-1. Table of main effects, two-way interaction, and three-way interaction effects on volatile compounds. Significance level was determined at $P \leq 0.05$

Volatile Compound	Effect/Interaction						
	QG ¹	Product Type	QG × Product Type	DOD ²	QG × DOD	Product Type × DOD	QG × Product Type × DOD
Acetaldehyde	X	X	X	X		X	
2-Propanone				X			
Carbon disulfide	X	X				X	
Acetic acid, methyl ester							X
2-methyl- Propanal							X
2,3-Butanedione	X	X	X	X	X	X	
3-methylbutanal							X
2-methyl-Butanal							X
1-Penten-3-ol	X	X	X	X	X		
3-Hydroxy-2-butanone	X	X	X	X	X	X	
Butanoic acid, methyl ester	X			X	X	X	
Dimethyl-Disulfide	X	X	X	X		X	
Butanoic acid	X	X	X	X		X	
Hexanal	X	X	X	X		X	
Methyl-Pyrazine							X
2-heptanone	X	X	X			X	
Heptanal	X	X				X	
2,5-dimethyl-Pyrazine							X
Benzaldehyde	X			X			
1-Octen-3-ol		X		X		X	
Octanal	X		X		X	X	
Trimethylpyrazine							X
Benzeneacetaldehyde							X
2-ethyl-3,5-dimethylpyrazine							X
Nonanal	X	X	X	X	X	X	
Octanoic acid	X			X		X	
Decanal	X	X		X		X	

¹QG = quality grade

²DOD = degree of doneness

Table 5-1a. The LS means of volatile compound concentrations (ng/g) in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Volatile Compound												
			Acetaldehyde	2-Propanone	Carbon disulfide	Acetic acid, methyl ester	2-methyl- Propanal	2,3-Butanedione	3-methyl- butanal	2-methyl-Butanal	1-Penten-3-ol	3-Hydroxy-2-butanone	Butanoic acid, methyl ester	Dimethyl-Disulfide	Butanoic acid
Prime	4	Steak	33.42	133.47	6.23	14.17	1.2	106.28	7.76	0.54	1.61	3372.11	25.55	0.38	18.45
Prime	25	Steak	20.99	47.8	2.28	11.75	0.28	61.82	8.52	0.26	1.73	1873.85	29.47	0.23	7.62
Prime	55	Steak	12.96	17.67	1.13	2.89	4.12	37.87	17.14	16.51	1.72	1587.98	12.96	0.25	11.01
Prime	60	Steak	12.78	15.4	1.45	2.26	4.34	29.06	15.71	17.15	1.47	1152.7	3.41	0.12	10.48
Prime	71	Steak	12.36	14.1	1.59	2.65	6.2	27.99	21.72	24.77	1.04	1202.83	1.02	0.23	8.81
Prime	77	Steak	9.35	15.55	1.94	2.93	8.55	23.12	28.39	37.1	1.55	972.45	1.03	0.32	7.64
Low Choice	4	Steak	18.17	23.73	2.13	6.11	0.35	34.76	6.26	0.17	0.66	1357.53	15.39	0.23	6.38
Low Choice	25	Steak	13.33	14.15	2.18	5.33	0.19	29.14	5.31	0.16	1.12	738.07	10.38	0.12	2.91
Low Choice	55	Steak	7.22	6.85	0.72	1.16	1.83	16.37	6.62	5.61	0.84	686.57	3.47	0.09	3.62
Low Choice	60	Steak	7.78	6.13	1.01	1.25	2.75	16.15	10.08	10.86	0.69	519.58	2.62	0.08	3.67
Low Choice	71	Steak	8.07	8.71	1.49	1.51	3.69	15.17	11.81	14.29	0.89	618.87	1.77	0.09	3.1
Low Choice	77	Steak	6.97	9.49	1.51	1.71	5.37	14.25	16.92	22.3	1.08	560.71	0.46	0.18	2.92
Standard	4	Steak	8.93	15.43	1.68	4.03	0.19	16.31	4.29	0.1	0.69	426.71	7.32	0.09	3.37
Standard	25	Steak	12	11.98	1.7	5.31	0.15	18.25	4.16	0.12	0.87	622.44	8.31	0.1	2.72
Standard	55	Steak	6.47	6.96	0.78	1.01	1.03	11.91	4.35	2.29	0.67	452.53	3.27	0.05	2.74
Standard	60	Steak	6.58	7.38	0.61	0.87	1.2	12.63	5.12	3.29	0.39	489.62	2.15	0.05	3.09
Standard	71	Steak	5.72	6.44	0.64	0.7	1.47	10.56	5.37	4.57	0.6	435.82	0.41	0.03	2.69
Standard	77	Steak	5.62	8.75	0.74	0.91	2.64	10.9	8.06	9.32	0.74	432.79	0.37	0.1	2.13
Prime	4	Ground	6.24	23.63	0.23	6.48	0.26	29.24	2.5	0.03	0.55	834.82	16.33	0.12	4.04
Prime	25	Ground	6.26	30.2	0.4	7.74	0.45	29.38	2.37	ND	0.73	973.67	20.85	0.08	3.34
Prime	55	Ground	9.51	15.92	1.2	2.95	1.97	25.98	8.64	4.8	0.41	907.25	13.81	0.19	5.16
Prime	60	Ground	5.47	13.18	0.69	2.68	1.02	20.03	4.68	1.98	0.25	609.9	10.72	0.14	3.64
Prime	71	Ground	8.65	20.82	1.39	5.2	3.42	22.17	8.42	5.66	0.27	534.24	5.54	0.26	3.9
Prime	77	Ground	5.5	11.68	0.97	1.8	1.74	17.35	5.33	3.03	0.18	459.03	4.11	0.18	2.86
Low Choice	4	Ground	5.31	13.72	0.21	3.74	0.39	14.85	1.78	0.1	0.31	371.1	7.21	0.09	1.38
Low Choice	25	Ground	4.92	15.29	0.23	4.85	0.21	18.2	2.28	ND	0.44	451.11	8.83	0.09	1.5
Low Choice	55	Ground	7.41	12.48	1.34	1.93	2.24	21	7.52	4.67	0.55	774.97	6.03	0.14	4.08
Low Choice	60	Ground	5.24	11.08	0.79	1.88	1.7	17.45	5.73	3.07	0.42	506.4	5.75	0.16	2.82
Low Choice	71	Ground	5.47	9.96	2.36	1.6	2.23	16.21	6.48	4.68	0.55	491.6	1.64	0.12	3.25
Low Choice	77	Ground	6.13	13.13	1.26	1.74	2.67	16.18	5.56	3.91	0.51	424.91	1.36	0.23	1.78
Standard	4	Ground	2.23	9.3	0.09	3.64	0.07	7.04	0.95	0.02	0.1	166.72	4.78	0.04	1.12
Standard	25	Ground	1.94	10.17	0.13	3.49	0.1	5.79	1.2	0.03	0.13	142.42	5.57	0.04	1.03
Standard	55	Ground	4.45	8.33	0.69	1.17	1.19	10.21	4.83	2.6	0.11	336.83	3.64	0.06	4.17
Standard	60	Ground	2.84	6.08	0.49	1.08	0.89	8.09	3.66	1.79	0.07	226.36	3.57	0.06	1.9
Standard	71	Ground	4	11.03	1.22	1.43	1.9	10.49	4.34	2.94	0.09	273.49	0.87	0.07	2.35
Standard	77	Ground	2.5	6.77	0.5	1.42	1.22	7.23	3.42	2.47	0.08	194.67	0.6	0.07	1.42
SEM			2.589	18.305	0.899	1.022	0.568	9.332	1.46	1.987	0.137	306.31	2.104	0.043	1.546
	QG		<0.001	0.087	0.047	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	2.00E-04	<0.001	<0.001	<0.001
	Product Type		<0.001	0.279	0.003	0.062	<0.001	0.004	<0.001	<0.001	<0.001	0.001	0.395	0.009	<0.001
	QG × Product Type		0.015	0.297	0.159	0.134	<0.001	0.045	<0.001	<0.001	<0.001	0.047	0.988	0.003	<0.001
P-values	DOD		<0.001	0.016	0.374	<0.001	<0.001	<0.001	<0.001	<0.001	4.00E-04	4.00E-04	<0.001	0.003	0.002
	QG × DOD		0.214	0.131	0.688	<0.001	4.00E-04	0.002	<0.001	<0.001	0.016	0.005	<0.001	0.2	0.175
	Product Type × DOD		<0.001	0.084	0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.162	0.002	<0.001	<0.001	0.002
	QG × Product Type × DOD		0.268	0.312	0.742	0.03	0.013	0.159	0.001	<0.001	0.499	0.213	0.057	0.548	0.538

Table 5-1b. The LS means of volatile compound concentrations (ng/g) in beef strip steaks and ground patties from three different quality grades (QG) and six degrees of doneness (DOD)

Quality Grade	DOD	Product Type	Volatile Compound											
			2-heptanone	Heptanal	2,5-dimethyl-Pyrazine	Benzaldehyde	1-Octen-3-ol	Octanal	Trimethylpyrazine	Benzeneacetaldehyde	2-ethyl-3,5-dimethyl-pyrazine	Nonanal	Octanoic acid	Decanal
Prime	4	Steak	0.3	3.49	0.01	2.04	0.56	4.01	0	0.19	0.02	6.99	0.58	0.53
Prime	25	Steak	0.13	1.81	6.22E-15	0.71	0.11	2.12	1.07E-14	0.08	2.22E-15	3.82	0.21	0.43
Prime	55	Steak	0.18	3.95	2.89	3.11	0.23	5.34	1.11	0.72	0.96	3.54	0.3	0.6
Prime	60	Steak	0.12	0.81	2.91	2.83	0.06	1.28	1.08	0.75	1	1.22	0.17	0.84
Prime	71	Steak	0.23	1.34	5.24	4.56	0.14	1.6	2.4	1.11	1.84	1.72	0.19	0.74
Prime	77	Steak	0.15	1.23	6.99	4.38	0.06	1.84	3.76	1.55	2.84	1.84	0.2	0.77
Low Choice	4	Steak	0.08	1.43	ND	0.84	0.2	1.28	ND	0.07	ND	1.84	0.21	0.22
Low Choice	25	Steak	0.05	0.71	0	0.53	0.1	1.02	0	0.05	0	1.24	0.05	0.12
Low Choice	55	Steak	0.04	0.83	0.7	2.14	0.08	1.37	0.28	0.23	0.35	1.37	0.11	0.46
Low Choice	60	Steak	0.09	0.74	1.88	2.63	0.06	1.24	0.91	0.45	0.96	1.23	0.1	0.73
Low Choice	71	Steak	0.08	0.65	2.7	3.07	0.07	1	1.24	0.47	1.21	1.02	0.09	0.54
Low Choice	77	Steak	0.07	0.89	3.15	3.96	0.07	1.26	1.61	0.76	1.51	1.26	0.11	0.56
Standard	4	Steak	0.06	1.07	2.44E-15	0.54	0.24	1.19	4.33E-15	0.03	ND	1.9	0.12	0.12
Standard	25	Steak	0.07	0.64	ND	0.46	0.12	0.91	ND	0.04	9.99E-16	1.21	0.06	0.29
Standard	55	Steak	0.04	0.7	0.25	1.9	0.12	1.1	0.1	0.12	0.15	1.31	0.08	0.47
Standard	60	Steak	0.07	0.76	0.24	1.89	0.13	1.07	0.12	0.16	0.19	1.22	0.07	0.56
Standard	71	Steak	0.06	0.73	0.45	2.3	0.09	1.12	0.22	0.22	0.27	1.23	0.07	0.54
Standard	77	Steak	0.06	0.92	0.96	2.94	0.08	1.14	0.49	0.28	0.51	1.24	0.08	0.55
Prime	4	Ground	0.19	2.63	0.01	1.12	1.7	1.94	0.01	0.13	0.05	2.83	0.27	1.29
Prime	25	Ground	0.24	2.37	0	1.57	2.26	1.99	ND	0.17	0.06	3.35	0.35	1.47
Prime	55	Ground	0.24	2.6	0.7	2.32	1.45	2	0.2	0.4	0.21	2.09	0.22	0.78
Prime	60	Ground	0.16	1.67	0.42	1.65	1.05	1.79	0.14	0.38	0.16	1.85	0.15	1.57
Prime	71	Ground	0.26	3.81	0.71	6.13	1.5	2.79	0.27	0.83	0.32	3.08	0.24	1.04
Prime	77	Ground	0.18	2.28	0.36	2.84	0.92	2.21	0.13	0.55	0.17	2.39	0.14	1.53
Low Choice	4	Ground	0.12	2.47	0	2.06	1.3	2.05	ND	0.12	0.03	2.65	0.14	0.84
Low Choice	25	Ground	0.16	2.16	ND	1.16	1.84	1.7	ND	0.1	0.02	2.82	0.14	0.89
Low Choice	55	Ground	0.23	2.21	0.43	2.81	1.48	1.68	0.15	0.29	0.16	1.52	0.16	0.64
Low Choice	60	Ground	0.19	1.99	0.3	2.65	1.1	1.7	0.12	0.33	0.16	1.61	0.12	0.78
Low Choice	71	Ground	0.25	2.86	0.61	3.47	1.41	2.52	0.25	0.49	0.29	2.78	0.16	1.18
Low Choice	77	Ground	0.21	3.35	0.38	5.76	1.33	2.56	0.15	0.45	0.19	2.9	0.13	1.27
Standard	4	Ground	0.07	0.74	0	0.49	0.8	1.36	ND	0.05	0.03	1.74	0.11	1.01
Standard	25	Ground	0.08	0.82	ND	0.66	0.78	1.35	6.27E-15	0.06	0.02	2.19	0.12	0.83
Standard	55	Ground	0.12	1.29	0.34	1.97	0.77	1.32	0.12	0.19	0.19	1.8	0.13	0.62
Standard	60	Ground	0.12	1.45	0.27	1.68	0.73	1.54	0.11	0.2	0.16	2.25	0.09	0.99
Standard	71	Ground	0.16	2.39	0.21	3.76	0.97	2.22	0.09	0.29	0.13	4.27	0.16	0.9
Standard	77	Ground	0.15	2.23	0.24	2.59	0.94	2.37	0.11	0.23	0.17	3.75	0.13	1.2
SEM			0.042	0.637	0.407	0.798	0.242	0.571	0.223	0.093	0.163	0.82	0.06	0.169
	QG		<0.001	0.001	<0.001	0.007	0.058	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	0.001
	Product Type		<0.001	2.00E-04	<0.001	0.355	<0.001	0.086	<0.001	0.002	<0.001	0.021	0.68	<0.001
	QG × Product Type		0.035	0.076	<0.001	0.144	0.068	0.001	<0.001	3.00E-04	<0.001	0.005	0.136	0.556
	DOD		0.143	0.07	<0.001	<0.001	0.007	0.113	<0.001	<0.001	<0.001	0.021	0.002	<0.001
	QG × DOD		0.165	0.056	<0.001	0.144	0.153	0.039	<0.001	<0.001	<0.001	0.022	0.051	0.621
	Product Type × DOD		0.029	0.006	<0.001	0.442	0.029	0.001	<0.001	<0.001	<0.001	0.002	0.007	0.001
	QG × Product Type × DOD		0.743	0.779	<0.001	0.669	0.432	0.158	<0.001	0.004	<0.001	0.57	0.599	0.461