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Effects of chilling duration on USDA quality grade of beef carcasses

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Two hundred and nine beef carcasses (361 ± 53 kg) from crossbred, grain-finished cattle harvested in a commercial packing plant and evaluated for marbling score, core temperature (n=1), pH, shrinkage, color, and aerobic plate count (n=50) for 24, 48, 72, and 96 h under spray chilling. There were minimal changes in shrinkage among time points (-0.4 to 1.2%; $P \leq 0.002$), pH (5.56 to 5.69; $P \leq 0.001$), and APC (0.1 to 0.7 log; $P < 0.001$). Marbling score values were converted to numeric values of 200 (Practically Devoid^{00}) to 1100 (Abundant^{00}). Carcasses with SM or greater marbling score at 24 h had a 34 to 60 points deduction after 96 h of spray chilling ($P \leq 0.042$), the SL carcasses had an increase marbling score, from 442 to 469 points. Moreover, SL carcasses had a greater percentage of PUFA ($P < 0.001$).

DEDICATION

This thesis is dedicated to my wonderful and encouraging family, friends and colleagues who have supported me throughout my life, career, and education. My parents Mr. L. Haines and Mrs. A Haines receive my heartfelt appreciation for always pushing me to achieve my goals and sticking by me when times were tough.

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CHAPTER I

LITERATURE REVIEW

Introduction

Consumer interest in beef carcass quality has existed from medieval times when providing food was essential for a family or community to today's inspection and grading systems focusing on food safety and quality. The development of the United States Department of Agriculture, the Food Safety and Inspection Service, and the Agriculture Marketing Service were keys in the regulation of meat safety and quality for both domestic and foreign trade, improvement of carcass characteristics, and market regulation. These entities paved the way for the current quality grading system with Prime, Choice, Select, and Standard grades and marbling score categories of Abundant, Moderately Abundant, Slightly Abundant, Moderate, Modest, Small, Slight, Traces, and Practically Devoid (Hale, Goodson, and Savell, 2013). Between 2005 and 2015, the percentage of quality grades doubled for Prime and Choice while Select and Standard carcass percentages declined (Boykin et al., 2016), which was needed to boost consumer acceptance of beef products. Consumers seek products of high quality that are priced accordingly (Claborn et al., 2011). If guaranteed to be more tender, flavorful, or juicy, beef consumers will spend more for a better eating experience (National Beef Quality Audit, 2015). Therefore, greater quality grades bring more financial incentives for beef packers. The USDA quality grade in beef carcasses can be improved through antemortem factors such as breeding and diet. However, postmortem processing has been used to improved marbling visibility to provide the

USDA graders with a better assessment of the degree of marbling. In the last 15 years, beef quality and yield grades have been improved along with quality attributes such as tenderness, flavor, and juiciness (Boykin et al., 2016; Hunt et al., 2014). Various breeding and finishing regimes as well as postmortem electrical stimulation have accentuated favorable carcass and quality traits (Savell, Smith, & Carpenter, 1978; Owens & Gardner, 2000; Jeong et al., 2011; Dias et al., 2019). Although professionals in the beef industry have observed that carcasses processed on Friday of the week may be graded better because of additional chilling time, such a phenomenon has not been researched as much as other factors. It is unclear how much prolonged chilling increases marbling visibility, thereby marbling score. Therefore, there is a need to determine whether the chilling duration of greater than 24 h improves carcass marbling score and how prolonged chilling affect carcass quality. Consumer interest in beef carcass quality has existed from medieval times when providing food was essential for a family or community to today's inspection and grading systems focusing on food safety and quality. The development of the United States Department of Agriculture, the Food Safety and Inspection Service, and the Agriculture Marketing Service were keys in the regulation of meat safety and quality for both domestic and foreign trade, improvement of carcass characteristics, and market regulation. These entities paved the way for the current quality grading system with Prime, Choice, Select, and Standard grades and marbling score categories of Abundant, Moderately Abundant, Slightly Abundant, Moderate, Modest, Small, Slight, Traces, and Practically Devoid (Hale, Goodson, and Savell, 2013). Between 2005 and 2015, the percentage of quality grades doubled for Prime and Choice while Select and Standard carcass percentages declined (Boykin et al., 2016), which was needed to boost consumer acceptance of beef products. Consumers seek products of high quality that are priced accordingly (Claborn et al., 2011). If guaranteed to be more tender, flavorful, or juicy, beef consumers will spend more for a better eating experience (National Beef Quality Audit, 2015). Therefore, greater quality grades bring more financial incentives for beef packers. The USDA quality grade in beef carcasses can be improved through antemortem factors such as breeding and diet. However, postmortem processing has been used to improved marbling visibility to provide the USDA graders with a better assessment of the degree of marbling. In the last 15 years, beef quality and yield grades have been improved along with quality attributes such as tenderness, flavor, and juiciness (Boykin et al., 2016; Hunt et al., 2014). Various breeding and finishing regimes as well as postmortem electrical stimulation have accentuated favorable carcass and quality traits (Savell, Smith, & Carpenter, 1978; Owens & Gardner, 2000; Jeong et al., 2011; Dias et al., 2019). Although professionals in the beef industry have observed that carcasses processed on Friday of the week may be graded better because of additional chilling time, such a phenomenon has not been researched as much as other factors. It is unclear how much prolonged chilling increases marbling visibility, thereby marbling score. Therefore, there is a need to determine whether the chilling duration of greater than 24 h improves carcass marbling score and how prolonged chilling affect carcass qua

History of Beef Quality Grading in the United States

Original Needs for a Beef Grading System

The beef grading system was developed to identify the two most important attributes: the quality of the product and the yield of lean meat from the carcass (Smith et al., 1987; Smith et al., 1984; Smith et al., 1982; Davis et al., 1979; Murphey, Hallet, Tyler, & Pierce, 1960). Beef cattle were introduced into North America by the Spanish explorers into parts of Florida, the lower

Mississippi Valley, and the Southwest in the 16th century (Kiehl & Rhodes, 1960). In addition, European colonists brought cattle from their countries to the settlements on the Atlantic Coast in the early 17th century. The main function of cattle during this time was farm work. The importance of cattle for meat production and carcass quality did not emerge until after the Revolutionary War (Kiehl & Rhodes, 1960). Most trading during this time was conducted at a local level and was heavily immersed in politics. Most organized markets during this period were unimportant and unregulated (Farnum, 1938). The need to raise and grow cattle to feed more of the population instead of single families prompted the rise of commercial cattle raising and opened up the corn growing area to the West, the rapid growth of the eastern coastline, the introduction of forage production like clover and gypsum after 1785, the start of county fairs and cattle shows in the early 1800s, and the expansion of an agricultural press in 1810 (Leavitt, 1933; US Census Bureau, 1864). This agricultural and industrial expansion inspired consumer efforts to establish regulations for beef quality that had been futile during the 18th century when the prevalence of unfair prices and unwholesome meat flooded the market. During this time, consumers needed a language that was consistent across all beef types; however, grading standards were rudimentary compared to the current system (Sheets, 1934). The early standardization of beef grading lends itself to the expansion of markets, economies, and societies. In the 1920s, consumers were influential in the establishment of a grading system. However, because the successful promotion of beef depends upon consumer acceptance, promoters were interested in establishing grades with a greater impact on consumer demand (Kiehl & Rhodes, 1960). Kiehl & Rhodes (1960) described that an adaptation toward consumer approval could be seen in the decrease in carcass weights and age since the early 20th century. At the International Livestock show in 1900, the average steer weight was 1,600 pounds which continuously decreased over the next 5 years into the 20th century. This trend for decreasing the weight of beef carcasses was prompted by consumers' demand for smaller portioned cuts due to household sizes, eating preferences, and affordability (Drouillard, 2014). Hiner and Hankins (1950) and Field, Nelms, and Schoonoer (1966) observed that as the age of the animal increased tenderness, flavor, and juiciness decreased. These early studies are consistent with research from Shorthose & Harris, (1990), Shackelford, Koohmaraie, & Wheeler, 1995, Lucero-Borja et al., 2014, and Moholisa, Hugo, Strydom, & van Heerden, 2017. The in-depth study of beef quality was established over the decades to provide consumers with a wholesome and consistent product.

Significant Events Shaping the Current Grading System in the United States

Classifying and grading cattle have been established since the early 17th century. In 1657, inspectors were appointed in New York to certify and inspect all meat for exportation. Moving forward to the 1800s, the expansion of railroads improved livestock transportation and the invention of refrigeration helped the meatpacking industry expand to year-round business (USDA, 2012). Export restrictions of US livestock became more stringent in foreign countries. A series of articles were written in the European countries in the late 19th century against the importation of American chilled beef and live animals (Clemen, 1924). Europe suspected that American products were being packed under unsanitary conditions and diseased and deceased animals were exported and sold into their markets. These actions were also stimulated by fears of competition from cheaper American livestock and meats, which had begun to be produced in large volume after 1875 (Clemen, 1924). Producers and packers in the U.S. implored the government to employ an inspection program that would allow them to compete in foreign markets. In the late 1800s, President Benjamin Harrison endorsed the first law which later became the Federal Meat Inspection Act of 1906 (USDA, 2018). The law required that the United States Department of Agriculture USDA, through the Bureau of Animal Industry, inspect pork products for exportation and was later amended to include live cattle and beef. Another justification for the implementation of meat inspection was the publication of Upton Sinclair's *The Jungle*. The book aimed at the working conditions in Chicago packing houses. However, even in the early 20th century, Aston (1948) reported that almost 50% of the meat consumed in America was uninspected. Improvements to meat inspection continued with the creation of agricultural research units, the passage of the Humane Slaughter Act of 1958 and the Humane Methods of Slaughter Act of 1978, and the creation of the Food Safety and Inspection System (FSIS; USDA, 2018). During the establishment of both safety and quality standards, the need for accurate market reporting arose because of the lack of uniformity in the grading system and the circulation of private market sheets.

Between 1901 and 1910, the University of Illinois initiated work to study market and grade classifications of livestock through the Union Stockyards and slaughtering plants in the Chicago area (Kiehl and Rhodes, 1960). Herbert Mumford in 1902 from the University of Illinois authored a series of bulletins titled “Market Classes and Grades of Cattle with Suggestions for Interpreting Market Quotations” (Cross, Harris, & Savell, 1996). These articles describe the need for established classes and grades for beef cattle that consumers and producers would understand and relate grades to the market and feedlot requirements. These also prompted Congress to allocate funding so that the USDA could study agricultural marketing and establish the Office of

Markets and Rural Organization. As a result of this study, Congress passed a law in 1916 establishing the National Livestock Market News Service. Cross, Harris, & Savell (1996) report that grading classes and nomenclature began to develop in 1916 with mimeographed versions being published in 1923 and 1924 and congress passing the United States Agricultural Products Inspection and Grading Act which authorized the federal grading of livestock and meat in 1927. During this time, it was also reported that Swift and Company began experimenting with a grading system in 1922, which was permanently implemented in 1923 (Cross, Harris, & Savell, 1996). Their grade classifications were a number sequence rather than descriptive nomenclature used today. The USDA (2018) reported that throughout the late 1920's that the Official United States Standards for Classes and Grades of Slaughter Cattle (1928) and Official United States Standards for Veal and Calf (1928) were implemented. Swine and ovine standards appeared later. The carcass grades adopted in 1926 were implemented as a free, voluntary service for a trial period despite the packers' contention that it was an unworkable program. During this trial period, the Institute of American Meat Packers (IAMP) published a "Standard Beef Grading System and Packers Guide for Grading as Developed by the Committee on Marketing (Beef) and Approved by the Executive Committee". The National Inquiry in 1935 stated that the Institute of American Meat Packers recommended a set of beef grading standards around 1930 but revised it 5 years later in 1935. The modest success of the initial trial period in 1926 efforts by the IAMP instigated the development of the beef grading system into its current form (Harris, Cross, & Savell, 1996). In 1939, the Agricultural Appropriations Act transferred the grading service from the Bureau of Agricultural Economics to the Agricultural Marketing Service (AMS) of the USDA (USDA, 2018). Their main focuses were on packers and how to standardize beef carcasses and improve the quality within a growing domestic market. Over time beef quality regulations and standards have developed from rather simple colonial standards to those of the present, having national significance and application.

Historical Development of Grading Standards

The development of grading standards is a phenomenon associated with the growth of organized markets, commerce, and urbanization of society (Keihl & Rhodes. 1960). The authors stated that quantity (yield) standards were emphasized in the beginning, but these standards also helped provide a minimum set of quality standards. Organized markets were nonexistent before the 18th century even with numerous laws trying to regulate the markets (Winter, 1939). In early 1900 only a small fraction of beef was being graded; however, within a decade that number had increased by 8-fold, indicating a steady growth of the service and by 1970, 65% of commercial beef was being graded in packing facilities. The grading of all dressed beef was divided into four classes: steers, heifers, bulls, and cows. Each class was further divided into segments such as conformation, quality, and finish. The grades were designed as a numerical system 1 through 9, with lower numbers indicating higher grades. Dowell and Bjorka (1941) reported that even with the success of this system, some major packers objected to establishing the first tentative U.S. standards of Prime, Choice, Good, Commercial, Utility, Cutter, and Canner for beef carcasses. The packers believed that beef could not be successfully sorted into as many as seven grades. Additional changes to the grading system were made between 1949 and 1965 to include lowering quality requirements by one grade so that Choice was moved into Prime, Good was moved into Choice and the top half of Commercial moved into Good and the other half was renamed Standard (Harris, Cross, & Savell, 1996). In 1962, one of the most significant changes in beef grading was the addition of cutability grades (Murphey, Hallet, Tyler, & Pierce, 1960) to create a dual grading system with cutability or yield grading and quality grading. At the origination, it was conducted on a trial basis and permanently adopted in 1965. The beef quality grades were also amended to place less importance on maturity in Prime, Choice, Good and Standard grades and to specify that all carcasses be ribbed before grading. Texas A&M University reported multiple changes in the standards for beef carcass grades in the late 19th century (Smith et al., 1987; Smith et al., 1984; Smith et al., 1982; Davis et al., 1979). Several changes did not go into effect until 1976, including maturity used to determine bullock, steer, heifer, and cow quality grades in maturity group A, an increase in marbling requirements for the Good grade, maximum maturity for steer, heifer, and cow beef in the Good, Standard, and Prime grades, and the elimination of carcass conformation. Dual grading is now applied for all graded beef. In 1987, the Good grade was changed to Select. The National Consumer Retail Beef Study found that this change was a better fit for consumer attitudes and perceptions (Savell et al., 1987, 1989). In April of 1989, yield grades and quality grades were separated so that all graded carcasses did not have to be graded for both yield and quality (Savell et al., 1989). In 1996, B-maturity carcasses with Slight or Small marbling must be graded as Standard. Grading standards have remained unchanged since the late 1990s.

USDA Quality Grades and Their Association with Sensory Attributes and Market Pricing

Overview

Beef carcass quality has progressively improved since the mid-2000s (NBQA, 2016). In the 2005 National Beef Quality Audit (NBQA), inadequate marbling was recognized as the greatest quality challenge by purveyors, retailers, and restaurateurs (Smith et al., 2006). Also, a shift away from trading of live cattle and movement toward the use of carcass-based transactions involving carcass quality premiums and discounts has strengthened quality-based market and the need for the production of cattle with higher grades (Tatum, 2015). Results of the cooler audit of the 2005 NBQA documented fed beef carcasses quality grade percentages of Select, Low Choice, High Choice, and Prime were approximately 40.1%, 33.7%, 18.2%, and 2.6%, respectively (Garcia et al., 2008). In 2015, the USDA showed improved quality grades for Select, Low Choice, High Choice, and Prime have averaged 20.6%, 42.3%, 27.0%, and 4.9%, respectively (Tatum, 2014). This improvement in grade distribution reflects the progress the industry has made in a decade. The 2016 NBQA discovered an increase in the rate of recurrence of Prime and Choice and a decrease in the frequency of Select. A reason for this was an increase in dairy-type carcasses of about 32%, with 8% grading USDA Prime. In NBQA 2016 vs. NBQA 2011, there was a 6% increase in dairy-type quality grading versus a 5.4% decrease in feedlot cattle quality grading. This increase in quality grade yielded the lowest lost opportunity margin since 1991. Steiner (2014) estimated that, between 2005 and 2013, added value through quality premiums to the beef industry was totaled nearly $4.5 billion. Graded beef pricing structure organizes carcasses and beef cuts of 30-month and younger steers and heifers into five marketing categories based on the amount of marbling and correlated differences in expected eating experience (Tatum, 2011). Beef carcasses with greater USDA quality grades produce a better eating experience for beef consumers (O'Quinn et al, 2012). The major function of USDA beef quality grades is to segment carcasses into groups that will produce cooked beef cuts with similar palatability attributes. A greater amount of intramuscular fat or marbling has been reported to increase consumer acceptance (Wheeler, Shackelford, & Koohmaraie, 1999). However, Jeremiah et al. (1970) reviewed early literature and found that USDA beef quality grades of Prime Choice, Good, and Standard were relatively inaccurate indicators of tenderness, flavor, and juiciness of cooked beef. Smith and Carpenter (1974) agreed with Jeremiah et al. by stating that quality grade had a low relationship to flavor and low to moderate relationships to juiciness and tenderness of cooked beef. Garcia-de-Siles et al. (1977) and Mata-Hernandez et al. (1981) compared steaks from Moderate (MD), Modest (MT), Small (SM), Slight (SL), and Traces (TR) marbling scores and found no differences in palatability after cooking. In recent years, Corbin et al. (2015) revealed that as the marbling level increased, so did the consumer acceptance of most sensory attributes and the descriptive panel scores of beef tenderness and flavor intensity. These authors selected beef strip loins from each USDA quality grade and different breeds of American Wagyu, Australian Wagyu, grass-fed, and Holstein in a predetermined, balanced order that are available to U.S. consumers. The authors observed that consumers favored higher fat content steaks due to greater tenderness, juiciness, and flavor. Trained panelists in the study showed a positive response to fat-like flavor for samples with greater marbling. Overall liking was also positively correlated with flavor and fat percentages. These findings agreed with what was reported by Lorenzen et al. (2003). These authors observed trained descriptive panelists and home consumers assessment overall tenderness, juiciness, flavor, and overall cooked tenderness, juiciness, and flavor. Steaks represented USDA Choice and Select quality grades and four cooking temperatures (60, 65, 70, and 75°C). Hunt et al. (2014) measured consumer acceptance of overall palatability and overall liking of USDA Upper 2/3 Choice and Select of *longissimus lumborum*, *gluteus medius*, *serratus ventralis*, and *semimembranosus*. Consumer observations show that both quality grade and muscle type influenced juiciness, flavor, and tenderness. The authors found that consumers liked beef with higher grades because of better tenderness (r = 0.75), juiciness (r = 0. 68), and better flavor (r = 0.86). Although there was early contradictory data because the USDA grading system was being developed and altered to better match consumer acceptance, in the past 30 years, numerous studies have found that greater quality grades and marbling scores increase overall palatability and consumer acceptance. The quality and value of beef have increased over time due to all the efforts of the industry to provide a premium product to consumers.

USDA quality grade and tenderness

Even though quality grades were initially established to predict tenderness, quality grades are an unreliable tool for tenderness prediction (USDA, 1997), especially in today's beef market because various postmortem processing technologies, like electrical stimulation and aging, have been developed to improve beef tenderness. Tenderness is the most important factor influencing consumer satisfaction, especially at the early development of the U.S. beef markets (Dikeman, 1987; Savell et al., 1987, 1989; Smith et al., 1987; Miller et al., 1995). In the 1987 National Consumer Retail Beef Study tenderness was not strongly demonstrated as an influence in affect palatability and consumer acceptance like it is today (Gonzalez and Phelps, 2018). Tenderness can vary even if antemortem factors such as age, sex, nutrition, stress, and postmortem factors such as chilling, aging, and pH are controlled (Field et al, 1970; Fergusen et al., 2001; Lawrie and Ledward, 2006). Huffman (1979) observed the difference in tenderness between a tenderometer, a non-destructive probe with ten 1/2-inch diameter needles used to press manually two inches into the ribeye surface after chilling (Hanson 1972), and Warner Bratzler shear force (WBSF) among USDA Prime, Choice, Good and Standard carcasses with significant differences being noted among Prime, Choice and Good grades and similar according to the taste panels and the WBSF; whereas Standard carcasses were less tender. The USDA Standard carcasses were less tender and their tenderness was most variable according to the taste panels and the WBSF data. However, the WBSF was not correlated with the USDA grades and marbling scores ($r = -0.14$, $P < 0.05$). Smith et al. (1984) observed marbling scores from practically devoid to abundant that as scores increased, 66% of loin steaks and 12.5% of round steaks were more palatable and had lower WBSF values. Carcasses with Moderately Abundant marbling produced loin, top round, bottom round, and eye of round steaks with better sensory panel ratings and less WBSF values than carcasses with lower marbling scores. A national beef tenderness survey by Morgan et al. (1991) observed WBSF values were small (3.05 to 4.15 kg) among beef chucks of various quality grades compared to round cuts (3.53 to 4.35 kg) and similar compared to rib and loin cuts (2.61 to 3.56). USDA Select and no-roll (below Select) grades had 10% more cuts measured at 4.0 kg or greater of WBSF than the USDA Choice. These authors found that USDA quality grade failed to regulate the variation in panel ratings or WBSF to guarantee tenderness consistency to the consumers. Although chemical, physical, and histological traits of the *longissimus* muscle influence physical attributes of beef carcasses and can be used to separate the USDA quality grade (Culler, Smith, & Cross, 1978; Dikeman et al., 1976; Joo, Kim, Hwang, & Ryu, 2013), consumers' ability to detect varying tenderness levels is vital for establishing the quality standards that place values on beef tenderness (Miller et al. 2001). Martinez et al. (2017) reported WBSF (reported in Newton (N)) and consumer sensory panel (1 = dislikes extremely; 10 = likes extremely) values for Prime, Top Choice, Low Choice, and Select steaks (top blade, ribeye, top loin, and top sirloin) from food service or retail establishments. These authors found that Prime steaks had lower WBSF (24.6 N) compared to other categories (28.5 to 30.3 N). Consumers identified tenderness level and overall tenderness differences ($P < 0.05$) between USDA quality grades with Prime being the most tender (7.2) compared to other categories (6.2 to 6.8). O'Quinn et al. (2018) findings agree with the above study. These authors evaluated

Prime, High Choice, Low Choice, Select, and Standards striploin steaks for consumer acceptance of overall tenderness, and found that Prime (95%; $P < 0.05$) steaks had a higher percentage of consumer acceptability compared to Choice, Select, and Standard (86%, 77%, and 74%, respectively). Miller et al. (2001) evaluated USDA Select strip loin steaks of known WBSF values, ranging from tough (> 5.7 kg) to tender (< 3.0 kg) for consumer acceptance based on monetary value. The authors divided beef consumption rates into light beef consumption (0 to 8 meals biweekly; 52%), 6% moderate beef consumption (9 to 12 meals/biweekly); and 41% heavy beef consumption (greater than 12 meals/biweekly) and found that consumers were able to differentiate strip steaks with various WBSF categories as well as their differences in tenderness. Greater consumer acceptability being associated with lower WBSF values and the consumers were willing to pay a higher price for more tender steaks. Platter et al. (2005) observed that in an auction model, if consumers knew steaks was USDA High Choice or Prime and WBSF values were less than 3.9 kg, marbling score alone accounted for less than 3% of the bids; however, when combined with known quality grade and WBSF, bids were increased by tenfold. However, variability exists in tenderness among carcasses of any given USDA quality grade; therefore, that is why the beef industry continues to use postmortem processing technologies such as electrical stimulation and aging to minimize tenderness variation (O'Quinn, Legako, Brooks, and Miller, 2018).

Colle et al. (2015) evaluated the influence of aging (2, 14, 21, 42, and 63 days) on WBSF and consumer sensory analysis (scale of 1 = not tender; 10 = extremely tender) of *gluteus medius* (GM) and *longissimus lumborum* (LL) steaks. WBSF values decreased with longer aging for the LL (3.42 to 2.26 kg), while no difference was observed for the GM (3.47 to 2.93 kg).

Consumer panel results agree that as muscles age tenderness increased, but in both LL and GM (4.92 to 5.88 and 4.53 to 6.20, respectively).

USDA Quality Grade and Flavor

Traditionally, beef tenderness, juiciness, and flavor have been considered the three major attributes of beef palatability (Gagaoua et al., 2018; Legeko et al., 2015; Watson et al., 2008). The combined effect of these attributes defines beef eating quality (Lucherk, et al., 2016; O'Quinn et al., 2012; Miller et al., 2001, Thompson, 2004). However, among these attributes, flavor is the most complex factor because it is the perceived aromas and tastes of hundreds of compounds formed in various chemical reactions during cooking (Mottram, 1998, Mottram, 1991). Intramuscular fat becomes the driver of beef palatability when tenderness is acceptable and off-flavors are not present (Corbin et al., 2015). The USDA quality grade is well associated with beef flavor in both consumer and descriptive panels (Corbin et al., 2015, Hunt et al., 2014, Lorenzen et al., 2003, Savell et al., 1986, Smith et al., 1984). Smith et al. (1984) reported top loin steaks with a marbling score of Moderate and higher had greater consumer flavor acceptance (55%) than Modest, Small, or Slight scores (32%, 38%, 23%, respectively). Hunt et al. (2014) observed that Top Choice steaks had more flavor was greater than Select ($P > 0.05$) regardless of muscle and consumer acceptance was highly correlated with flavor liking ($r = 0.85$). Corbin et al. (2015) found that as the fat level increased overall acceptability of flavor increased. Additionally, this was highly correlated with flavor liking ($r = 0.96$) and fat percentage ($r = 0.79$). The level of fat was a primary factor of beef flavor acceptability. Claborn et al. (2011) surveyed consumers who purchased certified Angus beef, Choice, and Select striploin steaks for tenderness, flavor, and juiciness. These authors reported that approximately

87% of consumers rated the flavor of certified Angus beef flavor as extremely or very desirable; whereas only 65 and 69% of consumers rated USDA Choice and Select beef as having acceptable flavor. The variation in consumer flavor analysis was greater for USDA Select beef. Lorenzen et al. (2003) reported a 0.2 correlation coefficient between consumer and descriptive panels. Consumers usually simplify and misevaluate a sensory attribute because of the positive perception of other attributes, being termed the halo effect (Roeber et al., 2000). This means consumers are more likely to rate flavor acceptable if tenderness is already acceptable. Therefore, asking consumers to provide scale-based responses on a sensory attribute is usually discouraged because consumer panels are not appropriate for such evaluation due to bias towards brands and other information influences (Amerine et al., 2013). Since flavor is a combination of taste and smell, both play a role in flavor detection the prevalence of volatile compounds like sulfur-containing compounds, furanthiols, disulfides, aldehydes, ketones, and other heterocyclic compounds, will factor into consumer perception (Mottram, 1991; Huffman et. al, 1996). Organic compounds in beef such as amino acids, reducing sugars, and fatty acids undergo chemical changes catalyzed by heat during cooking to produce non-volatile and volatile flavor compounds (Back, 2007; Cerny, 2007). An important reaction in volatile compound formation is the Maillard reaction which has three stages. First, condensation between an amino group and a reducing sugar leads to an *N*-glycosylamine or Heyns product if the reducing sugar is a ketose. Then sugar fragmentation releases the amino group and dehydration, fragmentation, cyclization, and polymerization reactions occur (Boekal, 2006). Strecker degradation is important because of flavor formation. In this reaction, amino acids are degraded by dicarbonyls formed in the Maillard reaction, leading to deamination and decarboxylation of the amino acid (Boekal, 2006). Legako et. al. (2015) reported consumer palatability scores and volatile compounds on four beef muscles (*Longissimus lumborum*, *Psoas major*, *Semimembranosus*, and *Gluteus medius*) and five USDA quality grades (Prime, Upper 2/3 Choice, Low Choice, Select, and Standard). No interactions were observed for differences between quality grades ($P > 0.05$); however, consumer flavor perception and volatile scores differed between muscles ($P \leq 0.001$; $P \leq 0.04$, respectively). Semimembranosus *and* Gluteus medius had increased lipid oxidation, Psoas major had increased sulfur-containing volatiles, and aldehydes were inversely related to fat percentage. Legako et al. (2016) confirmed that volatile compounds are primarily affected by muscle and not quality grade. Strecker degradation of amino acids is one pathway of the Maillard reaction which yields characteristic volatile compounds of beef (Cerny, 2007). Strecker aldehyde quantities, including valine, leucine, and isoleucine rely on muscle type ($P \leq 0.020$). Volatile compounds had negative flavor correlations ($P \leq 0.05$) with consumer palatability scores for *n*-aldehydes ($r \leq -0.318$) because these are lipid degradation products that specific flavoring depending on fatty acid composition (Kerth & Miller, 2015). However, evaluating a sensory attribute is challenging because of the intricate interrelationships among tenderness, juiciness, and flavor. Overall, perceived flavor by consumers is positively correlated with the amount of intramuscular fat, i.e., marbling score.

USDA Quality Grade as a Pricing Factor

Consumers associate many factors with quality when purchasing beef in retail settings and their choices will vary over time and by region. However, pricing has been an important factor in beef purchasing decisions (Caswell & Yaktine, 2013). Beef's flavor, juiciness, and tenderness cannot be determined before purchase; therefore, beef quality grades presented on packages are used for consumers to assess potential beef sensory characteristics and to convey marketing signals reflecting product performance and value (Holland & Loveday, 2013). The highest value is placed on marbling; however, research shows that consumers will choose a steak with lower marbling such as USDA Select instead of USDA Choice or higher because of a cheaper price (Killinger et al., 2014). The quality of the middle meats of the carcass is usually the driver of the market (Fausti & Qasmi, 1999). Variations in pricing are established between quality grade categories for boxed-beef and are reported by USDA - Agricultural Marketing Service (AMS) as "boxed-beef" cutout values, which are calculated using negotiated prices and estimated average yields of individual subprimals and credit items (USDA, 2015). In the USDA boxed-beef cutout calculation, the rib and loin primals account for only 1/3 carcass weight: however, cuts from the rib and the loin account for most of the inter-grade variation in cutout value. Within the past 15 years, the growth of USDA certified beef brands has influenced how USDA Choice beef is marketed in today's beef trade (McCully, 2010; Speer, 2013). For example, from 2009 through 2014, growth in consumer demand for Certified Angus Beef® (CAB) increased by 112%; whereas demand for commodity Choice beef declined by 2% (Zimmerman & Schroeder, 2013; Suther, 2015). Within the same period, Choice boxed-beef sales increased by 15% (Speer, 2013). In the past few years, as beef becomes more expensive, consumers also demonstrate a willingness to "trade up", meaning they will pay higher prices to obtain desired products and eating experiences (Tatum, 2015). However, consumers will also choose a lower grade or tougher steak when the price is a deciding factor (Claborn et al., 2011). These authors found that 61% of the consumers who purchased USDA Select grade steaks emphasized: "value in relation to price" or "total price" of the steaks in their purchase decision. Claborn et al. (2011) reported that 20% of consumers purchased Choice steaks for the value of the product, greater amount of marbling, and presented USDA quality grade. This indicates that tenderness, flavor, and juiciness of beef are being communicated along the beef chain primarily via price signals associated with USDA quality grades (USDA, 2015). According to the March 2015 Consumer Beef Index survey, 73% of consumers believe that steaks offered at retail "are priced just about right" or "are expensive but worth it" (The Beef Checkoff, 2015). In 2015, O'Quinn et al. (2012) revealed that customers' perception of beef quality rose 10 to 12% simply by knowledge of certain brands. These signals communicate supply-demand interactions for various grades of beef in the boxed-beef market through the supply chain and encourage the production of cattle, carcasses, and cuts that conform to industry targets (Smith et al., 2006) because today's quality-driven consumers are willing to pay more for beef if the eating experience justifies the price (NBQA, 2015).

Current USDA Beef Quality Grading Criteria

There are several classes of cattle that the industry will grade either internally or externally. However, the two classes of cattle associated with USDA quality grading are steers and heifers. Steers are castrated male cattle that do not show any bull characteristics. Heifers are female cattle that have not been bred. These two classes of cattle are eligible for USDA quality grade of Prime, Choice, Select, Standard, Commercial, Utility, Cutter, and Canner (Table 1). Prime is the highest quality grade, whereas Standard is the lowest. Cattle are also divided into maturity groups by the degree of ossification at the thoracic vertebrae. Animals with a maturity score of A will be assigned a quality grade, however, B-maturity carcasses will only be assigned a quality grade if the marbling score is sufficient (Traces or higher), and carcasses with C, D, and E maturity scores not eligible for USDA quality grading (USDA, 2015). The USDA Prime grade has marbling scores of Abundant, Moderately Abundant, or Slightly Abundant. The USDA Choice has less marbling scores, being Small, Modest, or Moderate. The USDA Select beef is leaner with a marbling score of Slight. It is somewhat tender but has less marbling and may lack the degree of juiciness and flavor of the beef graded USDA Choice and Prime. Both maturity and marbling scores are dictated on a 0–100-point scale with 0 being the least or youngest and 100 being the most or oldest of that score. Standard and commercial grades of beef are frequently sold as ungraded or as store brand meat. Marbling score would be Traces and Practically Devoid. Utility, cutter, and canner grades of beef are seldom if ever, sold at retail but are used instead to make ground beef and processed products (Meadows, 2019).

Maturity is a measurement of physiological age taken primarily at the cartilaginous buttons of the thoracic vertebrae. However, in the event that the backbone is damaged in that area, the sacral of lumbar vertebrae can be used as an assessment of age. In 1926, USDA maturity standards for cattle were considered to be 4 years of age or younger (USDA, 1926). By 1975 these standards revealed that carcasses passed a “magical line” (B+/C- maturity) palatability traits declined, and these carcasses would become ineligible for grading (Smith, Berry, Savell, & Cross, 1986). Through a succession of grade standard changes, the eligible age for beef to be graded by USDA is 42 months of age (USDA, 1975). Outside that range, beef is not eligible for Prime, Choice, Select or Standard grades. In recent times, the USDA/AMS employed new criteria that allow for the use of age documentation, dentition, or physiological maturity to categorize carcasses into maturity categories for quality grading (Sheets, 2017). Before this change occurred, the USDA/AMS only permitted the use of physical maturity as an indicator of overall carcass maturity when determining quality grade (USDA, 2016). When visually evaluating a carcass, the USDA graders base their assessment on vertebral ossification, rib shape and size, and lean color and texture (USDA, 2016). The USDA federal regulation §54.104 states that carcasses will be designated into the following groups based on physiological maturity: A^{0-100} (9 to 30 month), B^{0-100} (30 to 42 month), C^{0-100} (42 to 72 month), D^{0-100} (72 to 96 month), and E^{0-100} (> 96 months) (USDA, 2017). Most of today's conventionally raised steers and heifers fall into the A maturity category (Boykin et al., 2017). These cattle will have cherry red finely texture lean and no ossification of the sacral, thoracic, or lumbar vertebrae. Cattle of B maturity will have similarly textured lean but the beginning of partial ossification of all vertebrae. Cattle that are C, D, and E maturity will gradually have darker, more coarsely textured lean and have completely fused and ossified vertebrae. During the assessment of the carcass for quality grade, more emphasis is placed on skeletal maturity than lean maturity. The grading standards also require that overall maturity must not differ from the skeletal maturity by more than one full grade (USDA, 2016). However, estrogen may accelerate skeletal ossification, which increases the physical maturity of heifers over steers of the same age (Shackelford et al., 1995; Field et al., 1997; Grumbach and Auchus, 1999). Studies have shown that increased exogenous estrogen which is similar to increased endogenous estrogen increases skeletal maturity (Roeber et al., 2000; Reiling and Johnson, 2003). According to data from the 2005 NBQA, 3-14% of heifers and steer skeletal maturity was B or greater (Garcia et al., 2008). O'Connor et al. (2007) found that cattle between 22 and 24 months of age have a 9% probability of producing B maturity carcasses and a 3% probability of producing a C maturity carcass. Smith et al. (1982) confirmed that there are significant differences in palatability between young and mature beef carcasses, especially between A and E maturity. The palatability variation among A maturity carcasses (A^{00-100}) still exists but are much less severe.

Marbling is a term used to describe the white flecks or streaks of intramuscular fat between muscle bundles (Hocquette et al., 2010). Significant differences between animals, breeds, and muscle types exist not only in the degree of marbling but also in the morphological characteristics of marbling measured by computerized image analysis (Albrecht et al., 2006; Konarska et al., 2017). Marbling is assessed between the 12^{th} and 13^{th} rib of the *longissimus* muscle by the USDA graders to determine lean quality (USDA, 1997). The amount and distribution of marbling in the ribeye will determine whether a carcass is stamped with a corresponding quality grade (Savell, 2013). Marbling supports the visual evaluation of fat content; however high amounts of marbling could create a negative perception from the consumer because intramuscular fat cannot be trimmed like subcutaneous and intermuscular fat can be by the consumer (Killinger et al., 2004). Vierck et al. (2018) evaluated one hundred and seventeen beef strip loins for the effects of marbling texture on muscle characteristics. Steaks from Top Choice, Low Choice, and Select were select to equally represent differing marbling texture groups: fine, medium, and coarse. Marbling texture only affected fat cell size. There were no marbling texture × marbling score interactions. However, marbling score did affect adipocyte size, as high Choice and low Choice possessed larger adipocytes than Select steaks.

Factors Influencing USDA Beef Quality Grades

Breed

Currently, cattle producers and meatpackers in the US have little information regarding the role of breed influence on production or carcass traits (Drouillard, 2018). In an early study by Riley et al. (1986), breeds of bulls were evaluated to determine the association between breed and carcass traits, using British and British crosses, Brahman and Brahman crosses, Jersey and Jersey crosses, and Holstein and Holstein crosses. Jersey cattle had the lowest quality grades compared to the other breeds, whereas British-type cattle had more subcutaneous fat. Similarly, Ramsey et al. (1963) found that steers of the British type produced carcasses with greater external fat, marbling, and quality grades than steers of dairy-type and Brahman-type cattle. The data also revealed that bulls of the British type were fatter, had higher marbling scores, and higher USDA quality grades than young bulls of Jersey breeding. Studies of *Bos taurus* and *Bos indicus* steers and heifers comparing subcutaneous and intramuscular fat suggest that genetic and metabolic differences exist between the two species (Miller et al., 1991; St. John et al., 1991). Mateescu et al. (2015) evaluated Angus cattle for genetic correlations of sensory traits and marbling scores. These authors found heritability estimates for marbling score were 0.67 and genetic correlations of marbling scores with palatability traits were 0.57 to 1.00 for the Angus breed. Campbell et al. (2016) observed the effect of breed on fat deposition of Angus and Brahman crossbred cattle and found that cattle with more Angus influence had higher intramuscular fat volume compared to Brahman influenced cattle ($P < 0.001$). Boykin et al., 2017 reported that native cattle had lower USDA quality grades (705 points) and marbling scores (469 points) compared to dairy-type cattle (717 and 486 points, respectively); however native cattle had greater USDA quality grade and marbling score compared to *Bos* indicus cattle (667 and 382 points, respectively). Consumer acceptability of steaks by breed is detailed by McKenna et al. (2004). These authors used top loin steaks to evaluate consumer preference of USDA Low Choice, High Select, or Low Select and English, Continental European Cross, or Brahman Cross cattle breed. Neither breed type nor marbling score affected overall palatability (0.01 to 0.03 points separating each breed or quality grade; $P \geq 0.05$) cooking style indicated differences. Steaks from European cross cattle were more tender as marbling score increased (2.95kg) than English or Brahman cross cattle (3.25 and

3.30kg, respectively). In Brazil, Nellore or zebu cattle dominate as the major beef cattle breed because of their shorter growth period and pasture-based fattening resulting in beef with small intramuscular fat (3.08 to 3.86%) content (Silva et al., 2009) compared to purebred Angus and Angus crossed cattle that have intramuscular fat percentages from 4.6 to 13% (Graham et al., 2006; Gilles et al., 2004). Gotoh et al. (2018) stated that Wagyu has the ability to deposit a great amount of intramuscular fat (11 to 30%).

Diets

Cattle selection has been selected for feed/gain ratio (Cross & Dinius, 1978). In addition, cattle producing highly marbled beef are preferred because of consumers' demand for high-quality beef (Smith, Gotoh, & Greenwood, 2018). The body of literature clearly shows that diets change fat deposition and fatty acid composition of beef marbling. Owens & Gardner (2000) reported that cattle fed steam-flaked grain had larger longissimus muscle areas and greater backfat thickness but lower marbling and quality grades. Marbling score and quality grade was increased with carcass weight, slaughter age, and days on feed. However, a marked decrease was noted when initial weights increased. Changes greater than 2% added fat were related to lower quality grades and higher quality grades were more prevalent with added protein and heavier initial weights. Miller, Cross, Crouse, and Tatum (1987) found that steers backgrounded on high energy rations produced heavier hot carcass weights by 34 pounds and thicker adjusted back fat by .26 in along with greater marbling scores and quality grades ($P < 0.01$). Enhanced marbling deposition and improved palatability begin with intensive pre-slaughter feeding regimes (Ferrinho et al., 2020) Research by Pogge & Hansen, 2013; Pethick et al., 2004; Gregory et al., 1994; Blackwell et al. (1962) agree with the above statement. These processes target cattle that will deposit less external fat during the growing and finishing phases (Drouillard, 2018). Zinn, Durham, &

Hedrick, (1970) also suggested that marbling score and quality grade in steers and heifers on feed increased from 30 to 90, 120 to 180, and 210 to 270 days. Steers required a minimum of 120 d on feed to reach Select and 150 to 210 d to reach Low Choice. Heifers required 150 d on feed to reach Select and failed to reach Choice even after 270 d. Marbling score and carcass grade increased significantly up to 240 days on feed in steers.

Effects of Diets and Breed on Fatty Acid Composition

Lipid content in beef generally varies from 4 to 15%, depending on genotype, muscling, feed ration, and breed (Mapiye et al., 2013 and Smith et al., 2006). Fatty acid composition is important for beef quality grading because it decides the visibility of marbling for the USDA graders' visual assessment of the amount and distribution of intramuscular fat (Tume, 2004). The intramuscular in beef consists of mostly triglycerides, the melting point of which depends on the length and the number of double bonds of the carbon chain (Wood, 1984; Tume, 2004). Leaner carcasses have a greater proportion of PUFA because phospholipids of lean tissues are more predominant (Legako et al., 2015); whereas in fattier carcasses, more Saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) are being deposited in adipose tissues (Dinh et al., 2010). SFA solidifies at 20 to 22°C, whereas unsaturated fatty acids become solid below 20°C (Moorthy, 2018). Yang et al. (1999) found that subcutaneous fat in carcasses transitions to a solid-state between 8 and 15°C. The rate of fatty acid solidification depends on the length of the carbon chain and the introduction of double bonds (Wood, 1984). In leaner carcasses, phospholipids in lean tissues play a more important role in fatty acid composition. High MUFA concentrations can lead to a lower melting point contributing to softer beef fat (Smith et al., 2006). Saturated fatty acids melt at a higher temperature; whereas polyunsaturated fatty acids

(PUFA) melt at a lower temperature (Wood, 1984). Smith et al. (1998) reported that palmitic acid and stearic acid contribute to the overall fatty acid composition of beef and beef fat and increase the hardness. The concentration of stearic acid has a crucial effect on melting points of beef fat (Turk and Smith, 2009) because stearic acid has a melting point at approximately 70°C, whereas MUFAs have melting points below room temperature, at approximately 20°C (Smith, 2016). Beef fatty acid composition is characterized by a saturation index of 0.8 to 1.0 (Dinh et al., 2010), meaning saturated fatty acids predominated by palmitic and stearic acids (45and 46%, respectively) are slightly less than the total of MUFA, predominated by palmitoleic and oleic acids (49), and PUFA, predominated by linoleic and linolenic acids (4 and 6%, respectively). Breed and diet have been recognized to have the most influence on fatty acid composition of beef marbling. Dinh et al. (2010) reported approximately 45 to 47% SFA, 48% MUFA, 3 to 8% PUFA for Angus, Brahman, and Romosinuano cattle, with leaner carcasses having greater PUFA percentage. The accumulation of SFA and MUFA is due to the biohydrogenation in the rumen (Vahmani et al., 2015) and the Δ^9 desaturase activity and elongation in adipose and muscle tissues (Smith et al., 2006). The prevalence of PUFA is due to fatty acid synthesis (Voet et al., 2006). Lage et al. (2012) also suggested that *Bos* indicus genetics may decrease fat softness, whereas *Bos* taurus genetics may improve fat softness through greater content of unsaturated fatty acids. Bressan et al. (2016) found B*os* indicus cattle had similar SFA and PUFA concentration compared to *Bos* taurus x *Bos* indicus crossbreds (48 % and 9 to 10%, respectively). However, when finished on grain, *Bos* indicus cattle had greater SFA percentages (51%) compared to crossbred cattle (48%); whereas crossbred cattle had greater PUFA percentages (10%) compared to purebred cattle (7%).

Brugiapaglia et al. (2014) observed the fatty acid composition of Piemontese, Limousin, and Friesian bulls and observed breed differences in intramuscular fat content influenced the fatty acid composition. Piemontese animals exhibited low intramuscular fat, SFA, and MUFA content (46%, 32%, respectively), while Friesian animals demonstrated the higher intramuscular fat and MUFA content (42%). However, Piemontese cattle had the highest PUFA concentration (22%) compared to Limousin and Fresien cattle (15 and 11%, respectively). Zembayashi et al. (1995) suggested that Japanese cattle breeds have a genetic tendency for producing lipids with higher MUFA concentrations than other breeds. Zembayashi and Nishimura (1996) reported significant differences in SFA, MUFA, and PUFA concentrations of intramuscular triacylglycerols and phospholipids between the progeny of Japanese cattle. The Wagyu beef breed is known for its great amount of marbling, less subcutaneous fat, a greater percentage of MUFA and MUFA/SFA ratio than other breeds (Wang et al., 2005 and Smith et al., 2006). Dias et al. (2015) reported that fatty acids in various cuts from Nellore and Wagyu crosses had less than 50% SFA. Palmitic acid, at 26%, was the predominant SFA, followed by stearic acid. Wagyu beef had the least amount of SFA, only 38%. The Nellore and Jersey beef had 49 and 45% SFA, respectively. The Wagyu beef had the most unsaturated fatty acids, at 62%, much more than the Nellore beef at 51%. The Wagyu beef was richest in oleic acid, whereas the Nellore beef was richest in linoleic acid.

High proportions of MUFAs are suggested to be a result of the high concentrate rations fed to feedlot cattle. Muscles from animals fed high grain rations are reported to contain a greater proportion of n-6 PUFA; whereas those finished with forage have more n-3 PUFA concentrations (Daley et al., 2010; Wood et al., 2008). Smith et al. (2016) suggested that amount of marbling and MUFA concentrations were increased with time on feed and associated with the stearoyl-CoA desaturase activity. An increase in SCD activity lowers SFA proportions and increases MUFA proportions as seen in a study by Wang et al. (2005). These authors analyzed fat from the *longissimus dorsi* of Japanese black and Holstein cattle and found Japanese black cattle to have a higher expression due to more mature fat cells and MUFA synthesis. This phenomenon has been confirmed by Buchanan et al. (2013) and Albertí et al. (2014). Buchanan et al. (2013) observed the effect of concentrate finished compared to forage finished heifers. These authors found a greater total MUFA, primarily, heptadecenoic and oleic acid, in concentrate finished cattle (54%) compared to forage finished cattle (52%). Oleic acid is the most abundant fatty acid in bovine fat. Oleic acid concentration is dependent on Δ^9 desaturase activity by the SCD gene (Smith et al, 2006). Albertí et al. (2014) observed that bulls on concentrate diets who had more intramuscular fat at slaughter had higher percentages of MUFA (37%), mainly oleic acid, compared to bulls with less fat (29%). These authors suggest that it could be due to reduced levels of biohydrogenation in the rumen.

As previously reported, a greater percentage of MUFA leads to a lower fat-melting point, which positively contributes to the softness of beef fat and desirable beef flavor (Smith et al., 2006). Wood et al. (2004) suggested that muscles differing in the fat content and composition would have detectable differences in meat quality, thereby having differentiated consumer acceptance of not only sensory attributes such as tenderness and flavor but also color and texture (Devine, 2014).

Stress, pH, and Lean Color

Stressful situations, including transportation, social mixing, exposure to new environments, and personnel preslaughter, are prevalent in the beef industry (Ferguson & Warner, 2008; Miranda-de la Lama et al., 2014). Research indicates that temperamental or excitable cattle have a higher pH and poorer meat quality (Hall et al., 2011; Ponnampalam et al., 2017; Voisinet et al.,1997) due to their heightened sensitivity to stress. Stress causes high postmortem pH in muscles and dark cutters, which results in a financial loss for the meat industry (Teke et al., 2014). Carcasses are downgraded by a full grade if dark color negatively influences visual marbling score, and they are further discounted because of defects such as dark cutting. Although King et al. (2006) found no effect of animal temperament, and stress on lean color, marbling score, and quality grade, others such as Probst et al. (2012) reported that animals with excitable temperaments were more likely to show signs of borderline dark cutting lean. Stressors pre-slaughter like transport, lairage, and weaning increase production and concentration of cortisol (Russell et al., 2012) which depletes muscle glycogen and contributes to rapid glycolysis and increased lactic acid production early on in postmortem (Warriss, 2010), which leads to high ultimate pH and incidence of dark cutting beef (DFD) (Čobanović et al., 2016; Ouali et. al, 2006). However, Holdstock et al. (2014) stated that in the Canadian beef system that some dark cutting beef had pH < 6.0. Murray (1989) observed only 13.2% of the dark cutters had pH > 6.0; whereas 36.8%, 43.6%, and 6.4% had pH values of 5.8 to 6.0, 5.6 to 5.8, and less than 5.6, respectively. In a more recent study by Robertson et al. (2007), 56% of graded carcasses were deemed traditional dark cutters and 44% of graded cattle had a pH from 5.5 to 6.0. Consumers have signaled that color is an essential factor when selecting their beef (Vierck et al., 2018; Platter et al. 2003; Savell et al., 1989). Killinger et al. (2004), when studying consumer preferences for marbling and lean color in retail beef steaks, observed that 68 to 77% of consumer preference preferred bright, cherry-red lean and were willing to pay for such a color rather than dark red lean. High pH due to stress before the animal is harvested directly affects color development by reducing the layer of oxymyoglobin on the meat surface and increasing translucency (Ponnampalam et al., 2017). However, Hughes et al. (2014) found that pH and color are not as title coupled as previous research reports. These authors state that while glycogen production and pH are linked, this relationship does not always produce dark meat.

Temperature

Postmortem chilling of beef carcasses is to ensure food safety, maximize shelf-life, and reduce shrinkage with little emphasis on beef quality attributes (Savell, Mueller, & Baird, 2005). Rapid chilling of beef carcasses has numerous economic advantages including reduced chilling times, improved rate of product throughput, and reduced shrinkage (Liu et al., 2015; Neto, Beraquet, & Cardoso, 2013; Aalhus et al., 1994). However, rapid chilling causes cold shortening, resulting in tough meat (Locker & Hagyard, 1963). Moreover, rapid chilling and muscle shortening may lead to darker lean and decreased marbling visibility (Moeseke et al., 2001). Packers may delay chilling to 10°C before 10 h postmortem (Bendall, 1972) or before pH has fallen below a value of 6.0 (Davey & Gilbert, 1974). Merkel and Pearson (1975) and Lochner et al. (1980) subcutaneous and ~~IMF~~ intramuscular fat prevents heat from being dissipated during carcass chilling, which may impact marbling visibility. As an alternative, spray chilling is used to increase heat dissipation (Chen et al., 2019), decrease carcass shrinkage, and carry antimicrobial agents (Strydom & Buys, 1995; Liu et. al, 2016). Allen et al (1987) found that spraying chilled water on hot carcasses during the initial hours of chilling caused no change in muscle color, firmness, texture, and marbling visibility. Skeletal maturity was detected to be significantly younger on sides that were spray-chilled than conventionally chilled sides, notably in the thoracic chine bones because spray chilling delays the dehydration of cartilage, as usually observed on conventionally chilled carcasses. Therefore, spray chilling is more advantageous for improving USDA quality grade when chilling carcasses of greater than A maturity. Jeremiah, Martin, and Murray (1985) documented that among hot boned, delayed chilled, and spray chilled/electrically stimulated carcasses, hot boned carcasses had darker lean, delayed chilling produced paler and lighter colored lean with less perceived marbling, and spray chilled and electrically stimulated carcasses had higher perceived marbling scores. Fields et al. (1976), Jeremiah et al. (1984), and Babiker & Lawrie (1983), on the contrary, found that delayed chilling enhanced color in beef carcasses. Chilling practices are known for being vital in the mitigation of pathogens, improvement of shelf-life, and decrease in carcass shrinkage. However, more rapid chilling, to improve color, tenderness, and potentially quality grading of beef, requires postmortem interventions such as electrical stimulation to accelerate the conversion of muscle to meat.

Electrical stimulation is being used regularly in the beef industry (Devine et al. 2004) to accelerate postmortem pH decline and quicken rigor mortis to allow earlier postmortem fabrication of carcasses. Electrical stimulation also improves tenderness, color, and visibility of marbling (Adeyemi & Sazili, 2014). Electrical stimulation facilitates vigorous muscle contraction and increases glycogen usage, production of lactate, reduction of metabolites in oxidative pathways to improve ultimate pH and meat color (Lawrie and Ledward, 2006). Lawrie and Ledward (2006) noticed that the pH decline caused myofibrillar proteins to quickly reach their isoelectric point, causing oxidation of myoglobin. This phenomenon explains the quick development of brighter lean at 12 to 20 h postmortem with minimal color change after that. Savell, Smith, and Carpenter (1978) evaluated the effects of electrical stimulation on carcass quality characteristics of heifers with Practically Devoid and Traces marbling scores. These authors observed an improvement in lean maturity (6.6 points to 5.3 points) and tenderness (9.0 kg to 5.8 kg) but no effect on skeletal maturity, marbling score, and quality grade. Crouse, Sideman, and Cross (1985) and Roeber et al. (2000) confirmed improvement in lean maturity with minimal changes to quality grade. Roeber et al. (2000) used high voltage (550 to 600 V, 5 to 15 A) stimulation and reported carcasses with lower WBSF values and lighter, brighter lean than low voltage (20 to 90 V, less than 1 A). High voltage enabled pH to drop 0.25 units, more than 0.07 units by low voltage. These authors agree that high voltage yielded better carcass quality attributes and greater consumer acceptance. Nazli et al. (2010) findings agreed with what was reported by Aalhus et al. (1994) that high voltage electrical stimulation applied to beef carcasses produced more rapid pH decline (6.5 to 5.9 in 6 h) and brighter meat color (L* 27.8 to 30.1) than those of non-stimulated carcasses. However, Ledward et al. (1986) and Hector et al. (1992) suggested that although pH decline is faster with electrical stimulation, such an application may form more metmyoglobin, resulting in color loss over time. Overall, electrical stimulation is advantageous to beef packers as it can hasten pH decline, improve color and tenderness, and shorten carcass hanging time in the cooler. Brighter color and more visible marbling improve carcass grading.

Conclusion

From the development of key organizations in the early 20th century to the innovative research conducted today, the beef industry continues to make improvements to both the live animal and carcasses that profit the producers, packers, and consumers. By selecting excellent genetics, improving nutrients, and applying proper chilling and interventions such as electrical stimulation, premium quality grades like Choice and Prime have doubled in the last few decades and beef tenderness, juiciness, and flavor have been enhanced to satisfy the forevermore demanding consumers.

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CHAPTER II

EFFECTS OF CHILLING DURATION ON USDA QUALITY GRADE OF BEEF CARCASSES

Abstract

Two hundred and nine beef carcasses (361 ± 53 kg) from crossbred, grain-finished cattle harvested in a commercial packing plant were selected and evaluated for marbling score, core temperature (n=1), pH, shrinkage, and color, and aerobic plate count (n=50) for 24, 48, 72, and 96 h under spray chilling at 0 to 3°C with a wind speed of 3.1 m/s and 153-lux of fluorescent light in a commercial hot box. Carcasses reached 3ºC after 12 to 16 h of chilling. There were minimal changes in shrinkage among time points (-0.4 to 1.2%; $P \leq 0.002$), pH (5.56 to 5.69; $P \leq 0.001$), and APC (0.1 to 0.7 log; $P < 0.001$). Initial 24-h grading revealed a grade composition of 21.05% Slight (SL, n = 44), 33.97% Small (SM, n = 71), 17.22% Modest (MT, n = 37), 17.70% Moderate (MD, n = 36), and 10.05% Slightly Abundant (SA, n = 21). With marbling score in numeric values of 200 (Practically Devoid^{00}) belonging to USDA quality grade Standard carcasses to 1100 (Abundant^{00}) which is characteristic of USDA quality grade Prime carcasses, carcasses with SM or greater marbling score at 24 h had a 34 to 60 points deduction after 96 h of spray chilling ($P \leq 0.042$), the SL carcasses had an increase marbling score, from 442 to 469 points. Moreover, SL carcasses had a greater percentage of PUFA ($P < 0.001$).

Introduction

The current beef grading system underwent much revision to identify the most important factors: product quality and lean meat yield (Murphey et al., 1960; Smith et al., 1982; Smith et al., 1984; Schroeder et al., 2013; Steiner, 2014). These factors are to satisfy growing consumer demand for wholesome and high-quality beef. Quality grading is based on animal age and marbling of the *Longissimus* muscle at the interface of 12th and 13th ribs, which has been identified as an indicator of flavor, juiciness, and tenderness (Jeremiah et al., 1970; Smith and Carpenter, 1985; Shackelford et al., 1999; Schönfeldt, and Strydom, 2011; Bonny et al., 2016). Consumer acceptability and purchasing decisions of beef greatly depend on the above-mentioned sensory attributes and lean color (Huffman et al., 1996; Smith et al., 2000; Platter et al. 2003); therefore, beef consumers will choose steaks with greater marbling and brighter lean color even though they have to pay a premium for these products (Killinger et al., 2004). The Choice-Select spread is an important indicator in the strength of demand and relative supply of two major beef quality grades (McCully, 2017), a potential increase in marbling score in carcasses that have USDA Select and border USDA Choice will have a significant economic impact on beef packers and the price consumers pay at retail. The Choice-Select spread in October 2020 was $31.31/kg. In the summer months, the Choice-Select spread begins to narrow with an increase typically occurring in October through the end of the year (Lusk et al., 2001). However, the Choice-Select spread will begin to widen towards the end of October to December due to demand for middle meats from the rib and loin (Pruitt et al., 2013).

Many antemortem factors such as age, sex, nutrition, stress, and postmortem factors such as chilling, aging, and pH influence marbling content and meat color (Fergusen et al., 2001; Lawrie and Ledward, 2006; O'Neill et al., 2006; Loredo-Osti et al., 2019). Beef carcasses are normally chilled for 24 to 48 h after slaughter to achieve an internal temperature of 7°C or less (Savell, 2012). The two most common methods of chilling are blast chilling and spray chilling. In blast chilling, beef carcasses are placed in a “hot box” under circulated chilled air (Savell, 2012). This type of chilling may cause cold shortening, resulting in tough meat (Locker and Hagyard, 1963), and may lead to darker lean and decrease marbling visibility (Moeseke et al., 2001). Spray chilling uses water with or without antimicrobial agents to decrease the shrinkage caused by moisture evaporation and maintain the freshness and bloom of the meat (Savell et al., 2005). Cold water used in spray chilling has greater heat conductivity and decreases carcass through moisture evaporation compared with blast chilling. Spray chilling also maintains the freshness of the bone and bloomed color of the lean (Savell et al., 2005). A phenomenon that has been observed in many beef packing plants is that there is an increase in USDA quality grade if cattle are harvested on Friday and graded on Monday, which allows beef carcasses to be chilled 48 h longer than normal. Preliminary data collected by the authors of the current study from ten USDA Choice carcasses revealed a 20-point increase in marbling score after 96-h spray chilling (Practically Devoid^{00} = 200 points; Abundant^{00} = 1000 points). However, as beef carcasses are chilled, the carcass pH, lean color, and microbial growth may be influenced by the chilling duration. Depending on the saturation index of the fatty acid composition of the intramuscular fat, chilling duration may also influence marbling visibility on the background of lean color (Page et al., 2001; Hu and Willet, 2002; Wood et al., 2004), which is important for the grader to estimate the amount and distribution of intramuscular fat (Tan, 2004; Tume, 2004). Therefore, the objective of the current study is to determine the effects of spray-chilling duration on marbling score, carcass shrinkage, pH, aerobic plate count, and lean color of beef carcasses with differentiated marbling score categories and the fatty acid compositions of lean from these beef carcasses.

Materials and Methods

Experimental Design and USDA Grading

Two hundred and nine beef carcasses (361 ± 53 kg) were processed at a commercial facility for six non-consecutive weeks (32, 41, 42, 35, 25, and 34 heads, respectively). For each week, a group of Angus crossbred beef cattle from a commercial breeding and feeding facility in the Southeast was selected for processing. These cattle were housed in mono-slope barns and fed a diet of 48% corn, 20% corn silage, 10% sorghum silage, 7% wet corn gluten, 6% minerals, 5% dried distillers' grain, and 4% hay for 180 to 210 d. Cattle were selected solely by the packer's live weight requirement of 589 to 635 kg. The carcasses were spray-chilled in a hot box at 0 to 3°C with a wind speed of 3.1 m/s under 153-lux of fluorescent light for 96 h. The carcasses received 30-s spraying of cold water with 80 ppm of hypobromous acid every 5 min for 14 h. At 24 h, the carcasses were ribbed between the 12th and 13th ribs to expose the ribeye surface for a repeated evaluation by the same USDA grader for the yield grade (YG), quality grade (QG), and marbling score at 24, 48, 72, and 96 h. During each evaluation, the carcasses were randomized on the rail to prevent grader's bias. In addition, at 48, 72, and 96 h, the ribeye surface was refaced by approximately 3 mm with a boning knife to present a fresh surface to the USDA grader. The samples from resurfacing at 48 h was used for fatty acid analysis. The ribeye surface was allowed to bloom for 45 min before grading. The marbling score at 24 h (initial marbling score) was converted into a numerical value as follows: Practically Devoid (PD)^{00} = 200; Trace (TR)^{00} = 300, Slight (SL)^{00} = 400, Small (SM)^{00} = 500, Modest (MT)^{00} = 600, Moderate (MD)^{00}

= 700, Slightly Abundant (SA)^{00} = 800, Moderately Abundant (MA)^{00} = 900, and Abundant (AB)^{00} = 1000 (Senneke, 2005). The carcasses used in the current study did not have sufficient replication (at least 10 heads) for PD, TR, MA, and AB as determined by the preliminary study; therefore, these categories were removed. The marbling score categories at 24 h (SL, SM, MT, MD, or SA) were used to classify the carcasses into groups for statistical analysis. The USDA quality and yield grades at 96 h postmortem were recorded as the official grades.

Fatty Acid Analysis

The muscle samples from the ribeye surfaces collected at 48 h were placed in Whirlpak® bags, vacuum-packaged, and immediately frozen at -20ºC. The samples were transported to Mississippi State University in dry ice, thawed overnight, and trimmed of all external fat, connective tissues, and accessory muscles, leaving only the *longissimus* muscle. The *longissimus* muscle was cut into small pieces, frozen in liquid nitrogen, and pulverized into powder. A1-g homogenized sample was weighed into a 20-mL flat-bottom glass vial with a Teflon®-lined screw cap and 1.5 mg of methyl tridecanoate was added as an internal standard. Fatty acids (FA) were methylated by a direct transesterification method (O'Fallon et al., 2007) by first being saponified in the presence of potassium hydroxide and methanol and subsequently transesterified in the presence of sulfuric acid into FA methyl esters (FAME), which were extracted in hexane. Fatty acid methyl esters (FAMEs) were determined by a gas chromatography system (Agilent Technologies, Santa Clara, CA) equipped with an HP-88 capillary column (30 m × 0.25 mm i.d. × 0.2 µm film thickness; Supelco Inc., Bellefonte, PA) and an Agilent 5975C inert XL MSD with triple-axis mass detector. Peaks were identified by FAME standards in Supelco® 37 Component FAME Mix (Sigma-Aldrich, St. Louis, MO), FAME #21 Mix (AOCS #6; Restek, Bellefonte, PA), a customized 17-component FAME mix (Nu-Chek-Prep, Elysian, MN), and various individual FAME standards. All fatty acids were monitored by their target and quantitative ions in a selected ion monitoring mode (Quehenberger, Armando, Dumlao, Stephens, and Dennis, 2008). Fatty acid concentration (µg/g of sample) was quantified by an internal standard calibration method. Fatty acid percentage was subsequently calculated as g/100 g of total FAs. The saturation index (SI) was calculated by the ratio of SFA to the sum of MUFA and PUFA. The P/S was the ratio of PUFA to SFA. The iodine value (IV; g of iodine per 100 g of sample) was calculated as IV = [16:1] × 0.95 + [18:1] × 0.86 + [18:2] × 1.732 + [18:3] × 2.616 + [20:1] × 0.785 + [22:1] × 0.723 (IV; AOCS, 1998).

Temperature, Shrinkage, pH, and Aerobic Plate Count

Temperature data was recorded continuously for 96 h by inserting two portable temperature probes (Stainless Steel Pro Logger probe, ThermoWorks, American Fork, Utah) into the *longissimus* muscle on the left (not ribbed) and right (ribbed) sides and between the 12^{th} and 13^{th} ribs of an additional carcass chilled under the same conditions as others used in the current study. The hotbox cooler was also monitored for temperature, wind speed, and light during the entire study. Hot carcass weight and daily cold carcass weight were recorded by a Vandeburg static scale (ST-MONO-1000, VandeBurg Scales, Sioux Center, IA). The shrinkage was determined by the difference between hot carcass weight and cold carcass weight at each time point, divided by 100. The pH at 24, 48, 72, and 96 h was measured by laterally inserting a temperature compensated pH probe connected to a pH meter (model #HI99163, Hanna Instruments, Inc, Rhode Island) by approximately 1.3 cm deep into the *longissimus* muscle. The pH meter was calibrated using pH standards of 4, 7, and 10. Fifty carcasses were randomly selected the second to the sixth week (10 carcasses per week) to determine the aerobic plate count (APC). A sterile sponge wetted with buffered peptone water (3M Health Care, St. Paul, Minnesota) was used to swab a 12.5-cm × 12.5-cm area on the round and chuck of the carcass at 24, 48, 72, and 96 h. Sponges were transported in sterile Whirlpak® bags to a commercial laboratory (IEH Laboratories and Consulting Group, Lakeland, FL) for analysis. The APC was reported as log of colony-forming units per cm^2 (logCFU/cm^2 or log).

Instrumental Color

Lean color was measured by a HunterLab MiniScan 4500L Spectrophotometer (Hunter Associates Inc, Reston, VA) at 24, 48, 72, and 96 h on the ribeye surface, which had bloomed for 45 min. The CIE L^*, a^*, b^* values -were recorded with an illuminant A, 10° observer's angle, and 25-mm aperture size.

Statistical Analysis

Marbling score category at 24 h, animal, and time served as main factor, main plot, and split-plot (in time) factor, respectively, in a split-plot design. Marbling score, shrinkage, pH, L*, a*, b* data acquired at 24, 48, 72, and 96 h were analyzed by a generalized linear mixed model with marbling score category, time, and their interaction as fixed effects and animal within a marbling score category as random effect. The selection of the appropriate covariance structure for the repeated measurement was based on three default Information Criteria calculated by SAS in the smaller-is-better format (AIC, Akaike's Information Criteria; AICC, AIC Corrected; and BIC, Bayesian Information Criteria; (Kincaid, 2005)), resulting in a compound symmetry structure being used. Temperature data was interpreted from the instrument printout (no data extraction available) without statistical analysis. Fatty acid composition and APC were analyzed in the similar statistical model with only marbling category (FA composition) or time (APC) as fixed effect and animal as random effect. The analysis of variance was performed by the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, USA). Means, if differing, were separated by a protected t-test using the LSMEANS/PDIFF/SLICEDIFF statement of the GLIMMIX procedure. Actual probability values were reported.

Results and Discussion

Distribution of 24-h Marbling Score and Quality Grade and Fatty Acid Composition

The marbling score distribution (Fig. 1) was 21% Slight (SL, n = 44), 34% Small (SM, n = 71), 17% Modest (MT, n = 37), 18% Moderate (MD, n = 36), and 10% Slightly Abundant (SA, n = 21), resulting in a quality grade distribution of 21% Select, 69% Choice, and 10% Prime. The same commercial processing facility reported that within 7,574 steers and heifers harvested in 2020, there were 17% of USDA Select, 72% USDA Choice, and 8% USDA Prime carcasses. Compared with the National Beef Quality Audit of 9,106 fed steer and heifer carcasses (NBQA; Boykin et al., 2016), carcasses in the current study had greater USDA marbling scores than the NBQA distribution of 4% Prime, 67% Choice, 23% Select, and 5.6% Standard and lower. Marbling score distribution in the 2016 NBQA audit had 0.9% Slightly Abundant or greater, 7.6% Moderate, 23.5% Modest, 39.6% Small, 23.6% Slight and 0.8% Trace or less. The Trace and Practically Devoid marbling scores were rare in the cattle selected for the current study. Based on the structure of the marbling scores used to assign the USDA quality grade, the

USDA Choice and Prime grades was spread within a wide range of marbling scores than the USDA Select.

The fatty acid composition (Table 1) was typical of beef marbling with predominant fatty acids being 16:0 (27 to 32%), 18:1 cis9 (25 to 31%), 18:0 (13 to 18%), and 18:2 cis9,12 (4 to 5%). There were differences in fatty acid composition among marbling categories, with SL carcasses consistently having less saturated and monounsaturated fatty acids and greater polyunsaturated fatty acids ($P \leq 0.009$). Although the saturation index, P/S ratio, and iodine value were similar among marbling categories ($P \geq 0.269$), the SL carcasses had 2.5, 3.2, 3.9, and 4.0 % more PUFA than SM, MT, MD, and SA carcasses, respectively ($P < 0.001$). Moreover, there was approximately 6.4 to 8.4% branch-chained fatty acids, being reported for the first time in beef intramuscular fat.

The USDA FoodData Central reports that USDA Prime, Choice, and Select strip steaks have approximately 45 to 46% of SFA, 49% MUFA, and 4 to 6% of PUFA, respectively. Litwińczuk et al. (2015) also reported 49 to 56% of SFA, 38 to 49% of MUFA, and 2 to 7% of PUFA in intramuscular fat of Friesian Holstein heifers, cows, young bulls, and calves, similar to what were found in the current study. Dinh et al. (2010) reported 3 to 8% of PUFA for Angus, Brahman, and Romosinuano cattle, with leaner carcasses having greater PUFA percentage. In leaner carcasses, phospholipids in lean tissues play a more important role in fatty acid composition. Legako et al. (2015) reported smaller percentages of PUFA in neutral lipid fraction (triglycerides) compared with that in the polar lipid fraction. As ruminant animals deposit fat, more SFA and MUFA are accumulated because of the biohydrogenation in the rumen (Vahmani et al., 2015) and the elongation and the Δ^9 desaturase activity in adipose and muscle tissues (Smith et al., 2006). Although SI, P/S, and IV values did not differ among marbling categories, the greater PUFA percentage could delay the solidification of marbling in the SL carcasses.

Temperature, Shrinkage, pH, and Aerobic Plate Count

Regardless of whether a beef side was ribbed or not, the temperature of the *longissimus* muscle started at 37.5 to 39 °C and reached 3 °C after approximately 16 h (Fig. 2). The temperature did not change further up to 96 h. Postmortem chilling of beef carcasses is to ensure food safety, maximize shelf-life, and reduce shrinkage with little emphasis on beef quality attributes (Savell et al., 2005). Aalhus et al. (2001) reported conventionally chilled carcasses reached 4ºC at 24 h, similar to the current study. However, Sorheim et al. (2001) used similar chilling conditions to those in the current study and reported that carcasses reached 4 to 5°C at 10 h postmortem when being cut at the 10th and 11th thoracic vertebrae and behind the 5th sacral vertebrae, through the shaft of the *ilium* of the pelvic bone at approximately 2 cm cranial to the hip bowl. The data in the current study indicate that regardless of whether a carcass is ribbed, the pattern of temperature decrease remains the same during spray chilling with the final internal temperature was reached before 24 h. Therefore, the findings of fat solidification and marbling score in the current study are applicable to the normal production settings, in which a beef carcass remains intact until a specific time when it is ribbed for grading.

There was a two-way marbling category × time interaction for shrinkage (Table 2; $P = 0.002$). Carcasses in SA, MD, MT, and SM categories had similar shrinkage at 24 and 96 h ($P \geq 0.083$).

At 48 h, MD, MT, and SM shrinkage was 0.5% to 0.7% ($P \leq 0.007$), then shrinkage increased to 0.9% to 1.2% at 72 h ($P \leq 0.001$). The SL shrinkage differed slightly from other categories by 96 h (-0.4%, $P = 0.031$), although SL shrinkage at 48 and 72 h changes were similar to that of other categories ($P \leq 0.192$). There was a two-way marbling category × time interaction for pH (Table 2; $P \leq 0.001$). The MT and SL carcasses reached an average ultimate pH of 5.56 at 24 h and the pH of the *longissimus* muscle did not change during cold storage ($P \geq 0.079$). However, for SA, the pH declined from 5.64 at 24 h to 5.57 at 48 h ($P = 0.034$) but increased back to 5.64 and 5.69 at 72 and 96 h ($P = 0.025$ and < 0.001, respectively). For MD, the pH reached 5.57 at 24 h and increased to a final value of 5.65 to 5.68 in subsequent time points ($P \leq 0.001$). For SM, the pH slightly dropped to 5.57 at 72 h ($P = 0.010$) but increased back to 5.62 at 96 h ($P = 0.011$), which was similar to that at 24 h and 48 h ($P = 0.124$ and 0.981, respectively)**.** At 24 h, the APC value was 0.1 log, which gradually increased to 0.7 log by 96 h ($P < 0.001$; Fig. 3).

The Meat and Poultry Inspection Program (MPI) Regulations, Sections 301.2(c)(8) and 318.4(d), FSIS Directive 6340.1, and FSIS Directive 8830.1 state that cold carcass weight (CCW) may not increase by more than 2% of the hot carcass weight (HCW) if spray chilling is used. In the current study, carcass shrinkage ranged from 0.5 to 1.5% over a 96-h period with a rare -0.4% for SL carcasses at 96 h. This finding was consistent with reports from Jones and Robertson (1988), Strydom and Buys (1995), and Schweihofer (2011), who observed 0.8 to 2.0% shrinkage in North American carcasses in commercial operations that used spray chilling. During 48-h spray chilling, Kinsella et al. (2006) found that carcass shrinkage was 1.6 % of HCW, slightly more than what was observed in the current study. Greer and Jones (1997) found a linear relationship between the duration of spray chilling and carcass weight loss and concluded that carcass shrinkage only decreased by less than 0.1% for every hour of spray chilling. It is well known that the chilling system is successful at preventing 1 to 2% weight loss by evaporation, depending on how chilling cycles are arranged. However, weight gain may occur because of water absorption (Prado and Felicio, 2010). These findings assure that if prolonged chilling is needed to increase marbling score, there will be minimal impacts on cold carcass weight.

The normal pH for beef carcasses ranges from 5.40 to 5.59, which allows for the development of desirable meat quality traits (Page et al., 2001 and Viljoen et al., 2002). Van Moeseke et al. (2001) measured pH of beef carcasses at 1, 5, and 24 h postmortem at a commercial facility and reported pH values of 5.45 to 5.71, which are similar to the values reported in the current study. Reid et al. (2017) documented a decrease in pH from 6.17 to 5.57 from 24 to 96 h, which is slightly greater than the carcass pH values observed in the current study. However, the pH values observed in the current study are similar to what has been reported in the literature (Zhang et al., 2018; Emerson et al., 2013; Savell et al., 2005). Meat pH affects quality attributes such as color and water-holding capacity (WHC) (Jacob and Hopkins 2014; Kim et al., 2014). Meat with high pH due to the depletion of glycogen stores pre-harvest fails to develop bright cherry red lean color due to increased WHC (Zhang et al., 2018; Holman et al., 2016). Water-holding capacity affects the reflectance of light on the surface of meat and the visibility of marbling (Hughes et al., 2014; Swatland, 2013). Ijaz et al. (2020) observed the effect of high pH typically associated with dark, firm, and dry beef across multiple storage days. The authors reported that the pH deceased within the first 24 h but continued to increase as holding time increased. The authors observed an increase in lightness, redness, and chroma from days 1 to 3 in these carcasses. The pH in the current study was not as high as what was reported for DFD (dark, firm, and dry) beef and although there was a fluctuation of pH in some marbling categories, such changes was too minimal to produce effects on lean color.

The APC value observed in the current study was less than what has been reported for beef carcasses. Ahmad et al. (2013) sampled 100 cm^2 of the forequarter and hindquarter of the beef carcasses and reported an APC value of 2.8 log. Hauge et al. (2015) reported 4.3 to 4.5 log after 24-h chilling. However, these authors did not use spray chilling with antimicrobials. Reyes et al. (2018) used 1,3-dibromo-5,5-dimethylhydantoin in spray chilling and reported a reduction from 3.0 to less than 1.2 log CFU/cm^2 at 24 h. When other antimicrobials such as chlorine dioxide and peroxyacetic acid were used, there was a similar reduction of 2.6 to 4.0 log from 24 to 72 h (Kocharunchitt et al., 2020). Spray chilling is combined with antimicrobial interventions to control of bacterial growth on the wet surface of carcasses not only during chilling but also before fabrication (Dickerson and Anderson, 1992). The findings in the current study indicated that prolonged chilling up to 96 h did not any significant impacts on microbial growth on the surface of beef carcasses.

Lean Color

There was a two-way marbling category × time interaction for lightness (L*, $P = 0.007$). The SA, MD, MT, and SM categories had increased lightness from 24 h (41.6 to 43.5) to 96 h (45.1 to 46.2; $P \leq 0.037$). The SL category had similar lightness from 24 h to 96 h ($P > 0.170$) There were an overall marbling and time effect on redness (a*; $P = 0.002$ and 0.042, respectively; Fig. 4). Carcasses with SA, MD, MT, and SM marbling scores all had similar redness values from 29.1 to 30.4 ($P \leq 0.057$. Carcasses with SL marbling scores had the lowest redness value of 28.7 ($P \leq 0.001$). Carcasses at 48 and 72 h (30.0 and 29.9, respectively) had greater redness than that at 24 and 96 h (29.6 and 29.1, respectively; $P \leq 0.001$). There was a two-way marbling × time interaction for yellowness (b*; $P = 0.005$). Carcasses from SA, MD, MT, and SM all had similar yellowness from 24 to 96 h (28.5 to 30.3, 21.5 to 22.7, 22.1 to 23.1, 21.5 to 22.9, respectively, $P < 0.071$). The SL carcasses had a decreased yellowness by 1 to 1.5 units from 24 to 48 h and again from 72 h to 96 h ($P \leq 0.021$). The SA, MD, MT, and SM having similar chroma values of 36.3 to 37.9 ($P \leq 0.820$). The SL carcasses had the lowest chroma value of 35.5 ($P \leq 0.001$). No difference in the hue angle was found ($P = 0.082$).

Gagaoua et al. (2018) used illuminant D65, 8 mm diameter aperture, and 10° observer angle and reported 24-h L*, a*, b*, and chroma values of 32.4, 18.9, 18.7, and 26.6, respectively, under similar environmental conditions to those in the current study. The D65 illuminant simulates natural daylight and highlights blueish tones while subduing green and red tones (Salueña et al., 2019), yielding slightly less redness value compared with the A10 illuminant. Using the same instrumental settings as those in the current study to measure lean color of USDA Choice *longissimus* muscle under simulated retail display, King et al. (2012) found that L* decreased from 41 units at 24 h to 39 units at 96 h, that a* decreased from 34 units at 24 h to 30 units at 96 h. Chroma value also similarly decreased from 24 to 96 h. These values were similar to what was found in the current study. Although there were changes in lean color from 24 to 96 h, such changes are not detectable by consumers because of the small magnitude. Additionally, although myoglobin oxidation may affect surface color, the ribeye surfaces in the current study were refaced before grading and color measurement. Therefore, the effects of oxidation on lean color in the current study were minimal. Kirchofer et al. (2002) found that as chilling time increased a* and b* in carcasses at two commercial facilities. These authors reported an a* value of 32.5 at 24 h and 33.9 to 35.0 at 48 h, similar to those in the current study. However, King, Shackelford, and Wheeler (2011) documented a progressive decrease in redness as holding time increased, which was caused by oxidation, although such a phenomenon was unlikely to occur in the current study due to the refacing of evaluated ribeye surfaces. The color development is impacted by an array of carcass and plant operating practices (Kirchoferet al., 2002). The pH decline postmortem generally increases lightness (Hughes et al., 2014). However, changes in pH from 24 to 96 h in the current study were minimal. Orcutt et al. (1984) reported that L*, a*, and b* values did not differ between 24 and 48-h chilling durations in electrically stimulated beef carcasses; however, in those that had not been, the L* and b* values were higher and a* values were lower at 48 h compared with 24 h, which was similar to what occurred in SL carcasses in the current study (not electrically stimulated). Veirck et al. (2018) reported that Choice steaks with more marbling were darker in color than Select steaks at 24 h. Chilling duration up to 96 h induced minimal changes in lean color; however, such minimal changes in SL carcasses compared with carcasses in other marbling categories might influence the perception of the lean color background, upon which, marbling content was scored.

Effects of Chilling Time on Marbling Score

There was a two-way marbling × time interaction ($P < 0.001$; Fig. 5). For SA, the marbling score was 840 at 24 h, which decreased to 796 at 48 h and 797 at 96 h ($P = 0.004$). The MD was 743 at 24 h, which continued to decrease to 685 at 48 h, 694 at 72 h, and 683 at 96 h ($P \leq 0.001$). At 24 h, the MT was 635, which decreased to 597 at 48 h ($P < 0.001$), increased back to 621 at 72 h (*P*

= 0.032), then continued to decrease to 590 at 96 h ($P \leq 0.001$). For SM, marbling score was 539 at 24 h, decreasing to 523 at 48 h ($P = 0.042$) and finishing at 505 at 96 h ($P < 0.001$). The SL started at 442 at 24 h, increased to 450 at 48 and 72 h, and continued to increase to 469 at 96 h ($P < 0.001$).

Acheson et al. (2018) found six anatomical locations in the *longissimus* dorsi muscle with marbling varying from 504 to 565. Early studies (Blumer et al., 1962; Cook et al., 1964; Cross et al., 1975) indicated that variation in marbling located throughout the rib and loin sections of beef carcasses exists; however, the pattern has not been determined. Although the ribeye surface was refaced for each grading time, only 3 mm of lean was removed. Acheson et al. (2018) reported that marbling particles extended approximately 8 to 9 mm throughout the muscle. Thus, refacing the ribeye surface by approximately 9 mm over 96-h grading should not impact marbling pattern of the graded surface, especially given that marbling particles are distributed evenly throughout the *longissimus* muscle. Although dorsal marbling distribution in the beef *longissimus* muscle has not been researched, such distribution was reported to be even until the end of the thoracic vertebrae in pork *longissimus* muscle (Faucitano et al., 2004).

The overall hypothesis for the current study was that carcasses would continue to be chilled; thus, fat would continue to solidify and became more visible. As stated above, the internal temperature of the *longissimus* muscle was reach within 16 h, at 3°C, and remained constant after that. Historical temperature recording of carcass temperature in the processing facility (data not shown) indicated that final internal temperature was reached within 12 to 16 h postmortem and was not decreased further. Bowling et al. (1987) observed an increase in marbling score and a decrease in lightness in rapidly chilled carcass compared with conventionally chilled carcasses. Carcasses reaching -2 to 0°C in 5 h (rapid chilling) had more visible intramuscular fat, graded at MT^{95}, than those reaching the same temperature within 10 to 12 h (conventional chilling), graded at MT^{50}. Janz et al. (2004) used a similar chilling method to the one in the current study and reported a temperature of 5°C at 24 h postmortem. These authors observed an increase in marbling score of 10 to 110 points compared to the score of 527 at 48 h under modified chilling conditions of 5°C at 24 h and 0 to 2°C at 48 h. These authors also reported that carcasses of SM or lower marbling scores had a greater increase in marbling score than other greater marbling score categories, which was similar to the findings for SL category in the current study. The authors attributed such changes to an increase in chilling time; however, they could not conclude whether the longer holding time or the modified chilling method increased the solidification and visibility of the intramuscular fat.

In the current study, the decrease in marbling score in SA, MD, MT, and SM and the increase in marbling score in SL might be attributed to the slight changes in lightness and saturation (chroma) of the lean color background, as well as the less saturation of fatty acid composition in leaner carcasses. Page et al. (2001) and Wulf et al. (1994) observed that darker lean color is negatively correlated with the L* value. Bak et al. (2012) stated that the chroma and hue angle can be used to reflect the saturation and the shade (perceived color) of lean meat color. In the current study, there was no change in the perceived color (hue angle), which indicated the ribeye surface would be perceived in similar shade of redness. However, the redness saturation was decreased in the SL category, indicating redness was being diluted. Malau-Aduli et al. (2000) also reported less color saturation with lower marbling scores in Angus and crossbred steers and heifers, similar to what was found for SL carcasses in the current study. As discussed in fatty acid composition, leaner carcasses have a greater proportion of PUFA because phospholipids of lean tissues are more predominant (Legako et al., 2015); whereas in fattier carcasses, more SFA and MUFA are being deposited in adipose tissues (Dinh et al., 2010). Saturated fatty acids solidify at 20 to 22°C, whereas unsaturated fatty acids become solid at below 20°C (Moorthy, 2018). Yang et al. (1999) found that subcutaneous fat in carcasses transitions to a solid state between 8 and 15°C with many carcasses having fats transition from liquid to solid at 10°C for 18 to 20 h. The rate of fatty acid solidification depends on the length of the carbon chain and the existence of double bonds (Wood, 1984). The fatty acid analysis in the current study revealed a greater proportion of PUFA in SL carcasses. Although saturation index, P/S ratio, and iodine value indicated that most beef carcasses have their marbling solidified within 24 h of chilling at the current chilling rate, the more unsaturated marbling in SL carcasses might continue to solidify up to 96 h of chilling. This might explain the increase in marbling score in the SL carcasses in the current study over a 96-h duration.

Conclusion & Implication

Prolonged chilling had minimal effects on marbling score and other carcass quality measurements because carcasses reached a final internal temperature of 3°C by 16 h postmortem and arrived at the final pH within 24 h, with minimal fluctuation until 96 h. Our results differ from industry standard chilling times (36 to 48 h) and recent audits by allowing carcasses an extra 48 h of chilling duration (96 h) to greater solidification of unsaturated fatty acids, potentially affecting marbling visibility and score. Carcasses with a marbling score of USDA Select can potentially be graded higher; however, carcasses with greater marbling scores can be graded lower after prolonged spray chilling for up to 96 h. These findings do not favor prolonged chilling (greater than 48 h) of USDA Choice or higher beef carcasses but favor such a practice for carcasses with USDA Select marbling score as a method to improve marbling visibility and quality grade.

Tables and Figures

Table 1 Fatty acid composition (% of total fatty acids) of the *longissimus* muscle from beef carcasses having marbling score of Slightly Abundant (SA, n = 21), Moderate (MD, n = 36), Modest (MT, n = 37), Small (SM, n = 71), and Slight (SL, n = 44).

Fatty acids	SA	MD	MT	SM	SL	SE[d]	*P* value
SFA	49.63	51.23	49.48	49.59	48.42	1.80	0.486
10:0	0.07	0.07	0.11	0.06	0.05	0.02	0.192
12:0	0.08	0.08	0.09	0.07	0.06	0.01	0.060
14:0	3.01	3.30	3.36	2.85	2.35	0.26	< 0.001
15:0	0.37	0.37	0.35	0.31	0.35	0.03	0.069
16:0	31.72	31.38	30.66	29.46	26.69	1.01	< 0.001
17:0	0.93	0.88	0.79	0.70	0.70	0.06	< 0.001
18:0	13.34	15.01	13.99	15.98	18.05	1.13	< 0.001
19:0	0.05	0.05	0.05	0.06	0.08	0.01	< 0.001
20:0	0.07	0.08	0.08	0.09	0.12	0.01	< 0.001
22:0	0.01	0.01	0.00	0.01	0.02	0.01	0.314
MUFA	38.96	37.50	38.14	37.64	35.32	1.19	0.010
14:1 cis9	0.79	0.83	0.84	0.67	0.51	0.11	< 0.001
15:1 cis9	0.10	0.14	0.16	0.23	0.33	0.03	< 0.001
16:1 cis6	0.04	0.03	0.03	0.04	0.89	0.74	0.454
16:1 cis9	3.75	3.50	3.58	3.16	2.46	0.25	< 0.001
16:1 cis7	0.11	0.11	0.10	0.09	0.16	0.06	0.833
17:1 cis10	0.79	0.70	0.63	0.52	0.44	0.05	< 0.001
18:1 trans11	1.46	1.19	1.83	2.30	3.56	0.68	< 0.001
18:1 cis9	30.47	29.38	29.26	28.94	25.71	1.41	0.002
18:1 cis11	0.74	0.87	1.05	1.06	1.96	0.90	0.504
18:1 cis12	0.01	0.07	0.04	0.10	0.16	0.05	0.036
18:1 cis13	0.38	0.44	0.34	0.30	0.24	0.05	< 0.001
19:1 cis10	0.07	0.07	0.06	0.06	0.04	0.01	0.004
19:1 cis	0.03	0.02	0.02	0.01	0.01	0.00	< 0.001
20:1 cis11	0.23	0.15	0.17	0.16	0.11	0.03	0.002
PUFA	4.78	4.87	5.53	6.21	8.71	1.02	< 0.001
18:2 trans9,12	0.15	0.14	0.14	0.18	0.20	0.02	< 0.001
18:2 cis9,12	3.85	3.69	4.25	4.45	5.13	0.42	< 0.001
18:2 cis12,15	0.02	0.02	0.03	0.04	0.16	0.12	0.562
18:3 γ cis6,9,12	0.02	0.03	0.03	0.05	0.06	0.01	< 0.001
18:3 cis9,12,15	0.09	0.11	0.11	0.12	0.16	0.01	< 0.001
20:2 cis11,14	0.05	0.04	0.04	0.05	0.05	0.01	0.227
20:2 cis9,12	0.00	0.02	0.02	0.03	0.06	0.01	< 0.001
20:3 cis5,8,11	0.01	0.02	0.02	0.04	0.07	0.01	< 0.001
20:3 cis8,11,14	0.20	0.24	0.27	0.37	0.46	0.05	< 0.001
20:4 cis5,8,11,14	0.24	0.32	0.37	0.53	0.77	0.07	< 0.001
20:5 cis5,8,11,14,17	0.00	0.02	0.02	0.04	0.09	0.02	< 0.001
22:4 cis7,10,13,16	0.10	0.11	0.12	0.15	0.20	0.02	< 0.001
22:5 cis7,10,13,16,19	0.05	0.10	0.10	0.15	0.22	0.02	< 0.001
22:6 cis4,7,10,13,16,19	0.00	0.00	0.00	0.01	0.02	0.01	0.012
BCFA	6.63	6.40	6.85	6.56	8.42	1.02	0.068
14:0 13-methyl	0.05	0.07	0.09	0.07	0.14	0.06	0.308
15:0 14-methyl	0.09	0.09	0.09	0.10	0.12	0.01	0.001
16:0 15-methyl	2.56	2.58	3.01	2.95	3.52	0.41	0.057
16:0 14-methyl	3.75	3.50	3.58	3.16	2.46	0.16	0.041
P/S ratio	0.10	0.10	0.11	0.13	0.35	0.17	0.276
Saturation index	1.14	1.22	1.15	1.15	1.35	0.16	0.351
Iodine value	38.17	36.95	37.98	37.80	35.35	1.82	0.269

[a,b,c]: Within a row, means with different letters differ ($P \leq 0.05$)

SE[d]: pooled standard error

P: probability value

Table 2 Carcass shrinkage, pH, and lean color (lightness - L*, redness - a*, yellowness - b*, chroma, and hue angle) of beef carcasses with initial marbling score of Slightly Abundant (SA, n = 21), Moderate (MD, n = 36), Modest (MT, n = 37), Small (SM, n = 71), and Slight (SL, n = 44) at 24, 48, 72, and 96 h of spray chilling at 0 to 3°C with a wind speed of 3.1 m/s under 153-lux of fluorescent light in a commercial hot box.

Marbling category		Shrinkage	pH	L*	a*	b*	Chroma	Hue angle
SA	24 h	0.0^{b}	5.64^{a}	43.47^{b}	28.53^{ab}	21.27^{b}	35.63^{b}	36.59^{a}
	48 h	0.7^{a}	5.57^{b}	44.40^{ab}	29.81^{a}	22.18^{b}	37.16^{a}	36.63^{a}
	72 h	0.4^{b}	5.64^{a}	45.41^{a}	29.82^{a}	22.64^{a}	37.45^{a}	37.21^{a}
	96 h	0.1^{b}	5.69^{a}	45.34^{a}	30.28^{a}	23.56^{a}	38.39^{a}	37.86^{a}
MD	24 h	0.0^{b}	5.57^{b}	42.23^{c}	29.55^{a}	21.54^{b}	36.58^{a}	36.09^{a}
	48 h	0.7^{a}	5.68^{a}	44.33^{b}	30.00^{a}	22.53^{a}	37.53^{a}	36.88^{a}
	72 h	1.0^{a}	5.64^{a}	44.72^{b}	30.27^{a}	23.01^{a}	38.03^{a}	37.20^{a}
	96 h	0.3^{b}	5.65^{a}	46.15^{a}	29.69^{a}	22.66^{a}	37.36^{a}	37.31^{a}
MT	24 h	0.0^{b}	5.60^{a}	41.77^{b}	30.32^{a}	22.13^{a}	37.55^{a}	36.06^{a}
	48 h	0.5^{c}	5.65^{a}	44.25^{a}	30.70^{a}	22.44^{a}	38.07^{a}	36.28^{a}
	72 h	0.9^{a}	5.61^{a}	44.15^{a}	30.90^{a}	23.09^{a}	38.60^{a}	36.85^{a}
	96 h	0.0^{b}	5.62^{a}	45.52^{a}	29.85^{a}	22.49^{a}	37.38^{a}	36.96^{a}
SM	24 h	0.0^{c}	5.59^{ab}	41.63^{c}	29.51^{a}	21.53^{b}	36.54^{b}	36.11^{a}
	48 h	0.6^{b}	5.62^{a}	43.09^{b}	29.54^{a}	22.27^{a}	37.04^{ab}	36.93^{a}
	72 h	1.2^{a}	5.57^{b}	43.33^{b}	29.99^{a}	22.87^{a}	37.76^{a}	37.25^{a}
	96 h	-0.2^{c}	5.62^{a}	45.05^{a}	29.65^{a}	22.54^{a}	37.32^{ab}	37.15^{a}
SL	24 h	0.0^{c}	5.59^{a}	41.20^{a}	28.85^{a}	21.78^{a}	36.18^{a}	37.04^{a}
	48 h	0.5^{b}	5.58^{a}	42.05^{a}	28.80^{a}	20.64^{b}	35.47^{ab}	35.75^{a}
	72 h	1.0^{a}	5.56^{a}	41.54^{a}	29.26^{a}	21.91^{a}	36.65^{a}	36.55^{a}
	96 h	-0.4^{d}	5.56^{a}	41.62^{a}	27.77^{b}	20.22^{b}	34.37^{b}	36.02^{a}
SE		0.521	5.690	2.251	1.592	1.529	2.131	1.321
$P_{marbling}$		0.509	< 0.001	>0.001	0.002	0.003	< 0.001	0.175
P_{time}		< 0.001	0.673	>0.001	0.043	0.089	0.084	0.501
$P_{interaction}$		0.002	< 0.001	0.007	0.363	0.005	0.046	0.082

^{a,b,c}: Within a row, means with different letters differ ($P \leq 0.05$)

SE^{d}: pooled standard error

P: probability value

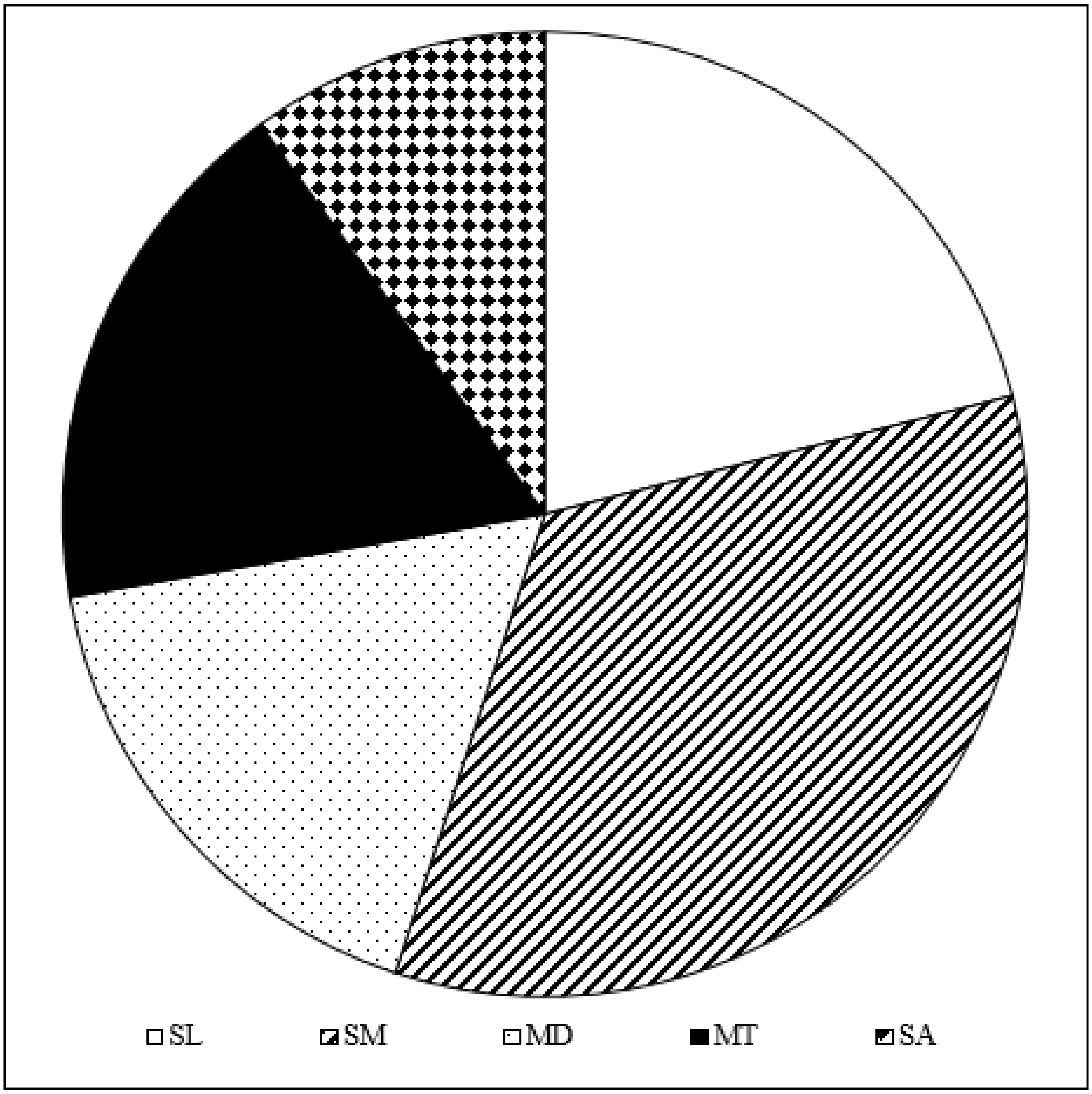

Figure 1 USDA marbling score composition 209 beef carcasses, including Slightly Abundant (SA, n = 21), Moderate (MD, n = 36), Modest (MT, n = 37), Small (SM, n = 71), and Slight (SL, n = 44), used to determine the effects of prolonged chilling on marbling score.

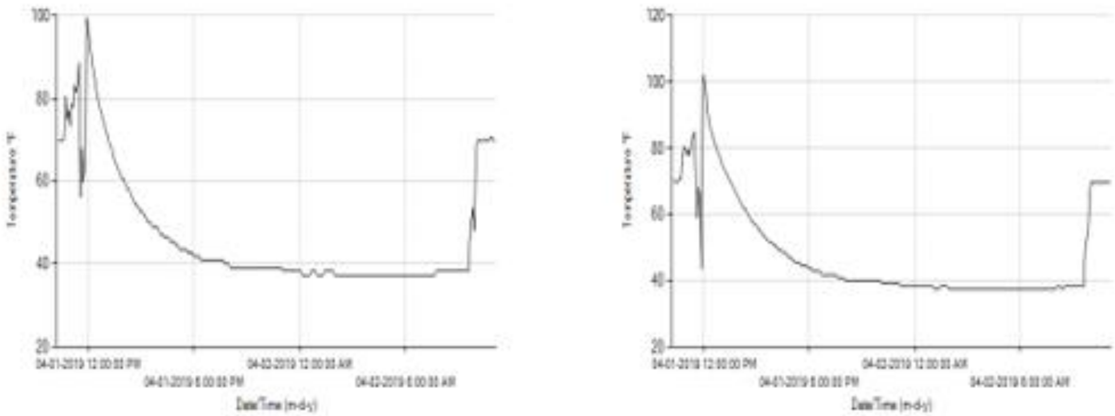

Figure 2 Core temperature of beef carcasses during chilling for 96 h at 0 to 3°C with a wind speed of 3.1 m/s under 153-lux of fluorescent light in a commercial hot box.

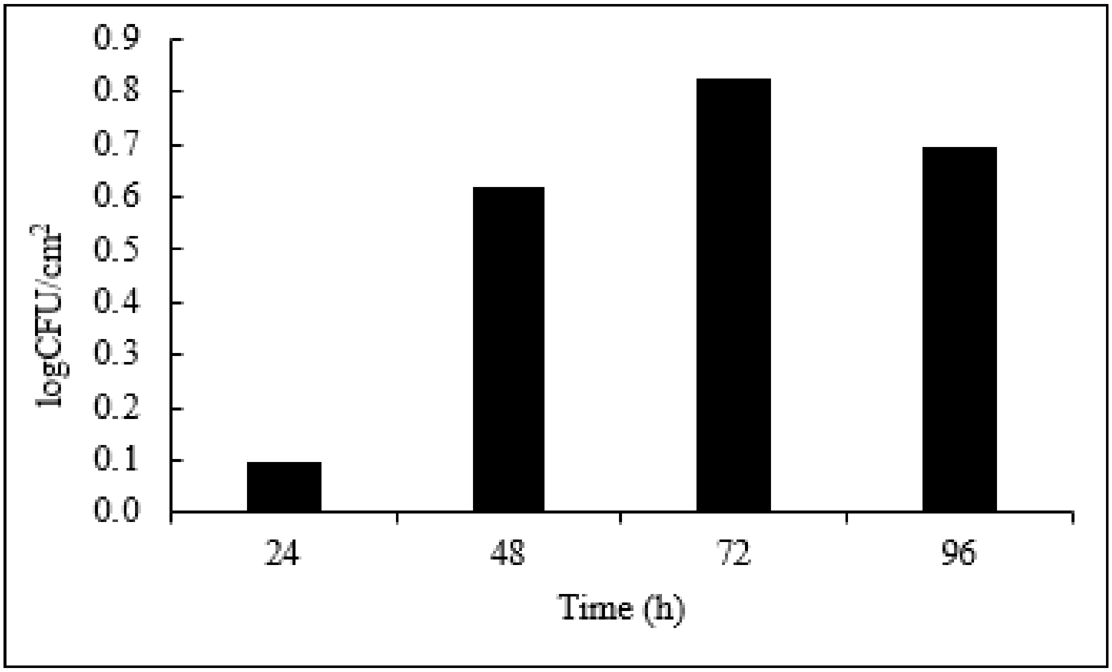

Figure 3 Aerobic plate count of 50 carcasses from data collection's 2 through 6 taken from a 12.5-cm × 12.5-cm area on the round and chuck of the carcass at 0 to 3°C with a wind speed of 3.1 m/s under 153-lux of fluorescent light in a commercial hot box at 24, 48, 72, and 96 h of spray chilling.

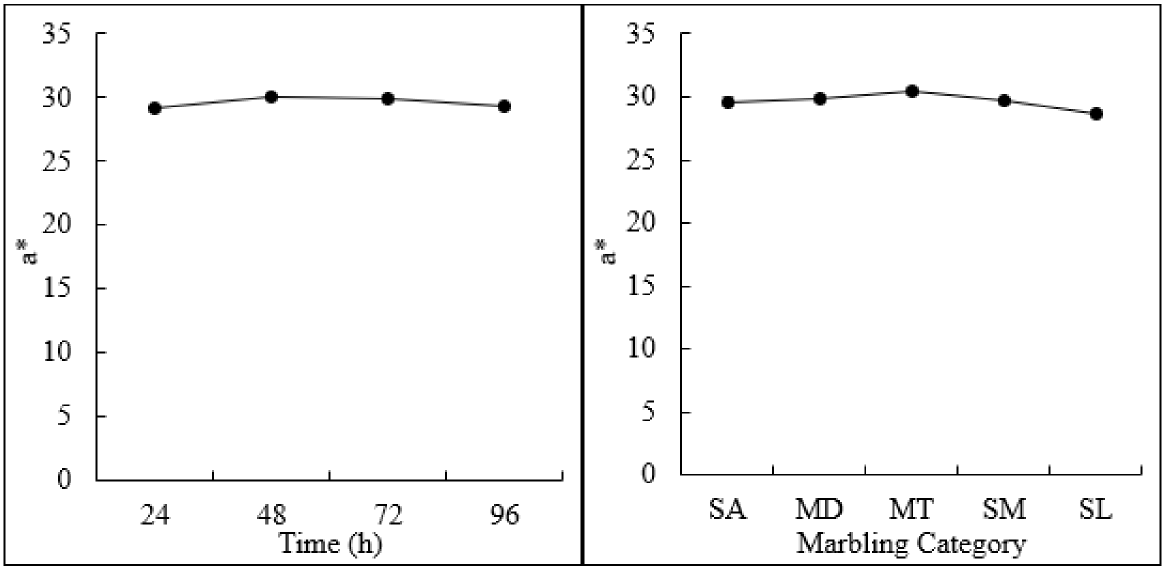

Figure 4 Redness (a*) of beef *longissimus* muscles of differentiated initial marbling scores, spray-chilled in a commercial hotbox under a wind speed of 3.1 m/s and 153-lux of fluorescent light, averaged across 24, 48, 72, and 96 h (5a) and across USDA quality grades of Slightly Abundant (SA, n = 21), Moderate (MD, n = 36), Modest (MT, n = 37), Small (SM, n = 71), and Slight (SL, n = 44).

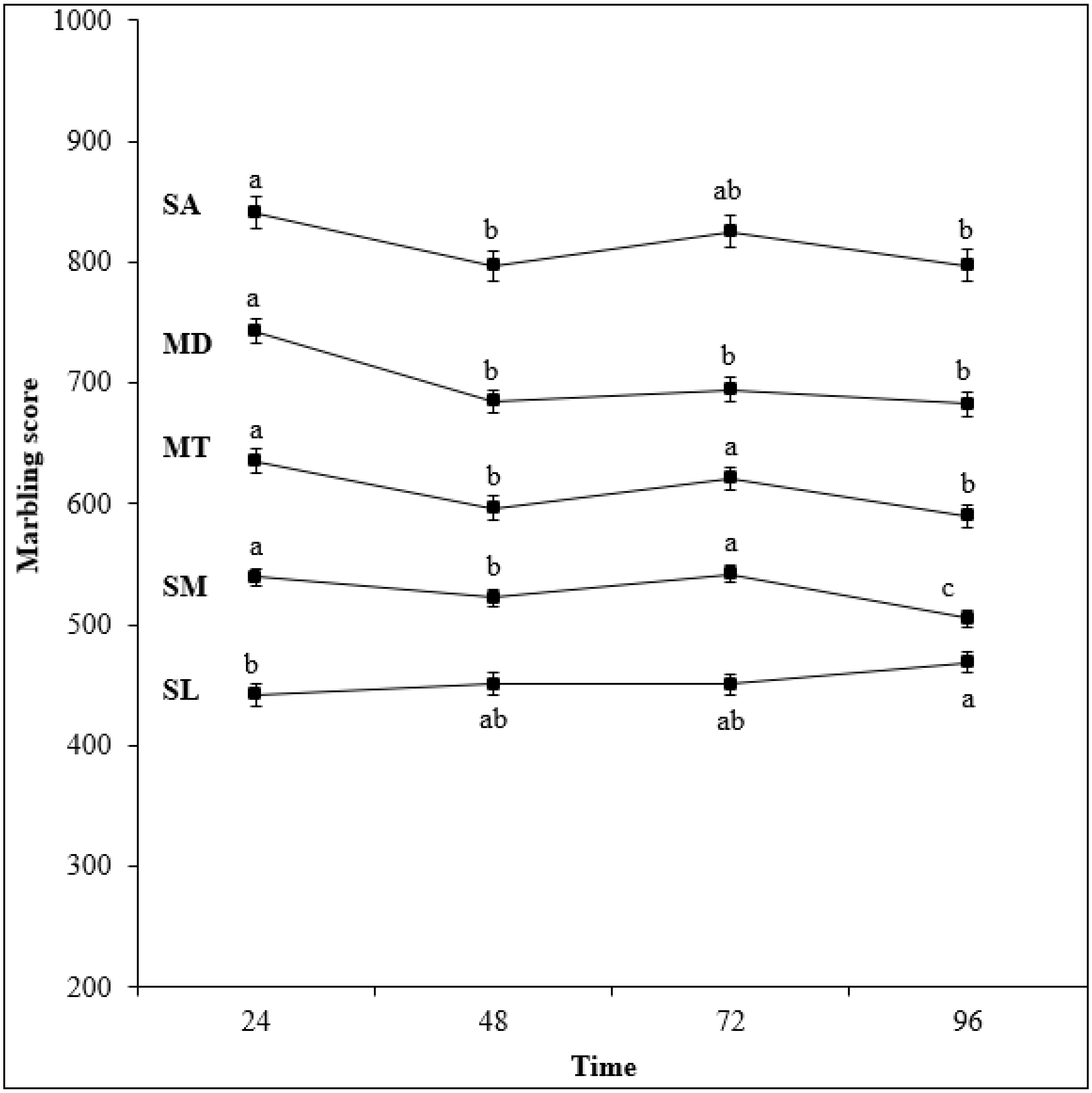

Figure 5 USDA marbling scores of beef carcasses graded Slightly Abundant (SA, n = 21), Moderate (MD, n = 36), Modest (MT, n = 37), Small (SM, n = 71), and Slight (SL, n = 44) at 24, 48, 72, and 96 h of spray chilling.

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