OPPORTUNITIES FOR ADDING VALUE AND INCREASING CONSISTENCY OF BEEF

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by

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Authorization to Submit Dissertation

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Abstract

Oversized beef carcasses and palatability improvements are an area of focus in the beef industry. The objectives of this research were to: 1) assess the effects of beef carcass size and its relationship to chill time, color, pH, and tenderness of the beef top round, 2) assess three aging periods of commercially available top rounds from varying carcass weights as it relates to retail shelf-life and color stability, 3) survey environmental parameters of commercial dry-aging facilities from selected regions of the United States, and 4) determine the effect of dry-aging parameter influences on eating quality of dry-aged beef. Eight industry average weight beef carcasses and eight oversized beef carcasses were evaluated for temperature and pH measurements for the initial 48 h postharvest. The top round anatomically deep location cooled at the slowest rates and had more rapid pH declines. Additionally, the deep location of top round steaks from oversized carcasses was the lightest and most yellow in color. Furthermore, the drastic color difference between the superficial and deep locations of the top round is further amplified as carcass weights increase. Retail characteristics varied with aging times, and differences were further amplified by carcass size. Alternative top round steak fabrication which separates the deep and superficial anatomical locations could be an effective means of providing consumers with more uniform steaks at the retail counter. Strip loins were aged at 11 treatment-aging locations for 45 days prior to being returned to the University of Idaho. Commercial dry-aging facility cooler conditions varied in cooler temperature, percent relative humidity, and wind speed. Consumer taste panel results identified differences in dry-aged steak acceptability, tenderness, and flavor based on location. The findings indicate that conditions within individual dry-aging facilities aid in

producing unique dry-aged beef flavors. Overall, the results of these studies indicate opportunities for adding value and increasing consistency of beef.

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Dedication

This dissertation is dedicated to my family. They have been a constant source of encouragement and support throughout my educational journey. Thank you for instilling in me the importance of hard work and passion for the education. I also want to thank my fiancé, Derek, for the constant support through graduate school and all that comes with it. Here is to the next great adventure!

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AD	Average Weight Beef Carcass – Deep Portion
AMSA	American Meat Science Association
APC	Aerobic Plate Count
AS	Average Weight Beef Carcass – Superficial Portion
AW	Average Weight Beef Carcass
CFU	Colony Forming Unit
CIE	Commission International de I'Eclairage
HCW	Hot Carcass Weight
IRB	Institutional Review Board
LSM	Least Squares Mean
MDA	Malondialdehyde
MMb	Metmyoglobin
MRA	Metmyoglobin Reducing Activity
OC	Oxygen Consumption Rate
OD	Oversized Beef Carcass – Deep Portion
OMb	Oxymyoglobin
OS	Oversized Beef Carcass – Superficial Portion
OW	Oversized Beef Carcass
SM	*Semimembranosus*
SSTC	Steak Section Treatment Combination
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
US	United States
USDA	United States Department of Agriculture

UV	Ultraviolent
WBSF	Warner-Bratzler Shear Force

CHAPTER 1

Review of Literature

Introduction

The 2016 National Beef Quality Audit identified the top six quality challenges to the beef industry as: food safety, eating satisfaction, lean fat and bone, weight and size, how and where cattle were raised, and visual meat characteristics (NCBA, 2016). Challenges associated with weight and size of carcasses and subsequent cuts has been identified in the five most recent National Beef Quality Audits (1995-2016) (NCBA, 2016).

The average beef carcass weight has increased over 61 kilograms (kg) at a linear rate over the past 30 years (USDA-ERS, 2019). The muscles of the round are noted as a problematic area that is challenging to uniformly cool due to mass (Hannula and Puolanne, 2004; Klauer, 2019). Personal communications with harvest facility personnel have indicated an increased prevalence of non-compliance with chilling regulations due to an uptick in oversized carcasses (personal communications, 2020). Additionally, the cooling challenge is further amplified by muscles in the round being noted for color and tenderness inconsistencies. At the retail level, top round steaks are noted for lacking color uniformity (Sammel et al., 2002b; Lee et al., 2007). It is essential to understand the implications of oversized carcasses on the cooling process and avenues to better accommodate the continuing trend of increased carcass size.

Eating satisfaction has ranked as the second top quality challenge in the two most recent National Beef Quality Audits (NCBA, 2016). Aging is a method to increase tenderness and allow for flavor development (FSIS, 2005). In the area of dry-aging, there are limited

publications and within those inconsistencies in experimental set up, analysis, and results exist. In addition, research on dry-aging has commonly compared dry-aged to wet-aged or evaluated the impact of the length of time product was dry-aged in a controlled meat research laboratory.

Further research is needed to 1) determine the temperature and pH declines of average versus heavy weight carcasses, 2) better understand the differences or lack thereof in top round steaks yielded for average and heavy weight carcasses, 3) optimize aging and fabrication strategies to make top round steaks more consistent in the retail case, and 4) determine the impact of the dry-aging environment on beef quality.

Transition of muscle to meat

During the harvest process, following exsanguination, the loss of circulating blood leads to several homeostatic changes: the shift of muscle cells from aerobic to anaerobic metabolism, loss of ability to remove waste products from tissues, loss of ability to regulate body temperature, and other disruptions to the entire biological system (Aberle et al., 2012). In the absence of oxygen, anaerobic metabolism is used to generate Adenosine Triphosphate (ATP) which is utilized by the cells for futile cellular functions (Ferguson and Gerrard, 2014). The first system to try and maintain ATP homeostasis is the phosphagen system using phosphocreatine (Matarneh et al., 2017). The phosphagen system does not directly contribute to pH decline, however, the system does produce metabolites (adenosine monophosphate, adenosine diphosphate, inorganic phosphate) that activate rate-limiting enzymes in the glycolytic pathway (Matarneh et al., 2017). Ultimately, the phosphagen system has limited capacity to maintain cellular ATP and glycolysis becomes the key pathway for ATP

production (Bendall, 1973). Glycolysis relies on glucose which is the predominant storage of carbohydrates in muscle. Lactate and hydrogen ions are products of glycolysis and ATP hydrolysis (Matarneh et al., 2017). Normally, these byproducts would be removed from functioning tissues via circulation and further metabolized in the liver, however, the loss of blood flow results in the accumulation of these products in the tissue (Matarneh et al., 2017) leading to pH decline.

Post-harvest the demand for ATP is high and demanded by several systems. Skeletal muscle utilizes ATP to break the actin-myosin bond in a contracted muscle and allows for muscle relaxation. As the ATP becomes limited, and eventually nonexistent, the ability to break the actin-myosin bonds is lost and rigor is obtained (Ferguson and Gerrard, 2014; Matarneh et al., 2017). The transition of muscle to meat and the intermediate metabolic pathways ultimately impact meat quality and eating experience.

Temperature Decline of the Beef Carcass and Top Round Subprimal

Chilling of carcasses is a time-sensitive process that is essential for food safety management, reducing carcass weight shrinkage, and providing an opportunity to optimize shelf-life (Savell et al., 2005). Thus, temperature declines are monitored as critical control points in Hazard Analysis Critical Control Points food safety systems utilized in the processing of beef carcasses (USDA-FSIS, 2017). Numerous factors intrinsic to the carcass (size, degree of muscling, amount of fat) and cooling environment (temperature, relative humidity, air movement, chilling methods) impact the chilling rate of beef carcasses (Savell et al., 2005).

Muscles within the carcass do not cool at a consistent rate. The chuck and round beef primals are two areas of the carcass that are the greatest in mass and volume (NCBA, 2014). The *Semimembranosus* (SM) cools at a slower rate than other muscles, such as the *Longissimus dorsi* (Hannula and Puolanne, 2004; Klauer, 2019). The SM starts at a higher temperature, cools at a slower chill rate, and remains at a higher final chill temperature than other muscles on the carcass (Klauer, 2019). Moreover, the rate at which cooling takes place can also be impacted by facility procedures and cooling parameters (Hannula and Puolanne, 2004; Klauer, 2019).

Muscle pH and pH Decline

The negative $\log_{10}$ of hydrogen is reported as pH (Lehninger et al., 2013). In living organisms pH is tightly regulated, and in general muscle pH is near 7.4 (Aberle et al., 2012). Muscle pH decline postmortem is not consistent across muscles and can be impacted by genetics, muscle fiber type, handling of the animal prior to harvest, and the management of the carcass (Matarneh et al., 2017). In the eight hours immediately postmortem the pH of the muscle in the carcass can drop from 7.2 to 5.8, and by 24 hours the pH is normally around 5.6 (Matarneh et al., 2017). However, the pH decline is not uniform within the carcass, at one hour postmortem the pH decreases to between 6.8 (1.5 cm from surface) and 6.5 (8 cm inside muscle) and will ultimately decline to around 5.5 to 5.6 (Tarrant and Mothersill, 1977). A final pH that deviates from normal (5.4 to 5.6) is unfavorable for meat quality (Matarneh et al., 2017).

Relationship of Muscle Temperature and pH

Temperature and pH are considered the largest sources of variation that impact meat quality (Aberle et al., 2012). The changes in temperature and pH in the first 24 hours postmortem visibly impact meat color as well as meat tenderness (Savell et al., 2005). Slow temperature declines after harvest can cause a more rapid pH decline which denatures muscle protein, and, therefore, reduced color stability (Tarrant, 1977; Seyfert et al., 2004; Ferguson and Gerrard, 2014). Color challenges include increased paleness (Sammel et al., 2002a; Sammel et al., 2002b) which has been partially attributed to an increase myosin denaturation (Hector et al., 1992) and myoglobin denaturation (Sammel et al., 2002b) due to the accelerated pH decline. Further, it has been demonstrated that high temperature and low pH conditions can disrupt enzyme activity, including calcium activated proteases (calpains), which could lead to a less-tender product (Koohmaraie et al., 1986; Melody et al., 2004; Barbut et al., 2008; Kim et al., 2018). It is well established that chemical reactions happen faster at higher temperatures. Thus, the slower temperature declines result in accelerated pH declines due to products and bi-products in the glycolytic pathway being used faster, which suggests temperature and pH are interrelated (Mohrhauser et al., 2014). Furthermore, heavier weight carcasses have greater mass to cool and pose challenges from a cooling standpoint with accelerated pH declines in combination with decelerated temperature declines compared to lighter weight carcasses (Aalhus et al., 2001; Agbeniga and Webb, 2018). Electrical stimulation, a method used to prevent cold shortening in beef carcasses, decreases the length of time glycolysis occurs and results in decreased pH (Ferguson and Gerrard, 2014). Given the additional mass and subsequent cooling challenges of the round, the use of electrical stimulation on oversized carcasses could further implicate meat quality challenges.

Meat Color

The color of beef is an important factor in purchasing decisions by consumers (Issanchou, 1996). Consumers associate a bright cherry-red lean color with freshness and quality in raw beef products (Suman et al., 2014). Products that deviate in color are less likely to be purchased by the consumer and cost the industry over a billion dollars annually (Smith et al., 2020). In general, the two methods for classifying meat color for research/quantitative purposes are visual evaluations and instrumental evaluations (Mancini, 2013). Visual color panels (subjective color) are used as a method of predicting consumer interpretation of meat color (Mancini, 2013). Evaluation of visual color utilizes established scales for each color category (AMSA, 2012). However, instrumental evaluations are often used as they are a more repeatable method that can quantify color observations (Mancini, 2013). Instrumental color evaluation is often reported in three dimensions which include units of L* (lightness), a* (redness), and b* (yellowness) (Mancini, 2013).

Color challenges associated with the beef top round have been attributed to myoglobin denaturation and decreased effectiveness of metmyoglobin reducing activity which results in greater discoloration at the deeper portion of the muscle, closer to the bone (Kim et al., 2010). When compared to other muscles from the chuck and round, the objective color scores of the SM were more red (a*) and more yellow (b*) (Von Seggern et al., 2005). Hunt and Henrick (1977) reported the deep portion of the top round subprimal was paler in appearance than the outer portion that had a darker red appearance. It is well established that consumers utilize meat color when making meat purchasing decisions.

Lipid Oxidation

Oxidation of lipids results in changes in color, odors, and ultimately sensory characteristics (Domínguez et al., 2019). Several factors influence the likelihood of lipid oxidation including the type of fat, presence of antioxidants and prooxidants, and storage conditions (Dave and Ghaly, 2011; Domínguez et al., 2019). The products of lipid oxidation are grouped into primary and secondary products. In primary oxidation there is an increase in hydroperoxides (Willian, 2013). Secondary lipid oxidation products include carbonyls, aldehydes, volatiles, and malondialdehyde (Domínguez et al., 2019). Malondialdehyde is a secondary product produced from the oxidation of unsaturated fats, and is considered the major marker of lipid oxidation in meat products (Domínguez et al., 2019). A challenge of measuring primary products is the volatility in the amounts of the products, thus measuring secondary products is more accurate and consistent (Willian, 2013; Domínguez et al., 2019).

Thiobarbituric acid reactive substances (TBARS) is a method commonly used for measuring lipid oxidation (Dave and Ghaly, 2011). In general, the method utilizes the color produced between the interaction of thiobarbituric acid and malondialdehyde (Domínguez et al., 2019). Several TBARS thresholds have been identified for detection of off-flavors (Tarladgis et al., 1960; McKenna et al., 2005; Campo et al., 2006). McKenna et al. (2005) suggested a value of 1.0 mg malondialdehyde/kg meat as a threshold for off-flavor detection in meat by consumers. Lipid oxidation has been reported to increase with length of retail shelf life (Sawyer et al., 2007) and aging of beef products (Colle et al., 2016). Despite this, research investigating top round steaks has indicated lipid oxidation is not likely a factor leading to

discoloration of top round steaks (Sawyer et al., 2007; Colle et al., 2016; Puga et al., 2019; Lancaster et al., 2020).

Meat Tenderness

Tenderness is generally defined as the amount of force required to bite through a piece of meat (Kerth, 2013). Beef tenderness is considered the most important sensory attribute to consumers (Huffman et al., 1996; Miller et al., 2001; Koohmaraie and Geesink, 2006). As reviewed by Koohmaraie and Geesink (2006) meat tenderness can be attributed to three factors: background toughness, toughening phase, and tenderization phase. Background toughness is attributed to connective tissue of the meat, and the toughening phase takes place during the transformation of muscle to meat during the establishment of carcass muscle rigor (Koohmaraie and Geesink, 2006; Kerth, 2013). The tenderization phase is the most variable of the tenderness factors and offers the greatest area for improvement and increased consumer satisfaction (Koohmaraie and Geesink, 2006).

An important factor in the tenderization phase is myofibrillar protein degradation (Kerth, 2013). Sacromeres are the smallest contractile unit of muscle and each sarcromere is designated by a z-line at the ends (Purslow, 2017). Titan and nebulin help to maintain elasticity and allignment within the sarcromere during skeletal muscle contaction (Horowits et al., 1986). Naturally occurring enzymes (calpains) degrade structural proteins, primarily titin and nebulin, at the connection point to the z-disc of the sarcomere (Kerth, 2013). Calcium-dependent proteolytic enzymes, calpains, are the primary enzyme type responsible for regulating skeletal muscle growth and the subsequent tenderization observed postmortem (Goll et al., 1998; Goll et al., 2003). Two types of calpains are of interest; calpain-1 and

calpain-2 and the calcium dependant inhibitor of calpains, calpastatin (Boehm et al., 1998; Goll et al., 2003; Koohmaraie and Geesink, 2006). Calpain-1 and calpain-2 were previously referred to as μ-calpain and m-calpain as a reflection of the amount of calcium required to activate the proteases (Goll et al., 1998). The original calpain naming system was based on the amount of calcium required for half maximal activity with μ-calpain needing 3 to 50 μM and m-calpain needing 400 to 800 μM of calcium (Goll et al., 1998). Additionally, in the process of tenderization calpains autolyze, break themselves down, limiting the extent of tenderization that can occur (Goll et al., 2003; Pomponio et al., 2008).

Carcasses that are chilled at a slower rate have decreased calpain-1 activity at 24 hours postmortem (Hwang and Thompson, 2001). The greatest percent of calpain-1 in the SM muscle is at day 2 (5.4%) and decreases to day 4 of aging, beyond four days the level becomes undetectable (Colle and Doumit, 2017). Reported levels of calpain-2 were constant from day two to four and then decreased out to day 28 but remained unchanged as aging extended out to day 42 (Colle and Doumit, 2017). Autolyzed calpain-2 begins to appear around d 3 and the percentage increases to day 42 (Colle and Doumit, 2017). Heavier weight carcasses have been observed to have a greater decline in calpain-1 activity from hour 1 to hour 24, but a slower decline in calpastatin levels (Agbeniga and Webb, 2018). Additionally, no difference has been observed between average and heavy weight carcass calpain-2 levels at 24 hours postmortem (Agbeniga and Webb, 2018).

Meat tenderness can be evaluated using objective or subjective measurements (Tornberg, 1996). Warner-Bratzler Shear Force (WBSF) is an objective means of measuring tenderness in meat, and the most widely utilized in the meat sciences (Kerth, 2013). Sample cores (1.27 cm) are removed parallel to muscle fibers of cooked steaks and sheared

perpendicular to muscle fibers (Wheeler et al., 2005). The force needed to shear through the meat is reflective of the tenderness (Wheeler et al., 2005). Though the WBSF methodology is intended for use on steaks from the *Longissimus*, the method can be utilized to classify other muscles (Shackelford et al., 1997).

Sensory evaluation by consumers is a method for better understanding consumer acceptance of meat products (Miller, 2017). Consumers have the ability to differentiate between steaks from different tenderness categories. Miller et al. (2001) reported when WBSF values go from 4.3 to 4.9 kg consumers reported steaks transitioned from slightly tender to slightly tough (Miller et al., 2001). A WBSF value of < 3.9 kg has been established by the USDA as the threshold for the USDA *Certified Very Tender* level (ASTM, 2011). Despite being able to measure tenderness both objectively and subjectively, additional factors like flavor and juiciness still play an important role in overall acceptability of steaks.

Nonconforming Beef Carcasses

Grid based marketing systems apply value to carcasses based on quality and yield grade and provide premiums or discounts based on alignment with industry targets (Tatum et al., 2006). These authors described carcasses that didn't fit within industry targets as "nonconforming carcasses". The category of nonconforming carcasses includes carcasses from animals that are extreme for carcass weight (light or heavy), have advanced maturity, dark cutters, and carcasses produced by nontraditional sources (bullocks, stags, heiferettes) (Tatum et al., 2006). Despite discounts being applied when carcasses exceed 408 kg, the average annual finished beef carcass weights continue to increase at a linear rate (USDA-NASS, 2020; Figure 1.1). Carcass weight provides the greatest influence of net carcass value

(Trenkle, 2001). Based off premiums and discounts applied through the grid based system, average sized carcasses have a greater value per hundred weight (USDA-NASS, 2020). However, when the total carcass value is taken into consideration, oversized carcasses often offer a greater net value (Table 1.1).

The percentage of beef carcasses exceeding 454 kg has increased to around 12.4% of the carcasses observed in the 2015 National Beef Quality Audit (Boykin et al., 2017). Thus, beef carcasses that are considered oversized in current research are much larger (≥ 431 kg) (West et al., 2011; Djimsa et al., 2018; Fevold et al., 2019; Foster et al., 2019; Klauer, 2019; Lancaster et al., 2020) than carcasses from previous decades (Savell et al., 1979). The current research in oversized beef carcasses aligns with the price deduction on grid-based marketing systems. Currently, discounts begin being applied when carcasses reach 408 kg, with the most extreme discount being applied when carcasses exceed 476 kg (USDA-AMS, 2020).

Challenges of heavy weight beef carcasses in packing plants have been reported in the chilling (Djimsa et al., 2018; Fevold et al., 2019; Klauer, 2019; Lancaster et al., 2020), fabrication (West et al., 2011), and final product stages (West et al., 2011; Foster et al., 2019). Heavy weight carcasses have been reported to have higher USDA yield grades (Fevold et al., 2019; Klauer, 2019), larger ribeyes (Fevold et al., 2019; Klauer, 2019; Lancaster et al., 2020), and longer carcass lengths (Klauer, 2019). Fabricating oversized carcasses takes a greater amount of time but results in greater saleable yields (West et al., 2011). Oversized carcasses pose challenges for retailers and the food service industries due to the larger steaks and cuts compared to those from more average weight carcasses (West et al., 2011). Despite size challenges from a merchandising standpoint, it has been reported that steaks from oversized carcasses have similar WBSF values (Agbeniga and Webb, 2018; Fevold et al., 2019; Foster

et al., 2019; Lancaster et al., 2020) or decreased WBSF values (Djimsa et al., 2018; Foster et al., 2019) compared to average weight carcasses.

Top Round Subprimal

The beef top round (NAMI #169; NAMI, 2014) can represent between 5.37 and 6.20% of the weight of a beef carcass (Kellermeier et al., 2009). The average weight of a beef carcass in 2020 was 411 kg (USDA NASS, 2021). If the top round makes up 6% of a beef carcass, a carcass weighing 411 kg would produce top rounds weighing 12.33 kg on average ([411 x .06]/2). The 2016 National Beef Quality Audit reported that 12.4% of beef carcasses observed had hot carcass weights exceeding 454 kg with a maximum weight of 615 kg (Boykin et al., 2017). Top rounds from carcasses ranging from 454 to 499 kg, at 6% of carcass weight, will weigh between 14 and 15 kg, respectively. Despite this increase in weight, little work has been completed on the impact of upper limit carcass size on meat quality especially with regards to retail presentation and eating characteristics.

In the classification of chuck and round muscles, Von Seggern (2005) found that carcass weight had a greater impact on objective muscle color than either quality or yield grade. Large muscles like the SM exhibit color variation within the muscle (Sammel et al., 2002a; McKenna et al., 2005; Lee et al., 2007; Fevold et al., 2019; Puga et al., 2019). The portion of the SM closer to the femur bone has been reported to be lighter (greater L*), less red (lower a*), and more yellow (greater b*) in color compared to the more superficial portion (Sammel et al., 2002a; Seyfert et al., 2006; Lancaster et al., 2020).

The National Beef Tenderness Survey indicated the average aging time for top rounds was 23.2 days, but ranged from eight days to 100 days (Henderson et al., 2016). Top round

steaks have a linear tenderness response to an increase in aging time ($r^2 = 0.82$), and should be aged at least 16 days to achieve optimal tenderness (Weatherly et al., 1998). The impact of days of aging varies as top rounds sourced from USDA Select carcasses increased in tenderness up to day 21, while those sourced from USDA Average and High Choice did not show improvement past day 14 (Gruber et al., 2006). Numerous studies have evaluated tenderness characteristics of the SM (Mc Keith et al., 1985; Reuter et al., 2002; Colle et al., 2016; Colle and Doumit, 2017; Puga et al., 2019; Lancaster et al., 2020). In a review of published WBSF data, Sullivan and Calkins (2011) concluded the SM was an intermediately tender muscle when using the previously established values of 3.9 to 4.6 kg established by Shackelford et al. (1991) and Huffman et al. (1996). Furthermore, there is variation within the SM muscle. More specifically, WBSF values are lower at the origin of the SM muscle and greater at the insertion of the SM (Reuter et al., 2002). In addition, the superficial location (more exterior) was more tender than the deep location of the steaks that is located closer to the femur bone (Reuter et al., 2002; Puga et al., 2019). Better understanding the characteristics of the SM muscle allows for additional opportunities in merchandising and consumer satisfaction.

Aging

Aging is the process of allowing for meat to become more tender and flavorful via enzymatic postmortem degradation of structural proteins (FSIS, 2005). The regulatory definition of aging requires the product to be held at refrigerated, nonfrozen, temperatures for a minimum of 14 days to allow for flavor development and enzymatic tenderization (FSIS, 2005). Aging is often a favorable process as it decreases mechanical tenderness values and

increases beef flavor and desirability without impacting juiciness of the product (Jeremiah and Gibson, 2003).

Two methods of aging are utilized in the meat industry: wet-aging (aged in protective packaging) and dry-aging (aged in the absence of protective packaging and exposed to a refrigerated environment). The topic of which aging process (wet-aging or dry-aging) is superior has been a continued debate that is influenced by production factors and consumer preferences (Bauer, 1983). Early research found wet-aging and dry-aging did not have differences in mechanical tenderness of products aged for the same length of time (Minks and Stringer, 1972; Oreskovich et al., 1988; Warren and Kastner, 1992). While dry-aged beef is noted for offering unique flavor attributes, it differs from the more conventional wet-aged beef flavor many consumers are used to (Bauer, 1983).

Dry-Aging Parameters

In a review by Dashdorj et al., (2016) dry-aging meat is impacted by a combination of important factors including the length of time the product is aged for, temperature at which the product is aged, relative humidity of the aging cooler, and wind velocity of the cooler. The length of time product is dry-aged for varies greatly in published literature and ranges from 7 to 60 days (Table 1.2). Reported cooler conditions for dry-aging meat have also greatly varied in temperature, relative humidity percentage, and wind velocity (Table 1.2). Minks and Stringer (1972) reported that dry-aging at 4°C resulted in taste panelists reporting a more tender eating experience than product dry-aged at 0°C, while no difference was observed in wet-aged counterparts. In addition, dry-aging product at a warmer temperature resulted in a lower percentage of weight loss over the aging period in comparison to product aged at 0°C

(Minks and Stringer, 1972). In addition, studies in the past have evaluated dry-aging using ultra-violet (UV) lights limits microbial growth (DeGeer et al., 2009; Lepper-Blilie et al., 2016; Capouya et al., 2019).

A compilation of analysis conducted in published dry-aged papers are reported in Table 1.3. Cooler conditions and aging time of previous studies have resulted in variations in yield losses between 4.37% and 17.00% (Minks and Stringer, 1972; Miller et al., 1985; Parrish et al., 1991; Warren and Kastner, 1992; Laster, 2007). Aging boneless subprimals has been noted to have greater moisture loss when compared to bone-in counterparts (DeGeer et al., 2009; Lepper-Blilie et al., 2016). As the aging period is extended there is greater trim loss on the product and ultimately a lower edible yield (Laster, 2007; Smith et al., 2008; DeGeer et al., 2009; Lepper-Blilie et al., 2016). In addition, fabrication of dry-aged products takes a greater amount of time than wet-aged counterparts (Laster, 2007; Smith et al., 2008). Ultimately, the calculated profit margin was estimated to be greater from product dry-aged 14 days compared to product aged for greater lengths of time (Smith et al., 2008).

There is often no mechanical tenderness difference between dry-aged and wet-aged product (Minks and Stringer, 1972; Oreskovich et al., 1988; Warren and Kastner, 1992; Smith et al., 2008; Berger et al., 2018; Oh et al., 2018; Kim et al., 2019). However, conflicting tenderness evidence exists. Laster (2007) reported that wet-aged product was more tender than dry-aged product and Kim et al. (2013) reported dry-aged beef was more tender than wet-aged counterparts. After the dry-aging period, products can be stored in a vacuum packaged environment (DeGeer et al., 2009) and frozen (da Silva Bernardo et al., 2020) without impacting product integrity. Steaks fabricated from dry-aged beef have been reported to have lower L*, a*, and b* values (Kim et al., 2013) and non-middle meat cuts increase L*

(Kim et al., 2019). Miller et al. (1985) reported greater retail case discoloration and slight to moderate odor in dry-aged steaks when compared to wet-aged steaks.

Method of aging had no impact on ultimate pH (Parrish et al., 1991; Berger et al., 2018; Oh et al., 2018; Kim et al., 2019; da Silva Bernardo et al., 2020), proximate composition (Parrish et al., 1991), chemical flavor desirability (Parrish et al., 1991), protein degradation (Parrish et al., 1991), free fatty acids (Oh et al., 2018) and glutamic acid levels (Oh et al., 2018). However, Sitz et al. (2006) and Berger et al., (2018) found dry-aged beef had a greater percentage of ash and protein while wet-aged counterparts had a greater percentage of moisture and fat post aging measured via proximate analysis. Meanwhile, there has been reported lower moisture (Kim et al., 2013; Berger et al., 2018; Oh et al., 2018; Kim et al., 2019) and protein content (Kim et al., 2013; Kim et al., 2019) in dry-aged beef as well as greater fat content (Kim et al., 2010; Kim et al., 2013). Proximate analysis varies by muscle location (Kim et al., 2019). Kim et al., (2013) reported dry-aged beef had a higher pH than wet-aged beef. Dry-aged beef had greater myofibrillar fragmentation index and in-vitro protein digestibility (Kim et al., 2013). Most volatile compounds increase in concentration as the aging window extends (Ha et al., 2019). Additionally, the dry-aging process results in greater levels of volatile compounds, alcohols, and nitrogen-containing compounds than wet-aged counterparts aged for a similar number of days (Ha et al., 2019).

Dry-aging of carcasses has been observed to produce a more tender and juicier product compared to wet-aged product from the same animal (Richardson et al., 2008). Subprimals wet-aged had preferred palatability characteristics over dry-aged carcasses aged for a similar period of time (Jeremiah and Gibson, 2003). One major disadvantage of dry-aging an entire carcass is the hanging space required in the cooler for an extended period of time (Kim et al.,

2017). A stepwise dry/wet-aging process has been investigated where product is first dry-aged as carcasses (10 d) followed by subsequent aging in vacuum packaging (7 d; 17 d total) (Kim et al., 2017). Stepwise aging in comparison to more traditional dry-aging techniques resulted in less drip loss, lower shear force values and no differences in consumer taste panel acceptability (Kim et al., 2017). The influence of the environment on the dry-aging process creates a need for continued understanding of the impacts of cooler conditions on dry-aged products.

Dry-Aging Taste Panels

Dry-aged steaks have a faster cook time (Warren and Kastner, 1992) and less cook loss than wet-aged counterparts (Oh et al., 2018; Kim et al., 2019). However, Berger et al., (2018) reported no difference in percent cook loss of wet-aged and dry-aged products. Freezing product prior to dry-aging has been reported to further increase cook loss, while decreasing evaporative loss, moisture content, and water activity (da Silva Bernardo et al., 2020).

Some consumer panelists found no difference between samples from wet-aged and dry-aged beef for overall acceptability (Sitz et al., 2006; Laster et al., 2008; Smith et al., 2008; Oh et al., 2018), flavor (Parrish et al., 1991; Sitz et al., 2006; Laster et al., 2008; Smith et al., 2008; Oh et al., 2018), tenderness (Sitz et al., 2006; Laster et al., 2008; Smith et al., 2008; Oh et al., 2018), or juiciness (Parrish et al., 1991; Sitz et al., 2006; Laster et al., 2008; Smith et al., 2008; Oh et al., 2018). However, consumers have been observed to prefer the juiciness of wet-aged beef over dry-aged beef (Smith et al., 2008). In addition, consumers have found more exterior muscles (*M. spinalis thoracics and M. gluteobiceps*) to have more favorable

sensory characteristics than muscles that are more interior (*M. Longissimus thoracis and M. gluteus medius*) in the aging process (Smith et al., 2008). When taking into consideration quality grade, consumers did not find a difference between wet-aged and dry-aged USDA Choice strip loins, but did prefer USDA Prime wet-aged strip loins over dry-aged USDA Prime strip loins for overall acceptability, tenderness and flavor (Parrish et al., 1991; Sitz et al., 2006). As expected greater quality grade (USDA Choice vs USDA Select) results in a more overall acceptability and juiciness (Laster, 2007).

Dry-aged flavor notes haves been observed beginning at 14 days of beef dry-aging (Campbell et al., 2001). Trained taste panels found wet-aged steaks from the rib and loin to be more tender and have better overall palatability than compared to dry-aged steaks but found no difference in juiciness, flavor intensity, or flavor desirability (Parrish et al., 1991). Trained taste panelists have determined dry-aged beef to have greater flavor intensity (Miller et al., 1985; Lepper-Blilie et al., 2016), a beefier flavor (Warren and Kastner, 1992; Smith et al., 2008), more roasted flavor (Warren and Kastner, 1992; Smith et al., 2008). Additionally, dry-aged beef has been noted to have less sour notes (Warren and Kastner, 1992), less serum flavor (Warren and Kastner, 1992; Smith et al., 2008), more metallic flavor (Smith et al., 2008), more musty and putrid (Smith et al., 2008) flavor notes than wet-aged beef. Dry-aged sirloins have been noted for having a more metallic flavor than other dry-aged middle meats (Smith et al., 2008). Fat from dry-aged beef has been reported to have greater frequency of off flavor notes (stale, old soapy) (Warren and Kastner, 1992). From a culinary standpoint it has been suggested 35 days of aging offers the greatest dry-aging flavor development and at 50 to 80 days of aging a unique texture develops (Perry, 2012). In general, as the aging timelines increases product becomes more tender, juicy, flavorful, and has a greater overall

acceptability (Ha et al., 2019). Researchers have focused on the differences between wet-aged and dry-aged beef and compared different aging timelines. However, to our knowledge, no research has compared dry-aging products aged at different aging facilities for product characteristics or taste panel analysis.

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Figure 1.1. Average beef carcass weight from 1974 to 2020 increased at a linear rate and continues to increase (Adapted from USDA NASS, 2020).

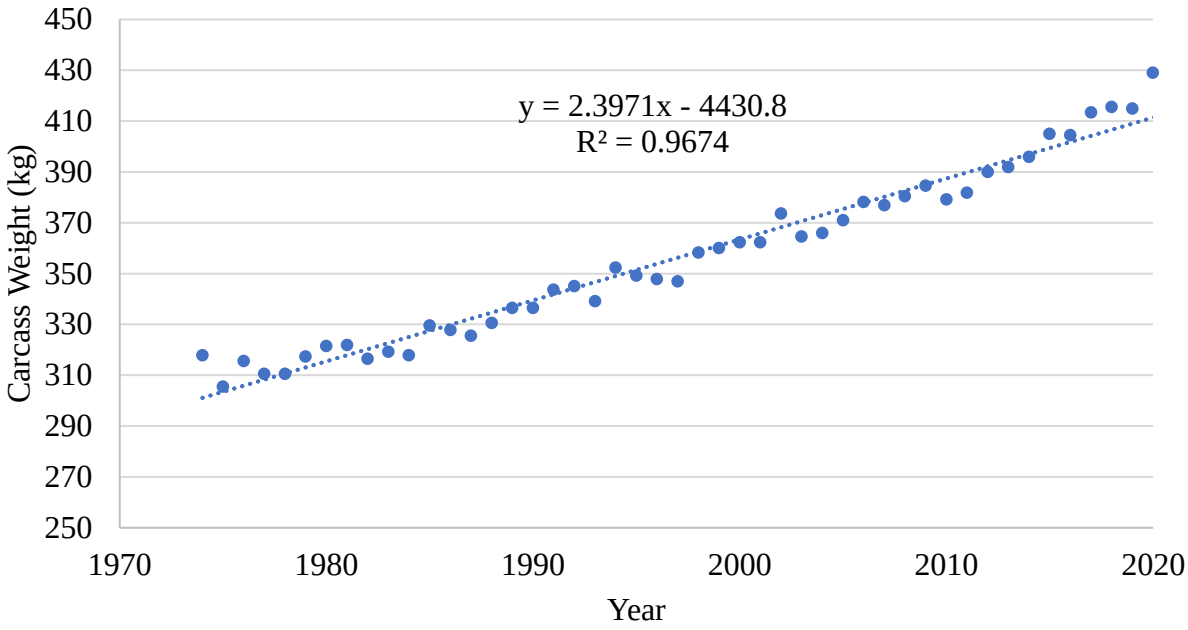

Table 1.1. Case-study value assessment of beef carcasses (N = 42) from average[1] (n = 21) and oversized[2] (n = 21) weight classifications based on 5-year average of premiums and discounts (USDA-NASS, 2020).

	Average	**Oversized**	**Difference**[3]
Average Carcass Weight[4]	829	1058	(229)
Base Price (/cwt)[5]	188.78	175.27	13.51
Average Net price/ head	1565.18	1851.37	(286.14)
Net Value of carcasses	32,868.84	38,878.92	(6,010.08)

[1] Average; Average-weight carcass (750-900 lbs.)

[2] Oversized; Oversized carcasses (≥1,000 lbs.)

[3] Calculated as Average – Oversized = Difference

[4] Average carcass weight of selected carcasses from commercial beef plant for average (n=21) and oversized carcasses (n=21)

[5] Base price per hundred weight (cwt) calculated based on yield grade (within yield grade 2 and 3) and weight discounts and premiums. Calculated on individual carcass basis and averaged across the weight group.

Table 1.2. Compilation of published dry-aging literature focusing on aging cooler parameters including days of aging, temperature of cooler, percent relative humidity, and wind speed.

Paper	Days	Temperature, °C	RH %	Wind Speed
Berger et al. (2018)	28	2	78	< 0.2
Campbell et al. (2001)	7, 14, 21	2	75	
Capouya et al. (2019)	49	35 – 39.4	75.9 – 90.1	
da Silva Bernardo et al. (2020)	28	2	70	2.5
DeGeer et al. (2009)	21, 28	0 – 1	50	
Dikeman et al. (2013)	21	2.2		
Ha et al. (2019)	20, 40, 60	2 – 4	65 – 85	
Hulánková et al. (2018)	21, 27	1 ± 1	85 ± 0.2	0.5 ± .2
Jeremiah and Gibson (2003)	7, 14, 21, 28	1 ± 1	60 – 80	1
Kim et al. (2013)	30	1	85	0.5
Kim et al. (2016)	21	1 or 3	73, 76, 49, 55	0.2 or 0.5
Kim et al. (2017)	17	1	78	1.5
Kim et al. (2019)	28	1 ± 1	85 ± 10	2 – 7
Laster et al. (2008)	14, 21, 28, 35	-0.6 ± 1.8	78 ± 9.3	
Lee et al. (2018)	28	4	75	2.5
Lee et al. (2019)	14, 28			
Lepper-Blilie et al. (2016)	14, 21, 28, 35, 42	1	70	
Oh et al. (2018)	28	1 ± 1	85 ± 10	2 – 7
Oh et al. (2019)	7, 14, 21, 35, 38	2 ± 1	75 ± 10	2.5
Parrish et al. (1991)	14, 21		80 – 85	0.5- 2.5
Richardson et al. (2008)	19	1		
Ribeiro et al. (2020)	42	2 ± 0.5	50, 70, 85	
Setyabrata et al. (2017)	28	2	75	
Setyabrata et al. (2019)	28	2	65	0.8
Sitz et al. (2006)	30			
Smith et al. (2008)	14, 21, 28, 35		83	
Smith et al. (2014)	35	4.0 ± 1.1	98.1	
Warren and Kastner (1992)	11	3.1 – 3.6	78 ± 3	

Table 1.3. Compilation of published dry-aging literature focusing on analysis conducted[1] on dry-aged products.

Paper	Shear Force	Sensory	Microbial	Microbiome	Composition	Yield	Volatile	Molecular	Retail	Color
Berger et al. (2018)		X	X		X	X				
Campbell et al. (2001)	X	X	X							
Capouya et al. (2019)				X						
daSilva Bernardo et al. (2020)	X		X				X			X
DeGeer et al. (2009)	X		X		X	X				
Dikeman et al. (2013)	X	X			X					X
Ha et al. (2019)		X					X			
Hulánková et al. (2018)			X							X
Jeremiah and Gibson (2003)	X	X							X	
Kim et al. (2013)	X									X
Kim et al. (2016)	X	X				X				X
Kim et al. (2017)	X	X								X
Kim et al. (2019)	X	X			X			X		X
Laster et al. (2008)	X	X								
Lee et al. (2018)		X	X						X	X
Lee et al. (2019)	X		X					X		
Lepper-Blilie et al. (2016)	X	X								
Oh et al. (2018)	X	X			X			X		
Oh et al. (2019)	X							X		
Parrish et al. (1991)	X	X	X					X		
Richardson et al. (2008)		X								
Setyabrata et al. (2017)									X	
Setyabrata et al. (2019)		X						X		
Sitz et al. (2006)	X	X			X					
Smith et al. (2008)	X	X								
Smith et al. (2014)		X								
Warren and Kastner (1992)		X				X				

[1]An X indicates the analysis was conducted and results were reported in the respective publication, while a blank box indicates analysis was not conducted/reported in publication.

CHAPTER 2

Impact of beef carcass size on chilling rate, pH decline, display color and tenderness of top round subprimals

"Impact of beef carcass size on chilling rate, pH decline, display color and tenderness of top round subprimals." Translational Animal Science, vol. 4 (4), 2020.

Abstract

Beef carcass weights in the United States have continued to increase over the past 30 years. As reported by the United States Department of Agriculture, grid-based carcass weight discounts begin when carcasses exceed 408 kg. Despite weight discounts, beef carcass weights continue to increase. At the same time, an increased prevalence of discoloration and color variability in top round subprimals has been observed throughout the industry which may be influenced by the increases in carcass weights. The objectives of this study were to assess the effects of beef carcass size and its relationship to chill time, color, pH, and tenderness of the beef top round. In the current study, eight industry average weight beef carcasses (AW, 341-397 kilograms) and eight oversized beef carcasses (OW, exceeding 432 kilograms) were evaluated. Temperatures and pH measurements were observed on both sides of all carcasses for the initial 48 h postharvest at a consistent superficial and deep anatomical location of the respective top rounds. Carcasses were fabricated into subprimals at 48 h and top rounds were aged at 2°C for an additional 12 d. The superficial location of both AW and OW carcasses cooled at a faster rate ($P < 0.01$) than the deep locations. The deep location of OW carcasses had a lower pH and a more rapid ($P < 0.01$) initial pH decline. Quantitative color of steaks from OW carcasses had greater mean L* (lightness; $P = 0.01$) and initial b*

(yellowness; $P < 0.01$) values. The delayed temperature decline and the accelerated pH decline of the deep location of the top round of OW carcasses occur at different rates than AW carcasses. Delayed rate of cooling leads to irreversible impacts on steak appearance of top round steaks fabricated from OW beef carcasses when compared with AW carcasses.

Introduction

The average beef carcass weight in the United States has increased over 61 kilograms (kg) in the past 30 years (USDA-ERS, 2019). On a grid-based marketing system, carcass weight discounts are applied once carcass weights reach the threshold of 408 kg (USDA-AMS, 2020). The 2016 National Beef Quality Audit reported that 32.2% of beef carcasses observed had hot carcass weights exceeding 408 kg with a maximum weight of 615 kg (Boykin et al., 2017). Even with the carcass weight discounts, the beef industry in North America continues to see incremental increases in fed beef carcass weights as producers can achieve greater gross profits on heavy weight carcasses because of the additional weight produced. As the number of heavy weight carcasses continue to increase in the beef industry, the researchers postulated that additional focus should be placed on thicker areas like the round to avoid potentially negative effects on meat quality associated with cooling.

The semimembranosus (SM) is a muscle that starts at the highest temperature and takes longer to dissipate heat than other muscles on the carcass (Hannula and Puolanne, 2004). Slower temperature declines postmortem result in accelerated pH declines, which suggests temperature and pH are not independent parameters (Mohrhauser et al., 2014). Furthermore, delayed temperature declines in combination with elevated pH results in meat that is paler in color with decreased protein functionality resulting in decreased water holding

capacity (Jacob and Hopkins, 2014). Discoloration has been observed in the SM at the location closest to the femur bone and can be attributed to myoglobin denaturation due to high temperature, low pH conditions, and resulting in enzyme denaturation leading to potential tenderness issues as well (Kim et al., 2010). The temperature gradient and pH decline between the deep and superficial locations can result in negative impacts to color and meat quality attributes. Thus, the objective of the current study was to assess the effects of beef carcass size and its relationship to chill time, color, pH, and tenderness of the beef top round. The researchers hypothesized that the top rounds from OW carcasses would cool at a slower rate with a more rapid pH decline and subsequently yield top round steaks that were less color stable and less tender than AW carcasses.

Materials and Methods

Product procurement

Between September 2018 and April 2019, beef steer carcasses that were within the parameters of United States Department of Agriculture (USDA) Yield Grade 2 or 3 were selected for the study. Steers were harvested at the University of Idaho Meat Laboratory under USDA-Food Safety Inspection Service inspection humane animal harvesting guidelines as outlined by Title 9 of the Code of Federal Regulations part 313. Carcasses were identified in the harvesting facility by the hot carcass weight (HCW) as “average weight” (AW) (n = 8, 335-387 kg), and “oversized” (OW) (n = 8, ≥ 432 kg). Carcasses considered AW in this study would not receive a discount for carcass weight on a grid-based value pricing system, whereas OW carcasses receive a discount because of HCW according to USDA carcass price reporting (USDA-AMS, 2020). All carcasses were from typical commercially raised concentrate-

finished Bos *taurus* beef cattle; due to the commercial nature of production and purchasing, cattle rations were not available.

Temperature and pH decline

Following USDA final inspection (approximately 35 min postmortem), a temperature monitoring data-logging probe (Multi-trip, Tempcord, Tulsa, OK) was inserted into the Semimembranosus (SM) at the approximate geometric center of the round medially until making contact with the femur bone of the respective carcass. This anatomical location was considered the ‘deep’ location. An additional temperature monitoring data-logging probe was inserted 2.54 cm from the surface of the top round in accordance with industry standard for temperature monitoring for Hazard Analysis and Critical Control Point plans (USDA-FSIS, 2017). This anatomical location was considered the ‘superficial’ location. Temperature monitoring data logging probes were placed in both sides of the carcass and positioned as described. Temperature decline was logged every 30 s and recorded over a 48-h period during the chilling process. Temperature and pH values were averaged from the two sides of each carcass. The pH of deep and superficial locations of the top round was measured with a portable pH meter (Apera Instraments SX811-SS, Colombus, OH) with a puncture-type probe every h for the first 12 h and then every 6 h until 48 h following initial entry into the cooler. Superficial and deep locations of the top round were measured on both sides of each carcass at all timepoints. Calibration standards (4.0, 7,0, 10.0) (Hanna Instruments, Woonsocket, RI) were utilized prior to measurements. Sides were evenly spaced in a 6.1 m by 2.1 m chill cooler (0°C) and placed to avoid touching other sides or infrastructure. Carcasses were ribbed between the 12th and 13th rib at 48 h postmortem. The pH of the ribeye at the 12th-13th rib interface was assessed to ensure that it was within the normal range of 5.4 to 5.7 (Matarneh et

al., 2017). Quality grade was determined on each side by trained University of Idaho personnel using USDA Quality Grade standards. Dentition was used to classify all carcasses as under 30 months of age. Ribeye area and external fat thickness (adjusted) were measured (external fat thickness, 3/4 of the length of the Longissimus dorsi muscle from the chine bone) on each side. Kidney, pelvic, and heart fat percentages were estimated using visual estimation.

Preparation of product

After 48 h chilling post-mortem at 0°C, carcasses were fabricated to obtain the top round subprimal (Institutional Meat Purchase Specification #168) from both sides of each carcass. Maximum circumference of the round (aitch bone removed) was recorded using a flexible tape measure. Top rounds were weighed, denuded, and the cap and side muscles were removed (NAMA #169A; NAMA, 2014). The trimmed top rounds were weighed, measured (length and width), vacuum packaged, and aged for an additional 12 d at 2°C. After the subprimal aging period, six steaks were cut to 2.54 cm thicknesses and were fabricated proximal to distal, perpendicular to the longitudinal axis of the cut. Ultimate pH was recorded at a point 2.54 cm from the deep and superficial side of each steak. All steaks were individually weighed. Four steaks from each subprimal were randomly and evenly assigned to a shelf-life treatment (d 1, d 2, d 3, or d 4 of shelf life). Each steak was placed in a commercially available rose-colored foam meat tray (23.8 cm x 31.4 cm; Bunzl; Riverside, MO) and overwrapped using an oxygen permeable polyvinyl chloride film (oxygen transmission: 1,450 cc/645 cm2 per 24 h, water vapor transmission rate: 17.0 g/645 cm2 per 24 h, Koch Industries, Inc #7500-3815; Wichita, KS). During steak fabrication, one steak was vacuum packaged and frozen at -20°C for later quantitative Warner-Bratzler shear force (WBSF) tenderness analysis. A 10 g sample was removed from deep and superficial locations

of the top round on d 2 and d 14 postmortem for calpain activity analysis. Samples from differing time periods were acquired independently. These samples were snap frozen in liquid nitrogen, stored in conical tubes (15 mL, VWR Centrifuge Tubes, Radnor, PA), and frozen at -76°C until measurement for calpain activity. Immediately following exsanguination, a single sample of the Sternocephalicus muscle was obtained from a reference steer carcass and snap frozen to be utilized as a reference sample for calpain analysis.

Retail color

Before objective color measurements, steaks were packaged and allowed to bloom for 60 min. Two objective color measurements per location (deep and superficial) were recorded using a portable hand-held spectrophotometer (MiniScan EZ, HunterLab, Restin, VA). Subsequent color measurements were taken on d 1, d 2, d 3, and d 4. Caution was taken during sampling to avoid large marbling flecks and connective tissue. The spectrophotometer was equipped with a 25 mm-diameter opening and a 10□ standard observer. The instrument was set to illuminant A and Commission International de I'Eclairage (CIE) L* (lightness), a* (redness) and b* (yellowness) values were recorded. Calibration of the spectrophotometer was performed daily utilizing calibration tiles (white and black) through the packaging film prior to analyzing color on the meat samples.

In addition to objective color measurements, steaks were visually evaluated on d 0, d 1, d 2, d 3, and d 4 of retail display. Parameters measured via visual evaluation of subjective color were oxygenated lean color, amount of browning, discoloration, surface discoloration, and color uniformity by at least three trained color evaluators following American Meat Science Association (AMSA) Meat Color Measurement Guidelines (AMSA, 2012). Steaks were

displayed in a glass-fronted, sliding door, retail display case (Model GDM-69, True Manufacturing Co., O'Fallon, MO) at 3°C for the duration of the retail display. The display case was lit with natural white Hg 40 W fluorescent lights which were on for the duration of the retail display with an average light intensity of 401 lx (Fisherbrand Traceable Dual-Range Light Meter, Fisher Scientific, Waltham, MA). To avoid potential confounding effects due to display case locations, steak positions were rotated after each measurement. Steaks from the front of the case were moved to the back and raised to the next shelf up while steaks on the top shelf would rotate to the bottom; steaks in sequential locations followed suit until the retail display period was finished.

Lipid oxidation

Thiobarbituric acid reactive substances (TBARS) were analyzed on d 0, d 1, d 2, d 3, and d 4 of retail display following the protocol provided in Appendix O of the Meat Color Measurement Guidelines (American Meat Science Association, 2012) (Appendix A). The samples (deep and superficial locations) were taken from the exposed surface of the steak, 1.27 cm deep into the steak, and avoided the edge of the steak following the procedure as previously described by Colle et al. (2016). Briefly, steaks assigned to the respective day were sampled by removing the edge of the steak (approximately 1 cm) and samples measuring 0.5 cm wide by 2.0 cm long by 1.27 cm thick were utilized for the analysis.

Warner-Bratzler shear force

Warner-Bratzler shear force (WBSF) steaks were tempered for 24 h at 4°C before cooking on a two-sided, clamshell style electric grill (Cuisinart Griddler Deluxe Model GR-150) to a target internal temperature of 71°C. Peak internal temperature was recorded using a Type K

thermocouple (Copper-Atkins 93230-K EconoTemp). Times were recorded for when steaks were placed on grills, removed from grills, and when steaks reached peak temperatures. Five cores (1.27 cm diameter) were removed parallel to the muscle fiber orientation for the deep and superficial locations of each steak. Each core was sheared once perpendicular to the muscle fiber orientation using a WBSF machine (G-R Manufacturing, Manhattan, KS). Peak shear force values were used to compute a mean peak shear force value of each steak. Steaks were weighed before and after cooking to determine percentage cook loss.

Calpain extraction and casein zymography

Extraction buffer (3mL, 100 mM Tris, 10 mM EDTA, 10 mM DTT, pH 8.3) was added to one gram of frozen sample previously stored at -75°C and homogenized (Polytron® PT 10-35 GT; PT-DA 12/2EC-B154) at 18,000 rpm on ice three times for 15 s with cooling (15 s) between bursts (Appendix B). The homogenate was pipetted into 2 mL microcentrifuge tubes. Subsequently, samples were centrifuged for 30 min at 8,800 x g at 4°C. The supernatant fluid was aliquoted and stored until calpain analysis at -75°C.

Calpain-1 and calpain-2 activity was determined utilizing casein zymography as originally described by Raser, Posner, and Wang (1995) and expanded upon by Pomponio et al. (2008) and Pomponio and Ertbjerg (2012) with minor modifications (Appendix C). Polyacrylamide gels (12.5%; 75:1 acrylamide to bisacrylamide) containing 0.2% casein were poured and overlaid with stacking gel (4%; 75:1 acrylamide to bisacrylamide) on the day the gels were run. Gels (8 x 10 x 0.1 cm) were pre-run with running buffer (25 mM Tris, 1 mM DTT, 192 mM glycine, 1 mM EDTA, pH 8.3) at 100 V for 15 min in an ice bath before loading samples. Sample buffer (10 µL) (150 mM Tris, 20% glycerol, 10 mM DTT, 0.02% bromophenol blue,

pH 6.8) was added to the supernatant (40 µL). Samples (20 µL) were added and the gels were run at 100 V for 6 h in an ice water bath. Gels were then placed in incubation buffer (~60 mL; 50 mM Tris, 10 mM DTT, 4 mM calcium chloride, pH 7.5) at room temperature with slow shaking for 17 h. Buffer was changed (~60 mL) at 30 min and (~130 mL) 60 min. Gels were stained in a commercially available, premixed, Coomassie Blue R-250 for 1 h and destained in a commercially available, premiex Coomassie Blue R-250 destaining solution for 3 h. The clear bands indicating calpain activity were quantified by inverting the image and then comparing the density of each band to the d 0 sample on each gel utilizing a ChemiDoc MPTM System (BioRad). Autolysis was used as an indicator of calpain activation (Geesink et al., 2006). Calpain activity was evaluated individually on for the deep and superficial portion of the top round from each side of each carcass at both d two and d 14. Calpain band densities were expressed as a percentage of the d 0 sample (collected immediately post exsanguination) of Sternocephalicus as an indicator of calpain activation.

Statistical Analysis

Temperature and pH data were modeled using nonlinear regression following the exponential form: eq (1) yi = a*exp(-b*houri) + c, where yi is the temperature or pH response at the ith hour, c is a lower asymptote, a + c represents the intercept term, and b is the rate of change over time (hour). This model describes a general decline in response over time starting at a value of a+c which eventually flattens out at a lower value c. Larger values of the rate parameter indicate a faster decline. Each treatment was initially estimated separately to ensure an adequate fit to eq(1) (Appendix D). Subsequently, a full model incorporating all treatments was fit allowing pair-wise comparison of model parameters across treatments.

All other data were analyzed using Mixed Model procedure of the Statistical Analysis System (SAS) assuming a randomized complete block design with treatments as fixed effects. Differences in the least squares means (LSM) were compared using pair-wise comparisons. Statistically significant p-values were evaluated at $P < 0.05$. Carcass served as the experimental unit with side as a replicate. Repeated measures were used for color analysis with day as the repeated measure and steak as the subject. Calpain activity is presented as a relative value and not in absolute terms. All data were analyzed using SAS version 9.4 (SAS Institute, Inc., Cary, NC).

Results

Temperature decline data for each treatment fit well to their respective models and all parameter estimates were significant ($P < 0.01$) (Appendix E). The overall difference between the initial top round temperatures, a+c, for AW and OW (36.72 and 36.77°C, respectively) was not different ($P = 0.70$), however the AW cooled at a faster rates ($P < 0.01$) than OW (Figure 2.1). The deep versus superficial starting temperatures (41.73 and 32.13°C, respectively) were different ($P < 0.01$) and the rate of change over time were also different ($P < 0. 01$) with the superficial locations having a greater rate of change over time than the deep locations (Figure 2.1). Furthermore, there was a difference ($P < 0.01$) in the temperature rate of change between the OW deep location that cooled at a slower rate than the AW deep location (Figure 2.1). There was not a difference ($P = 0.38$) between the rate of temperature change of the OW superficial and AW superficial locations (Figure 1). Temperature at 48 h was not different ($P = 0.55$).

The overall starting pH, a+c, of the top round for AW and OW (6.39 and 6.24, respectively) carcasses was different ($P < 0.01$) and the starting pH for deep versus superficial locations (6.04 vs 6.59) was different ($P < 0.01$; Figure 2.2). The pH rate of decline was different ($P = 0.02$) with the deep locations declining much more rapidly than the superficial locations (Figure 2.2). Furthermore, there was a difference ($P < 0.01$) in the rate of pH change between the OW deep location and the AW deep location, the OW deep location declining the fastest (Figure 2.2). However, ultimate pH (48 h) was not different ($P = 0.26$) between AW and OW carcasses (Figure 2.2).

Analysis of carcass traits are presented in Table 2.1. Hot carcass weights of the AW were lighter ($P < 0.01$) than the OW carcasses. 12th rib fat thickness and KPH fat percentage were not different between AW and OW carcasses ($P = 0.24$ and 0.79, respectively), but OW carcasses had larger ribeye areas ($P < 0.01$). In addition, calculated yield grade was not different ($P = 0.47$) between AW and OW carcasses. Intact round circumference of AW carcasses was smaller ($P < 0.01$) than the round circumference of OW carcasses. Top round length and width was greater ($P < 0.01$) for OW carcasses than AW carcasses. Untrimmed top round weight was greater ($P < 0.01$) for OW carcasses than AW carcasses. Trimmed top round weight tended to be greater for OW than AW carcasses however, it was not different ($P = 0.07$; Table 2.1).

Calpain-1 activity was observed in the reference muscle (Sternocephalicus, d 0 post-exsanguination), however calpain-1 activity was not observed at all in the SM samples (data not shown). Calpain-2 activity was greater ($P = 0.01$) for superficial locations than the deep locations based on values relative to the zero-hour reference standard (89.44% and 68.24%,

respectively; Table 2.2). Autolyzed calpain-2 activity was greater ($P = 0.04$) in OW carcasses compared to AW carcasses.

Analysis of lipid oxidation values indicated a two-way interaction ($P < 0.01$) between day and carcass weight (Table 2.3). Generally, all treatment combinations increased in lipid oxidation over days of retail display, with the AW carcasses having greater TBARS values starting at d 2.

There was a weight by day interaction for objective color scores for L* ($P = 0.01$), a* ($P < 0.01$), and b* ($P < 0.01$) (Table 2.4). The OW carcasses were lighter in color compared to the AW carcasses on d 0, d 2, and d 4. Redness (a*) decreased throughout the retail display for both AW and OW carcasses. The deep location of steaks from OW carcasses was lightest in color compared to all other locations (Table 2.6). On day one, steaks from OW carcasses were redder in color (a*; $P < 0.01$) whereas on day two they were less red compared to AW steaks. The superficial location on steaks remained more red (a*; $P < 0.01$) from day one to four of the retail display when compared to the deep location (Table 2.5). There was a difference in b* values ($P < 0.01$) with the steaks from OW carcasses being more yellow in appearance on the initial two days compared to steaks from AW carcasses (Table 2.4). Subjective color scores increased (favorable color to unfavorable color) from day zero to day four for: oxygenated lean color ($P < 0.01$), browning ($P < 0.01$), discoloration score ($P < 0.01$), degree of surface discoloration ($P < 0.01$), and steak uniformity ($P < 0.01$) (Table 2.7). Steaks from AW carcasses had greater oxygenated lean scores ($P = 0.05$) and less surface discoloration ($P = 0.02$) when compared to OW carcasses (Table 2.8).

Carcass weight did not impact cook time ($P = 0.64$), percent cook loss ($P = 0.48$), or WBSF ($P = 0.16$) values (Table 2.9). The location within the steak also did not impact WBSF ($P = 0.14$; Table 2.9).

Discussion

The top round from AW and OW carcasses started at similar temperatures, but AW carcasses cooled at a faster rate than OW carcasses. Similar results have been observed by others modeled in the Longissimus lumborum (Agbeniga and Webb, 2018; Fevold et al., 2019) and SM (Djimsa et al., 2018; Fevold et al., 2019; Klauer, 2019). The deep location had a greater temperature change from initial to final temperature than the superficial location, similar to data reported by Sammel et al. (2002b). In the present study, there was no difference in the magnitude of temperature change from initial to final temperature of deep locations within AW and OW carcasses (38.91 and 39.70 °C, respectively), however, the deep location of AW carcasses cooled at a faster rate than that of OW carcasses. Contrary to the current study, previous research reported no difference in the temperature decline of AW and OW carcasses at the center of the SM, measured 12.7 cm below the surface (Klauer, 2019). However, no difference was observed in the current research between the AW and OW superficial location temperature decline which is in agreement with previous research (Klauer, 2019). The superficial position of the SM is the location most often used for in-plant Hazard Analysis Critical Control Points temperature monitoring (USDA-FSIS, 2017); the data from the current study would indicate that it would still be an effective location for monitoring temperature decline regardless of carcass weight. Hot boning the top round has been suggested as an alternative to overcome challenges with chilling rate of the deep portion of the SM and the subsequent effects on pH decline (Sammel et al., 2002b), but this practice has not been widely

implemented in the industry (Troy, 2006). Disadvantages associated with hot boning have included: infrastructure or changes to current plant processes, increased microbial load, decreased product tenderness and subsequent impacts on eating experience (Troy, 2006).

The pH decline for the superficial location of the AW and OW carcasses was similar in the current study. However, the deep location for both carcass weights started at a lower initial pH with the OW carcasses starting lower than the AW carcasses (5.90 vs 6.18, respectively). Agbeniga and Webb (2018) reported similar trends in the decline of the Longissimus lumborum muscle pH where OW carcass pH decreased faster than that of AW carcasses. Djimsa et al. (2018) reported OW carcasses had a more rapid pH decline in the Longissimus dorsi, Psoas major, and SM compared with AW carcasses. Meanwhile, Fevold et al. (2019) reported no difference in pH decline among carcasses of different weights at h 0, h 4, and h 24 measured at the center of the SM. In addition, results from the current study indicate the deep location of the inside round started at a lower initial pH than the superficial location. Sammel et al. (2002b) also reported the deep location (10 cm below surface of muscle) pH declined more rapidly in the initial hours than the superficial location. Furthermore, in agreement with Sammel et al. (2002b), intermediate pH declines changing at different rates did not impact the ultimate pH and fell within a normal range of around 5.6 for ultimate muscle pH. Similar patterns have been reported in the biceps femoris; the inner location of the muscle dropped at a faster rate than the more exterior location (Kuffi et al, 2018). Temperature plays an important role in the decline of pH; higher temperature conditions result in a faster rate of energy substrate utilization, hence a greater accumulation of hydrogen ions reflected as a lowered pH (Matarneh et al., 2017). Previously, den Hertog-Meischke et al. (1997) reported that when pH values had reached below 6.0 while the temperature remained above 30°C, it

resulted in greater denaturation of myosin. For both the AW and OW carcass deep locations in the current study, a similar pH and temperature relationship was observed, with the pH being even more dramatically decreased in the OW carcasses. This decrease in pH could lead to further denaturation implications and negatively impact color, water holding capacity (Jacob and Hopkins, 2014) and enzymatic tenderness (Hwang and Thompson, 2001).

Despite differences in carcass weight, the back fat, KPH, and calculated yield grade were not different between the AW and OW carcass treatments. These results are different from previous research in that additional carcass weight was associated with increased back fat and KPH (Klauer, 2019). The current study was designed to target carcasses with similar yield grades in an effort to account for 12th rib fat thickness and muscling amount while previous work by Klauer (2019) and Fevold et al. (2019) did not select carcasses on these parameters but rather on HCW alone. On average, carcasses from the OW treatment had larger ribeyes compared with AW carcasses, as seen in previous research (Fevold et al., 2019; Klauer, 2019). Top round subprimals and subsequent trimmed, steak-ready subprimal weights were greater for OW carcasses than AW carcasses. Although top round subprimals were significantly larger for the OW carcasses, once the cap was removed and the SM was denuded, the differences between the respective muscles were no longer significant. It is possible that the increased size of the cap muscles caused a greater fat coverage on the medial section of the top round of the larger weight carcasses. Nevertheless, those measurements were not recorded in the present study.

Slower chilling rates of the Longissimus lumborum in previous studies have resulted in a decrease of extractable calpain-1 and calpastatin at 24-h postmortem as a result of rapid glycolysis (Hwang and Thompson, 2001). In the current study, calpain-1 activity was not

observed at all in the SM samples likely due to the collection of those samples occurring at 48-h postmortem. Native calpain-2 across all time points (2 and 14 d) was observed in greater activity in superficial locations compared with the deep locations. Colle and Doumit (2017) reported the relative percentage of native calpain-2 from the proximal location of the SM aged for 14 days was less than the superficial and deep locations examined in this study. In addition, a greater activity of autolyzed calpain-2 in OW carcasses were observed in the current study. The autolyzed calpain-2 activity in the AW carcasses of the current study was similar to that reported by Colle and Doumit (2017) which were obtained from carcasses that were not selected for specific weight criteria. The OW carcasses had almost triple the relative percentage of autolyzed calpain-2 activity previously observed at 14 days from commercially sourced product (Colle and Doumit, 2017). The relative percentage of autolyzed calpain-2 activity in the OW carcasses of the current study was more similar to observed levels in extended aged (42 d) product from the SM sourced from more traditional carcass sizes (Colle and Doumit, 2017). The elevated temperature for a greater length of time during the initial chilling process could have contributed to the increased presence of autolyzed calpain-2 activity in OW carcasses.

Several studies have reported TBARS value thresholds for off-flavor detection (Tarladgis et al., 1960; Campo et al., 2006) with a commonly used concentration of 1 malondialdehyde/kg meat (McKenna et al., 2005) being used as a benchmark. In the current study, there was an observed increase in TBARS values throughout the display time period, however the values were still relatively low and below the threshold of detectable off-flavors. Similar results were observed by McKenna et al. (2005), who reported a lag phase before the accumulation of oxidative rancidity by-products. Sawyer et al. (2007) also reported an increase in TBARS

values through the retail display, and similarly did not see a difference between the deep and superficial locations. Conversely, Puga et al. (2019) reported the deep location of the top round had greater TBARS values on average on d 4 compared to the superficial location, however all values were below the off-flavor threshold. Given low TBARS values, discoloration to top round steaks are likely not related to lipid oxidation.

Color variation within the SM has been reported, with the deep section being lighter in color, redder in appearance, and more yellow (Lee et al., 2007). The current research demonstrates a similar trend that is further amplified by carcass weight with the deep location of the steaks from OW carcasses being the palest of the locations evaluated across all days. The SM muscle often has elevated L* values compared with other muscles, but this trait alone does not fully account for decreased color stability (McKenna et al., 2005). Steaks from OW carcasses in the current study had greater average b* values through the first day of retail display, this is consistent with McKenna et al. (2005). In other work, Sammel et al. (2002b) reported the deep location maintained a more yellow appearance throughout the retail display period. In contrast, Fevold et al. (2019) reported steaks from heavy carcasses in their study were redder in appearance than those from the lightweight carcasses. In the current study, the superficial location of steaks remained redder throughout the retail display period, which is in agreement with previous research looking at the SM muscle (Sammel et al., 2002a; Seyfert et al., 2006). The difference in temperature and pH decline of the superficial and deep locations are contributing factors to the color difference between the two locations. The current study, similar to previously published work (Sammel et al., 2002a; Seyfert et al., 2006; Nair et al., 2016), reported steaks had more desirable color on the initial observations and the favorable appearance diminished throughout the retail display period. Hector et al. (1992) reported a

rapid pH decline postmortem to lead to protein denaturation and subsequently lighter colored muscle. Greater discoloration of the deep location is an effect of protein denaturation as a result of the delayed temperature and accelerated pH decline.

In the current study, it was observed that OW carcasses had a greater percentage surface discoloration over time based on subjective color evaluations. The observed difference in objective color scores averaged over the retail display between the deep and superficial locations of L* was 6.0 and a* had a difference of 3.83. Zhu and Brewer (1999) reported that a change in L* of 0.4 and a* of 0.6 were observable in subjective color evaluations, therefore it is likely that consumers would be able to distinguish between the two muscle locations.

Previous literature varies on the impact of carcass weight on shear force values of loin steaks from AW and OW carcasses. For instance, Foster et al. (2019) reported increased carcass weights (431.4-476.3 kg) and ribeye size can have negative impacts on tenderness compared with steaks sourced from AW cattle (340.7-385.6 kg). Moreover, Agbeniga and Webb (2018) reported no difference in tenderness between carcass weights (AW $\leq$ 260 kg vs OW $\geq$ 290 kg), whereas Djisma et al. (2018) reported steaks fabricated from OW carcasses were more tender. Similar to outcomes of with Fevold et al. (2019) in the current study, no differences were observed in the cook time, overall cook loss, and WBSF values of top round steaks sourced from AW and OW carcasses. In addition, the 2017 National Beef Tenderness Survey reported 65% of top round steaks in a retail setting had values that were considered very tender with WBSF less than 34.1 N (Martinez et al., 2017). Steaks from both the AW and OW treatments were below the threshold of USDA *Certified Very Tender* level of < 3.9 kg (ASTM, 2011). These findings differed from Sawyer et al. (2007) and Puga et al. (2019) where there were differences observed by location in the steak for WBSF values.

Conclusions

The current study suggested there are meaningful differences between the temperature and pH decline relationships in the top rounds among carcasses of different weights. Results indicate that OW carcasses present a challenge in terms of a delayed temperature declined combined with an accelerated pH decline in the top round subprimal. Alternative cooling or fabrication options may need to be considered to better accommodate the continuing trend of increasing beef carcass size in order to optimize the quality of the top round subprimal. Further research should be conducted to look at the impact of aging duration on top rounds from AW and OW carcasses for extended periods of time and the subsequent impacts on steak quality. Extending the aging period could amplify color differences of deep and superficial locations of AW and OW carcasses. Finally, research should be conducted to evaluate the difference in eating quality of the deep and superficial location of top round steaks.

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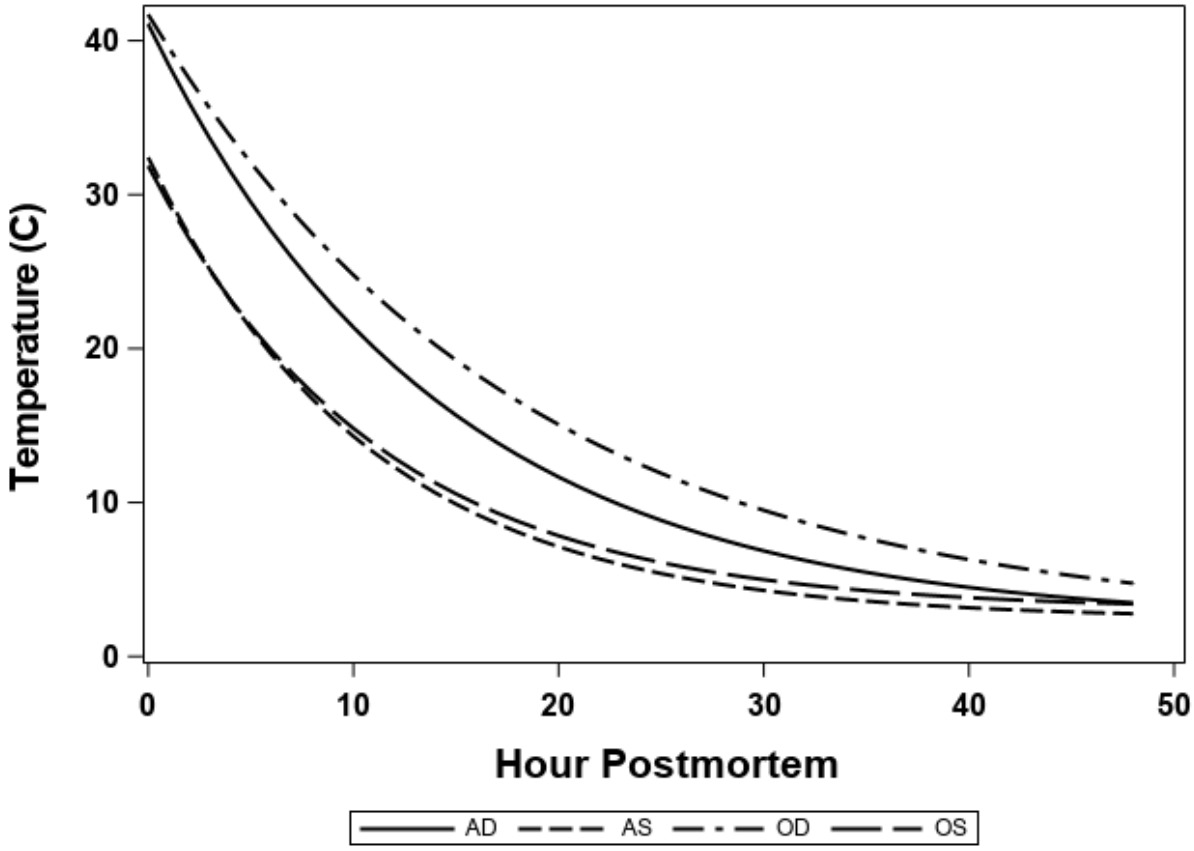

Figure 2.1. Estimated models of temperature (°C) of top round subprimals (Semimembranosus) decline over 48 h chill (starting immediately after final inspection, ~35 min postmortem) period for average and oversized carcasses monitoring the deep and superficial location. Individual lines [average superficial (AS), average deep (AD), oversized superficial (OS), oversized deep (OD)] estimated following the model: temperature = a*exp(-b*hour)+c.

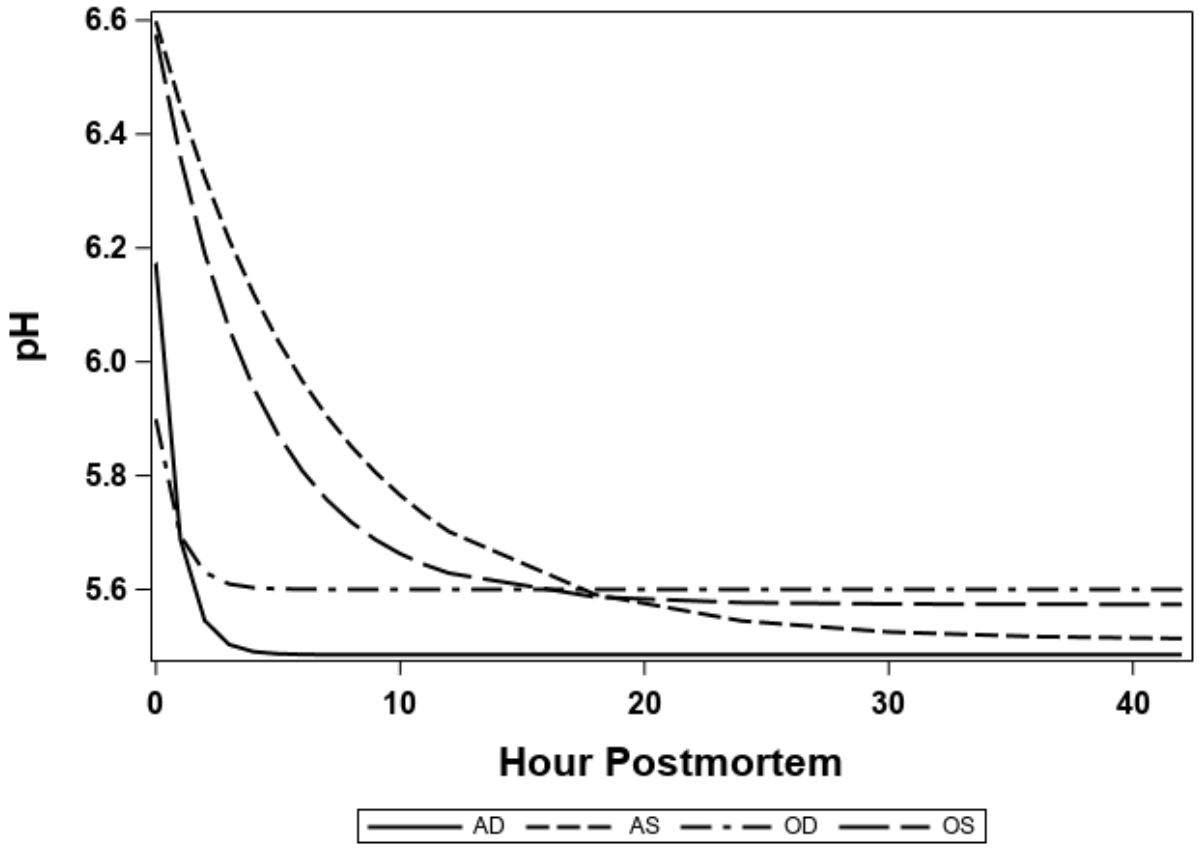

Figure 2.2. Estimated models of pH decline of top round subprimals decline over 48 h chill (starting immediately after final inspection, ~35 min postmortem) period for average and oversized carcasses monitoring the deep and superficial location. Individual lines [average superficial (AS), average deep (AD), oversized superficial (OS), oversized deep (OD)] estimated following the model: pH = a*exp(-b*hour)+c.

Table 2.1. Carcass trait means of steers (N = 16) and top round subprimal characteristics (n = 32).

	Average			Oversized			
Carcass	Min	Max	Mean	Min	Max	Mean	SEM
Hot carcass weight, kg	335	387	366[b]	432	500	462[a]	6
12th rib fat thickness, cm	0.76	2.03	1.32	1.02	2.29	1.50	0.12
Ribeye area, cm^2	68.4	103.2	85.9[b]	83.9	107.1	98.4[a]	2.5
Kidney, pelvic, and heart fat, %	1.5	2.5	2	1.5	2.5	2	0.1
Round Circumference, cm	109.2	128.3	117.3[b]	121.9	134.6	128.5[a]	1.3
Calculated yield grade	2.46	3.98	3.24	2.94	3.92	3.35	0.11
Top Round Subprimal							
Length, cm	40.6	53.3	49.5[b]	58.4	76.7	55.6[a]	0.8
Width, cm	52.8	63.5	57.7[b]	58.4	73.7	65.3[a]	1.0
Untrimmed wt., kg	9.04	11.62	10.32[b]	10.94	14.75	13.07[a]	0.24
Trimmed wt., kg	5.57	8.82	6.85	6.37	9.02	7.51	0.23
Ultimate pH	5.4	5.6	5.5	5.4	5.6	5.5	0.02

[ab] Within a trait, means without common superscripts differ ($P < 0.05$)

Table 2.2. Means of native calpain – 2 and autolyzed calpain – 2 activity of Semimembranosus from average and oversized carcasses (n = 32) across all aging periods.

	Average	Oversized	SEM
Native calpain-2 activity[1]	81.5	76.2	5.9
Autolyzed calpain-2 activity[1]	5.1[b]	14.0[a]	5.1
	Deep[2]	Superficial[2]	SEM
Native calpain-2 activity[1]	68.2[b]	89.4[a]	5.8
Autolyzed calpain-2 activity[1]	10.0	4.5	4.7

[1] As a percentage of day 0 calpain-2 activity

[2] Averaged among average and oversized carcasses

[ab] Within a trait, means without common superscripts differ ($P < 0.05$)

Table 2.3. Mean values of lipid oxidation[1] for top round steaks (n = 32) of average and oversized carcasses over a four-day simulated retail display.

	Day of Retail Evaluation					
	0	1	2	3	4	SEM
Average	0.17[c]	0.29[b]	0.36[b x]	0.56[a x]	0.64[a x]	0.04
Oversized	0.10	0.20	0.20[y]	0.23[y]	0.27[y]	0.04

[1] mg malondialdehyde/kg meat

[abc] Within a carcass weight, means without common superscripts differ ($P < 0.05$)

[xy] Within a day, means without a common superscript differ ($P < 0.05$)

Table 2.4. Objective color measurement means for L*, a*, and b* of top round steaks (n = 32) of average and oversized carcass over a four-day simulated retail display.

	Day of Retail Evaluation					
	0	1	2	3	4	SEM
L*						
Average	37.84$^{b\ w}$	37.50^{wx}	34.38$^{b\ y}$	37.07^{wxy}	36.16$^{b\ xy}$	0.87
Oversized	40.99$^{a\ w}$	39.15^{x}	39.23$^{a\ x}$	39.09^{x}	39.64$^{a\ wx}$	0.86
a*						
Average	25.67^{w}	24.22$^{b\ x}$	26.08$^{a\ w}$	20.90^{y}	20.60^{y}	0.63
Oversized	27.27^{w}	26.03$^{a\ w}$	22.88$^{b\ w}$	21.00^{y}	19.43^{z}	0.62
b*						
Average	22.54$^{b\ x}$	21.91$^{b\ x}$	26.22$^{a\ w}$	21.76^{x}	21.87^{x}	0.50
Oversized	27.00$^{a\ w}$	26.37$^{a\ w}$	23.45$^{b\ x}$	22.57^{xy}	21.82^{y}	0.50

^{abcd} Within carcass weight, means without common superscripts differ ($P < 0.05$)

^{wxy} Within a day within a trait, means without common superscripts differ ($P < 0.05$)

Table 2.5. Objective color measurement means for a* of the deep and superficial location of top round steaks (n = 32) averaged across average and oversized carcasses over a four-day simulated retail display.

	Day of Retail Evaluation					
	0	1	2	3	4	SEM
Deep	25.93^{a}	23.27$^{b\ y}$	22.07$^{b\ y}$	18.55$^{c\ y}$	17.65$^{c\ y}$	0.58
Superficial	27.01^{a}	26.99$^{a\ x}$	26.89$^{a\ x}$	23.35$^{b\ x}$	22.38$^{b\ x}$	0.56

^{abc} Within a steak location, means without common superscripts differ ($P < 0.05$)

^{xy} Within a day, means without common superscripts differ ($P < 0.05$)

Table 2.6. Objective color measurement means for L* of top round steaks (n = 32) from average and oversized carcasses averaged across a four-day simulated retail display period for the deep and superficial locations.

	Average	Oversized	SEM
Deep	38.98[a y]	43.23[a x]	0.78
Superficial	34.20[b]	36.00[b]	0.77

[ab] Within a carcass weight, means without common superscripts differ ($P < 0.05$)

[xy] Within a steak location, means without common superscripts differ ($P < 0.05$)

Table 2.7. Subjective color score means of top round steaks (n = 32) from average and oversized carcasses across a four-day simulated retail display period.

	0	1	2	3	4	SEM
Oxygenated lean[1]	4.12^{e}	4.30^{d}	4.56^{c}	5.32^{b}	5.81^{a}	0.14
Browning[2]	1.70^{e}	2.47^{d}	2.93^{c}	3.43^{b}	3.74^{a}	0.12
Discoloration Score[3]	2.03^{e}	2.67^{d}	3.05^{c}	3.53^{b}	3.93^{a}	0.11
Surface discoloration[4]	2.28^{e}	2.95^{d}	3.24^{c}	3.75^{b}	4.20^{a}	0.13
Uniformity[5]	2.58^{e}	2.84^{d}	3.20^{c}	3.58^{b}	3.87^{a}	0.12

[1]1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark cherry-red, 6 = moderately dark red, 7 = dark red, 8 = extremely dark red

[2] 1 = no evidence of browning, 2 = dull, 3 = grayish, 4 = brownish-gray, 5 = brown, 6 = dark brown

[3] 1 = none, 2 = slight, 3 = small, 4 = moderate, 5 = extreme

[4] 1 = no discoloration (0%), 2 = slight discoloration (1 – 20%), 3 = small discoloration (21 – 40%), 4 = modest discoloration (41 – 61%), 5 = moderate discoloration (61 – 80%), 6 = extensive discoloration (81 – 100%), reported as average of steaks from average and oversized carcasses

[5]1 = uniform, no two-toning, 2 = slight two-toning, 3 = small two-toning, 4 = moderate two toning, 5 = extreme two-toning, reported as average of steaks from average and oversized carcasses

[abcde] Within a trait, means without common superscripts differ ($P < 0.05$)

Table 2.8. Subjective color score means of top round steaks (n = 32) from average and oversized carcasses averaged across a four-day simulated retail display period.

	Average	Oversized	SEM
Oxygenated lean[1]	5.09[a]	4.70[b]	0.14
Surface discoloration[2]	3.04[b]	3.53[a]	0.14

[1]1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark cherry-red, 6 = moderately dark red, 7 = dark red, 8 = extremely dark red

[2] 1 = no discoloration (0%), 2 = slight discoloration (1 – 20%), 3 = small discoloration (21 – 40%), 4 = modest discoloration (41 – 61%), 5 = moderate discoloration (61 – 80%), 6 = extensive discoloration (81 – 100%)

[ab] Within a trait, means without common superscripts differ ($P < 0.05$)

Table 2.9. Mean percent cook loss, cook time, and WBSF of top round steaks (n = 32) from average and oversized carcasses.

	Average	Oversized	SEM
Percent cook loss	31.32	29.50	1.79
Cook time[1]	7.22	7.40	0.26
WBSF, kg	3.83	3.62	0.11
	Deep[2]	Superficial[2]	
WBSF, kg	3.84	3.61	0.11

[1]Min to 71 °C, measured at the geometric center

[2]Averaged among average and oversized carcasses

CHAPTER 3

Beef carcass size and subsequent aging time on retail display characteristics of top round steaks

Submitted to Meat and Muscle Biology

Abstract

Variation in cut size and weight of fabricated subprimals is a challenge of increased beef carcass weights. Subsequently, variation in carcass size has resulted in consistency challenges during retail display. The objective of this study was to assess the retail shelf-life of commercially available top rounds from varying carcass weights. In the current study, 21 industry average weight (AW, 340-409 kg; no industry discount) beef carcasses and 21 oversized (OS, exceeding 454 kg; receive a discount) beef carcasses were evaluated. Carcasses were selected at a commercial beef packing plant, where the left and right (paired) top round subprimals of each carcass were procured. Paired top rounds from respective carcasses were assigned to a short (8d), average (23d), or extended (42d) postmortem aging period. After wet-aging, subprimals were fabricated into steaks for additional analysis. Steaks were evaluated as whole top round steaks or further fabricated into “superficial” and “deep” portions at 5.08 cm from the superficial edge of the Semimembranosus. Top rounds and steaks from OS carcasses were larger ($P < 0.01$) than those from AW carcasses. Across all aging periods, subjective steak color browning ($P = 0.05$) and discoloration score ($P < 0.01$) were most favorable for the anatomically superficial locations of the steaks. Quantitative color of the anatomically deep locations of the OS steaks had the greatest mean L* (lightness; $P = 0.01$) and b* (yellowness; $P < 0.01$) values. Retail characteristics vary with aging times, and

differences are further amplified by carcass size. Alternative top round steak fabrication which separates the deep and superficial anatomical locations could be an effective means of providing consumers with more uniform steaks at the retail counter.

Introduction

Grid-based marketing systems provide premiums and discounts to beef carcasses as a reflection of carcass merit and merchandising ability. In the case of oversize carcasses, discounts in the United States (US) beef industry begin to be applied once carcasses reach the threshold of 408 kilograms (kg) (USDA-AMS, 2020). With the average finished beef carcass weight in the US continuing to rise (USDA-ERS, 2019), a greater percentage of carcasses are adversely assigned weight-based discounts when marketed on a grid-based pricing system. Despite the discounts applied for carcass weight, the US beef industry continues to see an uptick in carcass weights which could partially be attributed to the efficiencies gained by adding more weight per head.

The round is a high-volume beef primal which contains the *Semimembranosus* (SM) muscle. Due to its size, the SM has been observed to take additional time to cool compared to other muscles on the carcass (Klauer, 2019). The anatomically deep location of the SM, closest to the femur bone, cools at a slower rate than other parts of the carcass, while concomitantly the pH drops more rapidly than the anatomically superficial location (Lancaster et al., 2020). While the SM has been classified as “moderate” for color stability by McKenna et al. (2005), discoloration of top rounds at fabrication and during retail display has been observed by researchers (Sammel et al., 2002; Seyfert et al., 2006; Kim et al., 2010; Nair et al., 2016) and retailers. Kim et al. (2010) attributed the discoloration due to myoglobin denaturation and

noted potential tenderness challenges consequently. The anatomically deep location of steaks from oversized carcasses has been observed being lighter in color and more yellow in appearance compared to the anatomically superficial locations (Lancaster et al., 2020). The objective of this study was to assess three aging periods of commercially available top rounds from varying carcass weights as it relates to retail shelf-life and color stability.

Materials and Methods

Product Procurement

Carcasses were selected at Washington Beef (Toppenish, WA) in September 2019. Selected carcasses (N = 42) were commercially-sourced from youthful (determined to be less than 30 months of age physiologically by United States Department of Agriculture [USDA] grading protocol), traditional, concentrate-fed, *Bos taurus*, beef cattle. Due to the opportunistic nature of the carcasses selected at a commercial beef processing facility, concentrate cattle rations, and other management practices were not known. Two-carcass weight groups were identified; average sized (AW) (340-409 kg; n=21) and oversized (OS) (≥ 454 kg; n = 21) carcasses. Carcasses were included into the study if it met the requirements of USDA calculated Yield Grade 2 and 3. All carcasses selected were USDA Choice Quality Grade. During selection, carcass parameters were recorded (hot carcass weight, ribeye area, calculated yield grade, and quality grade) from a camera grading system (VGB2000, E+V Technology, Oranienburg, Germany). Carcasses were fabricated by plant personnel. The top rounds from both sides were collected and remained paired throughout the duration of the trial. Top rounds (NAMA #168) were vacuum packaged and transported under refrigerated conditions (4°C) to the University of Idaho Vandal Brand Meats Lab for further aging (1.6°C).

Product Preparation

All product was aged according to aging assignment (8 d, 23 d, or 42 d postmortem). Two aging times of the top rounds were identified as the minimum (8 d) and mean (23 d) aging time observed for top rounds at retail in the 2015 National Beef Tenderness survey (Martinez et al., 2017). The 42 d aging treatment time was observed in previous research as an optimal aging period for top rounds under extended aging conditions (Colle et al., 2016). Following the respective aging periods, top rounds were fabricated by removing the *Gracilis*, *Pectineus*, and *Sartorius* muscles at the natural seams to isolate the SM and *Adductor* muscle grouping to produce the equivalent of a NAMA #169A (NAMA, 2014). Subsequently, top rounds were denuded and trimmed to steak-ready level and then fabricated into steaks. Top rounds were faced perpendicular to the longitudinal axis of the cut at the distal edge from where the aitch bone was removed; subsequently, six 2.54 cm-thick steaks were cut proximal to distal using a scalloped boneless bandsaw blade (Walton's Inc, Wichita, KS) on a bandsaw (Butcher Boy SAE20, Walton's Inc., Wichita, KS). Steaks from the paired top rounds were fabricated and assigned for further analysis. Two steaks from the paired top rounds remained whole, while four steaks from the paired top round were fabricated to isolate the SM (from the *Adductor*) which was subsequently cut into an anatomically superficial and deep section (Fig 3.1). The outermost "superficial" steak was separated from the interior "deep" steak of the SM 5.08 cm from the anatomically superficial edge of the cut. Due to the small size of the *Adductor* muscle, the *Adductor* was chosen to be excluded from further assessment when the superficial and deep sections of the SM were fabricated into individual steaks; the adductor, however, did remain on the "whole" top round steaks. Steaks were systematically assigned by location within the subprimal to a length of retail display (0 to 4 d).

All steak samples were individually weighed (GFK 165a Bench Scale, Adam Equipment®, Columbia, MD). The pH of each steak sample was measured with a portable pH meter (Apera Instruments SX811-SS, Columbus, OH) utilizing a puncture-type probe at 2.54 cm from the anatomically superficial and deep edge of the SM of each whole steak prior to further fabrication into "deep" and "superficial" sections. Cut steaks were identified by steak section treatment combination (SSTC) with respect to carcass weight (AW whole, AW deep, AW superficial, OS whole, OS deep, and OS superficial). Steaks for retail display were overwrapped using oxygen permeable polyvinyl chloride film (oxygen transmission: 1,450 cc/645 cm^2 per 24 hr, water vapor transmission rate: 17.0g/645 cm^2 per 24h, Koch Industries, Inc. #7500-3815, Wichita, KS) and allowed to bloom for one hour prior to the initial color evaluation.

Retail Color Evaluation

Following steak fabrication, a simulated retail display with an average light intensity of 849 lux (Fisherbrand Traceable Dual-Range Light Meter, Fisher Scientific, Waltham, MA) was conducted for a period of 4 d at 2°C. Each day, steak subjective color was measured by four trained evaluators using the AMSA Color Evaluation Guidelines (AMSA, 2012). Subjective measurement color traits included: amount of browning, discoloration, and color uniformity. In addition, objective steak color (L* [lightness], a* [redness], b* [yellowness]) was scanned using a Nix Pro2 Color Sensor (Nix Sensor LTD, Hamilton, Ontario, Canada). Additionally, metmyoglobin reducing activity (MRA) (Appendix F) was observed on d 0 and all subsequent days of the retail display period utilizing one representative steak from each SSTC. Oxygen consumption (OC) (Appendix G) rate of steaks was assessed on d 0.Both oxygen consumption and metmyoglobin reducing activity were conducted in accordance with the

protocol provided in Section XI, and analyzed using formulas provided in Section IX, of the Meat Color Measurement Guidelines (AMSA, 2012). Metmyoglobin reducing activity was calculated using the percentage of metmyoglobin (MMb) as: $\mathrm{MRA} = \left(\frac{\text{Initial \% MMb} - \text{Final \% MMb}}{\text{Initial \% MMb}}\right) \text{x } 100$. The OC was calculated as a reflection of the percentage of oxymyoglobin (OMb) as: $\mathrm{OC} = \left(\frac{\text{Ititial \% OMb} - \text{Ending \% OMb}}{\text{Initial \% OMb}}\right) \text{ x } 100$.

Lipid Oxidation

Following color evaluation, thiobarbituric acid reactive substances (TBARS) were used to measure secondary lipid oxidation products daily throughout the retail display. Analysis was conducted on d 0, 1, 2, 3, and 4 of retail display following the protocol provided in Appendix O of the Meat Color Measurement Guidelines (American Meat Science Association, 2012) (Appendix A). The samples for the deep and superficial locations were obtained from the exposed side of the steak, avoiding the edge of the steak following the procedure as previously described by Colle et al. (2016). Briefly, samples were obtained by excluding the edge of the steak (approximately 1 cm), and subsamples were 0.5 cm wide, 2.0 cm long, and 2.54 cm thick were evaluated.

Statistical Analysis

All analyses were carried out using SAS V 9.4 (SAS Institute, Inc., Cary, NC). All data were analyzed using linear mixed models in the GLIMMIX procedure. A randomized complete block design was assumed with SSTC and aging period as fixed effects and carcass as the block. Differences in the least squares means (LSM) were assessed using pair-wise comparisons and a Tukey adjustment for multiple comparisons. Repeated measures were used

for color analysis with day as the repeated measure and steak as the subject. Statistically significant p-values were identified at $P \leq 0.05$.

Results and Discussion

Analysis of carcass traits are displayed in Table 3.1. Average hot carcass weights of carcasses in the OS group were heavier ($P < 0.01$) than carcasses in the AW group (480 vs 376 kg, respectively). In addition, OS carcasses had a greater ($P < 0.01$) average yield grade than the AW carcasses (USDA YG 3.6 vs 2.8, respectively). However, all carcasses fell within the parameter of USDA Yield Grade 2 or 3 as outlined in the carcass selection criteria. The average ribeye area in OS carcasses were larger ($P = 0.01$) than the AW carcasses (101.3 vs 94.88 cm^2, respectively). Mean marbling scores were not different ($P = 0.71$) between the AW and OS carcass groups, with the average of both weight groups being USDA Choice (marbling score: 501 vs 509, respectively). In agreement with previous research, ribeye area was larger for OS than AW carcasses (Fevold et al., 2019; Klauer, 2019; Lancaster et al., 2020). Due to the carcass data collection being derived from the online camera vision system, the 12^{th} rib fat thickness was not reported as it was directly included in the USDA Yield Grade automatically formulated by the camera. The researchers, however, postulate that it is likely the OW carcasses had a greater 12^{th} rib fat thickness measurement which led to the higher calculated USDA Yield Grade reported for that carcass treatment group.

Top Round Characteristics

Characteristics of top round subprimal fabrication yields and dimensions are displayed in Table 3.2. Top rounds from OS carcasses had a greater ($P < 0.01$) untrimmed weight (13.75 vs 10.95 kg, respectively), a greater ($P < 0.01$) trimmed weight (8.10 vs 6.89 kg,

respectively), and a greater ($P < 0.01$) amount of trim generated from the top round (4.06 vs 5.65 kg, respectively) compared to AW carcasses. In addition, trimmed OS top rounds were 5.11 cm longer ($P < 0.01$), on average, and 3.76 cm wider ($P < 0.01$), on average, than AW top rounds. Weights of all whole steaks, prior to subsequent separation, from OS top rounds were heavier ($P < 0.01$) than those from AW top rounds. Lancaster et al. (2020) previously reported a difference in untrimmed top round weight but did not see a difference in trimmed top round weight. The larger sample size in the current study likely contributes to the observed difference in the trimmed top rounds. Additionally, West et al. (2011), reported greater yields on boneless ribeye and strip loin subprimals fabricated from OS carcasses compared to AW carcasses.

pH

Previous research has established differences of pH by location throughout top rounds (Sammel et al., 2002; Lee et al., 2007), therefore, this study focuses on the differences between the deep and superficial steaks fabricated from the isolated SM top round steaks among hot carcass weight treatments. There was an aging period by SSTC interaction ($P = 0.02$) for steak pH (Table 3.3). At d 8 of aging no difference in pH was observed between SSTC's, while at d 23 and d 42 of aging differences became apparent. The AW deep and AW superficial sections of the SM had the greatest pH at d 23, and the AW deep section had the greatest pH at the d 42 aging period. In the current study the mean pH for all SSTC's was between 5.50 and 5.63 and fell within the normal range of 5.4 to 5.7 (Matarneh et al., 2017). However, the current data does suggest that the deep locations of the SM were consistently higher in pH than that of the superficial locations within carcass weight treatment group. This finding aligns with previous research of the deeper portion having a greater pH than the

superficial portion (Sammel et al., 2002; Lee et al., 2007). In addition, Lee et al. (2007) attributed variation in the pH of top round steaks to position in the subprimal (increased dorsal to ventral). In contrast, other research has indicated no difference in pH of the deep and superficial locations (Sawyer et al., 2007; Lancaster et al., 2020). Despite the positional differences, Colle et al. (2016) reported no difference in pH of steaks aged for varying lengths of time (d 2- d 63) measured at a consistent location on the exterior edge of the steak.

Lipid Oxidation

Analysis of lipid oxidation indicated a two-way interaction for SSTC by aging period ($P < 0.01$) (Table 3.3). Lipid oxidation values were greater ($P < 0.01$) for the steak sections from AW deep and superficial sections (0.63 and 0.77 mg malondialdehyde/kg meat, respectively) compared to OS deep and superficial sections (0.44 and 0.41 mg malondialdehyde/kg meat, respectively) during the 8 d aging period. All lipid oxidation values remained below 1 mg malondialdehyde per kg of meat regardless of aging period or SSTC. The threshold of 1 mg malondialdehyde per kg of meat was suggest by McKenna et al. (2005) for the detection of off-flavors linked to lipid oxidation and is one of several studies that have attributed elevated TBARS values to off-flavors (Tarladgis et al., 1960; Campo et al., 2006). Additionally, Colle et al. (2016) reported extended aging of top round steaks also resulted in TBARS values below the threshold of off-flavors. Meanwhile, Puga et al. (2019) reported the anatomically deep location of top round steaks had greater TBARS values at the end of the retail display.

Subjective Color

Interactions for subjective color evaluations are illustrated in Appendix H. Amount of browning values for SSTC of top round steaks by aging period are presented in Table 3.4. The

superficial section of steaks from the OS carcasses had the least amount of browning while the AW whole steaks had the greatest amount of browning ($P < 0.01$) observed. Similarly, steak discoloration scores were greatest ($P < 0.01$) for the AW and OS whole steaks and lowest for the AW and OS superficial steaks (Table 3.4). The OS whole SSTC had the greatest amount of two-toning ($P < 0.01$) across the three aging periods (Table 3.4). Limited work has been conducted looking at alternative fabrication of the top round and impacts of extended aging on the top round with respect to shelf-life. The current study found whole steaks had a greater amount of browning compared to the other SSTC portions. The authors postulate the alternative fabrication of top round steaks resulted in a more consistent appearance of the steaks compared to the whole steaks, as reflected in the more uniform subjective color score. Similarly, Lancaster et al. (2020) reported whole top round steaks from OS carcasses had greater surface discoloration across the retail display. The superficial steaks from AW and OS carcasses were most favorable in subjective color measurements for all aging periods versus the deep and whole steaks. The more favorable color characteristics of alternative fabrication agree with that reported by Puga et al. (2019) suggesting that separating top round steaks into deep and superficial portions resulted in more uniform steaks. In agreement with Colle et al. (2016), the was no impact of the time of aging on color uniformity.

Objective Color

Interactions for objective color evaluations are illustrated in Appendix H. The OS deep steak sections were the lightest (greatest L*) in color while the AW superficial steak sections were the darkest (lowest L*) in color ($P = 0.01$; Table 3.5). Redness (a*) was the greatest ($P < 0.01$) for OS superficial and whole at the d 8 and d 42 aging periods (Table 3.5). The OS deep

section was the most yellow (greatest b*) in color ($P < 0.01$) across all aging periods (Table 3.5). Top round steaks have been noted for having variation in color (Sammel et al., 2002; Seyfert et al., 2004; Lee et al., 2007; Fevold et al., 2019). Additionally, the SM muscle is often lighter (higher L*) than other muscles in the carcasses (McKenna et al., 2005). Delayed temperature decrease and more rapid pH decline of the deep portion negatively impact steak color (Lancaster et al., 2020). Previous research indicates more rapid pH declines increases protein denaturation which is partially attributed to the lighter colored muscle (Hector et al., 1992; Lancaster et al., 2020). MacDougall (1942) suggested denatured proteins have a more open structure which results in greater light scattering and negatively impacts meat color. Furthermore, the increased rate of glycolysis of the deep portion can result in negative color consequences and have similar appearance to pale, soft, and exudative pork in the beef muscle (Nair et al., 2016). Moreover, the OS deep section was the lightest and most yellow of all SSTC sections. The deep section of the top round has been attributed with being lighter in color, redder in appearance, and more yellow than the superficial section (Lee et al., 2007). In addition, Lancaster et al. (2020) found oversized carcasses (>432 kg) amplified the lightness and yellowness of the deep portion. Mancini and Ramanathan (2014) reported that beef aged for a longer time (45 d) has greater color intensity post bloom due to decreased oxygen consumption, but also decreased color stability over the shelf life of the product.

Metmyoglobin Reducing Activity

There is a two-way interaction observed with SSTC by aging period for metmyoglobin reducing activity ($P < 0.01$; Table 3.6). Metmyoglobin reducing activity was greatest for the short (8 d) aging period and all treatment combinations. Metmyoglobin reducing activity decreased as the aging periods progressed. The AW superficial section had the greatest

metmyoglobin reducing activity, while the OS deep and AW deep had the lowest metmyoglobin reducing activity. The advantage of the superficial anatomical locations of the SM in metmyoglobin reducing activity suggests benefits in terms of retail color stability. The SM has been previously identified as a muscle of intermediate color stability, with low metmyoglobin reducing activity (McKenna et al., 2005). Furthermore, the superficial location has been reported to have greater metmyoglobin activity than the deep location (Seyfert et al., 2006; Nair et al., 2017). Nair et al. (2017) reported the superficial portion of the top round had greater mitochondria functionality. These findings are partially attributed to the mitochondria's role in the reduction of metmyoglobin formation.

Oxygen Consumption Rate

All SSTC oxygen consumption rates were measured on the day steaks were fabricated, respectively. The OS deep section had the lowest oxygen consumption rate ($P < 0.01$) while consumption rate of the AW deep section were similar to that of the AW and OS superficial sections. (Fig 3.2). Nair et al. (2017) reported lower oxygen consumption rates, a reflection of decreased mitochondrial functionality, in the deep portion of the top round. Sammel et al. (2002) found the superficial location of the top round had greater oxygen consumption rates and total aerobic reducing activity.

Conclusion

Carcasses in the OS category (≥ 454 kg hot carcass weight) of the current study can receive heavyweight discounts on a grid-based marketing system. Oversized carcasses result in larger subprimals and, ultimately, larger steaks. Results study suggest that there are meaningful differences among the anatomical sections within a top round steak among carcasses of

different weights. The OS carcasses present a challenge in greater color variation for lightness and redness between superficial and deep sections of the steak. The altered color of steaks sourced from OS carcasses could lead to greater discoloration discounts at retail. Few adjustments are currently being made in processing and merchandising products from larger carcasses compared to average sized carcasses. This study suggests a lack of uniformity in steaks sourced from average and oversized beef carcasses. This research provides empirical data to help packers provide greater premiums for those cattle that fall within the more “ideal” range of hot carcass weight or increase the emphasis of alternative merchandising of carcasses exceeding 454 kg. Additionally, the research demonstrated that over-sized cattle may pose a greater challenge in producing top round steaks that are consistent throughout the entire steak.

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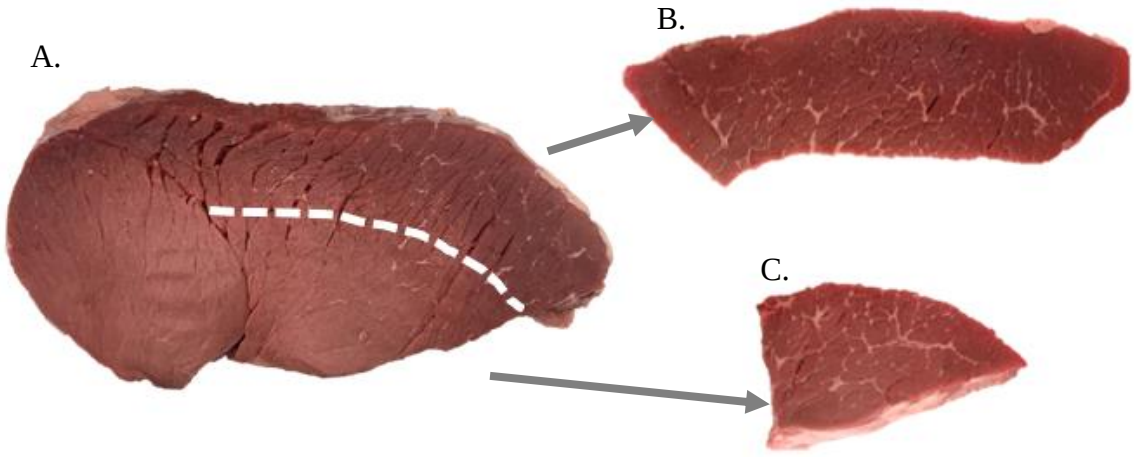

Figure 3.1. Image of traditionally fabricated top round steak (A.) compared to alternatively fabricated steaks (B.-C.). The alternative method is divided into superficial (B.) and deep (C.) portions based on anatomical locations from the *Semimembranosus*.

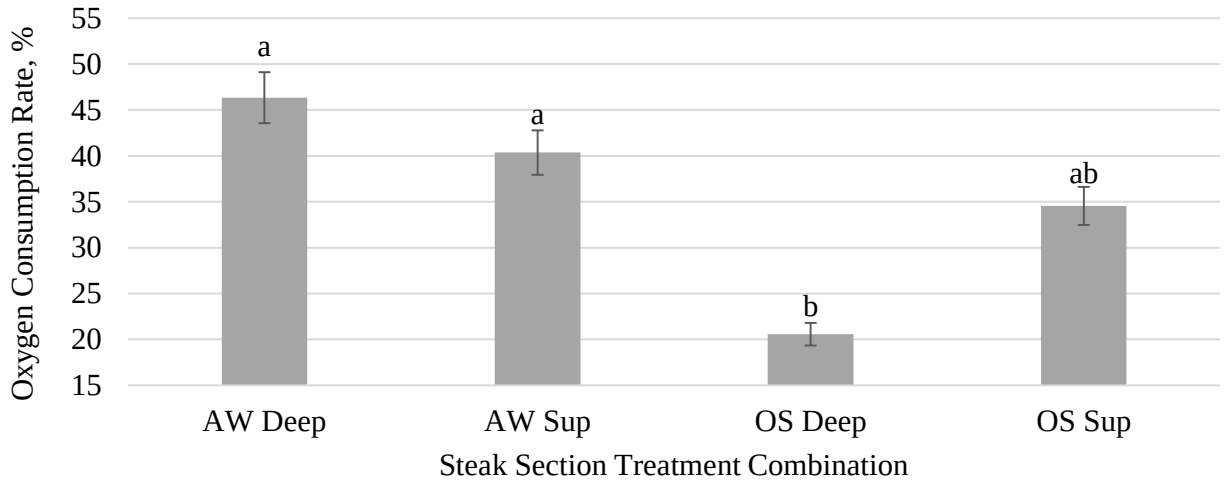

Figure 3.2. Oxygen consumption rate of beef top round steaks. Samples from average weight (AW; 340-408 kg) and oversized (OS; ≥ 454 kg) carcasses were analyzed by deep or superficial sections within the *Semimembranosus* muscle of steaks. Superficial sections were separated from the deep portion at 5.08 cm from the ventral side of the steak. Rates are means ± SEM from analyses evaluated in triplicate. Steak section treatment combinations with different superscripts differ ($P < 0.05$).

Table 3.1. Carcass trait means of AW[1] (*n* = 21) and OS[2] (*n* = 21) carcasses.

	AW			OS			
	Min	Max	Avg	Min	Max	Avg	SEM
Carcass Weight (kg)	345	407	376[b]	456	509	480[a]	4
Calculated Yield Grade	2.0	3.9	2.8[b]	2.26	3.9	3.6[a]	0.13
Ribeye Area (cm^2)	80.0	112.3	94.8[b]	89.0	118.7	101.3[a]	1.7
Marbling Score[4]	418	691	501	407	610	509	14

[1] AW; Average-weight carcass (340-409 kg)

[2] OS; Oversized carcass (≥ 454 kg)

[3] Marbling score: 400=Small, 500=Modest, 600= Moderate

[ab] Within a row, means without a common superscript differ ($P < 0.05$)

Table 3.2. Mean top round subprimal characteristics of AW[1] and OS[2] carcasses.

	AW (n = 42)	OS (n = 42)	SEM
Untrimmed Weight (kg)	10.95[b]	13.75[a]	0.20
Trimmed Weight[3] (kg)	6.89[b]	8.10[a]	0.10
Trim Weight[4] (kg)	4.06[b]	5.65[a]	2.05
Length (cm)	55.88[b]	60.99[a]	0.38
Width (cm)	45.34[b]	49.10[a]	0.33
Steak Weight (kg)	0.78[b]	0.89[a]	0.01

[1] AW; Average carcass (340-409 kg)

[2] OS; Oversized carcass (≥ 454 kg)

[3] Trimmed weight was measured on denuded top rounds and contained *Semimembranosus* and *Adductor* muscles

[4] Trim comprised of *Gracilis*, *Pectineus*, and *Sartorius* muscles and associated adipose tissue removed at the natural seams

[ab] Within a row, means without a common superscript differ ($P < 0.05$)

Table 3.3. Mean pH and lipid oxidation values (TBARS[1]) of deep and superficial top round sections of the *Semimembranosus* by hot carcass weight treatment and aging period.

Aging Period	Steak Section Treatment Combination (n = 630)					P-value
	AW[2] Deep[4]	AW[2] Sup[5]	OS[3] Deep[4]	OS[3] Sup[5]	SEM	
	pH					0.02
8d	5.55	5.52^{xy}	5.57	5.50	0.02	
23d	5.63^{a}	5.59$^{a\ x}$	5.55^{ab}	5.50^{b}	0.02	
42d	5.55^{a}	5.47$^{b\ y}$	5.52^{ab}	5.48^{ab}	0.02	
	TBARS[1]					<0.01
8d	0.63$^{a\ x}$	0.77$^{a\ x}$	0.44^{b}	0.41^{b}	0.06	
23d	0.43^{y}	0.44^{y}	0.52	0.43	0.06	
42d	0.56^{xy}	0.58^{y}	0.47	0.53	0.06	

[1] mg malondialdehyde/kg meat

[2] AW; steaks sourced from average carcass (340-409 kg)

[3] OS; steaks sourced from oversized carcass (≥ 454 kg)

[4] Deep; sample from the deep section of the SM

[5] Sup; sample from the superficial section SM

[ab] Within an aging period (row), means without a common superscript differ ($P < 0.05$)

[xy] Within a steak section treatment combination (column), means without a common superscript differ ($P < 0.05$)

Table 3.4. Subjective color amount of browning score, discoloration score, and color uniformity means of deep and superficial top round sections of the *Semimembranosus* and whole top round steak by hot carcass weight treatment and aging period.

	Steak Section Treatment Combination (n = 630)							
Aging Period	AW[1] Deep[3]	AW[1] Sup[4]	AW[1] Whole[5]	OS[2] Deep[3]	OS[2] Sup[4]	OS[2] Whole[5]	SEM	P-value
	Amount of Browning [6]							<0.01
8d	3.5[b]	3.1[bc]	4.1[a x]	3.0[bc]	2.6[c]	3.4[ab]	0.2	
23d	3.2[a]	2.7[b]	3.4[a xy]	3.0[ab]	2.3[c]	3.2[a]	0.2	
42d	2.7[a]	2.1[b]	3.0[a y]	3.0[a]	2.0[b]	3.0[a]	0.2	
	Discoloration Score[7]							<0.01
8d	3.4[a x]	2.5[bc]	3.8[a]	3.2[ab]	2.2[c]	3.4[a]	0.2	
23d	2.9[a xy]	2.2[b]	3.2[a]	3.4[a]	1.2[b]	3.6[a]	0.2	
42d	2.6[a y]	1.7[b]	2.9[a]	3.1[a]	1.7[b]	3.3[a]	0.2	
	Color Uniformity[8]							<0.01
8d	2.6[b]	1.8[c]	3.3[a]	2.5[b]	1.7[c]	3.3[a]	0.1	
23d	2.4[c]	1.8[cd]	3.0[ab]	2.5[bc]	1.6[d]	3.7[a]	0.1	
42d	2.1[c]	1.5[d]	2.8[ab]	2.5[bc]	1.5[d]	3.3[a]	0.1	

[1] AW; steaks sourced from average carcass (340-409 kg)

[2] OS; steaks sourced from oversized carcass (≥ 454 kg)

[3] Deep; sample from the deep section of the SM

[4] Sup; sample from the superficial section SM

[5] Whole; intact top round steak

[6] Amount of browning score; 1 = no evidence of browning, 2 = dull, 3 = grayish, 4 = brownish-gray, 5 = brown, 6 = dark brown

[7] Discoloration score; 1 = none, 2 = slight, 3 = small, 4 = moderate, 5 = extreme

[8] Color uniformity score; 1 = uniform, no two-toning, 2 = slight two-toning, 3 = small two-toning, 4 = moderate two toning, 5 = extreme two-toning

[abcd] Within an aging period (row), means without a common superscript differ ($P < 0.05$)

[xyz] Within a steak section treatment combination (column), means without a common superscript differ ($P < 0.05$)

Table 3.5. Objective color L* (0 = black; 100 = white), a* (-50 = green; 50 = red), and b* (-50 = blue; 50 = yellow) means of deep and superficial top round sections of the *Semimembranosus* (SM) and whole top round steak by hot carcass weight treatment and aging period.

	Steak Section Treatment Combination (n = 630)							
Aging Period	AW[1] Deep[3]	AW[1] Sup[4]	AW[1] Whole[5]	OS[2] Deep[3]	OS[2] Sup[4]	OS[2] Whole[5]	SEM	P-value
				L*				0.01
8d	37.93^{ab}	30.92^{e}	34.47^{cd}	40.68^{a}	32.39^{de}	35.86^{bc}	0.69	
23d	37.13^{b}	32.08^{d}	34.39^{c}	40.74^{a}	33.08^{cd}	37.33^{b}	0.71	
42d	38.95^{b}	32.28^{e}	36.85^{cd}	42.59^{a}	33.45^{de}	37.99^{bc}	0.71	
				a*				<0.01
8d	19.08$^{bc\ xy}$	18.98^{bc}	18.10^{c}	21.03$^{ab\ x}$	21.19$^{a\ xy}$	21.34$^{a\ x}$	0.45	
23d	17.56$^{b\ y}$	19.58^{a}	17.80^{ab}	18.19$^{ab\ y}$	19.21$^{ab\ y}$	18.46$^{ab\ y}$	0.46	
42d	20.26$^{ab\ x}$	20.77^{ab}	19.12^{b}	21.71$^{ab\ x}$	22.13$^{a\ x}$	22.01$^{a\ x}$	0.46	
				b*				<0.01
8d	16.99$^{ab\ x}$	14.13^{c}	14.54^{c}	18.25$^{a\ x}$	15.33^{c}	16.48^{b}	0.30	
23d	15.25$^{b\ y}$	14.33^{b}	14.11^{b}	16.69$^{a\ y}$	14.51^{b}	15.39^{b}	0.31	
42d	16.21$^{bc\ xy}$	14.44^{d}	14.86^{d}	18.21$^{a\ x}$	15.32^{cd}	16.44^{b}	0.30	

[1] AW; steaks sourced from average carcass (340-409 kg)

[2] OS; steaks sourced from oversized carcass (≥ 454 kg)

[3] Deep; sample from the deep section of the SM

[4] Sup; sample from the superficial section SM

[5] Whole; intact top round steak

[abcde] Within an aging period (row), means without a common superscript differ ($P < 0.05$)

[xy] Within a steak section treatment combination (column), means without a common superscript differ ($P < 0.05$)

Table 3.6. Metmyoglobin reducing activity means of deep and superficial top round sections of the *Semimembranosus* by hot carcass weight treatment and aging period.

	Steak Section Treatment Combination (n = 630)				
Aging Period	AW[1] Deep[3]	AW[1] Sup[4]	OS[2] Deep[3]	OS[2] Sup[4]	SEM
8d	$11.66^{x\ b}$	$24.76^{a\ x}$	$10.55^{b\ x}$	$22.52^{a\ x}$	1.40
23d	$7.99^{bc\ xy}$	$23.33^{a\ x}$	$3.84^{c\ y}$	$11.59^{b\ y}$	1.40
42d	$5.76^{c\ y}$	$14.23^{a\ y}$	$4.79^{c\ y}$	$9.67^{b\ y}$	1.40

[1]AW; steaks sourced from average carcass (340-409 kg)

[2] OS; steaks sourced from oversized carcass (≥ 454 kg)

[3] Deep; sample from the deep section of the SM

[4] Sup; sample from the superficial section SM

[abc] Within an aging period (row), means without a common superscript differ ($P < 0.05$)

[xy] Within a steak section treatment combination (column), means without a common superscript differ ($P < 0.05$)

CHAPTER 4

Assessment of Dry-Aged Beef from Commercial Aging Locations across the United States

Submitted to Foods

Abstract

Dry-aging is a practice that involves storing meat at refrigerated temperatures without protective packaging. The dry-aging process has been observed to create unique flavors. The objective of the current study was to survey commercial dry-aging facility environments and observe palatability differences related to consumer acceptance. Seventy-two bone-in beef strip loins (IMPS #175) were acquired. Strip loins were randomly assigned to each of ten commercial dry-aging facilities. Additionally, a set of strip loins were wet-aged at the University of Idaho meat laboratory. Strip loins were shipped overnight to respective aging locations and dry-aged for 45-days then returned overnight to the University of Idaho meat laboratory. Strip loins were fabricated into steaks, vacuum packaged, and then frozen until further analysis. Commercial dry-aging facility cooler conditions were observed to be different ($P < 0.01$) for temperature (0.74 – 5.26°C), percent relative humidity (64.87 – 99.21%), and wind speed (0.56 – 2.03m/s). Intrinsic quality parameters including pH and water activity were not different ($P > 0.05$) among treatment-locations. Consumer taste panels indicated a difference ($P < 0.01$) in acceptability (6.27 – 7.24), tenderness (6.65 – 7.54), and flavor (5.58 – 6.79) based on aging treatment-location. Overall, the findings indicate that conditions within individual dry-aging facilities aid in producing unique dry-aged beef flavors.

Introduction

Aging is the process of storing meat in refrigeration for a period of time to allow for meat to become effected by natural enzymatic activity [1]. Aging beef often results in improvements in tenderness and flavor desirability, without impacting juiciness of the product [2].

Dry-aging is the storage of meat under refrigeration in the absence of protective packaging for an extended period of time [1]. Dry-aged products are impacted by a combination of important factors including the length of time the product is aged, temperature at which the

product is aged, relative humidity of the aging cooler, and wind velocity within the cooler [3]. The effect of dry-aging on beef flavor is equivocal in the literature [4–7]. While dry-aged beef is noted for offering unique flavor attributes, it differs from the more conventional wet-aged beef flavor to which many consumers are accustomed [8]. Additionally, some consumers have reported no flavor difference between wet-aged and dry-aged beef [6,7,9,10]. Largely, studies have compared wet-aged beef to dry-aged beef treatments to determine which is a superior product [7,11–13]. Research around dry-aging has featured the effects of temperature, relative humidity, and wind speed, both independently and collectively [14–16]. Few studies have evaluated the effects of multiple commercial aging locations [17], cooler parameters, or subsequent impacts on sensory attributes [10].

The objectives of this study were to survey environmental parameters of commercial dry-aging facilities from selected regional locations of the United States. Furthermore, the study investigated the effect of dry-aging parameter influences on eating quality (acceptability, flavor, tenderness, and juiciness) of dry-aged beef.

Materials and Methods

Product Procurement

Certified Angus Beef® brand bone-in strip loins (n = 72; IMPS # 175), from a single production date, were sourced from a commercial beef harvesting facility (Wallula, Washington). Strip loins were transported under refrigerated temperatures (2°C) to the University of Idaho meat lab, Vandal Brand Meats, on the Moscow, Idaho campus of the University of Idaho. Strip loins were weighed, measured (length, width, depth), individually identified, and evenly sorted by weight and calculated surface area across eleven treatment-locations. Six strip loins were shipped overnight to nine different commercial dry-aging facilities each. Strip loins were shipped in insulated shipping containers (Uline Insulated Foam Shipping Kit S-13394, Pleasant Prairie, WI) and were packed with enough icepacks (Uline Cold Pack S-18253, Pleasant Prairie, WI) to maintain a chilled environment (< 4.0 °C) for the product being shipped., where strip loins were aged for a 45-day period using the individual facility's standard operating procedure for dry-aging. Furthermore, one set of six strip loins were assigned to be dry-aged at Vandal Brand Meats. An additional set of six strip

loins were assigned to a 45-day wet-aging treatment at Vandal Brand Meats. The remaining 6 strip loins represented the unaged product utilized for sensory analysis.

Cooler Conditions

A temperature and humidity logger (Onset® HOBO® temp/RH logger, Cape Cod, MA) accompanied the product to each respective dry-aging location in order to record environmental parameters throughout the shipping and aging periods. Loggers were activated prior to shipping, accompanied the product continuously through shipping and aging, and data were retrieved at the end of the trial by trained personnel at the University of Idaho. Aging facilities measured weekly wind speed using handheld anemometers (866B, HoldPeak, Zhuhai, China) positioned parallel to the cut anterior surface of the strip loin at a distance of 30.5 cm. Upon completion of the aging period, strip loins were individually vacuum packaged and shipped overnight in insulated shipping containers to the Vandal Brand Meats. Upon receiving, strip loins were weighed and dimensional measurements were recorded as previously described.

Fabrication and Sample Collection

Strip loins were deboned, the exterior dry-aged crust was removed on those dry-aged, and each strip loin was cut into ten steaks 2.54 cm thick beginning from the anterior end. Fabricated steaks were systematically assigned to subsequent analysis groups including consumer taste panels, Warner-Bratzler Shear Force, water activity, and additional analyses not reported in this manuscript. Weights were recorded throughout fabrication to determine yields of saleable product. The posterior face of the first steak cut from the anterior end of the strip loin was allotted 30 min to bloom prior to measuring color. Color was analyzed using a Nix Pro 2 Color Sensor (Nix Sensor Ltd., Hamilton, Ontario, Canada). Two readings were obtained, and the International Commission on Illumination (CIE) L*, a*, and b* measurements were averaged for each of the steaks. The geometric center of the 11th steak removed from the strip loin was used to measure pH and water activity. A puncture type pH meter (Apera Instruments SX811-SS, Columbus, OH) that was calibrated to pH standards of 4.0, 7.0, and 10.0 (Hanna Instruments, Woonsocket, RI) was used to determine pH of the product. Steaks were then vacuum packaged and frozen at -20°C until further analysis.

Water Activity Analysis

Water activity samples were obtained from the 11th steak fabricated from each strip loin to obtain comparable values for all strip loins and locations. A small sample (1.27 × 1.27 cm) was collected from the center of the steak, taking caution to avoid large areas of connective tissue and marbling. The sample was evaluated on an Aqualab water activity meter (model 3te, Meter Group, Pullman, WA) with standards (Meter Group, Pullman, WA) of 1.0, 0.92 and 0.75.

Warner-Bratzler Shear Force

Warner-Bratzler shear force (WBSF) steaks were tempered for 24 hours at 4°C prior to cooking on a two-sided electric grill (Cuisinart Griddler Deluxe Model GR-150) to a target peak internal temperature of 71°C. Times were recorded when steaks were placed on the grills, removed from the grills, and upon reaching their peak internal temperatures. Cook time was determined as the difference between when steaks were placed and removed from the grills. Peak internal temperature was recorded using a Type-K thermocouple (Copper-Atkins 93230-K EconoTemp). Steaks were allowed to equilibrate to room temperature prior to coring. A minimum of five cores (1.27 cm diameter) were removed parallel to the muscle fiber orientation from the Longissimus lumborum muscle of each steak. Each core was sheared perpendicular to the muscle fiber orientation using a WBSF machine (G-R Manufacturing, Manhattan, KS) at a cross-head speed of 225mm/sec. Peak shear force values were used to compute a mean peak shear force value for each steak. Steaks were weighed prior to, and immediately following, cooking to determine the percentage cook loss.

Consumer Taste Panels

The study was found to be Exempt by the University of Idaho Institutional Review Board (IRB #19-149) (Appendix I). All subjects provided informed, written consent for inclusion prior to their participation in the study (Appendix J). Panelists were informed they study was investigating dry-aged beef. Four steaks from randomly selected locations within each strip

loin were used for taste panels. Steaks were thawed overnight at 4°C and subsequently cooked to a medium rare degree of doneness (60°C). Four separate panels were conducted in accordance with American Meat Science Association guidelines [18]. Within each panel, treatment-location samples were allocated to panelists according to a balanced incomplete block design. Each panelist received four three-digit, blind coded samples for evaluation in a predetermined random order in an effort to reduce sample order bias. Prior to evaluating samples, all panelists received a sample of Semitendinosus, cooked in the same manner as the strip steaks, as a calibration sample. Each incomplete block represented all locations equally. Panelists were randomly assigned three-digit, blind-coded samples to evaluate in a preselected order to reduce sample order bias. Each sample was evaluated using a 10-point scale where 1 = dislike extremely and 10 = like extremely for the following attributes: overall acceptability, tenderness, juiciness, and flavor of the sample. In addition, consumers were asked to evaluate qualitatively the presence of an off flavor (no/yes) and consumer satisfaction (no/yes). Furthermore, panelists were asked to select the least favorable and most favorable trait (flavor, tenderness, juiciness, texture/mouth feel), when applicable. Lastly, consumers were provided with a list of flavor notes (brown/roasted, yeasty, metallic, earthy, nutty, aged cheese, sour, and sweet) and asked to select which were observed if any. Each panelist was provided with a ballot for each sample (Appendix K), along with a toothpick, napkin, expectorant cup, cup of room temperature water, and unsalted soda crackers (Nabisco, East Hanover, NJ). Prior to the commencement of the panel, the panelists were provided with verbal instructions on the process and forms. Before sampling, panelists were asked to complete a demographics survey.

Microbial Analysis

The posterior face of each strip loin (5 x 5 cm area) was swabbed at the end of the aging period using sterile cotton swabs and buffered peptone water (3M™, St. Paul, MN). All sampled swabs were vortexed (VWR Vortexer 2 G-550; Scientific Industries, Bohemia, NY), diluted 1:100 with buffered peptone water, vortexed a second time, and subsequently plated. Samples were plated on 3M™ Petrifilm™ Aerobic Count Plates (3M™, St. Paul, MN), and incubated (Model 10-140, Quincy Lab, Inc., Chicago, IL) at 35°C for 48 ± 2 h for growth of mesophilic organisms. Samples were also plated on 3M™ Petrifilm™ Rapid E.coli/ Coliform

Count Plates (3M, St. Paul, MN), and incubated at 35°C for 48 h. Colonies were counted following the 3M™ Interpretation Guides [18,19].

Statistical Analysis

A mixed model ANOVA assuming a randomized complete block design with treatment-location as a fixed effect was employed to analyze cooler conditions and intrinsic quality factors. Taste panel data were analyzed according to the predetermined balanced incomplete block design used for sample allocation where treatment-location was assumed as a fixed effect and panelist and the panelist*treatment-location interaction as random effects. Initial analysis also assessed the overall effects of panel and panel*location interactions; however, these were later omitted from the modeling as they showed minimal significance if any of the responses. Following model fitting, differences in least squares means (LSM) were compared using pair-wise comparisons with a Tukeys HSD adjustment. Plate count data were log10 transformed prior to analysis. A contingency table chi-squared analysis was used to assess treatment effects for observational taste panel traits. Pearson correlations were used to determine potential associations between cooler conditions and sensory characteristics. Statistically significant p-values were identified at $P < 0.05$. All data were analyzed using SAS version 9.4 (SAS Institute, Inc., Cary, NC).

Results and Discussion

Cooler Conditions

Cooler temperatures for the 45-day aging period differed ($P < 0.01$) among aging treatment-locations (Table 4.1). Aging location H had the lowest average temperature (0.74°C) whereas location C had the greatest mean temperature (5.26°C). Additionally, there was a difference ($P < 0.01$) in relative humidity across locations. Location A had the lowest average percent relative humidity (64.87%) and location I had the greatest average percent relative humidity (92.21%). Previous research conducted in laboratory environments has found that the percent relative humidity in a single treatment-location experiment has varied from as low as 49% to as high as 85% [10,14,21,22]. Average air speed over the 45-day aging period ranged from 0.56m/s (location J) to 2.03m/s (location E) and differed by aging treatment-location ($P < 0.01$). The air speed observed in the current study was within ranges of air speeds observed in

previously reported dry-aging experiments [23–25]. As expected, the percent aging weight loss was greater in the dry-aged strip loins compared with wet-aged strip loins ($P < 0.01$). Within the products that were dry-aged, there were differences ($P < 0.01$) in percent aging loss among treatment-locations; however, the majority of treatment-locations were near the range of 12-14% evaporative loss, similar to those previously reported [9]. Additionally, location E had both the greatest average wind speed and percent aging loss while location J had the lowest average wind speed and lowest percentage of aging loss. In agreement with Lee et al. (2019), aging facility locations are unique and can impact final product parameters [26].

Intrinsic Quality Parameters

Intrinsic quality factors for 45 day-aged strip loins are displayed in Table 4.2. There were no differences detected across aging treatment-locations for the following factors: pH ($P = 0.12$), water activity ($P = 0.08$), and the color metrics: L* ($P = 0.87$), a* ($P = 0.36$), and b* ($P = 0.09$). Kim et al. (2013) reported that pH of dry-aged beef was higher than wet-aged beef [21]. However, in agreement with the current study, it has previously been reported that dry-aged and wet-aged beef have similar ultimate pH [10,11,15,22]. Although Lee et al (2019) reported differences in pH of dry-aged beef at 42 days of aging, the raw product differed compared with the current study (Holstein steers vs Certified Angus Beef®) [26]. De-spite the lack of differences between wet-aging and dry-aging locations for water activity in the current study, Ribeiro et al. (2019) reported greater water activity in wet-aged bone-less strip loins compared to product dry-aged for 42 days [27]. The authors of the current study postulate that differences in the current study compared to previous work could be attributed to sampling location. Contrary to the current study, Dikeman et al. (2013), Kim et al. (2019) and Ribeiro et al. (2020) reported differences between wet-aged and dry-aged beef for L*, a*, and b* values [12,22,28]. The similarity in intrinsic quality factors (pH, water activity, L*, a*, and b*) of the current study suggest a uniform initial raw product and that sensory differences post aging can be attributed to aging conditions and locations.

Cook time ($P = 0.17$) and cook loss ($P = 0.41$) were not different among aging treatment-locations (data not shown). No difference ($P = 0.21$) was observed in WBSF values among the wet-aged, negative control, and the dry-aged locations (Table 4.2). Previous studies have

also reported no difference in WBSF of product aged for similar lengths of time [12,15]. In the current study, all steaks (1.59-2.21 kg WBSF) were well below the tenderness threshold of the USDA Certified Very Tender level of < 3.9 kg WBSF [29].

Consumer Taste Panels

Demographics for taste panel participants are listed in Table 4.3. Consumer taste panelists were recruited in the state of Idaho. Over half of the panelists had consumed dry-aged beef prior to participating in the panel; over 90% of the panelists reported that they eat beef twice a week or more. Gender of panel participants were distributed almost exactly in half. Taste panel outcomes are listed in Table 4.4. Consumers reported differences in overall acceptability of steaks ($P < 0.01$). The wet-aged treatment had the greatest consumer acceptability while locations D had the lowest consumer acceptability. Given the vast majority of beef consumed in the United States is wet-aged, consumer familiarity could contribute to the high acceptability rating of the wet-aged beef treatment [6]. Alternatively, some previous research has reported consumer sensory panelists found no difference in acceptability between dry-aged and wet-aged beef [6,10,13,30,31]. Despite all the samples having mean WBSF values within the threshold of USDA Certified Very Tender, and no observed mechanical differences, consumer panelists identified differences in tenderness between treatment-locations ($P = 0.01$). Consumers identified the wet-aged steaks and steaks aged at location C as the most tender and steaks from location I as the least tender. There were no identified differences in juiciness among aging treatment-locations for taste panels ($P = 0.20$). Conversely, Smith et al. (2008) reported consumers preferred the juiciness of wet-aged beef over dry-aged beef [13]. Flavor desirability differences among samples from different aging treatment-locations were identified by consumer panelists ($P < 0.01$). Consumers preferred the flavor of wet-aged steaks and found steaks from location D to have a less desirable flavor. Miller et al. (1985) and Lepper-Billie et al. (2016) reported that dry-aged beef had greater flavor intensity than wet-aged beef although these differences were not observed in the current study [32,33]. Other studies have also indicated no difference in flavor of dry-aged and wet-aged beef [6,7,12,13,34].

Overall acceptability and identifiable flavor notes reported by consumers are displayed in Table 4.5. Overall satisfaction of the samples ranged from 56.1% to 87.9% ($P < 0.01$) while

the observance of off flavors ranged from 5.3% to 44.6%. The observance of cheese ($P < 0.01$), sour ($P < 0.01$), and nutty ($P = 0.03$) flavors was different by aging treatment-location. Meanwhile, consumers reported no differences in the observance of yeasty ($P = 0.15$), earthy ($P = 0.42$), sweet ($P = 0.46$), and metallic ($P = 0.95$) notes. Trained taste panels using dry-aged beef samples have observed uniqueness of the samples as: beefier flavor [5,13], less sour notes [5], less serum flavor [5,13], and more musty/putrid notes [13] compared with wet-aged samples. In addition, Ribeiro (2020) reported lower humidity (50% relative humidity) had more favorable flavor notes, while product aged at higher relative humidity (70 and 85%) had fewer desirable flavors [15]. Furthermore, product dry-aged at higher humidity had sensory attributes with sourness, rancidity, and oxidation [13]. Panelists in the present study who indicated an unsatisfied eating experience most often (66%) indicated the reason was the flavor of the product ($P < 0.01$). When panelists reported off-flavors, the percentage of flavor attributes were observed as: yeasty (18.5%; $P < 0.01$), cheese (18.1%; $P < 0.01$), sour (19.3%; $P < 0.01$), metallic (14.2%; $P < 0.01$), and nutty (18.5%; $P = 0.06$). Despite these attributes being noted in tandem with off flavor, the authors of the current study postulate they contribute to other artisanal food products and could be highly sought after by some consumers. Consumer preferences of products is known to impact acceptability of a number of food products [35]. While the general United States Pacific Northwest consumer did not favor the distinct flavor notes associated with dry-aged beef, alternative population centers and niche consumers could savor the robustness of unique dry-aged flavors.

The Pearson correlation coefficients of refrigerated environment aging parameters and sensory attributes are displayed in Table 4.6. Percent relative humidity had a negative correlation with overall consumer panel acceptability (-0.79; $P < 0.01$) and flavor (-0.64; $P = 0.04$), whereas it had a positive correlation with the observance of off flavors (0.80; $P < 0.01$). Ribeiro (2020) reported that an increase in relative humidity can shift sensory attributes from neutral flavor notes (wet aged, 50% relative humidity) to sour and off-flavors (70 and 85% relative humidity) [15]. The percent aging loss also had a high negative correlation with sensory juiciness (-0.80; $P < 0.01$). However, results from the consumer taste panel should be interpreted cautiously. Consumers suggested differences in flavor intensity of sample on ballots of sample evaluations, but intensity of flavor was not evaluated. Additionally, around half of the consumers in the study indicated they had previously consumed dry-aged beef. The

relative novelty of flavors to the remaining consumer panelists could also be attributed to the taste panel results observed.

Microbial Growth

Aerobic plate count (APC) means were different by treatment-location ($P < 0.01$) and ranged from 0.18 to 4.00 colony forming unit (cfu)/cm2 (Table 4.7). Three of the eleven locations, not including the wet-aged treatment, had aerobic plate counts <1 cfu/cm2. Camp-bell et al. (2001) reported that duration of dry-aging did not impact APC, while Ryu et al. (2018) reported increased microorganism growth until day 50 of dry-aging [4, 36]. Berger et at. (2018) and Ribeiro (2020) reported dry-aged beef had lower APC than wet-aged counterparts [11,15]. In the current study, however, treatment-location J had a higher APC than the wet-aged treatment. The combination of lower humidity and greater wind speeds is likely contributing to drying of the dry-aged product surface and subsequently resulted in lower APC of dry-aged beef products. The coliform plate count means ranged from 0.59 to 4.00 cfu/cm2 and were different ($P < 0.01$) by treatment-location. No E. coli were observed. Location A had the lowest mean plate counts for both APC and Coliform assessments and was the only location with plate counts <1 cfu/cm2. Location A also had the lowest relative humidity in the refrigerated aging environment during the study. Mean-while, location J had the greatest mean plate counts for APC and coliform assessments. Location J also had one of the higher mean relative humidity (82.86%) and the lowest average wind speed (0.56 meters/second) observed. Conflicting results have been reported in previous studies on the abundance, or lack of abundance, [36] of coliforms on dry-aged beef. The relative dryness of the crust of dry-aged beef may create a hurdle for growth of E. coli as illustrated by this study with no E. coli found from the surface of the product.

Conclusion

Dry-aging is a meat production process influenced by an array of factors. Temperature, percent relative humidity, and wind speed are all aging conditions that vary across commercial aging facilities. Aging conditions in commercial dry-aging facilities contribute to unique differences in consumer eating preferences. Consumers can identify differences between dry-aged and wet-aged products. Within the dry-aged sector, the aging conditions of

the product provide individuality to dry-aged products. Moreover, consumer acceptability of dry-aged beef is likely influenced by previous eating experiences and interpretation of product flavor.

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Table 4.1. Mean environmental conditions and strip loin evaporative aging loss for aging treatment-locations for a 45-day aging period.

Aging Location	Temperature, °C[1]	Relative Humidity, %[1]	Wind Speed[2]	Aging Loss, %
A	4.09±0.58^{bc}	64.87±2.51^{d}	1.05±0.08^{cd}	13.26±0.01^{cd}
B	1.98±0.58^{ef}	72.52±2.51^{c}	1.13±0.08^{cd}	15.46±0.01^{ab}
C	5.26±0.77^{a}	72.07±3.32^{cd}	1.70±0.08^{b}	15.74±0.01^{ab}
D	2.81±0.58^{de}	85.47±2.51^{ab}	1.07±0.08^{cd}	13.30±0.01^{bcd}
E	3.64±0.58^{cd}	73.20±2.51^{c}	2.03±0.08^{a}	15.89±0.01^{a}
F	1.67±0.58^{fg}	71.38±2.51^{cd}	0.94±0.08^{de}	14.63±0.01^{abc}
G	4.26±0.58^{b}	77.22±2.51^{bc}	0.75±0.08^{ef}	12.95±0.01^{cd}
H	0.74±0.58^{h}	82.94±2.51^{ab}	1.09±0.08^{cd}	10.85±0.01^{e}
I	1.06±0.58^{gh}	92.21±2.51^{a}	1.23±0.08^{c}	11.62±0.01^{de}
J	1.33±0.58^{fgh}	82.86±2.51^{ab}	0.56±0.08^{f}	10.16±0.01^{e}
Wet[3]	0.76±0.58^{h}	-	-	0.55±0.01^{f}
Minimum[4]	0.74	64.87	0.56	0.55
Maximum[5]	5.26	92.21	2.03	15.89

[1] Parameters recorded via an Onset® HOBO® temp/RH logger at five-minute intervals

[2] Measured using handheld anemometers on a weekly basis, reported in m/s

[3] Cooler parameters not included due to protective packaging limiting impact

[4] Minimum mean observed

[5] Maximum mean observed

[a-h] Within a trait, means without a common superscript differ ($P < 0.05$)

Table 4.2. Mean intrinsic quality factors of 45-day dry-aged beef by aging treatment-location.

Aging Location	pH	AW[1]	L*[2]	a*[3]	b*[4]	WBSF[5], kg
A	5.66±0.24	0.99±0.002	37.01±0.71	20.18±0.73	14.81±0.56	1.92±0.14
B	5.68±0.24	0.99±0.002	36.21±0.71	20.46±0.73	13.61±0.56	1.95±0.15
C	5.67±0.24	0.98±0.002	37.01±0.71	21.51±0.73	15.61±0.56	1.59±0.15
D	5.67±0.24	0.99±0.002	37.03±0.71	20.04±0.73	14.68±0.56	2.08±0.15
E	5.65±0.24	0.98±0.002	36.77±0.71	19.19±0.73	13.77±0.56	1.84±0.16
F	5.73±0.24	0.99±0.002	36.16±0.71	19.71±0.73	13.79±0.56	2.19±0.16
G	5.66±0.24	0.98±0.002	35.96±0.71	20.63±0.73	13.99±0.56	1.87±0.14
H	5.66±0.24	0.99±0.002	37.43±0.71	19.40±0.73	14.61±0.56	2.21±0.15
I	5.61±0.24	0.99±0.002	37.03±0.71	21.48±0.73	15.73±0.56	1.97±0.14
J	5.67±0.24	0.98±0.002	36.66±0.71	20.97±0.73	14.50±0.56	1.87±0.15
Wet	5.64±0.24	0.99±0.002	37.56±0.71	20.66±0.73	15.20±0.56	1.83±0.15
Min[6]	5.61	0.98	35.96	19.19	13.61	1.59
Max[7]	5.73	0.99	37.56	21.58	15.73	2.21

[1] AW: Water Activity

[2] L*: objective color measurement (0=black; 100=white)

[3] a*: objective color measurement (-50=green; 50=red)

[4] b*: objective color measurement (-50=blue; 50=yellow)

[5] Warner-Bratzler Shear Force

[6] Minimum mean observed

[7] Maximum mean observed

Table 4.3. Consumer taste panel summary statistics of panelist demographics (N = 219).

	n	%
Age		
18-19	19	8.7
20-29	62	28.3
30-39	42	19.2
40-49	30	13.7
50+	66	30.1
Gender		
Male	111	50.7
Female	108	49.3
Beef meals/week [1]		
0 to 1	21	9.7
2 to 4	87	40.3
5 to 7	76	35.2
8+	32	14.8
Most consumed [2]		
Ground	132	57.6
Roast	16	7.0
Steak	78	34.1
Other	3	1.3
Dry-aged beef [3]		
Yes	114	54.0
No	97	46.0

For [1], [2], and [3], consumers were asked:

[1] Please indicate the number of meals a week in which you consume beef: 0-1, 2-4, 5-7, or 8+.

[2] Please indicate the form in which you most commonly consume beef: ground, roast, steak, or other.

[3] Have you ever consumed dry-aged beef?: yes or no.

Table 4.4. Consumer (N = 219) taste panel mean evaluations of 45-day aged steaks by aging treatment-location and unaged steaks.

Aging Location	Acceptability[1]	Tenderness[2]	Juiciness[3]	Flavor[4]
A	7.17±0.20^{ab}	7.35±0.18^{ab}	6.75±0.18	6.62±0.22^{ab}
B	6.82±0.20^{abc}	7.20±0.18^{ab}	6.64±0.18	6.26±0.22^{abc}
C	6.65±0.20^{abc}	7.47±0.18^{a}	6.76±0.18	6.10±0.23^{abc}
D	6.28±0.20^{c}	7.35±0.18^{ab}	6.88±0.17	5.52±0.22^{c}
E	6.52±0.21^{abc}	7.06±0.19^{ab}	6.65±0.19	5.92±0.23^{abc}
F	7.05±0.20^{abc}	7.13±0.18^{ab}	6.68±0.18	6.59±0.23^{ab}
G	6.82±0.20^{abc}	7.25±0.18^{ab}	6.72±0.18	6.56±0.22^{ab}
H	6.44±0.20^{bc}	6.79±0.18^{ab}	6.65±0.18	6.23±0.22^{abc}
I	6.54±0.21^{abc}	6.66±0.19^{b}	6.52±0.19	5.84±0.24^{bc}
J	6.38±0.21^{c}	7.05±0.19^{ab}	6.78±0.19	5.70±0.23^{bc}
Wet	7.26±0.20^{a}	7.51±0.18^{a}	7.21±0.18	6.86±0.22^{a}
Minimum[5]	6.28	6.66	6.52	5.52
Maximum[6]	7.26	7.51	7.21	6.86

[1] Overall Acceptability Score: 10= extremely acceptable; 1= extremely unacceptable

[2] Tenderness Score: 10= extremely tender; 1= not at all tender

[3] Juiciness Score: 10= extremely juicy; 1= extremely dry

[4] Flavor Score: 10= extremely flavorful; 1= extremely unflavorful

[5] Minimum mean observed

[6] Maximum mean observed

[a-c] Within a trait, means without a common superscript differ ($P < 0.05$)

Table 4.5. Percentage of consumers (N = 219) who noted the following flavors associated with dry-aged beef by aging location and unaged steaks.

Aging Location	Satisfaction	Off-flavor	Cheese	Sour	Nutty	Yeasty	Earthy	Sweet	Metallic
A	81.1	12.9	5.2	3.1	11.5	5.2	16.7	8.3	9.4
B	74.2	22.5	7.4	3.2	11.6	12.6	12.6	4.2	8.4
C	67.4	30.0	13.3	12.2	12.2	11.1	15.6	8.9	11.1
D	59.4	44.6	19.2	15.4	17.3	12.5	18.3	7.7	12.5
E	74.7	25.6	7.0	5.8	17.4	3.5	18.6	4.7	10.5
F	77.7	20.0	5.3	3.2	9.6	7.5	25.5	3.2	7.5
G	80.4	20.9	5.4	2.2	18.5	1.1	25.0	6.5	6.5
H	77.8	26.4	14.1	9.8	12.0	10.9	19.6	10.9	7.6
I	66.3	26.9	18.5	11.1	16.1	8.6	22.2	8.6	9.9
J	56.1	38.8	17.1	12.2	28.1	12.2	23.2	11.0	9.8
Wet	87.9	14.4	2.2	2.2	12.0	6.5	14.1	10.9	12.0
Unaged[5]	76.0	5.3	6.2	6.2	8.6	3.7	18.5	11.1	7.4
P - value	<0.01	<0.01	<0.01	<0.01	0.03	0.15	0.42	0.46	0.95

[1] Unaged steaks were frozen upon receiving of product

Table 4.6. Pearson correlation coefficients across refrigerated environment aging parameters and sensory attributes.

	Sensory Attributes				
Aging Parameters	Acceptability[1]	Tenderness[2]	Juiciness[3]	Flavor[4]	Off-Flavor[5]
Temperature	-0.04	0.31	-0.35	-0.02	0.01
Relative Humidity	-0.79**	-0.48	0.23	-0.64*	0.80**
Air Speed	-0.07	-0.04	-0.06	-0.11	-0.02
Aging Loss	-0.30	-0.21	-0.80**	-0.31	0.19

[1] Overall Acceptability Score: 10= extremely acceptable; 1= extremely unacceptable

[2] Tenderness Score: 10= extremely tender; 1= not at all tender

[3] Juiciness Score: 10= extremely juicy; 1= extremely dry

[4] Flavor Score: 10= extremely flavorful; 1= extremely unflavorful

[5] Off-flavor: observance of off flavor

* $P < 0.05$; ** $P < 0.01$

Table 4.7. Microbial growth of 45-day aged steaks by aging treatment-location.

Aging Location	Aerobic Plate Counts[2]	E. coli[1]/Coliform Plate Count[2,3]
A	0.18±0.44^{e}	0.59±0.67^{c}
B	1.11±0.41^{cde}	1.29±0.67^{c}
C	0.35±0.41^{de}	1.04±0.67^{c}
D	1.97±0.41^{bc}	3.60±0.67^{a}
E	0.73±0.41^{de}	2.21±0.74^{abc}
F	0.68±0.41^{de}	1.20±0.67^{c}
G	1.43±0.41^{bcd}	1.44±0.74^{c}
H	2.32±0.33^{b}	3.53±0.67^{ab}
I	2.36±0.41^{b}	2.08±0.74^{abc}
J	4.00±0.41^{a}	4.00±0.74^{a}
Wet	1.33±0.41^{bcd}	1.54±0.74^{bc}
Minimum[3]	0.18	0.59
Maximum[4]	4.00	4.00

[1] Zero E. coli were identified

[2] Plate counts estimated in accordance with 3M™ Interpretation Guides

[3] Log_{10} colony-forming units/cm^2

[4] Minimum mean observed

[5] Maximum mean observed

[a-e] Within a column, means without a common superscript differ ($P < 0.05$)

Appendix A

TBARS for oxidative rancidity - rapid, wet method

Adapted from Appendix O (AMSA, 2012)

Principle:

In the presence of thiobarbituric acid (TBA), malonaldehyde and other aldehyde products of lipid oxidation (TBA reactive substances; TBARS) form pink chromogens with maximum absorbance at 532-535 nm. However, in the presence of interfering sugars, a yellow chromogen forms, which can be avoided using the distillation method (Tarladgis, 1960).

Reagents:

1. TBA stock solution - 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25N HCl.
2. Stock solutions (100 mL) are sufficient for 20 individual tests. Stock solution may be stored at room temperature in the dark (foil-wrapped container).

Procedure:

1. Finely chop or mince a portion of the product of interest. Weigh out duplicate 0.25 g samples.
2. Add 1.25 ml TBA stock solution to each sample, giving a dilution factor of 6. Mix well.
3. Heat samples 10 min in boiling water in loosely capped 2.0 ml eppendorf tubes **Caution:** tightly capped tubes may burst during heating. Positive samples turn pink during heating.
4. Cool tubes in tap water.
5. Centrifuge at 5,000 × g for 10 min to obtain a clear supernatant.
6. Carefully pipette 200 µl of the supernatant to a 96 well plate. Take care that the solution remains clear.
7. Measure supernatant absorbance at 532 nm against a blank that contains all the reagents minus the meat.
8. Calculate the TBA value expressed as ppm malonaldehyde, using 1.56×10^5/M/cm as the extinction coefficient of the pink TBA chromogen (Sinnhuber and Yu, 1958), as follows:
   TBARS number (mg MDA/kg) = sample A_{532} × (1 M TBA chromagen/156,000) × [(1mole/L/M] × (0.003 L/0.5 g meat) × (72.07 g MDA/mole MDA) × 1000 mg/g) × 1000 g/kg) or **TBARS value (ppm)** = sample A_{532} × 2.77

References:

Buege, J.A. and Aust, S.D. 1978. Microsomal lipid peroxidation. Methods in Enzymology 52:302-304.

Sinnhuber, R.O. and Yu, T.C. 1958. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. Food Technology 12(1):9-12.

Appendix B

Calpain extraction

Solution:

Extraction buffer pH 8.3 (50ml)

Tris 0.6056g (100mM)
EDTA 0.1461g 10mM)
DTT 0.0772g (10mM)

Procedure:

1. Homogenize 1g muscle in 3mL extraction buffer in 15 ml centrifuge tubes three times on ice for 15s with 15s cooling between bursts.
2. Pipet the homogenate into 2ml microcentrifuge tubes. 1.0 ml/tube, 2 tubes per sample and centrifuge at 8,800xg for 30 min @ 4ºC
3. Pour supernatant into 1.5ml microcentrifuge tubes and freeze in -80 freezer.

Appendix C

Casein zymography

Solutions: *Those containing DTT Make fresh Daily*

1.5M Tris base pH 8.8
18.15g/100ml H2O – bring up to 100mL w/ water
pH to 8.8 with HCl. Filter and store at 4ºC

0.5M Tris base pH 6.8
6g/100ml H2O – bring up to 100mL w/ water
pH to 6.8 with HCl. Filter and store at 4ºC

Stock acrylamide 30%
25 ml of 75:1
7.4013g acrylamide
0.0988g bisacrylamide

10% ammonium persulfate
1g/10ml H2O – bring up to 10mL w/ water
Store in dark bottle @ 4ºC

Water saturated butanol (60ml)
50ml n-Butanol and 10ml H2O

Sample buffer pH 6.8 (10ml)
trisHCL 0.1817g (150mM)
glycerol 2ml (20%)
DTT 0.01543g (10mM)
Bromophenol blue (0.02%) 0.25ml of 0.8% bromophenol blue

Running buffer pH 8.3 (1 liter)
trisHCL 3.0275g (25mM)
DTT 0.1543g (1mM)
Glycine 14.4g (192mM)
EDTA 0.2922 (1mM)

Incubation buffer pH 7.5 (250ml)
Tris HCL 1.5138g (50 mM)
DTT 0.3856g (10mM)
CaCl2 0.111g (4mM)

Separating gel 12.5%	Enough for 4 1 mm Gels**
1.5M Tris pH 8.8	6.25ml
30% Acrylamide	10.44ml
H2O	7.94mL
Casein	0.05g
APS	125µl
Temed	12.5µl

Stacking gel 4%	Enough for 4 1 mm Gels**
0.5M Tris pH 6.8	1.88ml
30% Acrylamide	1.0ml
H2O	4.55ml
APS	50µl
Temed	7.5µl

Procedure:

1. The height of the separating gel is 5.5cm
2. Mix separating gel and degas 15 min. Add APS and TEMED and mix immediately before pouring gel (step 3)
3. Pour gel (5.5cm) overlay with water saturated butanol and allow to polymerize 1 hour
4. Make stacking gel and degas 15 minutes. Add APS and TEMED and mix immediately before stacker is to be poured (See step 5)
5. Pour off water saturated butanol and rinse well with distilled water. Remove any residual water with a kimwipe. Place comb in between plates. Pour the wells, allow to polymerize 30 min.
6. Remove comb and rinse wells with water, remove residual water with Kimwipe™.

Sample preparation

1. Thaw samples at room temp while pouring gels
2. Add 10µl of sample buffer to 40µl of supernatant

Running the gels

1. The casein minigels (1.0 mm) were run at 100V for 15min in an ice bath with running buffer before loading samples (The first gels in June 10µl was used in 0.75mm gels. We now need to use 20µl in 1.0mm gels)
2. Run gels at 100V for 6 hours in an ice water bath
3. Incubate in incubation buffer at room temp with slow shaking for 1 h (2 changes of buffer) followed by 16 h incubation in same buffer at room temp.
4. Stain for 1 hour in Coomassie blue R250
5. Destain for 3 hours (longer if necessary) in Coomassie blue R250 destaining solution
6. Remove, analyze using gel doc in biotech.

Dry using gel air drying frame and cellophane for 3h w/ no heat, 1h w/ heat.

Appendix D

Temperature and pH model estimation

Table D.1. Temperature decline parameter estimates (mean ± SEM) for the intercept term (a+c), rate of change over time (hour) (b), and lower asymptote(c). Temperature was modeled using nonlinear regression following the exponential form: $y_i = a*\exp(-b*hour_i) + c$ where y_i is the temperature. Estimates of oversized superficial (OS), oversized deep (OD), average weight superficial (AS), and average weight deep locations (AD) locations.

Parameter	AS	AD	OS	OD
A	30.00±0.42	38.91±0.39	28.86±0.39	39.72±0.41
B	0.092±0.003	0.070±0.002	0.089±0.003	0.056±0.002
C	2.41±0.43	2.18±0.32	3.02±0.22	2.00±0.43

Table D.2. pH decline parameter estimates (mean ± SEM) for the intercept term (a+c), rate of change over time (hour) (b), and lower asymptote(c). The pH was modeled using nonlinear regression following the exponential form: $y_i = a*\exp(-b*hour_i) + c$ where y_i is the pH. Estimates of oversized superficial (OS), oversized deep (OD), average weight superficial (AS), and average weight deep locations (AD) locations.

Parameter	AS	AD	OS	OD
A	1.09±0.05	0.69±0.06	1.00±0.05	0.23±0.12
B	0.15±0.01	1.22±0.27	0.24±0.02	1.15±0.90
C	5.51±0.03	5.49±0.02	5.57±0.02	5.60±0.05

Appendix E

Temperature and pH model confidence intervals

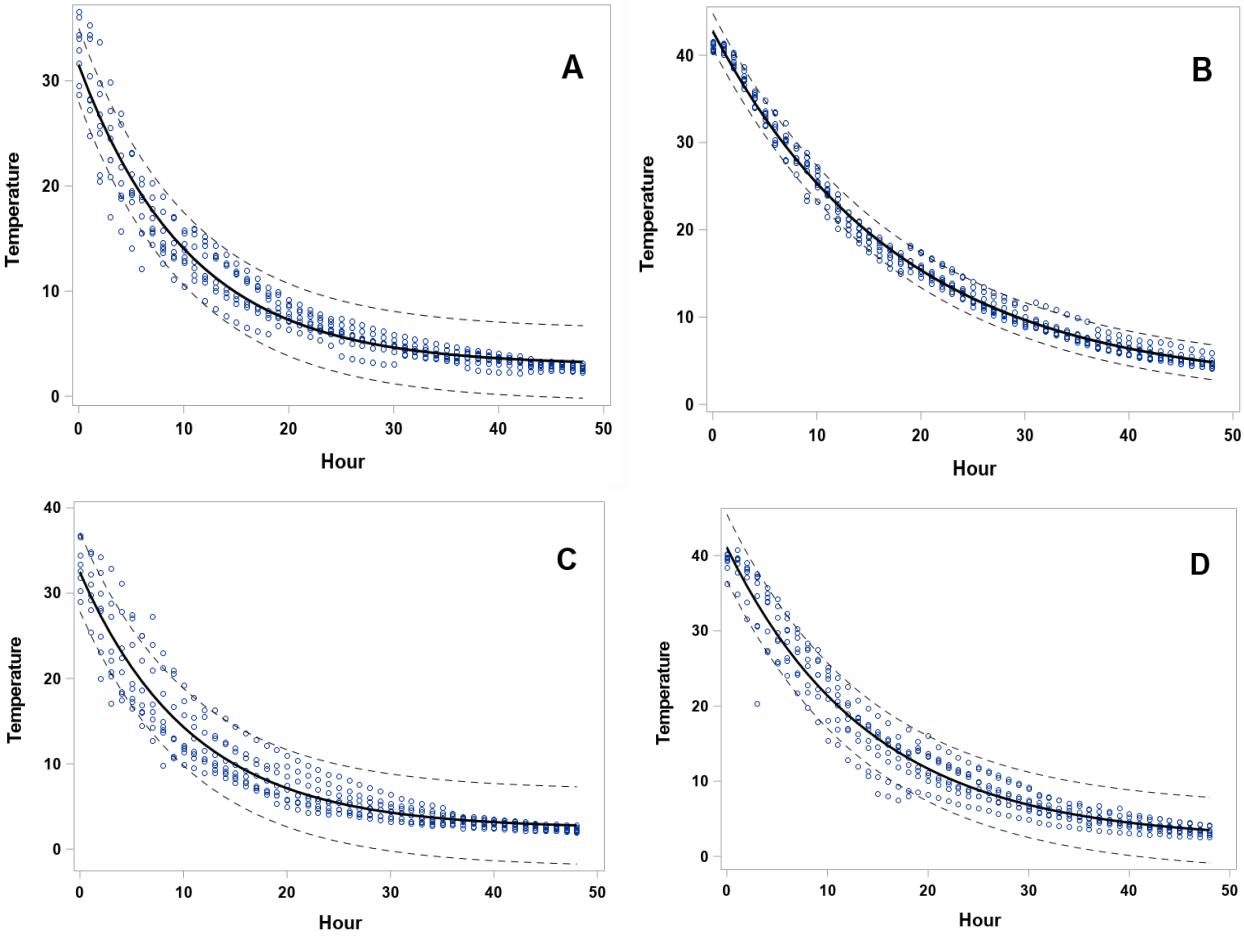

Figure E.1. Estimated models and error estimates of temperature (°C) of top round subprimals (Semimembranosus) decline over 48 h chill (starting immediately after final inspection, ~35 min postmortem) period for average and oversized carcasses monitoring the deep and superficial location. Individual lines [average superficial (AS), average deep (AD), oversized superficial (OS), oversized deep (OD)] estimated following the model: temperature = a*exp(-b*hour)+c.

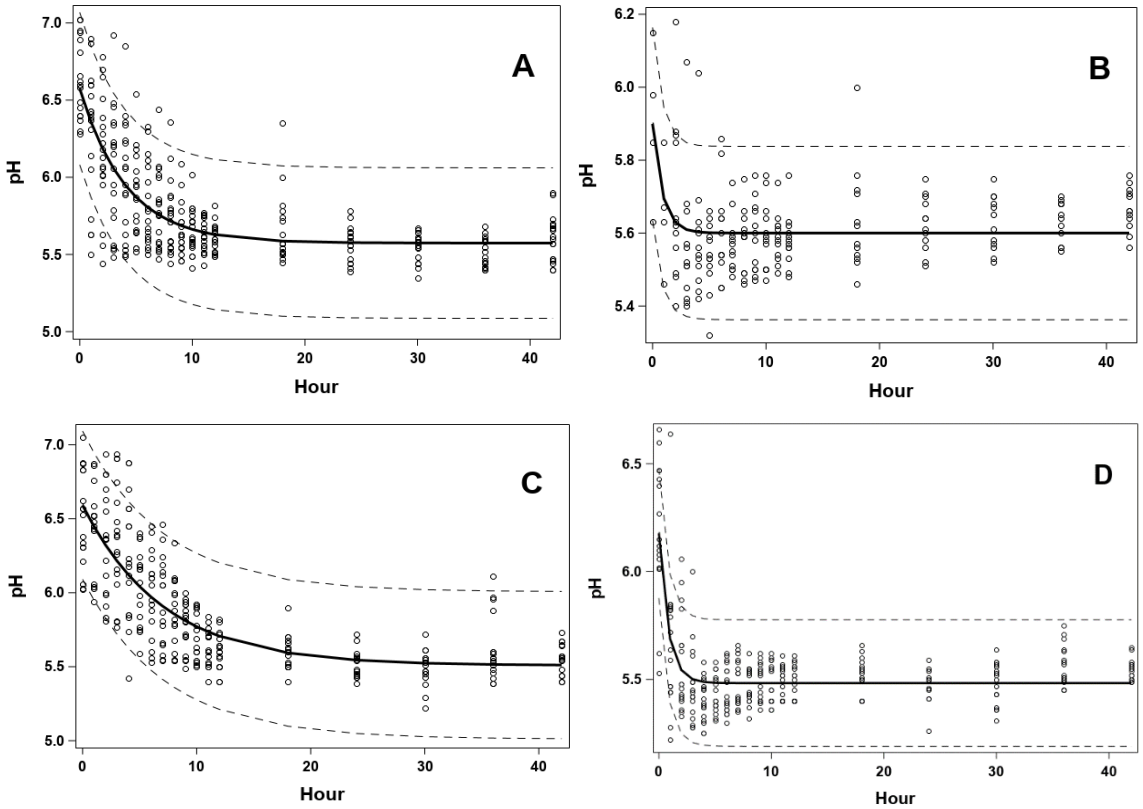

Figure E.2. Estimated models and error estimates of pH decline of top round subprimals decline over 48 h chill (starting immediately after final inspection, ~35 min postmortem) period for average and oversized carcasses monitoring the deep and superficial location. Individual lines [average superficial (AS), average deep (AD), oversized superficial (OS), oversized deep (OD)] estimated following the model: pH = a*exp(-b*hour)+c.

Appendix F

Metmyoglobin reducing capacity of intact or ground meat

Adapted from Appendix J (AMSA, 2012)

Principle

Surface pigments are initially oxidized to MMb by soaking the sample slice in a dilute sodium nitrite solution for 20 minutes. The slice (1.27 cm thick) is vacuum packaged, and surface % MMb is monitored for 2 hours at 30°C by measuring reflectance *K*/*S* ratios (572/525 nm). Sample reducing ability is defined as the percentage decrease in surface MMb concentration during the incubation period. The decline in MMb is assumed to reflect the tissue's ability to reduce ferric heme iron.

Reagent

0.3% (w/w) sodium nitrite solution: Tare a large beaker, and weigh 3.0 g $NaNO_2$ into the beaker and add distilled water to 1000 g. Make fresh daily. Incubate at room temperature.

Procedure

Remove a 3 cm × 3 cm × 2 cm sample of muscle tissue with no visible fat or connective tissue. For ground meat, use a similar sized sample that has been uniformly packed together to help avoid crumbling when the sample is immersed.

Be sure to orient sample to identify which surface will be evaluated later. This surface may be fresh cut or the surface that was displayed.

Submerge sample in 0.3% $NaNO_2$ solution for 20 minutes at room temperature to induce MMb formation. Ground samples can be placed on a small screen to help lower and raise the cube with minimal crumbling.

Remove sample from beaker, and blot to remove excess solution. Retain the 3-dimensional shape as much as possible and place the surface for evaluation up in an impermeable bag and vacuum package (a good, uniform vacuum). The vacuum may slightly flatten or round the samples.

Scan immediately for reflectance from 400 to 700 nm to determine the initial amount of MMb formed on the surface. Maintain surface integrity.

Place sample in an incubator at 30°C, and rescan after 2 hours to determine the remaining amount of MMb.

Calculations

%MMb = [*K/S*572 ÷ *K/S*525 (for 100% DMb)] − [*K/S*572 ÷ *K/S*525 (sample)] ÷ [*K/S*572 ÷ *K/S*525 (for 100% DMb)] − [*K/S*572 ÷ *K/S*525 (for 100% MMb)][× 100].

MRA (% of MMb reduced) = [(Initial %MMb − Final %MMb) ÷ Initial %MMb] × 100

or

Use the initial MMb formed as an indicator of MRA (see note below).

Notes

Some authors (McKenna et al., 2005; Mancini et al., 2008) indicate that the initial amount of MMb formed by oxidation in sodium nitrite solution is a good indicator of sample MRA. However King et al. (2011) found that percentage reduction was better than the initial amount of MMb formed. Thus, it is best to collect and statistically analyze both the initial amount of MMb formed, and the percentage of MMb reduced over the incubation time.

References

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Watts, B. M., J. Kendrick, M. W. Zipser, B. Hutchins, and B. Saleh. 1966. Enzymatic reducing pathways in meat. J. Food Sci. 32:855–862.

Appendix G

Oxygen consumption of intact muscle or ground meat

Adapted from Appendix I (AMSA, 2012)

Principle:
Freshly cut meat slices are oxygenated (allowed to bloom) for a standardized time and temperature and then vacuum packaged. The decline in OMb due to enzyme respiration is measured as an indicator of the tissue's ability to consume oxygen. Reflectance spectra over the range 400 to 700 nm are recorded immediately and a second time after 20 minutes in a water bath or incubator kept at 25°C. Oxymyoglobin levels are calculated using the ratio of the reflectance at 610 and 525 nm after *K*/*S* transformation as described in Section IX. Higher *K*/*S*610/*K*/*S*525 ratios indicate higher OMb levels. Oxygen consumption (OC) is reported as the difference in percentage from the first and last measurements.

Equipment and Supplies
1. Vacuum packaging machine
2. PVC film
3. Highly oxygen-impermeable vacuum bags (O2 permeability ≤0.6 g O2/625 cm2/24 hours at 0°C)
4. Spectrometer that can scan and record surface reflectance from 400 to 700 nm (see Section IX)

Procedure
1. All samples to be assayed must be the same temperature, 4°C, for instance. Otherwise oxygen consumption will be faster for samples at warmer temperatures and bloom development (oxygenation) will be less; it will be slower for those at colder temperatures and bloom development will be more.
2. Keep all samples at 2 to 4°C to help ensure uniform oxygenation. For intact, whole muscle, use a sharp knife to remove a 3 cm × 3 cm × 2 cm sample with minimal visible fat or connective tissue. For ground samples, prepare a comparable sized cube that has been uniformly packed. Again, the visible fat level should be typical of the lean portion of the sample. Avoid dull knives that disrupt surface structure. Also avoid excessive handling and pressing of the blooming surface of ground product (see Madhavi and Carpenter, 1993).
3. If the surface is not a fresh cut, then just before starting the bloom step, remove a thin surface layer to expose fresh tissue.
4. Cover the freshly cut surface with a small piece of oxygen-permeable film to avoid drying. Keep the film (polyvinyl chloride film is commonly used) in one, smooth layer to ensure uniform exposure of the surface to air. Make note of the film's oxygen permeability.
5. Bloom for 2 hours at 2 to 4°C (or some other standardized time). Take care to keep all samples at the same temperature during this step because blooming is very temperature dependent.

6. After bloom, remove the PVC film and place the sample in a pouch with very low oxygen permeability. Quickly vacuum package with high vacuum; keep the vacuum uniform from sample to sample.
7. IMMEDIATELY scan the surface of the sample for reflectance from 400 to 700 nm to determine the initial % OMb. The spectrophotometer must be calibrated through the vacuum bag film.
8. To speed up oxygen consumption, use an incubator or water bath at 25°C. Re-scan the same surface after 20 minutes (or some standardized time appropriate to the meat being used).

Calculations

%OMb = [*K/S*610 ÷ *K/S*525 (for 100% DMb)] − [*K/S*610 ÷ *K/S*525 (sample)] ÷ [*K/S*610 ÷ *K/S*525 (for 100% DMb)] − [*K/S*610 ÷ *K/S*525 (for 100% OMb)][× 100].

Oxygen consumption = [(Initial % OMb − Ending % OMb) ÷ Initial % OMb] × 100.

Notes

Madhavi and Carpenter (1993) described a reflectance procedure for measuring oxygen consumption (OC), using a spectrophotometer with reflectance attachment to measure surface OMb levels of vacuum packed samples initially, and at 5-minute intervals (20 minutes total) at 4°C. Samples were smaller (2.5 × 2.5 × 0.5 cm) to fit in the sample port of the reflectance unit.

Relative concentration of OMb was calculated using the method of Krzywicki (1979). However, that method was modified by Tang et al. (2004) and their revised wavelengths are recommended (see Section IX). OC was expressed as percentage of time-zero surface OMb consumed during

10 minutes in vacuum. Mancini, Hunt and Kropf (2003) reported a method using reflectance at 610 nm to directly determine OMb. This is possible because OMb has its unique reflectance at 610 while 610 is isobestic for both DMb and MMb (see Section IX for further discussion of meat surface reflectance measurements and calculation of *K/S* ratios). This method has been used successfully (see King et al., 2011). Some research has reported an actual "rate of oxygen consumption" using percentage changes of OMb per unit of time. This is more laborious and time consuming. With a large number of samples, "oxygen consumption" is often calculated as the "average percentage reduction of OMb" relative to the initial level of OMb formed on the sample. The time for deoxygenation of the sample must to be standardized. Usually, 20 minutes is sufficient to detect sample differences.

References

King, D. A., S. D. Shackelford, A. B. Rodriguez, and T. L. Wheeler. 2011. Effect of time of measurement on the relationship between metmyoglobin reducing activity and oxygen consumption to instrumental measures of beef longissimus color stability. Meat Sci. 87:26–32.

Madhavi, D. L., and C. E. Carpenter. 1993. Aging and processing affect color, metmyoglobin reductase and oxygen consumption of beef muscles. J. Food Sci. 58:939–942.

Mancini, R. A., M. C. Hunt, and D. H. Kropf. 2003. Reflectance at 610 nanometers estimates oxymyoglobin content on the surface of ground beef. Meat Sci. 64:157–162.

Mohan, A., M. C. Hunt, T. J. Barstow, T. A. Houser, and D. M. Hueber. 2010a. Near-infrared oximetry of three postrigor skeletal muscles for following myoglobin redox forms. Food Chem. 123:456–464.

Tang, J., C. Faustman, and T. A. Hoagland. 2004. Krzywicki revisited, equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. J. Food Sci. 69:C717–C720.

Appendix H

Interactions for subjective color and objective color evaluations

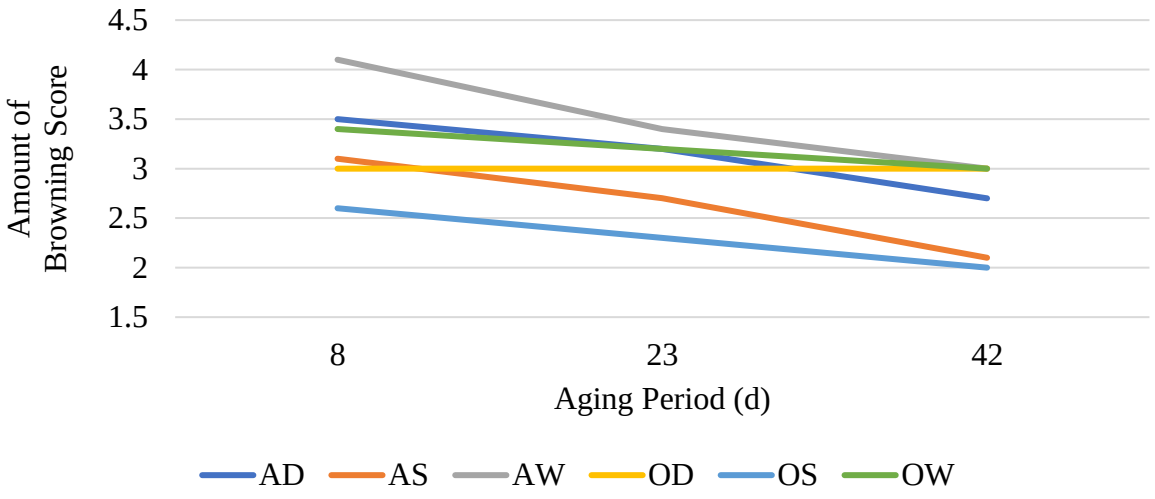

Figure H.1. Mean estimates for subjective amount of browning score for top round steaks from steak section treatment combinations (average weight deep [AD], average weight superficial [AS], average weight whole [AW], oversized deep [OD], oversized superficial [OS], and oversized whole [OW]) across three aging periods (8, 23, and 42 d).

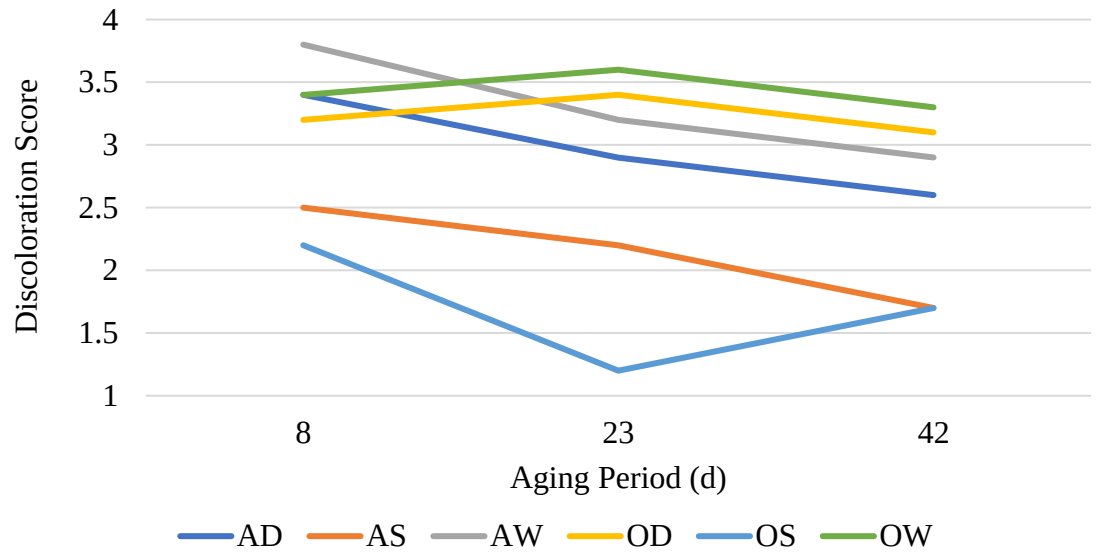

Figure H.2. Mean estimates for subjective discoloration score for top round steaks from steak section treatment combinations (average weight deep [AD], average weight superficial [AS], average weight whole [AW], oversized deep [OD], oversized superficial [OS], and oversized whole [OW]) across three aging periods (8, 23, and 42 d).

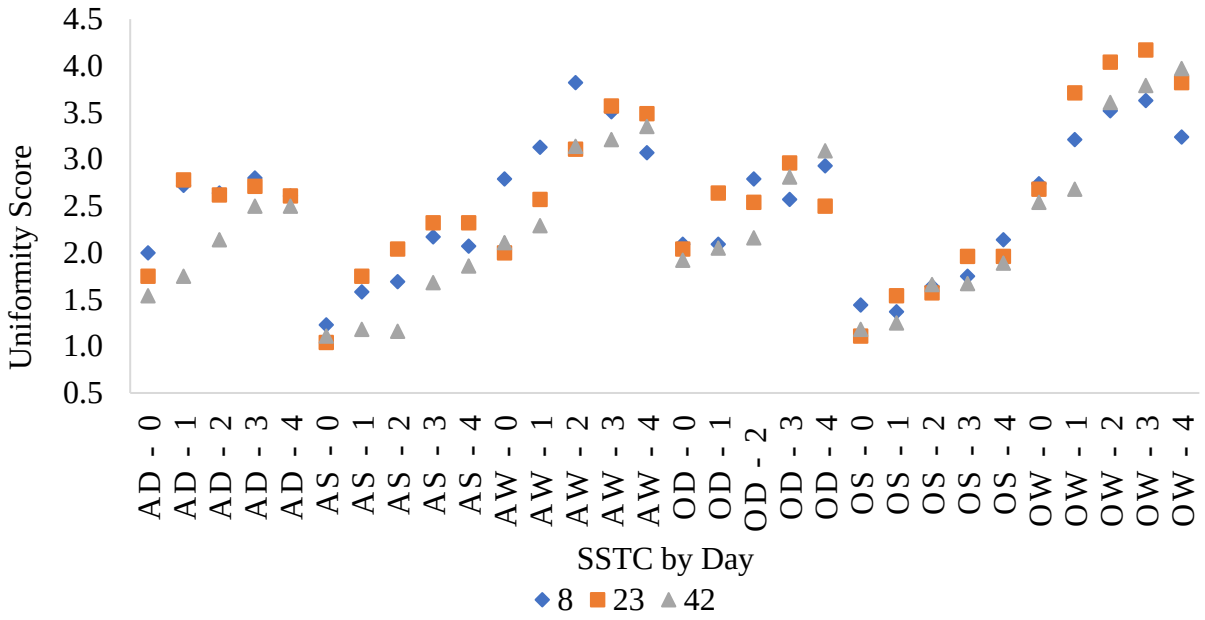

Figure H.3. Mean estimates for subjective color uniformity score for top round steaks from steak section treatment combinations (SSTC) (average weight deep [AD], average weight superficial [AS], average weight whole [AW], oversized deep [OD], oversized superficial [OS], and oversized whole [OW]) over simulated retail display (day 0-4) across three aging periods (8, 23, and 42 d).

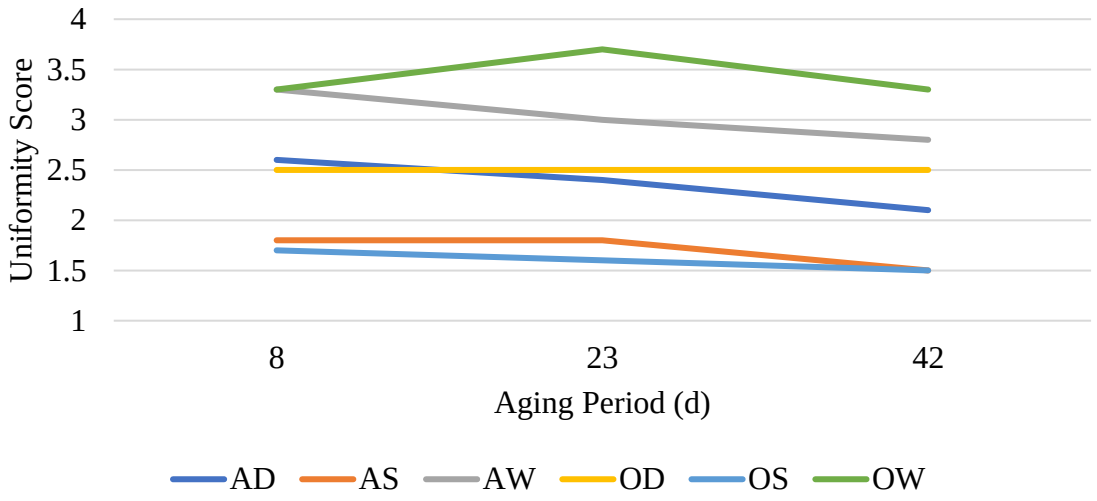

Figure H.4. Mean estimates for subjective color uniformity score for top round steaks from steak section treatment combinations (average weight deep [AD], average weight superficial [AS], average weight whole [AW], oversized deep [OD], oversized superficial [OS], and oversized whole [OW]) across three aging periods (8, 23, and 42 d).

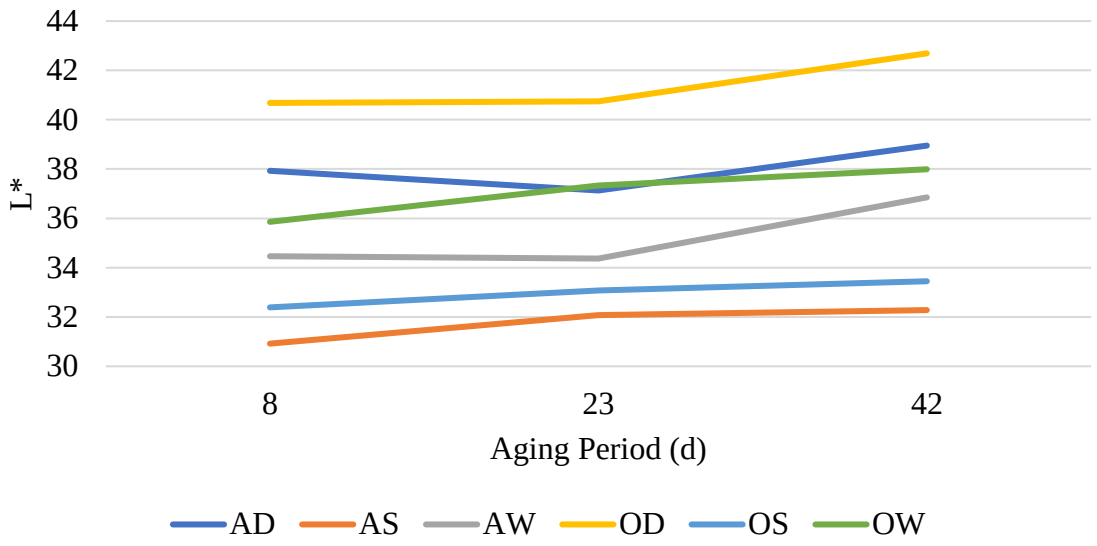

Figure H.5. Mean estimates for L* (lightness) objective color for top round steaks from steak section treatment combinations (average weight deep [AD], average weight superficial [AS], average weight whole [AW], oversized deep [OD], oversized superficial [OS], and oversized whole [OW]) across three aging periods (8, 23, and 42 d).

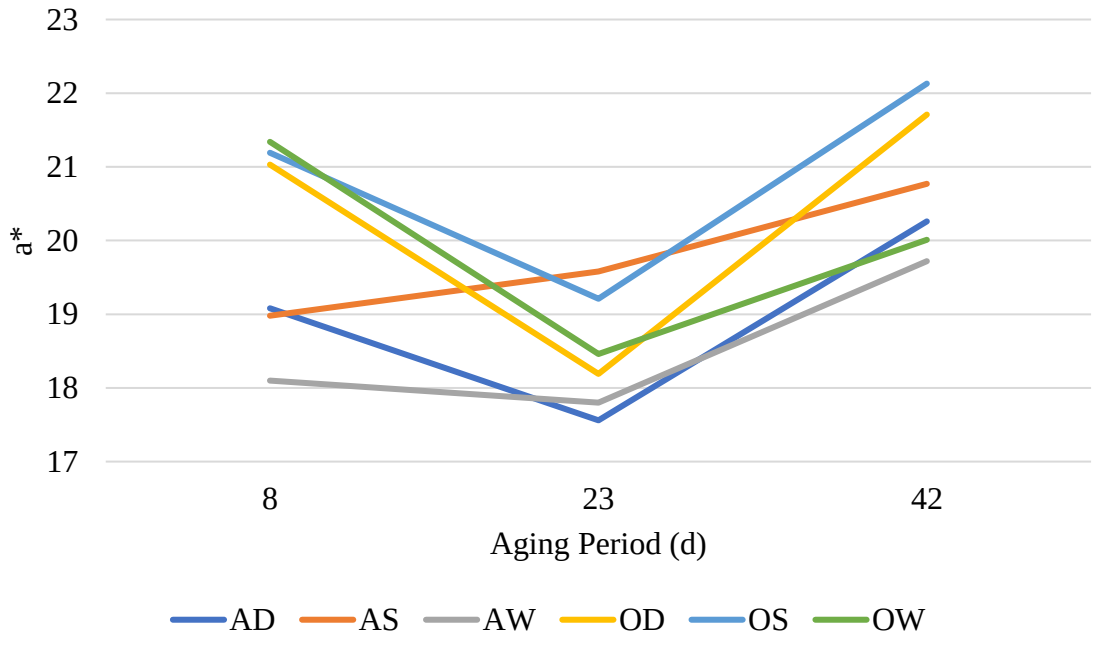

Figure H.5. Mean estimates for a* (redness) objective color for top round steaks from steak section treatment combinations (average weight deep [AD], average weight superficial [AS], average weight whole [AW], oversized deep [OD], oversized superficial [OS], and oversized whole [OW]) across three aging periods (8, 23, and 42 d).

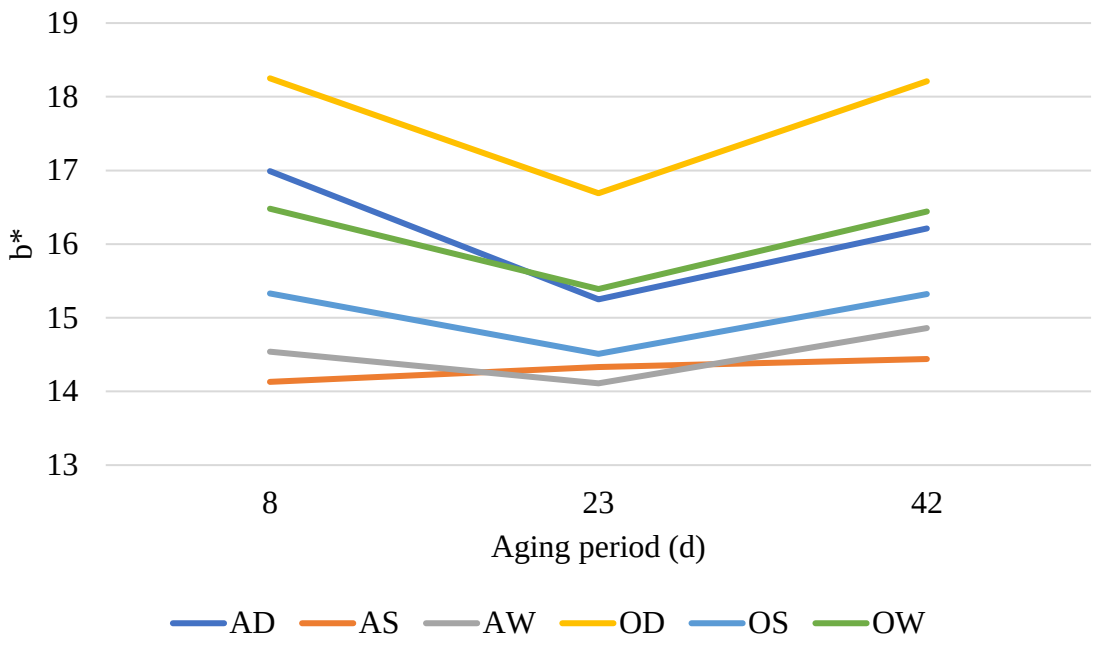

Figure H.6. Mean estimates for b* (yellowness) objective color for top round steaks from steak section treatment combinations (average weight deep [AD], average weight superficial [AS], average weight whole [AW], oversized deep [OD], oversized superficial [OS], and oversized whole [OW]) across three aging periods (8, 23, and 42 d).

Appendix I

Exempt certificate for IRB project number 19 – 149

University of Idaho
Office of Research Assurances

Institutional Review Board
875 Perimeter Drive, MS 3010
Moscow, ID 83844-3010
Phone: 208-885-6162
Fax: 208-885-6014
Email: irb@uidaho.edu

To: Phillip Bass, Ph.D.

From: University of Idaho Institutional Review Board

Date: October 04, 2019

Title: Functional importance of microbiota on sensory attributes of whole-muscle dry-aged beef
IRB #: 19-149

Submission Type: IRB Protocol Amendment Request Form

Review Type: Exempt

Protocol Approval Date: 06/21/2019

Study Status Check Date: None

The Institutional Review Board has reviewed and **approved** the amendment to your above referenced Protocol.

Appendix J

Sensory panel consent form

Sensory Panel Consent Form

1. The University of Idaho Institutional Review Board has reviewed and found this study to be exempt.

2. The objective of this study is to evaluate the effects of different dry aging environments on beef strip loins. The samples will be prepared under the Research Guidelines for Cookery, Sensory Evaluation, and Instrument Tenderness Measurements of Fresh Meat, as outlined by the American Meat Science Association.

3. You will be asked to evaluate 10 samples (approximately 1" x ½" x ½") per session for tenderness (1 = extremely tough to 10 = extremely tender), juiciness (1 = dry to 10 = juicy), and flavor (1 = bland to 10 = intense) using a 10 point scale. You will also be asked to describe any unique flavor notes you observe. It is not necessary that samples be ingested. The study should take approximately 15 to 20 minutes.

4. Although there are no or minimal risks associated with the project, it is possible that some samples will have one or more qualities that may not be appealing to you (e.g. flavor, tenderness or juiciness that is less than you would prefer).

5. With your help, society can benefit from our attempt to improve the understanding of dry-aged beef.

6. We anticipate that samples will be well received by panelists. However, if we find during the course of the taste panel that samples are unappealing, we will stop the evaluation process.

7. To maintain anonymity of the data collected during this evaluation, all the information you provide will be placed in a locked file with Dr. Bass.

8. If you have questions about the taste panel, you can ask the investigator during the evaluation, when the evaluation is complete or at a time you feel is appropriate.

9. Contact information for the University of Idaho faculty member leading this research:
   Dr. Phil Bass
   University of Idaho
   Department of Animal and Veterinary Science
   Moscow, ID 83844
   208-885-0990

10. During the course of this taste panel, you may terminate participation at any time. If you choose to do so, please notify the investigator that you no longer wish to participate.

11. If you choose to terminate participation in this evaluation, there will be no penalties associated with your withdrawal.

I have reviewed this consent form and understand and agree to its contents.

Participant Name: _____ Date: _____

Signature: _____

Appendix K

Sensory panel demographics questionnaire

CONSUMER EVALUATION OF BEEF QUALITY

Panelist #: _____ Date: _____

Age: _____ Gender: _____

Please indicate the number of meals a week in which you consume beef:

0-1 2-4 5-7 8+

Please indicate the form in which you most commonly consume beef:

Ground Roast Steak Other

Have you ever eaten dry-aged beef before:

Yes No

Please rank the importance of the following (High, Medium, Low) when purchasing meat:

_____Brand name of the product

_____Breed of the animal that produced the product

_____USDA Quality Grade

_____Nutrient content

_____Taste/eating experience

_____Visual appearance

_____Where and how the animal was raised

_____If the animal received growth promotants and/or antibiotics

_____If the animal was raised exclusively on pasture or fed grain in a feedlot for any period of time

Appendix L

Sensory panel sample questionnaire

CONSUMER SENSORY PANEL QUESTIONNAIRE

Sample ID #:_____

1. **OVERALL ACCEPTABILITY OF SAMPLE:** This is based on your overall acceptability of the sample

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

2. **TENDERNESS:** This is based on your overall opinion of the sample's tenderness

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

3. **JUICINESS:** This is based on your overall opinion of the sample's juiciness

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

4. **FLAVOR:** This is based on your overall opinion of the sample's flavor

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

5. **OFF-FLAVOR:** This is based on your ability to detect an off-flavor of the sample

NO YES

6. **CONSUMER SATISFACTION:** Would you be willing to purchase this product?

NO YES

7. **IF APPLICABLE,** please circle the trait you liked **least** about this product.

Flavor Tenderness Juiciness Texture/Mouth Feel

8. **IF APPLICABLE,** please circle the trait you liked **most** about this product.

Flavor Tenderness Juiciness Texture/Mouth Feel

9. **Please circle any flavor notes you observe in this sample:**

Brown/roasted Yeasty Metallic Earthy Nutty

Aged cheese Sour Sweet

Thank you for taking the time to participate in this sensory panel