INFLUENCE OF EXTENDED AGING ON BEEF QUALITY CHARACTERISTICS AND SENSORY PERCEPTION OF FOUR BEEF MUSCLES

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Authorization to Submit Thesis

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Abstract

The objective was to determine the influence of post-fabrication aging (2, 14, 21, 42, and 63 days) on beef quality characteristics and consumer sensory perception of top loin, top round, top sirloin, and bottom round steaks. The *longissimus lumborum* (LL), *semimembranosus* (SM), *gluteus medius* (GM), and *biceps femoris* (BF) were removed from their respective wholesale cuts, divided into 5 sections, and assigned to aging treatments. At the end of each aging period, two 2.54 cm-thick steaks were cut from designated sections for specific analyses and displayed in a glass-front retail display case at 3°C for up to 4 days. Thiobarbituric acid reactive substances to measure lipid oxidation were analyzed on days 0, 1, and 4 of retail display. Aerobic plate counts were taken on day 0 and 4 of retail display. Two color measurements (L*, a*, and b*) per steak were taken each day of retail display with a colorimeter. Oxygenated lean color, amount of browning, discoloration, surface discoloration, and color uniformity were also measured daily by two evaluators. Following retail display, Warner-Bratzler shear force was performed. Four consumer panels (n=60 per muscle) evaluated cooked steaks from each aging time for overall acceptability, tenderness, juiciness, and flavor. Data were analyzed using the Mixed Model procedure of the Statistical Analysis System and significance was determined at $P < 0.05$. Lipid oxidation increased ($P < 0.05$) with longer aging period and retail display time. Aerobic plate counts increased ($P < 0.05$) with longer aging periods of all muscles. Furthermore, an aging period by day of retail display interaction ($P < 0.05$) was observed for a* and b* values for all muscles and L* values for the LL. An aging period by day of retail

display interaction ($P < 0.05$) was observed for oxygenated lean color in the BF, GM, and LL; for amount of browning and discoloration in the BF, LD, and SM; for surface discoloration in the BF and LL; and for color uniformity in the BF, GM, and LL. Longer aging periods resulted in steaks that became darker, more discolored, and less uniform during retail display. Warner-Bratzler shear force values decreased ($P < 0.05$) with longer aging for the LD and SM, while there was no difference observed for the BF and GM. Sensory panel results demonstrated longer aging periods increased ($P < 0.05$) acceptability of the SM, tenderness of all muscles, and tended to increase ($P = 0.07$) the juiciness of the SM. Extended aging reduced retail shelf-life yet increased consumer perception of tenderness of all muscles and juiciness and overall acceptability of the top round.

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List of Abbreviations

AMSA	American Meat Science Association
BF	*Biceps femoris*
CIE	Commission International de l'Eclairage
CFU	Colony Forming Units
GM	*Gluteus medius*
IMPS	Institutional Meat Purchase Specifications
kDa	Kilodalton
LD	*Longissimus dorsi*
LL	*Longissimus lumborum*
LSM	Least Squares Means
MDA	Malondialdehyde
MMP	Matrix Metalloproteinase
NCBA	National Cattlemen's Beef Association
SAS	Statistical Analysis Software
SEM	Standard Error of the Mean
SM	*Semimembranosus*
SSF	Slice Shear Force
TBARS	Thiobarbituric Acid Reactive Substances
TC	Tendercut
TS	Tenderstretch
USDA	United States Department of Agriculture
WBSF	Warner-Bratzler shear force

CHAPTER 1

Review of Literature

Aging

Postmortem aging is the storage of beef at refrigeration temperatures. Aging is an important management practice that allows biochemical reactions to occur within the meat resulting in improved tenderness. Postmortem aging time required to ensure a tender product varies depending on USDA quality grade and variation between muscles within a carcass. Two aging methods are used in the industry; wet and dry aging. Wet aging is the main method of aging in the industry today. Product being wet aged is vacuum packaged and allowed to age at refrigeration temperatures. Dry aging is also done at refrigeration temperatures however, the product is not packaged. Humidity and air flow are critical to ensure a desirable dry aged end product (NCBA, 2008).

Aging is an important management practice that helps to ensure a tender product (Tatum et al., 1999). Tenderness is one of the most important palatability traits of beef. Therefore, consumers are willing to pay more for a product guaranteed to be tender. The 2010/2011 National Beef Tenderness Survey revealed that post-fabrication aging times for subprimal cuts in cold storage facilities ranged from 1 to 358 days and 9 to 67 days for retail and foodservice subprimals, respectively. The aging period for retail cuts in the 2005/2006 National Beef Tenderness Survey ranged from 3 to 83 days post-fabrication (Voges et al., 2007). Furthermore, the 2010/2011 survey found that 35.7% of subprimals were aged less

than 14 days as compared to 19.6% in the 2005/2006 survey. The aging period in the latest survey was much less consistent than that of the previous survey.

Research examining the effects of aging for less than 28 days is plentiful (Bratcher et al., 2005; Dixon et al., 2012; Eilers et al., 1996; Gruber et al., 2006). Both individual muscle and quality grade play an important role in the recommended aging time. USDA Select muscles require longer aging periods to ensure a more tender product than do USDA Choice muscles. Typically 20 days are required for USDA Select muscles to complete a majority of the aging response according to the Industry Guide for Beef Aging (Gruber et al., 2006).

Postmortem aging also plays a role in flavor development. Flavor changes that occur during aging contribute to final meat flavor (Spanier and Miller, 1996). Aging beef leads to a strong, savory, roasted odor, while unaged beef odor is weak and bland (Brewer, 2006). The positive flavor descriptors beefy, browned/caramel, and sweet decrease with aging, while negative flavor descriptors painty, cardboard, bitter and sour increase (Spanier et al., 1997). When aged for 21 or 35 days, Yancey et al. (2005) noted top sirloin, top blade, and tenderloin steaks tended to increase in metallic or rancid flavors.

A consumer's decision to purchase a meat product is largely influenced by appearance (AMSA, 2012; Mancini and Hunt, 2005). Discoloration causes meat to be perceived by the consumer as old and less wholesome (Faustman and Cassens, 1990). Postmortem aging has an influence on color stability. Madhavi and Carpenter (1993) found color stability was greater for steaks fabricated on days 4

and 7 than steaks fabricated on days 0, 1, 2, 14, and 21. These researchers noted that color stability improved until day 7 and then subsequently decreased.

Tenderness

Tenderness is the most important palatability trait according to consumer surveys (Mackintosh et al., 1936; Morgan et al., 1991; Koohmaraie et al., 1995; Huffman et al., 1996). Koohmaraie et al. (1995) reported consumers would pay more for a guaranteed tender product. Both the 1991 and 2010/2011 National Beef Tenderness Surveys noted that improvement in tenderness, specifically of beef round muscles was needed because the round muscles are consistently less tender than other muscles. Trained sensory panel evaluation of the *biceps femoris* (BF), *gluteus medius* (GM), *longissimus lumborum* (LL), and *semimembranosus* (SM) revealed the LL was the most tender followed by the GM, BF, and then the SM (Carmack et al., 1995).

Tenderness can be measure in several ways. Warner-Bratzler shear force (WBSF) and slice shear force (SSF) are two objective methods used to measure meat tenderness (Shackelford et al., 1999). Warner-Bratzler shear force is likely the more widely used method worldwide (Derington et al., 2011). Recently, some authors have suggested that SSF has some advantages. Shackelford et al. (1999) found SSF values of *longissimus dorsi* (LD) steaks had a greater correlation to trained sensory panel tenderness ratings than WBSF values. Interestingly, these researchers noted that SSF values taken promptly after cooking resulted in greater correlation to trained sensory panel tenderness ratings than SSF values recorded

after the steaks were allowed to cool to 4°C. Along with mechanical shear force, subjective evaluation by consumers in taste panels or more objective human measurement using trained taste panels can be utilized. Wheeler et al. (2004) showed that an untrained consumer panel was able to detect differences between steaks determined “tender,” “intermediate,” and “tough” by SSF. The authors suggested untrained laboratory consumer panels may be used to evaluate meat tenderness rather than using a more expensive trained sensory panel. This suggestion may also be supported by Chambers et al. (1981) who mentioned that panelists are able to score texture characteristics more precisely than flavor.

The four most influential aspects of meat tenderness to consider include intramuscular fat, connective tissue, contractile state of the muscle, and postmortem proteolysis (Belew et al., 2003). Similarly, Koohmaraie et al. (2002) reported connective tissue content, sarcomere length, and proteolysis to be responsible for most of the tenderness variation in meat. Interestingly, these components play a variable role depending on the muscle. The *psoas major* is very tender largely due to long sarcomeres and low connective tissue. The LL is tender mainly due to proteolysis, while the BF and SM lack tenderness because of higher connective tissue content. Belew et al. (2003) noted support muscles are generally considered to be more tender than locomotion muscles, however they observed that the *biceps brachii*, a locomotion muscle was more tender than the LL, a support muscle. Tenderness may vary within and between muscles due to differences in connective tissue, sarcomere length, and proteolysis (Rhee et al. 2004).

The bite theory suggests that, since intramuscular fat is less resistant to shear force than muscle protein, higher levels of marbling increase tenderness (Savell and Cross, 1988). Several studies show tenderness improves with higher degrees of marbling (Mcbee and Wiles, 1967; Jennings et al., 1978; Dolezal, 1982). Smith and Carpenter (1970) found that increases in the amount of intramuscular fat lead to significant improvements in several attributes, including tenderness, juiciness, and overall satisfaction in several lamb cuts. Conversely, other studies found marbling did not affect tenderness (Romans et al., 1965; Parrish et al., 1973; Garcia-de-Siles et al., 1977).

Connective tissue contributes to meat toughness (Blumer, 1963; Cross et al.,1973). Rhee et al. (2004) reported that collagen concentration is negatively correlated with sensory tenderness ratings and positively correlated with WBSF values. Similarly, Seideman (1986) concluded that both total and soluble collagen were negatively correlated to sensory panel tenderness ratings and positively correlated to shear force. Smith and Carpenter (1970) noted total collagen in lamb was inversely related to taste panel scores for tenderness, flavor, juiciness, and overall satisfaction. On the contrary, Cross et al. (1973) noted that elastin was generally not related to tenderness variation. As animals mature percent soluble collagen decreases (Smith and Carpenter, 1970). However, these authors found decreased soluble collagen was not related to differences in ovine muscle tenderness.

Sarcomere length is partially responsible for tenderness variation in meat (Koohmaraie et al. 2002). Smith and Carpenter (1970) noted that in ovine carcasses sarcomere length was positively correlated to sensory tenderness and overall satisfaction ratings and negatively correlated to shear force values. Furthermore, Hostetler et al. (1970) and Rhee et al. (2004) found that beef muscles with longer sarcomeres resulted in lower shear force values and improved sensory tenderness ratings.

Postmortem proteolysis of myofibrillar and associated proteins leads to improved tenderness of beef (Koohmaraie, 1996). Koohmaraie (1988) noted cathepsins and calcium-depended proteases (calpains) may be responsible for postmortem proteolysis. More recently, Koohmaraie and Geesink (2006) identified the multicatalytic proteinase complex (proteasome) as a third proteolytic system potentially involved in postmortem meat tenderization. Sentandreu et al. (2002) identified calpains, cathepsins, proteasomes, caspases, matrix metallopeptidases, and serine peptidases as muscle peptidases that may play a role in postmortem tenderization.

Calpains are broken into three groups: ubiquitous, tissue specific, and atypical calpains (Sentandreu et al., 2002). Calpains are heterodimers consisting of a unique 80 kDa subunit and a common 28 kDa subunit (Goll et al., 2003; Koohmaraie and Geesink, 2006). Ubiquitous calpains are believed to be the predominate peptidase involved in postmortem proteolysis of myofibrillar proteins (Delgado et al., 2001; Sentandreu et al., 2002; Nowak, 2011). Ubiquitous calpains

include; μ-calpain, m-calpain and μ/m-calpain (only found in poultry) which are activated at μM, mM, and intermediate calcium concentrations, respectively (Sentandreu et al., 2002). Calpains degrade desmin, filament, C-protein, tropomyosin, troponin T, troponin I, titin, nebulin, vimentin, gelsolin, and vinculin, but do not degrade α-actin, α-actinin, or myosin heavy chain (Huang and Forsberg, 1998).

Calpastatin is a specific inhibitor of ubiquitous calpains (Delgado et al., 2001; Sentandreu et al., 2002; Goll et al. 2003; Koohmaraie and Geesink, 2006: Underwood et al., 2008). The activity of calpains and interaction with calpastatin has been shown to influence tenderness values. Zamora et al. (1996) found μ- and m-calpain activity remained constant up to 9 hours postmortem. However, m-calpain had 97 and 50 percent of its original activity after 5 and 14 days of aging, respectively, while μ-calpain had 45, 20, and 0 percent of its original activity after 1, 3, and 14 days of aging. Calpastatin, the endogenous inhibitor of μ- and m-calpain, activity decreased over time but still had 17 percent of its original activity after 14 days of aging. Resistance of the myofibrillar structure decreased exponentially from day 1 to the end of storage following the decline in calpastatin, μ-, and m-calpain (Zamora et al., 1996). A concurrent decrease in myofibrillar structure resistance and calpain activity could indicate the relationship of the two to increased tenderness over aging time. Rhee et al. (2004) noted that percentage of desmin degraded is positively correlated to sensory tenderness ratings for the BF, GM, LD, and SM. Huff-Lonergan et al. (1996) found that myofibrals incubated with calpains produced the same proteolytic patterns that are observed in postmortem muscle.

Whipple et al. (1990) concluded that the LD of *Bos indicus* cattle was less tender than the LD of *Bos taurus* cattle due to reduced proteolysis from higher levels of the endogenous calpain inhibitor, calpastatin. Therefore, the calpain system is likely the main system involved in postmortem proteolysis (Koohmaraie and Geesink, 2006).

Proteosomes are large protein complexes found in muscle. The 26S proteosome degrades ubiquitinated proteins and consists of a 19S regulatory subunit and a 20S multicatalytic subunit (Nowak, 2011). When bovine muscle myofibrils were incubated with proteasomes, the 20S proteosome hydrolyzed nebulin, myosin heavy chain, actin, α-actinin, tropomyosin, and troponin (Robert et al., 1999). These authors hypothesize that calpains breakdown larger proteins into peptides, which are subsequently degraded by proteosomes. However, Koohmaraie (1992) found that proteosomes does not have a direct role in postmortem proteolysis.

Cathepsins are peptidases located in lysosomes and their action in postmortem proteolysis seems insignificant (Robert et al., 1999; Sentandreu et al., 2002; Nowak, 2011). Sancho et al. (1997) found that cathepsins degrade actin when muscle fibers are held at 20°C, but not at 4°C. The higher temperature likely disrupted lysosomes causing cathepsins to be released which then degraded actin. Sentandreu et al. (2002) notes that inhibition studies found cathepsin inhibitors did not stop proteolysis, however inhibitors of cathepsins and calpains did inhibit proteolysis.

Caspases function in apoptosis or programmed cell death (Sentandreu et al., 2002; Underwood et al., 2008). Caspase 3 is the caspase mainly responsible for protein hydrolysis (Underwood et al., 2008). These authors found that caspase 3 is active immediately postmortem but not further activated postmortem. Additionally, the caspase 3 activity and WBSF were not correlated. Therefore, caspase 3 did not likely contribute to postmortem proteolysis.

Matrix metallopeptidases (MMPs) are zinc-dependent proteases responsible for degrading and remodeling extracellular matrix proteins including intramuscular collagen (Sentandreu et al., 2002; Bode and Maskos, 2003; Snoek-van Beurden and Von den Hoff, 2005; Archile-Contreras et al., 2010; Purslow et al., 2012). Purslow et al. (2012) noted increasing connective tissue turnover in live animals would reduce the mature connective tissue structure allowing for easier collagen breakdown during cooking. Since collagen levels do not change significantly during postmortem aging, there is little evidence to support a role for MMPs in postmortem tenderization (Sentandreu et al., 2002).

Electrical stimulation leads to improved tenderness by hastening the onset of rigor mortis and thereby decreasing cold induced sarcomere shortening (Davey et al., 1976; Savell et al., 1978). Davey et al. (1976) noted that electrical stimulation eliminates meat toughness due to fast chilling, which is more desirable than slow chilling because it prevents bacterial growth. Furthermore, Savell et al. (1978) observed that along with improved tenderness, electrically stimulated carcasses had reduced formation of “heat ring” and brighter, more youthful lean color.

Eikelenboom et al. (1985) found that LD stimulated with high (300 V) or low (85 V) voltage and stored for 6 days had a brighter red color, higher drip loss, longer sarcomeres, lower shear force values, and improved sensory tenderness ratings when compared to the non-electrically stimulated control.

Carcass suspension also plays a role in beef tenderness due to increasing sarcomere length. Traditionally carcasses are suspended by the Achilles tendon, but the Tenderstretch (TS) or Tendercut (TC) method may be used to improve tenderness by increasing sarcomere length (Sorheim et al., 2001). Hostetler et al. (1970) demonstrated that suspension of the carcass from the obturator foramen (TS) rather than the Achilles tendon resulted in the LD and SM muscles having greater tenderness with little to no effect on other muscles. In the TC method, a prerigor cut is made through the 12th thoracic vertebra and the intercostals muscles, *multifidus dorsi*, and associated connective tissues are severed. Therefore the LD is the only dorsal attachment between the anterior and posterior portion of the carcass. This method leads to longer sarcomere lengths and improved sensory panel tenderness ratings of the LD, while not affecting yield grade, color, purge, or cooking loss (Ludwig et al., 1997). Wang et al. (1994) found longer sarcomere lengths and lower shear force values for the *vastus lateralis*, *rectus femoris* and *vastus medialis* when subjected to the TC treatment. They also found that the TC method did not affect soluble, insoluble, or total collagen, furthermore cooking or thawing loss were not affected. Sorheim et al. (2001) found chilling rates of the LD did not differ between TS, TC, and controls within treatments (fast or slow chilling), yet TS and TC sides had longer sarcomeres than control sides. Furthermore,

WBSF values and sensory tenderness scores of TS and TC sides were improved over the control for the fast chilling rate (2°C) but not for the medium chilling rate (10°C for 7 h, followed by 2°C). They noted that the WBSF values of all muscles were low at the medium chilling rate likely due to minimal cold shortening at the slower chilling rate.

Improved aging practices were recommended by the 2010/2011 National Beef Tenderness Survey to increase tenderness and consumer satisfaction. Postmortem aging effects on beef tenderness have been well studied (Jennings et al., 1978; Eilers et al., 1996; Bratcher et al., 2005; Gruber et al., 2006; Dixon et al., 2012). Bratcher et al. (2005) found that based on WBSF, beef in the upper two-thirds of USDA Choice need not be aged longer than 7 days postmortem, while beef from carcasses in the USDA Select grade should be aged a minimum of 14 days postmortem. Gruber et al. (2006) found an interaction between individual muscle, USDA quality grade, and aging period for WBSF. These authors demonstrated that muscles in the upper two-thirds of USDA Choice required shorter aging periods than muscles from USDA Select carcasses, longer aging periods decreased WBSF values, and muscles with higher 2 day WBSF values had a greater aging response. Dixon et al. (2012) studied beef round muscles and found an interaction between muscle and aging period but not between grade, muscle, and age. The WBSF values decreased with aging time but the aging response varied between round muscles. These authors contribute the lack of a grade interaction to the low levels intramuscular fat in round muscles. Jennings et al. (1978) found loins aged for 20 days had lower shear force values than steaks aged for 10 days. Eilers et al.

(1996) concluded that aging strip loin steaks 12 days should result in “acceptable” tenderness, whereas aging periods of 24 days would result in “superior” tenderness.

Flavor

Beef flavor is developed from the combination of taste and aroma (Brewer, 2006; Maughan et al., 2012). The formation of volatile compounds during heating is the major contributor to the flavor of meat (Mottram, 1998). Carbohydrates, lipids, and proteins play an important role in flavor development (Spanier et al., 1997; Mottram, 1998). Beef flavor may vary due to a variety of factors including breed and diet of animal, postmortem aging, muscle location, marination, storage type, and heat treatment (Brewer, 2006). Furthermore, Romans et al. (1965) noted steaks from less mature carcasses had preferable flavor over steaks from more mature beef carcasses. A few studies found increased marbling led to more desirable flavor scores (Mcbee and Wiles, 1967; Dolezal, 1982), while others found degree of marbling had no effect on flavor (Parrish et al. 1973; Garcia-de-Siles et al., 1977). Interestingly, Killinger et al. (2004) and Goodson et al. (2002) reported that when steaks were considered acceptable for tenderness, flavor became the major factor in overall consumer acceptability. Maughan et al. (2012) noted that that the terms brothy, umami, roast beef, juicy, browned, fatty and salty reflect positive flavor while oxidized, bitter, barny, gamey, grassy, livery, metallic, and astringent are used to describe negative flavor of beef based on results from a descriptive sensory panel.

Carmack et al. (1995) compared the BF, GM, LL, and SM steaks aged for 7 days and found the BF scored highest in beef flavor intensity followed by the GM, SM, and then LL steaks. These authors note hindquarter muscles generally display the most intense beef flavor, however a more intense flavor may not be more desirable. Beef top round aged up to 14 days led to a decrease in positive flavor attributes including beefy, brothy, browned/caramel and sweet, while negative flavors such as painty, cardboard, bitter, and sour increased (Spanier et al., 1997). Interestingly, in this study the largest flavor change for both positive and negative attributes occurred between 4 and 7 days of aging with little change in flavor from day 7 to 14. Hodges et al. (1974) found that off-flavors started to increase after 7 days of storage and continued to increase in wholesale beef cuts through 28 days of storage.

Juiciness

Juiciness is defined as the liquid detectable during the chewing of a bite of meat (Blumer, 1963). Therefore, juiciness plays a major role in mouth feel and meat texture (Dransfield et al., 1984; Hutchings and Lillford, 1988). Factors influencing juiciness include cooking method, meat characteristics such as marbling, sarcomere length, extent of protein denaturation, net protein charge, meat processing, and other factors (Winger and Hagyard, 1994).

Cooking method and final temperature have a large effect on juiciness (Honikel and Hamm, 1994). Microwave cooking is faster than a conventional oven but results in drier beef top round steaks (Moore et al., 1980). On the other hand

Ray et al. (1985) noted no differences in juiciness of lamb SM roasts when microwave cooked or roasted. Moore et al. (1980) also found cooking losses for top round steaks were greater for moist than dry heat. Parrish et al. (1973) and Kregel et al. (1986) found juiciness decreases as degree of doneness increases. Cross et al. (1979) revealed roasted beef LD steaks were juicier than broiled steaks but noted the difference may be too small for practical concern.

Marbling may play a role in juiciness. Savell and Cross (1988) noted that a minimum of 3 percent intramuscular fat in strip loins is needed for product to have acceptable palatability. Several studies note higher degrees of marbling lead to improved juiciness (Blumer, 1963; Romans et al., 1965; McBee and Wiles, 1967; Jennings et al., 1978; Dolezal, 1982; Muchenje et al., 2009). Other studies found no association between marbling and juiciness (Parrish et al., 1973; Garcia-de-Siles et al., 1977). Parrish et al. (1973) noted increased marbling led to increased cooking loss, however degree of marbling did not affect sensory panel juiciness scores.

During postmortem anaerobic glycolysis, lactic acid buildup causes a drop in muscle pH. As the pH approaches the isoelectric point of muscle proteins (~5.25) the net protein charge is reduced. At the isoelectric point there are an equal number of positive and negative charges, making the net charge zero. Because positive and negative charges are attracted to one another, this results in less space within in the myofibrils thus forcing water out (Huff-Lonergan and Lonergan,

2005). The reduced space in within the myofibrils decreases the water holding capacity of the steak.

Juiciness and water holding capacity are related. Juiciness is measured by sensory analysis of the product, while water holding capacity can be measured three main ways; applying no force, applying mechanical force, and/or applying thermal force with average fluid losses of approximately 3, 60, and 25-30 percent, respectively. The method of applying no force utilizes only gravity, which offers a precise but time consuming method. Using mechanical force such as centrifugation, filter paper press, or capillary volumeter provides a more rapid measurement, however the water released is greater than when no force is applied. Mechanical force allows differences to be measured but absolute values such as drip loss cannot be calculated. Cooking loss is the final way to measure water holding capacity and is critical since most meat is cooked prior to being consumed (Honikel and Hamm, 1994). Allison et al. (2003) reported that high-speed centrifugation, purge, and drip loss in pork are highly correlated.

Aging can affect juiciness in individual muscles (Winger and Hagyard, 1994). Percent purge has been shown to increase with increased storage time (Seideman et al., 1976; Boakye and Mittal, 1993). However, Wicklund et al. (2005) noted percent purge tended to decrease over 28 days of aging. Carmack et al. (1995) noted chuck and loin muscles were generally juicier than round muscles. These authors demonstrated that when aged for 7 days the LL scored highest for juiciness followed by the GM, BF, and then the SM.

Color

Color plays a critical role in a consumer's decision to purchase a meat product (AMSA, 2012; Mancini and Hunt, 2005). Discoloration causes meat to be perceived by consumers as old and less wholesome (Faustman and Cassens, 1990). Many extrinsic and intrinsic factors affect meat color. A few extrinsic examples include animal genetics, gender, age, diet, and stress. Intrinsic examples include pH, muscle type, location, water holding capacity, microbial growth, and temperature (AMSA, 2012).

Myoglobin is the main contributor to meat color, while hemoglobin and cytochrome C may play a minor role. Each of these heme proteins contain iron. The iron is able to form six bonds; four with the neighboring pyrrole nitrogen's, one with the proximal histidine, and one with a ligand. Meat color is determined by the ligand bound to the iron (Mancini and Hunt, 2005). Faustman and Cassens (1990) note three forms of myoglobin largely responsible for meat color. These chemical forms include oxymyoglobin, deoxymyoglobin, and metmyoglobin. Oxymyoglobin is formed when myoglobin comes into contact with oxygen and a bright cherry red color is subsequently formed. Deoxymyoglobin is the predominant form when the sixth potential binding site is not bound to a ligand. Purple is the predominant color associated with deoxymyoglobin. Metmyoglobin is the result of the ferrous iron (+2) being oxidized to the ferric state (+3). The resulting brown color is a deterrent to consumers. Mancini and Hunt (2005) report a fourth common form of myoglobin, carboxymyoglobin. Carboxymyoglobin is formed when carbon monoxide is bound

to the sixth coordination site. Meat exposed to low levels of carbon monoxide forms a bright red color similar to that of oxymyoglobin. Currently, low levels of carbon monoxide are used when packaging meat to increase shelf-life (Mancini and Hunt, 2005).

The AMSA Guidelines for Meat Color Evaluation (2012) note visual appraisal (subjective) and instrumental measurement (objective) as methods for color evaluation. Visual appraisal is critical since this is the method consumers use when selecting product to purchase. Furthermore, it sets the standard for instrumental measurement. Unfortunately, humans perceive colors differently making consistency of visual appraisal difficult. The use of color standards greatly improves the consistency and validity of visual appraisal. A colorimeter or spectrophotometer may be used to objectively measure color. These instruments are useful in tracking meat color changes over time. Both of these instruments are able to convert the reflected light to Commission International de l'Eclairage (CIE) L*a* and b* values. L* values range from 0 (black) to 100 (white), while a* and b* values range from -100 to +100. Positive a* and b* values are red and yellow, while negative values are green and blue, respectively (AMSA, 2012).

Beef muscles can be grouped depending on color stability (McKenna et al., 2005). Based on the accumulation of metmyoglobin, these researchers categorized muscles as "high", "intermediate", "low", and "very low" color stability. Metmyoglobin accumulation can be determined by the ratio of surface reflectance at 572 nm to 525 nm with higher ratios having less metmyoglobin formation (AMSA,

2012; McKenna et al., 2005). Both oxygen consumption rate and an enzyme reducing system (now known to be metmyoglobin reductase) are responsible for the rate of metmyoglobin accumulation (Ledward, 1985). Interestingly, McKenna et al. (2005) reported that the proportion of metmyoglobin reductase activity to oxygen consumption rate is a greater determinant of color stability than the level of one or the other. Madhavi and Carpenter (1993) found color stability was greater for steaks fabricated on days 4 and 7 than steaks fabricated on days 0, 1, 2, 14, and 21. These researchers demonstrated that color stability improved until day 7 at which point oxygen consumption rate reached its lowest point. They also noted color stability decreased with decreasing metmyoglobin reductase activity and nicotinamide adenine dinucleotide concentrations. Reddy and Carpenter (1991) found that muscles with the greatest metmyoglobin reductase activity were also muscles customarily designated as having the greatest color stability. Interestingly, McKenna et al. (2005) also noted that muscles categorized as being of “high” color stability typically have greater resistance to induced metmyoglobin formation, nitric oxide reducing ability, and oxygen penetration depth, while having lower oxygen consumption rates, myoglobin content, and oxidative rancidity than muscles categorized as less stable.

Microbial Growth

Meat provides an environment suitable for microbial growth. Microorganisms multiply quickly in the proper conditions and some can be involved in spoilage and foodborne illness. Contamination of meat varies based on antemortem conditions,

handling during harvest, sanitation, added ingredients, processing, storage, handling, and distribution (Sofos, 1994).

Psychrotrophic bacteria grow at refrigeration temperatures whereas mesophilic bacteria grow at temperatures above refrigeration (Francis et al., 1998). The ratio of psychrotrophs to mesophiles varies depending on temperature, time, air flow, and humidity. Microbial enzymatic action during storage causes off odors and flavors, discoloration, softening of the texture, and slime formation (Sofos, 1994).

Hodges et al. (1974) and Wicklund et al. (2005) found that both aerobic and anaerobic microbial growth of wholesale beef cuts increased during 28 days of storage under vacuum. The spoilage point of meat is 10^7 colony forming units (CFU)/g (Kraft, 1986) or 10^6 CFU/cm^2 (Jensen et al., 2003). Seideman et al. (1976) noted lactic acid production by lactobacilli may be responsible for brighter surface color with longer aging periods of the knuckle. These authors also partially attributed increased surface discoloration in beef knuckles packaged under low vacuum to greater bacterial activity because of the increased oxygen availability.

Effects of Aging on Shelf-life

Shelf-life is the time food stays stable and desirable (Doyle, 2007). Jennings et al. (1978) discovered loin steaks aged for 20 days as compared to 10 days had greater off-odor, increased fat discoloration, and greater bacterial counts. They also noted that subcutaneous fat and marbling did not influence shelf-life. Interestingly, Seideman et al. (1976) found surface discoloration of beef knuckle steaks, aged for 7, 14, 21, 28, and 35 days, decreased with longer aging periods. However,

although not statistically analyzed, surface discoloration increased over 4 days of retail display. Sofos (1994) noted shelf-life of vacuum-packaged meat at 0°C can be 10-12 weeks.

Summary

The current literature is focused on relatively short aging periods (< 35 days) of beef. To our knowledge no one has published research determining the influences of aging beef up to 63 days. Consequently, little is known about the effects of extended aging of beef on shear force or consumer acceptability. In addition to the paucity of information regarding the effects of extended aging on beef tenderness, relatively little is known about the effects of extended aging on beef color and flavor development. Top loin (*longissimus lumborum*), top round (*semimembranosus*), top sirloin (*gluteus medius*), and bottom round (*biceps femoris*) steaks derived from USDA Select carcasses have been shown to exhibit moderate to high aging responses, with potential to continue tenderizing beyond 28 days of aging. Consequently, peptides and amino acids generated by proteolysis may contribute to flavor development in these muscles during extended aging. Additionally, LD, SM, GM, and BF were categorized as “high”, “moderate”, “intermediate”, and “low” color stability muscles, respectively (McKenna et al., 2005). Therefore, these muscles reflect the broad diversity of beef cuts typically merchandized at retail. Our specific objective was to determine the influences of wet aging for 2, 14, 21, 42, and 63 days on retail color stability, microbial growth, WBSF, soluble and insoluble collagen levels, and consumer acceptability of top loin,

top round, top sirloin, and bottom round steaks. Based on the available literature, there is reason to believe that aging beef for longer periods could lead to a more tender and desirable product. I hypothesized that aging beef for up to 63 days would lead to reduction in shelf-life characteristics, but improvement in tenderness and consumer sensory attributes.

Aging is an extremely important tool for the beef industry to use in order to provide a high quality product to the consumer. While there is abundant research on aging beef for less than 28 days, little is known about the effects of extended aging (> 28 days) of beef on retail shelf-life or consumer acceptability. Extended aging may lead to improved tenderness and possibly alter the flavor profile. Both of which could potentially have an economic impact.

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CHAPTER 2

Influence of Extended Aging on Beef Quality Characteristics and Sensory Perception of Four Beef Muscles

Abstract

The objective was to determine the influence of post-fabrication aging (2, 14, 21, 42, and 63 days) on beef quality characteristics and consumer sensory perception of top loin, top round, top sirloin, and bottom round steaks. The *longissimus lumborum* (LL), *semimembranosus* (SM), *gluteus medius* (GM), and *biceps femoris* (BF) were removed from their respective wholesale cuts, divided into 5 sections, and assigned to aging treatments. At the end of each aging period, two 2.54 cm-thick steaks were cut from designated sections for specific analyses and displayed in a glass-front retail display case at 3°C for up to 4 days. Thiobarbituric acid reactive substances to measure lipid oxidation were analyzed on days 0, 1, and 4 of retail display. Aerobic plate counts were taken on day 0 and 4 of retail display. Two color measurements (L*, a*, and b*) per steak were taken each day of retail display with a colorimeter. Oxygenated lean color, amount of browning, discoloration, surface discoloration, and color uniformity were also measured daily by two evaluators. Following retail display, Warner-Bratzler shear force was performed. Four consumer panels (n=60 per muscle) evaluated cooked steaks from each aging time for overall acceptability, tenderness, juiciness, and flavor. Data were analyzed using the Mixed Model procedure of the Statistical Analysis System and significance was determined at $P < 0.05$. Lipid oxidation increased (*P*

< 0.05) with longer aging period and retail display time. Aerobic plate counts increased ($P < 0.05$) with longer aging periods of all muscles. Furthermore, an aging period by day of retail display interaction ($P < 0.05$) was observed for a* and b* values for all muscles and L* values for the LL. An aging period by day of retail display interaction ($P < 0.05$) was observed for oxygenated lean color in the BF, GM, and LL; for amount of browning and discoloration in the BF, LD, and SM; for surface discoloration in the BF and LL; and for color uniformity in the BF, GM, and LL. Longer aging periods resulted in steaks that became darker, more discolored, and less uniform during retail display. Warner-Bratzler shear force values decreased ($P < 0.05$) with longer aging for the LD and SM, while there was no difference observed for the BF and GM. Sensory panel results demonstrated longer aging periods increased ($P < 0.05$) acceptability of the SM, tenderness of all muscles, and tended to increase ($P = 0.07$) the juiciness of the SM. Extended aging reduced retail shelf-life yet increased consumer perception of tenderness of all muscles and juiciness and overall acceptability of the top round.

Introduction

The 2010/2011 National Beef Tenderness Survey revealed that post-fabrication aging times for subprimal cuts in cold storage facilities (temperature was not given) ranged from 1 to 358 days and 9 to 67 days for retail and foodservice subprimals, respectively. Effects of aging on beef tenderness have been well-documented (Bratcher et al., 2005; Dixon et al., 2012; Eilers et al., 1996; Gruber et al., 2006). Bratcher et al. (2005) concluded that based on WBSF, USDA Select

muscles should be aged at least 14 days postmortem, whereas beef from carcasses in the upper two-thirds of USDA Choice was tender by 7 days postmortem. Gruber et al. (2006) also demonstrated that most Select muscles require longer aging times than those from carcasses grading in the upper two-thirds Choice. To date, most research on beef tenderness, including the work cited above, has focused on the effects of relatively short term aging (28 days or less) on WBSF. Consequently, little is known about the effects of extended aging of beef on shear force or consumer acceptability.

In addition to the paucity of information regarding the effects of extended aging on beef tenderness, relatively little is known about the effects of extended aging on beef color and flavor development. McKenna et al. (2005) demonstrated that beef muscles can be classified based on color stability. These authors categorized the *longissimus, semimembranosus, gluteus medius,* and *biceps femoris* as “high”, “moderate”, “intermediate”, and “low” color stability muscles, respectively. Interestingly, Lee et al. (2008a) observed no interaction between aging up to 35 days and bloom development of beef ribeye steaks. However, these researchers reported that GM from top sirloin butts aged 14 days or less had more vivid color and a greater proportion of oxymyoglobin compared with steaks aged 28 to 35 days (Lee et al., 2008b). Additionally, color of beef LD was more stable than *psoas major* when product was stored from 8 hours to 21 days prior to steak fabrication (Madhavi and Carpenter, 1993). The retail display time to 20% metmyoglobin accumulation was similar across aging periods for LD steaks, despite

a decrease in metmyoglobin reducing activity with longer aging periods (Madhavi and Carpenter, 1993).

Aging alters the aroma and flavor precursors of beef and therefore may affect the sensory attributes of the product (Brewer, 2006). Aging influences numerous volatile compounds in beef muscles, and positive flavor compounds generally decrease while negative compounds increase with aging from 7 to 14 days (Stetzer et al., 2008). Likewise, Yancey et al. (2005) reported that wet-aging of top sirloin, top blade, or tenderloin steaks for 21 or 35 days tended to increase metallic or rancid flavors detected by a trained panel. Little is known regarding the effects of aging longer than 35 days on consumer perception of beef flavor.

Aging is an extremely important tool for the beef industry to use in order to provide a high quality product to the consumer. While there is abundant research on aging beef for less than 28 days on tenderness and 35 days on color and flavor, little is known about the effects of extended aging (> 28 days) of beef on retail shelf-life or consumer acceptability. Top loin (*longissimus*), top round (*semimembranosus),* top sirloin *(gluteus medius)*, and bottom round (*biceps femoris)* steaks derived from USDA Select carcasses have been shown to exhibit moderate to high aging responses, with potential to continue tenderizing beyond 28 days of aging. Consequently, peptides and amino acids generated by proteolysis may contribute to flavor development in these muscles during extended aging. Additionally, LD, SM, GM, and BF were categorized as “high”, “moderate”, “intermediate”, and “low” color stability muscles, respectively (McKenna et al.,

2005). Therefore, these muscles reflect the broad diversity of beef cuts typically merchandized at retail. Our specific objective was to determine the influence of wet aging for 2, 14, 21, 42, and 63 days on retail color stability, microbial growth, Warner-Bratzler shear force, soluble and insoluble collagen levels, and consumer acceptability of top loin, top round, top sirloin, and bottom round steaks.

Materials and Methods

Human subject participation in consumer panel

The University of Idaho Institutional Review Board certified this project as Exempt (Appendix A).

Product procurement

At 48 hours post mortem (fabrication = day 0), beef outside round (IMPS 171B), top (inside) round (IMPS 168), top sirloin butt (IMPS 184), and strip loin (IMPS 180) from the left side of USDA Select carcasses (n = 12) were purchased from AB Foods (Toppenish, WA) and transported to the University of Idaho Meat Science Laboratory.

Preparation of product

The BF, SM, GM, and LL were removed from their respective wholesale cuts for aging and subsequent analysis. The muscles were cut into five sections at least 5.1 cm-thick. Each section was randomly assigned to one of the five aging periods (2, 14, 21, 42, and 63 days post-fabrication). Sections were vacuum shrink

packaged (7 x 12 Durashrink bags, Winpak Films, Senoia, GA) and subsequently aged for the pre-determined time period at 0°C.

At the end of each aging period, designated sections were cut into two 2.54 cm-thick steaks, which were randomly assigned to determine either consumer acceptability or retail shelf-life followed by WBSF and collagen analysis. Steaks used for retail display were weighed, swabbed (3M Quick Swab) for microbial analysis, sampled for thiobarbituric acid reactive substances (TBARS) analysis, placed in white styrofoam trays, and overwrapped with an oxygen permeable PVC film (Koch Industries, Inc. #7500-3815; Wichita, KS) with the freshly cut surface exposed. Steaks were displayed in a glass-fronted retail display case (Model GDM-69, True Manufacturing Co., O'Fallon, MO) at 3°C for 4 days. The display case was equipped with natural white Hg 40W lights, and the average light intensity was 409 lux.

Fluid loss

Each section was weighed prior to vacuum packaging and after aging to determine percent purge. Steaks were weighed prior to and following 4 days of retail display to determine percent retail fluid loss.

Color stability

Steaks were allowed to bloom for at least 60 min, then two objective color measurements per steak were taken using a Hunter MiniScan EZ (Restin, VA). Each point was selected avoiding large marbling flecks, connective tissue, and the

very edge of the product. This represented day 0 of retail display, and subsequent color measurements were taken on days 1, 2, 3, and 4. The Hunter MiniScan is equipped with a 25 mm-diameter measuring area and a 10° standard observer. The instrument was set to illuminant A and Commission International de l'Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) values were recorded. Calibration of the machine was carried out each day by measuring against black and white calibration tiles.

The entire steak was evaluated daily during retail display for oxygenated lean color, amount of browning, discoloration, surface discoloration, and color uniformity by two evaluators following American Meat Science Association (AMSA) Meat Color Measurement Guidelines (AMSA, 2012). To avoid potential effects due to display case location, steaks were rotated after each measurement.

Microbial growth

Each steak was dry swabbed (5 cm x 5 cm area) twice on days 0 and 4 of retail display using 3M™ Quick Swabs. Letheen broth contained in the top of the swab was added and the samples were plated on 3M™ Petrifilm™ Plates (3M, St. Paul, MN). One sample was plated on a 3M™ Petrifilm™ Aerobic Count Plate and the other sample was plated on a 3M™ Petrifilm™ E. coli/Coliform Count Plate. The 3M™ Petrifilm™ Aerobic Count Plate was incubated at 35°C for 48 h to examine the growth of mesophilic organisms, while the 3M™ Petrifilm™ E. coli/Coliform Count Plate was incubated at 35°C for 24 h. Plates were counted by research personal following the 3M Interpretation Guide.

Lipid oxidation

TBARS were analyzed on days 0, 1, and 4 of retail display following the protocol provided in Appendix O of the Meat Color Measurement Guidelines (AMSA, 2012) (Appendix B).

Cooking

Following retail display, steaks were cooked on open-hearth broilers to an internal temperature of 40°C, then turned and cooked to a final internal temperature of 71°C. Temperature was monitored with hypodermic temperature probes (Omega Engineering Co., Stamford, CT) coupled with a 12-channel scanning thermocouple thermometer (Digi-Sense, Cole-Parmer Instrument Co., Vernon Hills, IL). Steaks were weighed before and after cooking to determine percent cook loss, and then refrigerated overnight for subsequent chemical and physical analysis.

Warner-Bratzler shear force

Six cores (1.27-cm diameter) were mechanically removed parallel with the muscle fiber orientation using a drill press-mounted coring device, and shear force was determined by shearing each core perpendicular to the muscle fibers using a Warner-Bratzler shear machine (GR Manufacturing, Manhattan, KS). The remaining portions of steaks were frozen at -20°C and used later to determine soluble and insoluble collagen.

Collagen

Soluble and insoluble collagen were determined following AOAC Method 990.26 (Kolar et al., 1990) with modifications from the procedure of Eilert and Mandigo (1993) for soluble collagen (Appendix C). Cooked steaks were thawed at 4°C and subsequently ground in a Rival 1.5 cup food chopper (Rival, Neosho, MO). Duplicate 4 g samples were placed in 50 mL Oak Ridge centrifuge tubes (Nalge Company, Rochester, NY). Ringer's solution (22 mL; 7.0 g NaCl, 0.026 g $CaCl_2$, and 0.35 g KCl) was added and the samples (4 g) were homogenized in a Polytron System PT 2500E (Polytron Technology Ltd. Kilbrittain, Co. Cork, Ireland) at 18,000 rpm for 20 sec, allowed to rest for 20 sec, and homogenized for another 20 sec. The samples were then heated in a water bath at 50°C for 15 min and subsequently centrifuged (Sorvall RC-5B Refrigerated Superspeed Centrifuge, DuPont Instruments, Polo Alto, CA) at 5900 x g at room temperature. The supernatant was filtered through Fisherbrand #5 filter paper (Thermo Fisher Scientific, Waltham, MA) into an erlenmeyer flask labeled as soluble collagen. The pellet was rinsed with ¼ strength Ringer's solution (10 ml) and centrifuged as previously described. The supernatant was filtered into the same flask and the pellet and filter paper were placed in a flask labeled as insoluble collagen. Sulfuric acid (3.5 M or concentrated) was added so the resulting solution (sulfuric acid and soluble or insoluble portion) was 3.5 M sulfuric acid. Flasks were then placed in a VWR 1370G drying oven (VWR International, Radnor, PA) at 105°C for at least 20 hours. The hot hydrolysate was diluted to 100 mL with water, mixed, and filtered through Fisherbrand #5 filter paper. Duplicate samples (0.12 mL diluted hydrolysate and

1.88 mL water) were pipetted into Fisherbrand borosilicate glass 13 x 100 mm test tubes (Thermo Fisher Scientific, Waltham, MA). One mL oxidant solution (water, 0.14 M citric acid monohydrate, 0.38 M sodium hydroxide, 0.66 M sodium acetate trihydrate, 3.88 M 1-propanol, and 0.05 M chloramine-t, pH = 6) was added and the samples were mixed and allowed to stand for 20 min. One mL color reagent (5.35 M perchloric acid, 0.67 M 4-dimethylaminobenzaldehyde and 8.49 M 2-propanol) was added and the resulting mixture was covered and heated at 60°C in a water bath for 15 min and then allowed to cool in a cold tap water bath for 3 min. Absorbance of samples was read at 558 nm using a BioTek Synergy 2 plate reader (BioTek, Winooski, VT). Hydroxyproline standards were used to generate a calibration curve and collagenous connective tissue content was calculated from hydroxyproline content. Hydroxyproline content was multiplied by 7.52 and 7.25 to determine soluble and insoluble collagen, respectively (Cross et al., 1973).

pH

Muscle pH was measured prior to the steaks being cooked for the consumer panel. A portable pH meter (Model SevenGo, Mettler Toledo, Woburn, MA) equipped with a InLab SolidsPro puncture-type electrode was used to measure pH. The pH meter was calibrated each day using standard pH 4.0 and 7.0 buffers.

Sensory panel

Consumer taste panels were conducted. One panel was conducted for each of the four muscles. Each consumer evaluated one cube from each of five steaks that represented all aging periods for a muscle.

Steaks designated for consumer acceptability were weighed and exposed to retail display conditions for 1 day, then reweighed, sampled for TBARS analysis, vacuum packaged and frozen at -20°C. For consumer sensory analysis, steaks were thawed overnight at 4°C and subsequently cooked as described above. A panel of consumers (n=60 per muscle) evaluated cooked steaks from each aging time for overall acceptability, tenderness, juiciness, and flavor using a 9-point scale (9 = like extremely, extremely tender, extremely juicy, and like flavor extremely, respectively; 1= dislike extremely, not at all tender, extremely dry, and dislike flavor extremely, respectively) (Appendix F). Five 1.27-cm x 1.27-cm x steak thickness cubes were obtained from each steak using a cutting die.

Statistical analysis

Data were analyzed using the Mixed Model procedure of the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Muscle sections served as the experimental units (n = 12 with the exception of soluble and insoluble collagen analysis; BF (n = 8), GM (n = 8), LL (n = 4), and SM (n = 6)). Individual muscle served as a random variable and day of aging, day of retail display, and the interaction between day of aging and day of retail display served as fixed variables. Color measurements were analyzed as a repeated measure. Aerobic Plate Counts were $\log_{10}$ transformed prior to analysis. Differences in least squares means (LSM) were compared by the DIFF option. P-values of ≤ 0.05 were considered statistically significant and p-values ≤ 0.10 were considered trends in the data. All data are presented as LSM ± SEM.

Results

Biceps femoris

Fluid-holding capacity during transportation and storage of wholesale cuts is important to both retailers and foodservice providers since weight lost during this time represents economic loss for the buyer. Furthermore, fluid loss during retail display is unappealing to the consumer. In the present study, percent purge was greater ($P < 0.001$) after 63 days of aging than the other aging periods for the BF (Table 2.1). Unlike percent purge, percent retail fluid loss decreased ($P < 0.001$) from day 2 to 14 and then remained constant (Table 2.1).

Color plays a critical role in a consumer's decision to purchase a meat product (AMSA, 2012; Mancini and Hunt, 2005). There was no aging period by day of retail display interaction ($P > 0.05$) for BF L* values (Figure 2.1). There were no differences ($P > 0.05$) in L* values across aging periods. However, L* values increased ($P < 0.001$) over retail display time. An aging period by day of retail display interaction ($P < 0.001$) was observed for a* and b* values for the BF (Figures 2.2 and 2.3). *Biceps femoris* a* values remained constant through the first 2 days of retail display of the first aging period and then decreased on days 3 and 4 of retail display. During the final 4 aging periods BF a* values decreased with retail display time. The BF b* value on day 2 of the first aging period was greater than days 0, 1, 3, and 4 of that aging period. During the 4 aging periods BF b* values decreased over aging periods and retail display time.

Subjective color measurements revealed changes in perceived color similar to those observed with objective color measurements. An aging period by day of retail display interaction was observed for BF oxygenated lean color ($P < 0.001$), amount of browning ($P < 0.001$), discoloration ($P < 0.001$), surface discoloration ($P < 0.01$), and color uniformity ($P < 0.02$) (Figures 2.4, 2.5, 2.6, 2.7, and 2.8, respectively). Oxygenated lean color became darker with longer aging periods and retail display times. Amount of browning did not change during the first 3 days of retail display of the first aging period but increased with longer aging periods and retail display time. Discoloration and surface discoloration did not change the first 2 days of retail display during the first two aging periods, but subsequently increased the final two days of retail display. Discoloration and surface discoloration increased with retail display during the final three aging periods. Furthermore, BF two toning remained relatively constant during the first aging period before increasing during retail display time of the final 4 aging periods.

An aging period by day of retail display interaction ($P < 0.001$) was observed for the number of aerobic organisms for the BF (Table 2.2). Aerobic microorganisms on day 0 of retail display increased from days 14 to 21, 21 to 42, and 42 to 63 days of aging. Aerobic microorganisms on day 4 of retail display increased from days 2 to 14, 14 to 21, and 21 to 63 days of aging. The day 4 counts were greater than day 0 counts for all aging periods except the first one where the day 0 counts were higher than day 4 counts. Coliform and E. coli counts were <1 cfu/5cm^2 over all aging periods.

The TBARS assay is commonly used as a measurement of lipid oxidation. An aging period by day of retail display interaction ($P < 0.001$) was observed for TBARS values in the BF (Figure 2.9). *Biceps femoris* lipid oxidation increased with retail display time except for the fifth aging period where TBARS values on days 1 and 4 of retail display were the same.

Cooking times increased ($P < 0.01$) with longer aging periods for the BF (Table 2.3). *Biceps femoris* steaks from the day 2 aging period cooked faster than the other aging periods. Percent cooking loss for the BF was less ($P < 0.001$) for the day 2 and 63 aging periods than the other three (Table 2.3).

Tenderness is the most important palatability trait according to consumer surveys (Mackintosh et al., 1936; Morgan et al., 1991; Koohmaraie et al., 1995; Huffman et al., 1996). Warner-Bratzler shear force is likely the most commonly used method to measure beef tenderness (Derington et al., 2011). Interestingly, there was no difference ($P > 0.05$) in shear force between aging periods for the BF (Table 2.3).

Connective tissue contributes to meat toughness (Blumer, 1963; Cross et al., 1973). *Biceps femoris* steaks from day 2 and 63 aging periods had less ($P < 0.01$) soluble collagen than steaks from the day 14 and 42 aging periods (Table 2.4). Similarly, BF steaks from day 2 and 63 aging periods had less ($P < 0.05$) insoluble collagen than steaks from the day 14 aging period (Table 2.4).

Muscle pH is negatively correlated to L*, a*, and b* values (Page et al., 2001) and positively correlated to microbial growth (Rhee et al., 1997). There were no differences ($P > 0.05$) in pH between aging periods for the BF.

One consumer sensory panel was held for each muscle following the completion of all aging periods. Panelists scored steaks for overall acceptability, tenderness, juiciness, and flavor. Demographics of consumer panelists are shown in Table 2.5. There were no differences in overall acceptability, juiciness, or flavor between aging periods for the BF (Table 2.6). Sensory tenderness was greater ($P < 0.05$) for steaks aged 21, 42, and 63 days than steaks aged 2 days (Table 2.6). Consumer preferences are shown in Table 2.7. Consumers liked flavor the most during the first four aging periods while juiciness was the most liked trait for the final aging period. Tenderness was the least liked during the first two aging periods, juiciness was least liked after 21 days of aging, while flavor was least liked after 63 days of aging. The percentage of consumers willing to purchase the BF after each of the five aging periods was 53, 63, 75, 59, and 67, respectively.

Gluteus medius

Fluid-holding capacity during transportation and storage of wholesale cuts is important to both retailers and foodservice providers since weight lost during this time represents economic loss for the buyer. Furthermore, fluid loss during retail display is unappealing to the consumer. In the present study, GM percent purge was greater ($P < 0.02$) after 42 and 63 days than 14 days of aging (Table 2.1).

Unlike percent purge, GM percent retail fluid loss decreased ($P < 0.001$) from day 2 to 14 and then from day 14 to 42 of aging (Table 2.1).

Color plays a critical role in a consumer's decision to purchase a meat product (AMSA, 2012; Mancini and Hunt, 2005). There was no aging period by day of retail display interaction for GM L* values (Figure 2.1). There also was no difference in L* values across aging periods. However, L* values increased ($P < 0.001$) over retail display time. An aging period by day of retail display interaction ($P < 0.001$) was observed for a* and b* values for the GM (Figures 2.2 and 2.3). *Gluteus medius* a* values decreased with retail display time over all aging periods. During the first aging period, b* values of the GM were greater on days 0 and 2 than days 3 and 4 of retail display. The GM b* values decreased with aging periods longer than 14 days and retail display time.

Subjective color measurements revealed changes in perceived color similar to those observed with objective color measurements. An aging period by day of retail display interaction was observed for oxygenated lean color ($P < 0.001$) and color uniformity ($P < 0.01$) for the GM (Figures 2.4 and 2.8). Oxygenated lean color became darker with longer aging periods and retail display times. Furthermore, GM color uniformity became less desirable with longer aging periods and retail display time. A trend was observed for the interaction between aging period and day of retail display for amount of browning ($P = 0.083$), discoloration ($P = 0.083$), or surface discoloration ($P = 0.063$) (Figures 2.5, 2.6, and 2.7, respectively). However, amount of browning, discoloration, and surface discoloration increased ($P < 0.001$)

with longer aging periods and retail display times (Figures 2.5, 2.6, and 2.7, respectively).

An aging period by day of retail display interaction ($P < 0.001$) was observed for the number of aerobic organisms for the GM (Table 2.2). On day 0 of retail display, aerobic counts increased from days 14 to 21, 21 to 42, and 42 to 63 of aging. While on day 4 of retail display aerobic counts increased from days 2 to 14 and 14 to 42 of aging. Coliform and E. coli counts were <1 cfu/5cm^2 over all aging periods.

The TBARS assay is commonly used as a measurement of lipid oxidation. An aging period by day of retail display interaction ($P < 0.001$) was observed for TBARS values in the GM (Figure 2.9). During the first aging period lipid oxidation was the lowest on day 1 of retail display. Lipid oxidation of steaks aged for 14, 21, and 42 days did not change from day 0 to 1 of retail display but increased from day 1 to 4 of retail display. Interestingly, after 63 days of aging lipid oxidation increased from day 0 to 1 of retail display but not from day 1 to 4 of retail display.

Cooking times increased ($P < 0.01$) from day 14 to 21 of aging for the GM (Table 2.3). Similarly, percent cooking loss increased ($P < 0.01$) from day 2 to 21 of aging (Table 2.3).

Tenderness is the most important palatability trait according to consumer surveys (Mackintosh et al., 1936; Morgan et al., 1991; Koohmaraie et al., 1995; Huffman et al., 1996). Warner-Bratzler shear force is likely the most commonly used method to measure beef tenderness (Derington et al., 2011). Interestingly,

there was no difference ($P > 0.05$) in shear force between aging periods for the GM (Table 2.3).

Connective tissue contributes to meat toughness (Blumer, 1963; Cross et al., 1973). In the present study, there were no differences ($P > 0.05$) in soluble or insoluble collagen between aging periods for the GM (Table 2.4).

Muscle pH is negatively correlated to L*, a*, and b* values (Page et al., 2001) and positively correlated to microbial growth (Rhee et al. 1997). There were no differences ($P > 0.05$) in pH between aging periods for the GM.

One consumer sensory panel was held for each muscle following the completion of all aging periods. Panelists scored steaks for overall acceptability, tenderness, juiciness, and flavor. Demographics of consumer panelists are shown in Table 2.5. There were no differences in overall acceptability, juiciness, or flavor between aging periods for the GM (Table 2.6). However, GM tenderness improved ($P < 0.01$) from day 14 to 42 of aging (Table 2.6). Consumer preferences are shown in Table 2.8. Consumers liked GM flavor the most during the first three aging periods while tenderness was the most liked trait for the final two aging period. Tenderness was the least liked trait during the first aging period, flavor was the least liked after 14 and 21 days of aging, while GM juiciness was the least liked trait after 42 and 63 days of aging. The percentage of consumers willing to purchase the GM after each of the five aging periods was 62, 63, 58, 70, and 65, respectively.

Longissimus lumborum

Fluid-holding capacity during transportation and storage of wholesale cuts is important to both retailers and foodservice providers since weight lost during this time represents economic loss for the buyer. Furthermore, fluid loss during retail display is unappealing to the consumer. In the present study, percent purge tended to increase ($P = 0.066$) over storage time for the LL (Table 2.1). Unlike percent purge, percent retail fluid loss decreased ($P < 0.001$) from days 2 to 14 and 14 to 21 of aging (Table 2.1).

Color plays a critical role in a consumer's decision to purchase a meat product (AMSA, 2012; Mancini and Hunt, 2005). An aging period by day of retail display interaction ($P < 0.001$) was observed for L*, a*, and b* values for the LL (Figures 2.1, 2.2, and 2.3, respectively). *Longissimus lumborum* L* values after the first aging period were greater on days 1, 3, and 4 than day 0 of retail display. After 14 days of aging L* values were greater on days 1 and 2 of retail display than day 4. After 21 days of aging LL L* values were greater on days 0 and 2 of retail display than days 1 and 4. Furthermore, during the final two aging periods LL L* values were generally greater on days 0, 1, and 2 of retail display than days 3 and 4. *Longissimus lumborum* a* values increased from day 1 to 2 of retail display before decreasing from day 2 to 3 of retail display after the first aging period. Furthermore, LL a* values decreased with aging periods longer than 14 days and retail display time. The LL b* values increased from day 1 to 2 of retail display and then decreased from day 2 to 3 of retail display during the first aging period.

Furthermore, LL b* values decreased with aging periods of 14 days or more and retail display time.

Subjective color measurements revealed changes in perceived color similar to those observed with objective color measurements. An aging period by day of retail display interaction ($P < 0.001$) was observed for oxygenated lean color, amount of browning, discoloration, surface discoloration, and color uniformity for the LL (Figures 2.4, 2.5, 2.6, 2.7, and 2.8, respectively). Oxygenated lean color became darker with longer aging periods and retail display times. Amount of browning did not change over the retail display time of the first three aging periods but increased with aging and retail display time during the final two aging periods. Discoloration did not change over retail display time over the first four aging periods but increased over retail display time of the final aging period. Surface discoloration did not change over the first two aging periods but increased during the final three aging periods and retail display times. Furthermore, LL color uniformity became less desirable with longer aging periods and retail display time.

An aging period by day of retail display interaction ($P < 0.01$) was observed for the number of aerobic organisms for the LL (Table 2.2). On days 0 and 4 of retail display, aerobic counts increased from days 2 to 14, 21 to 42, and 42 to 63 of aging. Coliform and E. coli counts were <1 cfu/5cm^2 over all aging periods.

The TBARS assay is commonly used as a measurement of lipid oxidation. An aging period by day of retail display interaction ($P < 0.01$) was observed for TBARS values in the LL (Figure 2.9). During the second aging period lipid oxidation

was the lower on day 1 of retail display than days 0 and 4. Lipid oxidation did not change from day 0 to 1 of retail display but increased from day 1 to 4 of retail display for steaks aged 2, 21, 42, and 63 days.

Cooking times increased ($P < 0.001$) from day 14 to 21 of aging for the LL (Table 2.3). Percent cooking loss also increased ($P < 0.02$) from day 14 to 21 of aging (Table 2.3).

Tenderness is the most important palatability trait according to consumer surveys (Mackintosh et al., 1936; Morgan et al., 1991; Koohmaraie et al., 1995; Huffman et al., 1996). Warner-Bratzler shear force is likely the most commonly used method to measure beef tenderness (Derington et al., 2011). In the present study, WBSF values decreased ($P < 0.001$) from days 2 to 14 and 21 to 63 post-fabrication for the LL (Table 2.3).

Connective tissue contributes to meat toughness (Blumer, 1963; Cross et al., 1973). In the present study, there were no differences ($P > 0.05$) in soluble or insoluble collagen between aging periods for the LL (Table 2.4).

Muscle pH is negatively correlated to L*, a*, and b* values (Page et al., 2001) and positively correlated to microbial growth (Rhee et al., 1997). *Longissimus lumborum* pH increased ($P < 0.001$) from days 2 to 42 and 42 to 63 of aging (Table 2.3). The LL pH increased by 0.11 units from day 2 to 63 of aging.

One consumer sensory panel was held for each muscle following the completion of all aging periods. Panelists scored steaks for overall acceptability,

tenderness, juiciness, and flavor. Demographics of consumer panelists are shown in Table 2.5. There were no differences in overall acceptability, juiciness, or flavor between aging periods for the LL (Table 2.6). Sensory panel tenderness scores improved ($P < 0.01$) from day 2 to 14 of aging (Table 2.6). Consumer preferences for the LL are shown in Table 2.9. Consumers liked LL juiciness the most after the first aging period and flavor the most after 14 and 21 days of aging, while tenderness was the most liked trait after the final 2 aging periods. Tenderness was the least liked trait after 2 and 21 days of aging, juiciness was the least liked after 14 and 42 days of aging, while flavor was the least liked trait after 63 days of aging. The percentage of consumers willing to purchase the LL after each of the five aging periods was 70, 73, 80, 62, and 68, respectively.

Semimembranosus

Fluid-holding capacity during transportation and storage of wholesale cuts is important to both retailers and foodservice providers since weight lost during this time represents economic loss for the buyer. Furthermore, fluid loss during retail display is unappealing to the consumer. In the present study, percent purge increased ($P < 0.001$) from day 21 to 42 of aging for the SM (Table 2.1). Unlike percent purge, percent retail fluid loss decreased ($P < 0.001$) from days 2 to 14 and 14 to 63 of aging (Table 2.1).

Color plays a critical role in a consumer's decision to purchase a meat product (AMSA, 2012; Mancini and Hunt, 2005). There was no aging period by day of retail display interaction ($P > 0.05$) for GM L* values (Figure 2.1). However, L*

values increased ($P < 0.001$) with aging period and over retail display time. An aging period by day of retail display interaction ($P < 0.001$) was observed for a* and b* values for the SM (Figures 2.2 and 2.3). *Semimembranosus* a* values were greater on days 0 and 2 of retail display than days 1, 3, and 4 of retail display during the first aging period. During the final 4 aging periods a* values decreased with retail display time. During the first aging period, b* values of the SM decreased from days 0 to 1 and 2 to 3 of retail display but increased from day 1 to 2 of retail display. *Semimembranosus* b* values decreased with aging periods of 14 days or more and retail display time.

Subjective color measurements revealed changes in perceived color similar to those observed with objective color measurements. There was an aging period by day of retail display interaction for amount of browning ($P < 0.05$) and discoloration ($P < 0.01$) (Figures 2.5 and 2.6). A trend was observed for the aging period by day of retail display interaction for oxygenated lean color ($P = 0.053$) and surface discoloration ($P = 0.052$) (Figures 2.4 and 2.7). There was no aging period by day of retail display interaction ($P > 0.05$) for color uniformity for the SM (Figure 2.8). There was however a difference ($P < 0.001$) between aging periods and days of retail display for oxygenated lean color, surface discoloration, and color uniformity. Amount of browning and discoloration increased to a greater extent with longer aging periods and retail display times. Oxygenated lean color became darker with longer aging periods and retail display times. Surface discoloration also increased with longer aging periods and retail display time. Furthermore, SM color uniformity became less desirable with longer aging periods and retail display time.

An aging period by day of retail display interaction ($P < 0.05$) was observed for the number of aerobic organisms for the SM (Table 2.2). On day 0 of retail display, aerobic counts increased from days 2 to 21, 21 to 42, and 42 to 62 days of aging. On day 4 of retail display, aerobic counts increased from days 2 to 14, 14 to 21, 21 to 42, and 42 to 63 of aging. Coliform and E. coli counts were <1 cfu/5cm^2 over all aging periods.

The TBARS assay is commonly used as a measurement of lipid oxidation. An aging period by day of retail display interaction ($P < 0.05$) was observed for TBARS values in the SM (Figure 2.9). During the second aging period lipid oxidation was lower on day 1 of retail display than days 0 and 4. Lipid oxidation did not change from day 0 to 1 of retail display but increased from day 1 to 4 of retail display for steaks aged 2, 21, 42, and 63 days.

No differences ($P > 0.05$) in cooking time or percent cooking loss between aging periods were observed for the SM (Table 2.3).

Tenderness is the most important palatability trait according to consumer surveys (Mackintosh et al., 1936; Morgan et al., 1991; Koohmaraie et al., 1995; Huffman et al., 1996). Warner-Bratzler shear force is likely the most commonly used method to measure beef tenderness (Derington et al., 2011). In the present study, WBSF decreased ($P < 0.001$) with longer aging periods for the SM (Table 2.3). The WBSF values were lower on days 42 and 63 than days 2 and 14 post-fabrication.

Connective tissue contributes to meat toughness (Blumer, 1963; Cross et al., 1973). In the present study, there were no differences ($P > 0.05$) in soluble or insoluble collagen between aging periods for the SM (Table 2.4).

Muscle pH is negatively correlated to L*, a*, and b* values (Page et al., 2001) and positively correlated to microbial growth (Rhee et al. 1997). There were no differences ($P > 0.05$) in pH between aging periods for the SM.

One consumer sensory panel was held for each muscle following the completion of all aging periods. Panelists scored steaks for overall acceptability, tenderness, juiciness, and flavor. Demographics of consumer panelists are shown in Table 2.5. Overall acceptability increased ($P <0.05$) with aging for the SM. Panelist scored steaks aged for 21, 42, and 63 days more acceptable than steaks aged for 2 days post-fabrication. Tenderness was greater ($P < 0.001$) for steaks aged 42 and 63 days than steaks aged for 2 and 14 days (Table 2.6). Juiciness scores tended to improved ($P = 0.07$) with aging for the SM (Table 2.6). No differences in flavor were observed between aging periods for the SM (Table 2.6). Consumer preferences are shown in Table 2.10. Consumers liked SM flavor the most after 2, 14, 21 and 63 days of aging, while tenderness was the most liked trait after 42 days of aging. Tenderness was the least liked trait after 2 and 14 days of aging and juiciness was the least liked after 21 and 42 days of aging, while favor was the least liked trait after 63 days of aging. The percentage of consumers willing to purchase the SM after each of the five aging periods was 35, 45, 57, 65, and 61, respectively.

Discussion

Biceps femoris

Tenderness is the most important palatability trait according to consumer surveys (Mackintosh et al., 1936; Morgan et al., 1991; Koohmaraie et al., 1995; Huffman et al., 1996). Koohmaraie et al. (1995) reported consumers would pay more for a guaranteed tender product. Both the 1991 and 2010/2011 National Beef Tenderness Surveys noted that improvement in tenderness, specifically of beef round muscles was needed because the round muscles are consistently less tender than other muscles. The BF is the largest muscle of the beef round.

Sensory panel tenderness scores for BF steaks were greater for product aged 21 days or longer than product aged for 2 days. However, no improvement in WBSF values was observed with aging. Gruber et al. (2006) found that WBSF of USDA Select BF muscles did not improve past 21 days postmortem. While, Smith et at. (1978) found WBSF values of USDA Choice BF did not improve after 11 days of aging. Koohmaraie et al. (2002) noted that tenderness improves due to degradation of specific myofibrillar proteins by calpain proteases. The rise in sensory panel tenderness scores in the current study is likely due to the breakdown of myofibrillar proteins. This tenderness improvement caused the willingness to purchase BF steaks to jump from 53 percent after 2 days of aging to 63 percent after 14 days of aging, to 75 percent after 21 days of aging. The 2010/2011 National Beef Tenderness Survey revealed the average postfabrication aging time for the bottom round was 17.2 days while 41.5 percent was aged for less than 14

days. Our results indicate that the USDA Select bottom round should be aged for at least 21 days.

Product aged for 2 and 63 days had less soluble collagen than product aged for 14 and 42 days. Furthermore, product aged for 2 and 63 days had less insoluble collagen than product aged for 14 days. These differences are likely due to the differences in cooking loss since steaks with less cooking loss had lower collagen values. Collagen solubility was not increased by extended aging suggesting a lack of postmortem collagen degradation. Cross et al. (1973) showed percent soluble collagen had a significant relationship to toughness when evaluated by a sensory panel. Unfortunately the BF has twice as much collagen as the LL and GM (Rhee et al., 2004). Silva et al. (1999) found no differences in collagen solubility over 13 days of aging the LD. Sentandreu et al. (2002) also noted that collagen does not change drastically at refrigeration temperatures. The lack of improvement in WBSF values could be attributed to the large background toughness of the BF caused by high levels of insoluble collagen.

Biceps femoris lipid oxidation generally increased with aging period and retail display time. Interestingly, the greatest TBARS value was observed after 21 days of aging and 4 days of retail display. McKenna et al. (2005) and Campo et al. (2006) likewise found that TBARS values increased with retail display time. Faustman and Cassens (1990) indicate a close relationship between lipid oxidation and myoglobin oxidation. The increase in lipid oxidation and the likely increase in myoglobin oxidation presumably played a role in the discoloration of steaks over the

retail display time. Steaks went from being bright to dark and became less red with increasing aging periods and retail display time. Furthermore, longer aging periods and retail display times increased the amount of browning, extent of discoloration, and percentage of the steak surface that was discolored.

Along with lipid oxidation, microbial growth may have also caused an increase in discoloration. Growth of mesophilic, aerobic organisms increased with longer aging periods. Similar to our results Hodges et al. (1974) and Wicklund et al. (2005) found that aerobic bacteria increased during 28 days of storage under vacuum. However, no steaks reached the spoilage point of 10^6 colony forming units/cm^2 (Jensen et al., 2003).

Other potential contributors to the reduced color stability observed between aging periods and retail display times include changes in reducing ability, oxygen consumption rate, oxygen penetration depth, and myoglobin content (Mckenna et al., 2005).

Besides affecting color, lipid oxidation can affect product flavor. No differences in sensory flavor scores were found across all aging periods. Hodges et al. (1974) noted there was greater evidence of off flavor as the storage time of rounds increased over 28 days. In beef, Campo et al. (2006) found that a TBARS value of approximately 2.3 mg MDA/kg meat was the threshold for lipid oxidation. In contrast, McKenna et al. (2005) used an arbitrary threshold value of 1.0 mg MDA/kg meat as the point at which off flavors can be detected in beef. Although there is a large range of reported thresholds for TBARS values (Tarladgis et al.,

1960; McKenna et al., 2005; Campo et al., 2006), the current study revealed that BF aged for 21 days and subjected to 4 days of retail display was the only product that reached the threshold of 1.0 mg MDA/kg of meat. Since, sensory panel steaks were only exposed to retail display conditions for one day, no steaks reached the threshold for lipid oxidation. Thus, we would not expect major changes in flavor due to lipid oxidation, regardless of aging period.

Johnson (1974) noted percent purge of 1 to 2 percent was acceptable, while greater than 4 percent would be excessive. In the current study, percent purge reached a maximum of 3.68 percent after 63 days of aging. Seideman et al. (1976) similarly found percent purge generally increased with increased storage time of beef knuckles aged for 7, 14, 21, 28, and 35 days. Hodges et al. (1974) also noted percent purge of vacuum packaged wholesale beef cuts increased with longer storage times. The increased purge during storage would be a concern for retail stores and foodservice due to a loss in weight of saleable product. Furthermore, purge contains the water soluble protein myoglobin along with other water soluble nutrients. Myoglobin is the pigment of meat (Huff-Lonergan, 2002). The increased purge and loss of myoglobin over storage time likely played a role in the decrease in a* values over longer aging periods.

Unlike percent purge, percent retail fluid loss decreased with retail display time. Cannon et al. (1996) similarly found retail drip loss decreased with longer storage times of pork loin chops. This would be an advantage to retailers since

retail fluid loss is unsightly to the consumer. Longer storage led to increased moisture loss, therefore less free water was available to be lost during retail display.

Gluteus medius

Sensory panel tenderness scores for the GM aged longer than 42 days were greater than scores for product aged 14 days or less. Interestingly, WBSF values did not statistically improve over the five aging periods. However, the values did numerically decrease, particularly from day 42 to 63 of aging. Gruber et al. (2006) found that WBSF of Select GM muscles improved up to 28 days postmortem. Tenderness has been shown to improve due to degradation of specific myofibrillar proteins by calpain proteases (Koohmaraie et al., 2002). There were no differences in soluble or insoluble collagen between aging periods for the GM. Likewise, Silva et al. (1999) found no differences in collagen solubility over 13 days of aging. Sentandreu et al. (2002) also noted that collagen does not change drastically at refrigeration temperatures. The improvement in sensory panel tenderness scores is therefore likely due to postmortem proteolysis. *Gluteus medius* sensory panel tenderness scores were greatest after 42 days of aging, likewise willingness to purchase the GM was also greatest after 42 days of aging.

Gluteus medius lipid oxidation increased with aging period and retail display time. McKenna et al. (2005) and Campo et al. (2006) likewise found that TBARS values increased with retail display time. Faustman and Cassens (1990) indicate a close relationship between lipid oxidation and myoglobin oxidation. The increase in

lipid oxidation and the likely increase in myoglobin oxidation presumably played a role in the discoloration of steaks over the retail display time.

Lee et al. (2008b) found no difference in GM L* values on day 0 of retail display after 0, 7, 14, 21, 28 and 35 days of aging. These authors also found a* and b* values increased from day 0 to 7 and then decreased from day 7 to 21 of aging. Our results also indicate no difference in day 0 L* values between aging periods, while a* and b* values increased from day 2 to 14 and then decreased from day 21 to 42 of aging.

Along with lipid oxidation, microbial growth may have also caused an increase in discoloration. Growth of mesophilic, aerobic organisms increased with longer aging periods. However, no steaks reached the spoilage point of 10^6 colony forming units/cm^2 (Jensen et al., 2003). Similar to our results Hodges et al. (1974) and Wicklund et al. (2005) found that aerobic bacteria increased during 28 days of storage under vacuum.

Other potential contributors to the reduced color stability observed between aging periods and retail display times include changes in reducing ability, oxygen consumption rate, oxygen penetration depth, and myoglobin content (Mckenna et al., 2005).

Besides affecting color, lipid oxidation can affect product flavor. No differences in sensory flavor scores were found across all aging periods. McKenna et al. (2005) used an arbitrary threshold value of 1.0 mg MDA/kg meat as the point at which off flavors can be detected in beef. Another study found a TBARS value of

approximately 2.3 mg MDA/kg meat as the threshold for lipid oxidation (Campo et al., 2006). Although there is a large range of reported thresholds for TBARS values (Tarladgis et al., 1960; McKenna et al., 2005; Campo et al., 2006), the current study revealed that GM beef subjected to extended aging and 4 days of retail display had less than 1 mg MDA/kg. Thus, we would not expect major changes in flavor due to lipid oxidation, regardless of aging period.

Percent purge of 1 to 2 percent is acceptable, while greater than 4 percent would be excessive (Johnson, 1974). In the current study, after 63 days of aging fluid losses were a maximum of 4.46 percent for the GM. Hodges et al. (1974) also noted percent purge of vacuum packaged wholesale beef cuts increased with longer storage times. The increased purge during storage would be a concern for retail stores and foodservice due to a loss in weight of saleable product. Furthermore, purge contains the water soluble protein myoglobin along with other water soluble nutrients. Myoglobin is the pigment of meat (Huff-Lonergan, 2002). The increased purge and loss of myoglobin over storage time likely played a role in the decrease in a* values over longer aging periods.

Unlike percent purge, percent retail fluid loss decreased with retail display time. Cannon et al. (1996) similarly found retail drip loss decreased with longer storage times of pork loin chops. This would be an advantage to retailers since retail fluid loss is unsightly to the consumer. Longer storage led to increased moisture loss, therefore less free water was available to be lost during retail display.

Interestingly, GM cooking times were longer for product aged for more than 21 days than product aged 14 days or less. Likewise, percent cooking loss was greater for product aged longer than 42 days than product aged 14 days or less. As expected longer cooking times lead to an increase in cooking loss.

Longissimus lumborum

Sensory panel tenderness scores of LL steaks improved from day 2 to 14 of aging but not thereafter. While WBSF values decreased from day 2 to 14 and 21 to 63. Gruber et al. (2006) found that WBSF of Select LD muscles improved up to 28 days postmortem. Tenderness has been shown to improve due to degradation of specific myofibrillar proteins by calpain proteases (Koohmaraie et al., 2002). No differences in soluble or insoluble collagen were observed. Likewise, Silva et al. (1999) found no differences in collagen solubility over 13 days of aging. Sentandreu et al. (2002) also noted that collagen does not change drastically at refrigeration temperatures. Therefore, the improved in LL sensory panel tenderness scores and decrease in WBSF is likely due to proteolysis of myofibrillar proteins by calpains. Our results indicate that USDA Select LL muscles do not need to be aged for longer than 14 days.

Longissimus lumborum lipid oxidation increased to a greater extent with longer aging periods and retail display time. McKenna et al. (2005) and Campo et al. (2006) likewise found that TBARS values increased with retail display time. Faustman and Cassens (1990) indicate a close relationship between lipid oxidation and myoglobin oxidation. The increase in lipid oxidation and the likely increase in

myoglobin oxidation presumably played a role in the discoloration of steaks over the retail display time. An increase in LL pH with longer aging periods may reduce the maximum discoloration, since higher pH values reduce myoglobin oxidation (Mckenna et al., 2005). Unfortunately, however, muscle pH is negatively correlated to L*, a*, and b* values (Page et al., 2001).

Lee et al. (2008a) found no difference in LL L*, a*, or b* values on day 0 of retail display after 0, 7, 14, 21, 28 and 35 days of aging. On the contrary, in the current study L* values on day 0 of retail display increased from day 2 to 14 of aging. While a* values increased from day 2 to 14 and then decreased from days 21 to 42 and 42 to 62 of aging. Lastly, b* values increased from day 2 to 14 and then subsequently decreased each of the final aging periods. McKenna et al. (2005) reported that at 72 hours postmortem LL a* values increased from day 0 to day 1 of retail display. They attributed these improved LL a* values to the high oxygen consumption rate early postmortem preventing the muscle from fully oxygenating. This closely follows our observation for the LL.

Growth of mesophilic, aerobic organisms significantly increased with longer aging periods. Similarly, Hodges et al. (1974) and Wicklund et al. (2005) found that aerobic bacteria increased during 28 days of storage under vacuum. This increase may have contributed to the increase in discoloration with longer aging periods. No steaks reached the spoilage point of 10^6 colony forming units/cm^2 (Jensen et al., 2003).

Other potential contributors to the reduced color stability observed between aging periods and retail display times include changes in reducing ability, oxygen consumption rate, oxygen penetration depth, and myoglobin content (Mckenna et al., 2005).

There were no differences in flavor between aging periods for the LL. With longer aging periods consumers scored a numerically higher percentage of steaks having an off flavor, although this does not appear to be associated with a decrease in willingness to purchase. Hodges et al. (1974) noted there was greater evidence of off flavor as the storage time of short loins increased over 28 days. McKenna et al. (2005) used an arbitrary threshold value of 1.0 mg MDA/kg meat as the point at which off flavors can be detected in beef. While another study found the threshold for lipid oxidation was a TBARS value of approximately 2.3 mg MDA/kg of meat (Campo et al., 2006). Although there is a large range of reported thresholds for TBARS values (Tarladgis et al., 1960; McKenna et al., 2005; Campo et al., 2006), the current study revealed that LL subjected to extended aging and 4 days of retail display had less than 1 mg MDA/kg of meat. Thus, we would not expect major changes in flavor due to lipid oxidation, regardless of aging period.

Johnson (1974) noted percent purge of 1 to 2 percent was acceptable, while greater than 4 percent would be excessive. In the current study, the LL fluid loss was highest after 42 days of aging and was 2.20 percent. This loss is higher than what would be expected in the industry since the wholesale cuts were divided into five sections for each of the five aging periods, thus increasing the surface area to

volume ratio during storage. Hodges et al. (1974) noted percent purge of vacuum packaged wholesale beef cuts increased with longer storage times. The increase of the LL pH over the aging periods helped to minimize purge loss. A higher pH leads to improved water binding since there are more negative charges to repel one another and increase myofilament spacing and more charges to bind to water. Boakye and Mittal (1993) noted LD pH increased through 16 days of aging. Mckenna et al. (2005) also found that pH increased over 5 days of retail display.

While, LL percent purge did not change with aging, percent retail fluid loss decreased over the aging periods. Cannon et al. (1996) similarly found retail drip loss decreased with longer storage times of pork loin chops. This would be an advantage to retailers since retail fluid loss is unsightly to the consumer.

In the present study, LL cooking time and percent cook loss generally increased with longer aging periods. As expected, longer cooking times led to greater cooking loss. Boakye and Mittal (1993) similarly found percent cooking loss increased for the LD from days 4 to 16 of aging. Jennings et al. (1978) found loins aged for 20 days had shorter cooking times than loins aged for 10 days. They attribute this difference to the increased purge during storage thus allowing for faster heat conduction. Wicklund et al. (2005) found no differences in LL percent cooking loss over 28 days of aging.

Semimembranosus

Tenderness is the most important palatability trait according to consumer surveys (Mackintosh et al., 1936; Morgan et al., 1991; Koohmaraie et al., 1995;

Huffman et al., 1996). Koohmaraie et al. (1995) reported consumers would pay more for a guaranteed tender product. Both the 1991 and 2010/2011 National Beef Tenderness Surveys noted that improvement in tenderness, specifically of beef round muscles was needed because the round muscles are consistently less tender than other muscles. The SM is the second largest muscle of the beef round.

Semimembranosus sensory panel tenderness scores and WBSF values were lower on days 42 and 63 than days 2 and 14 of aging. Furthermore the overall acceptability of SM steaks aged for 21 days or longer was greater than steaks aged for only 2 days. Gruber et al. (2006) found that WBSF of Select SM muscles did not improve past 21 days postmortem. Tenderness has been shown to improve due to degradation of specific myofibrillar proteins by calpain proteases (Koohmaraie et al., 2002). There were no differences in soluble or insoluble collagen between aging periods for the SM. Likewise, Silva et al. (1999) found no differences in collagen solubility over 13 days of aging. Sentandreu et al. (2002) also noted that collagen does not change drastically at refrigeration temperatures. Therefore, the improvement in sensory panel tenderness scores and decrease in SM WBSF is likely due to proteolysis of myofibrillar proteins by calpains. The large decrease in SM WBSF can be attributed to its high day 2 value. The 2010/2011 National Beef Tenderness Survey revealed the top round was aged for an average of 16.4 days while 46.6 percent was aged for 14 days or less. Our research suggests that the top round should be aged for at least 42 days to ensure a more tender product.

An increase in SM TBARS values was observed with longer aging periods and retail display times. The values never reached a level higher than 0.55 mg MDA/kg of meat. These low levels of lipid oxidation may allow the calpains to stay active longer, since oxidation decreases calpain activity (Huff-Lonergan and Lonergan, 2005). McKenna et al. (2005) and Campo et al. (2006) likewise found that TBARS values increased with retail display time. Faustman and Cassens (1990) indicate a close relationship between lipid oxidation and myoglobin oxidation. The increase in lipid oxidation and the likely increase in myoglobin oxidation presumably played a role in the discoloration of steaks over the retail display time. Furthermore, the observed decrease in a* values could partially be caused by the increased purge over storage time.

Along with lipid oxidation, microbial growth may have also caused an increase in discoloration. Growth of mesophilic, aerobic organisms increased with longer aging periods. Similar to our results Hodges et al. (1974) and Wicklund et al. (2005) found that aerobic bacteria increased during 28 days of storage under vacuum. However, no steaks reached the spoilage point of 10^6 colony forming units/cm^2 (Jensen et al., 2003).

Other potential contributors to the reduced color stability observed between aging periods and retail display times include changes in reducing ability, oxygen consumption rate, oxygen penetration depth, and myoglobin content (Mckenna et al., 2005).

Lipid oxidation can also cause a change in meat flavor. McKenna et al. (2005) used an arbitrary threshold value of 1.0 mg MDA/kg of meat as the point at which off flavors can be detected in beef. While Campo et al. (2006) found a TBARS value of approximately 2.3 mg MDA/kg of meat as the threshold for lipid oxidation in beef. Although there is a large range of reported thresholds for TBARS values (Tarladgis et al., 1960; McKenna et al., 2005; Campo et al., 2006), the current study revealed that SM subjected to extended aging remained below the lipid oxidation threshold. There were no differences in sensory panel flavor scores between aging periods. However, longer aging periods had a numerically higher percentage of steaks scored as having an off flavor, although this does not appear to be associated with a decrease in willingness to purchase. Hodges et al. (1974) noted there was greater evidence of off flavor as the storage time of rounds increased over 28 days. Seideman et al. (1976) found aging beef knuckles for 7, 14, 21, 35, and 42 days resulted in no differences in trained sensory panel evaluations in tenderness or flavor desirability. Interestingly, in the current study, willingness to purchase the SM increased from 35 percent on day 2 to 65 percent on day 42 of aging.

Purge is the fluid lost in fresh meat and is affected by pH and myofillament spacing (Huff-Lonergan, 2002). Johnson (1974) noted percent purge of 1 to 2 percent was acceptable, while greater than 4 percent would be excessive. In the current study, The SM fluid loss reached 6.11 percent after 63 days of aging. This loss is higher than what would be expected in the industry since the wholesale cuts were divided into five sections for each of the five aging periods, thus increasing the

surface area to volume ratio during storage. Nevertheless, a six percent loss is alarming. During the postmortem conversion of muscle to meat, cross-bridges between actin and myosin form and the sarcomere shortens. This coupled with a drop in pH allows entraped water to escape (Huff-Lonergan, 2002). Furthermore, the pressure from the vacuum package contributes to forcing free water out of the muscle. The high level of SM purge indicates large amounts of free water. Seideman et al. (1976) similarly found percent purge generally increased with increased storage time of beef knuckles aged for 7, 14, 21, 28, and 35 days. Hodges et al. (1974) also noted percent purge of vacuum packaged wholesale beef cuts increased with longer storage times. The increased purge during storage would be a concern for retail stores and foodservice due to a loss in weight of saleable product. Furthermore, purge contains the water soluble protein myoglobin which is the pigment of meat (Huff-Lonergan, 2002). The increased purge and loss of myoglobin over storage time likely played a role in the decrease in a* values over longer aging periods. Additionally, the high percent purge of the SM would indicate more free water on the steaks surface that would scatter and reflect light making the steak appear lighter. Thus explaining the increase in SM L* values with longer aging periods.

Unlike percent purge, percent retail fluid loss declined with longer aging periods. Cannon et al. (1996) similarly found retail drip loss decreased with longer storage times of pork loin chops. This would be an advantage to retailers since retail fluid loss is unsightly to the consumer. Longer storage led to increased moisture loss, therefore less free water was available to be lost during retail display.

Conclusions

Both subjective and objective color measurements revealed that longer aging periods led to decreased color stability of all four muscles examined. More research is needed to determine why aging affects color stability to varying degrees in different muscles. Potential contributors to the reduced color stability over time and differences between muscles include changes in reducing ability, oxygen consumption rate, oxygen penetration depth, myoglobin content, oxidative rancidity, and pH (Mckenna et al., 2005). These authors note color stability may best be determined by the ratio of reducing activity to oxygen consumption rate.

Our results indicate that extended aging has a negative impact on retail shelf-life yet positive effects on consumer perception of tenderness of all muscles, juiciness of round muscles, and overall acceptability of the top round. Because each muscle responds differently to extended aging, one overarching management strategy is not sufficient for retail and/or food service subprimals. Use of this information will lead to more effective product management for these beef subprimals and result in more consistent and desirable eating experiences for the consumer.

Future Research

Extended aging led to reduced color stability of both the SM and LL, but affected the SM to a greater extent than the LL. This is unfortunate because sensory characteristics of the top round improved substantially by 42 days of aging.

The use of an antioxidant during retail display could be a means to improve color stability and therefore shelf-life of long aged product, while maintaining the positive sensory attributes. Vitamin C and rosemary extract are two potential antioxidants that could increase the retail shelf-life of long aged top round and top loin steaks by improving color stability and reducing lipid oxidation. Furthermore, if the lower levels of lipid oxidation in the SM did contribute to the continued improvement in tenderness by inhibiting calpain action, the antioxidants may also improve muscle tenderness. The calpain system is likely responsible for the postrigor improvement in tenderness. Currently, it is believed that μ-calpain is responsible for the postmortem proteolysis (Koohmaraie and Geesink, 2006). Furthermore, Zamora et al. (1996) found that by day 14 μ-calpain activity is 0. Based on the improvement of SM tenderness from day 14 to day 42 in the present study, μ-calpain may be active for more than 14 days or m-calpain could potentially be activated later during the aging process. Therefore, determining μ- and m-calpain activity in extend aged beef is of utmost importance in future studies.

Improving the water holding capacity of the SM would increase its value, since after 63 days of aging the percent purge was 6.11 percent. Injecting the top round with a phosphate solution or some other solution that binds water would decrease purge and increase saleable product. This could lead to improved juiciness of the top round as well.

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Tables and Figures

Table 2.1: Percent purge and percent retail fluid loss of extended aged beef

	Day of aging				
	2	14	21	42	63
Percent purge					
Biceps femoris		2.28 ± 0.24[a]	2.49 ± 0.24[a]	2.63 ± 0.24[a]	3.68 ± 0.24[b]
Gluteus medius		3.13 ± 0.38[a]	3.51 ± 0.38[ac]	4.72 ± 0.38[b]	4.46 ± 0.38[bc]
Longissimus lumborum		1.52 ± 0.20	1.67 ± 0.20	2.20 ± 0.20	1.76 ± 0.20
Semimembranosus		3.78 ± 0.36[a]	4.08 ± 0.35[a]	5.64 ± 0.35[b]	6.11 ± 0.35[b]
Percent retail fluid loss					
Biceps femoris	1.95 ± 0.07[a]	0.88 ± 0.07[b]	0.78 ± 0.07[b]	0.74 ± 0.07[b]	0.70 ± 0.07[b]
Gluteus medius	1.85 ± 0.05[a]	1.00 ± 0.05[b]	0.94 ± 0.05[bc]	0.81 ± 0.05[c]	0.86 ± 0.05[c]
Longissimus lumborum	1.59 ± 0.04[a]	0.90 ± 0.04[b]	0.79 ± 0.04[c]	0.77 ± 0.04[c]	0.75 ± 0.04[c]
Semimembranosus	2.57 ± 0.16[a]	1.12 ± 0.16[b]	1.03 ± 0.16[bc]	0.78 ± 0.16[bc]	0.62 ± 0.16[c]

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

Table 2.2: Surface aerobic plate counts on extended aged beef

Muscle	Day of Display	Day of aging 2	14	21	42	63
Biceps femoris	0	0.4 ± 0.2^{ab}	0.2 ± 0.2^{a}	0.9 ± 0.2^{b}	1.3 ± 0.2^{c}	3.4 ± 0.2^{d}
	4	0.0 ± 0.2^{a}	1.2 ± 0.2^{b}	1.7 ± 0.2^{c}	2.1 ± 0.2^{c}	3.6 ± 0.2^{d}
Gluteus medius	0	0.5 ± 0.2^{ab}	0.2 ± 0.2^{a}	0.8 ± 0.2^{b}	1.6 ± 0.2^{c}	2.3 ± 0.2^{d}
	4	0.0 ± 0.2^{a}	0.8 ± 0.2^{b}	1.2 ± 0.2^{bc}	1.5 ± 0.2^{c}	1.4 ± 0.2^{c}
Longissimus lumborum	0	0.0 ± 0.2^{a}	1.1 ± 0.2^{b}	1.1 ± 0.2^{b}	1.9 ± 0.2^{c}	2.6 ± 0.2^{d}
	4	0.0 ± 0.2^{a}	1.0 ± 0.2^{b}	0.8 ± 0.2^{b}	2.3 ± 0.2^{c}	3.6 ± 0.2^{d}
Semimembranosus	0	0.0 ± 0.2^{a}	0.5 ± 0.2^{ab}	0.6 ± 0.2^{b}	1.6 ± 0.2^{c}	3.6 ± 0.2^{d}
	4	0.0 ± 0.2^{a}	1.0 ± 0.2^{b}	0.5 ± 0.2^{c}	2.1 ± 0.2^{d}	3.3 ± 0.2^{e}

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

[2]Plates were estimated following the 3M Interpretation Guide

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

Table 2.3: Physical characteristics of extended aged beef: cook time, percent cook loss, WBSF, and pH

	Day of aging				
	2	14	21	42	63
Cook time[1]					
Biceps femoris	24.4 ± 2.0^{a}	30.2 ± 2.0^{b}	35.2 ± 2.1^{b}	33.1 ± 2.0^{b}	29.9 ± 2.0^{b}
Gluteus medius	20.2 ± 1.8^{a}	20.9 ± 1.8^{a}	26.3 ± 1.8^{b}	29.6 ± 2.0^{b}	27.9 ± 1.8^{b}
Longissimus lumborum	19.5 ± 1.5^{ab}	17.9 ± 1.5^{a}	22.8 ± 1.5^{bc}	22.8 ± 1.5^{bc}	26.6 ± 1.5^{c}
Semimembranosus	26.0 ± 2.1	25.3 ± 2.1	28.6 ± 2.1	28.1 ± 2.1	31.2 ± 2.1
Percent cook loss					
Biceps femoris	25.3 ± 1.5^{a}	32.5 ± 1.5^{b}	33.2 ± 1.5^{b}	33.0 ± 1.5^{b}	26.3 ± 1.5^{a}
Gluteus medius	27.4 ± 1.6^{a}	28.8 ± 1.6^{ab}	33.2 ± 1.6^{cb}	33.9 ± 1.6^{c}	34.0 ± 1.6^{c}
Longissimus lumborum	24.2 ± 1.3^{a}	24.5 ± 1.3^{ab}	27.9 ± 1.3^{c}	28.7 ± 1.3^{c}	26.9 ± 1.3^{bc}
Semimembranosus	32.9 ± 1.4	36.3 ± 1.4	32.4 ± 1.4	33.6 ± 1.4	31.3 ± 1.4
WBSF (kg)					
Biceps femoris	2.97 ± 0.21	3.24 ± 0.21	3.13 ± 0.21	3.12 ± 0.21	3.14 ± 0.21
Gluteus medius	3.47 ± 0.18	3.10 ± 0.18	3.17 ± 0.18	3.23 ± 0.18	2.93 ± 0.18
Longissimus lumborum	3.42 ± 0.15^{a}	2.61 ± 0.15^{bc}	2.73 ± 0.15^{b}	2.62 ± 0.15^{bc}	2.26 ± 0.15^{c}
Semimembranosus	4.74 ± 0.22^{a}	4.31 ± 0.22^{ab}	3.78 ± 0.22^{bc}	3.58 ± 0.22^{c}	3.27 ± 0.22^{c}
pH values					
Biceps femoris	5.56 ± 0.02	5.56 ± 0.02	5.60 ± 0.02	5.61 ± 0.02	5.60 ± 0.02
Gluteus medius	5.56 ± 0.02	5.56 ± 0.02	5.57 ± 0.02	5.56 ± 0.02	5.59 ± 0.02
Longissimus lumborum	5.58 ± 0.02^{a}	5.60 ± 0.02^{ab}	5.60 ± 0.02^{ab}	5.63 ± 0.02^{b}	5.69 ± 0.02^{c}
Semimembranosus	5.55 ± 0.02	5.55 ± 0.02	5.56 ± 0.02	5.53 ± 0.02	5.52 ± 0.02

[1]Minutes to 71°C

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

Table 2.4: Physical characteristics of extended aged beef: soluble and insoluble collagen

	Day of aging				
	2	14	21	42	63
Soluble collagen[1]					
Biceps femoris	2.30 ± 0.10[a]	2.73 ± 0.10[b]	2.54 ± 0.10[ab]	2.67 ± 0.10[b]	2.28 ± 0.10[a]
Gluteus medius	0.97 ± 0.33	0.95 ± 0.33	0.95 ± 0.33	1.26 ± 0.33	1.30 ± 0.33
Longissimus lumborum	0.60 ± 0.31	1.18 ± 0.31	0.75 ± 0.31	0.51 ± 0.31	0.62 ± 0.31
Semimembranosus	0.54 ± 0.09	0.55 ± 0.09	0.39 ± 0.09	0.55 ± 0.09	0.43 ± 0.09
Insoluble collagen[1]					
Biceps femoris	9.70 ± 0.59[a]	11.86 ± 0.59[b]	10.49 ± 0.59[ab]	10.95 ± 0.59[ab]	9.76 ± 0.59[a]
Gluteus medius	6.15 ± 0.59	6.11 ± 0.59	6.24 ± 0.59	6.34 ± 0.59	6.48 ± 0.59
Longissimus lumborum	4.81 ± 0.57	5.65 ± 0.57	5.29 ± 0.57	5.32 ± 0.57	5.55 ± 0.57
Semimembranosus	8.12 ± 0.64	8.26 ± 0.64	6.67 ± 0.64	7.93 ± 0.64	7.09 ± 0.64

[1]mg collagen/g meat

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

Table 2.5: Demographics of consumer panelists

	Biceps femoris		*Gluteus medius*		*Longissimus lumborum*		*Semimembranosus*	
	n	%	*n*	%	*n*	%	*n*	%
Age								
18-19	4	6.7	4	6.8	4	6.7	1	1.7
20-29	30	50	42	71.2	35	58.3	32	53.3
30-39	7	11.7	4	6.8	3	5	6	10
40-49	7	11.7	4	6.8	6	10	5	8.3
50+	12	20	5	8.5	12	20	16	26.7
Gender								
Male	30	50	21	35.6	22	36.7	29	48.3
Female	30	50	38	64.4	38	63.3	31	51.7
Beef meals/wk[1]								
0 to 1	9	15	14	23.3	12	20.3	7	11.7
2 to 4	35	58.3	31	51.7	29	49.2	34	56.7
5 to 7	14	23.3	14	23.3	17	28.8	16	26.7
8+	2	3.3	1	1.7	1	1.7	3	5
Most consumed[2]								
Ground	40	62.5	32	45.7	42	65.6	39	52
Roast	2	3.1	6	8.6	4	6.3	5	6.7
Steak	19	29.7	28	40	17	26.6	27	36
Other	3	4.7	4	5.7	1	1.6	4	5.3

For 1 and 2, 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

Table 2.6: Sensory analysis by consumer panelists[1]

	Day of aging				
	2	14	21	42	63
Biceps femoris					
Acceptability	5.19 ± 0.29	5.65 ± 0.29	6.08 ± 0.29	5.95 ± 0.29	5.68 ± 0.29
Tenderness	4.50 ± 0.33[a]	5.15 ± 0.33[ab]	5.80 ± 0.33[b]	5.72 ± 0.33[b]	5.77 ± 0.33[b]
Juiciness	4.48 ± 0.39	5.28 ± 0.39	5.44 ± 0.39	5.70 ± 0.39	5.42 ± 0.39
Flavor	5.68 ± 0.29	5.93 ± 0.29	5.84 ± 0.29	5.78 ± 0.29	5.40 ± 0.29
Gluteus medius					
Acceptability	5.37 ± 0.28	5.16 ± 0.28	5.50 ± 0.28	5.78 ± 0.28	5.56 ± 0.28
Tenderness	4.92 ± 0.31[a]	4.72 ± 0.31[a]	5.38 ± 0.31[ab]	6.00 ± 0.31[b]	5.88 ± 0.31[b]
Juiciness	4.95 ± 0.32	4.53 ± 0.32	4.80 ± 0.32	4.98 ± 0.32	4.93 ± 0.32
Flavor	5.35 ± 0.27	5.15 ± 0.27	5.32 ± 0.27	5.23 ± 0.27	5.37 ± 0.27
Longissimus lumborum					
Acceptability	5.48 ± 0.28	6.03 ± 0.28	6.20 ± 0.28	5.78 ± 0.28	5.92 ± 0.28
Tenderness	4.53 ± 0.32[a]	5.72 ± 0.32[b]	6.03 ± 0.32[b]	6.18 ± 0.32[b]	6.20 ± 0.32[b]
Juiciness	5.00 ± 0.38	5.08 ± 0.38	5.77 ± 0.38	5.02 ± 0.38	5.07 ± 0.38
Flavor	5.35 ± 0.25	5.77 ± 0.25	6.07 ± 0.25	5.45 ± 0.25	5.39 ± 0.25
Semimembranosus					
Acceptability	4.33 ± 0.31[a]	4.86 ± 0.31[ab]	5.38 ± 0.31[b]	5.63 ± 0.31[b]	5.55 ± 0.31[b]
Tenderness	3.27 ± 0.33[a]	4.17 ± 0.33[ab]	4.82 ± 0.33[bc]	5.43 ± 0.33[c]	5.47 ± 0.33[c]
Juiciness	3.57 ± 0.34	4.05 ± 0.34	4.52 ± 0.34	4.75 ± 0.34	4.78 ± 0.34
Flavor	4.85 ± 0.26	5.30 ± 0.26	5.22 ± 0.26	5.30 ± 0.26	5.28 ± 0.26

[1]Scale, 9 = like extremely, extremely tender, extremely juicy, and like flavor extremely, respectively; 1= dislike extremely, not at all tender, extremely dry, and dislike flavor extremely, respectively.
[abcd]Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 2.7: Consumer preferences for the *biceps femoris*

	Days of aging									
	2		14		21		42		63	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Like most[1]										
Flavor	26	49.1	28	50.9	22	37.9	24	45.3	12	22.2
Tenderness	7	13.2	4	7.3	17	29.3	11	20.8	18	33.3
Juiciness	14	26.4	18	32.7	14	24.1	14	26.4	19	35.2
Texture	6	11.3	5	9.1	5	8.6	4	7.5	5	9.3
Like least[2]										
Flavor	5	9.1	4	7.3	11	22	13	24.5	18	33.3
Tenderness	26	47.3	31	56.4	15	30	16	30.2	14	25.9
Juiciness	16	29.1	11	20	16	32	16	30.2	12	22.2
Texture	8	14.5	9	16.4	8	16	8	15.1	10	18.5
Off flavor[3]										
Yes	11	18.3	9	15.3	9	15.3	14	23.3	20	33.3
No	49	81.7	50	84.7	50	84.7	46	76.7	40	66.7
Purchase[4]										
Yes	31	53.4	37	62.7	44	74.6	35	59.3	40	66.7
No	27	46.6	22	37.3	15	25.4	24	40.7	20	33.3

For 1 to 4, consumers were asked:

1. IF APPLICABLE, please circle the trait you liked most about this product. flavor, tenderness, juiciness, or texture/mouth feel
2. IF APPLICABLE, please circle the trait you liked least about this product. flavor, tenderness, juiciness, or texture/mouth feel
3. OFF-FLAVOR: This is your based on your ability to detect an off-flavor of the sample: NO/YES
4. CONSUMER SATISFACTION: Would you be willing to purchase this product? NO/YES

Table 2.8: Consumer preferences for the *gluteus medius*

	Days of aging									
	2		14		21		42		63	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Like most[1]										
Flavor	17	33.3	22	44.9	17	32.1	13	24.5	11	20
Tenderness	12	23.5	14	28.6	15	28.3	25	47.2	26	47.3
Juiciness	11	21.6	8	16.3	7	13.2	12	22.6	9	16.4
Texture	11	21.6	5	10.2	14	26.4	3	5.7	9	16.4
Like least[2]										
Flavor	13	26.5	18	32.7	21	38.2	17	31.5	16	29.1
Tenderness	18	36.7	11	20	18	32.7	9	16.7	10	18.2
Juiciness	10	20.4	17	30.9	14	25.5	18	33.3	22	40
Texture	8	16.3	9	16.4	2	3.6	10	18.5	7	12.7
Off flavor[3]										
Yes	13	22	13	21.7	15	25	19	31.7	15	25.9
No	46	78	47	78.3	45	75	41	68.3	43	74.1
Purchase[4]										
Yes	37	61.7	37	62.7	34	57.6	42	70	39	65
No	23	38.3	22	37.3	25	42.4	18	30	21	35

For 1 to 4, consumers were asked:

1. IF APPLICABLE, please circle the trait you liked most about this product. flavor, tenderness, juiciness, or texture/mouth feel
2. IF APPLICABLE, please circle the trait you liked least about this product. flavor, tenderness, juiciness, or texture/mouth feel
3. OFF-FLAVOR: This is your based on your ability to detect an off-flavor of the sample: NO/YES
4. CONSUMER SATISFACTION: Would you be willing to purchase this product? NO/YES

Table 2.9: Consumer preferences for the *longissimus lumborum*

	Days of aging									
	2		14		21		42		63	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Like most[1]										
Flavor	20	33.9	25	44.6	24	41.4	14	25	17	34
Tenderness	10	16.9	13	23.2	15	25.9	30	53.6	26	52
Juiciness	23	39	9	16.1	16	27.6	5	8.9	1	2
Texture	6	10.2	9	16.1	3	5.2	7	12.5	6	12
Like least[2]										
Flavor	12	21.1	14	26.4	14	26.9	16	30.8	23	42.6
Tenderness	26	45.6	16	30.2	16	30.8	10	19.2	6	11.1
Juiciness	12	21.1	17	32.1	14	26.9	19	36.5	16	29.6
Texture	7	12.3	6	11.3	8	15.4	7	13.5	9	16.7
Off flavor[3]										
Yes	12	20	7	11.9	11	18.3	11	18.3	15	25.4
No	48	80	52	88.1	49	81.7	49	81.7	44	74.6
Purchase[4]										
Yes	42	70	44	73.3	48	80	37	61.7	41	68.3
No	18	30	16	26.7	12	20	23	38.3	19	31.7

For 1 to 4, consumers were asked:
1. IF APPLICABLE, please circle the trait you liked most about this product. flavor, tenderness, juiciness, or texture/mouth feel
2. IF APPLICABLE, please circle the trait you liked least about this product. flavor, tenderness, juiciness, or texture/mouth feel
3. OFF-FLAVOR: This is your based on your ability to detect an off-flavor of the sample: NO/YES
4. CONSUMER SATISFACTION: Would you be willing to purchase this product? NO/YES

Table 2.10: Consumer preferences for the *semimembranosus*

	Days of aging									
	2		14		21		42		63	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Like most[1]										
Flavor	26	57.8	29	64.4	21	42	15	27.3	18	35.3
Tenderness	5	11.1	4	8.9	15	30	17	30.9	15	29.4
Juiciness	6	13.3	5	11.1	6	12	14	25.5	14	27.5
Texture	8	17.8	7	15.6	8	16	9	16.4	4	7.8
Like least[2]										
Flavor	4	6.7	6	9.8	10	19.2	15	26.8	19	34.5
Tenderness	36	60	25	41	16	30.8	16	28.6	14	25.5
Juiciness	16	26.7	23	37.7	21	40.4	17	30.4	13	23.6
Texture	4	6.7	7	11.5	5	9.6	8	14.3	9	16.4
Off flavor[3]										
Yes	12	20.3	14	23.7	17	28.8	16	27.1	20	33.9
No	47	79.7	45	76.3	42	71.2	43	72.9	39	66.1
Purchase[4]										
Yes	21	35	27	45	34	56.7	39	65	37	61.7
No	39	65	33	55	26	43.3	21	35	23	38.3

For 1 to 4, consumers were asked:
1. IF APPLICABLE, please circle the trait you liked most about this product. flavor, tenderness, juiciness, or texture/mouth feel
2. IF APPLICABLE, please circle the trait you liked least about this product. flavor, tenderness, juiciness, or texture/mouth feel
3. OFF-FLAVOR: This is your based on your ability to detect an off-flavor of the sample: NO/YES
4. CONSUMER SATISFACTION: Would you be willing to purchase this product? NO/YES

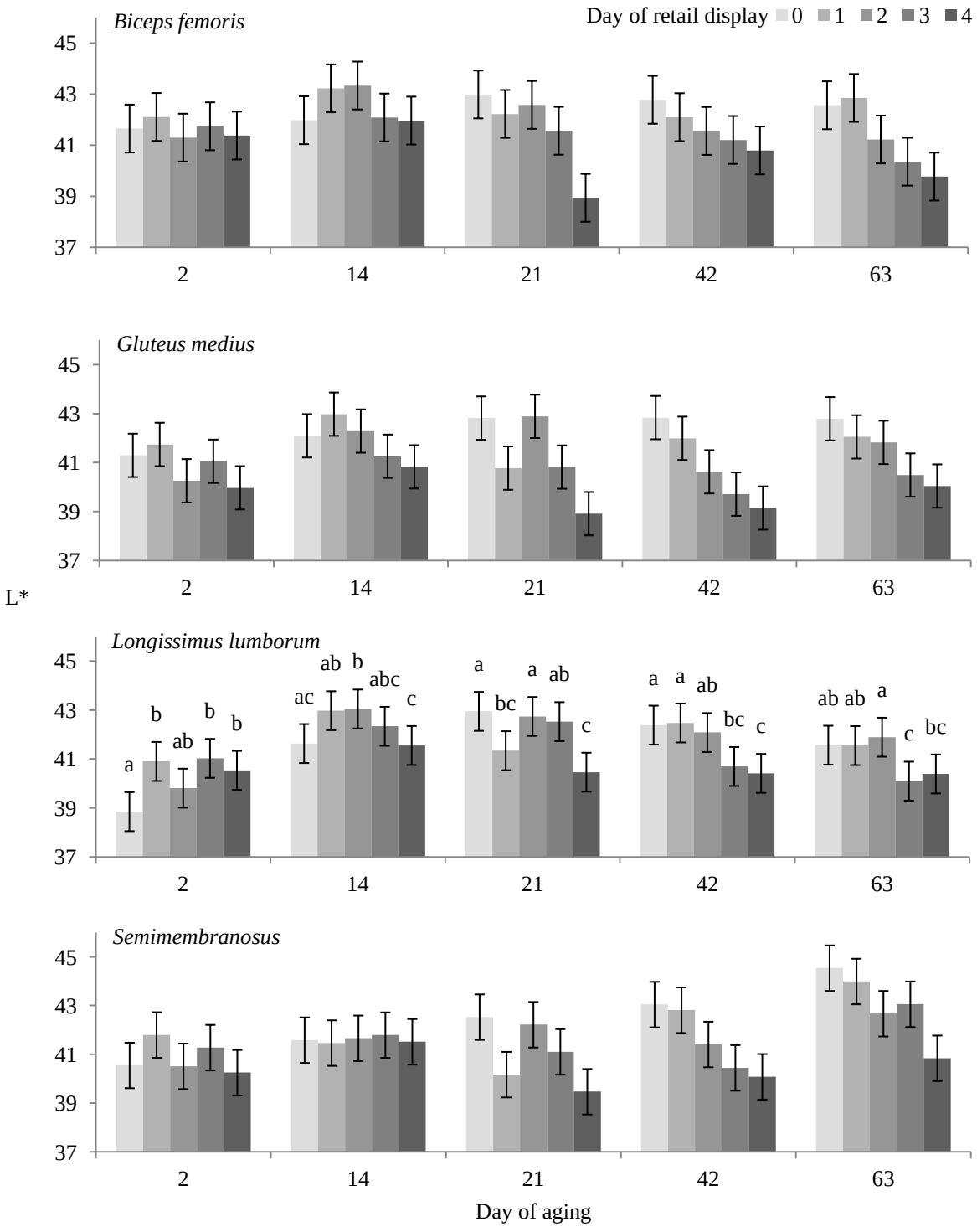

Figure 2.1: Effect of aging and retail display on Hunter L* values of beef *biceps femoris*, *gluteus medius*, *longissimus lumborum*, and *semimembranosus* steaks. Muscles were aged for 2, 14, 21, 42, and 63 days post-fabrication. At the end of each aging period, 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 4 days. Objective color measurements were taken daily. Bars represent the LSM ± SEM. Means within a muscle and aging period with different superscripts differ ($P < 0.05$).

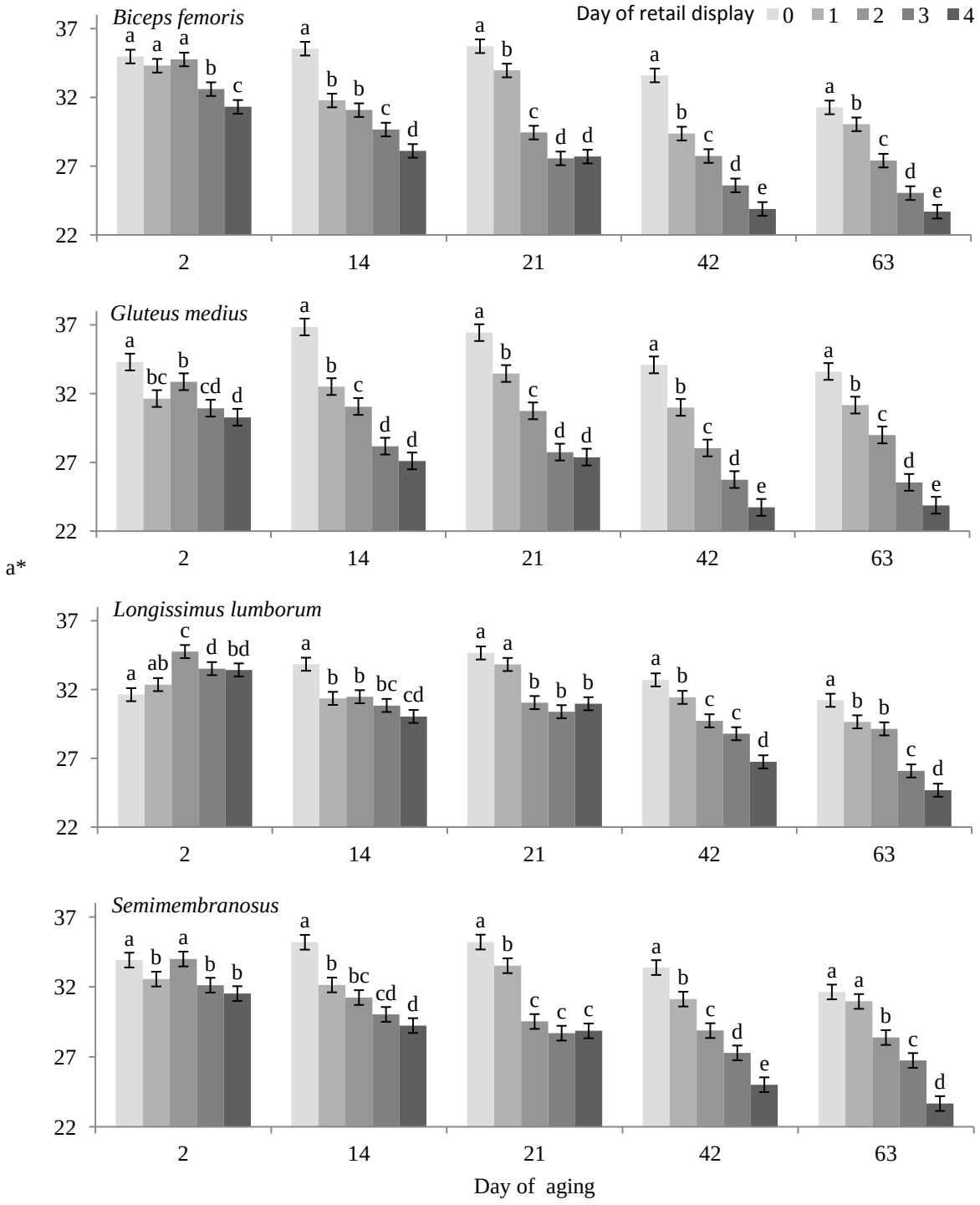

Figure 2.2: Effect of aging and retail display on Hunter a* values of beef *biceps femoris*, *gluteus medius*, *longissimus lumborum*, and *semimembranosus* steaks. Muscles were aged for 2, 14, 21, 42, and 63 days post-fabrication. At the end of each aging period, 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 4 days. Objective color measurements were taken daily. Bars represent the LSM ± SEM. Means within a muscle and aging period with different superscripts differ ($P < 0.05$).

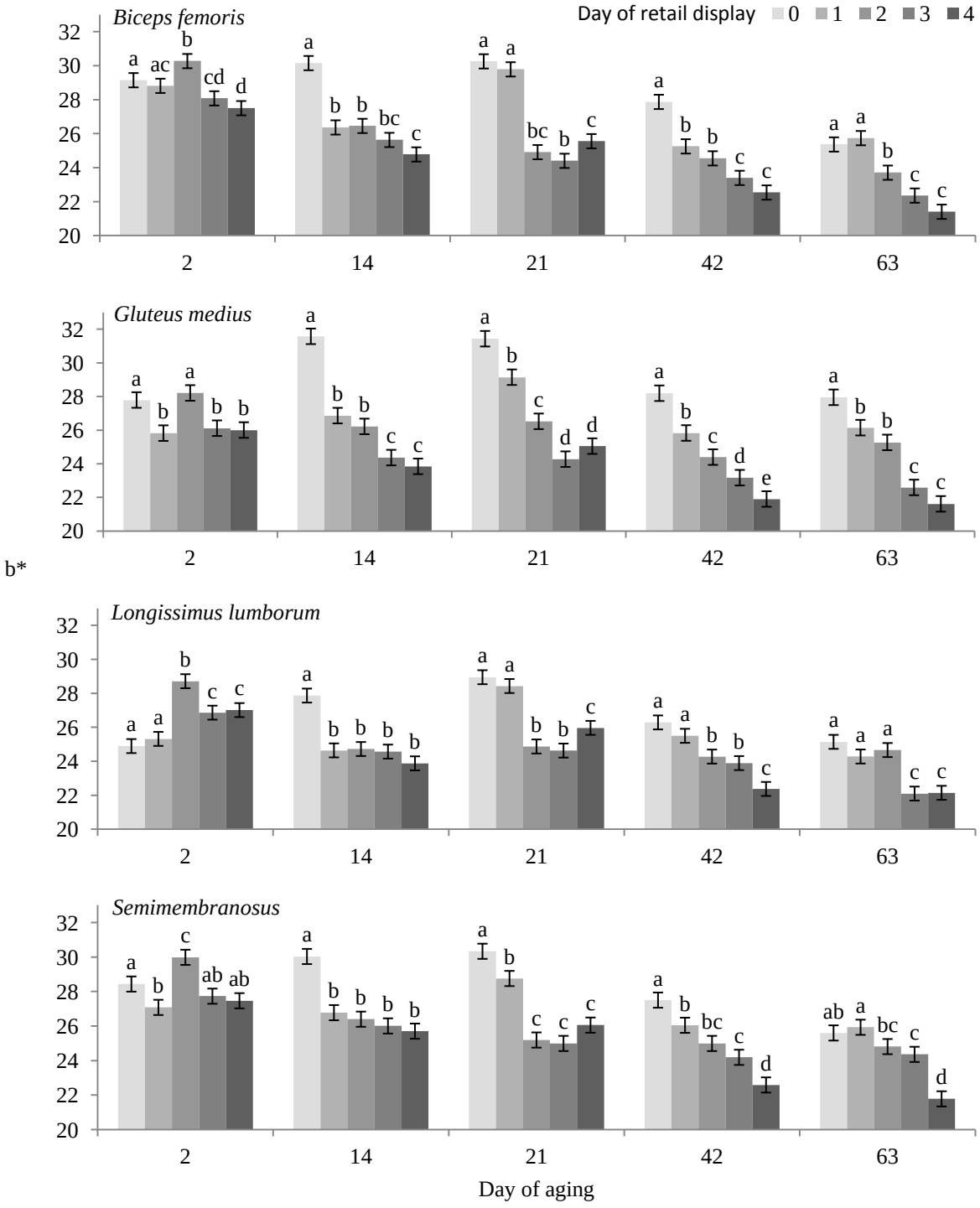

Figure 2.3: Effect of aging and retail display on Hunter b* values of beef *biceps femoris*, *gluteus medius*, *longissimus lumborum*, and *semimembranosus* steaks. Muscles were aged for 2, 14, 21, 42, and 63 days post-fabrication. At the end of each aging period, 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 4 days. Objective color measurements were taken daily. Bars represent the LSM ± SEM. Means within a muscle and aging period with different superscripts differ ($P < 0.05$).

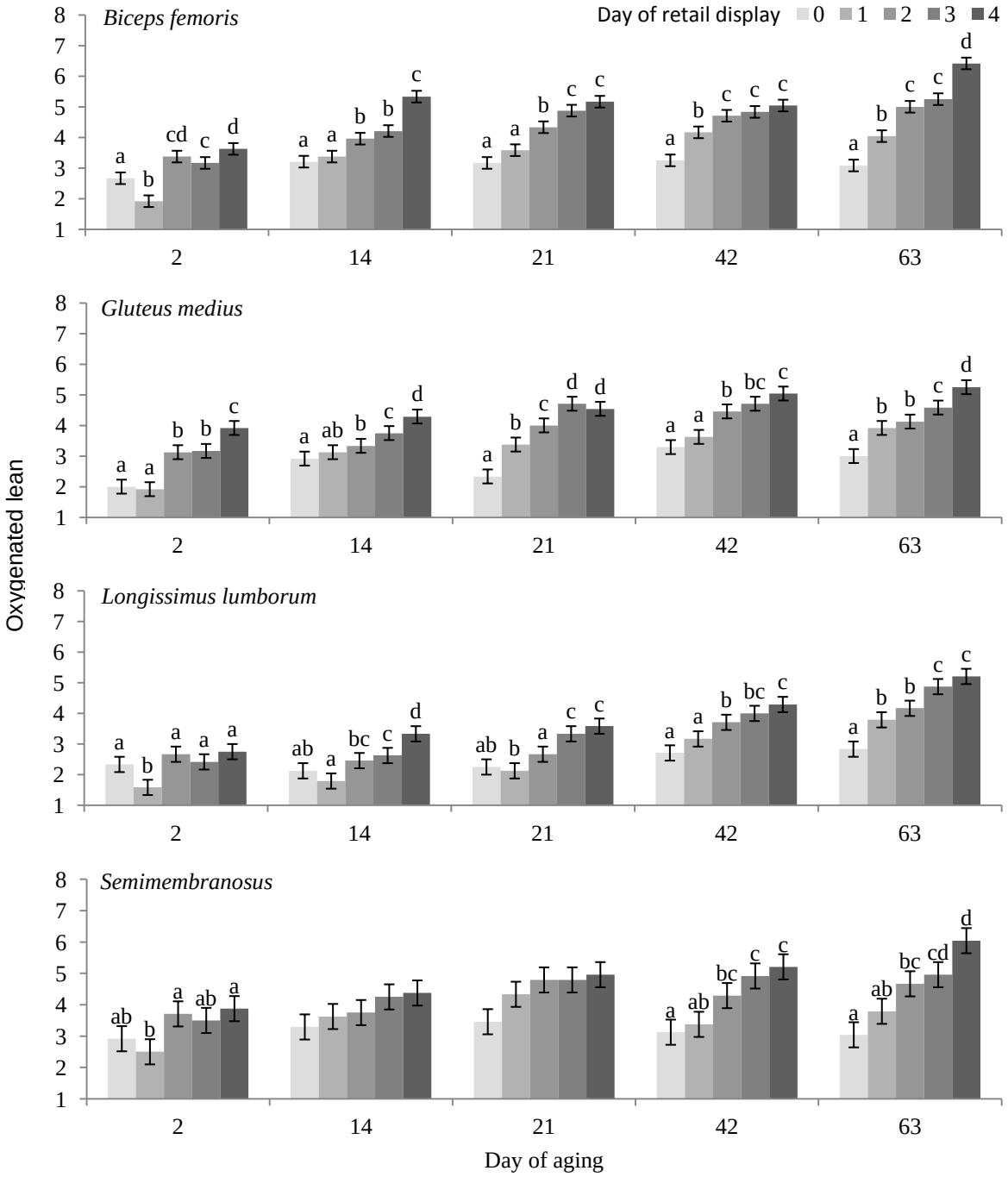

Figure 2.4: Effect of aging and retail display on oxygenated lean color of beef *biceps femoris*, *gluteus medius*, *longissimus lumborum*, and *semimembranosus* steaks. Muscles were aged for 2, 14, 21, 42, and 63 days post-fabrication. At the end of each aging period, 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 4 days. Two evaluators scored the steaks daily during retail display for oxygenated lean color using a 8 point scale (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). Bars represent the LSM ± SEM. Means within a muscle and aging period with different superscripts differ ($P < 0.05$).

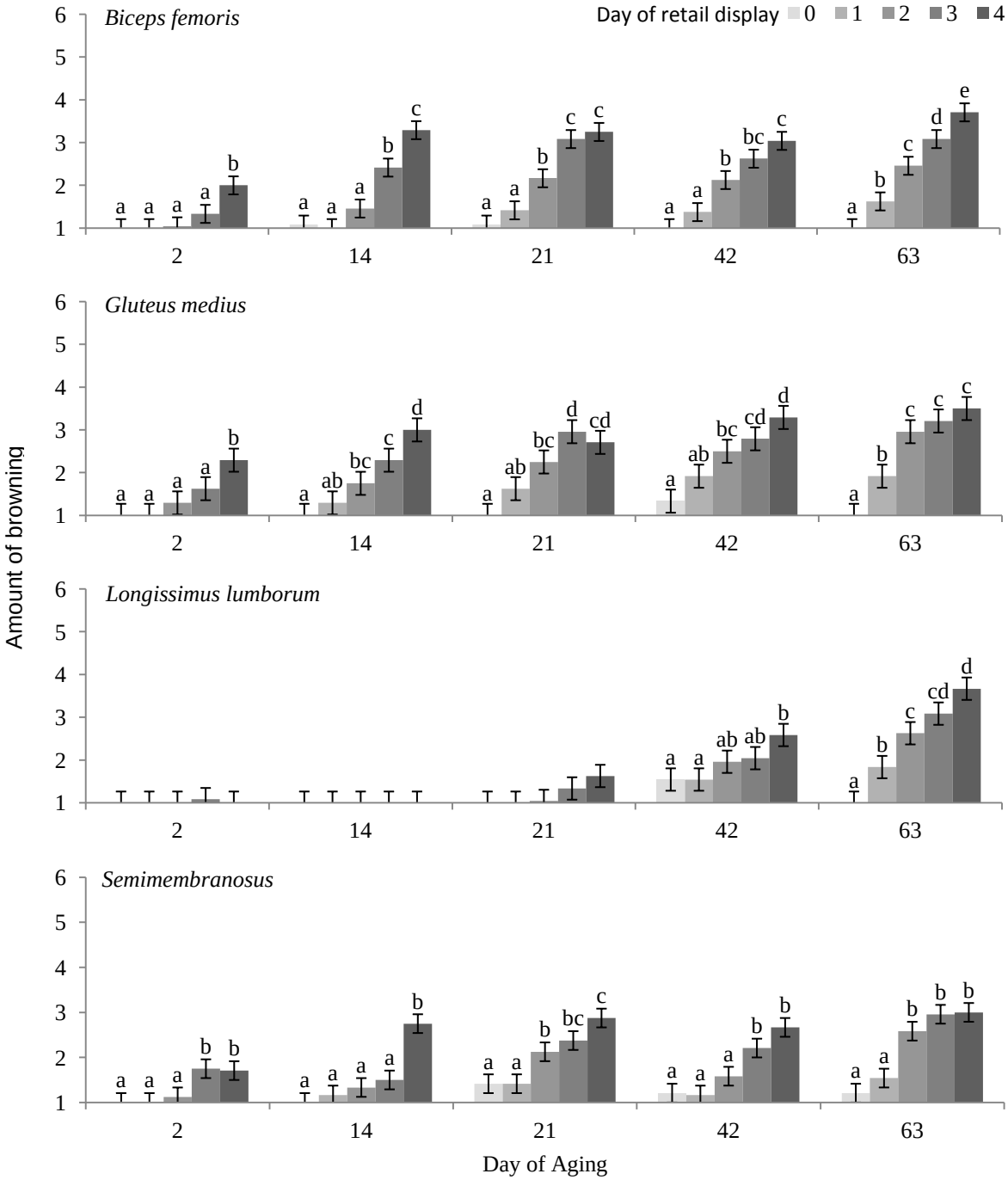

Figure 2.5: Effect of aging and retail display on amount of browning of beef *biceps femoris*, *gluteus medius*, *longissimus dorsi*, and *semimembranosus* steaks. Muscles were aged for 2, 14, 21, 42, and 63 days post-fabrication. At the end of each aging period, 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 4 days. Two evaluators scored the steaks daily during retail display for amount of browning using a 6 point scale (1 = no evidence of browning, 2 = dull, 3 = grayish, 4 = brownish-gray, 5 = brown, 6 = dark brown). Bars represent the LSM ± SEM. Means within a muscle and aging period with different superscripts differ ($P < 0.05$).

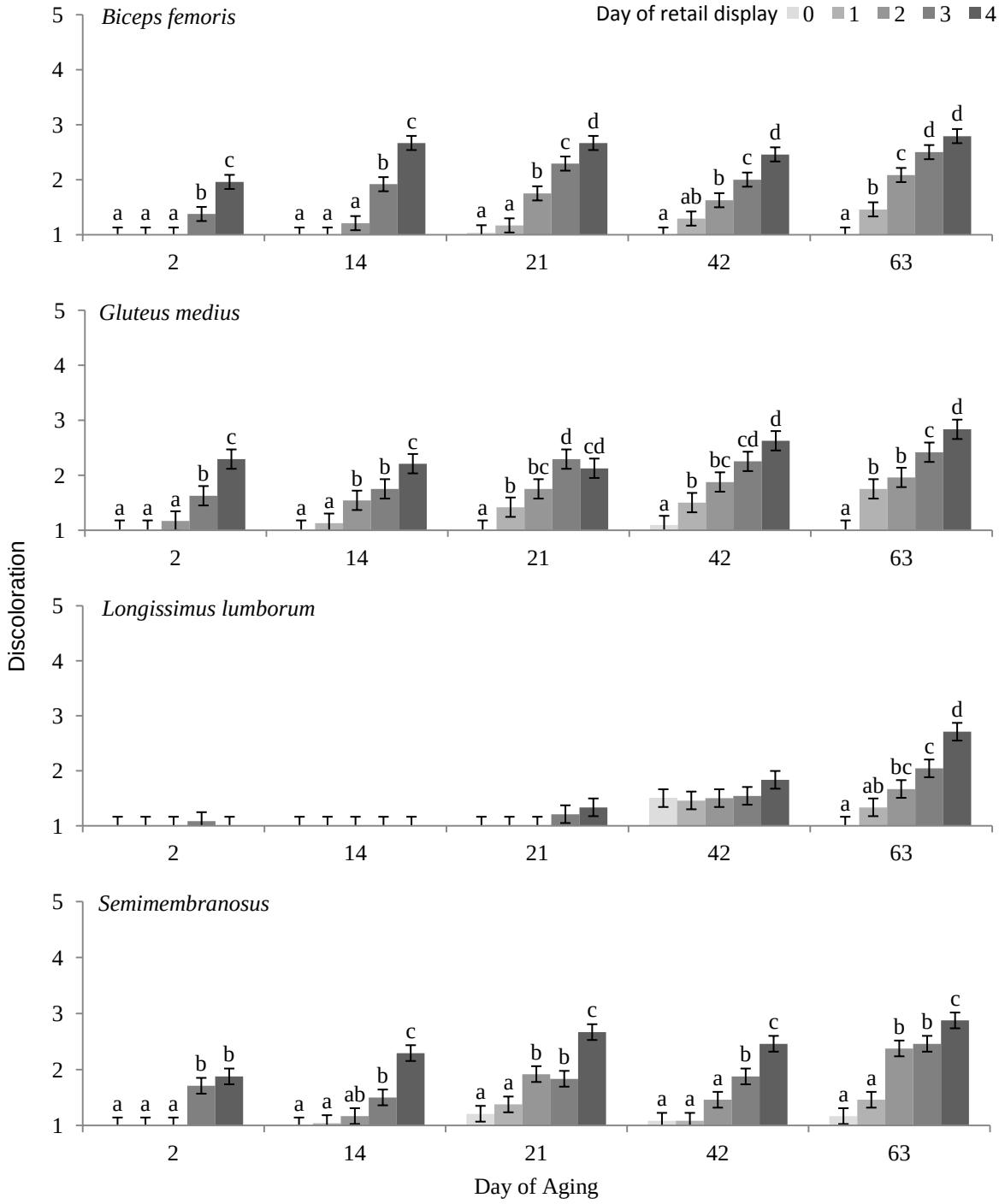

Figure 2.6: Effect of aging and retail display on discoloration of beef *biceps femoris*, *gluteus medius*, *longissimus dorsi*, and *semimembranosus* steaks. Muscles were aged for 2, 14, 21, 42, and 63 days post-fabrication. At the end of each aging period, 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 4 days. Two evaluators scored the steaks daily during retail display for discoloration using a 5 point scale (1 = none, 2 = slight, 3 = small, 4 = moderate, 5 = extreme). Bars represent the LSM ± SEM. Means within a muscle and aging period with different superscripts differ ($P < 0.05$).

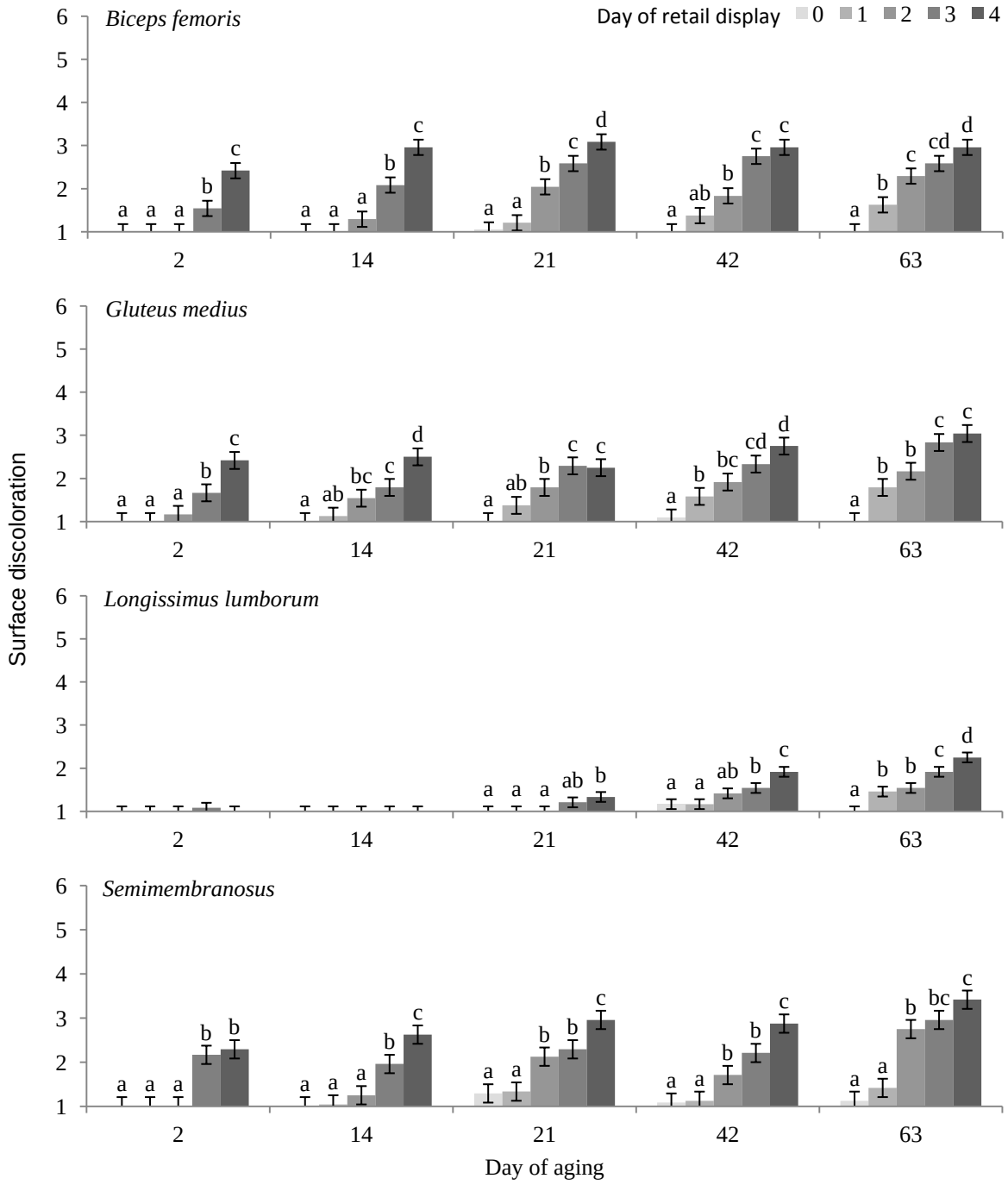

Figure 2.7: Effect of aging and retail display on surface discoloration of beef *biceps femoris*, *gluteus medius*, *longissimus lumborum*, and *semimembranosus* steaks. Muscles were aged for 2, 14, 21, 42, and 63 days post-fabrication. At the end of each aging period, 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 4 days. Two evaluators scored the steaks daily during retail display for percent surface discoloration using a 6 point scale (1 = none (0%), 2 = slight (1-20%), 3 = small (21-40%), 4 = modest (41-60%), 5 = moderate (61-80%), 6 = extensive (81-100%)). Bars represent the LSM ± SEM. Means within a muscle and aging period with different superscripts differ ($P < 0.05$).

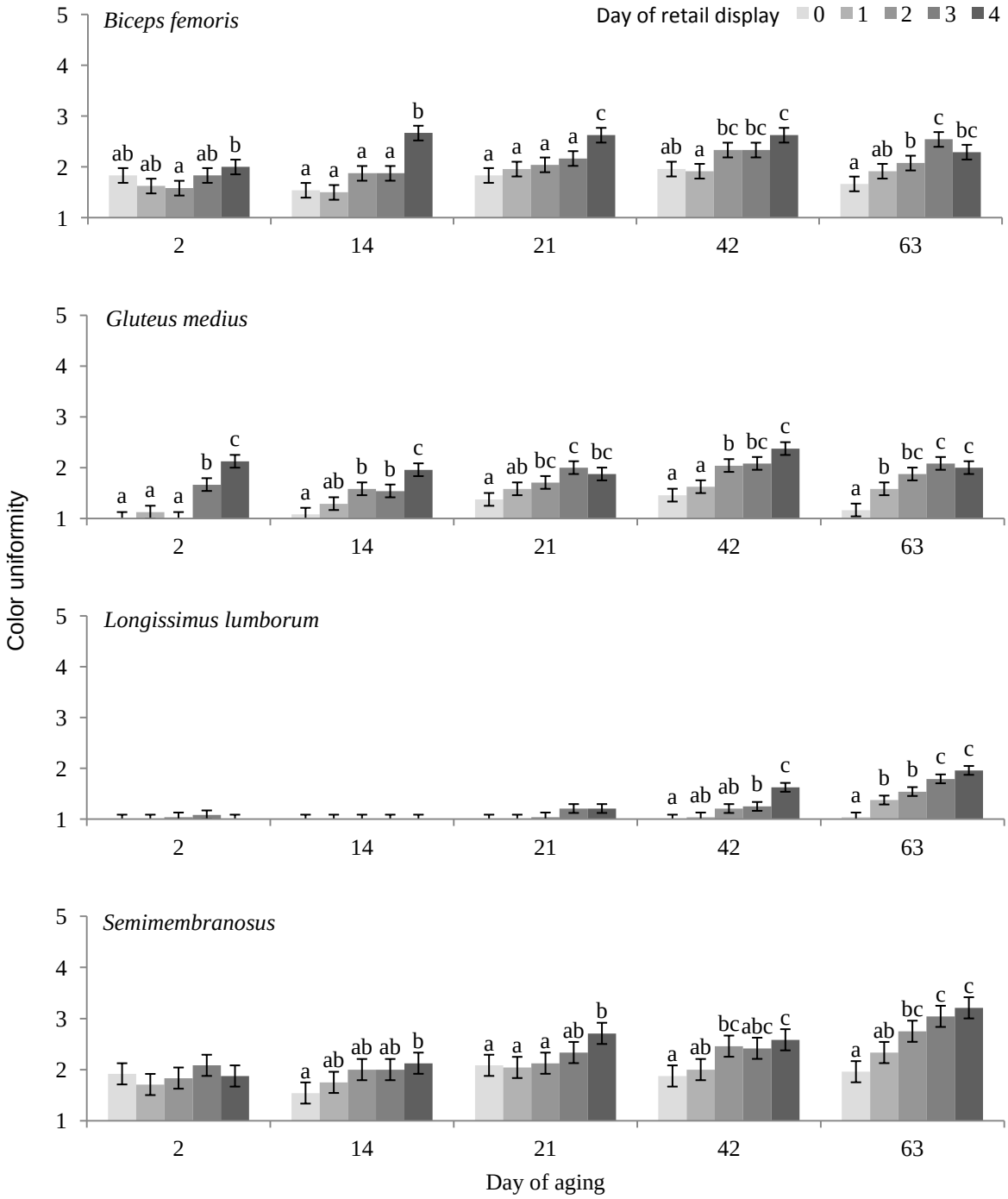

Figure 2.8: Effect of aging and retail display on color uniformity of beef *biceps femoris*, *gluteus medius*, *longissimus lumborum*, and *semimembranosus* steaks. Muscles were aged for 2, 14, 21, 42, and 63 days post-fabrication. At the end of each aging period, 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 4 days. Two evaluators scored the steaks daily during retail display for color uniformity using a 5 point scale (1 = uniform, 2 = slight two-toning, 3 = small amount of two-toning, 4 = moderate two-toning, 5 = extreme two-toning). Bars represent the LSM ± SEM. Means within a muscle and aging period with different superscripts differ ($P < 0.05$).

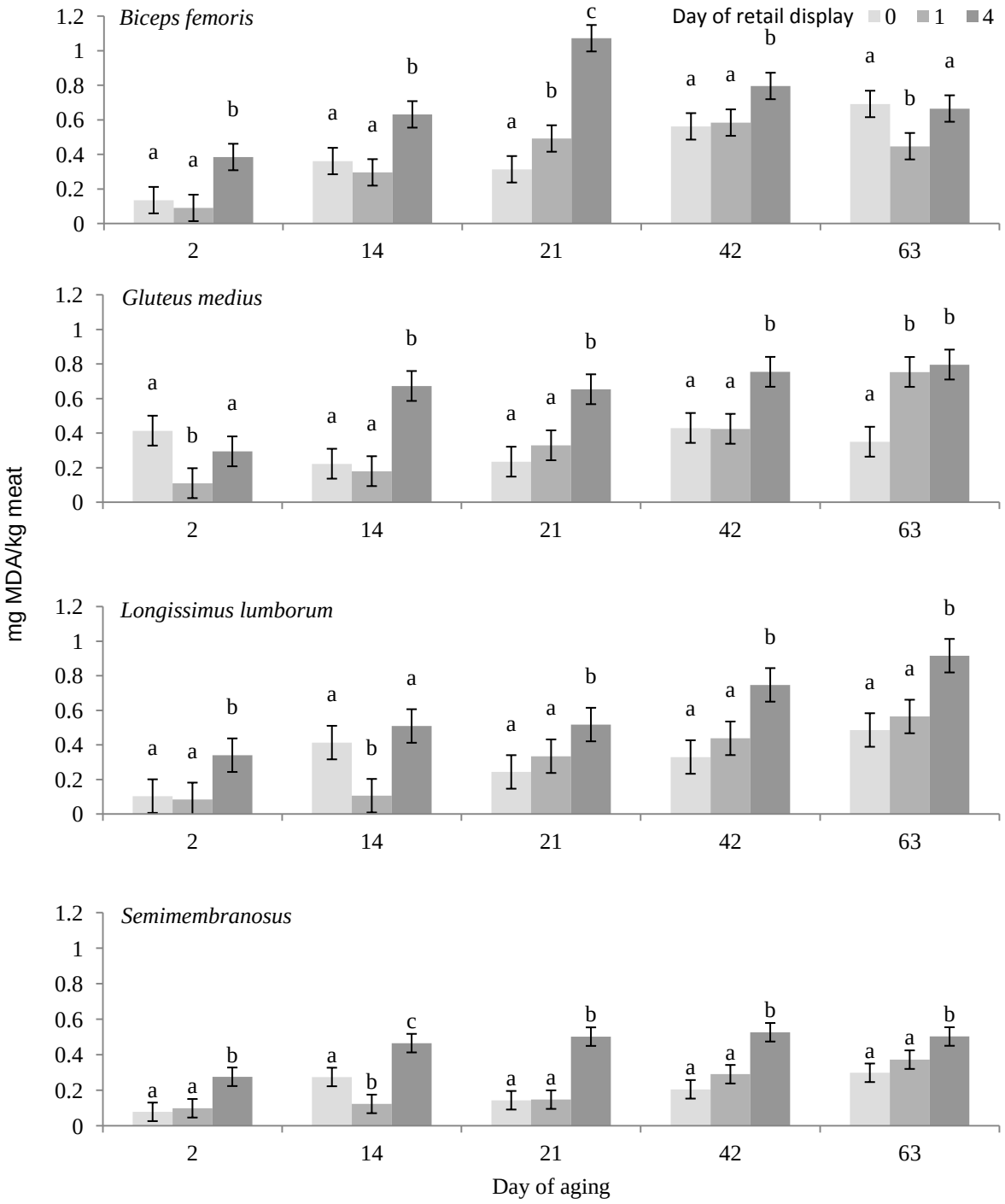

Figure 2.9: Effect of aging and retail display on lipid oxidation of beef *biceps femoris*, *gluteus medius*, *longissimus lumborum*, and *semimembranosus* steaks. Muscles were aged for 2, 14, 21, 42, and 63 days post-fabrication. At the end of each aging period, 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 4 days. Thiobarbituric acid reactive substances were quantified (mg MDA/kg of meat) in samples obtained from steaks on days 0, 1, and 4 of retail display. Bars represent the LSM ± SEM. Means within a muscle and aging period with different superscripts differ ($P < 0.05$).

Appendix A

Exempt Certification for IRB Project Number 12-238

June 22, 2012

University of Idaho
Office of Research Assurances (ORA)
Institutional Review Board (IRB)
PO Box 443010
Moscow ID 83844-3010

Phone: 208-885-6162
Fax: 208-885-5752
irb@uidaho.edu

To: Doumit, Matthew

From: IRB, University of Idaho Institutional Review Board

Subject: Exempt Certification for IRB project number 12-238

Determination: June 20, 2012
Certified as Exempt under category 6 at 45 CFR 46.101(b)(6)
IRB project number 12-238: Consequences of Extended Aging on Retail Shelf-Life and Consumer Acceptability of Four Beef Muscles

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This study may be conducted according to the protocol described in the Application without further review by the IRB. As specific instruments are developed, each should be forwarded to the ORA, in order to allow the IRB to maintain current records. Every effort should be made to ensure that the project is conducted in a manner consistent with the three fundamental principles identified in the Belmont Report: respect for persons; beneficence; and justice.

It is important to note that certification of exemption is NOT approval by the IRB. Do not include the statement that the UI IRB has reviewed and approved the study for human subject participation. Remove all statements of IRB Approval and IRB contact information from study materials that will be disseminated to participants. Instead please indicate, "The University of Idaho Institutional Review Board has Certified this project as Exempt."

Certification of exemption is not to be construed as authorization to recruit participants or conduct research in schools or other institutions, including on Native Reserved lands or within Native Institutions, which have their own policies that require approvals before Human Subjects Research Projects can begin. This authorization must be obtained from the appropriate Tribal Government (or equivalent) and/or Institutional Administration. This may include independent review by a tribal or institutional IRB or equivalent. It is the investigator's responsibility to obtain all such necessary approvals and provide copies of these approvals to ORA, in order to allow the IRB to maintain current records.

This certification is valid only for the study protocol as it was submitted to the ORA. Studies certified as Exempt are not subject to continuing review (this Certification does not expire). If any changes are made to the study protocol, you must submit the changes to the ORA for determination that the study remains Exempt before implementing the changes. The IRB Modification Request Form is available online at: http://www.uidaho.edu/ora/committees/irb/irbforms

Appendix B

TBARS for oxidative rancidity - rapid, wet method

Adapted from Appendix O: TBARS for Oxidative Rancidity (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 chromagen 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 acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. Food Technology 12(1):9-12.

Appendix C

Collagen Analysis

Collagen Analysis

SOLUTION RECEPIES

Ringers Solution (1L)
-7.0g NaCl
-0.026g $CaCl_2$
-0.35g KCl

Sulfuric Acid 3.5M (2L)
-1675mL H_20
-375mL sulfuric acid
    -Add sulfuric acid *slowly* to H_2O on ice while stirring.
**This must be re-calculated based on purity of sulfuric acid.

375/2000 = 0.1875 (correction factor for this calculation)

For example, 98% pure sulfuric acid:
    0.1875/0.98 = 0.1913

    2000*0.1913 = 382.6mL sulfuric acid + 1617.4ml H_2O

***This solution becomes *extremely hot* when adding acid to H_2O. Prepare *on ice* in the *fume hood*!!!!

Buffer Solution (1L) pH 6.0
-500mL H_2O
-30g citric acid monohydrate ($C_6H_80_7 \cdot H_2O$)
-15g NaOH
-90g sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$)
    -Stir all ingredients until dissolved, then add:
-290mL 1-propanol
    -Check pH – you will need to add more citric acid to bring pH down to 6.0.
    -Bring solution up to volume in graduated cylinder or volumetric flask.

**Solution is stable for 2 months @ 4°C in a dark bottle.

Oxidant solution (100mL)
-100mL Buffer Solution
-1.41g Chloramine-T (Sigma catalog #C9887)

**Solution is stable for 1 week @ 4°C in a dark bottle.

Color Reagent (100mL)
-35mL perchloric acid (chilled) (Sigma catalog #244252)
-10g 4-dimethylaminobenzaladehyde (Sigma catalog #156477)
-Stir until 4-dimethylaminobenzaldehyde is dissolved, then slowly add:
-65mL 2-propanol

***This solution becomes hot when adding 2-propanol to perchloric acid. Prepare on ice in the fume hood!!!!

****Solution is stable for no more than 24 hours.****

Hydroxyproline Standard Solution (100mL)
-60mg hydroxyproline (Sigma catalog #H5534)
-100mL H2O

**Solution is stable for 2 months @ 4°C.

Intermediate Solution (IS) (XmL)
-Intermediate solution is standard solution diluted.
-To make 10ml of IS, add 0.1mL of Standard to 9.9mL H2O.

**Make solution fresh on day of use.

Working Standards (20mL)

Standard	0.3μg/mL	0.6μg/mL	1.2μg/mL	2.4μg/mL	4.8μg/mL	6.0μg/mL
Intermediate Solution	1.0mL	2.0mL	4.0mL	8.0mL	16.0mL	20.0mL
H20	19.0mL	18.0mL	16.0mL	12.0mL	4.0mL	0mL

**Make solution fresh on day of use.

PROTOCOL

Sample Preparation

1. Use frozen samples left over from Warner-Bratzler shear force determination.
2. Temper frozen samples at 4°C overnight.
3. Grind meat sample in 1.5 cup food processor for 1 minute, stir sample, and grind for another 45 seconds.
4. Weigh out 4.0g in duplicate.
5. Add 22mL Ringers solution.
6. Homogenize sample for 20 seconds, rest for 20 seconds, then homogenize for 20 seconds at 18,000rpm.
   a. Collect homogenizer rinse, centrifuge at 5200xg, and place pellet in Erlenmeyer flask containing insoluble portion.
7. Heat in water bath at 50°C for 15 minutes stirring every 5 minutes
8. Centrifuge tubes at 5200xg for 10 minutes.

9. Decant supernatant into 125mL Erlenmeyer flasks through filter paper.
10. Add 10mL ¼ strength Ringers solution to pellet and stir.
11. Centrifuge again with same conditions, decant into same flasks.
12. Transfer pellet to another 125mL Erlenmeyer flask along with the filter paper.
13. Add 30mL 3.5M sulfuric acid to insoluble portion.
14. Add 8mL concentrated sulfuric acid to soluble portion.
    Add slowly on ice in the fume hood
15. Place in oven at ~105°C for at least 16 hours with watch glass on top.
16. Carefully remove flasks from oven.
17. Transfer hot hydrolysate to 100mL graduated cylinder.
18. Rinse flask 3 times with water and bring volume up to 100mL.
19. Mix sample by covering with parafilm and inverting 4 times.
20. Filter approximately 20mL into 50mL conical tube.

Assay

1. Pipet each 2mL sample in duplicate into test tubes. (0.12mL hydrolysate + 1.88mL H_2O, you may need to experiment with a dilution that keeps sample readings within the range of the standard curve.)
2. Pipet 2mL of each standard in duplicate into test tubes.
3. Add 1mL of Oxidant solution to each tube. Mix and let stand at room temperature for 20 minutes.
4. Add 1mL of Color reagent to each tube. Mix and heat covered with foil in 60°C water bath for 15 minutes.
5. Cool tubes with H_2O for at least 3 minutes.
6. Pipet 200μL of each sample (and standard) into 96 well plate.
7. Read samples at 558nm.

Appendix D

Sensory Panel Consent Form

EVALUATION OF BEEF QUALITY

1. The University of Idaho Human Assurance Committee has reviewed and found this study to be exempt.

2. The objective of this study was to evaluate the effects of extended aging of beef. 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. This taste panel is part of research funded by the Idaho Beef Council.

3. You will be asked to evaluate 5 samples (approximately 1" x ½" x ½") per session for tenderness (1 = extremely tough to 9 = extremely tender), juiciness (1 = dry to 9 = juicy), and flavor (1 = bland to 9 = intense) using a 9 point scale. 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. 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 aging 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. Doumit.

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. Matthew E. Doumit
   University of Idaho
   Department of Animal and Veterinary Science
   Moscow, ID 83844
   208-885-6007

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.

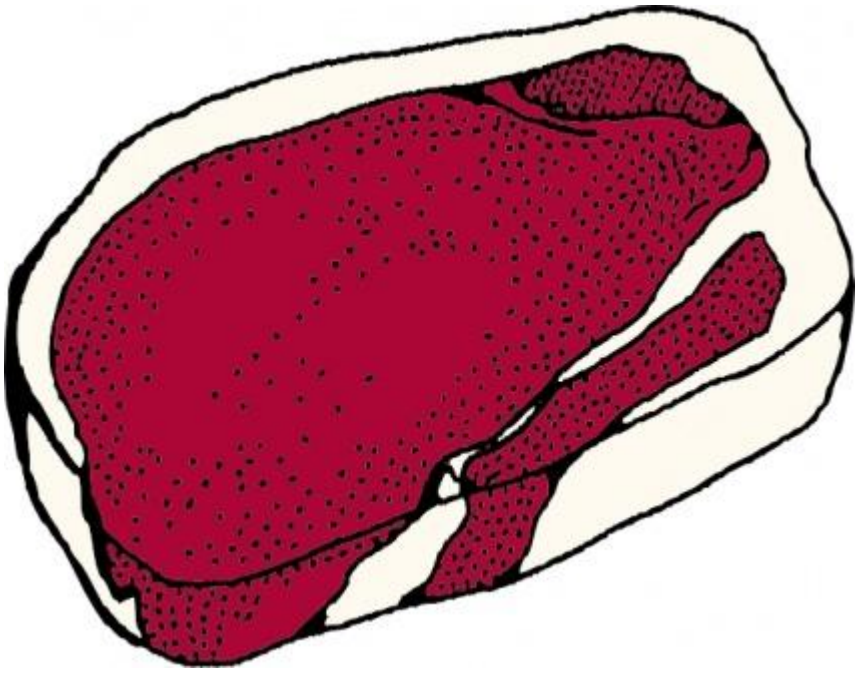
Participant Name: _____ Date: _____

Signature: _____ Date of Birth: _____

Appendix E

Sensory Panel Demographics Questionnaire

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

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

Appendix F

Sensory Panel Questionnaire

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. **Overall Comments on Product:**