COLOR AND FLAVOR STABILITY OF BEEF *GLUTEUS MEDIUS* AS INFLUENCED BY POSTMORTEM AGING TIME AND BLADE TENDERIZATION.

by

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B.S., Kansas State University, 2012

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2014

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Abstract

A total of 45 top sirloin butts (IMPS 184) were procured from three commercial beef processing facilities to determine the effects of post-mortem aging time and blade tenderization on the quality of beef *gluteus medius* (GM) steaks. Top sirloin butts were randomly assigned to five post-mortem aging periods (5, 19, 33, 47, and 61 days). One-half of each beef GM was randomly assigned to either a blade tenderized treatment or a non-blade tenderized treatment that was not blade tenderized. Steaks were then evaluated for thiobarbituric acid reactive substances (TBARS), metmyoglobin reducing activity (MRA), oxygen consumption rate (OCR), visual color panel, instrumental color, Warner-Bratzler shear force (WBSF), Lactic Acid Bacteria (LAB) enumeration, pH, and sensory properties. Aging × blade tenderization interactions ($P < 0.05$) were found for display color panel, discoloration panel, WBSF, overall tenderness, myofibrillar tenderness, bloody/serumy, metallic, overall sweet, and bitter ($P < 0.05$). As steaks were aged longer and blade tenderized they became more discolored during display and more tender. In addition, there were aging × display time interactions ($P < 0.05$) observed for L*, a*, b*, display color panel, and discoloration panel. As steaks were aged longer, they had increased L*, a*, b*, and hue angle values and display color panel scores when initially put into a retail case, but L*, a* and b* decreased and discoloration scores increased as display time increased. Furthermore, there were blade tenderization × display time interactions ($P < 0.05$) found for display and discoloration panels. Blade tenderized steaks discolored faster in retail display than non-blade tenderized steaks. With increased aging time, there was an increase ($P < 0.05$) in TBARS, OCR, initial color panel, LAB enumeration, and warmed-over flavor, as well as a decrease in MRA. Also, as aging increased there was a decrease ($P < 0.05$) in MRA, initial color

panel scores, and WBSF values. Blade tenderization significantly increased ($P < 0.05$) initial color panel scores, rancid flavor, and spoiled flavor. Increasing the aging time of the GM, produced steaks with decreased color stability, altered the flavor profile, and increased tenderness. Blade tenderization significantly increased tenderness, increased discoloration in a retail case, and produced more undesirable flavors.

Table of Contents

List of Figures

List of Tables

Chapter 1 - Review of Literature

Factors affecting fresh meat color

Fresh meat color is the most important factor in consumers purchasing meat at the retail level (Kropf, 1980). Consumers rely on fresh meat color as an indicator of product freshness (Mancini & Hunt, 2005). Consumers expect fresh beef to be bright-red in color (Kropf, 1980). When meat becomes discolored it causes significant monetary losses to the meat industry, as almost 15% of fresh beef sold in the retail setting becomes discounted in price due to discoloration (Smith et al., 2000).

There are many extrinsic and intrinsic factors, as well as their interaction that affect meat color (Kropf, 1993). It is widely accepted that as meat is placed into a simulated retail display case in aerobic packaging that it will become discolored (Apple et al., 2014). Visually meat becomes tannish-brown or otherwise known as the oxidized state of myoglobin (metmyoglobin).

Myoglobin State

Myoglobin and hemoglobin pigment reactions are important in determining fresh meat color (Fox, 1966). Myoglobin contributes the majority to fresh meat color (Faustman & Cassens, 1990). Myoglobin is a water soluble heme protein that has 8 α-helices that are linked by short non-helical sections (Mancini & Hunt, 2005). Myoglobin is also a carrier of oxygen to mitochondria (Tang et al., 2005; Wittenberg & Wittenberg, 1975). The deoxymyoglobin pigment occurs when the 6^{th} ligand of myoglobin is open and the heme iron in the reduced chemical state (ferrous) (Faustman & Cassesn, 1990). The deoxymyoglobin pigment is associated with the purplish-red color that is found in vacuum packaged fresh meat or a fresh cut surface of meat prior to being exposed to oxygen. When oxygen attaches to the 6^{th} ligand of the

myoglobin molecule and the valence does not change it becomes the oxymyoglobin pigment and is associated with the bright cherry-red color of fresh meat (Faustman & Cassens, 1990). The heme iron of both deoxymyoglobin and oxymyoglobin pigments can be oxidized to the ferric state to form the metmyoglobin pigment or the tanninsh-brown color associated with discolored meat (Faustman & Cassens, 1990). The metmyoglobin pigment can also form at low oxygen partial pressures (Mancini & Hunt, 2005). An example of the metmyoglobin pigment is the layer between the superficial oxymyoglobin layer and the interior deoxymyoglobin layer or in low oxygen packaging (Mancini & Hunt, 2005).

The pigment of oxymyoglobin can also be converted back to the deoxymyoglobin pigment. The conversion of the oxymyoglobin pigment into the deoxymyoglobin pigment requires the heme iron of myoglobin to first be oxidized by the loss of an electron and reduction in oxygen partial pressure and then reduced back into the deoxymyoglobin pigment through endogenous reductants (Mancini & Hunt, 2005). Therefore, the oxymyoglobin pigment cannot be directly converted into the deoxymyoglobin pigment. However, postmortem muscle must have NADH to fuel endogenous reductants to reduce metmyoglobin to deoxymyoglobin (Mancini & Hunt, 2005). Unfortunately, NADH decreases as postmortem time increases (Mancini & Hunt, 2005). In summary, meat color is greatly affected by the state of the heme iron and the occupancy of the 6^{th} ligand of the myoglobin molecule.

Extrinsic factors affect meat color

Postmortem Aging

It has been widely studied that postmortem aging affects color stability. The method of packaging can also play a role in color stability of fresh meat. Wet aging is the most common method of aging and refers to the aging of meat in vacuum packaging (Laster et al., 2008). In

general, increasing the postmortem aging time decreases the color stability of fresh meat. There have been several studies that have looked at how aging meat in vacuum packaging affects instrumental color values. A study done by Leisner et al. (1995) found that increasing beef postmortem aging time in vacuum packaging decreased a* values (redness) when placed into a simulated retail display. Similar results in lamb loins were shown as increasing aging time in vacuum packaging from 14 days to 63 days increased discoloration and decreased a* values (Kim et al., 2013). Lee et al. (2008) found that beef *gluteus medius* steaks from top sirloin butts aged in vacuum packaging for 7 and 14 days had increased chroma values, a* values, b* values, and oxymyoglobin percentages compared to steaks from top sirloin butts aged in vacuum packaging 28 and 35 days.

Other studies have shown that metmyoglobin increases both instrumentally and in visual color panels due to increased aging time. Lindahl (2011) found that steaks from beef *longisimus dorsi* and *semimembranosus* aged 5 days in vacuum packaging had less metmyoglobin percentage than steaks from *longisimus dorsi* and *semimebranosus* aged 25 days in vacuum packaging. Increasing aging time up to 35 days increased surface discoloration and decreased visual color scores in beef *biceps femoris* steaks (Smith et al., 1980).

Different packaging methods for aging have also been explored. Isdell et al. (1999) found that beef *gluteus medius* steaks displayed fresh and after 2, 4, and 6 weeks in modified atmosphere (gas flushed) had a color shelf life of 81, 33, 31, and 22 hours respectively. After 94 hours of lighted retail display, steaks were assessed for acceptability of visual color. Steaks that were held for 4 and 6 weeks in modified atmosphere packaging had lower visual acceptance scores than fresh steaks or steaks held for 2 weeks in modified atmosphere packaging. Greer, Dilts, and Jeremiah (1993) showed that for every 6 week increase in storage time of pork

longissimus dorsi stored in modified atmosphere packaging, there was a linear decrease of 1 day of color acceptance once chops were placed in a simulated retail display case. Overall, increasing postmortem aging regardless of packaging method, decreases color stability once meat is placed into a retail display case in aerobic packaging.

Blade Tenderization

There has been limited research that has looked at the effects of blade tenderization on meat color. Blade tenderization disrupts the muscle fibers, which may cause physical damage to mitochondria, ultimately affecting metmyoglobin reducing ability. Also, blade tenderization exposes more oxygen to the interior of the muscle and could cause an increase in metmyoglobin reducing enzymes to keep myoglobin in the reduced state. Ledward et al. (1977) found that ground beef *longissimus dorsi* had higher metmyoglobin formation than intact *longissimus dorsi*. Ledward et al. (1977) attributed the increased metmyoglobin formation in the ground product to the destruction of the metmyoglobin reducing system. However, Benito-Delgado et al. (1994) found that blade tenderization did not affect the color of beef *longisimus dorsi* and *infraspinatus* muscles.

Chilling Rate

Chilling rate is the rate at which the muscle chills following harvest. Chilling rate can be greatly affected by the location of the muscle within the carcass. Portions of the muscle that are located on the interior of the carcass would be chilled slower than portions of the muscle located on the exterior of the carcass. Tarrant (1977) reported that glycolysis increases in beef *semimembranosus* as the muscle gets closer to the femur bone or towards the interior. Muscles that are chilled faster during rigor have slower glycolysis, because the glycolytic enzymes become inactivated faster than if those same muscles were chilled slower (Apple et al., 2014).

Therefore, more rapid glycolysis results in an increased production of lactic acid and decrease in pH. Also, the amount of subcutaneous fat could also play a role as the exterior fat could act as insulation and decrease the chilling rate of the muscle. Apple et al. (2014) reported that the beef *gluteus medius* from yield grade 4 and 5 carcasses had lower pH and were more yellow than those from yield grade 1 and 3 carcasses. Higher yield grade carcasses have more external fat over the sirloin and would provide a layer of insulation and affect the chilling rate of the beef *gluteus medius*.

Chilling rate can influence color stability as it affects the pH decline of postmortem muscle. Hot-boning is a process of removing muscles from the carcass before the completion of rigor mortis. Hot-boning increases color stability of the interior portion of the muscle by decreasing the pH decline during chilling (Tarrant, 1977; Nichols & Cross, 1980; Sammel et al., 2002; Seyfert et al., 2004). An increase in pH decline denatures proteins and opens up muscle structure causing light scattering effects that are negative to meat color (MacDougall, 1982; Apple et al., 2014). Follet et al. (1974) found that the superficial portion or the portion closest to the exterior of the carcass of the beef *semimembranosus* chilled to 2°C 12 hours faster than the deep *semimembranosus*, or the portion closest to the bone. The interior portion of the beef *semimembranosus* has been reported to have a lighter and more washed out color than the exterior portion of the *semimembranosus* (Sawyer et al., 2007; Lee et al., 2008). Sammel et al. (2002) also reported that the interior portion of the beef *semimembranosus* was lighter and redder initially, but had lower color stability and became two-toned over its retail shelf life compared to the exterior portion. The decrease in color stability may be due to the decrease in metmyoglobin reducing ability of the interior portion of the beef *semimembranosus* compared to the exterior portion (Sammel et al., 2002). A study by Zhu and Brewer (1998) supports the theory of a

decrease in metmyoglobin reducing ability as a function of lower pH, as they reported that lower pH pork had a lower metmyoglobin reducing ability than normal pH pork at the same oxygen consumption rates. The decrease in metmyoglobin reducing ability due to lowering of pH is most likely due to the denaturing of the reducing enzymes (Zhu & Brewer, 1998).

Microbial Growth

Microbial growth can affect the discoloration of fresh beef (Stringer et al., 1969). Leisner et al. (1995) showed that beef inoculated with lactic acid bacteria, decreased a* values in aerobic storage. Leisner et al. (1995) also reported that beef inoculated with lactic acid bacteria and aged in vacuum packaging for 6 weeks had significantly lower a* values during retail display, compared to beef inoculated with lactic acid bacteria and aged in vacuum packaging for 0 to 1 week. Finally, Lesiner et al. (1995) showed that inoculating beef with *Lactobacillus sake* and *Carnobacterium maltaromicus* (lactic acid bacteria) and then storing in vacuum packaging for 6 weeks significantly deceased the time of aerobic color shelf life compared to sterile beef. Smith et al. (1980) showed that inoculating beef with *Lactobacillus* increased discoloration and decreased visual color scores. It is evident that lactic acid bacteria have a negative effect on color stability of beef.

Discoloration due to microbial contamination has also been seen in poultry products. Dhananjayan et al. (2006) found that ground turkey increased in total plate count and lactic acid bacteria count as retail display time increased. Dhananjayan et al. (2006) also found that hue angle increased and a* values decreased as retail display time increased.

Lipid Oxidation

Lipid oxidation has been positively correlated with myoglobin oxidation. Just like myoglobin has endogenous metmyoglobin reducing enzymes, there are endogenous antioxidants

enzymes that protect lipids from becoming oxidized; which include superoxide dismutase, catalase, and glutathione peroxidase (Decker & Mei, 1996). However, Renerre, Dumont, and Gatellier (1996), showed that the lower color stable muscles such as the *psoas major* and *diaphragm* have higher activities of superoxide dismutase, catalase, and glutathione peroxidase than more color stable muscles such as the *longissimus lumborum* and the *tensor fasciae latae.* Lower color stable muscles have a more oxidative metabolism than more color stable muscles, so in the living animal those more oxidative muscles would need more protection from oxidation. Lower color stable muscles have a greater concentration of lipofuscin, a product of lipid oxidation, compared to more color stable muscles. Gatellier (1996) concluded that even though the lower color stable muscles had a higher antioxidant enzyme activity, they could not overcome the higher amount of lipofuscin. It also cannot be forgotten that enzyme activity is limited in postmortem muscle. Enzyme activity decreases as time postmortem elapses (Spanier et al., 1990). Metmyoglobin reducing enzymes that reduce the metmyoglobin pigment must be active to prevent against oxidation of myoglobin (Kanner, 1994).

The products of lipid oxidation may catalyze the oxidation of myoglobin (Gray et al., 1996). Iron, which is readily found in meat, is capable of catalyzing free radical production that can oxidize myoglobin and lipids (Gray et al., 1996). During retail display, lipid oxidation and metmyoglobin formation increase (McKenna et al., 2005; Ponnampalam et al., 2001). Campo et al. also saw an increase in thiobarbituric acid reactive substances (TBARS) values as retail display days increased, as well as a degradation of meat color. Lipid oxidation aldehyde products (α-aldehyde and β-aldehyde) decrease color stability by covalently modifying myoglobin by altering histidine residues (Faustman et al., 1999; Lynch & Faustman, 2000; Alderton et al., 2003).

There have been many studies that have looked at feeding vitamin supplements to livestock that reduce lipid oxidation and increase color stability (Morrissey et al., 1998). Chu et al. (2004) found that feeding hanwoo steers vitamin C and E decreased metmyoglobin formation and lipid oxidation. Dietary vitamin E supplementation stabilized redness, decreased yellowness, and extended color display life in beef *gluteus medius, longissimus lumborum,*and *semimembranosus* (Liu et al., 1996). Monahan et al. (1994) reported similar results, as pork from pigs fed vitamin E had lower TBARS values and higher Hunter "a" values. Monahan et al. (1996) also found significant ($P < 0.01$) negative correlations between TBARS values and hunter "a" values. The decrease in lipid and myoglobin oxidation is due to the increase of the accumulation of α-tocopherol in muscle, which delays oxidation (Faustman et al., 1989).

Akamittath, Brekke, and Schanus (2006) presented that lipid oxidation and discoloration happen at the same time in pork and turkey. On the other hand, Akamittath, Brekke, and Schanus (2006) showed that beef discolors faster than lipid oxidation occurs. Lipid oxidation and meat color are related and the oxidation of myoglobin may be a catalyst for lipid oxidation or vice versa.

Intrinsic factors affecting meat color

Metmyoglobin Reducing Ability

During aging, there are several biochemical changes that happen that affect fresh meat color stability. Basic knowledge of myoglobin chemistry is needed to understand how discoloration of meat occurs. When the oxygenated form of myoglobin (oxymyoglobin) is oxidized it becomes the metmyoglobin pigment or discolored. Metmyoglobin pigment can be reduced back into the deoxymyoglobin pigment by endogenous reductants of the mitochondria otherwise known as metmyoglobin reducing enzymes. Echevarne, Renerre, and Labas (1989)

reported that the least color stable bovine muscles had the highest metmyoglobin reducing activity (MRA) compared to more color stable bovine muscles. The results of Echevarne, Rennerre, and Labas (1999) were also seen by Lanari and Cassens (1991), as the beef *gluteus medius* had a higher MRA than the beef *longissimus dorsi*, but they showed that MRA was not affected by postmortem aging time. This is because muscles with a higher oxidative metabolism have more mitochondria (Lanari & Cassens, 1991). The activity of metmyoglobin reducing enzymes relies on the amount of coenzyme nicotinimide dinucleotide (NADH) available to the enzymes (Echevarne, Renerre, & Labas, 1989). Unfortunately, it is well known that NADH concentration decreases as postmortem time increases (Mancini & Hunt, 2005). Madhavi and Carpenter (1993) reported a positive relationship between NADH concentration and metmyoglobin reducing activity, as they both decrease as postmortem aging time increases. Also, Echevarne, Renerre, and Labas (1989) reported that the catabolism of NADH was more rapid in color instable muscles, even though they had higher metmyoglobin reducing abilities.

It has been shown that NADH can be manufactured in postmortem muscle from the endogenous enzyme, lactate dehydrogenase (Mancini, Hunt, Kim, & Lawrence, 2004). The resurgence of NADH improved color stability through metmyoglobin reduction in beef (Mancini, Hunt, Kim, & Lawrence). Ultimately, the amount of NADH available is vitally important to metmyoglobin reducing ability in meat.

Oxygen Consumption Rate

Myoglobin is a transporter of oxygen to mitochondria in muscle tissue (Tang et al., 2005; Wittenberg & Wittenberg, 1975). Oxygen is consumed by mitochondria in postmortem muscle. The determining factor of oxygen consumption rate in postmortem muscle is attributed to the deterioration of the mitochondria via a decrease in pH (Bendall & Taylor, 1972; Devore &

Solberg, 1974). Several studies have shown that high oxygen consumption rates increase the rate of discoloration of beef (Tang et al., 2005; O'Keefe & Hood, 1982). O'Keefe and Hood (1982) showed that the *psoas major* reduced from oxymyoglobin into deoxymyoglobin two to three times faster than that of *longissimus dorsi*. To add to this, the *psoas major* is known to be a less color stable muscle than the *longissimus dorsi*, due to the psoas major having a higher oxygen consumption rate (Echevarne et al., 1989; O'Keefe & Hood, 1982). Renerre and Labas (1987) also reported that less color stable muscles such as the *diaphragma medialis* released more oxygen than that of more color stable muscles such as the *longissimus dorsi*. McKenna et al. (2005) proved that oxygen consumption rate decreases over retail shelf life, which in turn increases metmyoglobin percentage in beef. Finally, Lanari and Cassens (1991) reported that oxygen consumption rate was higher for the *gluteus medius* from crossbred and Holstein steers compared to the *longissimus dorsi* from those same animals. Lanari and Cassens (1991) also showed that increasing postmortem aging time, decreased oxygen consumption rate in beef *gluteus medius* and *longissimus dorsi*. O'Keefe and Hood (1982) reported that as aging time increased, there was a decrease in the oxygen consumption rate in beef *longissimus dorsi* and *psoas major* steaks. Overall, both oxygen consumption rate and the ability of the enzymes to reduce metmyoglobin affect discoloration in fresh meat.

pH

The ultimate pH and the rate of pH decline during rigor can affect the color stability of meat (Faustman & Cassens, 1990). There is a known relationship with pH and meat color as seen in the phenomenon of DFD (Dark, firm, and dry) and PSE (Pale, soft, and exudative) as ultimate meat pH decreases, the meat color becomes lighter in color. High ultimate pH allows for increased enzyme respiratory activity, whereas low ultimate pH decreases enzyme respiratory

activity resulting in more oxidation of myoglobin (Renerre, 1990). Fletcher et al. (2000) reported that there was a significant correlation ($R^2 = 0.59$) with L* values and pH of raw broiler breasts. Also, Qiao et al. (2001) found that there was a negative correlation ($R^2 = -0.9610$) with L* and pH of raw chicken breast fillets and ground breast. Seideman et al. (1984) showed that meat with lower pH had a lighter color due to a more open muscle structure that allowed more light scattering, whereas meat with higher pH had a darker color due to a more closed muscle structure allowing for less light scattering. Ockerman and Cahill (1977) also reported that pH altered by microorganisms over postmortem aging, caused undesirable color development.

pH may play a role in the biochemical processes of discoloration of meat. Ledward (1985) reported that beef *longissimus dorsi* and *semimembranosus* exposed to low pH had increased metmyoglobin formation. Echevarne, Renerre, and Labas (1990) showed that metmyoglobin reducing ability was highest at a pH of 7.4 and decreased for any pH that was higher or lower than 7.4. The results from Echevarne, Renerre, and Labas (1990) would imply that the metmyoglobin reducing enzymes are most active around a neutral pH and less active at more acidic or basic pH.

Muscle Fiber Type

Muscle fiber type varies throughout the carcass depending on the function of the particular muscle (locomotion vs. support). Slow twitch - oxidative muscle fiber types (Type I-red) have more myoglobin, mitochondria, ATP-ase activity, nuclei density, require more oxygen (aerobic metabolism), and have increased color stability than fast twitch – glycolytic muscle fiber types (Type II B – white). The *gluteus medius* is made up of a 52.6% type II B, 25.8% type I, and 21.7% type II A muscle fiber types (Hunt & Hedrick, 1977). Oksbjerg et al. (1995) presented that pork *gluteus medius* had more type II B muscle fiber types than pork *longissimus*

dorsi. Lanari and Cassens (1991) showed that beef *longissimus dorsi* was more color stable than the beef *gluteus medius.* The beef *gluteus medius* is an intermediate color stable muscle, whereas the *longissimus dorsi* is a high color stable muscle (McKenna, Mies, Baird, Pfeiffer, Ellebracht, & Savell, 2005). Lanari and Cassens (2005) have shown that the beef *gluteus medius* has a higher metmyoglobin reducing activity and oxygen consumption rate compared to the beef *longissimus dorsi*. Finally, the *gluteus medius* has been shown to have more metmyoglobin formation compared to the *longissimus dorsi*, due to less mitochondria density (Tang et al., 2005).

Factors affecting meat flavor

Uncooked meat has a mild, bloody flavor (Spanier et al., 1988). Upon the cooking of fresh meat, flavor-producing precursors such as water soluble proteins and lipids react due to thermal degradation to produce volatile flavor compounds associated with cooked meat (Spanier et al., 1990). More than 700 flavor and aroma volatiles are produced after cooking meat (Calkins & Hodgen, 2007). The most recognized reaction that produces flavor compounds is the Maillard reaction. The Maillard reaction occurs when the amino groups of fresh meat and the carbonyls of a reducing sugar are heated. The Maillard reaction produces heterocylic compounds that are associated with meaty flavor (Mottram, 1985). More specifically lactones, acyclic sulfur containing compounds, nonaromatic heterocylic sulfur, nitrogen, and oxygen containing compounds, as well as, aromatic heterocyclic sulfur, nitrogen, and oxygen containing compounds impact meat flavor (Chang & Peterson, 1977). Another reaction that occurs when thermally processing meat is Strecker degradation. Strecker degradation is the reaction of α-amino acids and dicarbonlys to produce strecker aldehydes (Schonberg & Moubacher, 1952). Strecker aldehydes have a positive impact on meat flavor. Strecker degradation is responsible

for non-oxidative thermal degradation of lipids (Huang & Ho, 2001). Heating triglycerides and phospholipids causes dehydration, decarboxylation, hydrolysis, and cleavage of carbon-carbon bonds which releases free fatty acids (Huang & Ho, 2001).

Postmortem Aging

Increasing postmortem aging changes the flavor of meat products. Aging beef has shown to increase flavor desirability and overall palatability (Smith, Culp, & Carpenter, 1978). Jeremiah and Gibson (2003) showed that increasing postmortem aging time to 28 days for vacuum packaged beef ribs and short loins increased beef flavor intensity, flavor desirability, and overall palatability, without affecting juiciness.

Other researchers have reported no differences in flavor due to postmortem aging. Increasing postmortem aging time of beef *gluteus medius* steaks from between 18 and 35 days compared to between 4 and 7 days showed no significant differences for flavor or juiciness in sensory panels (George-Evins et al., 2004; Harris et al., 1992; Savell et al., 1982). Similarly, increasing aging time from 0 to 15 days had no significant affect for juiciness or beef flavor for beef *longissimus dorsi* (Minks & Stringer, 1972).

Aging meat can also have detrimental effects on meat flavor. More pronounced off-flavors can occur when postmortem aging increases for vacuum packaged beef *longissimus dorsi* (Hodges, Cahill, & Ockerman, 1974; Ockerman & Cahill, 1977; Blixt & Borch, 2002). Juarez et al. (2010) showed that increasing aging time in vacuum packaging up to 42 days for beef strip loins, blade eye, outside round, inside round, eye of round, and chuck tender increased off-flavors and decreased flavor intensity. Yancey et al. (2005) reported that increasing aging time of beef in vacuum packaging longer than 21 days significantly decreased beef flavor identity and increased sour flavor. Yancey et al. (2005) also showed that increasing aging time to 35 days in

vacuum packaging significantly increased metallic flavor compared to aging for 7 or 14 days. Yancey et al. (2005) concluded that beef aged to 21 or 35 days had more metallic and rancid flavor. Spanier et al. (1997) showed that increasing postmortem aging of beef, decreased beefy, brothy, browned-caramel, and sweet flavor, and increased bitter and sour flavor. In addition, Monson et al. (2005) also saw beef *longissimus thoracis et lumborum* aged to 35 days in vacuum packaging had higher bitterness sensory scores than those same cuts aged 1 or 3 days. Finally, Jeremiah and Gibson (2003) reported that beef ribs and short loins aged in vacuum packaging for 28 had higher bitterness sensory scores than if they were aged 0, 7, or 21 days.

Stetzer et al. (2008) found that beef *gluteus medius* had the highest livery off-flavor in a sensory panel compared to beef *rectus femoris, vastus lateralis, vastus medialis, teres major, infraspinatus, complexus, serratus ventralis, psoas major,* and *longissimus dorsi.* The beef *gluteus medius* also has more beef flavor intensity than the *infraspinatus*, *semitendinosus*, *pectoralis profundus*, and *supraspinatus* (Carmack et al., 1995). Additionally, Yancey et al. (2005) found that beef *gluteus medius* had significantly higher brown/roasted and metallic flavor compared to beef *infraspinatus* and *psoas major*. Furthermore, beef *gluteus medius* was less rancid when compared to the beef *infraspinatus* (Yancey et al., 2005).

Volatile compounds can become more or less pronounced as aging increases. Stetzer et al. (2008) showed that increasing wet (vacuum packaging) aging from 7 to 14 days of beef subprimals decreased butanoic acid. Stetzer et al. (2008) also found positive correlations for livery off-flavors with pentanal, hexanal, 3-hyroxy-2-butanone, and hexanoic acid, as well as positive correlations for rancid off-flavors and pentanal and 2-pentyl furan. Gorraiz et al. (2002) showed that 2-propanone was related with livery flavor. Furthermore, Gorraiz et al. (2002) presented that increasing aging time increased characteristic beef flavor and aftertaste intensity,

and increased 2, 3, 4-trimethylpentane, 2, 3, 3-trimethylpentane, 3-methyl-2-heptane, 2, 5-dimethylheptane, and 2, 2, 4, 6, 6-pentamethylheptane and decreased ethanol volatile compounds.

Blade Tenderization

There has been little research on the effects of blade tenderization on meat flavor. Blade tenderization causes concerns in terms of juiciness as the disruption of the muscle fibers may decrease the ability for the muscle to hold water. Jeremiah et al. (1999) showed that although blade tenderization did not affect cooking loss, it did decrease perceived juiciness by a sensory panel. Blade tenderized beef *longissimus dorsi* steaks were significantly less juicy than non-blade tenderized steaks (Bidner et al., 1985; Savell et al., 1977). However, beef top sirloin steaks blade tenderized once or twice had no significance difference in juiciness compared to steaks that were not blade tenderized (George-Evins et al., 2004). Additionally, Savell, Smith, and Carpenter (1977) showed that blade tenderized beef *semimembranosus*, *biceps femoris*, and *gluteus medius* steaks were not significantly less juicy than non-blade tenderized steaks.

There has been mixed results on how beef flavor is affected by blade tenderization. Savell, McKeith, Murphey, Smith, and Carpenter (1982) showed that although blade tenderized strip loins and top sirloin butts steaks from young bulls were less juicy than non-blade tenderized steaks, there were no significant differences for flavor. George-Evins et al. (2004) reported that there were no flavor differences between blade tenderized and non-blade tenderized beef top sirloin butts. In contrast, Jeremiah et al. (1999) reported that blade tenderized beef top sirloin butts had less flavor intensity than non-blade tenderized beef top sirloin butts, but there were no significant differences in flavor desirability. According to the literature reviewed, blade tenderization has little impact on meat flavor.

Lipid Oxidation

Lipid oxidation plays a large role in meat flavor. Lipid oxidation produces off-flavors by catalytic oxidation of unsaturated fatty acids (Kanner, 1994). Lipid oxidation has been shown to be closely related to the production of undesirable warmed-over flavor (St. Angelo et al., 1987). Warmed-over flavor is described as cardboardy, rancid, stale, and metallic (St. Angelo et al., 1987). Campo et al. (2006) showed that TBARS or lipid oxidation, abnormal flavors, and rancid flavors increased and beef flavor and overall liking decreased as retail display days increased. Furthermore, Campo et al. (2006) found strong positive correlations with TBARS compared with rancid and abnormal flavors (R^2= 0.84 and 0.80 respectively) and negative correlations with TBARS compared with overall liking, beef flavor, livery, and bloody flavor (R^2= -0.84, -0.80, -0.60, and -0.60 respectively). In addition, Smith et al. (1987) found a very strong correlation (R^2= 0.96) with TBARS and warmed-over flavor in poultry. Finally, St. Angelo et al. (1987) showed that warmed-over flavor correlated highly with TBARS values in cooked and recooked beef top round roasts (*semimembranosus* and *adductor*).

The changes in meat flavors from lipid oxidation are due to changes in lipid precursors that react in the cooking process. Stetzer et al. (2008) showed that increasing aging time of beef subprimals increased flavor volatiles that are also increased with increases in lipid oxidation. St. Angelo et al. (1987) saw that both warmed-over flavor and TBARS values were correlated with hexanal, pentanal, 2, 3-octanedione in beef. Similarly, Ahn, Olson, Lee, Jo, Wu, and Chen (1998) reported that propanal, pentanal, hexanal, 1-pentanol, and total volatiles were highly correlated with TBARS values of cooked meats, with hexanal and total volatiles being the best representation of lipid oxidation. Also, Jo and Ahn (2000) showed that as lipid oxidation increased there was an increase in aldehydes, ketones, and alcohols including propanal, hexanal,

3-heptanol, 1-pentanol, cyclohexanone, 1-heptanol, and total volatiles in aerobically packaged pork sausage. Likewise, Kim, Nam, and Ahn (2002) also determined that increased TBARS values showed an increase in total volatiles in meat. Gorraiz et al. (2002) showed that branched alkanes such as octane, 2-octene, and 3-octene that are typically associated with lipid oxidation, increased with increased aging time in beef.

Hood and Allen (1971) explained that the products of lipid oxidation are a result of the lipolytic enzyme activity. The most classic example to understand how lipid oxidation affects meat flavor is from Cross and Ziegler (1965) as they showed that adding the strong antioxidant, nitrite to ham reduced the volatile compounds associated with lipid oxidation, hexanal and valeraldehyde. Ramarantham et al. (1991) also reported higher levels of hexanal in uncured pork, poultry, and beef compared to their cured counterparts. Hexanal is known to be a major product of lipid oxidation, thus has an effect on meat flavor (Ramarantham et al., 1991).

With increased postmortem aging time, free fatty acids increase in beef. The free fatty acid fraction also changes as C16:0 and C14:0 fatty acid decrease, C18:1 increase, and there is no change in cholesterol and phospholipids (Hood & Allen, 1971). Ultimately, the increase in unsaturated fatty acids increase lipid oxidation and leads to lower flavor and aroma sensory scores (Hood & Allen, 1971).

pH

When postmortem muscle pH is drastically changed from normal, flavor changes may occur. Yancey et al. (2005) showed that high pH beef (dark cutting beef) had less beef flavor identity and brown-roasted flavor than normal pH beef. Yancey et al. (2005) also showed that dark cutting meat had a more rancid flavor and if aged to 35 days was significantly more sour than normal pH beef. The authors concluded that sour flavor is most likely due to an increase in

microbial load. High pH pork was sweeter, less sour, less bitter, less salty, and received higher overall quality scores than normal and low pH pork (Flores et al., 1999).

Low pH can also have negative effects on meat flavor. Decreased pH due to postmortem aging decreased flavor desirability and overall palatability, as well as increasing off-odors in beef *biceps femoris* (Smith et al., 1980). Ahn et al. (2001) showed that pale, soft, and exudative (PSE) pork which is typically associated with a low muscle pH, had a lower concentration of total sulfur-containing volatiles compared to high pH and normal pH pork. Ockerman and Cahill (1977) reported similar results as pH was altered by microbial growth over postmortem aging, there was a decrease in flavor scores and an increase off-flavor scores, which ultimately lowered general acceptability.

Factors affecting meat tenderness

Tenderness has been determined to be a major contributor to eating experience in beef (Miller et al., 2001). Tenderness can be manipulated by either mechanical action or by enzyme activity. Blade tenderization offers a mechanical solution to marketers of beef to guarantee tenderness (George-Evins, 1999). Aging meat in vacuum packaging at refrigerated temperatures offers purveyors a way to increase tenderness (Guelker et al., 2013; Voges et al., 2007). Endogenous enzymes alter myofibrillar proteins to increase tenderness in postmortem muscles (Longergan, et al., 2010; Kemp et al., 2010; Koohmaraie, 1992; Ouali, 1990). Both blade tenderization and postmortem aging are effective ways to increase palatability of meat.

Postmortem Aging

It is widely known that postmortem aging increases tenderness in meat. The 2010 National Beef Tenderness Survey reported that retail and food service establishments age beef on an average of 20.5 and 28.1 days with a range of 1 to 358 days and 9 to 67 days, respectively

(Guelker et al., 2013). The beef top sirloin butt, which is primarily made up of the *gluteus medius,* is aged on the average of 20.3 days (Guelker et al., 2013). The beef *gluteus medius* from retail establishments had no significant differences in Warner-Bratzler shear force values compared to the beef *longissimus dorsi*, but from the food service sector the *gluteus medius* had higher Warner-Bratzler shear force values than the *longissimus dorsi* (Guelker et al., 2013). Interesting enough, Belew et al. (2003) reported that the *gluteus medius* from retail establishments had 0% in the "tough" category for Warner-Bratzler shear force values, whereas the *gluteus medius* from food service establishments had 4.05% that was defined as "tough" (WBSF > 4.6 kg).

Gruber et al. (2006) found that increasing time of postmortem aging, decreased Warner-Bratzler Shear Force values in beef *biceps femoris* – long head, *complexus, gluteus medius, infraspinatus, longissimus dorsi, psoas major, rectus femoris, semimembranosus, semitendinosus, serratus ventralis, spinalis dorsi, supraspinatus, tensor fasciae latae, triceps brachii* – long head, *vastus lateralis,* and *vastus medialis* muscles. More specifically, increasing aging time from 2 to 28 days in beef *gluteus medius* decreased Warner-Bratzler Shear Force by 1.54 kg and 1.31 kg from USDA Select and USDA Choice carcasses respectively. Most research has focused efforts on the *longissimus dorsi*, due to its high value in relation to the rest of the carcass. Increasing postmortem aging time increases tenderness in beef *longissimus dorsi* muscles (King et al., 2009; Jeremiah & Gibson, 2003; Monson et al, 2004; Monson et al., 2005; Bidner et al., 1985; Savell et al., 1982). More specifically, King et al. (2011) showed beef *longissimus lumborum* aged for 40 days was more ($P < 0.001$) tender than being aged only 26 or 12 days. Jeremiah and Gibson (2003) showed that increased postmortem aging time of beyond 14 days, increased tenderness for beef *longissimus dorsi,* compared to not aging or only aging for

7 days. Juarez et al. (2010) reported increasing ageing time up to 56 days increased tenderness (decreased shear force values and increased tenderness sensory scores) for beef strip loin, eye of round, blade eye, and chuck tender. Minks and Stringer (1972) showed that aging beef *longissimus dorsi* for 15 days increased tenderness both instrumentally and through a sensory panel compared to aging the beef *longissimus dorsi* for 0 or 7 days. Monson et al. (2004 & 2005) presented that aging beef *longissimus dorsi* for at least 35 days increased tenderness (instrumentally, trained sensory panel, and consumer sensory panel) compared to aging under 35 days. Similarly, *longissimus dorsi* aged for 21 days were significantly more tender than *longissimus dorsi* that were not aged (Bidner et al., 1985). Finally, Savell et al. (1982) showed similar results as beef *gluteus medius* and *longissimus dorsi* steaks aged 18 days were significantly more tender than steaks aged 4 days.

Recently, more attention to tenderness has been given to the beef *gluteus medius*, as there is opportunity to improve tenderness in this muscle. The beef *gluteus medius* is known to be less tender than the *longissimus dorsi* (Carmack et al., 1995). George-Evins et al. (2004), found that beef *gluteus medius* steaks aged 21 days had significantly lower Warner-Bratzler Shear Force values compared to *gluteus medius* steaks aged for 14 and 7 days. King et al. (2011) showed similar results for aging in the beef *gluteus medius* as the beef *longissimus lumborum*, as steaks from the beef *gluteus medius* aged for 40 days were significantly more tender than if aged for only 26 or 12 days.

Connective tissue plays a significant role in meat tenderness (Bailey, 1989). Collagen is the connective tissue that supports muscle tissues (Bailey, 1989; Weston et al., 2002). Collagen and collagen cross-links have an important role in meat tenderness. As collagen becomes less soluble and more cross-linked, meat becomes tougher (Weston et al., 2002). Harris et al. (1992)

reported that the beef *longissimus dorsi* was more tender than *gluteus medius*, due to having less total collagen and having higher collagen solubility. In addition, beef *gluteus medius* steaks aged 21 days had significantly less perceived connective tissue and were more tender in a sensory panel compared to *gluteus medius* steaks aged 7 days. Harris et al. (1992) also showed that aging the beef *gluteus medius* for 28 and 35 days significantly reduced total collagen content and were more tender than steaks from the *gluteus medius* aged less than 21 days. Jeremiah and Gibson (2003) saw similar results in the beef *longissimus dorsi*, in that increasing aging time beyond 21 days significantly decreased shear force values and the amount of perceived connective tissue, and increased initial tenderness and overall tenderness compared to aging less than 21 days. The mechanisms behind the decrease in collagen content during aging are still under investigation.

Tenderization from postmortem aging is due to the biochemical process. Several studies have shown that calpain enzymes are largely responsible for meat tenderization (Goll et al., 1992; Lametsch et al., 2004; Pomponio et al. 2008; Taylor et al., 1995). There are 3 proteases of the calpain system (μ-calpains, m-calpains, and calpain 3) that are inhibited by calpastatin. Calpains cleave myofibrillar proteins when activated by calcium. Unfortunately, calpains inhibitor, calpastatin also requires calcium to bind to calpains (Huff-Lonegran et al., 2010). M-calpains require between 300 and 1000 μM of calcium for half-maximal activity, whereas μ-calpains require between 5 and 65 μM of calcium (Goll et al., 1992). Lametsch et al. (2004) showed that in vitro μ-calpains degrade actin, myosin heavy chain, myosin light chain I, desmin, troponin T, tropomyosin α1, tropomyosin α4, thioredoxin, and CapZ.

Myofibrillar and cytoskeletal proteins are the main myofibers that are degraded during aging. These include troponin-I, troponin-T, desmin, vinculin, meta-vinculin, dystrophin,

nebulin, and titin. Attachments from the Z-line to the Z-line via desmin, as well as from the Z-line and M-line to the sarcolemma via titin, have thought to be the reason for tender meat (Taylor et al., 1995). However, Geesink and Koohmarie (1999) showed after two weeks postmortem, there was little degradation of desmin and titin in non-callipyge and callipyge lamb, but callipyge lamb is known to be tougher. Although, Geesink and Koohmaraie (1999) did see increases in I-band and Z-disk breaks in non-callipyge lamb compared to callipyge lamb. The result from Geesink and Koohmaraie (1999) supports that I-band and Z-disk attachment degradation are more important for meat tenderness than cytoskeletal protein attachment degradation. In conclusion, postmortem aging increases tenderness in meat through degradation of myofibrillar proteins by calpain enzymes.

Blade Tenderization

Blade tenderization is a physical mechanism to disrupt the muscle structure and increase tenderness in meat. Jeremiah et al. (1999) describes the process as the penetration of blades or needles into the meat, which causes severing of myofibrillar proteins and connective tissue. King et al. (2009) and Jeremiah et al. (1999) found that steaks from blade tenderized *gluteus medius* were significantly more tender than steaks from non-blade tenderized *gluteus medius*. George-Evins et al. (2004) also showed that steaks from beef *gluteus medius* that had been blade tenderized twice had significantly lower Warner-Bratzler Shear Force values than that of steaks from beef *gluteus medius* that had not been blade tenderized or blade tenderized once. George-Evins et al. (2004) suggested that blade tenderizing twice and aging top sirloin butts for 21 days was the best method to optimize tenderness. Similarly, Savell et al. (1982) suggested that blade tenderizing and aging top sirloin butts for 18 days was the best method to optimizing tenderness.

Pietrasik and Shand (2011) showed that blade tenderizing steaks increased sensory tenderness and amount of connective tissue in beef inside round steaks. Seideman, et al. (1977) showed blade tenderizing beef *psoas major* and *semitendinosus* increased tenderness instrumentally and organoleptically, while also decreasing the amount of connective tissue perceived. Similarly, blade tenderized beef *longissimus dorsi* were significantly more tender and had less perceivable connective tissue compared to non-blade tenderized beef *longissimus dorsi* (Bidner et al. 1985). Liu et al. (2006) showed that blade tenderized top round steaks from Bos indicus bovine were significantly more tender than steaks that were not blade tenderized. Bos indicus cattle are known to be tougher and have more connective tissue than Bos taurus cattle (Crouse et al., 1989; Whipple et al., 1990). These studies all show that by utilizing blade tenderization, there are positive affects for both muscle fiber tenderness and connective tissue tenderness.

pH

The change in pH alters enzyme activity in postmortem muscle. Geesink and Koohmaraie (1992) showed that caplain activity was slower at postmortem muscle pH values compared to a neutral pH. Hwang et al. (2001) reported μ-calpain activity decreased with slow chilling with a faster pH decline. Postmortem aging has also been shown to decrease muscle pH (Smith et al., 1980). Koohmaraie (1992) reported that even though calpain activity decreases with decreased pH, calpains are still active at the pH of postmortem muscle.

Factors affecting meat microorganisms

Contamination from bacteria affects meat quality. Microorganisms of concern in meat products are either pathogenic or spoilage microorganisms. Pathogenic microorganisms found in meat can be a potential threat to public health (Mor-Mur et al., 2010). Spoilage microorganisms

cause not only color shelf life deterioration, but also deterioration of flavor and odor (Smith, et al., 1980; Ockerman & Cahill 1977). In vacuum packaging, anaerobic and facultative anaerobic microorganisms can grow more efficient, due to the lack of oxygen. Postmortem aging and blade tenderization of meat can affect the amount and location of microorganisms.

Postmortem aging

Anaerobic spoilage microorganisms increase as postmortem aging time increases for vacuum packaged beef (Hodges, Cahill, & Ockerman, 1974; Nissen, Sorheim, & Dainty, 1996; Minks & Stringer, 1972; Hanna et al., 1980; Hanna et al., 1977). Lactic acid producing bacteria such as *Lactobacillus* are known to be a spoilage microorganism in vacuum packaged meats (Newton & Gill, 1978; Hanna et al., 1977). Shaw and Nicol (1969) reported that *Lactobacillus* and *B. thermosphacta* were the major spoilage microorganisms in meat stored in oxygen-impermeable bags. Others note that *Leuconostoc* is the main spoilage microorganism in vacuum packaged beef (Nissen, Sorheim, & Dainty, 1996). Lactic acid bacteria also have been shown to have gas production that can cause loss of vacuum in vacuum packaged beef (Chaves et al., 2012). Lactic acid bacteria metabolize carbohydrates more efficiently than other spoilage microorganisms in anaerobic conditions and they also reduce pH due to the production of lactic acid which also inhibits growth of other microorganisms (Newton & Gill, 1978; Nissen, Sorheim, & Dainty, 1996; Hanna et al., 1979). Greer, Dilts, and Jeremiah (1993) showed that lactic acid bacteria were the only detectable microorganism in fresh pork loins stored in modified atmosphere packaging for 168 days. The initial level of *lactobacillus* sp. significantly affected the final level of *lactobacillus* sp. in beef after 35 days of vacuum packaging (Hanna et al., 1979; Hanna et al., 1980).

Blitz and Borch (2002) reported that there was a significant increase in both lactic acid bacteria and aerobic microorganisms as postmortem time increased for vacuum packaged beef and pork *longissimus dorsi*. Lee et al. (1984) showed that increasing the temperature of storage of vacuum packaged and modified atmosphere packaged meat loaves significantly increased the production of *Lactobacillus* over a 49 day shelf life. Furthermore, Li et al. (2013) showed that aging beef in vacuum packaging increased lactic acid bacteria counts compared to aging in a dry aging bag.

Blade Tenderizing

Disruption of muscle structure due to blade tenderization has been widely studied. There has been heightened concern over the translocation of pathogenic microorganisms into the interior of muscle via blade tenderization. Blade tenderization creates a problem for intact muscle cuts that are cooked to various degrees of doneness, as meat cooked to a rare degree of doneness would not reach 60°C and could increase the risk for a foodborne illness. Therefore, in 1999 the USDA-FSIS defined any beef that was mechanically tenderized as non-intact muscle. In 2013, the USDA-FSIS proposed a new rule to label all uncooked blade tenderized beef as "mechanically tenderized."

Nonetheless, some research (Bentio-Delgado et al., 1994; Ray et al., 2010; Sporing, 1996) has shown that blade tenderization does not affect the microbial load of the interior of meat. Benito-Delgado et al. (1994) reported that blade tenderized beef *longisimus dorsi* and *infraspinatus* steaks did not have a significantly higher microbial load than non-blade tenderized steaks. Furthermore, Sporing (1999) reported that blade tenderized beef top sirloin butts had the same *Escherichia coli* 0157:H7 microbial load than their non-blade tenderized counterparts. Also Johns et al. (2011) demonstrated that beef loins inoculated with *Escherichia coli* that were

processed through a blade tenderizer had higher levels of *Escherichia coli,* than non-inoculated loins that were processed through the blade tenderizer after the inoculated loins were processed. In addition, the loins that were processed through the blade tenderizer more than three loins after the inoculated loin had significantly less *Escherichia coli* than the one that was processed right after the inoculated loin. The results from John et al. (2011) indicate that if a high level of *Escherichia coli* was on one loin, there would be minimal effect on the loins that were subsequently processed. Finally Ray et al. (2010) confirmed the thought that the initial microbial load plays the largest role in translocation, as they saw a decrease in *Escherichia coli* in beef strip loins that had been injected with a brine solution using needle injection compared to strip loins that had been injected with a brine solution using needle free injection. Ray et al. (2010) concluded that the difference in the treatments was mainly a factor of the initial microbial load on the exterior of the subprimals.

Blade tenderization has been reported to increase the microbial load into the interior of meat compared to not blade tenderizing. Wen and Dickson (2013) reported that injecting pork loins inoculated with *Esherichia coli* with a brine solution contaminated the interior and exterior of the four loins that were subsequently processed. Furthermore, increasing the injection pump percentage from 10 to 30 had no significant effect on the amount of Escherichia coli found on the surface or interior of pork loins. Wen and Dickson (2013) also found that the injecting needles used in their study had 1 to 2 log CFU/cm^2 of *Escherichia coli* and the recirculating brine solution contained 4.6 log CFU/50 ml. Gill and Mckinnis (2004) reported that mechanically tenderized steaks purchased from retail establishments that were cooked to a medium rare degree of doneness was sufficient enough to kill microorganisms. Gill et al. (2005) reported similar results in a commercial plant, as they found translocation into the deep tissue of the muscle due

to mechanical tenderization, when cooked to a medium rare degree of doneness caused microbial cell lysis. Another study by Chancey et al. (2013) showed that cores from blade tenderized beef strip loins that were inoculated with high levels of *Escherichia coli 0157:H7* had presence of *Escherichia coli 0157:H7* when cooked to 55, 60, and 70°C, compared to blade tenderized beef strip loins inoculated with low levels of *Escherichia coli 0157:H7* had no presence of *Escherichia coli 0157:H7* cooked to those same temperatures. The results from Chancey et al. (2013) indicate that if strip loins have a high level of *Escherichia coli 0157:H7* prior to blade tenderization, there may be a risk for contamination in the interior of the steak after cooking. At the same time, Chancey et al. (2013) also found that using an organic acid intervention has potential to decrease the initial microbial load. Chancey et al. (2013) finally showed that treating strip loins with 5% lactic acid spray reduced *Escherichia coli 0157:H7* by 1.0 log CFU/g. Yoon et al. (2009) showed that the addition of organic acids to a ground beef system decreased the presence of *Escherichia coli 0157:*H7 cooked to a rare or medium-rare degree of doneness compared to control treatments with no added organic acids.

Lemmons et al. (2011) showed that trimming the exterior surface prior to blade tenderization of inoculated top sirloin butts that had been aged up to 28 days, was more effective at reducing Escherichia coli 0157:H7 than washing with water. Blade tenderization can translocate microorganisms to the internal portion of meat, but can be controlled by the level of contamination on the exterior portion of meat through organic acid washes or trimming of the exterior of the sub-primal. Finally, pathogenic microorganisms can be controlled through cooking to the correct degree of doneness of non-intact muscles.

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Chapter 2 - Color and flavor stability of beef *gluteus medius* as influenced by postmortem aging time and blade tenderization.

Abstract

A total of 45 top sirloin butts (IMPS 184) were procured from three commercial beef processing facilities to determine the effects of post-mortem aging time and blade tenderization on the quality of beef *gluteus medius* (GM) steaks. Top sirloin butts were randomly assigned to five post-mortem aging periods (5, 19, 33, 47, and 61 days). One-half of each beef GM was randomly assigned to either a blade tenderized treatment or a non-blade tenderized treatment that was not blade tenderized. Steaks were then evaluated for thiobarbituric acid reactive substances (TBARS), metmyoglobin reducing activity (MRA), oxygen consumption rate (OCR), visual color panel, instrumental color, Warner-Bratzler shear force (WBSF), Lactic Acid Bacteria (LAB) enumeration, pH, and sensory properties. Aging × blade tenderization interactions ($P < 0.05$) were found for display color panel, discoloration panel, WBSF, overall tenderness, myofibrillar tenderness, bloody/serumy, metallic, overall sweet, and bitter ($P < 0.05$). As steaks were aged longer and blade tenderized they became more discolored during display and more tender. In addition, there were aging × display time interactions ($P < 0.05$) observed for L*, a*, b*, display color panel, and discoloration panel. As steaks were aged longer, they had increased L*, a*, b*, and hue angle values and display color panel scores when initially put into a retail case, but L*, a* and b* decreased and discoloration scores increased as display time increased. Furthermore, there were blade tenderization × display time interactions ($P < 0.05$) found for display and discoloration panels. Blade tenderized steaks discolored faster in retail display than non-blade tenderized steaks. With increased aging time, there was an increase ($P < 0.05$) in

TBARS, OCR, initial color panel, LAB enumeration, and warmed-over flavor, as well as a decrease in MRA. Also, as aging increased there was a decrease ($P < 0.05$) in MRA, initial color panel scores, and WBSF values. Blade tenderization significantly increased ($P < 0.05$) initial color panel scores, rancid flavor, and spoiled flavor. Increasing the aging time of the GM, produced steaks with decreased color stability, altered the flavor profile, and increased tenderness. Blade tenderization significantly increased tenderness, increased discoloration in a retail case, and produced more undesirable flavors.

Introduction

The *gluteus medius* (GM) has shown to have variation in tenderness and color stability (Apple et al., 2014). Tenderness plays a large role in consumers' satisfaction of beef products (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). Postmortem aging is an effective way to increase tenderness in beef. Aging GM steaks for 40 days decreased slice shear force values compared to GM steaks aged 12 or 26 days (King et al. 2009). As a result, top sirloin butts are typically aged an average of 20.3 days and 24.7 days in retail and food service operations, respectively (Guelker et al., 2013). Furthermore, top sirloin butts are commonly blade tenderized to significantly increase tenderness. George-Evins, Unruh, Waylan, and Marsden (2004) reported that blade tenderization decreased Warner-Bratzler Shear Force (WBSF) values and increased sensory tenderness ratings for top sirloin steaks compared with steaks receiving no blade tenderization.

Consumers rely on their eating experience (flavor and tendereness) to repurchase fresh meat. Aging periods of 14 to 28 days have been reported to increase the palatability of beef when contrasted with shorter or no aging period (Brewer & Novakofski, 2008). Not only is postmortem aging beneficial for tenderness, but it has been shown to benefit beef flavor.

Jeremiah and Gibson (2003) reported that beef flavor intensity progressively increased up to 28 days of postmortem age. However, Juarez et al. (2010) reported that extended aging times up to 42 days increase off-flavor intensity. The increase in off-flavors is due to the increase in precursors for meat flavor such as free amino acids and lipid oxidation products (Mottram, 1998). Lipid oxidation can be evaluated through thiobarbituric acid reactive substances (TBARS). St. Angelo et al. (1987) found that volatile compounds found from TBARS were also identified in warmed-over flavor in beef top round roasts. Warmed-over flavor was described as cardboardy, rancid, stale, and metallic (St. Angelo et al., 1987).

Increased product aging under anaerobic conditions (vacuum) is known to increase the production of lactic acid bacteria (LAB). Lee and Yoon (2001) showed LAB significantly increased with increased postmortem aging of beef products. Juraez et al. (2010) reported an increase in sour and lactate flavors due to an increase in LAB growth in vacuum packaged beef.

Consumers rely on color to assess freshness of fresh meat at the retail meat case. Bright-red color is the most desirable fresh meat color to consumers (Kropf, 1980). It is known that the GM has a more limited color shelf life compared to *longissimus dorsi* (LD). O'Keefe and Hood (1982) reported that GM steaks had a 2 day shorter shelf life in comparison to LD steaks. Additionally, aging beef decreases color stability under simulated retail display conditions (Madhavi & Carpenter, 1993). Decreases in color shelf life from the combination of the inherent properties of the GM and postmortem aging results in a higher percentage of retail cuts being marked down in price or removed from the retail case (Smith, Belk, Sofos, Tatum, & Williams, 2000), which in turn results in significant monetary losses across all facets of the beef industry.

Color stability has been widely studied using metmyoglobin reducing activity (MRA) and oxygen consumption rate (OCR). Higher rates of MRA result in an increased color shelf life, as

MMb is reduced to DMb which allows for regeneration of OMb. Postmortem mitochondria in muscle continue to respirate oxygen (Faustman & Cassens, 1990). Mitochondria require the same substrates that metmyoglobin reducing enzymes require to metabolize oxygen (O'Keefe & Hood, 1982). Therefore, higher OCR depletes the necessary substrates needed for MRA.

Research highlighting the effects of blade tenderization, as well as postmortem aging past 30 days on beef color and flavor stability is limited. Therefore, the objectives of this study were to determine color and flavor stability of the beef *gluteus medius* during extended postmortem aging times with and without blade tenderization.

Materials and Methods

Sample Handling and Preparation

Forty-five top sirloin butts (IMPS #184) were transported to the Kansas State University Meat Laboratory from three commercial beef harvest facilities (15 top sirloin butts from each facility) and were randomly assigned to 5 different aging periods. The five postmortem aging treatments were 5, 19, 33, 47, and 61 days. Each beef top sirloin butt, was then stored in the original anaerobic package at 2 to 4°C throughout the aging period. On the final day of each postmortem aging treatment, top sirloin butts were removed from their anaerobic packages and the *gluteus medius* (GM) was removed and trimmed of excess fat. The GM was then separated into approximately two equal pieces yielding a ventral and dorsal piece. The ventral and dorsal portions of the GM were randomly assigned to either a blade tenderization or a non-blade tenderized (control) treatment. The blade tenderized treatment was processed twice through a commercial blade tenderization machine (Ross model T7001 Tenderizer, Midland, VA, USA). After tenderization treatment, GM pieces were sliced perpendicular to the muscle fibers into seven 2.54 cm steaks. The first, fourth, and fifth steaks, starting at the anterior end were

immediately vacuum packaged with oxygen impermeable film (Prime Source Vacuum Pouches; 3mil, STP Barrier, Nylon/PE Vacuum Pouch; oxygen transmission rate 0.04g/254cm^2/24 hours at 0ºC; water vapor transmission rate 0.2cc/254cm^2/24 hours at 0ºC at 0% relative humidity) and frozen at -80°C and were stored for subsequent assessment of thiobarbituric acid reactive substances (TBARS), sensory analysis, and WBSF, respectively. The second steak was used for metmyoglobin reducing activity (MRA) and oxygen consumption rate (OCR). The third steak was used for simulated retail display. The sixth steak was placed in a sterilized bag (Whirl Pack, Nasco, Modesta, CA, USA) and immediately used for analyze lactic acid bacteria enumeration (LAB). The seventh steak was used to evaluate pH.

Thiobarbituric Acid Reactive Substances (TBARS) for Oxidative Rancidity

Steak samples were thawed at 2 to 4°C for 24 hours. TBARS analysis were completed as described by Buege and Aust (1978) and modified according to the AMSA (2012). Samples were trimmed of excess fat and connective tissue and then frozen in liquid nitrogen and pulverized (model 51BL32, Waring Commercial, Torrington, CT, USA) to produce a homogenous powder. Next, 2.5 mL of thiobarbituric acid (TBA) stock solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25N HCl (Fisher Scientific, Pittsburg, PA, USA) was vortexed (Vortex Mixer-Standard 120V, Fisher Scientific, Waltham, MA, USA) with 0.5 g of duplicate pulverized sample in a conical tube which was heated in a 100°C water bath for 10 minutes. After the tubes were cooled, samples were centrifuged (Centrifuge 5810R, Eppendorf International, Hauppauge, NY, USA) at 5,000 x *g* for 10 min at 4°C. The clear supernatant from the sample was pipetted into a spectrophotometer cuvette (Fisher Scientific Disposable Plastic Cuvette, Pittsburg, PA, USA, Semimicro Style Methacrylate, 10 mm lightpath,1.5 mL) and measured at 532 nm against a blank

spectrophotometer (EON265489, Biotek Instruments, Inc., Winooski, VT, USA) cuvette that contained all reagents. The TBARS value was calculated according to AMSA (2012) procedures and expressed as ppm of malonaldehyde.

Color Stability

Metmyoglobin Reducing Activity (MRA)

Duplicate steak samples (2.54 x 2.54 x 2.54 cm) were cut from steaks designated for MRA and OCR, so that a fresh surface was exposed to oxygen. Metmyoglobin reducing ability was evaluated using methods described by the American Meat Science Association (AMSA) Color Guidelines (2012). Samples were cut so they were free of fat and connective tissue and then were tagged to identify which surface was to be evaluated. Samples were submerged into 100 mL of 0.3% $NaNO_2$ solution for 20 minutes at 20°C to induce MMb formation. Samples were then removed from solution and blotted to remove excess solution. The 3-dimensional shape was retained and then samples were vacuum packaged in an oxygen impermeable bag and immediately scanned in triplicate for reflectance from 400 to 700 nm to determine the initial amount of MMb formed on the surface. Samples were then incubated using a Thelco incubator (Precision Scientific, model 31488, GCA Corporation, Chicago, IL, USA) at 30°C placed and rescanned in triplicate for reflectance from 400 to 700 nm at 2 h to determine the remaining amount of MMb. MRA was calculated as the percentage reduction of MMb from the initial sample to final incubated sample. Surface MMb percentage was calculated using the equations defined by the AMSA Color Guidelines (2012).

Oxygen Consumption Rate (OCR)

Duplicate steak samples (2.54 x 2.54 x 2.54 cm) from the same steaks used to evaluate MRA were used to determine OCR according to methods described by the AMSA Color

Guidelines (2012). Samples were cut so they were free of fat and connective tissues. Then, samples were covered by polyvinylchloride film (Prime Source, Houston, TX, USA, oxygen transmission rate 0.6g/254cm^2/24 hours at 0ºC; water vapor transmission rate 0.6cc/254cm^2/24 hours at 0ºC and 0% relative humidity) and allowed to bloom for 2 hours at 2 to 4°C. After the samples bloomed, samples were immediately vacuum packaged with oxygen impermeable film and scanned in triplicate for reflectance from 400 to 700 nm to determine initial %OMb. The samples were then incubated in a Boekel incubator (Boekel Industries, Model 132000, Feasterville, PA, USA) at 25°C for 20 minutes and rescanned in triplicate for reflectance from 400 to 700 nm. Oxygen consumption rate was expressed as %OMb reduced. Color standards were made and oxymyoglobin was calculated using AMSA Color Guidelines (2012).

Visual Color

After cutting, steaks were packaged onto foam trays (2S, Cryovac Sealed Air, Duncan, SC, USA) with moisture absorbent pads (Dri-Loc Pad, Sealed Air Corporation, Elmwood Park, NJ, USA) and overwrapped with oxygen permeable polyvinylchloride overwrap (Prime Source, Houston, TX, USA, oxygen transmission rate 0.6g/254cm^2/24 hours at 0ºC; water vapor transmission rate 0.6cc/254cm^2/24 hours at 0ºC and 0% relative humidity). Steaks were placed into coffin style refrigerated display cases (Unit Model DMF8, Tyler Refrigeration Corp., Niles, MI, USA) under continuous florescent lighting (3500K, 2,100 lux and CRI=85, Bulb Model F32T8/ADV830/Alto, Phillips, Bloomfield, NJ) at 2-4ºC. A minimum of 8 trained visual were screened using the Farnsworth-Munsell 100 Hue Color Vision Test. Panelists assessed visual color once daily from 0 to 4 days. Panelists evaluated Initial color and Display color of samples using an 8 point scale with 0.5 increments (The initial color scale consisted of: 1=Bleached red, 2=Very light cherry-red, 3=Moderately cherry-red, 4=Cherry-red, 5=Slightly dark red,

6=Moderately dark red , 7=Dark red, and 8=Very dark red, The display color scale consisted of: 1=Very bright red, 2=Bright red, 3=Dull red, 4=Slightly dark red, 5=Moderately dark red, 6=Dark red to dark reddish tan, 7=Tannish red, and 8=Tan to brown). Panelists also evaluated surface discoloration using a 7 point scale with 1.0 increments (1=0% Surface discoloration, 2=1 to 10% Surface discoloration, 3=11 to 25% Surface discoloration, 4=26 to 50% Surface discoloration, 5=51 to 75% Surface discoloration, 6=76-99% Surface discoloration, and 7=100% Surface discoloration).

Instrumental Color

Samples placed in display cases for visual color analysis were also used for instrumental color analysis during simulated retail display. Instrumental color readings were taken immediately after placing into cases and every 12 hours thereafter for 96 hours. Steaks were scanned in quadruplet for CIE L* (lightness), a* (redness), and b* (yellowness) using a HunterLab MiniScan EZ (Model 4500; MSEZ0115; Reston, VA, USA, Illuminant A, 31.8 mm aperture, and a 10° observer). CIE a* and b* values were used to calculate hue angle $[(b^*/a^*)\tan^{-1}]$ at display hour 0 (AMSA, 2012). The average of the quadruplet readings were used for the overall mean value in statistical analysis. *Gluteus medius* aged 61 days were not taken after hour 0 of display due to instrumental malfunction.

Tenderness

Warner-Bratzler Shear Force (WBSF)

Steaks were thawed at 2-4°C for 24 hours, cooked to 35°C, turned, and then cooked to an internal temperature of 65°C in a dual flow, forced-air convection gas oven (Blodgett, model DFC-102 CH3; G.S. Blodgett Co.; Burlington, VT, USA) preheated at 163°C. Internal steak temperatures were monitored with copper-constantan thermocouples (Omega® Engineering;

Stamford, CT, USA) inserted into the approximate geometric center of each steak and attached to a Doric temperature recorder (model 205; Vas Engineering; San Francisco, CA, USA). Samples were chilled overnight at 2-4°C before six round cores (1.27-cm diameter) free of fat and connective tissue were taken from each sample parallel to the long axis of the muscle fibers using a 1.27-cm corer (G-R Manufacturing Co., Manhattan, KS, USA). Each core was sheared once perpendicular to the direction of the muscle fibers using a Warner-Bratzler V-shaped blunt blade (G-R Manufacturing Co., Manhattan, KS, USA) attached to an Instron Universal Testing Machine (model 4201, Instron Corp., Canton, MA, USA) with a 50-kg compression load cell and a crosshead speed of 250 mm/minutes. The average of the six cores were used for statistical analysis for each sample.

Microbiology

Lactic Acid Bacteria (LAB) Enumeration

Lactic acid bacteria were enumerated using the 3M Aerobic Method for lactic acid bacteria (3M Microbiology Products; St. Paul, MN, USA). An aseptically sterilized knife, cutting board, tweezers, and gloves were used to weigh out and place 25 g of steak sample into a stomacher bag (Filtra-Bag, no. 01-002-57, Fisher Scientific, Waltham, MA, USA). Using a sterile pipette, 225 mL of 0.1% sterile peptone (Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) was added to the sample which was then stomached (Stomacher 400 Lab Blender, Seward Medical, London, UK) for 1 minute to produce a 1:10 original dilution. Three 1:10 serial dilutions were made from the original dilution. Then, 1 mL of diluted sample was combined with 1 mL of deMan, Rogasa, Sharpe broth (MRS) (Aerobic Lactic Acid Bacteria Broth, Laboratory Media Corporation, Montgomery, IL) into a sterile test tube and vortexed. Next, 1mL of homogenous MRS and diluted sample mixture was plated on 3M Aerobic

Pertrifilm (3M Microbiology Products; St. Paul, MN, USA) using a 1 mL sterile pipette. Plates were incubated (Forced Air General Incubator, VWR International, Radnor, PA, USA) for 48 hours at 37°C. Immediately after incubation LAB were enumerated and recorded.

pH

After cutting, each steak was immediately measured in duplicate for pH by inserting a pH probe (Hanna Instruments; H199163; Woonsocket, RI, USA) attached to an Accumet Basic pH Meter (Fisher Scientific, Waltham, MA, USA) into the sample. pH measurements were processed in triplicate for each experimental unit and were averaged for statistical analysis.

Sensory Analysis

Steaks used for sensory analysis were thawed at 0-2°C for approximately 12 h and cooked (Adhikari, 2011) using a George Foreman Grill (Model GRP100, Miramar, FL, USA). Grills were set on high and preheated for 10 minutes. Steaks were then placed horizontally on the middle of the bottom grill. The grill was then closed and steaks were cooked to 70°C. Steaks were rested at room temperature (21-23°C) for 4 minutes before they were cut into 1.27 cm cubes. Six highly trained descriptive analysis panelists from the Sensory Analysis Center at Kansas State University evaluated steaks using the lexicon for beef flavor described by Adhikari et al. (2011).

Panelists were exposed to all sample types and chose 15 flavor attributes ("Beef Identity", "Brown/Roasted", "Bloody/Serumy", "Liver-like", "Metallic", "Fat-like", "Green", "Rancid", "Warmed-Over", "Overall sweet", "Sour", "Bitter", "Salty", "Umami", "Spoiled") and 2 tenderness attributes ("Overall Tenderness" and "Myofibrillar Tenderness") during 3, 1.5 hour orientation periods. Each sample was prepared, placed into warmed glass jelly jars (placed onto foil wrapped clay bricks that were heated at 120°C for 2 hour), randomly coded, and

evaluated (resulting in a total of 90 evaluations). Panelists randomly evaluated each of the five samples in a 1.5 hour session over an 18 d testing period. Panelists scored samples using a 0 to 15-point numerical scale with 0.5 increments (0 representing "none" to 15 representing "extremely strong"). Panelists used unsalted crackers (Unsalted, Tops Premium saltine crackers, Nabisco, East Hanover, NJ, USA) and reverse osmosis, de-ionized, carbon-filtered water to clean their palates to reduce build-up of any flavors from one sample to the next. The Sensory Analysis Center used Compusense 5 (Compusense, Guelph, ON, CA) for data collection.

Statistical Analysis

For TBARS, MRA, OCR, initial color panel, WBSF, LAB, and pH the experimental design was a completely randomized design in a split-plot arrangement, where the 5 aging periods (5, 19, 33, 47, and 61 days) were the whole plot and the 2 blade tenderization treatments (Control vs. blade tenderized) were the sub plot, with 3 replications. Fifteen top sirloin butts from each block were randomly assigned to one of five aging periods, after which each top sirloin butt was split into two pieces and randomly assigned to one of two blade tenderization treatments. A repeated measure was added for instrumental color, display color panel, and discoloration panel. The experimental design for sensory analysis was a completely randomized design in a split-plot arrangement representing a whole plot of 5 aging periods (5, 19, 33, 47, and 61 days) and a sub plot of 2 blade tenderization treatments (Control vs. blade tenderized) with 6 panelists and 3 replications.

Data were analyzed using the PROC MIXED procedure in SAS (Statistical Analysis System 9.2, SAS Institute Inc., Cary, NC, USA). Analysis of variance was used to determine significant differences among treatments and interactions at an α-level of 0.05. Degrees of

freedom were estimated with the Satterthwaite method. Principal component analysis (PCA) was used for further analysis utilizing Unscrambler® (version 10.2; Camo Inc., Oslo, Norway).

Results and discussion

Thiobarbituric Acid Reactive Substances (TBARS) for Oxidative Rancidity

There was no aging × tenderization interaction ($P > 0.05$) detected for mean values of TBARS (table A.1). In general, increased days of aging, increased ppm malonaldehyde (figure 2.1) as steaks aged 47 and 61 days had increased ($P < 0.05$) ppm malonaldehyde than all other aging treatments. Additionally, steaks aged 5 days had the lowest ($P < 0.05$) ppm malonaldehyde compared to all other aging treatments. Malonaldehyde is a product of lipid oxidation (AMSA, 2012). Therefore the increase in ppm malonaldehyde indicates an increase in lipid oxidation due to increased aging time. Ismail, Lee, Ko, and Ahn (2008) reported similar results, as increasing the aging of beef inside rounds from 7 days to 21 days increased TBARS values. There were no significant differences ($P > 0.05$) between tenderization treatments for TBARS values (table A.3).

Color Stability

Metmyoglobin Reducing Activity (MRA)

No aging × tenderization interaction ($P > 0.05$) was found for MRA (table A.1). *Gluteus medius* steaks aged 5 days had the highest metmyoglobin reducing ability and steaks aged 33 and 61 days had the lowest metmyoglobin reducing ability (figure 2.2). The results from the current study are in alignment with results reported by Highfill (2012). Highfill (2012) reported that ground beef patties from beef primals aged 42 days had a lower MRA than ground beef patties from beef primals aged 7 or 21 days. The results from the current study and study by Highfill

(2012) are in contrast with Lanari and Cassens (1991) findings, as they reported that postmortem aging time had no effect on MRA in the beef *gluteus medius* and *longissimus dorsi*. However, Madhavi and Carpenter (1993) reported that MRA was correlated with NADH concentration and that they both decrease as postmortem time increases. NADH is used by the mitochondria to consume oxygen, thus decreasing the available NADH to reduce MMb. Therefore, steaks with increased aging periods would have less color stability.

Metmyoglobin reducing ability was higher for GM steaks aged 47 days compared to steaks aged 33 days. This may be due to the fact that steaks aged 33 days had significantly lower pH than all other aging treatments. pH has been shown to play a role in the biochemical processes of discoloration of meat. Echevarne, Renerre, and Labas (1989) reported that metmyoglobin reducing ability was highest at a pH of 7.4 and decreased for any pH that is higher or lower than 7.4. This indicates that metmyoglobin reducing enzymes are most active around a neutral pH and less active at more acidic or basic pH. Tenderization treatment did not affect MRA (table A.3).

Oxygen Consumption Rate (OCR)

There were no aging × tenderization interactions ($P > 0.05$) for mean values of OCR (table A.1). *Gluteus medius* steaks had an increase in %OMb reduced when aging time was increased. GM aged to 61 days had ($P < 0.05$) a higher %OMb reduced when compared to all other aging treatments (figure 2.3). GM aged to 61 days had a mean %OMb reduced of 83.55, whereas GM aged to 5 days had a mean %OMb reduced of 48.44. Highfill (2012) also reported that ground beef patties from beef subprimals aged 47 days had a higher %OMb reduced than ground beef patties from beef subprimals aged 7 or 21 days. The increase in %OMb due to aging was apparent as GM aged 5 days had a higher final %OMb due to an increase in the

muscle's ability to reduce MMb back into OMb compared to GM aged 61 days. Increased %OMb reduced due to enzyme respiration is indicative of decreased color stability (O'Keefe & Hood, 1982; Renerre & Labas, 1987; Tang et al., 2005). After the 20 minute incubation period, there was a decrease ($P < 0.05$) in metmyoglobin % for steaks aged 5 days compared to steaks aged 47 and 61 days (figure 2.4). Color stability is decreased because NADH is used for respiration which reduces the amount available for metmyoblobin reducing enzymes to reduce metmyoglobin. Therefore, GM steaks enduring extended aging periods (61 days) may have reduced color stability due to higher OCR. There were no significant differences ($P > 0.05$) between tenderization treatments for OCR (table A.3).

Visual Color

Gluteus medius steaks aged 19 days had ($P < 0.05$) lower initial color scores than steaks aged 5, 47, and 61 days, indicating steaks aged 19 days were lighter red in color (table 2.1). Also, steaks aged 5 days had ($P < 0.05$) the highest initial color panel scores compared to all other aging treatments, or were the darkest red in color. Additionally, initial color panel scores for control steaks were significantly lower than blade tenderized steaks indicating that control samples had a lighter red color (figure 2.5).

No interactions ($P > 0.05$) for aging × tenderization × display for display color panel (table A.12), or discoloration panel scores were found (table A.13). Aging × tenderization interactions ($P < 0.05$) for display color panel (figure 2.6) and discoloration panel scores (figure 2.7). In general, as postmortem aging time increased and steaks were blade tenderized, steaks became darker and more discolored. Non-tenderized treatments had substantially ($P < 0.001$) lower display color panel scores at all aging time periods compared with tenderized GM steaks. As a result, tenderized steaks appeared to be darker red and more tan in color compared with

steaks that were not blade tenderized ($P < 0.001$). Non-blade tenderized steaks also had ($P < 0.05$) lower discoloration scores over the entire aging period compared to tenderized steaks. This would indicate that more brownish-red (metmyoglobin) appearing pigment was present on the surface of steaks that were tenderized compared with controls at all aging periods. It can be concluded that non-tenderized controls would have less discoloration and be brighter red in color compared to tenderized steaks when displayed in a fresh retail meat case given similar aging periods. Aging × display interactions ($P < 0.05$) were observed for display color (figure 2.8) and discoloration panel (figure 2.9). Increasing the aging time and display time made steaks more discolored and dark red to tan colored. Kim et al. (2013) saw similar results as discoloration scores increased as aging time of vacuum packaged lamb loins increased from 14 days to 63 days and an increase in display time from 0 to 8 days.

A tenderization treatment × display day interaction ($P < 0.05$) was observed for display color (figure 2.10) and discoloration panel (figure 2.11). With an increase of display days, steaks that were blade tenderized became darker red to tan in color and more discolored compared to non-blade tenderized steaks.

Instrumental Color

There were no aging × blade tenderization interactions for initial color results (table A.7). *Gluteus medius* steaks aged 47 and 61 days had ($P < 0.05$) higher L*, a*, and b* values than steaks aged 5, 19, and 33 days at 0 hours of display (table 2.1). These results are in alignment with visual color analysis, as steaks aged 47 and 61 days had higher initial color panel scores than 19 and 33 days as they redder in color, but had lower initial color panel scores than steaks aged 5 days as they were lighter in color (table 2.1). In addition, steaks aged 19 days had ($P < 0.05$) decreased a* values compared to all other aging treatments at hour 0 of display (table 2.1).

This was confirmed by the initial color panel results as steaks aged 19 days had the lightest red color (table 2.1).

No three way interaction ($P > 0.05$) between aging × tenderization × display day were found for CIE L*(table A.17), a* (table A.18), and b* values (table A.19). Aging × display interactions ($P < 0.05$) time were found for CIE L* (figure 2.12). L* values were the highest at 0 hours of display for steaks aged 47 days compared to all other aging times. Lagerstedt, Lundstrom, and Lindahl (2011) reported similar results as beef *longissimus dorsi* steaks aged 15 days had higher L* values compared to steaks that had not been aged at 0 hours of display. Vitale, Pérez-Juan, Lloret, Arnau, and Realini (2014) also reported that L* values were the lowest for beef *longissimus thoracis et lumborum* steaks that were not aged compared to aging steaks for 3 to 21 days at 0 hours of display. In the current study, L* values decreased ($P < 0.05$) from 0 to 96 hours of display for GM steaks aged 5 and 47 days, but did not effect steaks aged 19 and 33 days. However, Vitale et al. (2014) reported that L* values increased during display time for steaks aged between 0 and 21 days. Vitale et al. (2014) only let steaks bloom for 30 minutes compared to 120 minutes in the current study. Lee, Apple, Yancey, Sawyer, and Harris (2008) reported that GM steaks bloomed 30 minutes had significantly lower L* values than GM steaks bloomed 120 minutes.

Furthermore, aging × display time interactions ($P < 0.05$) were found for CIE a* (figure 2.13). *Gluteus medius* steaks aged 47 days had increased ($P < 0.05$) a* values than steaks aged 5, 19, and 33 days at 0 hours of display. However, as display time increased to 96 hours steaks aged 47 days became ($P < 0.05$) less red in color. Lagerstedt et al. (2011) also reported that beef *longissimus dorsi* aged for 15 days had ($P < 0.05$) higher a* values than steaks aged only 3 or 5 days in vacuum packaging at 0 hours of display, but became ($P < 0.05$) the lowest at 120 hours

of display. MacDougall (1972) attributes the increase in redness to the deeper layer of oxymyoglobin from increased oxygen penetration. Furthermore, steaks aged 5 and 33 days decreased ($P < 0.05$) in a* values as display time increased. Apple et al. (2014) also reported beef *gluteus medius* aged 14 days decreased in a* values as display time increased. Although in the current study, steaks aged 19 days had decreased ($P < 0.05$) a* values at 0 hours of display compared to steaks aged 5, 19, and 47 days, steaks aged 19 days did not change in a* values over the entire display period.

Aging × display interactions ($P < 0.05$) were also found for CIE b* (figure 2.14). *Gluteus medius* steaks aged 47 days had the highest b* values compared to all other steaks aged 5, 19, and 33 days at 0 hours of display, but decreased ($P < 0.05$) at 60 hours of display. These results are in alignment with Lagerstedt et al. (2011) as they saw that beef *longissimus dorsi* steaks aged for 15 days had higher b* values compared to steaks aged 3 or 5 days in vacuum packaging at 0 hours of display. Apple et al. (2014) also reported that GM steaks are more yellow in color when initially placed into a retail display case, but become less yellow as display time increased. Steaks aged 5, 19, and 33 days were similar ($P > 0.05$) in b* values throughout the entire display period, until hour 84 of display where steaks aged 33 days increased in b* values. In conclusion, an increase in aging caused steaks to be more lighter, redder, and more yellow in color initially, darker, less red, and less yellow in color as time in the display case increased. No aging × tenderization (table A.1) or tenderization × display day (table A.16) interactions ($P > 0.05$) were found for L* a*, and b* values.

Tenderness

Warner-Bratzler Shear Force (WBSF)

There was an aging × tenderization interaction ($P < 0.05$) for WBSF measurements (figure 2.15). As expected, steaks that were blade tenderized and aged longer had lower WBSF values, except for steaks aged 61 days as blade tenderized and non-blade tenderized steaks were similar. As steaks are aged longer and blade tenderized they become more tender (George-Evins et al., 2004; Savell, McKeith, Murphey, Smith, & Carpenter, 1982). King et al. (2009) also found a blade tenderization x aging time interaction in beef *longissimus lumborum* steaks, but not in beef *gluteus medius* steaks. King et al. (2009) reported that blade tenderization of beef *longissimus lumborum* steaks had ($P < 0.05$) lower slice shear force values compared to their non-blade tenderized counterparts. However in the present study, non-blade tenderized GM steaks aged 61 day were not only more tender than control GM steaks aged 5 days, but also more tender than blade tenderized GM steaks aged 5 days, as well as being similar to blade tenderized steaks aged 19, 33, and 47 days. In addition, King et al. (2009) also reported that aging only improved tenderness for non-blade tenderized beef *longissimus lumborum* steaks. In the current study, it was shown that blade tenderized GM steaks aged 19, 33, 47, and 61 days were all significantly more tender than blade tenderized steaks aged 5 days.

Both aging and blade tenderization have been previously reported to decrease shear-force values. As the beef *longissimus dorsi* is aged longer, shear force values decrease (Bidner, Montgomery, Bagley, & McMillin, 1985; Jeremiah & Gibson, 2003; King et al., 2009; Monson, Sañudo, & Sierra, 2004; Monson, Sañudo, & Sierra, 2005; Savell et al., 1982). Blade tenderization has been shown to significantly decrease shear force values in beef top sirloin butts (George-Evins et al., 2004; Savell, et al., 1982).

Microbiology

Lactic Acid Bacteria (LAB) Enumeration

There was no significant aging × tenderization interaction found for LAB numeration (table A.1). Blade tenderization did not ($P > 0.05$) affect the number of lactic acid bacteria, indicating that there was not a significant amount of translocation of lactic acid bacteria into the interior of the steaks (table A.3). These results follow the findings of Benito-Delgado, Marriott, Claus, Wang, and Graham (1994) as they saw there was ($P > 0.05$) no difference in microbial load between blade tenderized beef *longissimus dorsi* and *infraspinatus* steaks and non-blade tenderized steaks. The reason for not seeing translocation of LAB may be due to the trimming of excess fat of the top sirloin butts prior to blade tenderizing in the present study. Lemmons, Lucia, Hardin, Savell, and Harris (2011) reported that trimming the exterior surface prior to blade tenderization of inoculated beef top sirloin butts aged for 28 days decreased ($P < 0.05$) the amount of translocated *Escherichia coli 0157:H7* into the interior of the steaks.

However, aging treatment significantly affected LAB enumeration ($P < 0.05$), as longer aging periods increased LAB counts (figure 2.16) from 1.17 log CFU/g at 5 days compared with 3.85 log CFU/g at 61 days. Other studies have also showed that increasing aging time of vacuum packaged beef increases LAB counts (Hanna, Vanderzant, Carpenter, & Smith, 1977; Hanna, Hall, Smith, & Vanderzant, 1980; Hodges, Cahill, & Ockerman, 1974; Minks & Stringer, 1972; Nissen, Sorheim, Dainty, 1996). Several studies have shown that the initial lactic acid bacteria counts have the greatest impact on the final enumeration of sub-primals, whether they are aged or blade tenderized (Hanna, Smith, Hall, & Vanderzant, 1979; Hanna et al., 1980; Ray et al., 2010).

pH

There was no aging × tenderization interaction ($P > 0.05$) for mean values of pH (table A.1). There were significant differences ($P < 0.05$) between aging treatments for pH (figure

2.17). Top sirloins aged for 33 days had ($P < 0.05$) the lowest pH (5.42) compared to all other aging treatments. Furthermore, GM steaks aged 47 days had ($P < 0.05$) lower pH than steaks aged 5 or 61 days. There were no significant differences ($P > 0.05$) between tenderization treatments for pH (table A.3).

Sensory Analysis

There was an aging × tenderization interaction ($P < 0.05$) for overall tenderness, myofibrillar tenderness, bloody/serumy, metallic, overall sweet, and bitter (table 2.2). Blade tenderized steaks aged 61 days received ($P < 0.05$) the highest overall tenderness scores compared to all other treatments. Furthermore, steaks that were blade tenderized had ($P < 0.05$) higher overall tenderness scores at each aging period compared with their non-blade tenderized counterparts. In addition, non-blade tenderized (control) steaks aged 5, 19, and 33 days had ($P < 0.05$) the lowest scores for overall tenderness. George-Evins et al. (2004) and Savell et al. (1982) also reported that blade tenderized steaks were more tender than non-blade tenderized steaks at all aging periods. The increase in overall tenderness may be due to the decreased toughness perceived from panelists by the disruption of connective tissue by blade tenderization (Bidner et al., 1985; Seideman, Smith, Carpenter, & Marshall, 1977). Additionally, blade tenderized steaks aged 5, 19, 33, and 47 days had ($P < 0.05$) higher myofibrillar tenderness scores compared to their non-blade tenderized counterparts. Non-blade tenderized steaks aged 61 days received ($P < 0.05$) higher myofibrillar tenderness scores than steaks aged 5, 19, 33, and 47 days. Non-blade tenderized steaks aged 47 days had ($P < 0.05$) lower bloody/serumy flavor scores than non-blade tenderized steaks aged 5 and 61 days, as well as blade tenderized steaks aged 5 and 19 days. Blade tenderized steaks aged 19 and 61 days had ($P < 0.05$) higher metallic flavor scores than blade tenderized steaks aged 5 days, whereas the non-blade tenderized steaks

stayed relatively constant and displayed no differences across all aging periods. Also, blade tenderized steaks aged 61 days had the lowest overall sweet flavor scores compared to blade tenderized and non-blade tenderized steaks aged 5, 19, and 33 days. Additionally, bitter flavors increased ($P < 0.05$) for blade tenderized steaks aged 5 days compared to blade tenderized steaks aged 19 days and longer. Non-blade tenderized steaks aged 33 days had ($P < 0.05$) lower bitter flavor scores compared to non-blade tenderized steaks aged 47 days.

There were significant differences ($P < 0.05$) between aging treatments for sensory warmed-over flavor and fat like flavors (table 2.3). Steaks aged 61 days had ($P < 0.05$) lower fat like flavor than steaks aged 19 and 33 days. In addition, steaks aged 5 days had decreased warmed-over flavor in comparison to steaks aged 33 days and longer. Stetzer et al. (2008) also saw as aging increased of beef sub-primals there was an increase in flavor volatiles that are associated with lipid oxidation. Finally, warmed-over flavor has been associated with an increase of TBARS values, as malonaldehyde is a product of lipid oxidation and can alter lipid precursors of meat flavor (Smith, Salih, & Morgan, 1987; St. Angelo et al., 1987).

Steaks that were blade tenderized had ($P < 0.05$) increased rancid and spoiled flavors compared to control steaks (table 2.4). This may be due to translocation of products of phospholipids found in cell membranes to the interior of the steak which would cause a rancid and spoiled flavor note upon cooking. Campo et al. (2006) reported as TBARS values increased, that rancid flavors increased in beef *longissimus dorsi*.

Conclusions

An increase in aging time of beef *gluteus medius* increased lipid oxidation. Also, as beef top sirloin butts were aged longer, *gluteus medius* steaks became lighter, more red, and more yellow in color initially, but became darker, less red, less yellow, and more discolored as display

time increased. This could be caused by decreased metmyoglobin reducing activity and increased oxygen consumption rate for steaks aged extended periods. Furthermore, alternative packaging methods besides aerobic overwrap packaging, should be considered for steaks from top sirloin butts aged 47 days or longer.

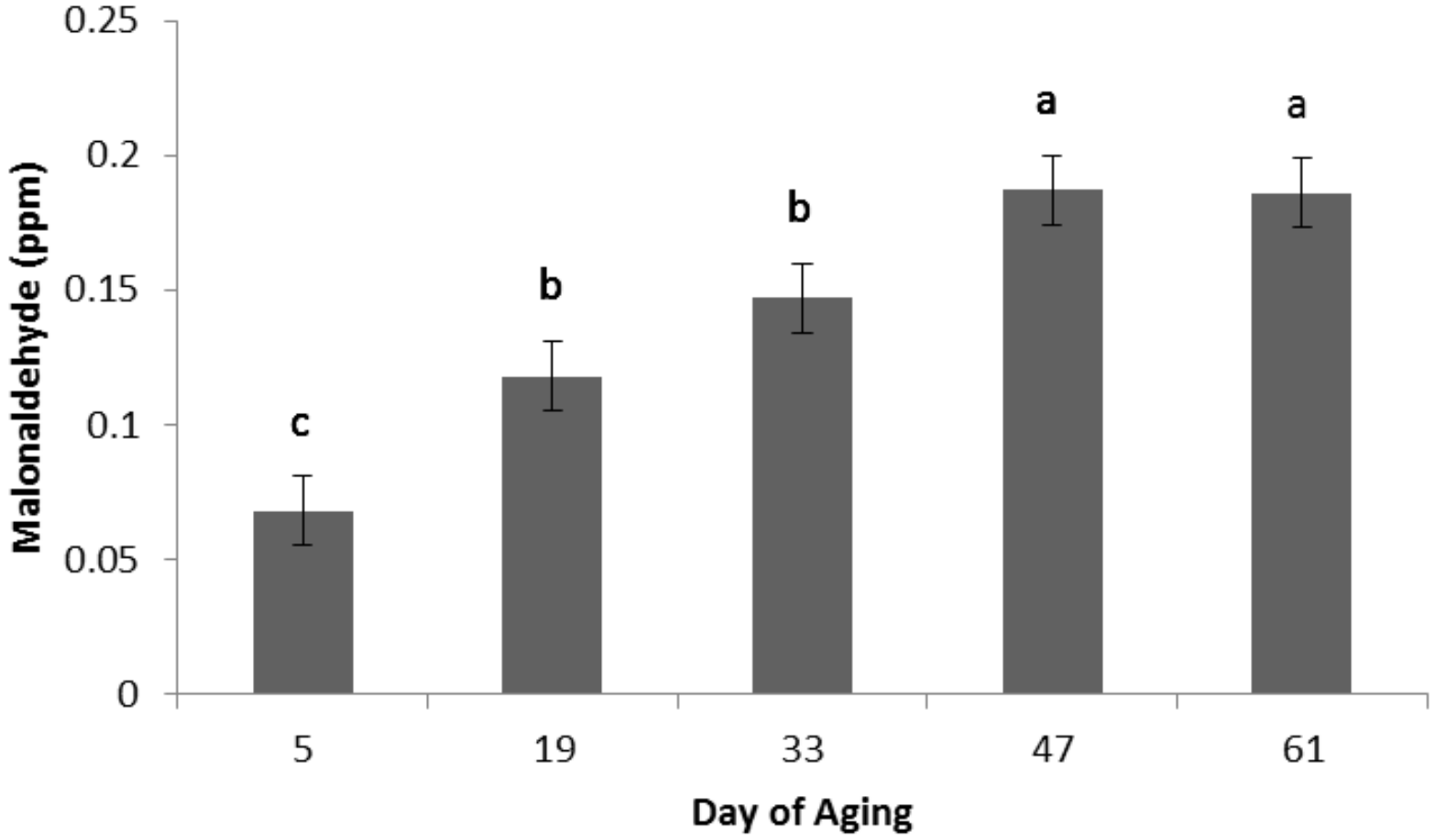
Although blade tenderization did not affect metmyoglobin reducing ability or oxygen consumption rate, GM steaks that were blade tenderized became more discolored than non-blade tenderized steaks, in a retail case. To our knowledge, this is the first time to show that blade tenderization has a negative effect on color stability. Further research is necessary to evaluate the mechanics associated with the more rapid discoloration of blade tenderized steaks.

Postmortem aging proved to be an effective way to tenderize the beef *gluteus medius,* while blade tenderization provided additional tenderization at each aging period. Non-blade tenderized steaks aged for 61 days were more tender than control and blade tenderized steaks that were aged for 5 days, both instrumentally and in the descriptive sensory analysis panel. There was no tenderization benefit to age blade tenderized steaks past 19 days.

Bitter flavors increased while bloody/serumy and overall sweet flavors decreased as aging time increased for blade tenderized steaks. Additionally, warmed-over flavors increased with an increase in aging time. Finally, in this study blade tenderization increased the level of rancid and bitter flavors compared with controls. Processors looking to age top sirloin butts for extended periods of time or blade tenderize for benefits of tenderness should be aware of the negative effects on color and flavor stability. In conclusion, *gluteus medius* steaks destined for the retail sector should be aged as short as possible and not blade tenderiezed to increase color stability throughout display, whereas *gluteus medius* steaks utilized in the food service sector

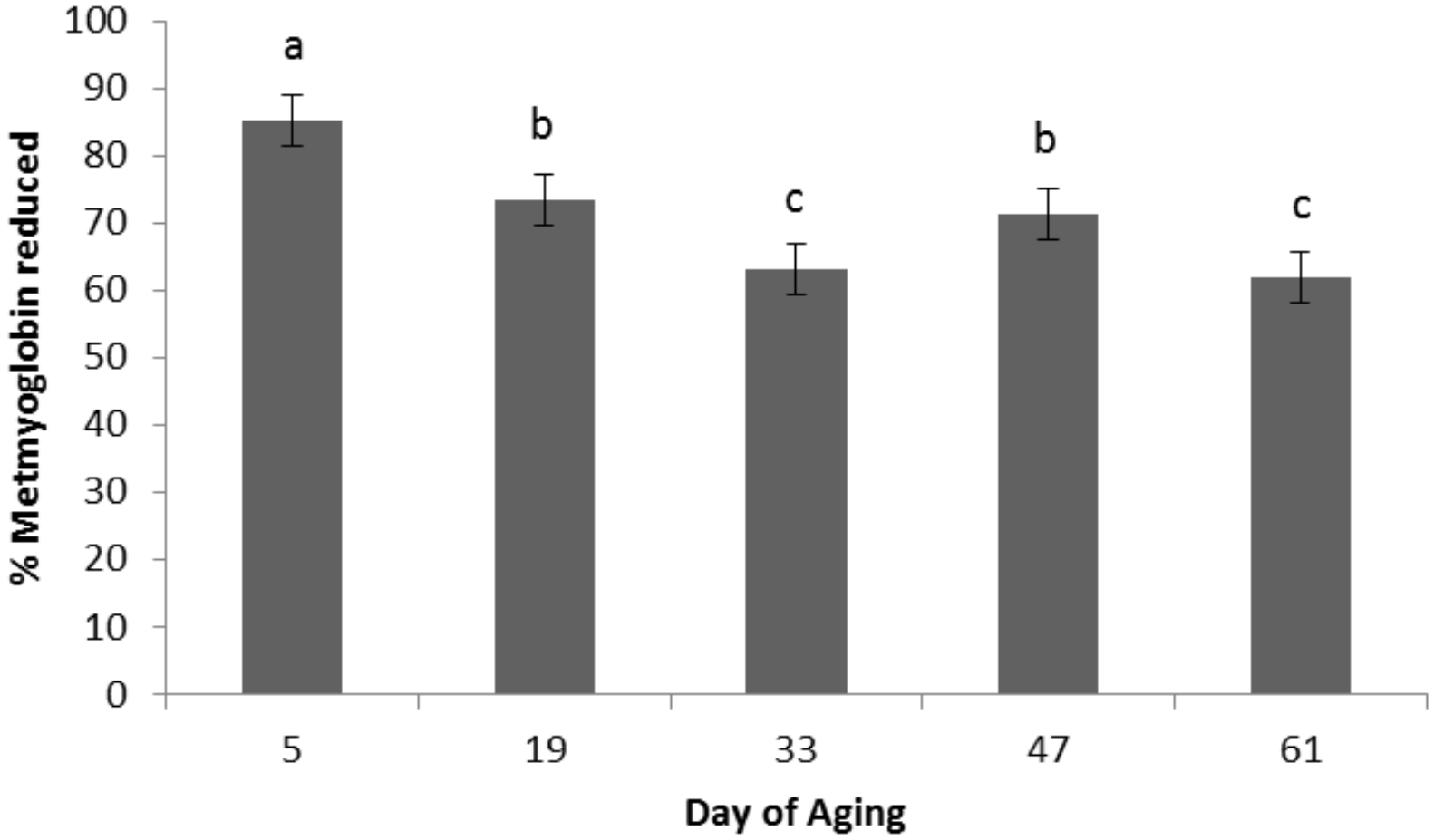
should be aged for 19 days then blade tenderize to increase tenderness, but avoid the development of undesirable flavors.

Figure 2.1 Least squares means of Thiobarbituric acid reactive substances (TBARS) values for beef *gluteus medius* steaks over 5 different postmortem aging treatments (SEM = 0.0179).



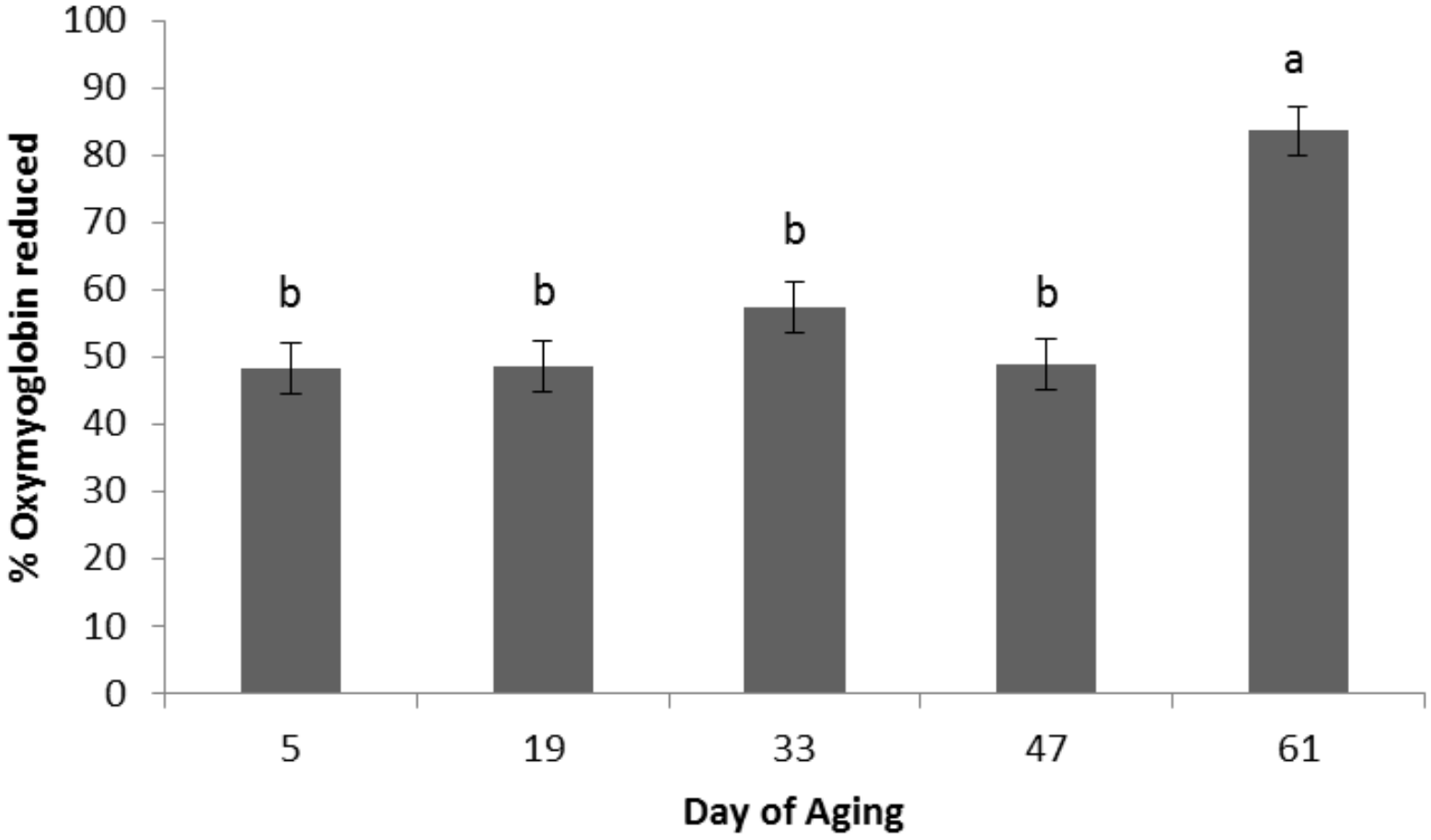
[a-c]Means with different superscripts differ ($P<0.05$).

Figure 2.2 Least squares means of metmyoglobin reducing ability (MRA) values for beef *gluteus medius* steaks over 5 different postmortem aging treatments (SEM = 3.78).



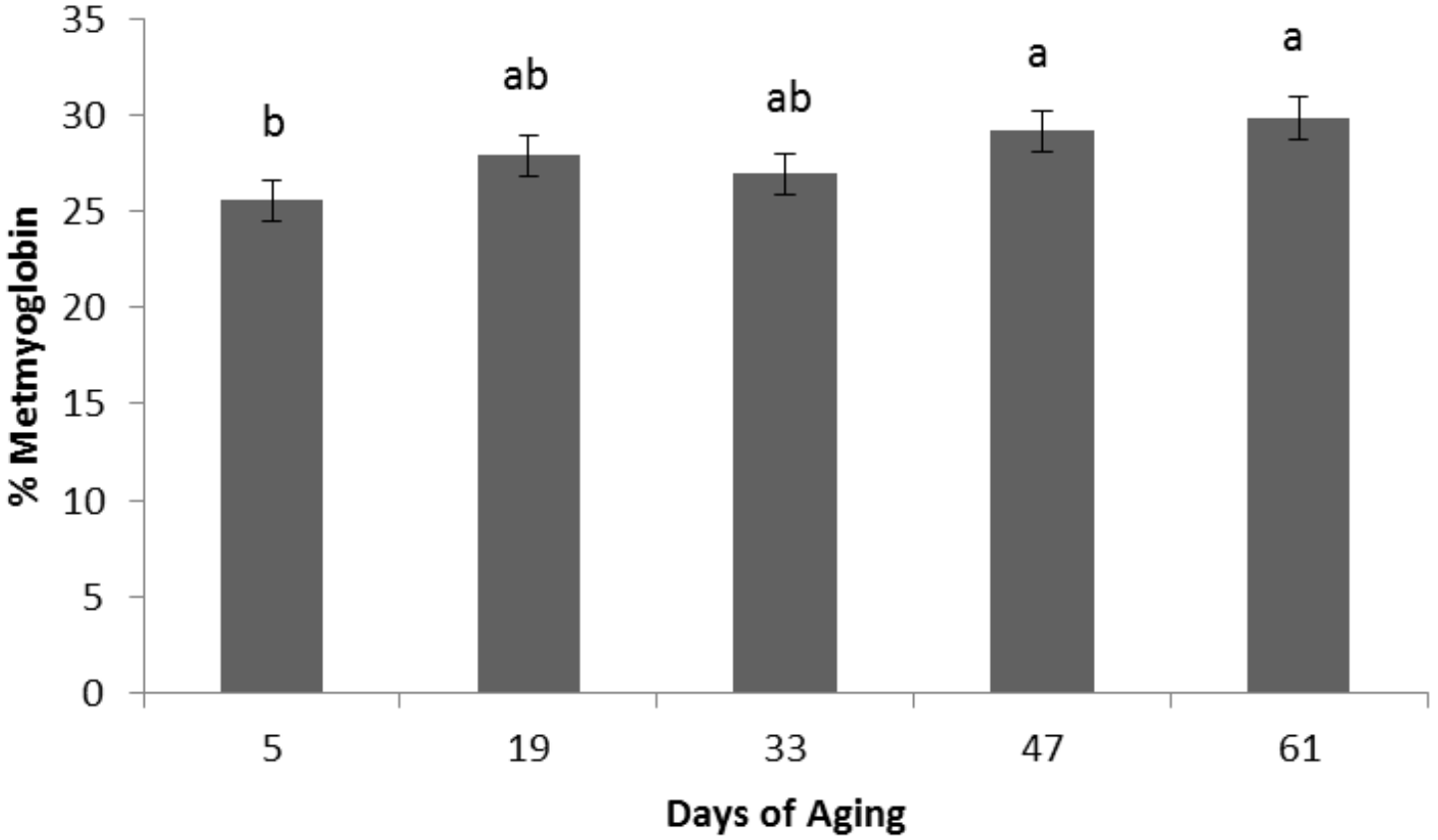
[a-c]Means with different superscripts differ ($P<0.05$).

Figure 2.3 Least squares means of oxygen consumption rate (OCR) values for beef *gluteus medius* steaks over 5 different postmortem aging treatments (SEM = 4.42).



[a-b]Means with different superscripts differ ($P<0.05$).

Figure 2.4 Least squares means of final metmyoglobin % for oxygen consumption rate (OCR) assay for beef *gluteus medius* steaks over 5 different postmortem aging treatments (SEM = 1.085).



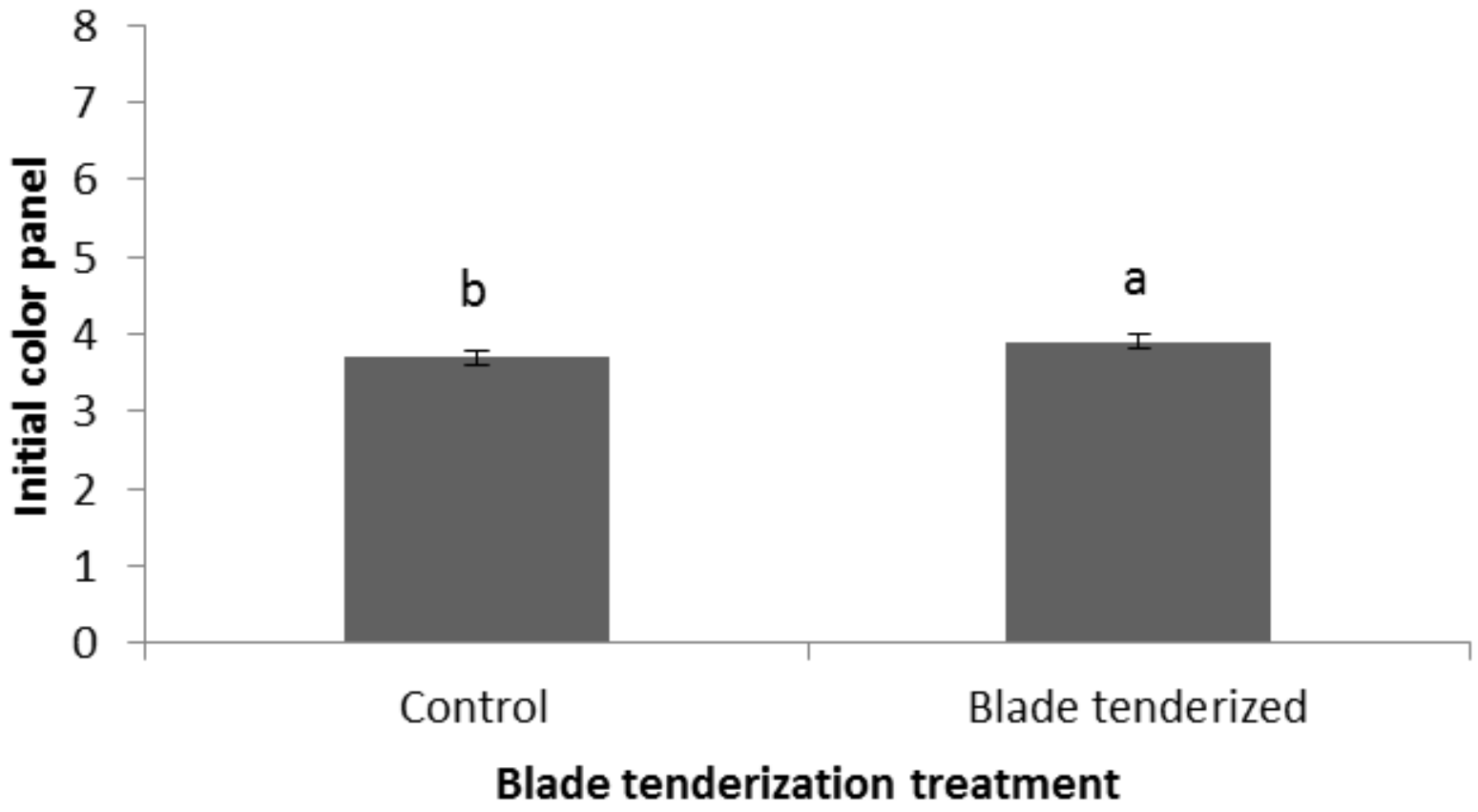
[a-b]Means with different superscripts differ ($P<0.05$).

Table 2.1 Least squares means of aging for initial color results of beef *gluteus medius* steaks subjected to different postmortem aging treatments.

Trait	Aging Period					SEM[1]
	5	19	33	47	61	
Initial color panel[2]	4.5[a]	3.5[d]	3.6[cd]	3.9[b]	3.7[bc]	0.12
Display color panel[3]	2.9[b]	2.9[b]	2.7[b]	5.9[a]	5.7[a]	0.19
L*[4]	48.1[b]	46.9[b]	49.3[b]	56.1[a]	56.6[a]	1.10
a*[5]	30.8[c]	24.9[d]	32.5[c]	36.8[b]	40.4[a]	1.20
b*[6]	22.6[b]	22.5[b]	25.6[b]	38.6[a]	38.5[a]	1.54
Hue angle[7]	36.3[c]	42.2[b]	37.9[c]	46.4[a]	43.1[b]	0.861

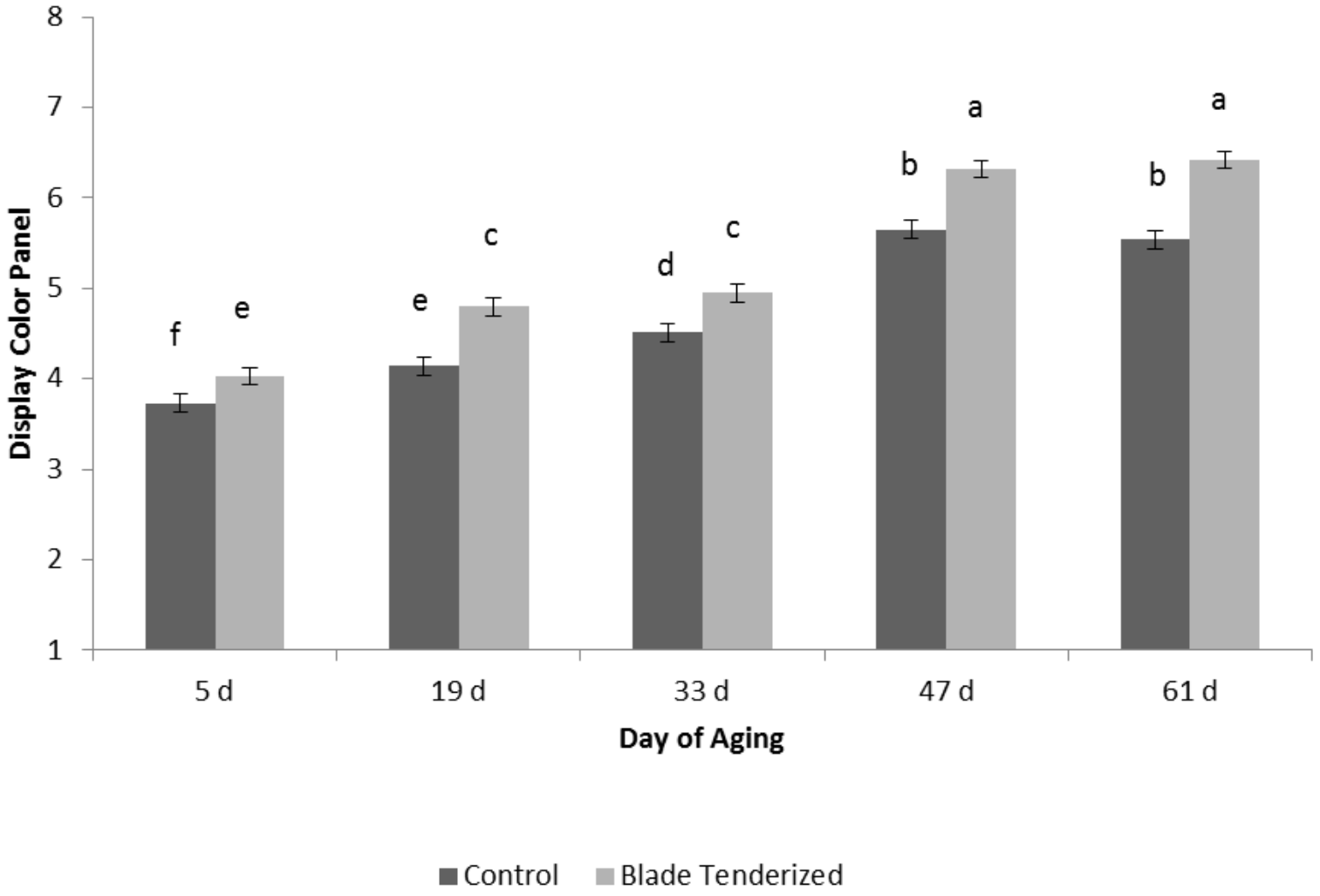
[a-d]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]1=Bleached Red, 2=Very light cherry-red, 3=Moderately cherry-red, 4=Cherry-red, 5=Slightly dark red, 6=Moderately dark red , 7=Dark red, 8=Very dark red
[3]1=Very bright red, 2=Bright red, 3=Dull red, 4=Slightly dark red, 5=Moderately dark red, 6=Dark red to dark reddish tan, 7=Tannish red, 8=Tan to brown
[4]L* lightness (0=black, 100=white)
[5]a* redness/greenness (positive values = red, negative values = green)
[6]b* yellowness/blueness (positive values= yellow, negative values=blue)
[7]Hue angle (reported in degrees) represents the change from the true red axis (a larger hue angle indicates a greater shift from red to yellow)

Figure 2.5 Least squares means of initial color panel score for beef *gluteus medius* steaks with and without blade tenderization (SEM = 0.095).



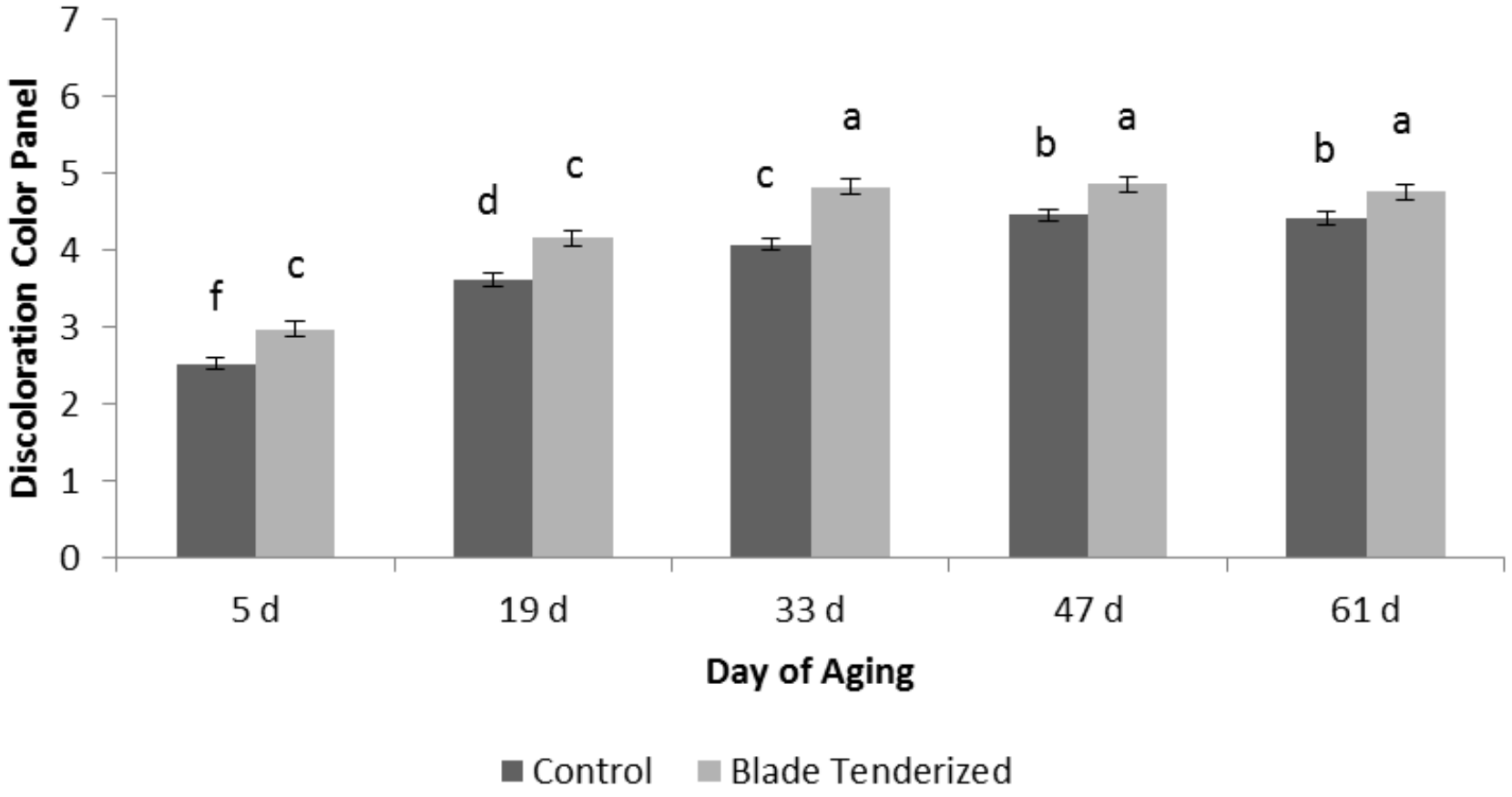
[a-b]Means with different superscripts differ ($P<0.05$).

Figure 2.6 Least squares means for aging × blade tenderization interactions for display color panel scores of beef *gluteus medius* steaks (SEM = 0.099).



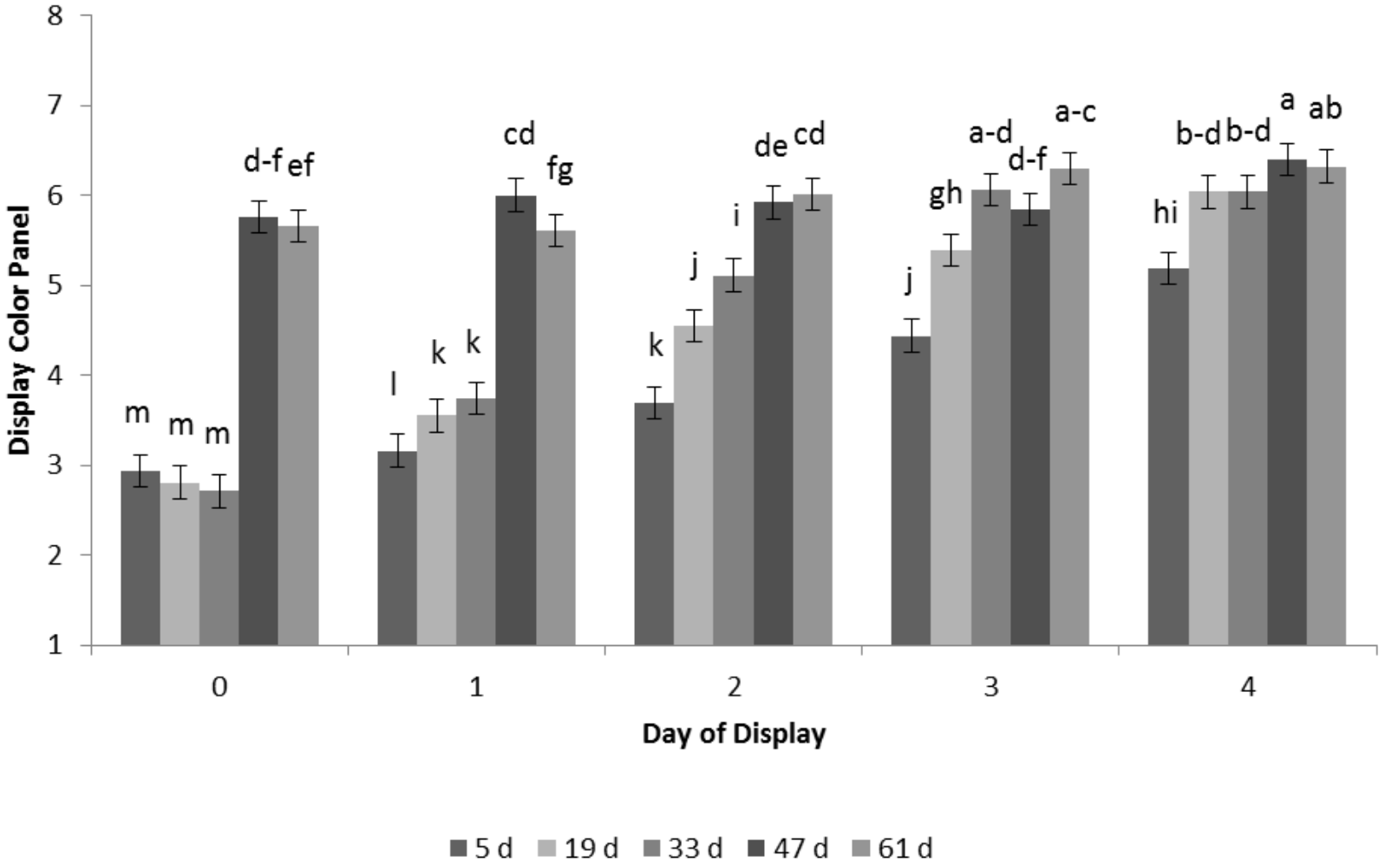
[a-f]Means with different superscripts differ ($P<0.05$).

Figure 2.7 Least squares means for aging × blade tenderization interactions for discoloration panel scores of beef *gluteus medius* steaks (SEM = 0.099).



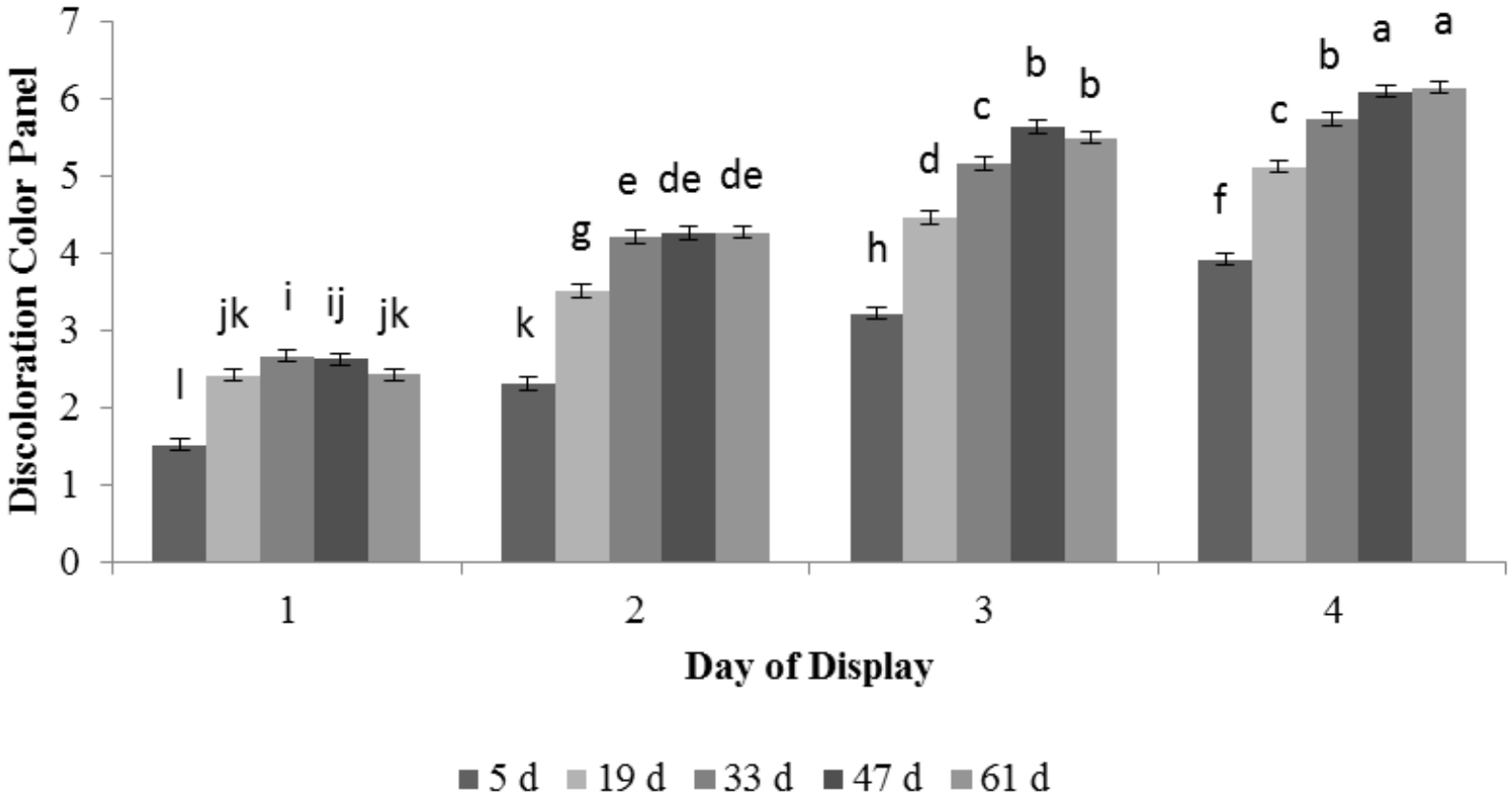
[a-f]Means with different superscripts differ ($P<0.05$).

Figure 2.8 Least squares means for aging × display interactions for display color panel scores of beef *gluteus medius* steaks (SEM = 0.22).



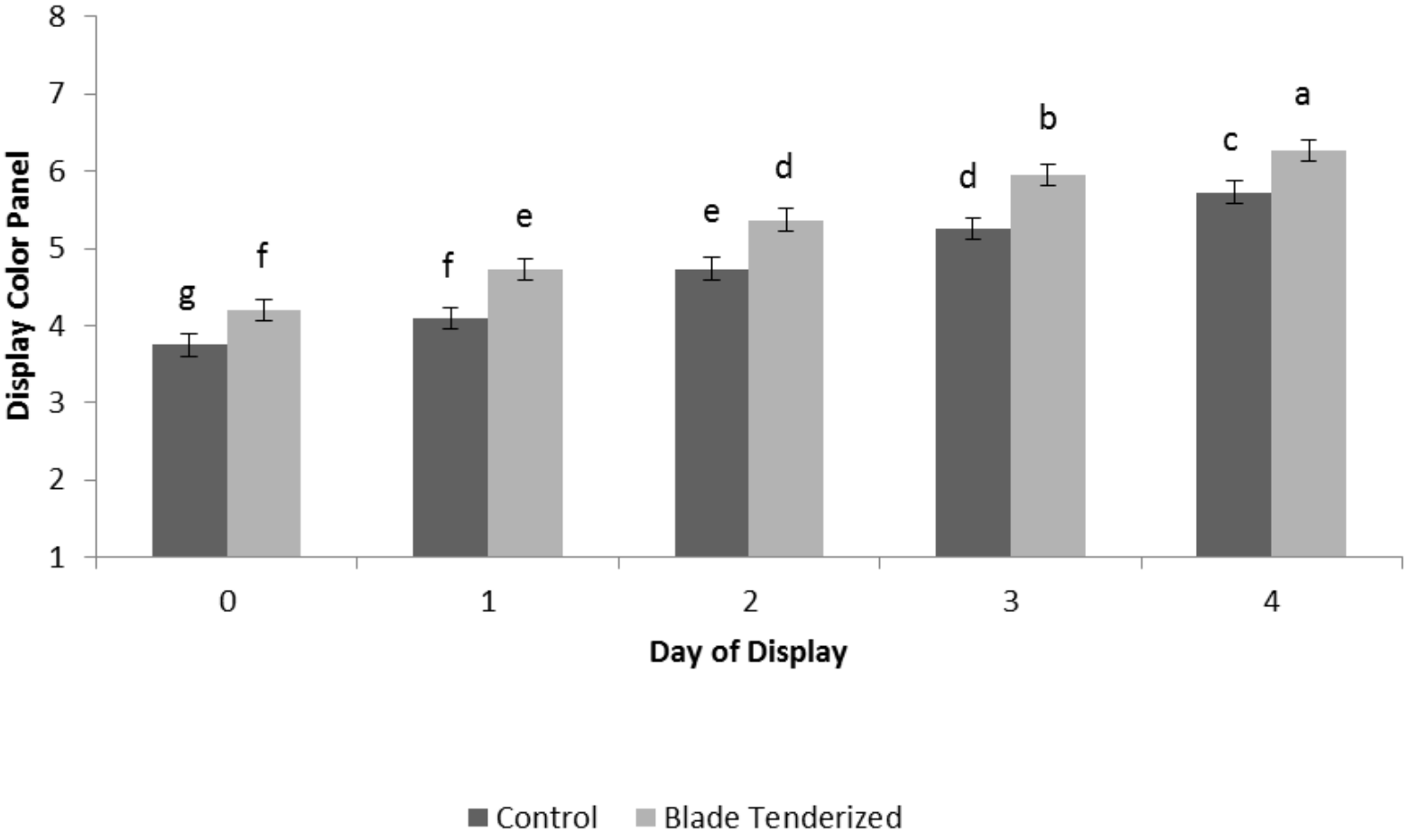
[a-m]Means with different superscripts differ (P<0.05).

Figure 2.9 Least squares means for aging × display interactions for discoloration color panel scores of beef *gluteus medius* steaks (SEM = 0.17).



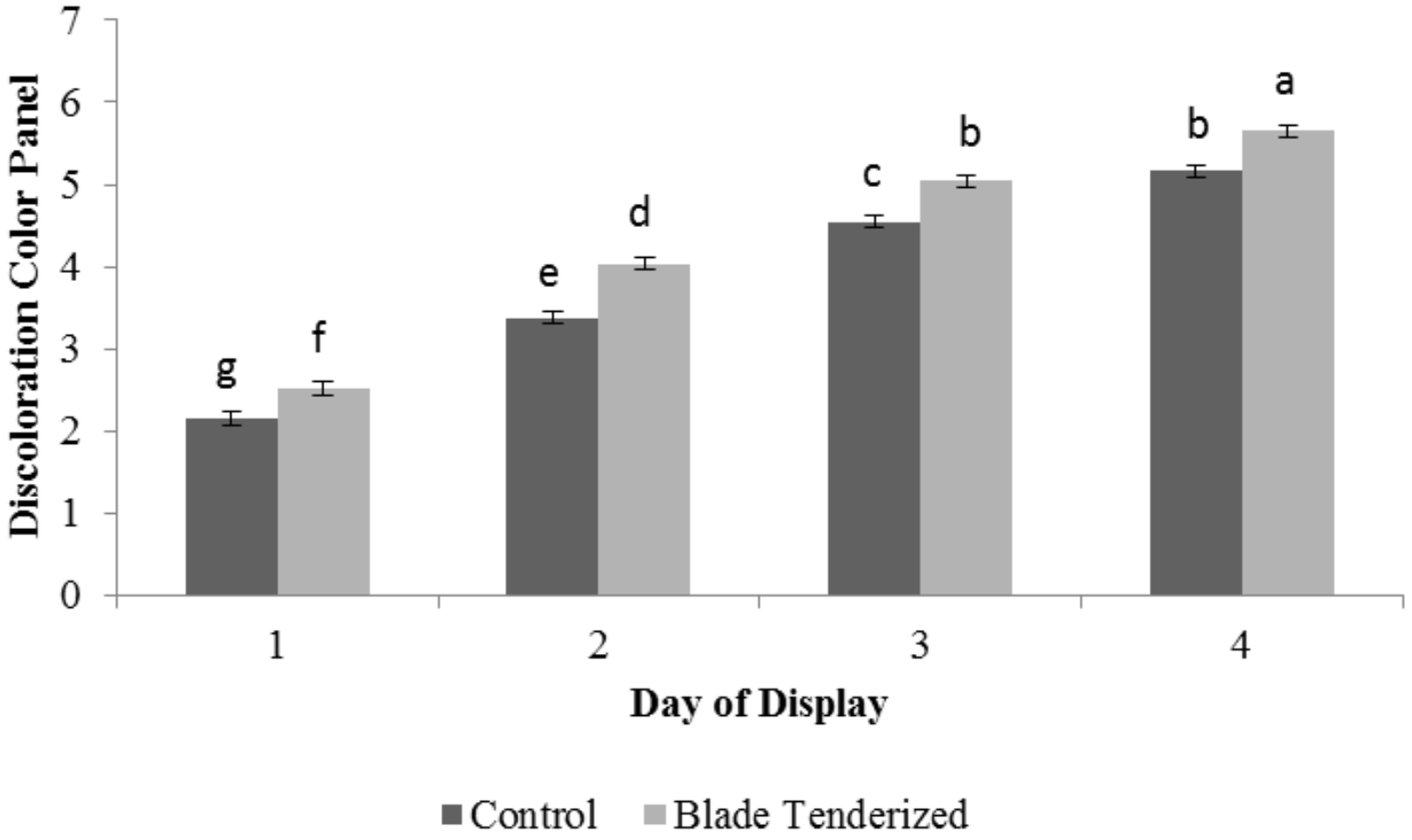
[a-l]Means with different superscripts differ ($P<0.05$).

Figure 2.10 Least squares means for tenderization × display interactions for display color panel scores of beef *gluteus medius* steaks (SEM 0.14).



[a-g]Means with different superscripts differ ($P<0.05$).

Figure 2.11 Least squares means for tenderization × display interactions for discoloration color panel scores of beef *gluteus medius* steaks (SEM = 0.095).



[a-g]Means with different superscripts differ ($P<0.05$).

Figure 2.12 Least squares means for aging × display interactions for L* values of beef *gluteus medius* steaks (SEM = 1.27).

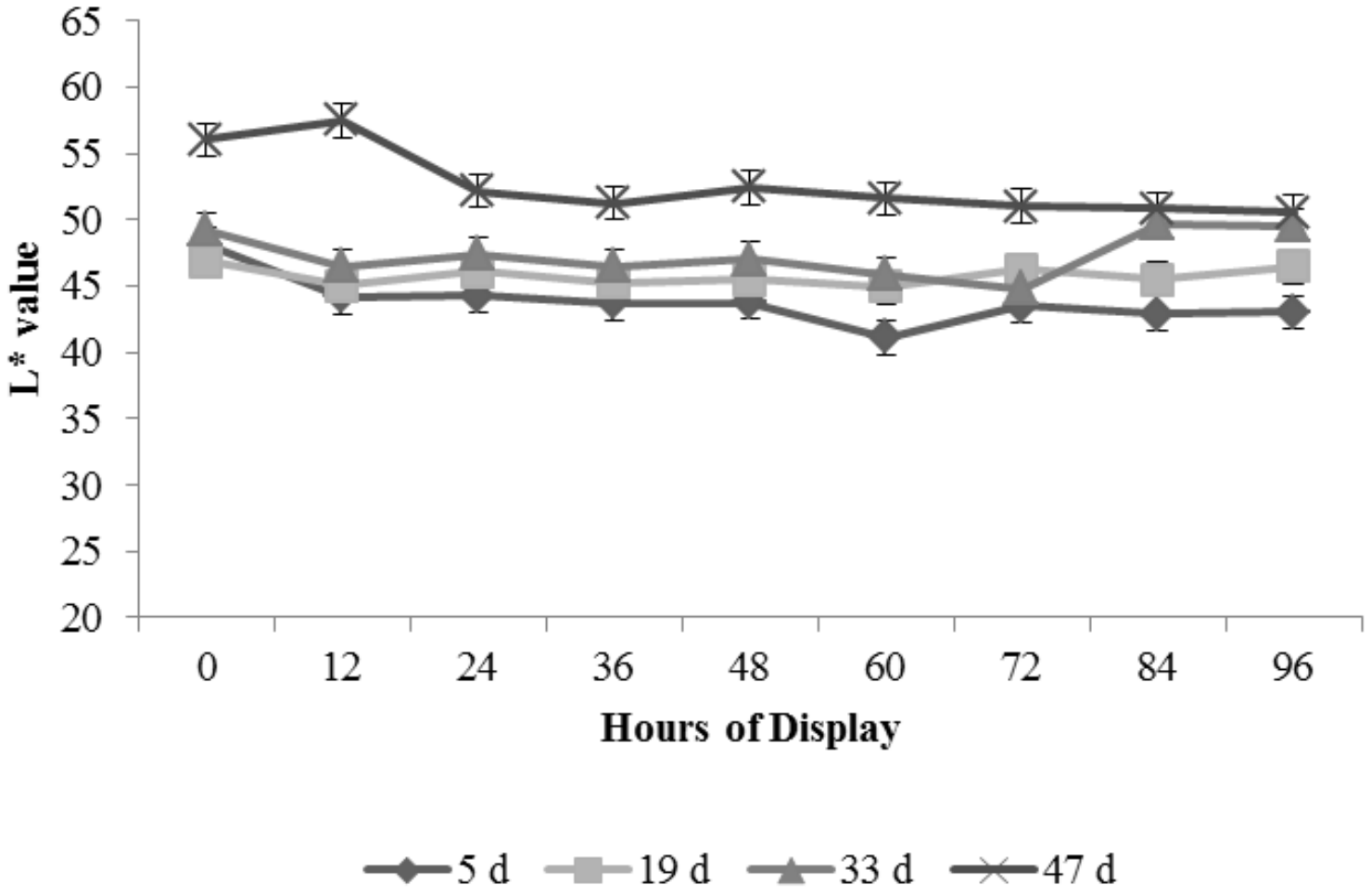


Figure 2.13 Least squares means for aging × display interactions for a* values of beef *gluteus medius* steaks (SEM = 1.13).

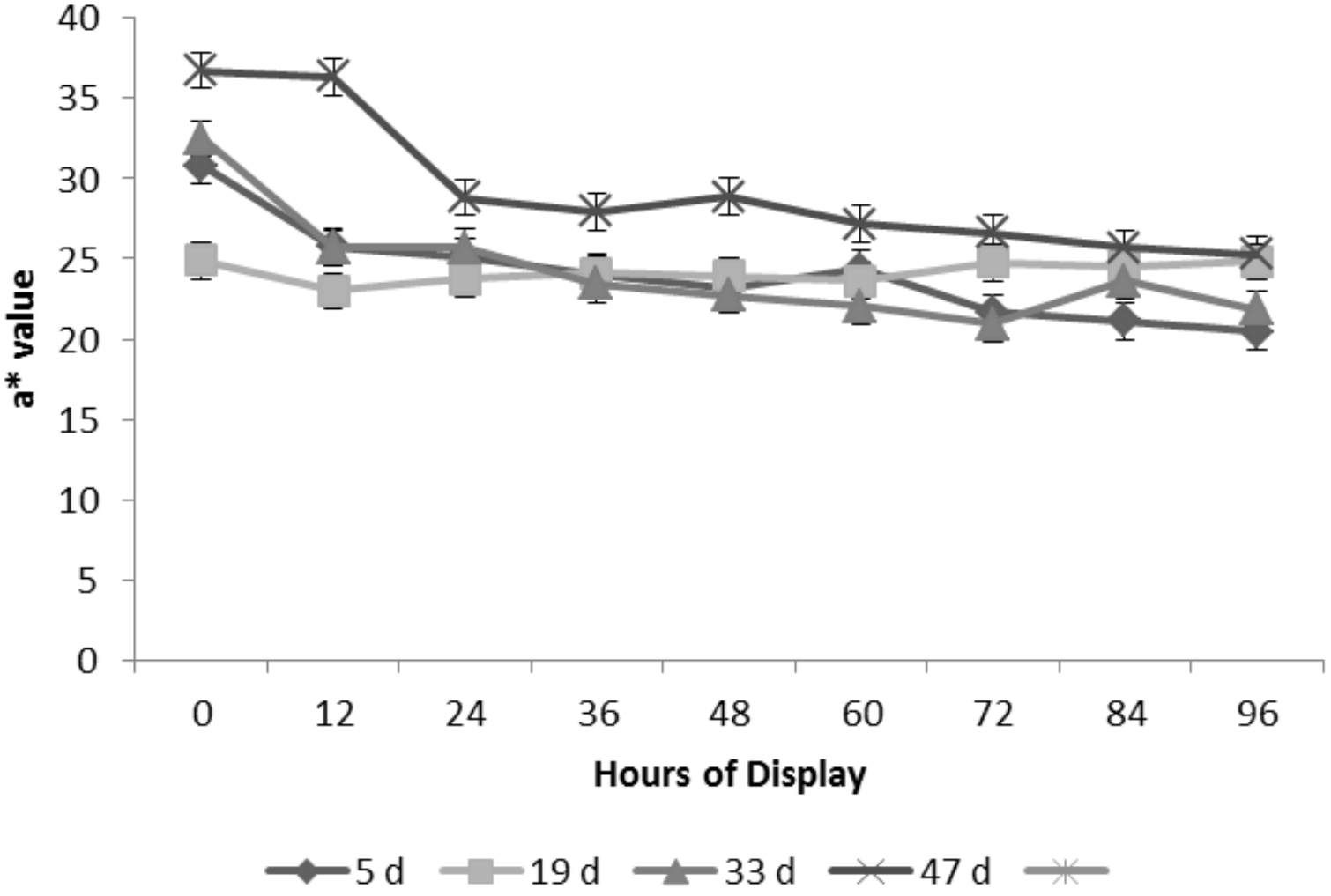


Figure 2.14 Least squares means for aging × display interactions for b* values of beef *gluteus medius* steaks (SEM = 1.67).

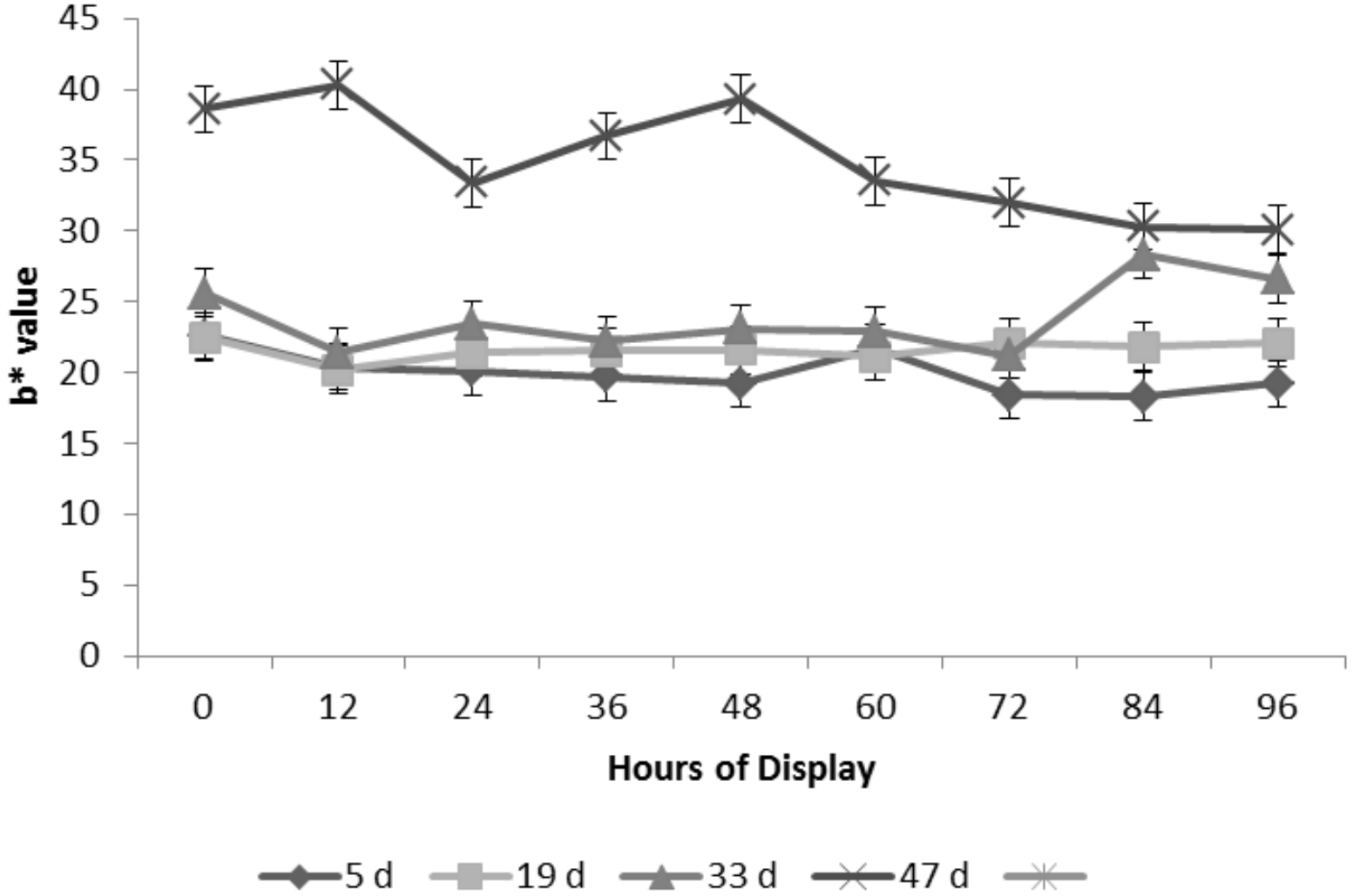
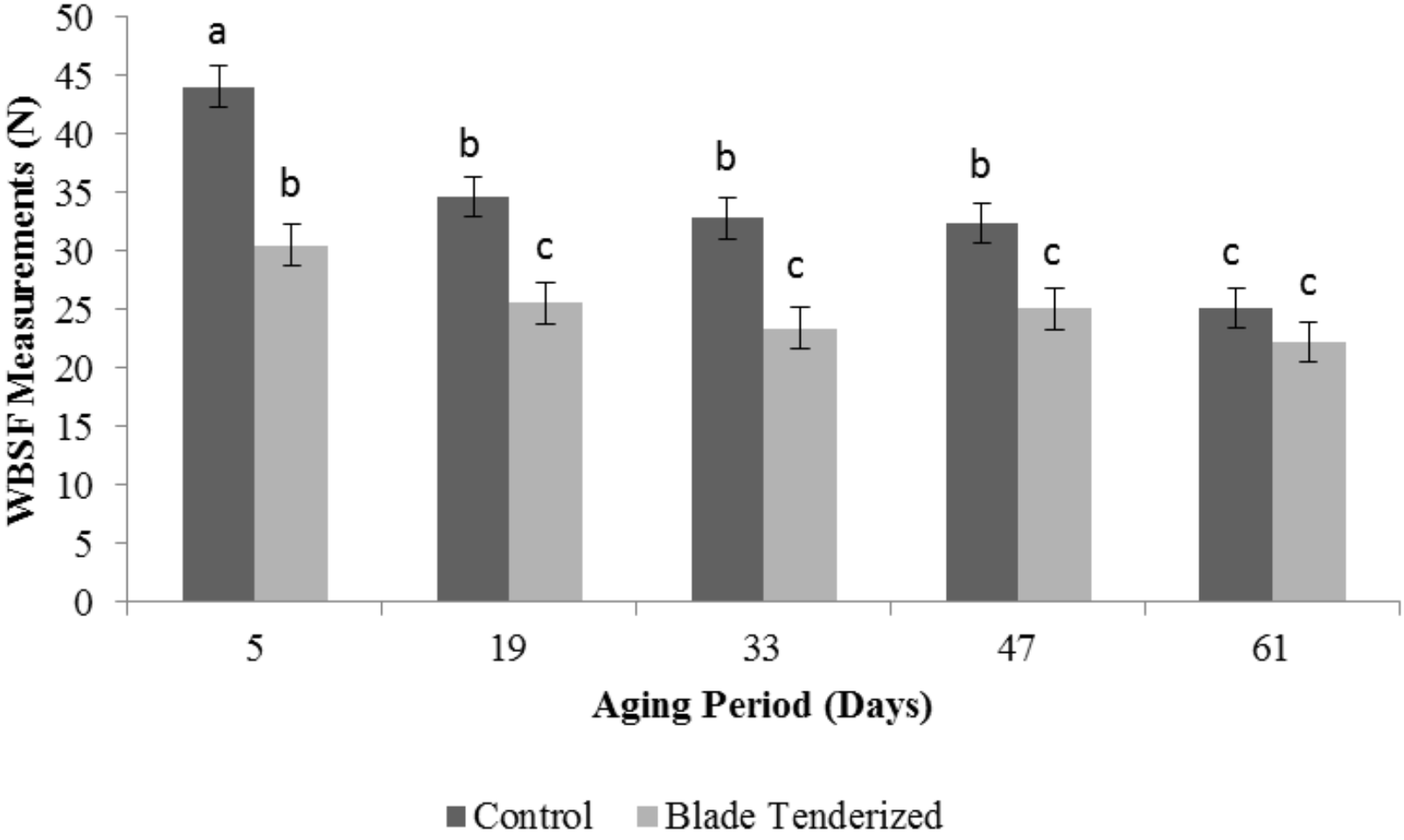
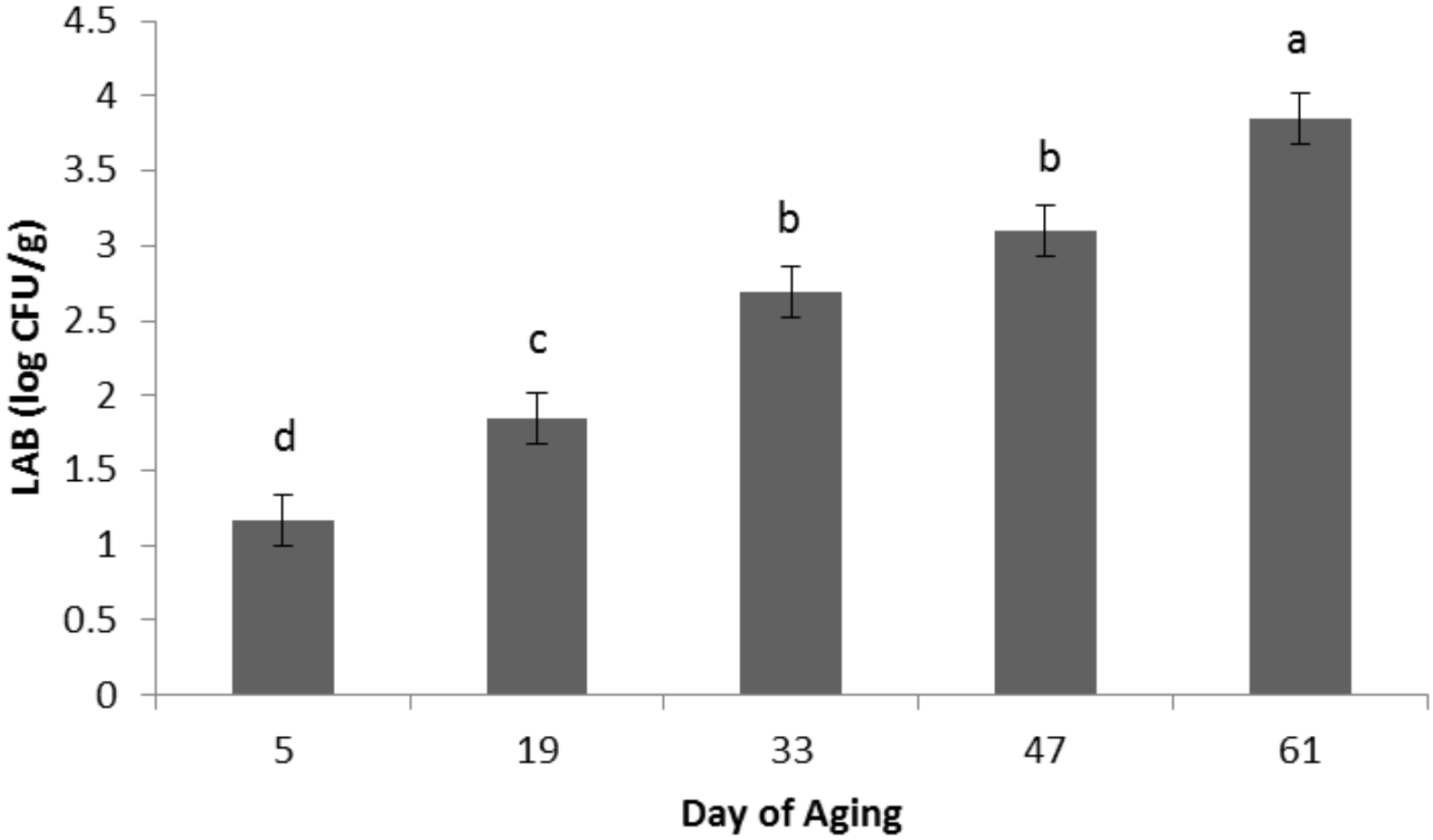


Figure 2.15 Least squares means for aging × tenderization interactions for Warner-Bratzler shear force values of beef *gluteus medius* steaks (SEM =1.74).



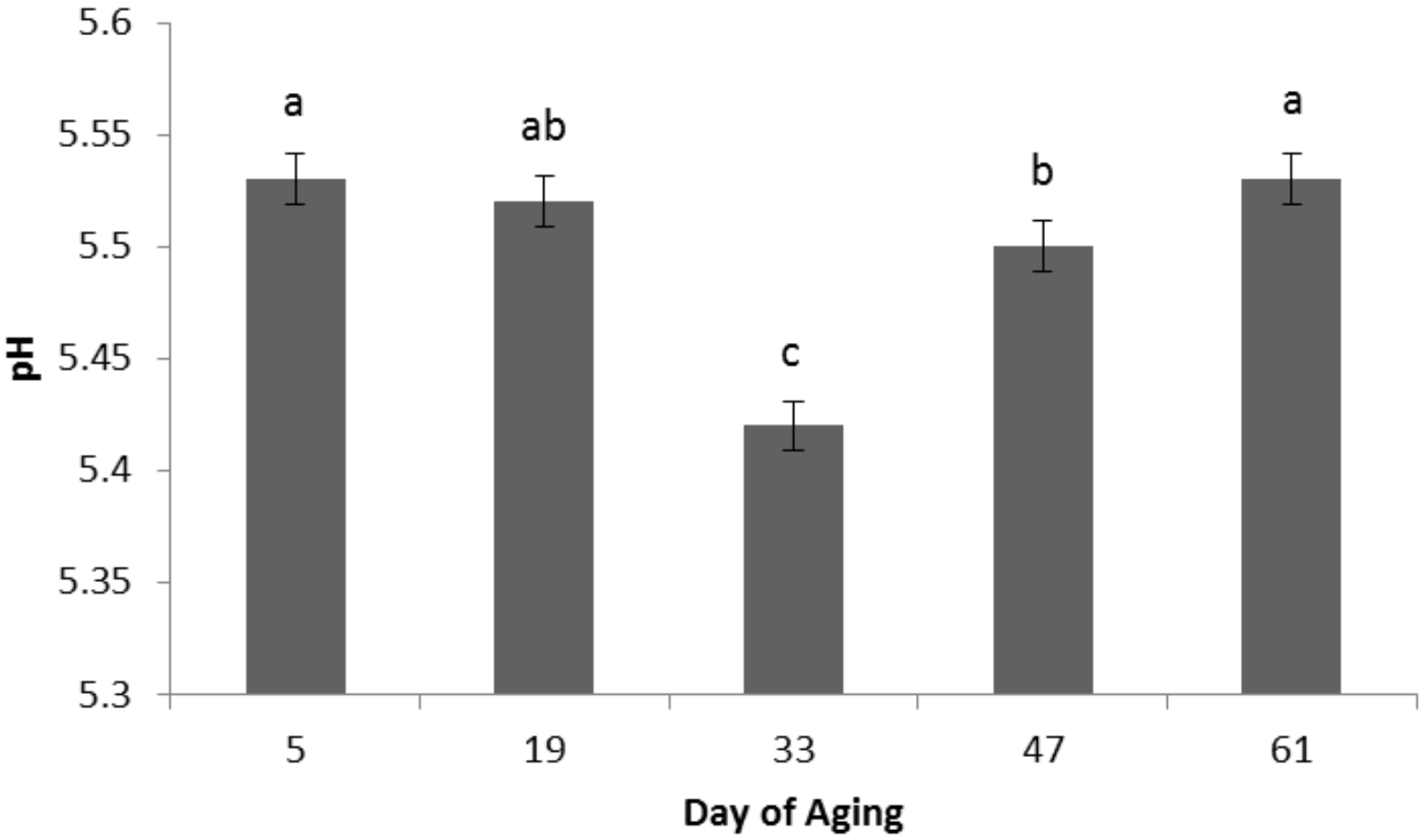
[a-c]Means with different superscripts differ ($P<0.05$).

Figure 2.16 Least squares means for lactic acid bacteria (LAB) enumeration for beef *gluteus medius* steaks over 5 different postmortem aging treatments (SEM = 0.172).



[a-d]Means with different superscripts differ ($P<0.05$).

Figure 2.17 Least squares means for pH for beef *gluteus medius* steaks over 5 different postmortem aging treatments (SEM = 0.0112).



[a-c]Means with different superscripts differ ($P<0.05$).

Table 2.2 Least squares means of aging × tenderization interaction for sensory traits of beef *gluteus medius* steaks.

	Control					Tenderized					
	Aging Days					Aging Days					
Sensory trait	5	19	33	47	61	5	19	33	47	61	SEM[1]
Overall Tenderness[2]	9.08^{c}	9.21^{c}	9.01^{c}	9.21^{c}	10.03^{b}	9.42^{b}	10.26^{ab}	10.09^{b}	10.17^{b}	10.51^{a}	0.2675
Myofibrillar tenderness[2]	9.36^{b}	9.56^{b}	9.32^{b}	9.61^{b}	10.44^{a}	9.67^{a}	10.58^{a}	10.45^{a}	10.57^{a}	10.81^{a}	0.3219
Bloody/serumy[3]	3.57^{ab}	3.34^{bcd}	3.38^{abcd}	2.98^{d}	3.47^{abc}	3.53^{ab}	3.76^{a}	3.23^{bcd}	3.36^{abcd}	3.11^{cd}	0.474
Metallic[3]	1.97^{abc}	1.76^{bc}	1.89^{abc}	1.88^{abc}	1.89^{abc}	1.68^{c}	2.18^{a}	2.00^{ab}	1.97^{abc}	2.08^{a}	0.196
Overall sweet[3]	1.41^{bc}	1.58^{a}	1.44^{abc}	1.35^{cd}	1.52^{ab}	1.47^{abc}	1.41^{bc}	1.45^{abc}	1.38^{bcd}	1.22^{d}	0.232
Bitter[3]	3.27^{abcd}	3.10^{cd}	3.00^{d}	3.36^{ab}	3.11^{bcd}	3.07^{cd}	3.37^{ab}	3.30^{abc}	3.49^{a}	3.42^{a}	0.295

[a-d]Means within a row with different superscripts differ (P<0.05).
[1]Standard error of the mean
[2]15=Very Tender, 1=Very Tough
[3]15=Extremely Strong, 0=None

Table 2.3 Least squares means of sensory characteristics of beef *gluteus medius* steaks subjected to different postmortem aging treatments.

	Aging Days					
Sensory Characteristic	5	19	33	47	61	SEM[1]
Fat like[2]	1.67^{abc}	1.79^{a}	1.73^{ab}	1.60^{bc}	1.57^{c}	0.136
Warmed over[2]	1.77^{b}	1.86^{ab}	2.08^{a}	2.07^{a}	2.01^{a}	0.480

[a-b]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]15=Extremely Strong, 0=None

Table 2.4 Least squares means of sensory characteristics for beef *gluteus medius* steaks with and without blade tenderization treatment.

Sensory Characteristic	Treatment		SEM[1]
	Control	Blade Tenderized	
Rancid[2]	0.66[b]	0.82[a]	0.18
Spoiled[2]	0.34[b]	0.46[a]	0.12

[a-b]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]15=Extremely Strong, 0=None

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Appendix A - Additional Results

Table A.1 Least squares means of aging × tenderization interaction for quality traits of beef *gluteus medius* steaks.

	Control					Tenderized					
	Aging period in days					Aging period in days					
Trait	5	19	33	47	61	5	19	33	47	61	SEM[1]
TBARS (ppm)[2]	0.074	0.097	0.144	0.187	0.171	0.062	0.139	0.151	0.187	0.202	0.0179
Metmyoglobin reducing activity[3]	85.1	72.2	64.9	69.1	62.3	85.2	74.5	61.3	73.2	61.3	3.78
Oxygen consumption rate[4]	49.3	42.2	57.5	44.8	77.5	47.5	55.2	57.1	52.9	89.6	6.25
L*[5]	44.1	46.4	47.7	53.5		43.6	45.2	47.2	51.8		0.422
a*[6]	24.3	24.4	22.8	29.9		23.8	23.9	25.8	28.7		0.566
b*[7]	19.9	21.8	22.9	35.3		19.8	21.4	24.8	34.5		0.786
Initial color panel[8]	4.4	3.3	3.5	3.8	3.7	4.5	3.6	3.7	4.0	3.6	0.16
Display color panel[9]	3.7^{f}	4.1^{e}	4.5^{d}	5.6^{b}	5.5^{b}	4.0^{e}	4.8^{c}	5.0^{c}	6.3^{a}	6.4^{a}	0.099
Discoloration color panel[10]	2.5^{f}	3.6^{d}	4.1^{c}	4.5^{b}	4.4^{b}	3.0^{e}	4.2^{c}	4.8^{a}	4.9^{a}	4.8^{a}	0.099
Warner-Bratzler shear force (N)	44.1^{a}	34.6^{b}	32.8^{b}	32.4^{b}	25.1^{c}	30.5^{b}	25.6^{c}	23.4^{c}	25.1^{c}	22.2^{c}	1.74
Lactic acid bacteria (log CFU/g)	1.07	1.79	2.69	3.14	3.76	1.27	1.91	2.70	3.05	3.95	0.243
pH	5.52	5.52	5.43	5.50	5.53	5.54	5.51	5.41	5.49	5.53	0.0159

[a-f]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]Thiobarbituric acid reactive substances, ppm malonaldehyde
[3]% Metmyoglobin reduced
[4]% Oxymyoglobin reduced
[5]L* lightness (0=black, 100=white)
[6]a* redness/greenness (positive values = red, negative values = green)
[7]b* yellowness/blueness (positive values= yellow, negative values=blue)
[8]1=Bleached red, 2=Very light cherry-red, 3=Moderately cherry-red, 4=Cherry-red, 5=Slightly dark red, 6=Moderately dark red , 7=Dark red, 8=Very dark red
[9]1=Very bright red, 2=Bright red, 3=Dull red, 4=Slightly dark red, 5=Moderately dark red, 6=Dark red to dark reddish tan, 7=Tannish red, 8=Tan to brown
[10]1=0% Surface Discoloration, 2=1 to 10% Surface Discoloration, 3=11 to 25% Surface Discoloration, 4=26 to 50% Surface Discoloration, 5=51 to 75% Surface Discoloration, 6=76-99% Surface Discoloration, 7=100% Surface Discoloration

Table A.2 Main effect least squares means of quality traits for beef *gluteus medius* steaks aged over 5 different postmortem aging treatments.

Trait	Aging Period in Days					SEM[1]
	5	19	33	47	61	
TBARS (ppm)[2]	0.068[c]	0.118[b]	0.147[b]	0.187[a]	0.186[a]	0.0127
Metmyoglobin reducing activity[3]	85.2[a]	73.3[b]	63.1[c]	71.2[b]	61.8[c]	3.78
Oxygen consumption rate[4]	48.4[b]	48.7[b]	57.3[b]	48.9[b]	83.6[a]	4.42
L*[5]	43.9[e]	45.8[d]	47.4[c]	52.6[b]	53.9[a]	0.422
a*[6]	24.1[b]	24.2[b]	24.3[b]	29.3[a]		0.400
b*[7]	19.9[d]	21.6[c]	23.9[b]	34.9[a]		0.556
Initial color panel[8]	4.5[a]	3.5[d]	3.6[cd]	3.9[b]	3.7[bc]	0.12
Display color panel[9]	3.9[d]	4.5[c]	4.7[b]	6.0[a]	6.0[a]	0.13
Discoloration color panel[10]	2.7[d]	3.9[c]	4.4[b]	4.7[a]	4.6[a]	0.081
Warner-Bratzler shear force (N)	37.3[a]	30.1[b]	28.1[b]	28.7[b]	23.7[c]	1.20
Lactic acid bacteria (log CFU/g)	1.17[d]	1.85[c]	2.69[b]	3.10[b]	3.85[a]	0.172
pH	5.53[a]	5.52[ab]	5.42[c]	5.50 [b]	5.53[a]	0.0112

[a-e]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]Thiobarbituric acid reactive substances, ppm malonaldehyde
[3]% Metmyoglobin reduced
[4]% Oxymyglobin reduced
[5]L* lightness (0=black, 100=white)
[6]a* redness/greenness (positive values = red, negative values = green)
[7]b* yellowness/blueness (positive values= yellow, negative values=blue)
[8]1=Bleached Red, 2=Very light cherry-red, 3=Moderately cherry-red, 4=Cherry-red, 5=Slightly dark red, 6=Moderately dark red , 7=Dark red, 8=Very dark red
[9]1=Very bright red, 2=Bright red, 3=Dull red, 4=Slightly dark red, 5=Moderately dark red, 6=Dark red to dark reddish tan, 7=Tannish red, 8=Tan to brown
[10]1=0% Surface Discoloration, 2=1 to 10% Surface Discoloration, 3=11 to 25% Surface Discoloration, 4=26 to 50% Surface Discoloration, 5=51 to 75% Surface Discoloration, 6=76-99% Surface Discoloration, 7=100% Surface Discoloration

Table A.3 Least squares means of quality traits for beef *gluteus medius* steaks with and without blade tenderization treatment.

Trait	Treatment		SEM[1]
	Control	Blade Tenderized	
TBARS (ppm)[2]	0.135	0.148	0.00802
Metmyoglobin reducing activity[3]	70.7	71.1	1.69
Oxygen consumption rate[4]	54.3	60.5	2.70
L*[5]	48.9	48.5	0.255
a*[6]	25.4	25.8	0.242
b*[7]	25.7	26.3	0.335
Initial color panel[8]	3.7[b]	3.9[a]	0.095
Display color panel[9]	4.7[b]	5.3[a]	0.12
Discoloration color panel[10]	3.8[b]	4.3[a]	0.068
Warner-Bratzler shear force, (N)	33.8[a]	25.4[b]	0.74
Lactic acid bacteria (log CFU/g)	2.49	2.58	0.109
pH	5.50	5.50	0.00711

[a-b]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]Thiobarbituric acid reactive substances, ppm malonaldehyde
[3]% Metmyoglobin reduced
[4]% Oxymyoglobin reduced
[5]L* lightness (0=black, 100=white)
[6]a* redness/greenness (positive values = red, negative values = green)
[7]b* yellowness/blueness (positive values= yellow, negative values=blue)
[8]1=Bleached red, 2=Very light cherry-red, 3=Moderately cherry-red, 4=Cherry-red, 5=Slightly dark red, 6=Moderately dark red , 7=Dark red, 8=Very dark red
[9]1=Very bright red, 2=Bright red, 3=Dull red, 4=Slightly dark red, 5=Moderately dark red, 6=Dark red to dark reddish tan, 7=Tannish red, 8=Tan to brown
[10]1=0% Surface discoloration, 2=1 to 10% Surface discoloration, 3=11 to 25% Surface discoloration, 4=26 to 50% Surface discoloration, 5=51 to 75% Surface discoloration, 6=76-99% Surface discoloration, 7=100% Surface discoloration

Table A.4 Least squares means of aging × tenderization interaction for sensory traits of beef *gluteus medius* steaks.

	Control					Tenderized					
	Aging Days					Aging Days					
Sensory trait	5	19	33	47	61	5	19	33	47	61	SEM[1]
Overall Tenderness[2]	9.1^{c}	9.2^{c}	9.0^{c}	9.2^{c}	10.0^{b}	9.4^{b}	10.26^{ab}	10.1^{b}	10.2^{b}	10.5^{a}	0.267
Myofibrillar tenderness[2]	9.4^{b}	9.6^{b}	9.3^{b}	9.6^{b}	10.4^{a}	9.7^{a}	10.6^{a}	10.5^{a}	10.6^{a}	10.8^{a}	0.322
Beef identity[3]	9.2	9.5	8.9	9.1	9.3	9.2	9.3	9.3	9.2	8.9	0.24
Brown/roasted[3]	7.8	8.1	8.1	8.0	8.3	8.0	8.1	8.1	8.0	7.9	0.71
Bloody/serumy[3]	3.6^{ab}	3.3^{bcd}	3.4^{abcd}	3.0^{d}	3.5^{abc}	3.5^{ab}	3.8^{a}	3.2^{bcd}	3.4^{abcd}	3.11^{cd}	0.47
Liver like[3]	1.3	1.2	1.5	1.4	1.7	1.3	1.6	1.5	1.5	1.6	0.27
Metallic[3]	2.0^{abc}	1.8^{bc}	1.9^{abc}	1.9^{abc}	1.9^{abc}	1.7^{c}	2.2^{a}	2.0^{ab}	2.0^{abc}	2.1^{a}	0.20
Fat like[3]	1.7	1.7	1.8	1.5	1.7	1.7	1.9	1.7	1.7	1.5	0.15
Green[3]	0.6	0.6	0.6	0.5	0.6	0.6	0.5	0.6	0.5	0.6	0.2
Rancid[3]	0.8	0.5	0.6	0.8	0.6	0.7	0.8	0.9	0.8	0.9	0.2
Spoiled[3]	0.3	0.2	0.5	0.2	0.5	0.3	0.5	0.4	0.5	0.6	0.1
Warmed over[3]	1.8	1.9	2.0	2.0	2.0	1.7	1.8	2.1	2.1	2.0	0.49
Overall sweet[3]	1.4^{bc}	1.6^{a}	1.4^{abc}	1.4^{cd}	1.5^{ab}	1.5^{abc}	1.4^{bc}	1.5^{abc}	1.4^{bcd}	1.2^{d}	0.23
Sour[3]	2.5	2.6	2.5	2.6	2.6	2.5	2.7	2.7	2.7	2.8	0.15
Bitter[3]	3.3^{abcd}	3.1^{cd}	3.0^{d}	3.4^{ab}	3.1^{bcd}	3.1^{cd}	3.4^{ab}	3.3^{abc}	3.5^{a}	3.4^{a}	0.29
Salty[3]	1.9	1.9	1.8	1.8	1.8	1.9	2.0	1.8	1.9	1.7	0.23
Umami[3]	2.0	2.2	1.9	2.1	2.2	1.9	2.2	2.0	2.2	1.9	0.22

[a-d]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]15=Very Tender, 1=Very Tough
[3]15=Extremely Strong, 0=None

Table A.5 Least squares means of sensory characteristics of beef *gluteus medius* steaks subjected to different postmortem aging treatments.

	Aging Days					
Sensory Characteristic	5	19	33	47	61	SEM[1]
Overall tenderness[2]	9.3[c]	9.6[b]	9.7[b]	9.8[b]	10.3[a]	0.242
Myofibrillar tenderness[2]	9.5[c]	10.1[b]	9.9[b]	10.1[b]	10.6[a]	0.294
Beef identity[3]	9.2	9.4	9.1	9.1	9.1	0.21
Brown/roasted[3]	7.9	8.1	8.1	8.0	8.1	0.70
Bloody/serumy[3]	3.6[a]	3.6[a]	3.3[ab]	3.2[b]	3.3[ab]	0.46
Liver like[3]	1.3	1.4	1.4	1.5	1.6	0.25
Metallic[3]	1.8	2.0	1.9	1.9	2.0	0.18
Fat like[3]	1.7[abc]	1.8[a]	1.7[ab]	1.6[bc]	1.6[c]	0.14
Green[3]	0.6	0.6	0.6	0.5	0.6	0.2
Rancid[3]	0.7	0.7	0.8	0.8	0.8	0.2
Spoiled[3]	0.3	0.3	0.4	0.4	0.6	0.1
Warmed over[3]	1.8[b]	1.9[ab]	2.1[a]	2.1[a]	2.0[a]	0.48
Overall sweet[3]	1.4	1.5	1.4	1.4	1.4	0.23
Sour[3]	2.5	2.6	2.6	2.6	2.7	0.13
Bitter[3]	3.2[b]	3.2[b]	3.2[b]	3.4[a]	3.3[ab]	0.28
Salty[3]	1.9	1.9	1.8	1.8	1.8	0.22
Umami[3]	2.0	2.2	2.0	2.1	2.1	0.21

[a-b]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]15=Very Tender, 1=Very Tough
[3]15=Extremely Strong, 0=None

Table A.6 Least squares means of sensory characteristics for beef *gluteus medius* steaks with and without blade tenderization treatment.

Sensory Characteristic	Treatment		SEM[1]
	Control	Blade Tenderized	
Overall tenderness[2]	9.4[b]	10.1[a]	0.228
Myofibrillar tenderness[2]	9.7[b]	10.4[a]	0.279
Beef identity[3]	9.2	9.2	0.19
Brown/roasted[3]	8.0	8.0	0.70
Bloody/serumy[3]	3.4	3.4	0.45
Liver like[3]	1.4	1.5	0.24
Metallic[3]	1.9	2.0	0.16
Fat like[3]	1.7	1.7	0.13
Green[3]	0.6	0.6	0.2
Rancid[3]	0.7[b]	0.8[a]	0.2
Spoiled[3]	0.4	0.5	0.1
Warmed over[3]	2.0	2.0	0.48
Overall sweet[3]	1.5[a]	1.4[b]	0.23
Sour[3]	2.6	2.7	0.12
Bitter[3]	3.2[b]	3.3[a]	0.28
Salty[3]	1.8	1.8	0.22
Umami[3]	2.1	2.0	0.20

[a-b]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]15=Very Tender, 1=Very Tough
[3]15=Extremely Strong, 0=None

Table A.7 Least squares means of aging × tenderization interaction for initial color results of beef *gluteus medius* steaks.

	Control					Tenderized					
	Aging Period					Aging Period					
Trait	5	19	33	47	61	5	19	33	47	61	SEM[1]
Initial Color Panel[2]	4.4	3.3	3.5	3.8	3.7	4.5	3.6	3.7	4.0	3.6	0.16
L*[3]	48.8^{de}	48.7^{de}	50.5^{cd}	57.6^{ab}	53.5^{bc}	47.4^{de}	45.0^{e}	48.1^{de}	54.5^{bc}	59.7^{a}	1.55
a*[4]	30.5^{de}	26.7^{ef}	33.3^{d}	39.4^{ab}	37.9^{bc}	31.1^{de}	23.1^{f}	31.7^{d}	34.1^{cd}	42.9^{a}	1.70
b*[5]	22.3^{cd}	24.7^{cd}	27.2^{c}	42.3^{a}	34.4^{b}	23.0^{cd}	20.3^{d}	24.0^{cd}	34.9^{b}	42.7^{a}	2.18
Hue angle	37.8	36.5	43.1	58.0	51.2	38.6	30.8	39.8	49.0	60.6	2.57

[a-e]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]1=Bleached Red, 2=Very light cherry-red, 3=Moderately cherry-red, 4=Cherry-red, 5=Slightly dark red, 6=Moderately dark red , 7=Dark red, 8=Very dark red
[3]L* lightness (0=black, 100=white)
[4]a* redness/greenness (positive values = red, negative values = green)
[5]b* yellowness/blueness (positive values= yellow, negative values=blue)
[6]Hue angle (reported in degrees) represents the change from the true red axis (a larger hue angle indicates a greater shift from red to yellow)

Table A.8 Least squares means of blade tenderization for initial color results of beef *gluteus medius* steaks subjected to different blade tenderization treatments.

Trait	Control	Blade Tenderized	SEM[1]
Initial Color Panel[2]	3.7[b]	3.9[a]	0.095
L*[3]	51.8	50.9	0.663
a*[4]	33.6	32.6	0.727
b*[5]	30.2	29.0	0.929
Hue angle[6]	45.3	43.7	1.10

[a-e]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]1=Bleached Red, 2=Very light cherry-red, 3=Moderately cherry-red, 4=Cherry-red, 5=Slightly dark red, 6=Moderately dark red , 7=Dark red, 8=Very dark red
[3]L* lightness (0=black, 100=white)
[4]a* redness/greenness (positive values = red, negative values = green)
[5]b* yellowness/blueness (positive values= yellow, negative values=blue)
[6]Hue angle (reported in degrees) represents the change from the true red axis (a larger hue angle indicates a greater shift from red to yellow)

Table A.9 Least squares means of aging × display interaction for color panel results of beef *gluteus medius* steaks.

Trait	Aging Period	Display Day					SEM[1]
		0	1	2	3	4	
Discoloration[2]	5		1.5^{l}	2.3^{k}	3.2^{h}	3.9^{f}	0.13
	19		2.4^{jk}	3.5^{g}	4.5^{d}	5.1^{c}	0.13
	33		2.7^{i}	4.2^{e}	5.2^{c}	5.7^{b}	0.13
	47		2.6^{ij}	4.3^{de}	5.6^{b}	6.1^{a}	0.13
	61		2.4^{jk}	4.3^{de}	5.5^{b}	6.1^{a}	0.13
Display Color[3]	5	2.9^{m}	3.2^{l}	3.7^{k}	4.4^{j}	5.2^{i}	0.18
	19	2.8^{m}	3.6^{k}	4.6^{j}	5.4^{h}	6.0^{bcd}	0.18
	33	2.7^{m}	3.7^{k}	5.1^{i}	6.1^{bcd}	6.0^{bcd}	0.18
	47	5.8^{def}	6.0^{cd}	5.9^{de}	5.8^{def}	6.4^{a}	0.18
	61	5.7^{efg}	5.6^{fg}	6.0^{cd}	6.3^{abc}	6.3^{ab}	0.18

[a-m]Means within a row with different superscripts differ ($P<0.05$).

[1]Standard error of the mean

[2]1=0% Surface discoloration, 2=1 to 10% Surface discoloration, 3=11 to 25% Surface discoloration, 4=26 to 50% Surface discoloration, 5=51 to 75% Surface discoloration, 6=76-99% Surface discoloration, 7=100% Surface discoloration

[3]1=Very bright red, 2=Bright red, 3=Dull red, 4=Slightly dark red, 5=Moderately dark red, 6=Dark red to dark reddish tan, 7=Tannish red, 8=Tan to brown

Table A.10 Least squares means of aging × display interaction for color panel results of beef *gluteus medius* steaks.

Trait	Treatment	Display Days					SEM[1]
		0	1	2	3	4	
Discoloration[2]	Control		2.2[g]	3.4[e]	4.6[c]	5.2[b]	0.095
	Blade Tenderized		2.5[f]	4.0[d]	5.0[b]	5.7[a]	0.095
Display Color[3]	Control	3.8[g]	4.1[f]	4.7[e]	5.3[d]	5.7[c]	0.14
	Blade Tenderized	4.2[f]	4.7[e]	5.4[d]	6.0[b]	6.3[a]	0.14

[a-g]Means within a row with different superscripts differ ($P<0.05$).

[1]Standard error of the mean

[2]1=0% Surface discoloration, 2=1 to 10% Surface discoloration, 3=11 to 25% Surface discoloration, 4=26 to 50% Surface discoloration, 5=51 to 75% Surface discoloration, 6=76-99% Surface discoloration, 7=100% Surface discoloration

[3]1=Very bright red, 2=Bright red, 3=Dull red, 4=Slightly dark red, 5=Moderately dark red, 6=Dark red to dark reddish tan, 7=Tannish red, 8=Tan to brown

Table A.11 Least squares means for display for color panel results of beef *gluteus medius* steaks subjected to different display time treatments.

Trait	Display Days					SEM[1]
	0	1	2	3	4	
Discoloration[2]		2.3[d]	3.7[c]	4.8[b]	5.4[a]	0.078
Display Color[3]	4.0[e]	4.4[d]	5.1[c]	5.6[b]	6.0[a]	0.13

[a-e]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]1=0% Surface discoloration, 2=1 to 10% Surface discoloration, 3=11 to 25% Surface discoloration, 4=26 to 50% Surface discoloration, 5=51 to 75% Surface discoloration, 6=76-99% Surface discoloration, 7=100% Surface discoloration
[3]1=Very bright red, 2=Bright red, 3=Dull red, 4=Slightly dark red, 5=Moderately dark red, 6=Dark red to dark reddish tan, 7=Tannish red, 8=Tan to brown

Table A.12 Least squares means of aging × tenderization × display interaction for display color panel results of beef *gluteus medius* steaks.

Trait	Aging Period	Treatment	Display Days					SEM[1]
			0	1	2	3	4	
Display Color[2]	5	Control	2.9	3.0	3.6	4.2	5.0	0.22
	5	Tenderized	3.0	3.3	3.8	4.7	5.4	0.22
	19	Control	2.6	3.3	4.3	5.0	5.6	0.22
	19	Tenderized	3.0	3.9	4.9	5.8	6.5	0.22
	33	Control	2.6	3.4	4.8	5.7	6.1	0.22
	33	Tenderized	2.8	4.1	5.4	6.5	6.0	0.22
	47	Control	5.5	5.7	5.5	5.5	6.1	0.22
	47	Tenderized	6.1	6.3	6.3	6.2	6.8	0.22
	61	Control	5.2	2.3	4.0	5.4	6.0	0.17
	61	Tenderized	6.1	2.6	4.5	5.6	6.3	0.17

[1]Standard error of the mean

[2]1=Very bright red, 2=Bright red, 3=Dull red, 4=Slightly dark red, 5=Moderately dark red, 6=Dark red to dark reddish tan, 7=Tannish red, 8=Tan to brown

Table A.13 Least squares means of aging × tenderization × display interaction for discoloration panel results of beef *gluteus medius* steaks.

Trait	Aging Period	Treatment	Display Days					SEM[1]
			0	1	2	3	4	
Discoloration[2]	5	Control		1.3	2.1	3.0	3.6	0.17
	5	Tenderized		1.7	2.5	3.4	4.2	0.17
	19	Control		2.3	3.2	4.2	4.8	0.17
	19	Tenderized		2.6	3.9	4.7	5.4	0.17
	33	Control		2.4	3.7	4.8	5.4	0.17
	33	Tenderized		3.0	4.8	5.5	6.0	0.17
	47	Control		2.6	4.0	5.4	5.9	0.17
	47	Tenderized		2.7	4.5	5.9	6.3	0.17
	61	Control		2.3	4.0	5.4	6.0	0.17
	61	Tenderized		2.6	4.5	5.6	6.3	0.17

[1]Standard error of the mean

[2]1=0% Surface discoloration, 2=1 to 10% Surface discoloration, 3=11 to 25% Surface discoloration, 4=26 to 50% Surface discoloration, 5=51 to 75% Surface discoloration, 6=76-99% Surface discoloration, 7=100% Surface discoloration

Table A.14 Main effects least squares means of display for instrumental color results of beef *gluteus medius* steaks subjected to different display time treatments.

Trait	Hours of Display									SEM[1]
	0	12	24	36	48	60	72	84	96	
L*[2]	51.4[a]	49.7[bc]	51.1[ab]	49.5[c]	51.2[a]	45.0[e]	46.2[de]	47.4[d]	47.0[d]	0.540
a*[3]	33.1[a]	29.1[b]	28.5[b]	26.9[c]	22.1[e]	22.8[de]	22.8[de]	23.6[d]	21.9[e]	0.513
b*[4]	29.6[a]	28.0[a]	29.8[a]	28.9[a]	23.1[b]	23.1[b]	23.1[b]	24.9[b]	24.0[b]	0.712

[a-e]Means within a row with different superscripts differ ($P<0.05$).
[1]Standard error of the mean
[2]L* lightness (0=black, 100=white)
[3]a* redness/greenness (positive values = red, negative values = green)
[4]b* yellowness/blueness (positive values= yellow, negative values=blue)

Table A.15 Least squares means of aging × display interaction for instrumental color results of beef *gluteus medius* steaks.

Trait	Aging Period	Hours of Display									SEM[1]
		0	12	24	36	48	60	72	84	96	
L*[2]	5	48.1^{fghijk}	44.2^{lmnop}	44.3^{lmnop}	43.8^{mnop}	43.8^{mnop}	41.1^{p}	43.5^{nop}	42.9^{op}	43.0^{op}	1.27
	19	46.9^{hijklmn}	45.0^{klmno}	46.1^{jklmno}	45.2^{klmno}	45.5^{klmno}	45.0^{klmno}	46.2^{ijklmno}	45.5^{klmno}	46.5^{hijklmn}	1.27
	33	49.3^{efghij}	46.5^{hijklmn}	47.4^{ghijkl}	46.5^{hijklmn}	47.0^{hijklm}	45.9^{klmno}	44.9^{klmno}	49.7^{efgh}	49.6^{efghi}	1.27
	47	56.1^{c}	57.5^{bc}	52.2^{de}	51.3^{ef}	52.5^{de}	51.6^{e}	51.0^{ef}	50.9^{ef}	50.6^{efg}	1.27
a*[3]	5	30.8^{ef}	25.7^{hijkl}	25.2^{ijklm}	24.0^{klmno}	23.2^{lmnop}	24.4^{jklmn}	21.7^{nopq}	21.17^{opq}	20.5^{pq}	1.13
	19	24.9^{ijklmn}	23.0^{lmnopq}	23.8^{klmno}	24.1^{jklmno}	23.9^{klmno}	23.7^{klmnop}	24.8^{ijklmn}	24.5^{jklmn}	24.8^{ijklmn}	1.13
	33	32.5^{ed}	25.7^{hijkl}	25.7^{hijkl}	23.4^{lmnop}	22.7^{lmnopq}	22.1^{mnopq}	20.9^{opq}	23.6^{klmnop}	21.9^{nopq}	1.13
	47	36.8^{c}	36.4^{c}	28.8^{fgh}	28.0^{fghi}	28.9^{fg}	27.2^{ghij}	26.6^{ghijk}	25.7^{hijkl}	25.3^{ijkl}	1.13
b*[4]	5	22.6^{ijkl}	20.4^{klmn}	20.1^{klmn}	19.7^{klmn}	19.2^{klmn}	21.7^{jklm}	18.4^{lmn}	18.3^{lmn}	19.2^{mn}	1.67
	19	22.5^{ijklm}	20.2^{klmn}	21.4^{jklm}	21.5^{jklm}	21.6^{jklm}	21.1^{klm}	22.1^{ijklm}	21.8^{jklm}	22.1^{ijklm}	1.67
	33	25.6^{hij}	21.4^{jklm}	23.4^{ijk}	22.2^{ijklm}	23.1^{ijk}	22.9^{ijk}	21.2^{klm}	28.3^{fgh}	26.6^{ghi}	1.67
	47	38.6^{c}	40.3^{bc}	33.4^{cd}	36.7^{c}	39.3^{c}	33.5^{de}	32.0^{ef}	30.3^{efg}	30.1^{efg}	1.67

[c-u]Means within a row with different superscripts differ ($P<0.05$).

[1]Standard error of the mean

[2]L* lightness (0=black, 100=white)

[3]a* redness/greenness (positive values = red, negative values = green)

[4]b* yellowness/blueness (positive values= yellow, negative values=blue)

Table A.16 Least squares means of tenderization × display interaction for instrumental color results of beef *gluteus medius* steaks.

		Hours of Display									
Trait	Treatment	0	12	24	36	48	60	72	84	96	SEM[1]
L*[2]	Control	51.8	50.3	50.7	49.5	51.5	45.3	46.3	47.3	47.7	0.764
	Tenderized	50.9	49.1	51.4	49.5	51.0	44.6	46.0	47.6	46.4	0.764
a*[3]	Control	33.6	29.5	28.2	26.7	22.0	22.6	22.2	22.7	21.7	0.764
	Tenderized	32.6	28.7	28.7	27.1	22.2	22.9	23.4	23.4	22.2	0.764
b*[4]	Control	30.2	28.4	29.2	27.4	22.8	23.0	22.5	23.8	24.4	1.00
	Tenderized	29.0	27.6	30.3	30.4	23.5	23.1	23.6	26.0	23.5	1.00

[1]Standard error of the mean

[2]L* lightness (0=black, 100=white)

[3]a* redness/greenness (positive values = red, negative values = green)

[4]b* yellowness/blueness (positive values= yellow, negative values=blue)

Table A.17 Least squares means of aging × tenderization × display interaction for L* values of beef *gluteus medius* steaks.

			Hours of Display									
Trait	Aging	Treatment	0	12	24	36	48	60	72	84	96	SEM[1]
L*[2]	5	Control	48.8	44.3	44.5	43.7	43.7	41.2	43.8	43.2	43.5	1.80
	5	Tenderized	47.4	44.1	44.1	43.8	43.8	41.1	43.3	42.7	42.5	1.80
	19	Control	48.7	46.1	46.8	45.4	47.0	45.7	46.0	45.7	46.1	1.80
	19	Tenderized	45.0	43.9	45.5	45.1	44.0	44.2	46.5	45.3	46.9	1.80
	33	Control	50.5	46.2	46.9	47.1	47.3	45.9	44.9	48.8	51.5	1.80
	33	Tenderized	48.1	46.8	48.0	46.0	46.8	45.8	44.8	50.7	47.6	1.80
	47	Control	57.6	61.2	52.7	53.9	51.7	52.1	51.1	49.7	51.1	1.80
	47	Tenderized	54.5	53.9	51.6	48.6	53.2	51.1	51.0	52.0	50.1	1.80

[1]Standard error of the mean

[2]L* lightness (0=black, 100=white)

Table A.18 Least squares means of aging × tenderization × display interaction for a* values of beef *gluteus medius* steaks.

			Hours of Display									
Trait	Aging	Treatment	0	12	24	36	48	60	72	84	96	SEM[1]
a*[2]	5	Control	30.5	26.0	25.4	24.2	23.6	24.8	22.0	21.4	20.7	1.70
	5	Tenderized	20.7	31.1	25.4	23.8	22.7	23.9	21.3	20.8	20.2	1.70
	19	Control	26.7	24.4	24.9	24.2	24.2	23.8	24.1	23.7	23.7	1.70
	19	Tenderized	23.1	21.7	22.7	24.0	23.6	23.5	25.5	25.3	26.0	1.70
	33	Control	33.3	24.3	23.6	21.7	21.4	19.8	18.8	21.0	21.4	1.70
	33	Tenderized	31.7	27.1	27.8	25.2	24.1	24.3	23.1	26.3	22.4	1.70
	47	Control	39.4	38.9	29.2	29.9	28.6	28.0	24.9	24.2	25.6	1.70
	47	Tenderized	34.1	33.8	28.4	26.0	29.2	26.4	28.3	27.2	25.0	1.70

[1]Standard error of the mean

[2]a* redness/greenness (positive values = red, negative values = green)

Table A.19 Least squares means of aging × tenderization × display interaction for b* values of beef *gluteus medius* steaks.

			Hours of Display									
Trait	Aging	Treatment	0	12	24	36	48	60	72	84	96	SEM[1]
b*[2]	5	Control	22.3	20.5	20.2	19.7	19.3	21.9	18.7	18.4	18.2	2.36
	5	Tenderized	23.0	20.3	20.1	19.7	19.1	21.4	18.2	18.2	18.2	2.36
	19	Control	24.7	20.9	22.3	20.9	21.7	21.5	21.4	21.5	21.5	2.36
	19	Tenderized	20.3	19.5	20.6	22.1	21.5	20.6	22.8	22.1	22.7	2.36
	33	Control	27.2	20.5	21.1	21.6	21.6	21.2	19.8	25.5	27.6	2.36
	33	Tenderized	24.0	22.4	25.6	22.8	24.5	24.7	22.6	31.1	25.5	2.36
	47	Control	42.3	44.7	34.9	34.7	38.6	34.2	30.1	27.0	31.2	2.36
	47	Tenderized	34.9	36.0	31.8	38.7	39.9	32.8	33.8	33.6	28.9	2.36

[1]Standard error of the mean

[2]b* yellowness/blueness (positive values= yellow, negative values=blue)

Figure A.1 Least squares means for tenderization × display interactions for L* values of beef *gluteus medius* steaks.

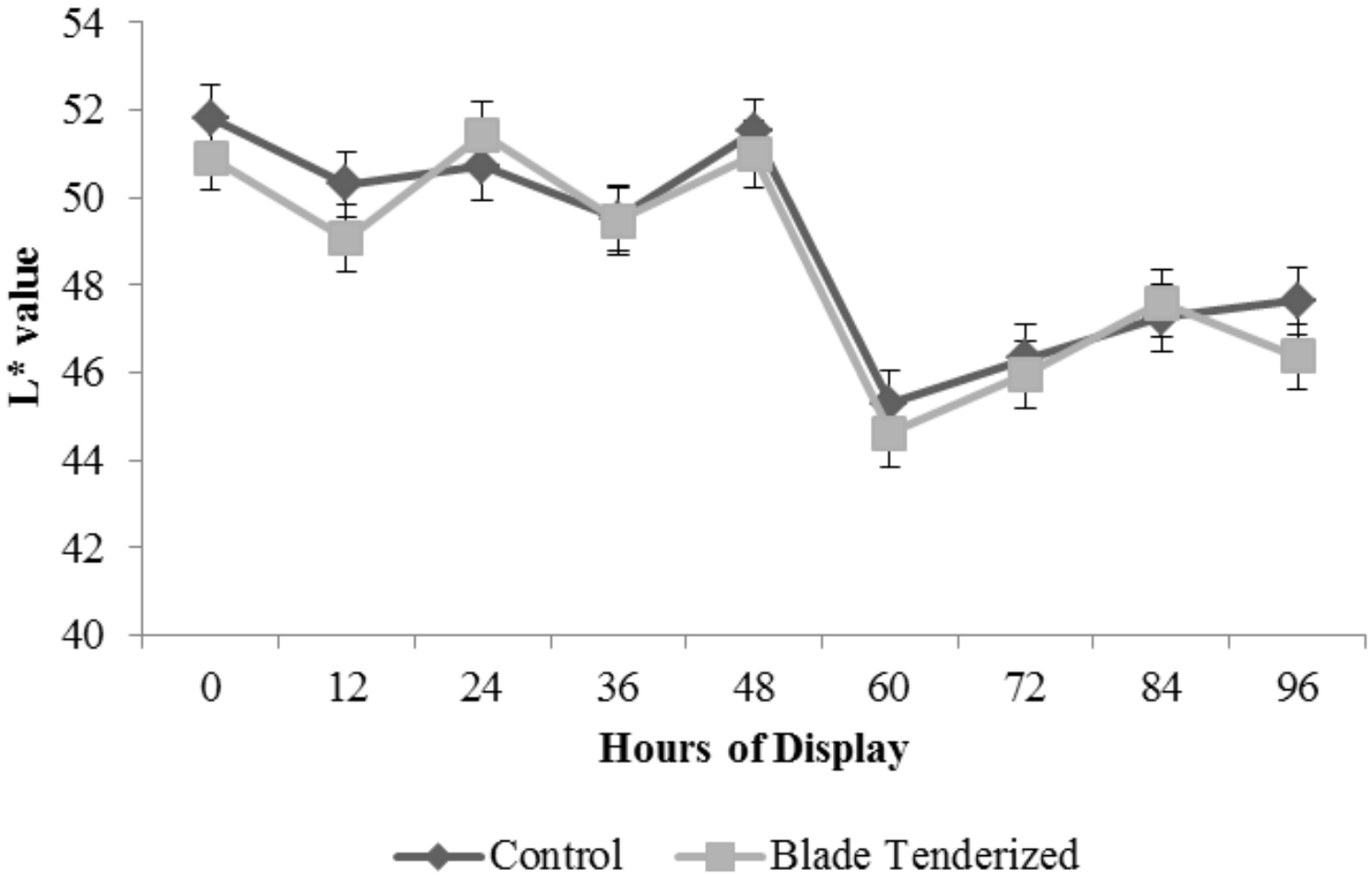


Figure A.2 Least squares means for tenderization × display interactions for a* values of beef *gluteus medius* steaks.

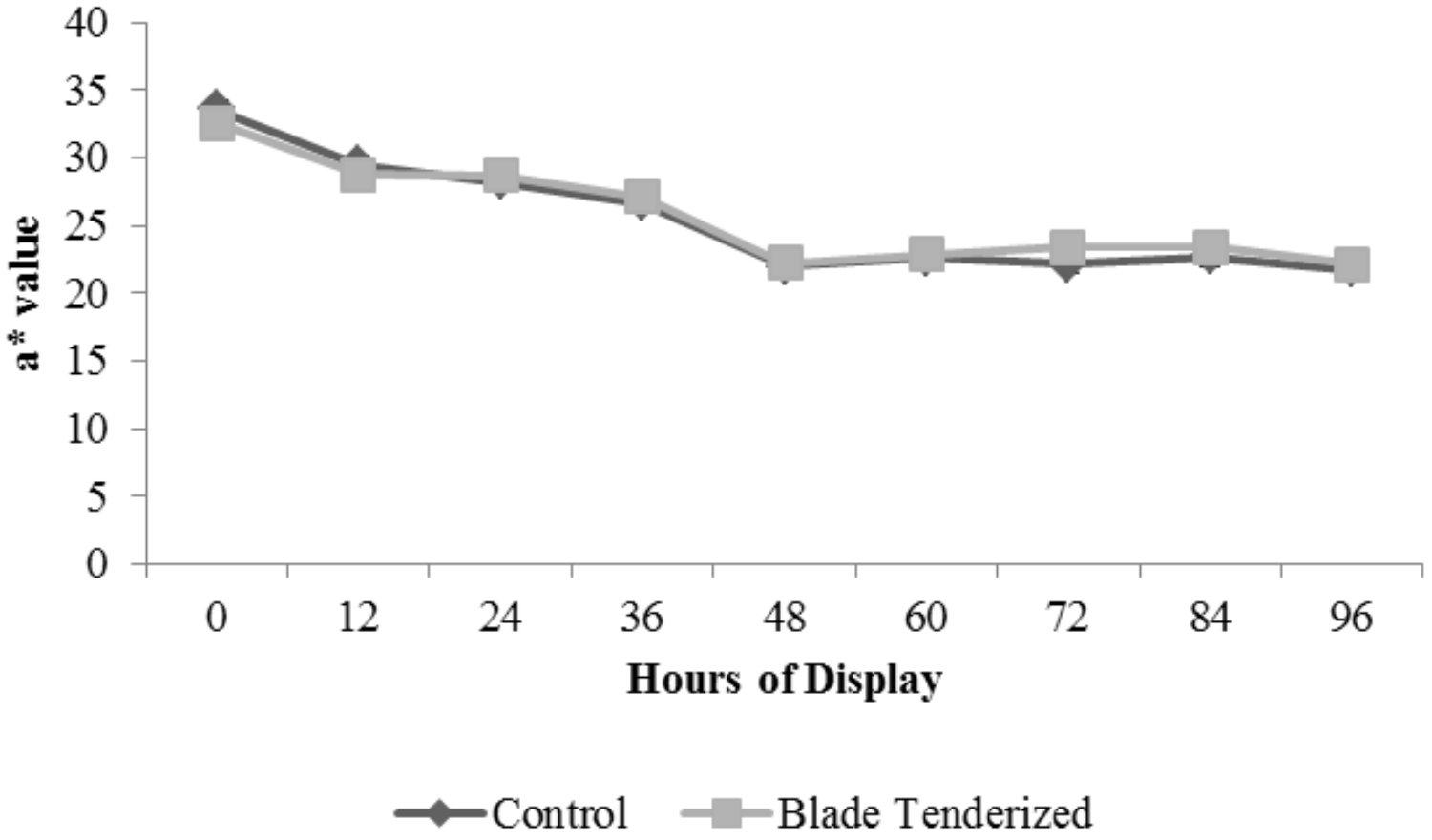


Figure A.3 Least squares means for tenderization × display interactions for b* values of beef *gluteus medius* steaks.

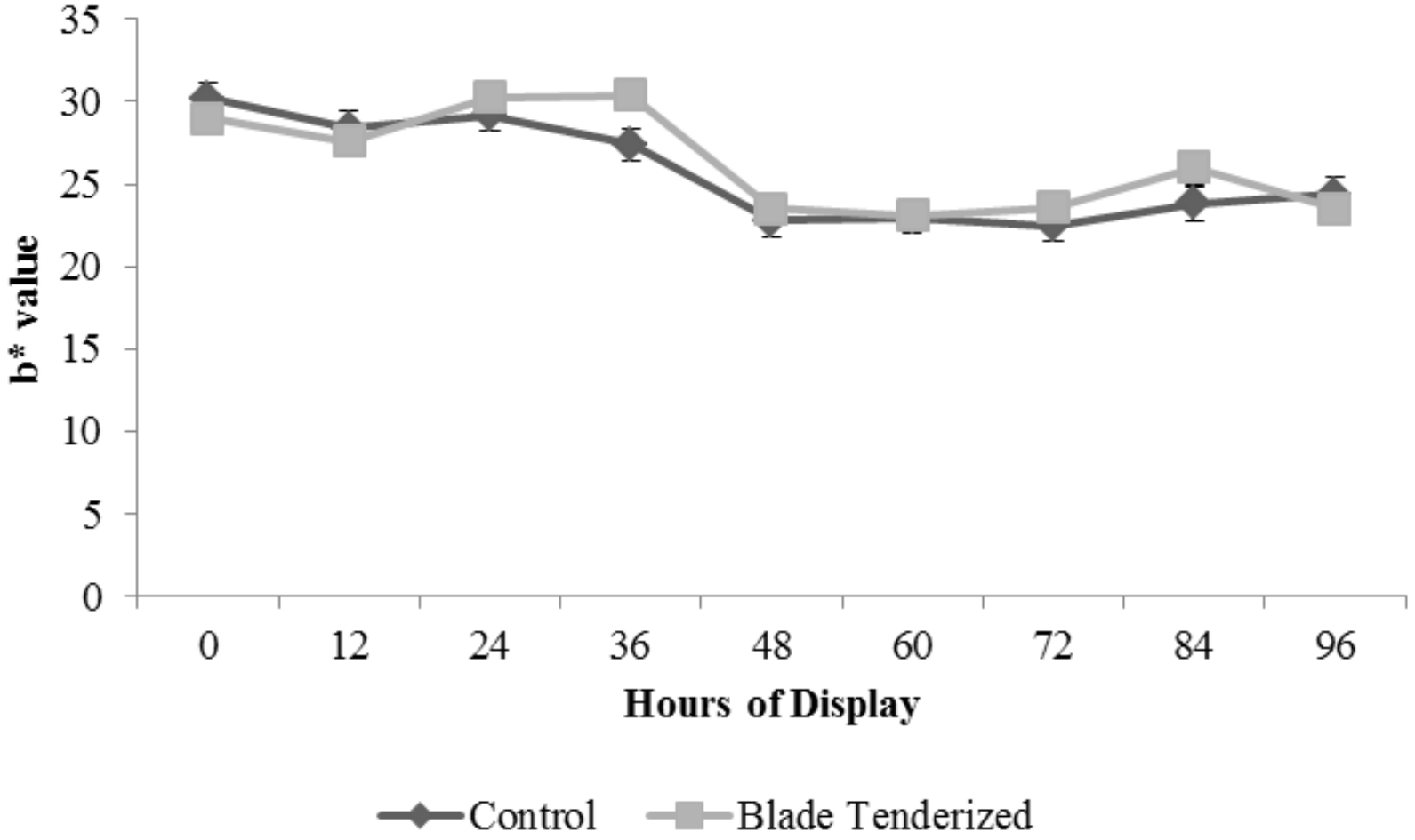


Table A.20 Main effects and interactions statistical significance p-value < 0.05.

Trait	Tenderization	Aging Period	Tenderization × Aging Period
pH	NS	*	NS
TBARS	NS	*	NS
Lactic Acid Bacteria (log CFU/g)	NS	*	NS
MRA	NS	*	NS
OCR	NS	*	NS
L*	NS	*	NS
a*	NS	*	NS
b*	NS	*	NS
Initial Color Panel	*	*	NS
Display Color Panel	*	*	*
Discoloration Color Panel	*	*	*
Warner-Bratzler Shear Force	*	*	*

*= Significant P < 0.05
NS= Not Significant P > 0.05

Table A.21 Main effects and interactions statistical significance p-value < 0.05.

Trait	Tenderization	Aging Period	Tenderization × Aging Period
Overall Tenderness	*	*	*
Myofibillar Tenderness	*	*	*
Beef ID	NS	NS	NS
Brown/Roasted	NS	NS	NS
Bloody/Serumy	NS	*	*
Liver Like	NS	NS	NS
Metallic	NS	NS	*
Fat Like	NS	*	NS
Green	NS	NS	NS
Rancid	*	NS	NS
Spoiled	*	NS	NS
Warmed Over	NS	*	NS
Overall Sweet	*	NS	*
Sour	NS	NS	NS
Bitter	*	*	*
Salty	NS	NS	NS
Umami	NS	NS	NS

*= Significant P < 0.05
NS= Not Significant P > 0.05

Table A.22 Statistical effects.

Trait	Tenderization	Aging Period	Tenderization × Aging Period
pH	NS	****	NS
TBARS	NS	****	NS
Lactic Acid Bacteria (log CFU/g)	NS	****	NS
MRA	NS	****	NS
OCR	NS	****	NS
L*	NS	****	**
a*	NS	****	****
b*	NS	****	*
Initial Color Panel	**	****	NS
Display Color Panel	****	****	****
Discoloration Color Panel	****	****	**
Warner-Bratzler Shear Force	****	****	**

NS > 0.10, *P < 0.1, **P < 0.05, ***P < 0.01, ****P < 0.001

Table A.23 Statistical effects.

Trait	Tenderization	Aging Period	Tenderization × Aging Period
Overall Tenderness	****	****	**
Myofibillar Tenderness	****	****	**
Beef ID	NS	NS	NS
Brown/Roasted	NS	NS	NS
Bloody/Serumy	NS	**	**
Liver Like	NS	*	NS
Metallic	NS	NS	**
Fat Like	NS	**	*
Green	NS	NS	NS
Rancid	**	NS	NS
Spoiled	**	*	NS
Warmed Over	NS	**	NS
Overall Sweet	**	NS	***
Sour	NS	*	NS
Bitter	***	**	**
Salty	NS	NS	NS
Umami	NS	NS	NS

NS > 0.10, *P < 0.1, **P < 0.05, ***P < 0.01, ****P < 0.001

Table A.24 Color main effects and interactions statistical significance p-value < 0.05.

Trait	Tenderization	Aging	Tenderization × Aging	Display	Tenderization × Display	Aging × Display	Tenderization × Aging × Display
L*	NS	*	NS	*	NS	*	NS
a*	NS	*	NS	*	NS	*	NS
b*	NS	*	NS	*	NS	*	NS
Display Color Panel	*	*	*	*	*	*	NS
Discoloration Panel	*	*	*	*	*	*	NS

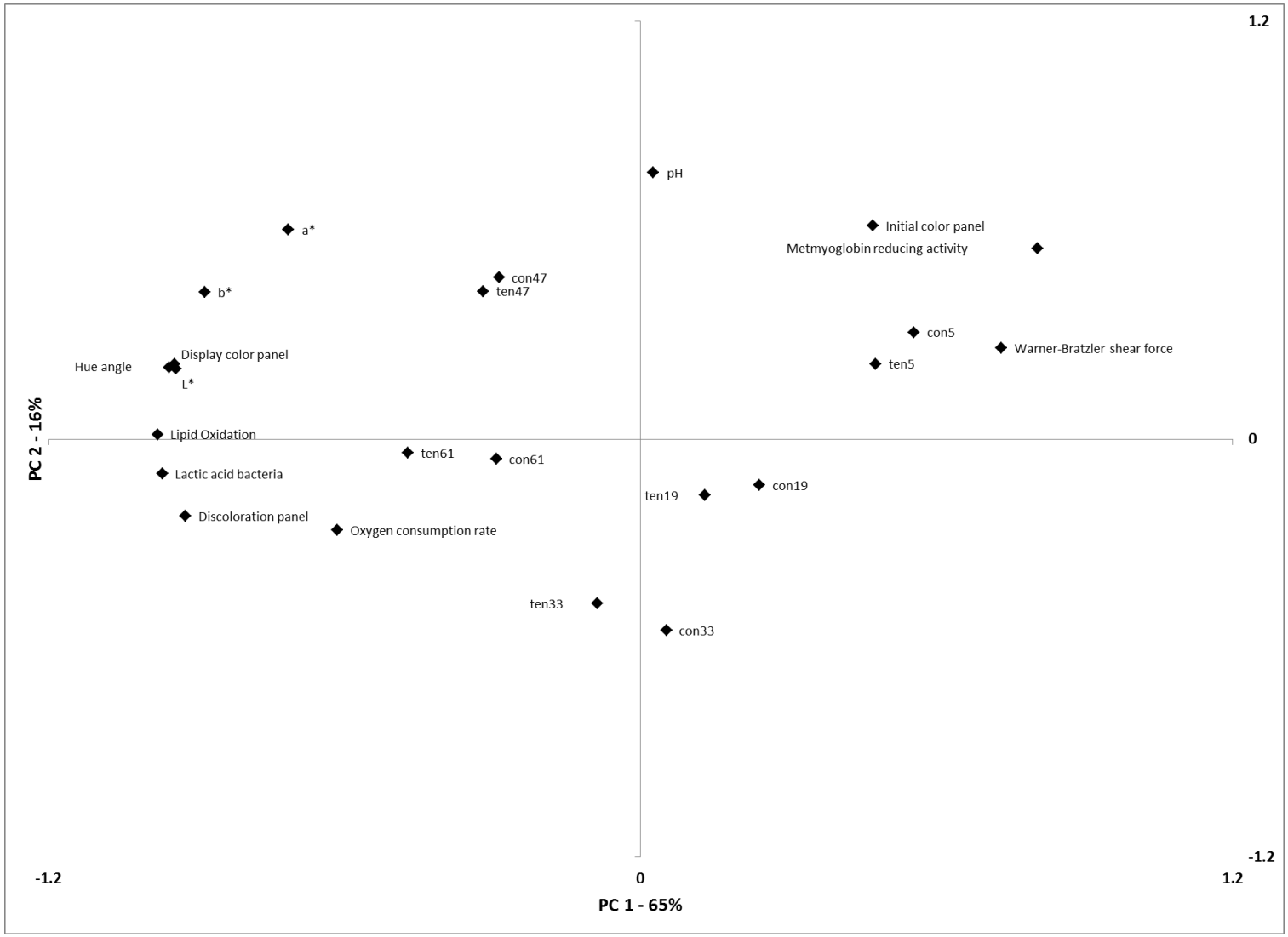
*= Significant P < 0.05
NS= Not Significant P > 0.05

Table A.25 Color Statistical effects.

Trait	Tenderization	Aging	Tenderization × Aging	Display	Tenderization × Display	Aging × Display	Tenderization × Aging × Display
L*	NS	****	NS	****	NS	****	NS
a*	NS	***	NS	****	NS	****	NS
b*	NS	****	NS	****	NS	****	NS
Display Color Panel	****	****	****	****	**	****	NS
Discoloration Panel	****	****	**	****	**	****	NS

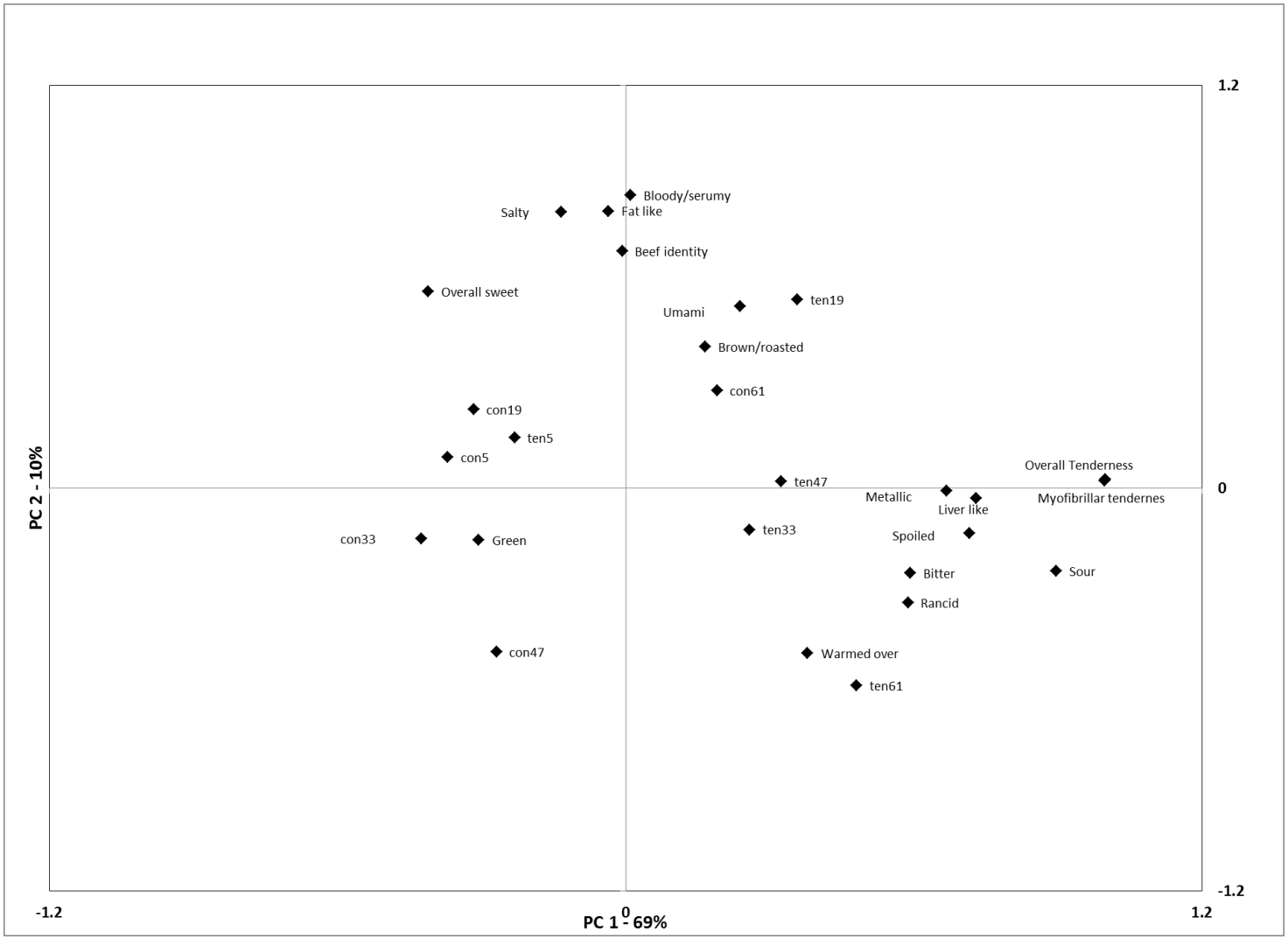
NS > 0.10
*P < 0.10
**P < 0.05
***P < 0.01
****P < 0.001

Figure A.4 Principal Component Analysis chart for all samples and quality traits.



control aged 5 d (con5); control aged 19 d (con19); control aged 33 d (con33); control aged 47 d (con47); control aged 61 d (con61); tenderized aged 5 d (ten5); tenderized aged 19 d (ten19); tenderized aged 33 d (ten33); tenderized aged 47 d (ten47); tenderized aged 61 d (ten61).

Figure A.5 Principal Component Analysis chart for all samples and sensory analysis traits.



control aged 5 d (con5); control aged 19 d (con19); control aged 33 d (con33); control aged 47 d (con47); control aged 61 d (con61); tenderized aged 5 d (ten5); tenderized aged 19 d (ten19); tenderized aged 33 d (ten33); tenderized aged 47 d (ten47); tenderized aged 61 d (ten61).

Appendix B - Methodology

Figure B.1 Initial color panel evaluation sheet.

Visual Color Panel

Gluteus medius steaks

NAME: _____ DATE: _____ Time: _____

Initial Color Scale:

1 = Bleached Red
2 = Very light cherry-red
3 = Moderately cherry-red
4 = Cherry-red
5 = Slightly dark red
6 = Moderately dark red
7 = Dark red
8 = Very dark red

Score to half-point increments

Package ID	Color Score		Package ID	Color Score

Figure B.2 Initial color panel scale.

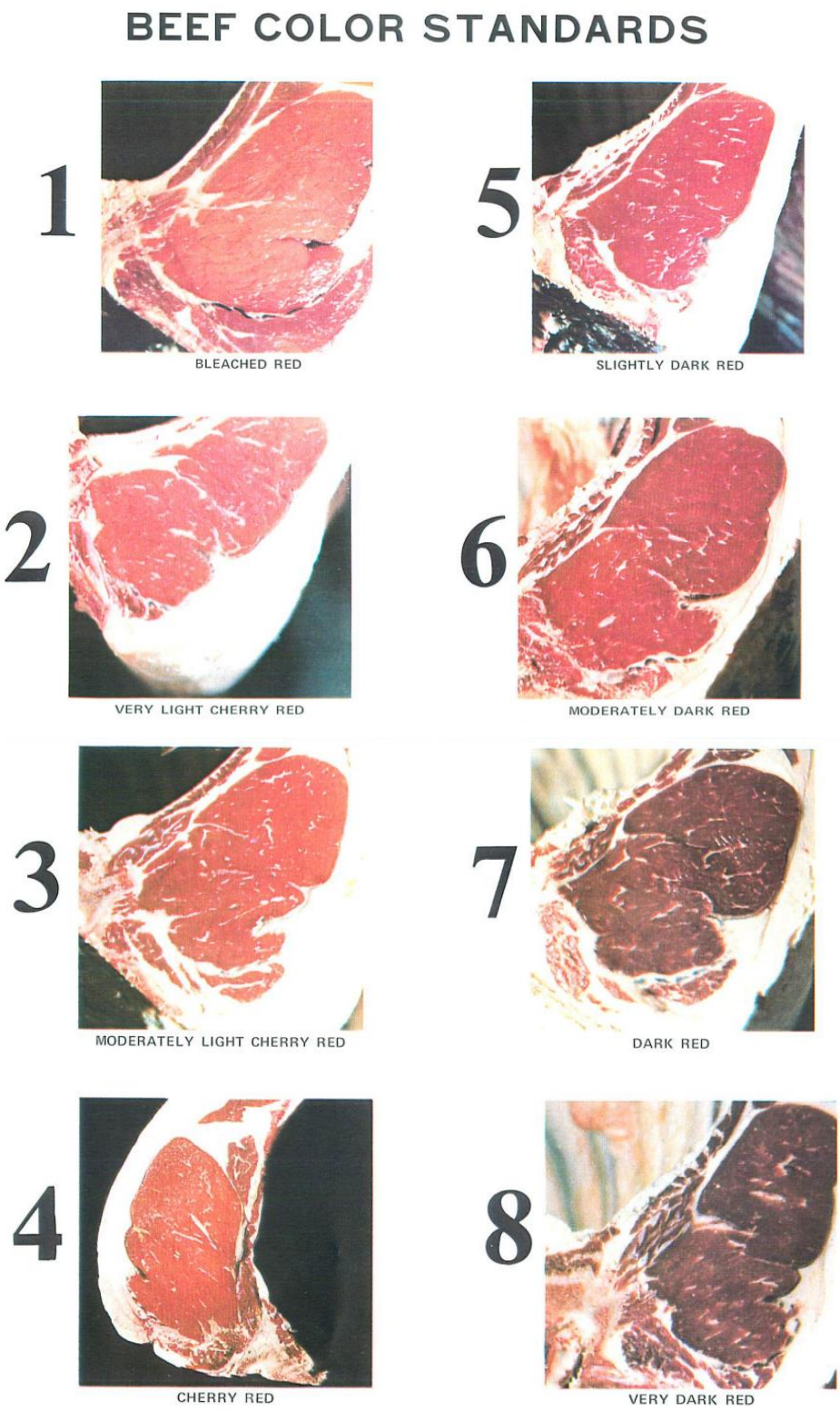


Figure B.3 Display color panel evaluation sheet.

Visual Color Panel

Gluteus medius steaks

NAME: _____ DATE: _____ Time: _____

Color Scale: To characterize retail color shelf-life

1 = Very bright red
2 = Bright red
3 = Dull red
4 = Slightly dark red
5 = Moderately dark red
6 = Dark red to dark reddish tan
7 = Tannish red
8 = Tan to brown

Score to half-point increments

Package ID	Color Score		Package ID	Color Score

Figure B.4 Discoloration panel evaluation sheet.

Visual Color Panel - Surface Discoloration

Gluteus medius Steak

NAME: _____ DATE: _____ Time: _____

Discoloration Color Scale:

1 = 0% Surface Discoloration

2 = 1 to 10% Surface Discoloration

3 = 11 to 25% Surface Discoloration

4 = 26 to 50% Surface Discoloration

5 = 51 to 75% Surface Discoloration

6 = 76-99% Surface Discoloration

7 = 100% Surface Discoloration

Package ID	Color Score		Package ID	Color Score

Figure B.5 Definition and references of sensory evaluation attributes.

Overall tenderness: Ease with which the sample can be cut through with molars on first bite. Chew the samples lengthwise, i.e. teeth cut through the middle of the sample and not the surfaces that have been grilled (long way on your teeth)

TEXTURE:

Reference: Beef Eye of Round Steak (leached overnight) = 5.0
Beef Tenderloin = 11.0

Preparation: Soak thawed eye of round steak overnight in water. Grill on high until internal temperature reach 76.7^{o}C. Discard edges and cut into 1.27 cm cubes. Place three cubes into 3.25 oz cups. Serve warm.
Cut tenderloin into 1 inch thick steaks. Grill on high until internal temperature reach 71.1^{o}C. Discard edges and cut into 1.27 cm cubes. Place three cubes into 3.25 oz cups. Serve warm.

Myofibrillar tenderness: Evaluate after first 5 chews. The ease with which the sample's muscle fibers (excludes connective tissue) can be broken down. Chew the samples lengthwise, i.e. teeth cut through the middle of the sample and not the surfaces that have been grilled (long way on your teeth).

TEXTURE:

Reference: Beef Eye of Round Steak (leached overnight) = 4.0
Beef Tenderloin = 12.0

Preparation: Soak thawed eye of round steak overnight in water. Grill on high until internal temperature reach 76.7^{o}F. Discard edges and cut into 1.27 c cubes. Place three cubes into 3.25 oz cups. Serve warm.
Cut tenderloin into 1 inch thick steaks. Grill on high until internal temperature reach 71.1^{o}F. Discard edges and cut into 1.27 cm cubes. Place three cubes into 3.25 oz cups. Serve warm.

For the following attributes, chew the samples lengthwise to the point of swallowing.

Beef flavor ID: Amount of beef flavor identity in the sample.
AROMA and FLAVOR:
Reference: 80% Lean Ground Chuck = 7.0
Beef Brisket = 11.0
Preparation: lean ground chuck on a pan to 71.1°C. Serve warm into 1 oz cups.

Cut brisket into 1 inch thick steaks. Grill on High until internal temperature reach 71.1°C. Let it sit for 2 minutes. Discard edges and cut into 1.27 cm cubes. Place three cubes into 3.25 oz cups. Serve warm.

Brown/Roasted A round, full aromatic generally associated with beef suet that has been broiled.
AROMA and FLAVOR:
Reference: 80% Lean Ground Chuck = 10.0
Preparation: lean ground chuck on a pan to 71.1°C. Serve warm into 1 oz cups.

Bloody/Serumy: An aromatic associated with blood on cooked meat products. Closely related to metallic aromatic.
AROMA and FLAVOR:
Reference: USDA choice Strip Steak = 5.5
Beef Brisket = 6.0
Preparation: Grill Strip Steak on "High" until internal temperature reach 60.0°C. Let it sit for 2 minutes. Discard edges and cut into 1.27 cm cubes. Place three cubes into 3.25 oz cups. Serve warm.
Cut brisket into 2.54 cm thick steaks. Grill on High until internal temperature reach 71.1°C. Let it sit for 2 minutes. Discard edges and cut into ½'' cubes. Place three cubes into 3.25 oz cups. Serve warm.

Liver-like: Aromatics associated with cooked organ meat/liver.
AROMA and FLAVOR:
Reference: Beef Liver (broiled) = 7.5
Preparation: Pan-fry liver on a skillet on medium high until internal temperature reaches 71.1°C or liver is brown throughout. Cut into 1"square. Place two pieces into 1 oz cups. Serve warm.

Metallic: The impression of slightly oxidized metal, such as iron, copper, and silver spoons.

AROMA and FLAVOR:

Reference: 0.10% Potassium Chloride Solution = 1.5 (flavor)
USDA choice Strip Steak = 4.0 (aroma and flavor)

Preparation: Grill Strip Steak on "High" until internal temperature reach 60.0^{o}C. Let it sit for 2 minutes. Discard edges and cut into 1.27 cm cubes. Place three cubes into 3.25 oz cups. Serve warm.

Fat-like: Aromatics associated with cooked animal fat.

AROMA and FLAVOR:

Reference: Hillshire Farm lit'l Beef Smokies = 7.0

Preparation: Place 1/2 package of smokies in 1.9 cm cup of water in the crockpot.

Green: Sharp slightly pungent aromatics associated with green/plant/vegetable matters such as parsley, spinach, pea pod, fresh cut grass, etc.

AROMA and FLAVOR:

Reference: Fresh parsley water = 9.0 (flavor)

Preparation: Rinse and chop 25 g of fresh parsley. Add 300 ml of water. Let sit for 15 min. Filter and serve the liquid part in 1 oz cup.

Rancid: An aromatic commonly associated with oxidized fat and oils. These aromatics may include cardboard, paint, varnish, and fishy.

FLAVOR:

Reference: Wesson Vegetable Oil (3 min) = 7.0

Preparation: Microwave ½ cup oil on high power for 3 minutes. Let cool and pour into 1 ounce cups. Serve covered.

Warmed-over: Perception of a product that has been previously cooked and reheated.

FLAVOR:

Reference: 80% lean ground beef (reheated) = 6.0 (flavor)

Preparation: Cook ground beef in a skillet, on medium-high temperature, to 71.1^{o}C. Drain grease. Refrigerate overnight in a covered glass container. The next day, place cooked ground beef in a in an 20.3 cm x 20.3 cm glass baking dish. Pre-heat oven on bake at 204.4^{o}C. Heat ground beef until internal temperature reaches 71.1^{o}C (approximately 7 min). Fill 1 oz. cup with the reheated ground beef. Serve warm.

Overall Sweet: A combination of sweet taste and sweet aromatics. The aromatics associated with the impression of sweet.
FLAVOR:
Reference: Post Shredded Wheat Spoon Size = 1.5
Hillshire Farms Lit'l Beef Smokies = 3.0
Preparation: Place 7 Post shredded wheat in 3.25 oz cup.

Sour: The fundamental taste factor associated with a citric acid solution.
Reference: 0.015% Citric Acid Solution = 1.5
0.050% Citric Acid Solution = 3.5
0.080% Citric Acid Solution = 5.0

Bitter: The fundamental taste factor associated with a caffeine solution.
Reference: 0.01% Caffeine Solution = 2.0
0.02% Caffeine Solution = 3.5
0.035% Caffeine Solution = 5.0

Salty: A fundamental taste factor of which sodium chloride is typical.
Reference: 0.15% NaCl Solution = 1.5
0.25% NaCl Solution = 3.5

Umami: Flat, salty, somewhat brothy. The taste of glutamate, salts of amino acids and other molecules called nucleotides.
Reference: 0.035% Accent Flavor Enhancer Solution = 7.5

Spoiled: The presence of inappropriate aromatics and flavors that is commonly associated with the products. It is a foul taste and/or smell that indicates the product is starting to decay and putrefy.
AROMA and FLAVOR:
Reference: Dimethyl disulfide in propylene glycol (10,000 ppm) = 12.0 (aroma)
Preparation: Dip a Orlandi Perfumer Strip #27995 (2.2 cm) in the solution to the second marking line and place dipper strip (marking line up) in a glass test tube with screw cab.

Figure B.6 Sensory evaluation sheet.

Panelist _____ Sample _____ Date _____

Overall tenderness	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	**5**	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	**11**	11.5	12	12.5	13	13.5	14	14.5	15
Myofib. tenderness	0	0.5	1	1.5	2	2.5	3	3.5	**4**	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	**12**	12.5	13	13.5	14	14.5	15
Beef flavor ID	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	**7**	7.5	8	8.5	9	9.5	10	10.5	**11**	11.5	12	12.5	13	13.5	14	14.5	15
Brown/Roasted	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	**10**	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Bloody/Serumy	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	**5.5**	**6**	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Liver-like	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	**7.5**	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Metallic	0	0.5	1	**1.5**	2	2.5	3	3.5	**4**	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Fat-like	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	**7**	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Green	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	**9**	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Rancid	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	**7**	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Warmed-over	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	**6**	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Overall sweet	0	0.5	1	**1.5**	2	2.5	**3**	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Sour	0	0.5	1	**1.5**	2	2.5	3	**3.5**	4	4.5	**5**	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Bitter	0	0.5	1	1.5	**2**	2.5	3	**3.5**	4	4.5	**5**	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Salty	0	0.5	1	**1.5**	2	2.5	3	**3.5**	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Umami	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	**7.5**	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
Spoiled	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	**12**	12.5	13	13.5	14	14.5	15
_____	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
_____	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15