EFFECTS OF EXTENDED AGING ON BIOCHEMICAL PROPERTIES OF DARK CUTTING BEEF

By

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Abstract:
Elevated muscle pH in dark cutting beef can enhance mitochondrial activity and stability of enzymes involved in oxygen consumption. Both processes can influence the myoglobin oxygenation, hence beef color. Limited studies have characterized the effects of extended aging on biochemical properties of dark cutting beef. Therefore, the objectives were: (1) to determine the effects of extended aging on biochemical factors involved in dark cutting beef, and (2) to quantify the mitochondrial content in normal pH and dark cutting beef using real time quantitative polymerase chain reaction (q-PCR). Ten USDA choice (pH = 5.6) and ten no-roll dark cutter (pH > 6.4) strip loins (IMPS #180) were obtained from a commercial packing plant within 3 d of harvest. Loins were cut into four equal sections, vacuum packaged, and aged for 21, 42, and 62 d at 2°C. Following aging of normal pH and dark cutting loin sections, two 2.5-cm thick steaks were cut and used to determine blooming properties, oxygen consumption, metmyoglobin reducing activity, lipid oxidation, mitochondrial quantification, and myoglobin concentration. Surface color readings were measured using a HunterLab Miniscan XE Plus spectrophotometer. Oxygen consumption rate and metmyoglobin reducing activity were measured as oxygenation capacity and initial metmyoglobin formation, respectively. q-PCR was used to quantify mitochondrial concentration. The experiment was replicated 10 times. The data were analyzed using the Mixed Procedure of SAS and significance was determined at $P < 0.05$. A muscle pH type x blooming time x aging interaction ($P < 0.05$) was found for a* values. Normal pH beef had greater a* values at all bloom time points. Metmyoglobin reducing capacity was greater ($P < 0.05$) for dark cutting beef compared with normal pH beef. Aging improved blooming properties of both normal and dark cutting beef. There was no effect on lipid oxidation. Mitochondria content was 2.04 fold greater in dark cutting beef compared with normal pH beef. Better understanding the biochemical properties will help to design strategies to improve blooming properties in dark cutting beef.

Keywords: dark cutter, aging, bloom, mitochondria

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CHAPTER I

INTRODUCTION

Meat purchasing decisions are influenced by color more than any other quality factor because consumers use discoloration as an indicator of freshness and wholesomeness. As a result, nearly 15% of retail beef is discounted in price due to surface discoloration, which corresponds to annual revenue losses of $1 billion (Smith et al., 2000).

Dark cutting beef is the result of a depletion of glycogen reserves prior to slaughter, and is often described as meat that fails to bloom after the cut surface has been exposed to oxygen. Dark cutting beef is one of the most prominent meat quality issues worldwide. The National Beef Quality Audit reported that the US Beef Industry lost approximately $165-$170 million dollars in 2000 due to dark cutting carcasses. More specifically, this loss results from discounted price in beef carcasses. In Canada, it has been estimated that approximately $1.4 million are loss per year to beef producers (McKenna et al., 2002).

When the pH of meat remains elevated, the physical state of the proteins will be above the iso-electric point, proteins will then retain more water and, therefore, will cause the muscle fibers to be more tightly packed. Consequently, the meat is then dark in color because its surface does not scatter light to the same extent as the more open surface of meat with a lower ultimate pH (Seideman et al., 1984). Increased pH also enhances mitochondria activity (Ashmore et al., 1972). Mitochondrial oxygen consumption can influence myoglobin oxygenation. It has been reported that oxidative enzymes are more active and oxygen diffusion is reduced in dark cutters due to the high ultimate pH (Lawrie, 1958). Dark cutting beef is unacceptable because it is visually unappealing to consumers and high pH allows spoilage bacteria to grow readily reducing its shelf life (Tarrant, 1989). Hence, developing post-harvest methods to reverse the dark cutting problems will have significant impact on the meat industry.

Postmortem aging has been shown to decrease oxygen consumption. Mac Dougall et al. (1982) reported that increased aging would improve bloom by decreasing the competition for oxygen between myoglobin and mitochondria. More specifically, the substrates for mitochondria respiration are continually depleted during extended aging. As a result, oxygen can penetrate faster into tissue as time postmortem increases.

Modified atmosphere packaging has also been shown to be a useful technique for extending shelf life of fresh beef. For example, packaging meat in high-oxygen atmosphere will promote the formation of oxymyoglobin and hence a brighter red color. Mancini & Hunt (2005) reported that carbon monoxide can improve the color of meat by binding to myoglobin to form a very stable bright red color. Similarly, enhancement-technology has allowed the beef purveyors to inject meat with ingredients that can improve color life. For, example there has been research involving the use of lactic acid enhancement to alter the muscle pH to reverse the dark red color in high pH beef.

Although it is known that aging, and modified atmosphere packaging can increase the blooming properties; limited research has determined its effects during extended aging. Similarly, limited knowledge is currently available on the effects of aging on the biochemical properties of dark cutting beef. Thus, the objectives of the current research were:

1. To determine the effects of extended aging and modified atmospheric packaging on surface color of beef *longissimus*.
2. To evaluate the effects of aging and freeze thawing on oxygenation properties of dark cutting beef.

REFERENCES

Ashmore, C. R., Parker, W., & Doerr, L. (1972). Respiration of mitochondria isolated from dark-cutting beef: postmortem changes. *Journal of Animal Science*, 34:1.

Lawrie, R. A. (1958). Physiological stress in relation to dark-cutting beef. *Journal of Food Science Agriculture*, 11:721

Mac Dougall, D., (1982). Changes in colour and opacity of meat. *Food Chemistry,* 9,75-88.

Mancinin, R. A., Kropf, D. H., Hunt, M. C., & Johnson, D. E. (2005). Effects of endpoint temperature, pH, and storage time on cooked internal color reversion of pork longissimus chops. *Journal of Muscle Foods*, 16, 16-26.

Mckenna, D. R., D. L. Roeber, P. K. Bates, T. B. Schmidt, D. S. Hale, D. B. Griffin, J. W. Savell, J. C. Brooks, J. B. Morgan, T. H. Montgomery, K. E. Belk, & G. C. Smith. (2002). National Beef Quality Audit-2000: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. *Journal of Animal Science,* 80:1212.

Seideman, S. C., Cross, H. R., Smith, G. C., & Durland, P. R., (1984). Factors affecting fresh meat colour: review. *Journal of Food Quality,* 6(3), 211-237.

Smith, G. C., Belk, K. E., Sofos, J. N., Tatum, J .D., & Williams, S. N., (2000). Economic implications of improved color stability in beef. In E. A. Decker, C. Faustman, & C. J. Lopez-Bote (Eds.), Antioxidants in muscle foods: Nutritional strategies to improve quality (pp. 397-426). New York: Wiley Interscience.

Tarrant, P. V., (1989). Animal behavior and environment in the dark-cutting condition in beef-A review. Irish *Journal of Food Science & Technology*, 13, 1-21.

CHAPTER II

REVIEW OF LITERATURE

Meat Color

Meat color is the most important parameter influencing marketability of fresh beef because at the point of sale consumers often associate bright cherry-red color of fresh beef and the purplish-red color of vacuum-packaged beef as an indicator of wholesomeness (Hood & Riordan, 1973; Faustman & Cassens, 1990). As a result nearly 15% of retail beef is discounted in price due to surface discoloration, which corresponds to annual revenue losses of $1 billion (Smith et al., 2000).

Myoglobin is the principle protein responsible for meat color, although other heme proteins such as hemoglobin and cytochrome C may also play a role in beef, lamb, pork, and poultry color. Myoglobin is a water-soluble protein containing 8 α-helices (A-H) linked by short non-helical sections. Myoglobin also contains a prosthetic group located within the protein's hydrophobic pocket. The heme ring has a centrally located iron atom that can form six bonds. A 6^{th} site is available to reversibly bind with ligands. A distal histidine also influences color dynamics by affecting the spatial relationship within the hydrophobic heme pocket. The ligand present and the valence of iron dictate meat color. Three major forms of myoglobin are primarily responsible for meat color. Deoxymyoglobin occurs when no ligand is present at the 6^{th} coordination site and the heme iron is ferrous (Fe^{2+}). This results in the purplish-red or purplish-pink color typically associated with vacuum packaged product and/or muscle immediately after cutting. Oxygenation occurs when myoglobin is exposed to oxygen and is characterized by the development of a bright cherry-red color. No change in iron's valence occurs during oxygenation although the 6^{th} coordination site is occupied by diatomic oxygen. Depth of oxygen penetration and thickness of the oxymyoglobin layer depend on the temperature, oxygen partial pressure, pH, and competition for oxygen by other respiratory processes. Discoloration results from oxidation

of both ferrous myoglobin derivatives to ferric iron (Livingston & Brown, 1982; Wallace et al., 1982). Discoloration is often referred to as the amount of surface area covered by metmyoglobin. Subsurface myoglobin forms also play a role in product appearance. This is because metmyoglobin beneath the surface (located between superficial oxymyoglobin and interior deoxymyoglobin) gradually thickens and moves toward the surface. Reduction of metmyoglobin is crucial to meat color life and greatly depends on muscle's oxygen scavenging enzymes, reducing enzyme systems, and the NADH pool, which is limited in postmortem muscle. Carboxymyoglobin is a relevant chemical state of myoglobin because of the current increased interest in packaging with low levels of carbon monoxide (Luno et al. 2000; Hunt et al., 2004; Sorheim et al., 2001). Carbon monoxide can bind to the vacant 6th position of deoxymoylobin and form a very bright-red color that is very stable. The FDA now approves the usage of 0.4% carbon monoxide gas in a modified atmospheric package.

There are numerous other factors that affect the color stability of meat from livestock and poultry (Suman et al., 2014). Modern-day agricultural industry must utilize scientific principles, with both pre- and/or post-harvest strategies, to improve the appearance and color stability of fresh as well as cooked beef to satisfy consumer needs (Troy & Kerry, 2010).

Dark Cutting Beef

Dark cutting beef is characterized by high postmortem pH, increased water binding capacity, sticky texture, and the inability to bloom when exposed to air. Bloom is the development of a bright red color when meat is exposed to air, due to the oxygenation of myoglobin to form oxymyoglobin. Since dark cutting meat will not bloom when exposed to air, it is discounted at the retail level (Price & Schweigert, 1978). In normal meat, postmortem

glycolysis reduces pH to 5.8 or lower which impairs mitochondrial oxygen consumption (Ashmore et al., 1972) and allows normal bloom on meat surfaces exposed to air. Lawrie (1958) found that mitochondrial cytochrome oxidase was more active at pH values above 6.0, characteristic of dark cutting beef muscle, and concluded that increased oxygen consumption of dark cutting meat could increase the concentration of deoxygenated myoglobin, thus resulting in the dark color.

Ashmore et al., (1973) showed that dark cutting meat could be experimentally prevented by injecting the live animal with propranolol a competitive inhibitor of epinephrine, thus preventing ante-mortem depletion of muscle glycogen. Egbert and Cornforth (1986) reported that the incidence of dark cutting beef might be experimentally reduced by the use of drugs to reduce ante-mortem glycolysis depletion or by proper handling conditions that reduce ante-mortem stress. Cornforth and Egbert (1985) showed that normally dark pre-rigor muscle would turn bright red when blended with the mitochondrial respiratory inhibitor, rotenone, lowering pH, and/or when thin meat slices were chilled in an oxygen-rich atmosphere.

Hedrick et al., (1959) described dark cutting beef as a dark, sometimes a blackish, color when cut and fails to develop a cherry-red color expected by the meat trade. The basis for dark cutting beef is the activation of muscle glycolytic metabolism culminating in low muscle glycogen stores at the time of slaughter and therefore, leading to a high ultimate pH. Zerouala and Stickland (1991) tested different fiber type proportions in dark cutting and normal animals that had been slaughtered under normal abattoir conditions. Results demonstrated a shift to more oxidative metabolism in the muscle of dark cutting cattle with some evidence for a greater shift in dark cutting bulls. Confirming that cattle exhibiting a relatively high level of oxidative metabolism with their muscles are more susceptible to the development of dark cutting beef.

In midwestern beef processing plants 1.1-1.4% of cattle slaughtered in August, September, and October were dark cutters, with incidence of 0.4-0.7% during the other months (Kreikemeier et al., 1998). According to Wulf (1998) most meat packers discount dark cutters substantially. In Canada, the proportion of beef carcasses that grades Canada B4 has increased noticeably from an average of 0.8% of total carcasses processed in 1998/99 to 1.3% in 2010/11 (Wulf, 1998). The increase in dark cutting carcasses in Canada represents about $1.4 million in lost carcass value each year and is of significant concern to the Canadian beef industry. Canada B4 carcasses shelf life is shortened by its increased pH being conducive to microbiological growth; however, this can be managed through packaging and cold-chain control, but also because the toughness of the cooked product is unpredictable (Holdstock et al., 2014). The ability to predict the likelihood of an animal producing dark meat is important in the Canadian beef industry because of the economic penalty assessed to dark cutting carcasses (Wulf, 1998).

When the first National Beef Quality Audit (NBQA) was conducted in 1991, Smith et al., (1992) concluded that 5.0 percent of all carcasses surveyed were dark cutters and, 3.4 percent were discounted one-third of a quality grade, 1.1 percent were discounted two thirds of a quality grade and 0.5 percent were discounted one full quality grade. During the 1995 NBQA, the incidence of dark cutting beef carcasses in the United States was 2.7% and cost the beef industry $172 million USD annually (Smith et al., 1995), and 2.7 % of the carcasses audited were classified as dark cutters (Boleman et al., 1998). According to the 2000 NBQA, discounts as high as $240 USD per carcass are associated with dark cutting beef and it cost the industry an estimated $5.81 USD per head. In 2000, 2.3% of all steer and heifer carcasses were dark cutters, resulting in a loss of $165 - $170 million USD on dark cutting carcasses alone. In NBQA-2011, 3.2% of the carcasses assessed were dark cutters.

Effects of Increased pH on Meat

Dark cutters result from pre-slaughter stress, which depletes muscle glycogen. If glycogen is depleted by chronic long-term stress before slaughter then less lactic acid is formed and the meat does not acidify normally, hence the ultimate pH remains high. Dark cutting meat has a pH of > 5.8 (Viljoen et al., 2002). When postmortem glycolysis decreases muscle pH, making it brighter, and superficially more wet (Swatland, 1989). If the ultimate meat pH is high, the physical state of the proteins will be above their iso-electric point. Proteins will associate with more water in the muscle and therefore fibers will be tightly packed. Therefore, the meat is then dark in color because its surface does not scatter light to the same extent as the more open surface of meat with a lower ultimate pH (Seideman et al., 1984). Dark cutting beef is unacceptable because it is visually unappealing to consumers and high pH allows spoilage bacteria to grow readily thus reducing its shelf life (Tarrant, 1989).

Mitochondria and Oxygen Consumption

Mitochondria and myoglobin are highly correlated, myoglobin provides oxygen to mitochondria that then consumes more than 80% of the oxygen in muscle (Neely et al., 1974). Mancini & Ramanathan (2010) stated that in postmortem muscle, diffusion of oxygen from the surface is the major pathway for obtaining oxygen. Additionally, mitochondrial oxygen consumption can influence both color development and color stability. The specific cause of the dark color of the meat from dark cutters is equivocal. It has been reported that oxidative enzyme are more active and oxygen diffusion is reduced in dark cutters due to the high ultimate pH (Lawrie, 1958). Ashmore (1971) observed that increased oxygen consumption, and the decreased availability of oxygen results in the conversion of bright-red oxymyoglobin to dark-red

deoxymyoglobin. Ashmore et al., (1971) assessed the functional capacity of mitochondria isolated from muscle of dark cutters and normal animals at the time of slaughter. The primary objective of their study was to investigate the effects of epinephrine administration on oxidative metabolism in bovine muscle. Dark cutting beef was produced in heifers by subcutaneous injection of epinephrine. No significant differences were found in mitochondrial protein yield, mitochondrial function, or in specific activity of succinic dehydrogenase between preparations from control and experimental animals. It has been proposed that the dark color of the meat results from enhanced oxygen consumption, impaired oxygen permeability of the carcass, or a combination of both (Lawrie 1958).

Oxygen consumption in situ requires a functional chain of mitochondrial enzymes (Ashmore et al., 1972). Ashmore et al., (1972) examined the relationships of the functional capacity of mitochondria, isolated from control and epinephrine injected animals, with time after death, and with pH. Mitochondria isolated from the muscles of dark cutters showed the same trend as those from the control animals. There was a decrease in functional ability with time after death, and mitochondria isolated and assayed at the higher pH performed better than those which has been subjected during isolation, assay, or both to the lower pH. However, each hour after death, and at each set of pH conditions mitochondria from dark cutters performed better than those isolated from the control animals. Five days after death, mitochondria isolated and assayed from dark cutters at the higher pH exhibited reasonably good respiration control ratios, and rates of state, 3 respiration were higher than those obtained from control animals only 24 hours after death. Furthermore, the dark color of meat from dark cutters is a cut surface phenomenon. The meat of control animals is also dark when freshly cut, but the surface brightens rapidly when exposed to air. The meat from the dark cutters fails to brighten appreciably. The results in

Ashmore et al., (1972) showed that the failure of the bright color to develop in dark cutters results from the maintenance of a relatively high rate of oxygen consumption at the surface of the meat where oxygen is available, thereby maintaining the myoglobin in the reduced state. Inactivation of mitochondria in muscle fibers of control animals inhibits the myoglobin to remain oxygenated and bright red in color.

Mitochondria are less active at lower temperatures (Atkinson, 1969). An increased storage temperature can augment oxygen consumption, as well as limit the formation and thickness of oxymyoglobin beneath the surface. When the enzyme activity is greater, pH then declines more rapidly due to more enzyme activity at a greater temperature This will reduce oxygen consumption within the deep portion of the *semimembranosus*, creating the two tone appearance of the inside round muscle. Intra-muscle differences in temperature and pH decline also cause variation in color stability between the deep and superficial portions of the *semimembranosus* by influencing NADH regeneration, metmyoglobin reduction and protein denaturation (Mancini & Ramanathan, 2010). Postmortem aging has been shown to decrease oxygen consumption. Mac Dougall et al., (1982) reported that increased aging would improve bloom by decreasing competition for oxygen between myoglobin and mitochondria because substrates for mitochondria respiration are continually depleted postmortem. As a result, oxygen can penetrate faster and further into tissue as time postmortem increases. Although, aging improves initial myoglobin oxygenation, it can be detrimental to color stability because the activity of reducing enzymes and NADH content also are depleted postmortem.

Extended Aging and Beef Color

Mancini & Ramanathan (2014) reported that meat color is significantly influenced by mitochondrial activity via two mechanisms: oxygen consumption and metmyoglobin reducing activity. Oxygen consumption decreases initial red color development and intensity when mitochondrial respiration outcompetes myoglobin for oxygen. This maintains myoglobin in a deoxy-state and results in dark colored muscle (Ledward, 1992). Mancini & Ramanathan (2014) investigated the effects of aging time (0, 15, 30, and 45d) and temperature (0 or 5°C) on beef mitochondrial oxygen consumption and metmyoglobin reduction, as well as steak color intensity and color stability. Results indicated that as aging time increased, mitochondrial oxygen consumption decreased (Mancini & Ramanathan, 2014). As aging time increased, the ability of mitochondria to convert oxymyoglobin to deoxymyoglobin also decreased when succinate and lactate were used as substrates for mitochondrial respiration. Mitochondria-mediated metmyoglobin reduction tended to decrease as aging time increased (Mancini & Ramanathan, 2014).

After beef is exposed to air it slowly turns to a bright cherry-red color, this process is typically referred to as blooming. Blooming is the results of oxygen binding to the iron atom and, in this state, the myoglobin molecule is called oxymyoglobin and the amount of time it takes to oxygenate the cut surface deoxymyoglobin to oxymyoglobin is know as the blooming time. Instrumental color measurements have been used to measure muscle color and characterize bloom time (Lee et al., 2008). Lee et al. (2008) evaluated the effect of postmortem vacuum-aging period on bloom development of the *gluteus medius* (GM) muscle from top sirloin butts. Results indicated that 50%, or more, of the color development in the GM occurred within the first 10 min post-cutting for L*, a*, and b* values. Instrument color results (a* and b* values), as well as

calculated chroma values and oxymyoglobin percentages, demonstrated that the extent to which GM steaks bloomed was affected by the duration of vacuum-aging with steaks from top sirloin butts aged 14 days or less, having a greater proportion of oxymyoglobin and a more vivid, redder, and more yellow color when compared to steaks from top sirloin butts aged 28 or 35 days. Wulf and Wise (1999) reported that L*, a* and b* values of the *longissimus thoracis* (LT) increased during the first 12 min after ribbing beef carcasses, but did not change thereafter, furthermore that the bloom time has a greater effect on a* and b* values then on L*. Rentfrow et al. (2004) found similar results in that a* and b* values for beef LT steaks were impacted more than L* values across a 93-min bloom time, with all changes in instrumental color measures occurring within the first 9-12-min after cutting.

Lee et al. (2008) determined that the activities of the oxygen-consuming enzymes likely contributed to the differences in color development among the aging period treatments. Oxygen consumption rates decrease with increasing days of retail display for all beef muscles (McKenna et al., 2005). Ledward (1992) stated that meat that has been aged for several weeks in vacuum packaging prior to exposure to air blooms more rapidly and intensely than fresh meat owing to some loss of activity of the oxygen-consuming enzymes. These results were confirmed by Zhu and Brewer (1998), in which the oxygen consumption rate of pork *longissimus thorais et lumborum* declined exponentially during storage at 4°C.

Research has indicated that postmortem aging improves the tenderness of the LT (Shorthose & Harris, 1990; Gruber et al., 2006); however, there is limited information concerning the effect of aging on color development/blooming. Studies dealing with bloom development in beef have been conducted on LT of carcasses after ribbing, and very little, research has been focused on the factors affecting color development in vacuum-aged subprimals

(Lee et al., 2008). Lee et al. (2008) investigated the duration of postmortem vacuum/wet-aging of beef ribeye rolls on bloom development in the LT. They found that despite the duration of postmortem aging beef there was virtually no effect on bloom development, with the exception, that the rate of oxymyoglobin formation in the LT appears to be quickened by extending the wet-aging time of ribeye rolls to 28 or 35 d. Additionally, 90% of the change in instrumental color measurements was achieved within 60 min of steak fabrication; results indicated that LT color would stabilize sometime beyond the 2 h observation period (Lee et al., 2008).

Altering Dark Cutting Fresh Beef Color

Subcutaneous injections of epinephrine administered for 24 to 48 h prior to slaughter has been shown to mimic the condition produced by natural stress conditions. Epinephrine acts to accelerate glycogen metabolism in muscle tissue (Ashmore et al., 1973). Ashmore et al. (1973) utilized a propranolol, a beta-adrenergic blockade agent and a competitive inhibitor of epinephrine to prevent the occurrence of dark cutting carcasses in sheep. Sheep were injected at 44, 24, and 16 h prior to slaughter. Propranolol was injected simultaneously and at 48, 40 and 20 h prior to slaughter. Animals that were injected with epinephrine alone showed 48 h post-mortem pH averaging 6.6, and depleted muscle glycogen at slaughter. Isolated mitochondria respired actively 48 h after slaughter. Sheep injected with epinephrine and with propranolol exhibited 48 h postmortem pH averaging 5.8, and normal muscle glycogen content at slaughter. Isolated mitochondria respired at less than half the rate of mitochondria isolated from sheep injected with epinephrine alone. There were no significant differences between the control sheep and those injected with epinephrine and propranolol. Animals injected with epinephrine at levels, which consistently produce dark cutters, instead produced carcasses that were normal in all parameters tested if they were simultaneously injected with propranolol. These data suggest that other beta-

adrenergic blockade agents might also be effective in prevention of dark cutting meat (Ashmore et al., 1973).

When muscle pH values remain elevated, mitochondrial respiration remains high, myoglobin is deoxygenated, and a dark red color results (Ashmore et al., 1973). Elevated muscle pH curtails metmyoglobin accumulation by protecting the protein from oxidizing conditions (Pan & Solberg, 1972). Sawyer et al. (2009) investigated the effect of lactic acid (LA) enhancement on pH, water-holding capacity, fresh color stability, and cooked color of dark cutting beef. Results from Sawyer et al. (2009) indicated that enhancing dark cutting beef strip loins with 0.25% lactic acid can effectively lower postmortem muscle pH, thereby improving fresh and cooked beef color similar to that of normal pH beef. Furthermore, the results indicate that not only can the traditional dark cutting color associated with muscles from high pH (> 6.2) carcasses be beneficially altered, but also the stability of the color is comparable to normal pH beef during retail display.

Research has shown that enhancing post-rigor dark cutting beef with 0.25% to 0.50% lactic acid could reduce muscle pH, thereby eliminating the persistent red pink color typically observed in cooked dark cutting beef and producing an internal cooked color similar to that of normal pH beef (Sawyer et al., 2008, 2009). Lactic acid enhancement has the potential to add value to dark cutting beef in the food-service industry; where consumers are concerned more with cooked rather than fresh beef color (Apple et al., 2011).

Apple et al. (2011) conducted tests to determine the effects of lactic acid enhancement of dark cutting beef on fresh color during 5 d of simulated retail display, cooked beef color, and cooked steak palatability. Fresh and cooked color of dark cutting beef was only minimally

altered when enhanced with 0.35% lactic acid at 105% of the fresh product weight; however, when dark cutting beef was enhanced with 0.35 and 0.50% of lactic acid at a target of 112%, fresh and cooked color were improved close to that of choice beef. The persistent red or pink cooked color of dark cutting was virtually eliminated by 0.50% lactic acid enhancement.

Sawyer et al. (2008) described that the use of organic acids in meat systems have been shown to increase collagen solubility, reduce shear force, and muscle pH, and improve fresh beef color. The objective of their study was to compare the effects of lactic acid enhancement on muscle pH, water-holding capacity, and the resultant cooked color in dark cutting beef. Results from Sawyer et al. (2008) indicated that the addition of lactic acid to dark cutting beef strip loins would lower postmortem muscle pH, allowing for improvements in cooked steak color similar to that of normal pH beef. However, the addition of lactic acid to dark cutting strip loins at concentrations exceeding 1.0% is extremely detrimental to fresh and cooked meat characteristics. Lactic acid enhancement at concentrations of 0.5%-1.0% has the potential to add value to dark cutting beef products by the prospective marketing of such products through food service outlets (Sawyer et al., 2008).

Sawyer et al. (2007) investigated the effects of utilization of acid marinate on muscle pH, cooked color and thermal denaturation of myoglobin in dark cutting beef. Results from the study showed that the addition of lactic acid to dark cutting beef strip loins will lower postmortem muscle pH, resulting in an improved cooked steak color of dark cutting muscle that typically maintains a consistent pinking, regardless of the endpoint temperature (Sawyer et al., 2007).

Modified Atmospheric Packaging

Modified atmospheric packaging (MAP) has proven to be successful in extending the shelf life of fresh meat by the inclusion of oxygen to prolong its bloomed, cherry-red appearance and the inclusion of carbon dioxide to decrease the microbial activity (McMillin, 2008). Mancini & Hunt (2005) determined that carbon monoxide has also been shown to improve the color of meat by binding to myoglobin to form a bright pink red color. The carbon monoxide also acts to decrease the redox potential of the environment in which the meat is packaged, thus decreasing microbial activity.

However, there has been limited research on the effects of extended aging, modified atmosphere packaging, and lactic acid enhancement to reverse dark cutting color. The current study further investigated the effects of extended aging at 21, 42, and 62 d, then displayed in PVC, high-oxygen, or CO packaging and put under simulated retail display for six days. Lactic acid enhancement was also utilized along with MAP packaging to further evaluate the capabilities to reverse dark cutting color. Dark cutting beef strip loins were enhanced with lactic acid then packaged in either PVC, high-oxygen, or CO and placed in simulated retail display for 4 d.

Freeze Thawing

Freezing meat to extend shelf life has been utilized for thousands of years, although most improvements in freezing technologies have occurred in the past century. This is imperative as the meat export industry is currently worth more than US $13 billion and freezing plays an essential role in the industry in ensuring the safety of the meat products being supplied to all regions of the world (Leygonie et al., 2012). As the water freezes, the concentration of the

remaining solutes (proteins, carbohydrates, lipids, vitamins, and minerals) increases, thereby disrupting the homeostasis of the complex meat system (Lawrie, 1998). The changes that happen in the immediate environment of the meat fibers affect the cell membrane characteristics, which in turn affect the quality of the meat (Fellows, 2000). Freezing and thawing alter both the content and the distribution of moisture in meat tissue. Freezing and thawing cause damage to the ultrastructure of the muscle cells with the ensuing release of mitochondrial and lysosomal enzymes, haem iron and other pro-oxidants. These increase the degree and rate of protein oxidation (Xiong, 2000). It has been reported that denaturation of the globin moiety of the myoglobin molecules takes place at some stage during freezing, frozen storage and thawing (Calvelo, 1981).

The pH of meat that has been frozen and thawed tends to be lower than prior to freezing (Leygonie et al., 2011). As pH is a measure of the amount of free hydrogen ions (H^+) in a solution, it is possible that freezing with subsequent exudate production could cause denaturation of buffer proteins, the release of hydrogen ions and a subsequent decrease in pH. The loss of fluid from the meat tissue may cause an increase in the concentration of the solutes, which results in a decrease in the pH (Leygonie et al., 2012).

Mitochondria are important subcellular organelles involved in energy metabolism. It has been reported that freeze-thaw treatment of isolated liver mitochondria disrupted mitochondria and increased state IV oxygen consumption rate (OCR). In postmortem muscle, mitochondria respire under decreased or exhausted ADP, a situation similar to state IV respiration of isolated mitochondria. The objective of Tang et al. (2006) was to investigate the effect of freeze-thaw and sonication treatments on mitochondrial oxygen consumption, ETC-dependent metmyoglobin reducing activity, lipid oxidation, and myoglobin redox stability. Freeze-thaw treatment of

mitochondrial preparations had no effect on state IV OCR, lipid oxidation, ETC-dependent metmyoglobin reducing activity, or myoglobin redox stability. There has been limited to no research on the effects of freeze thawing to limit mitochondrial activity in dark cutting beef to improve retail display color.

CONCLUSION

Various studies have been conducted to better understand the mechanism of dark cutting beef. It is know that decreased glycogen levels before slaughter can have an impact on postmortem muscle pH, which will influence both physical and biochemical factors affecting beef color. However, limited research has utilized beneficial effects of extended aging. Modified atmospheric packaging, altering mitochondrial function by freeze thawing. Therefore, the overall objective of this research was to better understand the mechanism of the failure to bloom in dark cutting beef and limit the darkening effect by aging, packaging, and altering mitochondrial function.

REFERENCES

Apple, J. K., Sawyer, J. T., Meullenet, J. F., Yancey, J. W. S., & Wharton, M. D., (2011). Lactic acid enhancement can improve the fresh and cooked color of dark-cutting beef. *Journal of Animal Science*, 89, 4207-4220.

Ashmore, C. R., L. Doerr, G. Foster & F. Carroll. (1971). Respiration of mitochondria isolated from dark-cutting beef. *Journal of Animal Science*, 33:574.

Ashmore, C. R., Parker, W., & Doerr, L., (1972). Respiration of mitochondria isolated from dark-cutting beef: postmortem changes. *Journal of Animal Science*, 34:1.

Ashmore, C. R., Carroll, F., Doerr, L., Tompkins, G., Stokes, H., & Parker, W., (1973). Experimental prevention of dark-cutting meat. *Journal of Animal Science*, 36:33.

Atkinson, J., Follett, M., & Ratcliff, P., (1969). Postmortem changes in the oxygen uptake and NAD content of lamb semimembranosus. Nature. 223, 1372-1373.

Boleman, S. L., S. J. Boleman, W. W. Morgan, D. S. Hale, D. B. Griffin, J. W. Savell, R. P. Ames, M. T. Smith, J. D. Tatum, T. G. Field, G. C. Smith, B. A. Gardner, J. B. Morgan, S. L. North- cutt, H. G. Dolezal, D. R. Gill, and F. K. Ray. 1998. National beef quality audit—1995: Survey of producer-related defects and carcass quality and quantity attributes. *Journal of Animal Science,* 76:96–103.

Calvelo, R. J. (1981). Recent studies on meat freezing. In R. Lawrie (Ed.), Developments in meat science-2 (pp.125-158). London: Elsevier Applied Science Publishers.

Cornforth, D. P., & Egbert, W. R., (1985). Effect of Rotenone and pH on the color of pre-rigor muscle. *Journal of Food Science*, 50, 34-35.

Egbert, W. R., & Cornforth, D. P., (1986). Factors influencing color of dark cutting beef muscle. *Journal of Food Science*, 51, 57-59.

Faustman, C., & Cassens, R. G., The biochemical basis for discoloration in fresh meat: A review. (1990). *Journal of Muscle Foods*, 1, 217-243.

Fellows, P. (2000). Freezing. Food processing technology, principles and practice (pp. 418-440). (2 ed.). London: Woodhead Publishing Limited.

Gruber, S. L., Tatum, J. D., Scanga, J. A., Chapmant, P. L., Smith, G. C., & Belk, K. E., (2006). Effects of postmortem aging and USDA quality grade on Warner-Bratzler shear force values of seventeen individual beef muscles. *Journal of Animal Science*, 84, 3387-3396.

Hedrick, H. B., Boillot, J. B., Brady, D. E., & Naumann, H. D., (1959). Etiology of dark-cutting beef. Missouri Agriculture Experimental Station Res. Bull 717.

Holdstock, J., Aalhus, J. L., Uttlaro, B. A., Lopez-Campos, O., Larsen, I. L., & Bruce, H. L., (2014). The impact of ultimate pH on muscle characteristics and sensory attributes of the longissimus thoracis within the dark cutting (Canada B4) beef carcass grade. *Meat Science*, 98. 842-849.

Hunt, M. C., Mancini, R. A., Hachmeister, K. A., Kropf, D. H., Merriman, M., DelDuca, G., et al. (2004). Carbon monoxide in modified atmosphere packaging affects color, shelf life, and microorgansims of beef steaks and ground beef. *Journal of Food Science*, 69(1), C45-C52.

Kreikemeier, K. K., & Unruh, J. A., Eck, J. A., (1998). Factors affecting the occurrence of dark-cutting beef and selected carcass traits in finished beef cattle. *Journal of Animal Science*, 76(2), 388-395.

Lawrie, R. A. (1958). Physiological stress in relation to dark-cutting beef. *Journal of Food Science Agriculture*, 11:721

Lawrie, R. A., (1998). In Anonymous (Ed.), Lawrie's meat science (pp. 1-336). (6^{th} ed.). Lancaster, PA: Technomic Publishing Inc.

Ledward, D.A., D. E. Johnston, & M. K. Knight, (1992). The Chemistry of Muscle-Based Foods. pp 128-139. The Royal Society of Chemistry, Thomas Graham House, Science Park, Cambridge, U. K.

Lee, M. S., Apple, J. K., Yancey, J. W. S., Sawyer, J. T., & Johnson, Z. B., (2008). Influence of vacuum-aging period on bloom development of the beef gluteus medius from top sirloin butts. *Meat Science*, 80: 592-598.

Leygonie, C., Britz, T. J., & Hoffman, L. C., (2011). Oxidative stability of previously frozen ostrich M. Iliofibularis packaged under different modified atmospheric conditions. *International Journal of Food Science and Technology*, 46, 1171-1178.

Leygonie, C., Britz, T. J., & Hoffman, L. C., (2012). Meat quality comparison between fresh and frozen/thawed ostrich M. iliofibularis. *Meat Science*, provisionally accepted.

Livingston, D. J., & Brown, W. D., (1982). The chemistry of myoglobin and its reactions. *Food Technology*, 35(5), 244-252.

Luno, M., Roncales, P., Djenane, D., & Beltran, J. A., (2000). Beef shelf life in low O_2 and high CO_2 atmospheres containing different low CO concentration. *Meat Science*, 55(4), 413-419.

Mac Dougall, D., (1982). Changes in colour and opacity of meat. *Food Chemistry,* 9,75- 88.

Mancini, R. A., Hunt, M. C. (2005). Current research in meat color. *Meat Science,* 71, 100-121

Mancinin, R. A., Kropf, D. H., Hunt, M. C., & Johnson, D. E. (2005). Effects of endpoint temperature, pH, and storage time on cooked internal color reversion of pork longissimus chops. *Journal of Muscle Foods*, 16, 16-26.

Mancini, R. A., & Ramanathan, R., (2010). Out-competing myoglobin for oxygen; The role of mitochondria in terms of oxygen consumption and balance in postmortem muscle. *Fleischwirtschaft Inter.* 2, 73-74.

Mancini, R. A., & Ramanathan, R., (2014). Effects of postmortem storage time on color and mitochondria in beef. *Meat Science*, 98:65-70.

Mckenna, D. R., D. L. Roeber, P. K. Bates, T. B. Schmidt, D. S. Hale, D. B. Griffin, J. W. Savell, J. C. Brooks, J. B. Morgan, T. H. Montgomery, K. E. Belk, & G. C. Smith. (2002). National Beef Quality Audit-2000: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. *Journal of Animal Science,* 80:1212.

McKenna, D., Mies, P., Baird, B., Pfeiffer, K., Ellebracht, J., & Savell, J., (2005). Biochemical and physical factors affecting discolouration characteristics of 19 bovine muscles. *Meat Science,* 70, 665-682.

McMillin, K. W., (2008). Where is MAP Going? A review and future potential of modified atmosphere packaging for meat. *Meat Science*, 80, 43-65.

Neely, J., & Morgan, H., (1974). Substrate and energy metabolism of the heart. *Annual Review in Physiology*, 36, 413-459.

Pan, B. S., & Solberg, M., (1972). The effect of pH on bovine oxymyoglobin structure and stability. *Journal of Food Science*, 37, 29-32.

Price, J. F., & Schweiegert, B. S., (1987). The science of meat and meat products. 3rd ed. Food and Nutrition Press, Westport CT.

Rentfrow, M. L., Linville, M. L., Stahl, C. A., Olsen, K. C., & Berg, E. P. (2004). The effects of the antioxidant lipoic acid on beef longissimus bloom time. *Journal of Animal Science,* 82, 3034-3037.

Sawyer, J. T., Apple, J. K., & Johnson, Z. B., (2007). The impact of lactic acid concentration and sodium chloride on pH, water-holding capacity, and cooked beef of injection-enhanced dark-cutting beef. *Meat Science*, 79:317-325.

Sawyer, J. T., Apple, J. K., & Johnson, Z. B. (2008). The impact of lactic acid concentration and sodium chloride on pH, water-holding capacity, and cooked color of injection-enhanced dark-cutting beef. *Meat Science*, 79, 317-325.

Sawyer, J. T., Apple, J. K., Johnson, Z. B., Baublits, R. T., & Yancey, J. W. S. (2009). Fresh and cooked color of dark-cutting beef can be altered by post-rigor enhancement with lactic acid, *Meat Science*, 83, 263-270.

Seideman, S. C., Cross, H. R., Smith, G. C., & Durland, P. R., (1984). Factors affecting fresh meat colour: review. *Journal of Food Quality,* 6(3), 211-237.

Shorthose, W. R., & Harris, P. V., (1990). Effect of animal age on the tenderness of selected beef muscles. *Journal of Food science*, 55, 1-8.

Smith, G. C., Sewell, J. W., Dolezal, H. G., Field, T. G., Gill, D. R., Griffin, D. B., Hale, D. S., Morgan, J. B., Northcutt, S. L., & Tatum, J. D., (1995). The Final Report of the Second Blueprint for Total Quality Managemnet in the Fed-Beef (Slaughter Steer/Heifer) Industry: National Beef Quality Audit-1995. National Cattlemen's Association, Englewood, CO.

Smith, G. C., Belk, K. E., Sofos, J. N., Tatum, J .D., & Williams, S. N., (2000). Economic implications of improved color stability in beef. In E. A. Decker, C. Faustman, & C. J. Lopez-Bote (Eds.), Antioxidants in muscle foods: Nutritional strategies to improve quality (pp. 397-426). New York: Wiley Interscience.

Sorheim, O., Nissen, H., Aune, T., & Nesbakken, T., (2001). Use of carbon monoxide in retail meat packaging. In 54th Proceedings Reciprocal Meat Conference (pp. 47-51), Indianapolis IN, USA.

Suman, S. P., Hunt, M. C., Nair, M. N., & Rentfrow, G., (2014). Improving beef color stability: Practical strategies and underlying mechanisms. *Meat Science*, 98, 490-504.

Swatland, H. J., (1989). A review of meat spectrophotometry (300 to 800 nm). Canadian Institute of Food Science and Technology, 22(4), 390-402.

Tang, J., Faustman, C., Mancini, R. A., Seyfert, M., & Hunt, M. C., (2006). The effects of freeze-thaw and sonication on mitochondrial oxygen consumption, electron transport chain-linked metmyoglobin reduction, lipid oxidation, and oxymyoglobin oxidation. *Meat Science*, 74, 510-515.

Tarrant, P. V., (1989). Animal behavior and environment in the dark-cutting condition in beef-A review. Irish *Journal of Food Science & Technology*, 13, 1-21.

Troy, D. J., & Kerry, J. P., (2010). Consumer perception and the role of science in the meat industry. *Meat Science*, 86, 214-226.

Wallace, W. J., Houtchens, R. A., Maxwell, J. C., & Caughey, S., (1982). Mechanism of autoxidation for hemoglobins and myoglobins. *Journal of Biological Chemistry*, 257(9), 4966-4977.

Viljoen, H. F., Kock, H. L., & Webb, E. C., (2002). Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. *Meat Science*, 61, 181-185.

Wulf, D., (1998). Ohio State University Extension Meat Specialist. Personal Communitcation.

Wulf, D. M., & Wise, J. W., (1999). Measuring muscle color on beef carcasses using the L*a*b* color space. *Journal of Animal Science*, 77, 2418-2427.

Xiong, Y. L., (2000). Protein oxidation and implications for muscle food quality. In: Antioxidants in muscle foods (edited by E. Decker & C. Faustman). Pp. 3-23, 85-111, 113-127, Chichester: John Wiley & Sons.

Zerouala, A. C., & Stickland, N. C., (1991). Cattle at risk for dark-cutting beef have a higher proportion of oxidative muscle fibres. *Meat Science*, 29, 263-270.

Zhu, L. G., & Brewer, M. S., (1998). Metmyoglobin reducing capacity of fresh normal, PSE and DFD pork during retail display. *Journal of Food Science*, 63, 390-393.

CHAPTER III

EFFECTS OF EXTENDED AGING AND MODIFIED ATMOSPHERIC PACKAGING ON BEEF *LONGISSIMUS* COLOR

ABSTRACT

Beef color has a critical influence on beef purchasing decisions; hence it is important for the meat industry. There has been extensive research assessing the effects of postmortem aging on meat quality and tenderness of *longissimus* muscle. However, limited research has determined the effects of extended aging and modified atmospheric packaging on beef color. Therefore, the objective of this study was to evaluate the effects of extended aging at 21, 42, and 62 d, on bloom characteristics, metmyoglobin reducing activity (MRA), oxygen consumption rate (OCR), and lipid oxidation of *longissimus* steak color packaged in carbon monoxide-modified atmospheric packaging (CO-MAP), high-oxygen (HiOx), and polyvinyle chloride overwrap (PVC) film. Each beef strip loin was divided equally into 3 sections. Using a randomized complete block, each section within a loin was assigned to 1 of 3 aging periods 21, 42, or 62 d. After respective aging, each sections were then sliced into four 2.5 cm steaks and randomly assigned to 1 of 3 packages, PVC, CO-MAP (0.4% CO), and HiOx MAP (80% O2,), or were displayed under continuous fluorescent lighting for 6 d. The surface color was measured using a HunterLab Miniscan XE Plus spectrophotometer. The fourth steak was used to characterize blooming properties and metmyoglobin reducing activity (MRA). The experiment was replicated 10 times. The data were analyzed using the Mixed Procedure of SAS and were considered significant at $P < 0.05$. Extended aging improved the blooming properties of beef *longissimus* muscle, but had a negative effect on shelf life and MRA ($P < 0.05$). CO-MAP was the most effective in maintaining the beef color when aged for 42 d compared with 62 d aging ($P < 0.05$). The results suggest that metmyoglobin reducing property of steaks is critical for the stability including carboxymyoglobin form. By better understanding the mechanism(s) involved in beef color during aging will help the meat processors to develop strategies to improve meat color life.

Keywords: beef color, modified atmospheric packaging, aging, oxygen consumption, metmyoglobin reducing activity

INTRODUCTION

Meat color is an important quality attribute that influences consumers' decision to purchase meat. Metmyoglobin reducing activity (MRA) and oxygen consumption (OC) of meat are the two important biochemical characterististics that determines color stability (Mancini & Ramanathan, 2014). More specifically, mitochondria plays an important role in bloom development and color stability. Hence, processes factors that can affect mitochondria function will have an impact on myoglobin redox state. Extended aging can improve bloomed color intensity because mitochondrial oxygen consumption decreases with postmortem aging (Mac Dougall et al., 1982). This will increase oxygen availability to myoglobin by minimizing competition between myoglobin and mitochondria. Although aging can improve initial color intensity, postmortem storage is detrimental to color stability because the activity of mitochondrial reducing enzymes are depleted postmortem, resulting in less metmyoglobin reducing activity (MacDougall et al., 1982).

Wet aging has been used to improve tenderness and also to improve blooming properties. A recent study has suggested that steaks aged for 45 d and packaged PVC discolored faster compared with aged for 30 days, but had brighter red color (Mancini & Ramanathan, 2014). However, limited studies have assessed the effects of extended aging followed by packaging in high-oxygen (HiOx-MAP) or carbon monoxide modified atmospheric conditions (CO-MAP) on color stability. Therefore, the objective of the study was to determine the effects of extending aging and modified atmospheric packaging on beef *longissimus lumborum* color.

MATERIALS AND METHODS

Raw materials and processing

Ten USDA Choice beef strip loins (IMPS #180, NAMP 2002) were purchased from a local purveyor within 3 d of harvest. Loins were transported in ice to Oklahoma State University. Following arrival, each loin was cut into three equal sections, vacuum packaged, and randomly assigned to three aging periods: 21 d, 42 d, or 62 d. Loin sections were then stored at 2°C in the dark for the respective aging period. Following aging, each section within a loin was cut into four 2.5 cm think steaks. The first steak was used to characterize 0 d color after respective aging, the second steak was assigned to PVC, the third steak for HiOx, and the fourth one for CO-MAP.

Packaging and display conditions

Steaks were randomly assigned to PVC, HiOx (80% oxygen and 20% carbon dioxide), and CO (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide) packaging and placed in a coffin-style display case maintained at 2°C ± 1, under continuous lighting (1612 to 2152 lx) for 6 d. Both HiOx and CO modified atmosphere packaging were performed using a MAP system utilizing Rock-Tenn DuraFresh™ rigid trays sealed with clear, multi-layer barrier film in a Mondini semi-automatic tray-sealing machine and certified gas blends (Stillwater Steal, Stillwater, OK). For PVC packaging, steaks were placed onto foam trays with absorbent pads, over-wrapped with a PVC film. Using a headspace analyzer (Bridge 900131 O_2/CO_2/CO, Illinois Instruments, Ingleside, IL), the percentage O_2 and CO_2 in extra modified atmosphere packages were determined 6 h after packaging. Packaging concentrations were O_2 78-80%, CO_2 18-20% and CO 0.2-0.4%. Each steak was evaluated for color attributes (visual and instrumental) at 24 h intervals during the retail display for 0, 1, 2, 3, 4, 5, and 6 d.

Color measurements

Surface color was measured on the steaks assigned for display at two random locations on each steak using a HunterLab MiniScan XE Plus spectrophotometer (HunterLab Associates, Reston, VA, USA) with a 2.5 cm diameter aperture, Illuminant A, and 10° standard observer. Reflectance at isobestic wavelengths from 400 – 700 nm was used to quantify myoglobin redox forms on the surface of steaks. Myoglobin 100% reference standards were determined using extra steaks and substituted into the appropriate equation outlined in AMSA (2012). Reflectance at 474, 525, 572, and 610 nm for each myoglobin form were converted to K/S values using the following equation: $K/S = (1-R)^2/ 2R$. These values were then substituted into the appropriate equation (AMSA, 2012) to calculate the percentage of DMb, OMb, or MMb. These values also were used to calculate metmyoglobin reduction (metmyoglobin reducing activity) and oxygen consumption.

To assess initial color intensity, the fresh-cut surface of each steak was scanned immediately after aging (21, 42, or 62 d). Each roast was bisected to determine pre-oxygenation a* and oxymyoglobin values. Steaks were then oxygenated at 4°C for 2 h. During this time, a* and L* were measured 0, 15, 30, 60, 90, and 120 min. The increase in a* and L* that occurred during the 2-h oxygenation period was used to determine color changes during bloom. Surface color, including a* and visual color, were measured daily for 6 d after packaging.

After bloom measurement, 10 g of sample was used to measure pH and lipid oxidation. Each steak was then cut in half and was used to measure oxygen consumption and metmyoglobin reducing activity of steaks prior to display. In order to record, lipid oxidation, OCR, and MRA, steaks immediately after surface color measurements were used.

Visual color analysis

A trained panel (n = 6) conducted daily visual color evaluations. All panelists passed the Farnsworth Munsell 100-hue test. Panelists scored each steak to assess muscle darkening using a 7-point scale (1 = no darkening, 7 = very dark), muscle color using a 9-point scale (1 = extremely dark brown or black , 9 = extremely bright cherry red), overall meat color using a 9-point scale (1 = extremely undesirable , 9 = extremely desirable) and for discoloration using a 6-point scale (1 = no discoloration 6 = total discoloration).

Metmyoglobin Reducing Activity

Metmyoglobin-reducing activity was determined after aging for 21, 42, 62 d, and on 6 d after packaging in PVC, HiOx, and CO according to the procedures described by Sammel et al., (2002). Samples from the interior of steak halves were submerged for 20 min in a 0.3% solution of sodium nitrite (Sigma, St. Louis, MO) to facilitate metmyoglobin formation, and then removed, blotted dry, vacuum packaged (Prime Source Vacuum Pouches, 4 mil, KOCH Supplies Inc., Kansas City, MO), and scanned with a HunterLab MiniScan XE Plus Spectrophotometer to determine pre-incubation metmyoglobin values (AMSA, 2012). Each sample was incubated at 30°C for 2 h to induce metmyoglobin reduction. Upon removal from the incubator, samples were rescanned to determine the percentage of remaining surface metmyoglobin. The following equation was used to calculate metmyoglobin reducing activity: [(% surface metmyoglobin pre-incubation - % surface metmyoglobin post-incubation) ÷ % surface metmyoglobin pre-incubation] x 100. Increased metmyoglobin reducing activity is associated with improved color stability.

Oxygen consumption rate

Oxygen consumption rate (OCR) was determined according to a modified procedure of Madhavi and Carpenter (1993) on the fresh-cut surface of the bottom half portion of the cube removed prior to MRA analysis. The samples were allowed to oxygenate for 30 min at 1°C, were vacuum packaged, and then were scanned twice (as described in the MRA procedure) on the bloomed surface (representing the previously unexposed interior of the original cube) to measure oxymyoglobin (OMb). Oxygen consumption (measured by conversion of OMb to DMb) was induced by incubating samples at 30°C for 30 min. Samples were rescanned immediately upon removal to determine remaining surface DMb as a percentage by using K/S ratios and equations (AMSA, 2012). To calculate OCR, changes in deoxymyoglobin values pre- and post-incubation were used.

Muscle pH

Samples from steaks assigned to 21, 42, and 62 d of storage, visually devoid of fat and connective tissue, were blended in a Sorvell onmi tabletop mixer (Newton, CT, USA). To determine pH, 10 g of pulverized sample was combined with 100 mL of deionized water and mixed for 30 s, and the pH values were obtained by using an Accumet combination glass electrode connected to an Accumet 50 pH meter (Fisher Scientific, Fairlawn, NJ).

Proximate analysis

Samples for compositional analysis (protein, moisture, fat, and collagen) were obtained prior to packaging. Analysis was conducted using an AOAC-approved (Official Method 2007.04) near-infrared spectrophotometer (FOSS Food Scan™ 78800; Dedicated Analytical Solutions, DK-3400 Hillerod, Denmark). Compositional values are reported on a percent (%) basis.

Lipid oxidation

Thiobarbituric acid reactive substances values were measured according to the procedure of Witte, Krause, and Bailey (1970). From each steak, 5 g of sample was blended with 25 mL TCA solution (20%) and 20 mL distilled water. Samples were homogenized using a Sorvell onmi tabletop mixer (Newton, CT, USA) for 1 min and filtered through Whatman (#1) filter paper. One milliliter of filtrate was mixed with 1 mL TBA solution (20 mM), incubated in a boiling water bath for 10 min. The samples were cooled in and absorbance at 532 nm was measured using a Shimadzu UV-2600 PC spectrophotometer (Shimadzu Inc., Columbia, MD, USA). Blank samples for the spectrophotometer consisted of 2 mL TCA/distilled water (1:1 v/v) and 2 mL TBA solution.

Statistical analysis

A split plot design was used to evaluate the effects of extended aging and packaging on display color, bloom, metmyoglobin reducing activity, oxygen consumption, and lipid oxidation. The whole plot consisted of a randomized complete block, with USDA Select *Longissimus* lumborum (NAMP 180; NAMP, 2002; n = 10) muscles serving as blocks. Data were analyzed using the Mixed Procedure of SAS (SAS, 2011). Fixed effects included aging time, post-aging/post-packaging time, and their interactions. For the split plot, random effects included loin, loin × whole plot treatments (Error A), and residual error (Error B). For color stability data, the random term included loin and the repeated option was used to assess covariance–variance structure amongst the repeated measures. The most appropriate structure was determined using AIC and BIC output. Least square means for protected F-tests ($P < 0.05$) were separated using the pdiff option and were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Surface color

A significant aging x packaging x display day interaction ($P < 0.05$; Tables 3.1,3.2) resulted for a* value (redness), chroma, and visual color (muscle color, surface discoloration, and overall acceptability). However, results show packaging x display color within the aging periods. Irrespective of the aging period, CO-MAP had the least discoloration. As the aging time increased, HiOx-MAP had the most discoloration compared with other packaging. Steaks aged for 62 d and packaged in HiOx and PVC had shorter shelf-life compared with CO-packaging. Mancini & Hunt (2005) showed that utilizing carbon monoxide and form a very stable bright red color. Interestingly, steaks aged for 62 d and packaged in CO had lower a* value on 6 d of display compared to 21 or 42 d. Visual color also had a same trend like the instrumental color.

Oxygenation properties

A significant aging x incubation time interaction resulted for bloom a* value. However, for L*, only main effects of aging and blooming incubation time were significant. Changes in a* value of pre- and post-incubation were greatest for steaks aged for 62 d. Previous studies also reported that aging increased bloom color intensity (Lee et al., 2008). Steaks aged for 62 d were lighter in color compared with steaks aged for 21 or 42 d. There was significant increase in lightness of steaks as the blooming time increased from 0 to 120 min ($P<0.05$).

Oxygen consumption

There was a significant aging time x packaging interaction for OCR. Loin sections aged for 21 d had the greatest OCR and it decreased when aged for 42 and 62 d. Steaks displayed for 6 d and packaged in CO-MAP had the greatest OCR compared with HiOx and PVC. Greater oxygen content in HiOx steaks might have resulted in an oxidative condition; hence

mitochondrial enzymes will be less active to consume oxygen. Previous studies also reported less oxygen consumption in steaks packaged in HiOx (McMillin, 2008). There were significant differences in oxygen consumption of steaks packaged in CO and displayed for 6 d. More specifically, extended aging decreased both oxygen consumption and MRA of steaks aged for 42 and 62 d compared with aged 21 d.

Metmyoglobin reducing activity

There was a significant aging x packaging x display time interaction for metmyoglobin reducing activity. There was a significant effect on aging time on MRA. Aging time decreased ($P < 0.05$) MRA. Steaks packaged in CO had greater MRA after 6 d of display. Anaerobic condition created by CO packaging limited the oxidation of enzymes involved in metmyoglobin reduction. Steaks packaged in HiOx had the least MRA after 6 d of display. On day 6 of display, there was no effect of aging on MRA on steaks packaged in HiOx. However for steaks packaged in PVC, as aging time increased, there was decrease in MRA after 6 d display. Decreased MRA might be responsible for decreased color stability of steaks in three packaging conditions. The results indicate that extended aging can decrease MRA, which can significantly affect the color stability of steaks packaged in PVC and HiOx. The decreased color stability from extended aging can be a result of the depletion of substrates for mitochondria respiration resulting in decreased competition for oxygen between myoglobin and mitochondria Aging is also detrimental to color stability, because the activity of reducing enzymes and NADH content are depleted postmortem (Mac Dougall et al., 1982).

Lipid oxidation

There was no effect of aging on lipid oxidation when loin sections were aged for 21 or 42 d. However, steaks had significant effects when packaged in different atmospheric conditions.

For example, steaks packaged in HiOx>PVC> CO. Previous studies also have reported that a high oxygen condition can increase oxidative reactions.

CONCLUSION

Extended aging can improve the color intensity (redness) of steak, but has a detrimental effect on color stability. HiOx condition will result in shorter shelf life where as steaks packaged in CO-MAP had the greatest color stability. This decreased color stability resulted from decreased MRA and OCR.

Figure 3.1a

Effects of extended aging, packaging, and display time on oxygenation of beef *longissimus* steak.

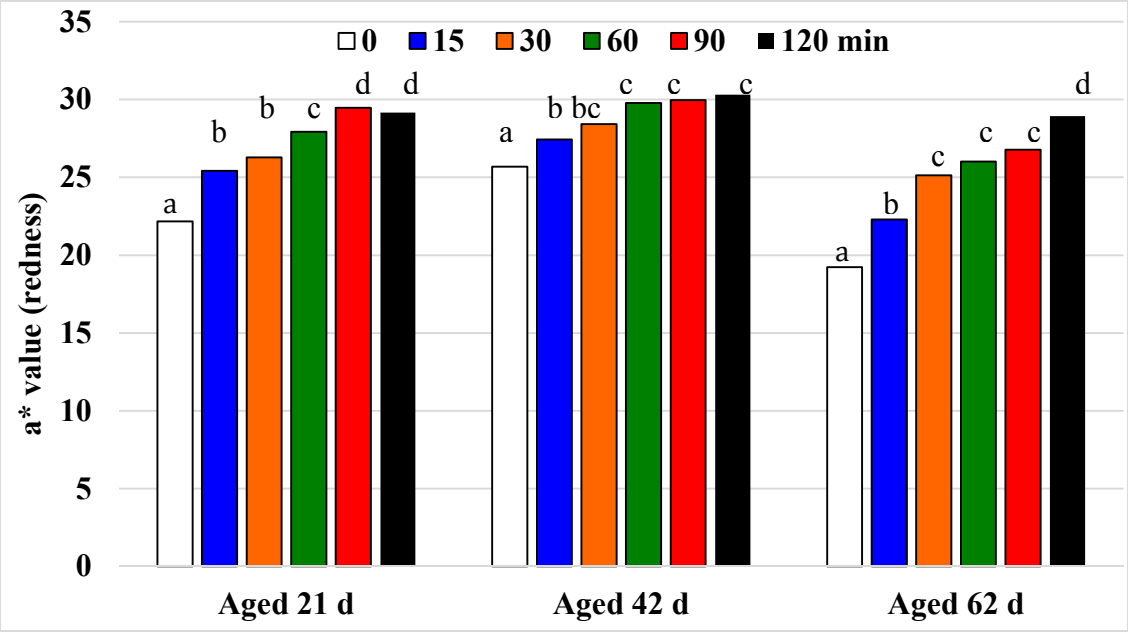
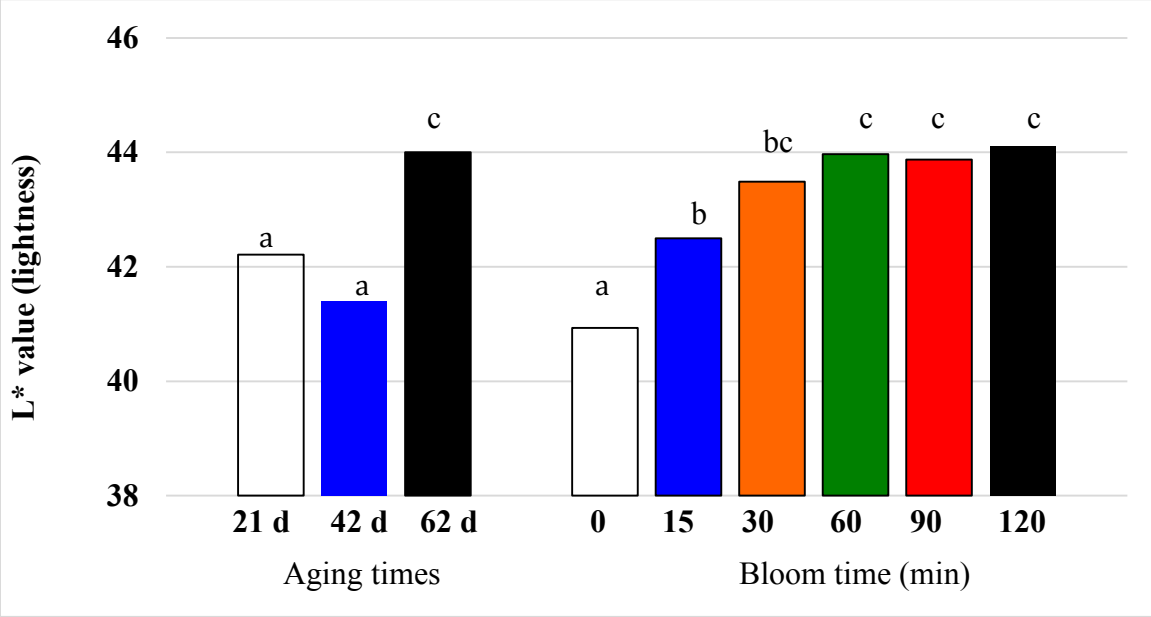


Figure 3.1b



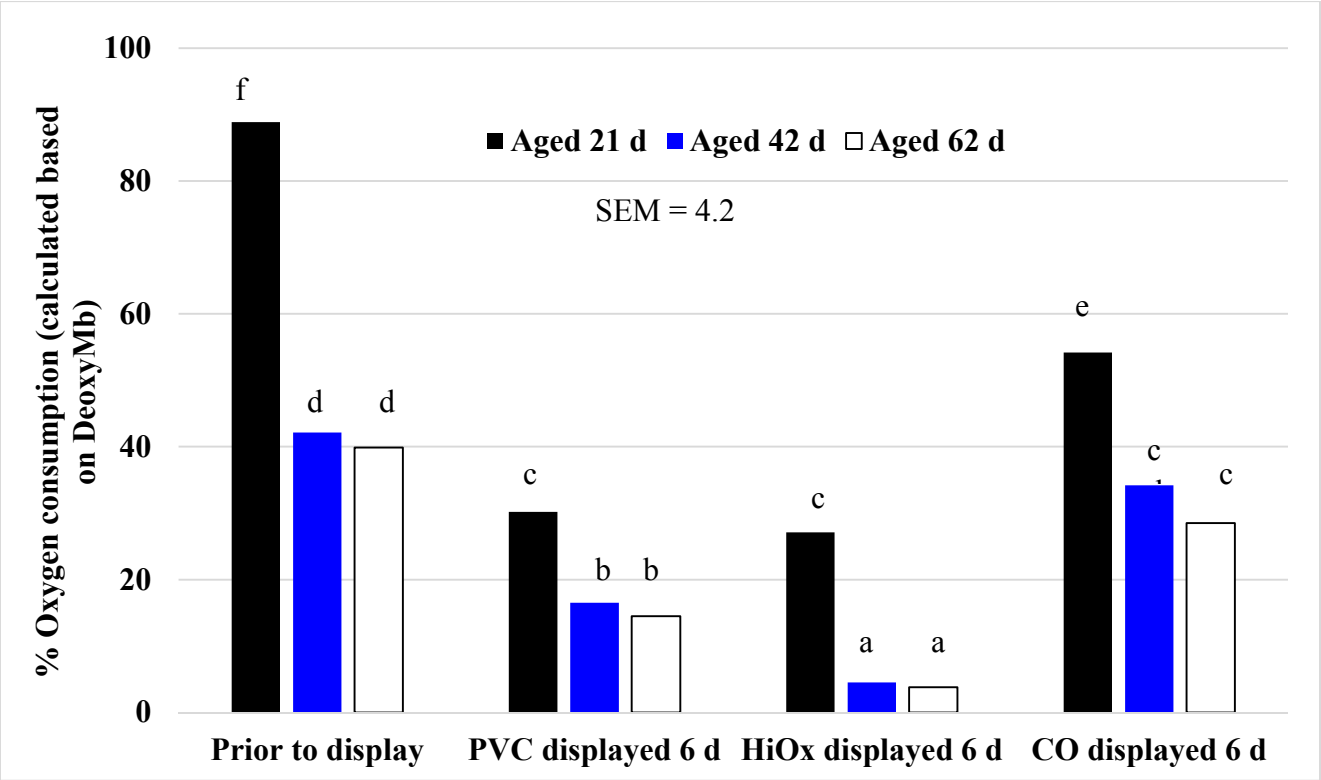
Least square means with different superscripts are significantly different (P<0.05)

Standard error for a* value (aging x packaging x display time) = 0.59

Standard error for the main effects of aging and oxygenation time for L* value = 0.97

Figure 3.2

Effects of extended aging, packaging, and display time on oxygen consumption of beef *longissimus* steak.

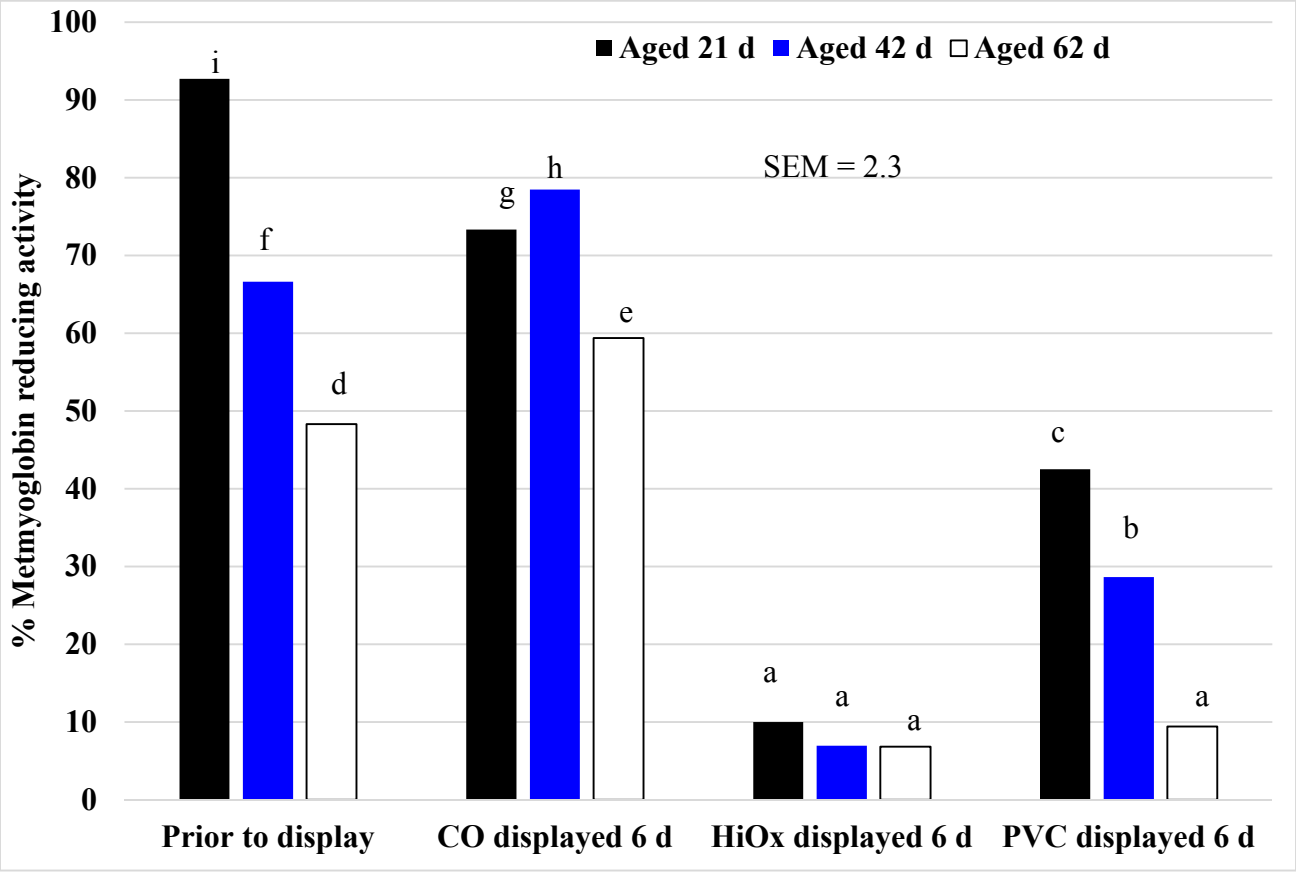


Least square means with different superscripts are significantly different (P<0.05)

n=40

Figure 3.3

Effects of extended aging, packaging, and display time on metmyoglobin reducing activity of beef *longissimus* steak.

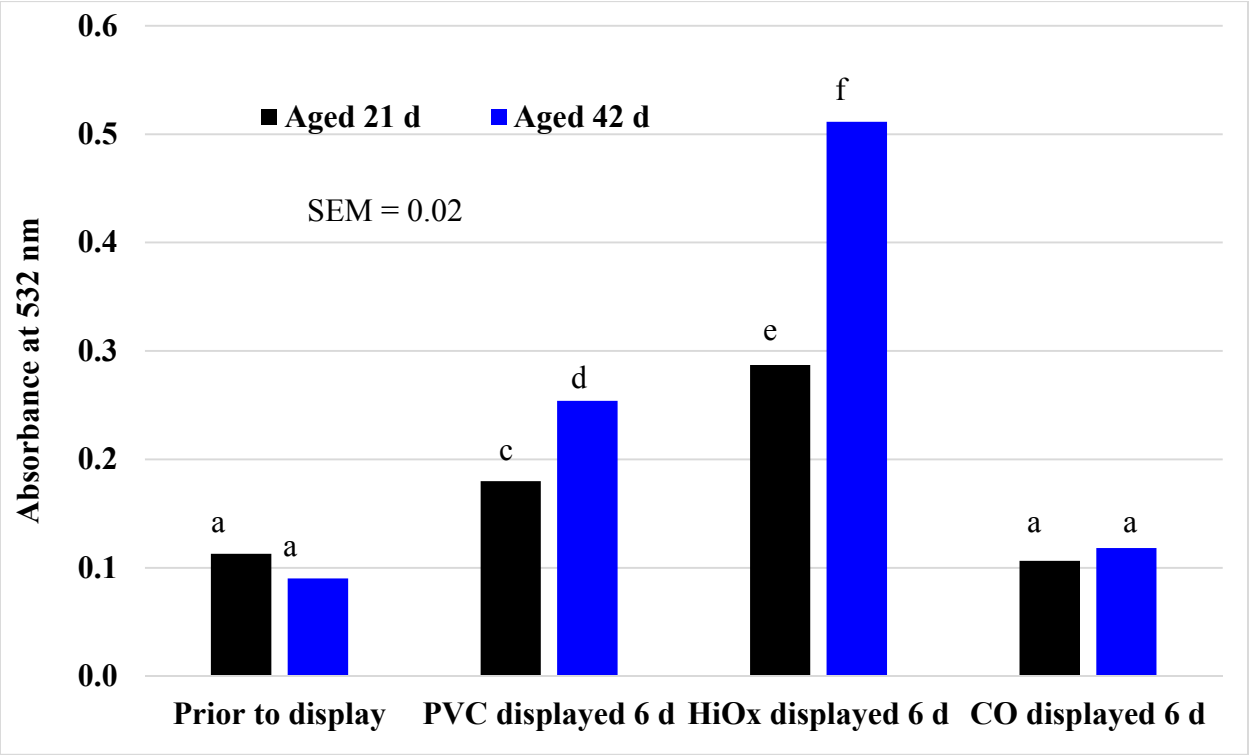


Least square means with different superscripts are significantly different (P<0.05)

n=120

Figure 3.4

Effects of extended aging, packaging, and display time on lipid oxidation of beef *longissimus* steak.



Least square means with different superscripts are significantly different (P<0.05)

n=80

Table 3.1 Least squares means for a* and chroma (aging x packaging x day interaction) of beef steaks displayed for 6 d

Parameter[1]	Aging period[2] (d)	Packaging[3]	Display (d) 0	1	2	3	4	5	6
a* value	21	PVC	$34.2^{a,s}$	$23.8^{b,u}$	$24.1^{b,tu}$	$20.9^{c,u}$	$20.2^{c,v}$	$18.7^{d,t}$	$18.1^{d,u}$
		HiOx	$32.1^{a,t}$	$25.9^{b,t}$	$26.7^{b,s}$	$23.2^{c,t}$	$23.3^{c,u}$	$16.2^{d,uv}$	$11.5^{e,x}$
		CO	$22.9^{a,x}$	$20.4^{b,w}$	$23.6^{a,v}$	$24.7^{bc,s}$	$27.8^{d,s}$	$28.3^{de,s}$	$29.5^{e,s}$
	42	PVC	$30.6^{a,u}$	$24.0^{b,u}$	$20.8^{c,x}$	$16.2^{de,w}$	$15.2^{ef,x}$	$17.3^{d,u}$	$15.1^{f,vv}$
		HiOx	$31.1^{a,tu}$	$28.2^{b,s}$	$25.4^{c,st}$	$21.6^{d,u}$	$17.5^{e,w}$	$12.9^{f,uv}$	$9.6^{g,y}$
		CO	$23.1^{a,w}$	$23.4^{a,u}$	$23.5^{ab,v}$	$24.7^{b,s}$	$27.1^{c,s}$	$28.2^{c,s}$	$29.7^{d,s}$
	62	PVC	$27.1^{a,v}$	$22.3^{b,v}$	$16.3^{c,y}$	$14.2^{d,x}$	$13.0^{de,y}$	$13.7^{ef,v}$	$13.1^{f,w}$
		HiOx	$29.9^{a,u}$	$26.4^{b,t}$	$21.4^{c,wx}$	$17.6^{d,v}$	$10.7^{e,z}$	$8.1^{f,w}$	$7.2^{f,z}$
		CO	$22.2^{a,x}$	$23.0^{ab,uv}$	$24.0^{bc,u}$	$25.0^{c,s}$	$25.3^{c,t}$	$27.0^{d,st}$	$27.4^{d,t}$
Chroma	21	PVC	$44.3^{a,s}$	$29.1^{b,uv}$	$30.6^{c,t}$	$26.5^{d,t}$	$27.5^{d,v}$	$25.0^{e,u}$	$23.9^{e,u}$
		HiOx	$39.0^{a,t}$	$30.9^{b,u}$	$32.3^{c,s}$	$28.0^{d,s}$	$28.9^{d,u}$	$21.1^{e,v}$	$16.8^{f,w}$
		CO	$26.3^{a,w}$	$23.3^{b,x}$	$26.9^{ab,u}$	$28.2^{c,s}$	$32.1^{d,s}$	$32.3^{d,s}$	$33.7^{e,s}$
	42	PVC	$38.4^{a,t}$	$30.5^{b,u}$	$27.2^{c,u}$	$22.4^{d,u}$	$21.7^{d,w}$	$22.1^{de,v}$	$19.3^{f,v}$
		HiOx	$37.7^{a,u}$	$34.2^{b,s}$	$30.9^{c,t}$	$26.7^{d,t}$	$22.7^{e,w}$	$18.6^{f,w}$	$15.7^{g,w}$
		CO	$26.2^{a,w}$	$27.0^{a,w}$	$27.1^{ab,u}$	$28.3^{b,s}$	$30.8^{c,st}$	$32.2^{d,s}$	$34.0^{e,s}$
	62	PVC	$33.8^{a,v}$	$28.5^{b,v}$	$22.4^{c,v}$	$20.5^{d,v}$	$18.9^{e,x}$	$19.5^{ef,w}$	$18.4^{e,v}$
		HiOx	$36.4^{a,u}$	$32.1^{b,t}$	$26.2^{c,uv}$	$22.4^{d,u}$	$16.1^{e,y}$	$14.3^{f,x}$	$13.8^{f,x}$
		CO	$25.4^{a,x}$	$26.4^{ab,w}$	$27.6^{bc,u}$	$28.7^{cd,s}$	$29.1^{de,tu}$	$30.7^{ef,t}$	$31.2^{f,t}$

[a–h]Within a row, read as two way interaction (packaging x day) means without a common superscript differ ($P < 0.05$).

[s–z]Within a column, read as two way interaction (packaging x day) means without a common superscript differ ($P < 0.05$).

n=90

Standard error for a* value = 1.3

Standard error for chroma value = 1.4

Table 3.2. Least squares means for visual color evaluation (aging x packaging x day interaction) of beef steaks displayed for 6 d

Parameter	Aging period (d)	Packaging	Display (d)						
			0	1	2	3	4	5	6
Surface discoloration	21	PVC	$1.0^{a,s}$	$1.3^{b,st}$	$2.3^{c,t}$	$2.4^{c,v}$	$3.3^{d,u}$	$4.1^{e,vw}$	$5.0^{f,uv}$
		HiOx	$1.0^{a,s}$	$1.1^{a,s}$	$1.4^{b,vw}$	$1.7^{c,u}$	$2.0^{d,v}$	$3.8^{e,w}$	$5.6^{f,s}$
		CO	$1.0^{a,s}$	$1.1^{a,s}$	$1.2^{a,u}$	$1.2^{a,x}$	$1.2^{a,x}$	$1.2^{a,y}$	$1.1^{a,x}$
	42	PVC	$1.2^{a,st}$	$1.6^{b,u}$	$2.2^{c,t}$	$3.3^{d,u}$	$4.5^{e,t}$	$4.4^{e,uv}$	$4.8^{f,v}$
		HiOx	$1.2^{a,st}$	$1.5^{b,tc}$	$1.5^{b,v}$	$2.2^{c,v}$	$3.6^{d,u}$	$4.7^{e,u}$	$5.3^{f,tu}$
		CO	$1.0^{a,s}$	$1.2^{ab,s}$	$1.4^{bc,v}$	$1.5^{cd,wx}$	$1.6^{cd,u}$	$1.7^{d,x}$	$2.1^{e,w}$
	62	PVC	$1.4^{a,u}$	$1.6^{a,u}$	$3.3^{b,s}$	$4.9^{c,s}$	$5.8^{d,s}$	$5.5^{e,t}$	$5.5^{e,st}$
		HiOx	$1.3^{a,tc}$	$1.4^{a,t}$	$2.1^{b,tu}$	$3.8^{c,t}$	$5.6^{d,s}$	$5.9^{e,s}$	$5.7^{e,s}$
		CO	$1.3^{a,tc}$	$1.5^{a,tu}$	$1.8^{b,u}$	$2.1^{cd,v}$	$1.9^{bc,vu}$	$1.8^{b,x}$	$1.9^{bc,u}$
Muscle color	21	PVC	$3.6^{a,uv}$	$3.6^{a,v}$	$4.9^{b,v}$	$5.3^{c,u}$	$5.5^{c,u}$	$6.5^{d,u}$	$7.0^{c,u}$
		HiOx	$3.5^{a,v}$	$3.3^{a,v}$	$4.0^{b,x}$	$4.5^{c,v}$	$4.6^{c,v}$	$6.3^{d,u}$	$7.3^{e,t}$
		CO	$4.3^{b,t}$	$4.0^{a,u}$	$5.0^{c,uv}$	$4.7^{c,v}$	$4.1^{ab,u}$	$3.8^{a,w}$	$4.2^{b,u}$
	42	PVC	$3.7^{a,u}$	$4.9^{b,t}$	$5.5^{c,t}$	$6.2^{d,st}$	$6.1^{d,t}$	$6.8^{e,t}$	$7.0^{e,u}$
		HiOx	$3.8^{a,u}$	$4.2^{b,u}$	$4.5^{b,w}$	$5.2^{c,u}$	$5.8^{d,tu}$	$7.0^{e,t}$	$7.4^{f,st}$
		CO	$4.2^{a,t}$	$5.2^{cd,st}$	$5.4^{d,t}$	$5.2^{c,u}$	$4.7^{b,v}$	$4.5^{ab,v}$	$4.8^{b,v}$
	62	PVC	$3.8^{a,u}$	$5.1^{b,t}$	$6.0^{c,s}$	$6.5^{d,s}$	$7.3^{e,s}$	$7.0^{e,t}$	$7.2^{e,tu}$
		HiOx	$3.5^{a,v}$	$4.3^{b,u}$	$4.9^{c,v}$	$6.0^{d,t}$	$7.1^{e,s}$	$7.6^{f,s}$	$7.7^{f,s}$
		CO	$5.3^{bc,s}$	$5.5^{c,s}$	$5.3^{b,tu}$	$5.3^{b,u}$	$4.5^{a,v}$	$4.6^{ab,v}$	$4.4^{a,w}$

Table 3.3 Least squares means for visual color evaluation (aging x packaging x day interaction) of beef steaks displayed for 6 d

Overall acceptability	21	PVC	$8.3^{a,s}$	$7.8^{b,t}$	$5.8^{c,u}$	$4.9^{d,t}$	$4.9^{d,v}$	$3.3^{e,v}$	$1.9^{f,v}$
		HiOx	$8.3^{a,s}$	$8.3^{a,s}$	$7.5^{b,s}$	$5.6^{c,s}$	$6.0^{c,u}$	$3.7^{d,v}$	$1.9^{e,v}$
		CO	$6.9^{a,t}$	$7.0^{a,u}$	$7.1^{a,s}$	$5.6^{b,s}$	$7.3^{a,s}$	$7.7^{c,s}$	$7.1^{a,s}$
	42	PVC	$8.1^{a,s}$	$6.1^{b,v}$	$5.4^{c,uv}$	$3.4^{d,uv}$	$3.1^{de,x}$	$2.7^{ef,w}$	$2.3^{f,uv}$
		HiOx	$8.3^{a,s}$	$7.2^{b,u}$	$6.6^{c,t}$	$4.8^{d,t}$	$3.7^{e,w}$	$2.5^{f,w}$	$2.0^{g,v}$
		CO	$6.6^{ab,s}$	$6.0^{bc,vw}$	$6.2^{b,t}$	$5.9^{bc,s}$	$6.6^{a,t}$	$6.8^{a,t}$	$5.8^{c,t}$
	62	PVC	$7.3^{a,t}$	$6.2^{b,v}$	$4.2^{c,v}$	$3.1^{d,v}$	$2.1^{e,y}$	$1.8^{f,x}$	$2.5^{e,u}$
		HiOx	$6.9^{a,t}$	$6.9^{a,u}$	$5.5^{b,u}$	$3.8^{c,u}$	$1.9^{d,y}$	$1.4^{e,x}$	$1.7^{de,v}$
		CO	$6.1^{bc,u}$	$5.8^{cd,w}$	$5.5^{d,u}$	$5.5^{e,s}$	$6.1^{bc,u}$	$6.2^{b,u}$	$6.7^{a,s}$

$^{a–h}$Within a row, interaction for (packaging x day) means without a common superscript differ ($P < 0.05$).

$^{s–z}$Within a column, interaction for (packaging x day) means without a common superscript differ ($P < 0.05$).

n=90

Standard error for surface discoloration = 0.17

Standard error for muscle color = 0.24

Standard error for overall acceptability = 0.38

REFERENCES

American Meat Science Association. 2012. Meat Color Measurement Guidelines. American Meat Science Association, Chicago, IL.

Mac Dougall, D., (1982). Changes in colour and opacity of meat. Food Chemistry 9,75-88.

Mancini, R. A., & Ramanathan, R., (2014). Effects of postmortem storage time on color and mitochondria in beef. *Meat Science*. 98:65-70.

Witte, V. C., G. F. Krause, & M. E. Bailey. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *Journal of Food Science.* 35:582-585.

Sammel, L. M., M. C. Hunt, D. H. Kropf, K. A. Hachmeister & D. E. Johnson. (2002). Comparison of Assays for Metmyoglobin Reducing Ability in Beef Inside and Outside Semimembranosus Muscle. *Journal of Food Science*. 67:978-984.

Madhavi, D. L., Carpenter, C. E., (1993). Aging and processing affect color, metmyoglobin reductase and oxygen consumption of beef muscle. *Journal of Food Science.* 58: 939-942.

Mancini, R. A., Hunt, M. C. (2005). Current research in meat color. *Meat Science*, 71, 100-121.

Mac Dougall, D., (1982). Changes in colour and opacity of meat. *Food Chemistry*, 9,75-88.

CHAPTER IV

EFFECTS OF AGING AND FREEZE THAWING ON BIOCHEMICAL PROPERTIES OF DARK CUTTING BEEF

Abstract

Elevated muscle pH in dark cutting beef can enhance mitochondrial activity and stability of enzymes involved in oxygen consumption. Both processes can greatly influence the myoglobin oxygenation, hence the beef color. Limited studies have characterized the effects of extended aging on biochemical properties of dark cutting beef. Therefore, the objectives were: (1) to determine the effects of extended aging on biochemical factors involved in dark cutting beef, and (2) to evaluate the effects of aging and freeze thawing on oxygenation properties of dark cutting beef quantify. Ten USDA choice (pH = 5.6) and ten no-roll dark cutter (pH > 6.4) strip loins (IMPS #180) were obtained from a commercial packing plant within 3 d of harvest. Loins were cut into four equal sections, vacuum packaged, and aged for 21, 42, and 62 d at 2°C. Following aging of normal pH and dark cutting loin sections, two 2.5 cm thick steaks were cut and used to determine blooming properties, oxygen consumption, metmyoglobin reducing activity, lipid oxidation, mitochondrial quantification, and myoglobin concentration. Surface color readings were measured using a HunterLab Miniscan XE Plus spectrophotometer. Oxygen consumption rate and metmyoglobin reducing activity were also measured, respectively. q-PCR was used to quantify mitochondrial concentration. The experiment was replicated 10 times. The data were analyzed using the Mixed Procedure of SAS and significance was determined at $P < 0.05$. A muscle pH type x blooming time x aging interaction ($P < 0.05$) resulted for oxymyoglobin level and a* values. Normal pH beef had greater oxymyoglobin and a* values at all bloom time points. Initial metmyoglobin formation was lower ($P < 0.05$) for dark cutting beef compared with normal pH beef, indicating more metmyoglobin reducing activity. Aging improved blooming properties of both normal and dark cutting beef. No differences were found for lipid oxidation of normal pH and dark cutting beef ($P>0.05$). Mitochondria content was 2.04 fold greater in dark

cutting beef compared with normal pH beef. Freeze thawing dark cutting beef significantly ($P < 0.05$) increased blooming values compared to non-freeze thawed dark cutting beef across all bloom times. Hence, better understanding the biochemical properties will help to design strategies to improve blooming properties in dark cutting beef.

Keywords: dark cutter, aging, bloom, mitochondria

Introduction

Dark cutting beef is the result of a depletion of glycogen reserves prior to slaughter, and is often described as meat that fails to bloom after the cut surface has been exposed to oxygen. Dark cutting beef is a substantial meat quality issue worldwide. According to the National Beef Quality Audit the US Beef Industry lost approximately $165-170 million in 2000 due to dark cutting carcasses (McKenna et al., 2002).

When the pH of meat remains elevated, the physical state of the proteins will be above the iso-electric point, proteins will then retain more water, therefore causing the muscle fibers to be more tightly packed. Consequently, the meat is then dark in color because its surface does not scatter light to the same extent as the more open surface of meat with a lower ultimate pH (Seideman et al., 1984). Increased pH also enhances mitochondria activity (Ashmore et al., 1972). Mitochondrial oxygen consumption can also influence myoglobin oxygenation (Lawrie, 1958).

Postmortem aging has been shown to decrease oxygen consumption Mac Dougall et al. (1982) reported that increased aging would improve bloom by decreasing the competition for oxygen between myoglobin and mitochondria. Specifically, the substrates for mitochondria respiration are depleted during extended aging; therefore oxygen can penetrate faster into tissue as postmortem time increases. However, limited information is currently available on the effects of aging on biochemical properties of dark cutting beef.

As water freezes, the concentration of the remaining solutes increases, thereby disrupting the homeostasis of the meat system (Lawrie, 1998). The changes that happen in the immediate environment of the meat fibers affect the cell membrane characteristics, which in turn affect the quality of the meat (Fellows, 2000). It has been reported that freeze-thaw treatment of isolated

liver mitochondria disrupted mitochondria and increased state IV oxygen consumption rate (OCR). There has been little to no research investigating the effects of freeze thawing to limit mitochondrial activity. Therefore, the objectives of the current study were 1) to determine the effects of extended aging on biochemical properties, and 2) to assess the effects of extended aging and freeze thawing on oxygenation properties of dark cutting beef.

MATERIALS AND METHODS

Experiment 1: Effect of extended aging on the biochemical factors of dark cutting beef

Raw materials and processing

Ten USDA beef strip loins (IMPS #180) were selected at random and ten no-roll dark cutting beef carcasses were selected and individually identified and tagged prior to carcasses fabrication from the Fresh Beef Tyson Plant in Garden City, KS, 72 h after slaughter. The selected dark cutting strip loins, once fabricated were vacuum packaged and transported to Oklahoma State University for further analysis. Upon arrival at the Food and Agriculture Product Center located on the Oklahoma State University campus, normal pH strip loins and dark cutting strip loins were equally divided into four sections and randomly assigned to three aging periods 21, 42, and 62 d, the fourth section was later used for freeze thawing analysis. Samples were allowed to age for the respective storage period at refrigeration temperature (2°C) in prime source vacuum pouches 12 x 18 cm, 3 mil high barrier. Following aging periods, each strip loin section was cut into four 2.5-cm thick steaks per aged section using a meat slicer (Bizerba 1 phase model SE12US, Bizerba USA Inc. Piscataway, New Jersey). Steaks were used to measure oxygenation properties (bloom), oxygen consumption rate, metmyoglobin reducing activity, lipid oxidation, proximate analysis, pH, myoglobin and mitochondrial quantification.

Muscle pH

pH was obtained using a model Corning pH meter 320 (Corning Incorporated, Corning, NY, USA). pH was measured by taking triplicate readings of each sample, inserting the probe directly into the sample. The probe prior to readings was standardized using 4.0 and 7.0 buffers.

Proximate analysis

Excess external fat from each steak was trimmed and ground using a commercial grinder (LEM Big Bite Grinder, LEM Products, West Chester, OH, USA) to obtain approximately 200-g of sample. Analysis of protein, fat, moisture, and collagen was measured using a near-infrared spectrophotometer (FoodScan 78800, FOSS, Laurel, MD).

Surface color measurement

All instrumental color measurements were performed using a HunterLab MiniScan XE Plus spectrophotometer (Model 45/0 LAV, 2.54-cm diameter aperture, illuminant A, 10° observer). Both reflectance spectra from 400 to 700 nm (10 nm increments) and CIE L*, a*, and b* were measured in duplicates for each steak and subsamples were averaged for statistical analyses. Reflectance data from 400 to 700 nm also was used to estimate % myoglobin forms during storage (AMSA, 2012).

Oxygenation properties

After respective aging period, from each loin, a 2.5 cm think steak was cut and color readings were recorded immediately (time 0). Following initial color reading, steaks were wrapped with polyvinyl chloride film and incubated at 4°C. Bloom measurements were taken using a HunterLab Miniscan repeatedly on the steak at 30, 60, 90, and 120 min.

Metmyoglobin reducing activity

Metmyoglobin reducing activity was measured on fresh meat samples once aging periods were met and were cut to remove any visible fat or connective tissue. Surface pigments were initially oxidized to metmyoglobin (MMb) by submerging in 0.3% $NaNO_2$ solution (Sigma-Aldrich, St. Louis, MO, USA) for 20 min in a 30°C incubator. Samples were removed from the solution, blot dried to remove excess solution and immediately placed in an impermeable vacuum package and scanned for reflectance from 400 to 700 nm with a Hunter Lab Miniscan to determine initial MMb formation on the surface. MMb was monitored for 2 h at 30°C in an incubator and rescanned to measure the remaining MMb (AMSA, 2012). The following equation was used to calculate metmyoglobin-reducing activity: [(% surface metmyoglobin pre-incubation−% surface metmyoglobin post-incubation)/% surface metmyoglobin preincubation]×100.

Oxygen consumption rate

Once aging periods were met, meat samples were sliced to expose a fresh meat surface and cut where minimal fat or connective tissue was present. Then samples were allowed to oxygenate for 120 min at 4°C covered by an oxygen-permeable PVC film to minimize drying of the sample. After bloom, the samples were vacuum packaged and incubated at 30°C. Steaks were scanned immediately after vacuum packaging and after 30 min of incubation. The surface was then scanned using a HunterLab Miniscan for reflectance from 400 to 700 nm to determine the initial % OMb. Differences in oxymyoglobin level before and after incubation was used to calculate %oxygen consumption (AMSA, 2012).

Myoglobin quantification

Five gram of sample from both normal and dark cutting beef samples were weighed separately and placed in homogenizing tubes. A 25-mL of ice cold phosphate buffer at pH 6.8 (40 mM) per 5-g sample was added and then homogenized in a Sorvell onmi mixer (Newton, CT, USA) for 45 sec at low speed. Samples were held in ice (0 to 4°C) for 1 h. After 1 h 1.5-mL of homogenate was then transferred to a 1.5-mL Eppendorf tube (safe-lock, Eppendorf North America, USA) and centrifuged at 16,000 *x g* for 15 min. Supernatant was filtered through a 0.45 µM syringe (Covidien Mansfield, MA, USA) filter (Phoenix Research Products, USA) to a cuvette (1.5 mL semi-mirco fisher scientific, USA) then absorbance was measured at 525 nm to calculate total myoglobin concentration (AMSA, 2012).

Quantification of mitochondrial DNA

DNA Isolation

Total DNA was isolated using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA) and using manufacturer's instructions with following modifications. Briefly, 0.5 g of dark cutter or normal pH steaks were cut into small pieces and ground in liquid nitrogen. Approximately, 0.3 g of the ground muscle was used for further processing. The final elution of DNA was performed using 100 µL of deionized water and stored at -20°C.

Quantitative real-time PCR

A 274 bp amplicon of mitochondrial cytochrome b gene (F – 5'GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA3', R – 5'CTAGAAAAAGTGTAAGACCCGTAATATAAG3') was used to evaluate the mitochondrial

copy number (Matsunaga et al., 1999) and a 119 bp amplicon based on the 18S rRNA gene (F-5'TTCGAACGTCTGCCCTATTAA3', R – 5'GATGTGGTAGCCGTTTCTCAGG3') was used as the normalizer. Muscle samples from each muscle type (dark cutter or normal) were assayed separately in duplicate for each muscle using the SYBR green1 reporter assay (Roche Diagnostics, Indianapolis, IN).

A 15-µL reaction mixture contained 400 nM of each forward and reverse primer, 1X master mix (Roche Diagnostics, Indianapolis, IN), and 10 ng of template DNA. Cycling conditions were; 95 °C for 10 min followed by 50 cycles of 94 °C for 15 sec, 58 °C for 15 sec, and 72 °C for 20 sec. Relative mitochondrial copy number was calculated using the comparative CT method as previously described (Schmittgen & Livak, 2008).

Lipid Oxidation

Thiobarbituric acid reactive substances (TBARS) values were measured according to the procedure of Witte, Krause, and Bailey (1970) as an indicator of lipid oxidation. Five grams from each steak were blended with 25 ml tricholoracetic acid (TCA) solution (20%) and 20 ml distilled water. The mixture was homogenized using a Sorvell onmi mixer (Newton, CT) for 30 s and filtered through Whatman (42, ashless, 125 mm, circle, filter paper, Fisher Scientific, Bohemia, NY) filter paper. One milliliter of filtrate was mixed with 1 ml thiobarbituric acid (TBA) solution (20 mM) and incubated at 100°C water bath (Isotemp, Fisher Scientific, Bohemia, NY, USA) for 10 min. After incubation, absorbance was measured using a Shimadzu UV-2600 spectrophotometer (Shimadzu Inc., Columbia, MD, USA) at 532 nm against a blank consisting of 2 ml acid/water mix (TCA/water 1:1 v/v) and 2 ml TBA solution.

Experiment II: Effect of freeze thawing on oxygenation properties of dark cutting beef

Raw materials and processing

The fourth section of the dark cutting and normal pH strip loins as identified in Experiment l were used for Experiment ll. The loins were vacuum packaged and aged for 62 d at 2°C. Following aging, from each normal and dark cutting loin section, a 2.5 cm thick steak was cut (without freeze thawing) and the remaining portion of dark cutting section was vacuum packaged and subjected to three cycles of freeze thawing at -20°C and thawed in a controlled 15°C water bath.

Oxygenation properties

Once freeze thawing was complete steaks were bisected and readings with a HunterLab Miniscan spectrophotometer were immediately taken to measure 0 min and continuously through times 15, 30, 60, 90, and 120 min. The reference standards for 100% myoglobin redox forms were prepared for steaks from normal and dark cutters. The reflectance values were converted to K/S ratios to calculate % oxymyoglobin levels.

Muscle pH

pH was obtained using a model corning pH meter 320; Corning Incorporated, Corning, NY, USA. pH was measured by taking triplicate readings of each sample, inserting the probe directly into the sample. The probe prior to readings was standardized using pH 4.0 and 7.0 buffers.

Statistical Analysis

Data were analyzed as a split plot design. Within the whole plot, 10 longissimus lumborum and 10 no-roll longissimus lumborum psoas major muscles were considered experimental units in order to compare biochemical properties of two muscles types (n = 10 for each muscle and N = 20 total subprimals). Within the subplot, each longissimus was divided into three sections, resulting in three experimental units per subprimal. One of the three experimental units within each subprimal was assigned to one of three aging treatments (aged 21 d, 42 d, and 62 d). The analysis of variance was generated using the mixed models procedure of SAS (SAS Inst., Inc., Cary, NC, USA). Least squares means were generated, and, when significant ($P<0.05$) *F*-values were observed, least squares means were separated using a pair-wise t-test (PDIFF option).

RESULTS AND DISCUSSION

Muscle pH

There was a significant ($P < 0.0001$) muscle effect on pH. The average muscle pH for normal and dark cutting beef were 5.5 and 6.4, respectively. Dark cutters result from pre-slaughter stress, which depletes muscle glycogen. If glycogen is depleted by chronic long-term stress before slaughter then less lactic acid is formed and the meat does not acidify normally, hence the ultimate pH remains high. Dark cutting meat has a pH of > 5.8 (Viljoen et al., 2002).

Proximate analysis

Proximate analysis demonstrated no significant differences for dark cutter and controls in protein and fat percentages shown in Table 4.4. However, there were significantly ($P < 0.0001$)

greater moisture and collagen percentages for dark cutters verses controls. A greater pH in dark cutting beef can hold more water than normal pH.

Oxygenation properties

Figure 4.4 and Table 4.1 shows there was a significant ($P < 0.0001$) interaction for muscle by blooming time. Choice treatments across blooming times had significantly ($P < 0.0001$) greater oxymyoglobin formation compared to the dark treatment. As blooming time increased oxymyoglobin formation significantly ($P < 0.0001$) increased for both dark and control treatments from time 0 min to 120 min. Wulf and Wise (1999) stated that L* values of beef *longissimus* did not change following 30 min of the total 93 min of blooming following ribbing carcasses; but a* and b* values continued to increase until the 75 min post ribbing. Haas and Bratzler (1965) reported that a* values of beef *longissimus* continued to increase up to 180 min of bloom time. Meat that has been aged for several weeks prior to exposure to air blooms more rapidly and intensely than fresh meat because of the loss of activity of the oxygen-consumption enzymes according to Ledward (1992). In normal meat, postmortem glycolysis reduces pH to 5.8 or lower which impairs mitochondrial oxygen consumption (Ashmore et al., 1972) and allows normal bloom on meat surfaces exposed to air. Lawrie (1958) found that mitochondrial cytochrome oxidase was more active at pH values above 6.0, characteristic of dark cutting beef muscle, and concluded that increased oxygen consumption of dark cutting meat could increase the concentration of deoxygenated myoglobin, thus resulting in the dark color. Ledward et al. (1992) explained that color in muscle tissue is based on the reflectance of light off free water and on oxygenation of the myoglobin. At increased muscle pH, proteins are able to bind stronger with water, allowing less free water. When there is more water bond to the proteins, the muscle fibers are swollen, leaving less space between the muscle fibers. So meat that has an elevated pH

will be darker in color because less free water is available to reflect light (Ledward et al., 1992). Also, at higher muscle pH, enzymes that use oxygen are more active, resulting in less oxygenation of the surface myoglobin, leaving a darker color (Price and Schweigert, 1987; Ledward et al., 1992).

Metmyoglobin reducing activity

Figure 4.2 shows there was a significant ($P < 0.0001$) interaction for muscle by aging time for both a* value and the initial metmyoglobin formation. The dark treatment a* values for the percent of metmyoglobin reducing activity were significantly ($P < 0.0001$) greater across aging periods than control steaks. Aging period for 21 d dark and control steaks were significantly ($P < 0.0001$) greater than aging periods 42 d and 62 d. There were no significant differences between aging periods 42 d and 62 d for dark and control steaks. As muscles age, mitochondrial activity decreases, leading to elimination of energy conversion and, dramatic reduction in metmyoglobin reducing activity (Faustman & Cassens, 1990). Oxygen in meat is used for oxygenation of meat pigments and/or mitochondrial respiration; therefore, as mitochondrial activity decreases, oxygen is then consumed at a higher rate by myoglobin (Lanari & Cassens, 1991). Greater pH in dark cutting muscles will result in greater MRA compared with normal pH beef.

Oxygen consumption rate

There was a significant ($P < 0.0001$) difference for muscle, and a significant ($P < 0.0001$) difference with aging, however no significant ($P < 0.1096$) interaction between muscle and aging. As to be expected there was a significantly ($P < 0.0001$) greater oxygen consumption rate on aging period 21 d for dark cutters and controls. On aging times 42 d and 62 d dark cutters had

significantly ($P < 0.0001$) oxygen consumption rates compared to controls. Lee et al., (2008) determined that the activity of oxygen-consuming enzymes were likely contributed to the differences found in color development among the aging period treatments, similar to what was found in the current study. Oxygen consumption rates decline with increased days of retail display for all beef muscles (McKenna et al., 2005). According to Mckenna et al. (2005) color stability was not solely dependent on reducing activity or oxygen consumption rate, but rather color stability is determined by the proportion of these two components.

Myoglobin and mitochondrial quantification

Dark cutters had an average of (7.6 mM) and control steaks had an average of (5.6 mM) (Table 4.2). Dark cutters compared to normal pH steaks were significantly greater ($P < 0.05$) in myoglobin concentration. In addition, mitochondria content was 2.04 fold greater in dark cutting beef compared with normal pH beef. Zerouala and Stickland (1991) tested different fiber type proportions in dark cutting and normal animals that had been slaughtered under normal abattoir conditions. Their results demonstrated a shift to more oxidative metabolism in the muscle of dark cutting cattle with some evidence for a greater shift in dark cutting bulls. Confirming that cattle exhibiting a relatively high level of oxidative metabolism with their muscles are more susceptible to the development of dark cutting beef. Increased amount of oxidative muscle might be responsible for the greater myoglobin and mitochondrial content.

Lipid oxidation

There were no significant differences between treatments, or aging time by treatment. Average lipid oxidation (Figure 4.3) for aging period 21 d for dark cutting and normal pH meat were 0.119 and 0.1089, respectively. Similarly, for aging period 42 d dark samples had 0.107

and control steaks had 0.09. Packaging the loin sections in vacuum and dark storage might be responsible for no significant changes in TBARS values.

Experiment II: Effect of freeze thawing on oxygenation properties of dark cutting beef

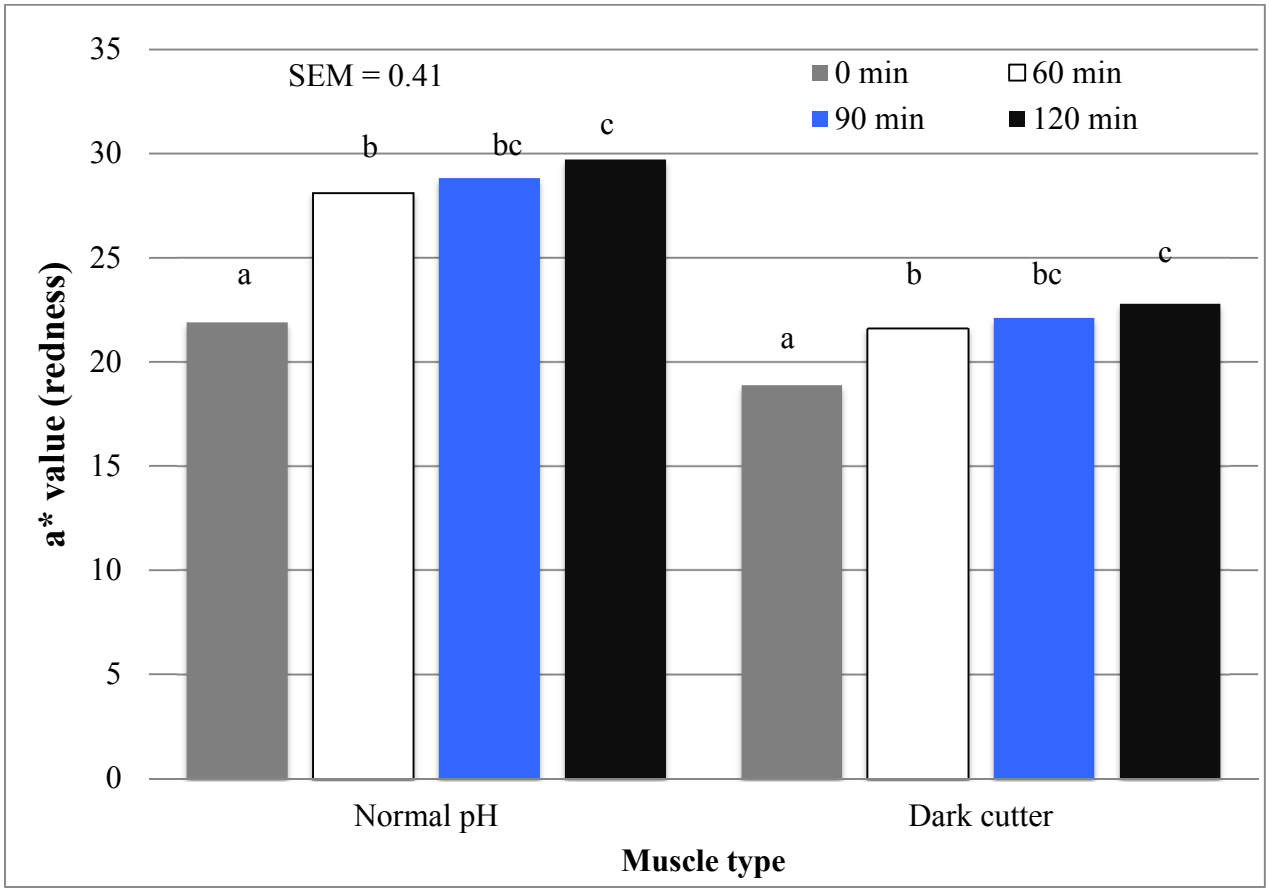
Oxygenation properties

There was a significant ($P < 0.05$) interaction between aging and bloom time for a*, and oxymyoglobin level. a* value and percent oxymyoglobin level was significantly greater ($P < 0.05$) in normal pH steaks compared with dark cutting for all bloom times. Dark cutters with freeze thawing cycles compared to dark cutters without freeze thawing had greater ($P < 0.05$) a* values for all bloom times. Dark cutters with freeze thawing had greater ($P < 0.05$) oxymyoglobin at all bloom times when compared to dark cutters without freeze thawing. Freezing and thawing cause damage to the ultrastructure of the muscle cells with the ensuing release of mitochondrial and lysosomal enzymes, heme iron and other pro-oxidants (Leygonie et al., 2012). Mac Dougall et al. (1982) reported that increased aging will improve bloom by decreasing competition for oxygen between myoglobin and mitochondria, because substrates for mitochondrial respiration are continually depleted postmortem. Oxygen can then penetrate further into tissue as time postmortem increases (Mancini and Ramanathan, 2010). Mitochondria are important subcellular organelles involved in energy metabolism. It has been reported that freeze-thaw treatment of isolated liver mitochondria disrupted mitochondrial function, hence there will be less competition for oxygen between mitochondria and myoglobin.

CONCLUSION

Extended aging can affect the blooming properties and oxygen consumption rate of dark cutting beef. Furthermore, freeze thawing and aging of dark cutting beef can be an effective method to increase blooming values comparable to normal pH beef *longissimus* muscle. Hence, better understanding the biochemical properties can help the processors to design strategies to improve surface color of dark cutting beef.

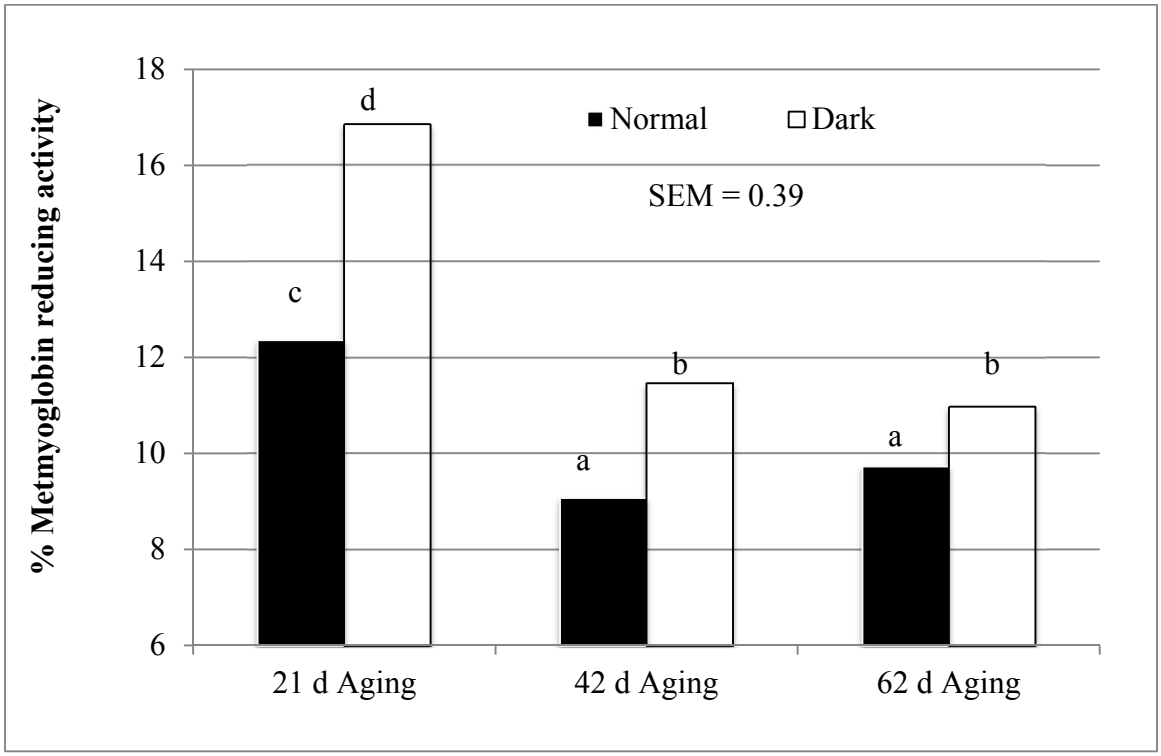
Figure 4.1 Effects of muscle pH on blooming properties (a* value) of normal and dark cutting beef.



[abcd]Least square means within a muscle that do not have a common superscript letter differ (P < 0.05).

n = 20

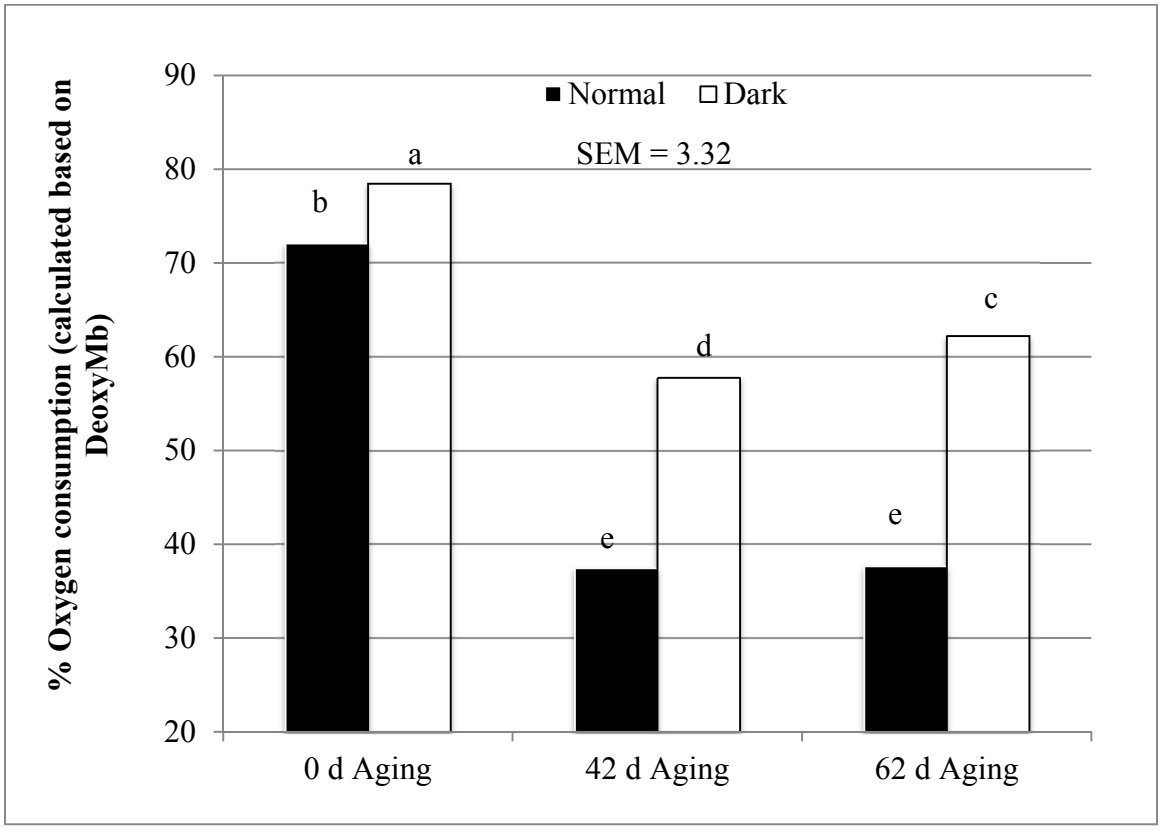
Figure 4.2 Effects of extended aging and muscle pH on metmyoglobin reduction activity of beef *longissimus* steak.



[abcd]Least square means across aging times that do not have a common superscript letter differ ($P < 0.05$).

n = 60

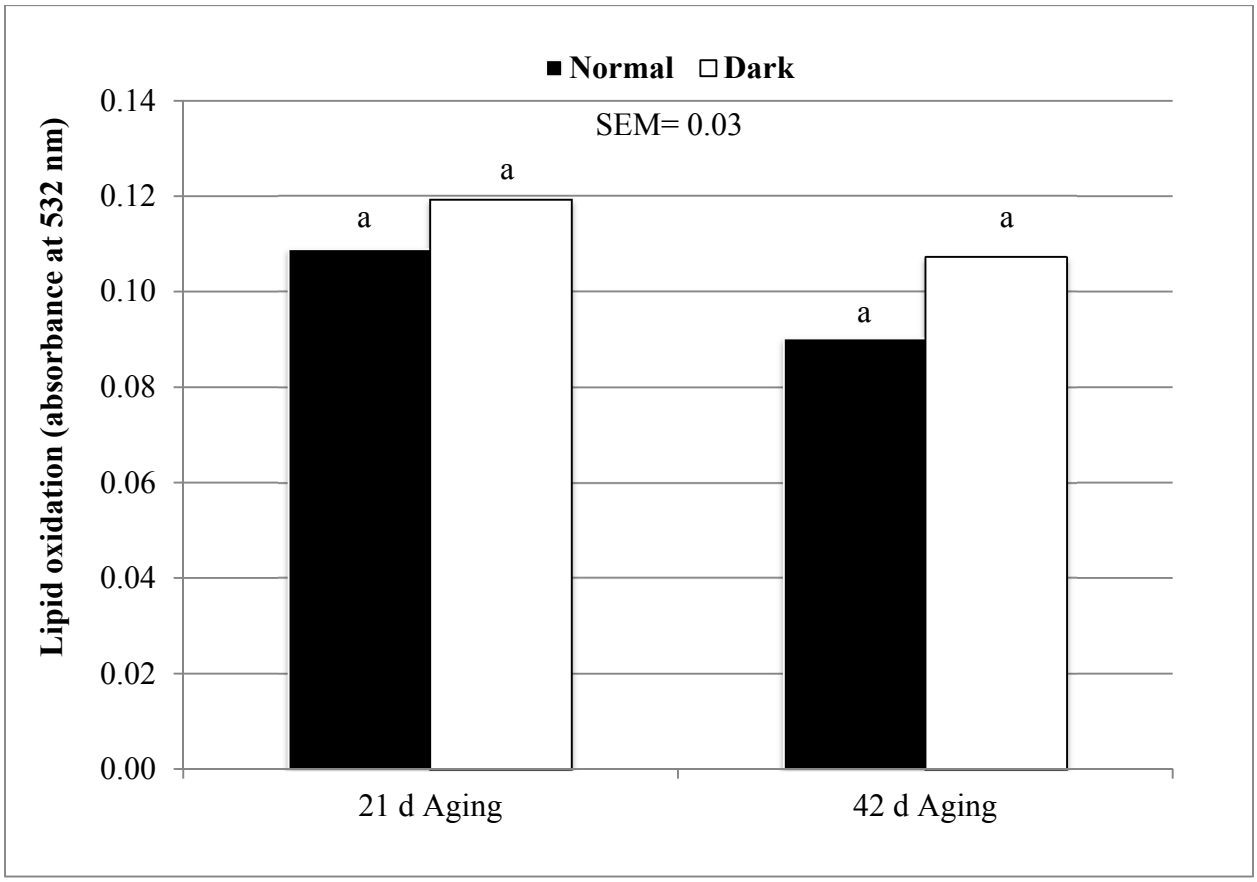
Figure 4.3 Effects of extended aging and muscle pH on oxygen consumption of beef *longissimus* steak.



[abcde]Least square means across aging times that do not have a common superscript letter differ ($P < 0.05$).

n = 60

Figure 4.4 Effects of extended aging and muscle pH on lipid oxidation of beef *longissimus* steak.



[a]Least square means within aging times that do not have a common superscript letter differ (P < 0.05).

n = 40

Table 4.1

Least square means for L* (treatment x time) of bloomed beef steaks

Parameter	Aging Period (day)	Treatment	Bloom Time (min)			
			0	60	90	120
L*	21	Normal	$40.3^{a,u}$	$43.2^{a,w}$	$42.3^{a,u}$	$43.1^{a,u}$
		Dark	$31^{a,v}$	$31.5^{a,u}$	$30.9^{a,u}$	$31^{a,u}$
	42	Normal	$37.1^{a,v}$	$40.9^{b,w}$	$41.6^{b,v}$	$41.8^{b,v}$
		Dark	$26.2^{a,w}$	$30.2^{b,u}$	$31.3^{b,w}$	$31.2^{b,w}$
	62	Normal	$45.4^{a,x}$	$48^{a,x}$	$47.6^{a,w}$	$47.4^{a,w}$
		Dark	$33.7^{a,y}$	$36.4^{ab,v}$	$37.1^{b,x}$	$38.2^{b,x}$

^{abcd}Least square means within a muscle, and aging time that do not have a common superscript letter differ ($P < 0.05$).

^{uvwxy}Least square means within a bloom time that do not have a common superscript letter differ ($P < 0.05$).

Standard error = 1.52

Table 4.2

Quantification of mitochondrial concentration in normal pH and dark cutting beef using real-time polymerase chain reaction

Sample	18S-1	18S-2	Avg.18S	Act.-1	Act-2	Avg.Act.	Cyt.B-1	Cyt.B-2	Avg.Cyt.	DCT-18S	DCT-Act	Avg.DCT-18S	Avg.DCT-Act
D100	22.01	22.45	22.23	26.04	26.47	26.26	18.70	18.98	18.84	-3.39	-7.42	-3.17	-7.38
D200	21.06	21.76	21.41	25.48	25.66	25.57	18.19	19.12	18.66	-2.76	-6.92		
D300	21.44	21.48	21.46	26.13	26.48	26.31	18.98	19.23	19.11	-2.36	-7.20		
D400	20.73	21.32	21.03	26.50	24.97	25.74	16.74	17.13	16.94	-4.09	-8.80		
D500	21.92	20.91	21.42	24.98	24.48	24.73	17.90	18.44	18.17	-3.25	-6.56		
N100	22.76	22.25	22.51	26.93	27.73	27.33	20.03	20.87	20.45	-2.06	-6.88	-2.14	-6.77
N200	23.49	23.49	23.49	28.02	28.28	28.15	21.94	21.66	21.80	-1.69	-6.35		
N300	21.28	21.02	21.15	25.60	25.60	25.60	18.34	18.16	18.25	-2.90	-7.35		
N400	21.70	21.46	21.58	25.05	26.97	26.01	19.01	17.99	18.50	-3.08	-7.51		
N500	22.33	22.40	22.37	27.17	27.17	27.17	21.58	21.25	21.42	-0.95	-5.76		
D-N	**DDCT-18S:**	**-1.03**		**Folds-18S:**	**2.04**	**D/N**							
D-N	**DDCT-Act:**	**-0.61**		**Folds-Act:**	**1.53**	**D/N**							

Table 4.3

Least square means for a* and OxyMb (treatment x time) of freeze thawed bloomed beef steaks.

Parameter	Treatment	Bloom Time (min)					
		0	15	30	60	90	120
a*	Normal	$19.2^{a,x}$	$22.3^{b,y}$	$25.1^{c,z}$	$26.1^{c,y}$	$26.8^{cd,z}$	$28.8^{d,z}$
	Dark	$17.5^{ab,x}$	$17.4^{a,x}$	$16.8^{a,x}$	$21.5^{b,x}$	$20.6^{b,x}$	$19.5^{b,x}$
	Dark freeze	$18.1^{a,x}$	$21.6^{b,y}$	$22.9^{b,y}$	$22.4^{b,x}$	$23.3^{b,y}$	$23.7^{b,y}$
OxyMb	Normal	$31.6^{a,y}$	$59.8^{b,z}$	$74.5^{c,z}$	$78.6^{d,z}$	$83.9^{e,z}$	$91.8^{f,z}$
	Dark	$2.9^{a,x}$	$11.4^{b,x}$	$10^{b,x}$	$54.4^{e,x}$	$40.9^{c,x}$	$46.7^{d,x}$
	Dark freeze	$1^{a,x}$	$46.5^{b,y}$	$46.5^{b,y}$	$62.9^{c,y}$	$62.9^{c,y}$	$78.6^{d,y}$

^{abcd}Least square means within a muscle and aging that do not have a common superscript letter differ ($P < 0.05$).

^{xyz}Least square means within a bloom time and aging that do not have a common superscript letter differ ($P < 0.05$).

Standard error = 1.16

Table 4.4

Proximate analysis and myoglobin concentration averages for control and dark cutting steaks.

Aging Period (d)	Treatment	Parameters				
		Protein	Fat	Moisture	Collagen	Mb Concentration (mM)
21	Normal	23.3[a]	10.8[a]	65.6[a]	3[a]	5.6[a]
	Dark	22.5[b]	7[a]	70.5[b]	1.8[b]	7.9[b]
	SEM	0.3795	1.35	0.9087	0.2165	0.0345

[ab]Least square means within a muscle that do not have a common superscript letter differ ($P < 0.05$).

REFERENCES

American Meat Science Association. 2012. Meat Color Measurement Guidelines. American Meat Science Association, Chicago, IL.

Ashmore, C. R., Parker, W., & Doerr, L., (1972). Respiration of mitochondria isolated from dark-cutting beef: postmortem changes. *Journal of Animal Science*. 34:1.

Faustman, C., & Cassens, R. G., The biochemical basis for discoloration in fresh meat: A review. (1990). *Journal of Muscle Foods*. 1, 217-243.

Fellows, P. (2000). Freezing. Food processing technology, principles and practice (pp. 418-440). (2 ed.). London: Woodhead Publishing Limited.

Lanari, M. C., & Cassens, R. G. (1991). Mitochondrial activity and beef muscle color stability. *Journal of Food Science*, 56, 1476-1479.

Lawrie, R. A. (1958). Physiological stress in relation to dark-cutting beef. *Journal of Food Science Agriculture*. 11:721

Lawrie, R. A., (1998). In Anonymous (Ed.), Lawrie's meat science (pp. 1-336). (6^{th} ed.). Lancaster, PA: Technomic Publishing Inc.

Ledward, D.A., D. E. Johnston, & M. K. Knight, (1992). The Chemistry of Muscle-Based Foods. pp 128-139. The Royal Society of Chemistry, Thomas Graham House, Science Park, Cambridge, U. K.

Lee, M. S., Apple, J. K., Yancey, J. W. S., Sawyer, J. T., & Johnson, Z. B., (2008). Influence of vacuum-aging period on bloom development of the beef gluteus medius from top sirloin butts. *Meat Science*. 80: 592-598.

Leygonie, C., Britz, T. J., & Hoffman, L. C., (2012). Meat quality comparison between fresh and frozen/thawed ostrich M. iliofibularis. *Meat Science*, provisionally accepted.

Mac Dougall, D., (1982). Changes in colour and opacity of meat. Food Chemistry 9,75-88.

Mancini, R. A., & Ramanathan, R., (2010). Out-competing myoglobin for oxygen; The role of mitochondria in terms of oxygen consumption and balance in postmortem muscle. *Fleischwirtschaft Inter.* 2, 73-74.

Mckenna, D. R., D. L. Roeber, P. K. Bates, T. B. Schmidt, D. S. Hale, D. B. Griffin, J. W. Savell, J. C. Brooks, J. B. Morgan, T. H. Montgomery, K. E. Belk, & G. C. Smith. (2002). National Beef Quality Audit-2000: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. *Journal of Animal Science.* 80:1212.

McKenna, D., Mies, P., Baird, B., Pfeiffer, K., Ellebracht, J., & Savell, J., (2005). Biochemical and physical factors affecting discolouration characteristics of 19 bovine muscles. *Meat Science.* 70, 665-682.

Price, J. F., & Schweiegert, B. S., (1987). The science of meat and meat products. 3rd ed. Food and Nutrition Press, Westport CT.

Seideman, S. C., Cross, H. R., Smith, G. C., & Durland, P. R., (1984). Factors affecting fresh meat colour: review. *Journal of Food Quality.* 6(3), 211-237.

Schmittgen, T. D., & Livak, K. J., (2008). Analyzing real-time PCR data by the comparative C_T method. *Nature Protocols* 3, 1101-1108.

Witte, V. C., G. F. Krause, & M. E. Bailey. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *Journal of Food Science*. 35:582-585.

Wulf, D. M., & Wise, J. W., (1999). Measuring muscle color on beef carcasses using the L*a*b* color space. *Journal of Animal Science*. 77, 2418-2427.

Zhu, L. G., & Brewer, M. S., (1998). Metmyoglobin reducing capacity of fresh normal, PSE and DFD pork during retail display. *Journal of Food Science*

CONCLUSION

The current research demonstrates that by extended aging of dark cutting beef can improve the color intensity (redness) of the steaks, but it does in fact have a detrimental effect on the color stability. CO-MAP however did have the greatest color stability and HiOx having the least color stability during retail display, a result of decreased MRA and OCR. Furthermore, by freeze thawing in combination with extended aging can in fact be an effective method to increase blooming values which are comparable to normal pH beef *longissimus* muscle. Hence, by having a better understanding of the biochemical properties can help processors to design effective strategies to improve surface color of dark cutting beef.

APPENDICES

Meat Color Score Sheet

Evaluator: **Date:** **Time:**

Muscle Darkening (MD)	Muscle Color (MC)	Surface Discoloration (SD)	Overall Meat Color (OMC)
1 No darkening	1 Purplish red	1 No discoloration 0%	9 Extremely desirable
2	2 Reddish purple	2 Slight discoloration 1 - 20%	8 Very desirable
3 Slight Dark	3 Extremely bright cherry red	3 Small discoloration 21- 40%	7 Moderately desirable
4	4 Moderately bright cherry red	4 Modest discoloration 41 - 60%	6 Slightly desirable
5 Moderately dark	5 Slightly bright cherry red	5 Moderate discoloration 61 - 80%	5 Neither acceptable or unacceptable
6	6 Slightly dark red or tan-brown	6 Extensive discoloration 81 - 100%	4 Slightly undesirable
7 Very dark	7 Moderately dark red or tan-brown		3 Moderately undesirable
	8 Extremely dark red or tan-brown		2 Very undersirable
			1 Extremely undersirable

ID	MD	MC	SD	OMC
1A				
2A				
3A				
4A				
5A				
6A				
7A				
8A				
9A				
10A				
1B				
2B				
3B				
4B				
5B				

ID	MD	MC	SD	OMC
6B				
7B				
8B				
9B				
10B				
1C				
2C				
3C				
4C				
5C				
6C				
7C				
8C				
9C				
10C				

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