CONTEMPORARY LIGHT SOURCES AND THEIR IMPACT ON THE DISCOLORATION AND OXIDATION OF FRESH BEEF PRODUCTS

A Thesis
Presented to
the Faculty of the Graduate School
at the University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by
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MAY 2017

The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

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ACKNOWLEDGEMENTS

First and foremost, I want to thank my major professor, Dr. Carol Lorenzen for providing me with an immeasurable amount of opportunities over the last three years. Without your continued support, guidance, and encouragement none of this would have been possible. Thank you for believing in me, sacrificing for me, and for doing everything in your power to ensure I got the most out of my education. You truly are an inspiration to me, and I hope I continue to make you proud throughout the rest of my career.

Dr. Bryon Wiegand, thank you for being both an educational and personal support system during this process. I feel truly blessed to have worked in an environment with multiple mentors to turn to at any point I needed. Thank you for treating me as one of your own students, even when you didn't have to. I couldn't have done this without you. (P.S. Thank you for not taking the couch out of the lab until after I was done with lab work. Sleeping on the lab floor all night wouldn't have been near as comfortable).

To my remaining committee member, Dr. Leon Schumacher, thank you for your wisdom and help with any and all things engineering related to this work. I can undoubtedly say that without your expertise, knowledge, and patience this work would not have went as smoothly as it did.

There are multiple people remaining who I owe a great deal of gratitude to, but none come close to Zach Callahan. You have helped me keep my head above water countless times. I don't feel like the words thank you are enough to express how grateful I am for all of the work and sacrifice you put in to these projects. I will cherish the long hours in the lab accompanied by multiple MLB games playing on all devices, life chats,

advice, long drives to Kentucky, and HACCP training memories forever. You truly are the glue that holds us all together, and I hope you never forget that.

Rick Disselhorst and all of the gang at the Meat Lab—thank you for being so helpful and accommodating for all of these projects. You all are wonderful!

I want to extend a special thank you to Drs. Bulent Koc and Surendranath Suman. Your assistance with portions of this work are much appreciated and only made my work stronger.

To my fellow and former graduate student colleagues along with the numerous undergraduates that assisted me with lab work, THANK YOU. There are numerous nights I got to sleep in my bed instead of on the lab couch because of you and I am so appreciative of that. Hats off to all of you: Maggie Shane, Libby Martin, Courtney Spencer, Lauren Moss, Audrey Anderson, Katy Shircliff, and Ty Peckman.

To you, Danielle, thank you for your help, support, guidance, and encouragement, for being a shoulder to cry on and an ear to listen whenever I need you. You are the eternal Ray to my Willie. This program has blessed me in so many ways, and your friendship is one of them.

Jacquelyn, thank you for being an incredible roommate and an even better friend. I don't know what bug bit me when I decided to reply to that email about an incoming graduate student needing a place to live, but I am so glad it did. Your friendship is such a blessing to me and I can't wait to watch you succeed in the next step of your life.

To my parents and the rest of my family, thank you for your continued support and encouragement from 9 hours away. I am blessed to know I have people I can call when

things are good, when things are bad, and everything in between. You all are my rock, and I couldn't have done this without you. I hope I make you proud each and every day.

Jordan, you haven't been here through it all- but you have been here through the part I needed you the most. Your guidance, encouragement, patience, and support is something that will always be precious to me. Thank you for believing in me even when I didn't believe in myself.

Last but certainly not least, I want to thank God and the Dr. Pepper Snapple Group. Without my faith and an unhealthy amount of Diet Dr. Pepper each day, there is absolutely no way any of this would have been possible.

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ABSTRACT

Discolored fresh meat products in a retail setting are often perceived negatively by consumers when making purchasing decisions. Prolonging fresh meat color is critical for the retail industry in regard to sales and consumer perception. Variation in retail display settings can impact meat color shelf life from both a discoloration and oxidation standpoint. Lighting technologies are known to impact meat discoloration due to light intensities and temperature variations. A growing demand for the use of energy efficient lighting sources such as light emitting diode (LED) bulbs is being adopted in the United States. Therefore, it is imperative to know the impact these new lighting technologies have on fresh meat quality. Three trials were conducted to determine the impact of LED lighting technologies in comparison to commonly used fluorescent (both low [FLO] and high – UV [HFLO]) bulbs, and no light source (DRK). In a study conducted on ground beef from the *Semimembranosus* (SM) (n = 20) patties at two different fat levels (5 and 25%) had superior a* values, oxymyoglobin concentrations (MbO_2), and lipid oxidation levels (TBARS) than those treated with LED or FLO light sources over 7 days of retail display. Patties displayed under LED bulbs had higher a* values and MbO_2 concentrations than patties displayed under FLO light sources. Data indicated that the use of LED bulbs on ground beef promoted greater red color retention and less oxidation than FLO bulbs in a retail display setting. To evaluate retail display settings and fresh beef cuts further, two whole muscle cuts one color labile cut (*Triceps brachii* [TB]) (n = 20) and steaks from the SM (n = 20), known to be moderately color stable were evaluated

under HFLO, FLO, and LED light sources over 7 days of retail display. For both whole muscle cuts, the use of HFLO light sources promoted greater redness retention as indicated by MbO_2 and a* values, less lipid oxidation as indicated by TBARS values, and less undesirable metmyoglobin (MMb) formation in comparison to steaks displayed under FLO or LED light treatments. The use of LED bulbs for prolonged ground beef retail display was superior to other light sources. However, ground beef is rarely displayed in retail settings for an extended period of time. The use of LED bulbs showed no advantages when displaying whole muscle cuts. Therefore, data from this study suggests while from an energy efficiency standpoint LED bulbs can be beneficial to retail settings. However, from a fresh meat quality standpoint, there is no advantage from color or oxidative standpoint for using LED lights in a retail display setting for fresh beef products.

Chapter 1

Introduction

Consumer purchasing intent is heavily influenced by meat color which is indicative of freshness and quality in fresh meat products (O'Grady et al., 2000; Carpenter et al., 2001; Mancini and Hunt, 2005; Rogers et al., 2014). Prolonging the ability for fresh beef products to retain the bright cherry red color of oxymyoglobin (MbO_2) is a driving factor for meat quality research. There are numerous factors that contribute to the oxidation of MbO_2 to an undesirable brown metmyoglobin (MMb) which include temperature, pH, metmyoglobin reducing activity (MRA), lipid oxidation, myoglobin concentration within each muscle, product manipulation such as grinding or slicing, partial oxygen pressure, retail display time, packaging materials, and lighting conditions (Renerre, 2000.; Bekhit and Faustman, 2005; Faustman et al., 2010).

The formation of MMb in fresh meat products often leads to a decrease in purchasing intent by consumers leading to discounted prices for discolored products. Smith et al. (2000) reported that 15% of total retail sales of fresh meat were discounted due to discoloration; these discounts resulted in the loss of $1 billion annually. Prolonging retail display life can help reduce the amount of food waste in the United States, along with reducing revenue loss for retail stores.

With consumer purchasing decisions being so heavily dependent on appearance, finding and implementing strategies to improve color stability and product appearance are critical for retail settings (Montgomery, 2003). Any changes that occur in a retail display environment can impact product quality and appearance, therefore, demands for

change in retail environments result in demand for product quality research. In recent years, a push for the transition from commonly used fluorescent bulbs to newer, more efficient technologies such as light emitting diode (LED) bulbs has occurred both in the United States and globally (Schleich et al., 2014; US DOE, 2016; Steele et al., 2016). Evaluating the impact this transition has on both product quality and appearance is necessary to determine the impact this transition could have on the meat industry.

Bercík et al. (2016) reported that light influences behavior for specific product purchases in retail settings such as meat, bakery items, and produce. Brighter lights within retail stores lead to more positive impressions on consumers and has been reported to prolonged time spent evaluating products. Bercík et al. (2016) also found that LED light sources had the highest amount of positive consumer emotions related to retail food settings. As the transition from fluorescent (FLO) to LED lights occurs in retail settings, it is important to not only know how LED lights will impact the objective measurements of visual perception of fresh products within a retail case, but understanding the impact, if any, that these light sources will have on meat quality in relation to retail display life in order to maximize retail display life and product value.

Preliminary data (Cooper et al., 2015) found that ground beef patties displayed under high ultraviolet (UV) FLO lights had a greater amount of discoloration than patties displayed in chambers under LED lights or no light, as indicated by a* values. Patty surface temperature indicated that patties under FLO display were continually higher than LED or no light displayed patties. It was unclear if the increased discoloration was due to light source or temperature within the chamber. Therefore, the objective of these studies were to evaluate the impact of different light sources on oxidation and color change of

ground beef at two different fat percentages and steaks produced from both low and high color stability muscles in a controlled temperature environment.

Chapter 2

Literature Review

2.1 Myoglobin

Function of Myoglobin. Myoglobin is a heme protein, that along with hemoglobin, contributes to meat color. It is reported that myoglobin is responsible for 80-90% of total meat color pigment (Aberle et al., 2012). Myoglobin is most commonly known for being the protein responsible for binding and transporting of oxygen to mitochondria in living muscle cells (Ramanathan et al., 2013). Myoglobin structure consists of a globular protein portion and heme ring, which is the nonprotein portion and contributes heavily to meat color. Within the heme ring of myoglobin molecules lies an iron atom which can exist in one of two states, the reduced, ferrous (Fe^{2+}) form or the oxidized, ferric (Fe^{3+}) form. This iron atom interacts with six ligands; four nitrogen atoms and a histidine side chain occupy five of the six ligands (Ordway and Garry, 2004). The type of molecule attached at the binding site at the sixth ligand and the redox state of the central iron atom determines the state of myoglobin and ultimately meat color (Bekhit and Faustman, 2005).

Chemical State of Myoglobin. Myoglobin commonly exists in three chemical states in regard to fresh meat products, deoxmyoglobin (DMb), oxymyoglobin (MbO_2), and metmyoglobin (MMb). When the sixth ligand of the iron atom is not occupied, myoglobin is in the DMb state. Deoxymyoglobin is a purple-red, purple-pink color due to lack of O_2 binding at the sixth ligand. As oxygen is introduced to myoglobin, oxygen binding at the ligand occurs and meat color begins to transition from purple to a bright

"cherry" red MbO_2 which occurs during initial oxygen exposure (Mancini and Hunt, 2005; AMSA, 2012). This phenomenon is often referred to as "bloom". As oxidation occurs and the ferrous iron becomes ferric, H_2O occupies the ligand binding site causing the desirable red meat color to transition to the undesirable brown color of MMb (Baron and Andersen, 2002; Bekhit and Faustman, 2005; Faustman et al. 2010). Myoglobin is capable of reversible oxygenation where the O_2 molecule is released and MbO_2 reverts to DMb. Metmyoglobin can be enzymatically reduced through MMb reductase to ferrous Mb (Faustman and Bekhit, 2005).

2.2 Meat Color

Objective measurements. Instrumental color analysis can be conducted with numerous colorimeters and spectrophotometers. Colorimeters can be used to measure color at different wavelengths and indirectly determine heme pigment concentration (Renerre, 2000). Instrumental color measurements are beneficial from repeatability, user friendly and cost effective standpoints in meat research (Holman et al., 2016). It is recommended to utilize the CIE (1976) L*, a*, b* color scale to measure lightness, redness and yellowness, respectively (AMSA, 2012). L* values indicate variation in color ranging from black to white. The a* values indicate a color range from red to green, with higher a* values indicating a redder product. Decreases in a* values indicate discoloration and often oxidation of MbO_2 to MMb. Variation in b* values indicate a color range from blue to yellow. Changes in L*, a*, and b* values indicate changes in color of fresh meat products. Decreasing a* values indicate that product is losing redness, and ultimately discoloring. Utilizing L*, a*, and b* values, hue angle (Tan^{-1} b*/a*) and saturation index ($(a^{*2}+b^{*2})^{1/2}$) can be calculated (AMSA, 2012). Increases in hue angle

values indicates loss of redness in meat products; conversely, a decrease in saturation index values indicates a decrease in color intensity of the product (Renerre, 2000; Steele et al., 2016).

Subjective measurements. Consumers associate fresh meat product quality with visual appearance. Any deviations from a bright cherry red color in beef products is often discriminated against in a retail setting (Djenane et al., 2001; Mancini and Hunt, 2005; Suman et al., 2014; Holman et al., 2016). Therefore, the use of visual color assessment by both trained and untrained sensory panels allow for the most accurate description of consumer color perception, acceptability, purchasing intent, and product desirability. When evaluating acceptance, room environmental factors play a key role in product assessment, mimicking retail settings (lighting, packaging, product temperature) as much as possible allows for the most accurate results from consumers (Renerre, 2000). Holman et al. (2016) utilized pictures of fresh beef muscle to determine relationships between instrumental color data and consumer color acceptability of beef steaks. Preliminary data showed variation in acceptability was found with L* and b* values (Holman et al., 2016). Due to a* values indicating redness, and no differences being found, further research with larger sample sizes needs to be conducted to establish a relationship between instrumental color values and consumer acceptability of product appearance.

2.3 Factors Affecting Meat Color Stability

Pre-Harvest:

Physiological age, breed, and sex class. Numerous variables play a role in variation in myoglobin concentration within the muscle. Boccard et al. (1979) reported that an increase in physiological age of an animal correlated with darker colored lean.

This agrees with Aberele et al. (2012) who reported that an increase in animal age leads to an increase in muscle myoglobin content. In the United States, many older beef animals, mainly cows, are slaughtered long after the average slaughter age. Cuts of beef from these animals are normally combined with cuts from other beef animals, and are used to produce ground beef due to both color and tenderness issues with increased physiological age (Suman et al., 2014). Raines et al. (2009) found that ground beef from dairy type cattle appeared darker and had higher pH values than that from beef type cattle with values of 5.8 and 5.5, respectively.

Girard et al. (2012) found that age at slaughter had a significant impact on lightness, mean hue angle and chroma values in steaks from the *semitendinosus* when comparing calf and yearling fed steer carcasses. Girard et al. (2012) also found that *semitendinosus* steaks from yearling fed steers had higher MMb relative content values 7 d after slaughter than carcasses of calf fed steers with values of 0.12 and 0.14, respectively. These values indicate a greater amount of MMb in yearling fed steer carcasses.

Xiong et al. (2007) found that lipid oxidation rates subsequently increased with age in ground beef patties made from mature cows with ages of 10-12 > 6-8 > 2-4 years. Lipid oxidation and the formation of MMb are closely related to one another, therefore, this increase in lipid oxidation rate with age could potentially impact the rate of MMb formation in fresh beef products depending on physiological age of animals at harvest.

Diet. Variations in muscle and fat color can occur with variation in diets. Fat from grass finished animals often appears yellow as a result of β-carotene found in the forage (Aberle et al., 2012). Bruce et al. (2004) found that muscle from pasture fed

steers was darker than steers finished on grain due to the dietary impact of increased subcutaneous fat levels, slower postmortem chill time, and lower muscle pH which ultimately increases protein denaturation in grain finished animals. Altering the antioxidant and pro-oxidant components of animal muscle via the diet can play a substantial role in muscle color by altering susceptibility of muscle to oxidative deterioration (Pouzo et al., 2016). The addition of antioxidants such as vitamin E, a fat-soluble vitamin, into the diet can retard lipid oxidation in fresh meat, prolonging retail display life (Descalzo et al., 2007; Juarez et al., 2011).

Stress. Animals experience increased levels of stress and anxiety throughout the duration of harvest. Transportation, loading, abattior lairage, mixing of unfamiliar animals, and inadequate environmental monitoring and upkeep can lead to both physical and psychological stress in an animal prior to harvest (Pearce et al., 2011; Aberle et al. 2012; Hayes et al., 2015). As a result of stressors, there are often increases in muscle activity leading to increases in muscle contraction. Contraction contributes to an increase in internal muscle temperature; after harvesting, this increased temperature can impact carcass chilling rates which can impact the rate of pH decline during glycolysis leading up to rigor, this is often associated in animals experiencing greater levels of short term stress, such as swine, resulting in pale, soft, and exudative (**PSE**) meat (Falowo et al., 2014). Romero et al. (2013), found that bull carcasses presented higher pH values 24 hours post slaughter than steer carcasses indicating that increased levels of circulating testosterone in the body due to lack of castration could lead to increased excitement and stress levels prior to slaughter. Meat quality impacts are often thought to be related to stress intensity, duration and animal susceptibility (Ferguson and Warner, 2008). Dark

cutting (**DFD**) beef is often related to long term, or chronic stress conditions (McKeith et al., 2016). These conditions are known to deplete muscle glycogen levels, ultimately impacting glycolysis during the transition of muscle to meat (Ferguson and Warner, 2008;). Dark cutting beef often results in cuts with undesirable color characteristics, high ultimate pH, increased water holding capacity (**WHC)**, along with potential off flavors and tenderness variation (McKeith et al., 2016).

Post-Harvest:

pH and carcass temperature decline. Muscle pH declines post-harvest due to accumulation of hydrogen atoms resulting from glycolysis as glycogen is converted to lactic acid. Rate and extent of muscle pH decline is one of the most significant factors in meat quality characteristics (Aberle et al. 2012). A normal pH decline will begin at the living muscle pH of approximately 7.0 and over roughly 24 hours will decline to an endpoint between 5.3 – 5.7 in beef carcasses (Aberle et al. 2012). Kapper et al. (2014) reported that in general, carcasses with higher drip loss, indicating lower WHC capabilities exhibited accelerated postmortem pH declines and lower ultimate pH values. Conversely, carcasses with higher WHC capabilities generally had a decreased rate of pH decline and higher ultimate pH values.

Decline in muscle pH and decline in internal carcass temperature correlate with regard to meat quality. If muscle pH declines at a more rapid rate than carcass cooling has occurred, proteins will begin to lose shape and breakdown will occur, this is often known as denaturation (Liu et al., 2016a). Denaturation of these proteins can attribute to decreased water and protein binding capacity, decreased protein solubility and decreased color intensity in highly pigmented muscle (Aberle et al. 2012). Conversely, if muscle pH

fails to decline at an appropriate rate, meat may appear very dark and dry due to tight binding of water to proteins.

Myoglobin oxidation. Myoglobin oxidation has been reported to impact meat quality issues such as color, WHC, and textural issues (Utrera et al., 2014). Oxidation of myoglobin is impacted by many factors such as processing, storage temperature, and retail display on fresh meat products (Estevez, 2011). Oxidation of the central iron atom within the heme group is responsible for product discoloration as the ferrous iron atom associated with red MbO_2 oxidizes to its ferric form which causes the development of brown MMb with exposure to oxygen (Renerre, 2000). As the ferrous form of the heme iron oxidizes to its ferric form, the oxygen is released from the sixth ligand binding site and an H_2O molecule takes its place resulting in undesirable MMb formation (Aberle et al., 2012).

Lipid oxidation. Animal fats are highly susceptible to lipid oxidation for multiple reasons; a relatively high proportion of polyunsaturated fatty acids (PUFA) in cell membranes (Wood et al., 2003; Jiang and Xiong, 2016), low levels of antioxidants and high levels of proxidants, addition of oxygen during further processing and manipulation. Raines et al. (2009) indicated that higher levels of saturated and monounsaturated fatty acids in beef from cows had lower lipid oxidation levels than beef containing higher PUFA content.

Autoxidation of lipids is a three-stage process starting with initiation, propagation and finally termination. Initiation occurs as molecular oxygen attacks a fatty acid double bond which in turn cleaves the bond to produce free radicals (Morrissey et al., 1998; Kilic et al., 2014). Free radicals produced during the initiation stage produce additional

molecular oxygen atoms which continue the cleaving process of double bonds and the subsequent formation of free radicals, also known as the propagation stage (Aberle et al., 2012).

As the propagation stage continues, an increase in free radical formation increases the momentum of the propagation stage which will continue as long as oxygen and double bonds are available for reaction (Aberle et al., 2012). Propagation is most likely to occur immediately pre-slaughter and in early post slaughter stages of meat production (Morrissey et al., 1998). Once the availability of double bonds and potentially the oxygen concentrations decline, unpaired electrons carried by free radicals begin reacting with one another, thus producing a non-reactive product, or reaching the termination stage of oxidation.

Termination only naturally occurs after a considerable amount of oxidation has occurred, most often occurring during handling, processing and storage of fresh meat products. This type of termination leads to products with numerous quality defects on appearance, odor, and flavor (Morrissey et al., 1998; Beltran et al., 2003;). Inclusion of a free radical scavenger or removal of oxygen exposure can lead to an increased rate of termination of lipid oxidation (Renerre, 2000).

Lipid oxidation is commonly measured by analyzing thiobarbituric acid reactive substances (**TBARS**) (Beltran et al., 2003; Ferioli et al., 2008). Malonaldeyhde (MDA), a byproduct of unsaturated fatty acid decomposition and oxidation interacts with TBA reagent to produce a pink pigment which can be read by a spectrophotometer to determine the degree of lipid oxidation in meat products (Faustman et al., 2010). Diaz et

al. (2014), reported that distillation and aqueous acid extraction are the most widely used TBARS quantification methods today.

Relationship between myoglobin and lipid oxidation. Biochemical reactions that are directly responsible for oxidation of myoglobin and oxidation of lipids each generate products that can further accelerate oxidation for its counterpart (Morrissey et al., 1998; Baron and Andersen, 2002; Faustman et al., 2010; Jiang and Xiong, 2016). Kilic et al. (2014) reported that fresh meat products with higher myoglobin content had higher TBARS values than samples containing lower amounts of myoglobin; suggesting that free iron, as a result of myoglobin oxidation, is a catalyst for lipid oxidation to occur in fresh meat products. The inclusion of antioxidants in both diet and post-harvest has further supported the theory that oxidation rates between myoglobin and lipids are interdependent. McKenna et al. (2005), reported that muscles that are generally more color stable had lower TBARS values than muscles with low color stability. Colle et al. (2016) reported similar findings with steaks from the *biceps femoris* (low color stability) having higher TBARS values over a retail display period than steaks from the *semimembranosus* (moderate – high color stability). Min et al. (2010) reported that MMb at numerous concentrations, induced lipid oxidation resulting in increasing TBARS values in phospholipid liposome model systems. Martin et al. (2013) reported that increases in lipid oxidation correlated with trained panelist reports of product discoloration in ground beef patties. Agreeing with multiple reports of lipid oxidation impacting meat color (Renerre, 2000; Wood et al., 2003; Faustman et al., 2010).

Muscle location. Muscle color stability is highly muscle specific. Muscles within a beef carcass are categorized as color-stable or color-labile in regard to color

stability during retail display as each muscle demonstrates different postmortem biochemistry (Renerre and Labas, 1987; McKenna et al., 2005; Faustman et al., 2010; Joesph et al., 2012). Muscle location is also impacted by animal movement and exercise rates. Increased exercise in an animal increases oxygen levels in the muscle, which can accelerate lipid oxidation and meat discoloration rates for that animal's carcass (Dunne et al. 2005). Joesph et al. (2012) investigated the role of sarcoplasmic proteomes on variation in beef color stability and determined that color stable beef muscles had a greater abundance of antioxidant proteins than muscles with less color stability; these proteins were positively correlated to surface redness, color stability and MMb reducing activity.

Ground product is often made from a combination of muscles that are normally less tender or carcass trim; often meat from older animals such as cows and bulls is utilized. Production of large scale batches of ground beef require muscles and added adipose tissues of multiple animals to be combined and can impact color stability. Blending of color-stable muscle with color-labile muscles in ground beef blends can prolong retail display life of ground beef. Suman et al. (2014) reported that enhanced color stability occurs in products limited to 25% or less of color-labile muscle within the ground beef batch.

Muscle fiber type. Differences in myoglobin concentrations between muscles are due to the ratio of red to white muscle fibers within that muscle. Muscles with 30-40% of red muscle fibers appear darker than those with lesser values (Aberle et al., 2012). Generally, McKenna et al. (2005) found that steaks manufactured from muscles that were low in myoglobin content had higher L* values, which could be an

indicator of muscle fiber variation within muscles of an animal. Wegner et al. (2000) found that variation of different fiber types in the *Semitendinosus* of Angus, Belgian Blue, and Holstein bulls impacted meat color. White muscle fibers contain less myoglobin than red muscle fibers, therefore they are a much lighter color (Wegner et al., 2000). Muscles that contain larger proportions of red muscle fibers and therefore a greater amount of phospholipids appear to discolor at a faster rate than muscles containing greater amounts of white muscle fibers (Faustman et al., 2010; Kilic et al., 2014). This could be attributed to increased lipid and myoglobin oxidation occurring in red muscle fibers.

Metmyoglobin reducing activity. Metmyoglobin reducing activity (MRA) is one of the primary endogenous processes within the muscle by reducing ferric MMb to ferrous redox myoglobin forms (Nair et al. 2016). Subsequent oxygenation of reduced myoglobin will prolong the bright cherry red MBO_2 which consumers prefer (McKenna et al., 2005). Dean and Ball (1960), were the first to report MMb reduction in meat. Over time, there have been multiple conflicting reports about the significance of the MMb reducing system on color stability (Renerre and Labas, 1987; Echevarne et al., 1990; Bekhit and Faustman, 2005). McKenna et al., (2005) reported that beef muscles with the highest color stability had the highest MRA values. This supports the findings of Suman et al. (2014) that low color stability muscles reportedly demonstrate lower rates of MMb reduction. However, conflicting findings by Lanari and Cassens (1991) reported that the least color stable muscles had the highest reducing activity. Raines et al., (2010) found that ground beef patties containing 50% or more color-stable muscle had greater MRA abilities, and brighter red color throughout retail display. Bekhit and Faustman (2005)

reported that the extent that MRA contributes to fresh meat color stability is still unknown. Indicating there is still work to be done regarding the role of MRA in meat color stability.

Aging. Storage of carcasses in refrigerated temperatures after harvest for extended periods of time is known as aging. Aging of carcasses is one of the most common tenderization methods used today. There are numerous variations in aging methods for beef products. Prolonged aging, while beneficial for tenderness and palatability of beef, is detrimental to beef color stability during retail display (Suman et al., 2014). Mancini and Ramanathan (2014) found that a* values decreased as aging time increased from days 4-7 of retail display. English et al. (2016) reported that beef steaks packaged with oxygen permeable overwrap and wet aged 21 days had a 16.1-unit decrease in a* values through six days of retail display. Colle et al. (2016) reported that extended aging periods (above 21 days) for bottom and top rounds had a negative impact on retail display life due to increased browning and extent of surface discoloration on beef steaks.

Steaks from the *biceps femoris* and *semimembranosus* had increased lipid oxidation levels with increased aging periods (Colle et al. 2016). This agrees with the findings of Mancini and Ramanathan (2014) who found that TBARS values of beef steaks increased as aging time increased. With the known relationship between lipid oxidation and MMb formation it is apparent that these factors, brought on by the longer aging periods, can contribute to discoloration of steaks over a retail display period.

Garner et al. (2014) found that ground beef patties from muscles aged 21 and 42 d had higher TBAR values than patties produced from muscles aged for 7 d, incidentally

patties aged for 42 d had lower a* values than patties aged 7 and 21 d, indicating that aging time can impact lipid oxidation and discoloration rates on ground product.

Fat content. Differences in fat content often contribute to differences in L* values as an increase in fat content contributes to greater amounts of lightness to be detected. Martin et al. (2013) reported that ground beef patties containing lean:fat ratios of 73:27, 81:19 and 91:9 had similar a* values through the first 14 days of retail display. At 21 days 81:19 and 91:9 ground beef had higher a* values than the higher fat content 73:27 ground beef. Garner et al. (2009) found that ground beef patties from the chuck roll and premium choice subprimals had higher L* values than ground beef patties from select grade subprimals and the knuckle. Further indicating that a higher fat content contributes to higher L* values and a lighter color. Raines et al (2009) reported that ground beef fattened with young beef trim had higher L* values, indicating a lighter color, than ground beef patties with beef cow trim.

Product manipulation and variation. Grinding of meat products creates conditions for increased lipid oxidation levels compared with whole muscle cuts due to the grinding process increasing oxygen exposure, reducing particle size, redistributing and mixing reactive components and potentially causing an increase in muscle temperature during grinding (Aberle et al., 2012). All of these factors are known to increase the rate of oxidation of MbO_2 to MMb, thus decreasing the retail display life of that product.

Retail Storage Environment.

Retail display time. Oxygen exposure over retail display leads to the formation of MMb. Ground beef is commonly packaged with an oxygen-permeable

packaging material. Demos and Mandigo (1996) found that ground beef packaged in an oxygen-permeable system and kept in refrigerated temperatures over retail display contained approximately 65% MMb. Xiong et al. (2007) and van den Oord and Wesdorp (1971) produced similar results with MMb values of 70 and 50%, respectively.

Along with MMb formation, decreases in a* values occur over retail storage time (Garner et al. 2014; Lavieri and Williams, 2014; Cooper et al., 2015; Nair et al., 2016; English et al., 2016; Colle et al., 2016) Decreases in a* values indicate a loss of redness and ultimately, discoloration. Jeremiah and Gibson (2001), found that redness decreased over time during storage but was impacted greatly by storage temperature.

Storage temperature. Storage temperature control is critical for retail display life of fresh meat products. High temperatures favor scavenging of and other oxygen consuming processes such as lipid oxidation and ultimately low oxygen tension resulting in the autoxidation of myoglobin (Renerre, 2000). Low display temperatures suppress enzyme activity, oxidation, and discoloration (Aberle et al. 2012). Supporting findings Jeremiah and Gibson (2001) which state that MMb formation and product discoloration increased as storage temperature increased. Jeremiah and Gibson (2001) reported that steaks stored at lower temperatures were generally redder and contained more MbO_2 than steaks stored in higher temperatures. Martin et al. (2013) found that ground beef stored at -1.7°C had higher a* values than ground beef stored at 2.3°C. It was also reported that ground beef stored at 2.3°C had an increased rate of discoloration and MMb formation reported by trained sensory panelists. Consumers reported less desirable color scores for ground beef patties during temperature abuse in both modified atmosphere and polyvinyl chloride overwrapped packages (Rogers et al., 2014). Mancini

and Ramanathan (2014) found that aging product at 5°C decreased color stability compared to products aged at lower temperatures with steaks aged at 5°C having 3.4% more MMb and a 1.3 unit decrease in a* value than steaks aged at 0°C before retail display.

Packaging. Numerous options exist for packaging fresh meat products. Three major packaging options are used in fresh meat products: polyvinyl chloride overwrap (PVC), modified atmosphere packaging (MAP), and vacuum packaging (VP). Packaging type often reflects storage plans for fresh meat products. As oxygen exposure is a key contributor to discoloration each packaging system offers different exposure and environmental factors to meat products.

Polyvinyl chloride overwrap (PVC). Fresh meat packaged in PVC is often used for retail storage as it is extremely permeable to oxygen and has a relatively short retail display life (Lavieri and Williams, 2014; Rogers et al. 2014). High oxygen permeability helps maintain the bright cherry red color associated with MbO_2 that consumers associate with fresh meat. When low levels of oxygen are present, MMb formation and the associated undesirable brown color will occur (Mancini and Hunt, 2005; Aberele et al. 2012).

Modified atmosphere packaging (MAP). The addition of 20% CO_2 has been shown to be sufficient in preventing bacterial growth on fresh meat in high oxygen MAP (McMillin, 2008). Use of high oxygen MAP promotes anaerobic spoilage, lipid oxidation and discoloration in meat products due to high (80%) O_2 levels in the packaging environment (Renerre, 2000; Mancini and Hunt, 2005; Lund et al., 2007; Kim et al., 2010; Lyte et al., 2016). In recent years, COMAP packaging (69.6% N, 0.4% CO,

and 30% CO_2) has been found to be one of the most effective methods for extending ground beef retail display life (Renerre, 2000; Carpenter et al., 2001; Limbo et al., 2010; Lyte et al., 2016). However, consumer acceptance of carbon monoxide exposure has limited the acceptability of the use of COMAP for fresh meat products (McMillin, 2008).

Vacuum packaging (VP). Vacuum packaging provides an anaerobic environment for storage of meat products. The use of VP extends both shelf due to oxidation prevention and microbiological shelf life of the meat (Strydom and Hope-Jones, 2014). Removal of oxygen results in a dark purplish color on the surface of the product due to the formation of DMb. Unfortunately, consumers often find the appearance of this dark color unappealing (Renerre, 2000; Carpenter et al., 2001).

Surface bacteria. Surface bacteria on whole muscle products can contribute to product discoloration as microbes compete for available oxygen, thus decreasing MbO_2 retail display life (Aberle et al. 2012). Lavirei and Williams (2014) found that surface bacteria levels increase with retail display time. These findings agree with Rogers et al. (2014) who reported that microbial levels increased during retail display day and with temperature abuse.

Lighting. Display lighting type and intensity impacts meat appearance during storage along with retail display life (Steele et al. 2016). Variations in light wavelengths, intensities, and temperature impact meat discoloration and fading of processed products (Kropf, 1980; Aberle et al., 2012;). Meat display cases are predominantly illuminated with fluorescent (FLO) light bulbs in today's retail settings (Aberle et al., 2012; Steele et al., 2016; DOE, 2016). However, transitions to more energy

efficient illuminants in a retail setting are beginning to occur (Steele et al., 2016; DOE, 2016).

Lighting impact on product appearance. It is known that supermarket lighting can impact customer purchasing decisions regarding meat products (Kropf, 1980). This statement is supported by Steele et al. (2016) who found that both beef steaks and ground beef products had higher color stability, as identified by a trained consumer panel, under LED lights over retail display in comparison to FLO lights. Paul et al. (2014) found that consumers preferred the appearance of PVC packaged ground beef under warm white LED bulbs over cool white LED and soft white FLO bulbs. Barbut (2001) found that cool white fluorescent bulbs resulted in a more desirable product appearance than warm white fluorescent bulbs. As the use of LED lighting in retail settings increases, more research needs to be done to assess consumer acceptance of product appearance in retail display.

Lighting impact on meat quality. Ultraviolet (UV) radiation from FLO bulbs has a negative impact on meat color stability (Djenane et al., 2001; Martinez et al., 2007; Steele et al., 2016). Light emitting diode lights are a newer lighting technology known to use 25-80% less energy than incandescent bulbs (US DOE, 2016). Steele et al. (2016), found that LED bulbs produced less heat than FLO bulbs in retail meat cases. Cooper et al. (2016) reported similar findings with deli cases equipped with LED lights had lower average temperatures than cases equipped with FLO lights. Steele et al. (2016) reported lower product temperature for meat displayed under LED bulbs. These findings agree with those in Cooper et al. (2015) who found that ground beef patties under LED retail display had lower surface temperatures than patties under FLO

lights. The use of LED lights during retail display has been reported to extend retail display life of pork loin chops, beef *semimembranosus* steaks, beef *longissimus lumborum* steaks (Steele et al., 2016) and ground beef (Cooper et al., 2015; Cooper et al., 2016; Steele et al., 2016). Djenane et al. (2001) found that TBARS values and microbial counts were lower for ground beef displayed in the absence of UV lights in comparison to those displayed under UV lights in a retail setting.

Display Lighting in Retail Settings.

Commercial energy use. Electric lighting is estimated as 20-40% of the total electricity used in commercial buildings in the United States (Liu et al., 2016). In 2016, the Department of Energy reported that roughly 6% of all installed lighting stock in the United States were LED lights. However, by 2035 it is anticipated that 86% of installed lighting stocks in the United States will be LED lights resulting in a 75% decrease in energy usage for lighting (DOE, 2016). These figures indicate that major transitions will occur in both retail and household settings regarding light technologies.

Retail lighting transitions. High up-front costs for retailers and consumers regarding the transition from FLO to LED lighting systems is a major cause for hesitation to switch (Chappin and Afman, 2013). However, Martin et al. (2016) reported that there is a trend in the United States for retailers to replace FLO lights with LED lights in dairy display cases, indicating that a push for transition in the United States is occurring. The European Union has implemented a plan that will ultimately phase out incandescent lighting completely (Chappin and Afman, 2013). This indicates that globally, energy efficiency in retail is becoming a high priority.

Chapter 3

Impact of contemporary light sources on oxidation of fresh ground beef

As printed in: doi:10.2527/jas2016-0728

ABSTRACT: Meat color is considered one of the driving factors in consumer purchasing decisions. The objective of this study was to determine the impact of 2 different lighting sources on color and lipid oxidation of ground beef patties in a controlled environment. USDA Select top rounds (n = 20) were processed to produce ground beef at 2 different fat levels (5 and 25%) and made into patties (113.4 g). Patties were packaged with oxygen permeable polyvinyl chloride, assigned to one of three lighting treatments (low UV fluorescent [FLO], light emitting diode [LED], and no light [DRK, negative control]), and placed within deli cases at 5°C. Patty removal for evaluation occurred on retail display d 1, 3, 5, and 7. Objective color measurements were obtained using a HunterLab MiniScan 45/0 LAV. These values were utilized to determine myoglobin redox forms as a measure of myoglobin oxidation. Additionally, thiobarbituric acid reactive substances (TBARS) were measured to indicate lipid oxidation. Objective color measurement for a* (redness), decreased for all light treatments by retail display day ($P < 0.0001$). Oxymyoglobin values for all light treatments decreased daily but showed no differences between treatments until d 5 ($P < 0.0001$) where DRK > LED > FLO. Conversely, metmyoglobin values increased daily ($P < 0.0001$), but showed no differences between treatments until d 5 where FLO > LED > DRK. TBARS values increased by day for each fat percentage ($P < 0.0001$) with 5% fat patties having higher TBARS values indicating great oxidation occurring in the phospholipids than adipose

tissues. Results indicate that light treatment affected discoloration and metmyoglobin formation in ground beef patties; LED lighting may lead to increased meat quality shelf life in a retail setting.

Key words: color, ground beef, lighting, myoglobin, oxidation

3.1 Introduction

Color is one of the driving factors in consumer purchasing decisions regarding meat products in a retail setting (Djenane et al., 2001). As oxymyoglobin (MbO2) transitions to metmyoglobin (MMb), meat color subsequently changes from a desirable bright cherry red to an undesirable brown color. This color change often results in a decreased willingness of consumers to purchase meat products; leading to discolored meat products being sold at discounted rates. Smith et al. (2000) reported that 15% of total retail sales of meat products were discounted due to product discoloration; resulting in a loss of $1 billion of revenue annually. Oxygen tension, temperature, surface microbial growth, and lighting conditions are the major factors that play a role in the alteration of meat color shelf life (Renerre, 1990). Fluorescent bulbs are the most common light source utilized within meat cases in retail settings today. However, Paul et al. (2014) reported that light emitting diodes (LED) are evolving quickly within retail settings. LED bulbs have the advantage of a longer life span, and an increased energy efficiency (Lee et al., 2011). A pilot study, conducted at the University of Missouri meat lab, evaluated the effect of no light, high ultraviolet (UV) fluorescent light, and LED lights on ground beef patties in a simulated retail setting. Data showed fading of redness over time as indicated by a decrease in a* values (Cooper et al., 2015). The surface temperature of patties within the fluorescent chamber were continually higher than LED displayed patties (Cooper et al., 2015). With temperature having an impact on oxidation in fresh products, controlling that variation would allow for the impact of the light source on discoloration to be studied independently. Therefore, the objective of this study was to

determine the impact of low UV fluorescent and LED light sources on color and lipid oxidation of ground beef patties in a controlled temperature environment.

3.2 Materials and Methods

Deli case preparation. Three deli cases (TDBD-72–4, True Food Service Equipment, O'Fallon, MO) were installed at the University of Missouri meat lab. All windows were blacked out to eliminate exposure to outside light sources. One deli case was equipped with factory installed low UV fluorescent bulbs (F25T8 TL741, Philips, Amsterdam, the Netherlands) with an average light intensity of 244 lux, color temperature of 4,100 K, and a CRI of 78, a second case was equipped with LED bulbs (L36/40/15W Market Lite LED, Interlectric Corporation, Warren, PA)with an average light intensity of 732 lux, color temperature of 4,000 K, and CRI of 85, and bulbs were removed from the final case (negative control). Light intensities were measured with a TES 1335 Digital Light Meter (TES Instrument, Shanghai, China). Lights in the deli cases were on continually for the duration of retail display. Deli cases were set to a constant temperature of 3 ± 1°C. Temperature and humidity were monitored within each individual case with data loggers placed in the center of each case (EL-USB-W-LCD, Dataq Instruments, Akron, OH) recording values every 15 min.

Ground beef patty manufacture. USDA Select top rounds (n = 20) with remaining subcutaneous fat were purchased. Rounds were then trimmed and processed to remove all visible external fat from the surface. Lean was ground through a 10-mm grinding plate, blended and divided in half. One-half of the lean was ground through a 4.5 mm grinding

plate (#8 Meat Grinder .35 HP, LEM Products, West Chester, OH) before being formed into 113.4 g patties of a 5% fat premium ground beef. The remaining one-half was supplemented with a portion of the trimmed subcutaneous fat, blended and ground through a 4.5-mm grinding plate, and formed into 113.4 g patties containing 25% fat. Patties were placed in Styrofoam trays, overwrapped with oxygen permeable polyvinyl chloride (UltraWrap Stretch Product #7021860 PVC#3, Anchor Packaging, St. Louis, MO), and assigned to 1 of 3 light treatments (low-UV fluorescent [**FLO],** LED, no light [**DRK]**).

Fat and moisture percentage determination. This method was done as described by Dow et al. (2011). Using the CEM SMART Trac rapid fat analysis system, 2 sample pads were dried and a 3.75 – 4.5 g sample was spread across the first pad. The second pad was placed on top of the first and the sample was sandwiched between both pads. Moisture percentages of samples were determined on weight basis using the CEM Moisture/Solids Analyzer. Following moisture determination, dried sample pads were then wrapped in TRAC paper and inserted into a CEM TRAC tube. The tube was placed into the CEM Rapid Fat Analyzer and fat percentage was then determined on a dry basis using NMR and converted to wet basis. To increase accuracy of fat and moisture determinations, samples were analyzed in triplicate. Values of these readings were averaged to determine an overall fat percentage for each sample batch.

Objective color determination. Instrumental measurements of color were preformed to assess color change in relation to overall acceptability within treatments. A HunterLab MiniScan 45/0 LAV (Hunter Associates Laboratory, Reston, VA) with a 25-mm aperture, D65 light source and physical standard was utilized to measure color (L*, a*, b*)on each

patty on their assigned display removal day of d 1, 3, 5, or 7. Hue angle (**HA**), saturation index (**SI)**, and a/b ratios were determined according to AMSA (2012). Color measurements were taken on patties immediately after removal from their respective cases. To obtain greater accuracy of patty surface color, samples were evaluated in triplicate and averaged as an indicator of total patty surface color.

Myoglobin concentrations. Myoglobin concentrations were determined using selected wavelengths described by AMSA (2012). Reflectance was measured at the isobestic wavelengths 470, 530, 570, and 700 nm. Wavelength values were obtained in triplicate on retail display d 1, 3, 5, and 7 of the study. Deoxymyoglobin (DMb), MbO2, and MMb values are calculated using the equations provided in AMSA (2012). Oxymyoglobin values were determined after both MMb and DMb values were calculated.

Lipid oxidation. Lipid oxidation was measured using the method described by Tarladgis et al. (1960) with modifications from Fernando et al. (2013). Duplicate 5-g samples of each patty were obtained from both the surface and interior portion of each patty and blended for 2 min with 25 mL of distilled water with a hand blender. Following homogenization, the cup was rinsed with an additional 25 mL of distilled water and poured into a Kjeldahl flask. 2.5 mL of HCl was added to the flask to balance the pH between 1.5 and 1.6 along with 2 drops of antifoam solution. 25 mL of each sample was distilled through a water-cooled distillation apparatus. Following distillation, 5 mL of each sample was pipetted into a glass tube followed by 5 mL of thiobarbituric acid (TBA) reagent. Samples were then placed in a boiling water bath for 35 min; once pulled

samples were immediately placed into an ice bath for 10 min. Color absorbance was measured at 538 nm using a Spectronic 20 (Bausch & Lomb, Rochester, NY) spectrophotometer. Values of each reading were recorded and averaged for further use. Concentrations were calculated using the recorded averages, and the standard curve equation, thiobarbituric acid reactive substances (TBARS) values are expressed in mg of malonaldehyde/kg of product.

Statistical analysis. All experiments were replicated 20 times for each fat percentage. Data was analyzed as a randomized complete block design, the model included the fixed effects of light (DRK, LED, FLO), fat percentage (5 and 25%), length of retail display time (1, 3, 5, or 7 d), and all possible interactions. Statistical analysis for objective color, myoglobin concentrations, and TBARS values were analyzed using the GLIMMIX function of SAS (SAS Inst. Inc., Cary, NC) to obtain LS means and SE estimates. Significance was determined at $P < 0.05$.

3.3 Results and Discussion

Deli case environments and patty characteristics. Light intensity measurements taken for each light treatment resulted in means of 0, 244, and 732 lux for DRK, FLO, and LED, respectively. Mean deli case temperatures for DRK, FLO, and LED lighting treatments were 3.41, 3.65, and 3.37°C, respectively. Data indicates that although LED bulbs had a higher light intensity than FLO bulbs, they produced less heat within the case which agrees with the previous studies results (Cooper et al., 2015). Differences in temperature due to lighting treatment in the current study were smaller than the

differences in our previous study (Cooper et al., 2015), which was expected as the deli cases were a controlled environment. Steele et al. (2016) also reported cases equipped with LED lights produced lower overall mean temperatures than those equipped with FLO lights. Decrease in storage temperature can reduce the rate of product discoloration and oxidation, in turn prolonging retail shelf life. Mean fat contents were 5.7 and 18.74% for the low and high fat patties. These values did not match the desired fat percentages of 5 and 25%, but are accurate representations for examining a low and high fat content ground beef patties. Values for mean moisture content for low and high percent fat patties were 61.64 and 71.09%, respectively (data not presented in tabular form). These values are expected as protein contains 3 to 4 times more water than fat. Therefore, patties with a higher protein content would in turn have a higher moisture content than patties with low fat percentages.

Objective color determination. L* values for all light treatments showed no differences ($P > 0.05$) over the duration of retail display as seen in Table 3.1. These findings agree with those reported in Steele et al. (2016) which reported no differences in mean L* values for ground beef displayed in FLO or LED lights. Values for a* decreased over time for all treatments where d $1 > 3 > 5 > 7$, indicating a decrease in the amount of redness over display time in agreement with previous findings (Jeremiah and Gibson, 2001). As seen in Table 3.1, each day of the study, patties with no light exposure had higher a* values than both lighting treatments, indicating that

Table 3.1: Means of light source and retail display day on objective color values and myoglobin concentration percentages in ground beef

Item	Retail Display Day 1			3			5			7				
	Lighting Treatment													
	DRK	FLO	LED	DRK	FLO	LED	DRK	FLO	LED	DRK	FLO	LED	SEM	P-Value[1]
L*	46.54	46.59	46.43	47.05	45.82	46.59	46.11	45.49	45.75	46.35	45.65	46.13	0.52	0.4468
a*	24.57^{a}	23.67^{b}	23.39^{b}	20.35^{c}	18.98^{d}	19.00^{d}	17.50^{e}	14.01^{g}	15.49^{f}	13.95^{g}	10.47^{h}	10.75^{h}	0.41	<0.0001
b*	22.37^{a}	21.97^{ab}	21.94^{b}	20.32^{c}	19.74^{d}	19.63^{d}	18.95^{c}	17.47^{g}	17.89^{f}	17.69^{fg}	16.41^{h}	16.26^{h}	0.25	0.0003
DMb	4.60^{a}	4.43^{a}	4.71^{a}	4.40^{a}	3.73^{b}	4.35^{a}	3.52^{bc}	2.37^{d}	3.14^{c}	2.42^{d}	2.15^{d}	2.11^{d}	0.19	0.0282
MbO_2	58.08^{a}	58.14^{a}	57.84^{a}	57.60^{ab}	57.84^{a}	57.34^{ab}	57.52^{ab}	55.94^{dc}	56.75^{bc}	55.41^{d}	52.24^{e}	51.57^{e}	0.43	<0.0001
MMb	37.32^{f}	37.43^{ef}	37.45^{ef}	38.00^{de}	38.43^{d}	38.32^{de}	38.96^{d}	41.70^{b}	40.10^{c}	42.17^{b}	45.63^{a}	46.33^{a}	0.40	<0.0001
TBARS	0.53	0.56	0.48	1.28	1.39	1.30	1.88	1.95	1.91	2.24	2.42	2.28	0.22	0.9960
SI	33.25^{a}	32.32^{b}	32.09^{b}	28.78^{c}	27.41^{d}	27.35^{d}	25.82^{e}	22.49^{g}	23.70^{f}	22.63^{g}	19.55^{h}	19.59^{h}	0.40	<0.0001
HA	42.36^{f}	42.89^{f}	43.19^{f}	45.00^{e}	46.14^{e}	45.95^{e}	47.35^{d}	51.78^{b}	49.27^{c}	52.34^{b}	57.83^{a}	56.99^{a}	0.63	<0.0001

$^{abcdefgh-}$ Means within a row lacking a common superscript differ ($P < 0.05$)

$^{1-}$P-Value light treatment x retail display day interaction

light exposure contributes to discoloration. It is important to note that on d 5, LED and FLO displayed patties differed from one another ($P < 0.05$) with mean a* values of 15.48 and 14.01, respectively. Values indicate that LED displayed patties retained more redness on d 5 of retail display life than FLO displayed patties. Steele et al. (2016) reported a decrease in visual color score for ground beef displayed under LED lights in comparison to ground beef displayed under FLO lights indicating more red color. Values for b* mimicked a* values in the decrease over retail display time with d 1 > 3 > 5 > 7 as seen in Table 3.1. These decreases in b* values over retail display time support data found in Raines et al. (2009) and Rogers et al. (2014) indicating an increase in discoloration over retail display time. However, Jeremiah and Gibson (2001) found that decreases in b* values had no relation to display time on whole muscle steaks in vacuum or CO2 packaging, possibly indicating that grinding and packaging types impact changes in b* values over retail display time.

Differences ($P < 0.05$) in L* values were found between low and high fat patties over retail display time. As seen in Table 3.2, patties composed of 25% fat had higher L* values ($P < 0.05$) than patties containing 5% fat. Additionally, L* values were higher ($P < 0.05$) for patties containing 25% fat on each retail display day. These values were expected as L* is measuring darkness to lightness. Fat within the patties was white, therefore a higher fat content would contribute to an increase in lightness compared to a lower fat content. Differences were found in a* values with fat percentage by day changes ($P < 0.05$). Values for a* decreased by retail display day for both fat percentages with d 1 > 3 > 5 > 7, respectively. Patties with 25% fat had lower ($P < 0.05$) a* values than patties containing 5% fat, with the exception of d 5 which had no difference ($P >$

0.05). These results were expected due to patties with lower fat content containing more lean, therefore a greater amount of redness to be detected. Fat percentage also played a role in b* values as patties containing 25% fat had higher ($P < 0.05$) b* values than 5% fat patties. These values are to be expected as an increase in fat content would cause an increase in detectable yellowness within the patties.

Decreases in a* and b* values over time indicate the loss of redness that occurs during the oxidation of bright red MbO2 and its transition to brown MMb. Data indicates that oxidation and discoloration of ground beef are impacted by light treatment and fat percent over retail display time. Steele et al. (2016) reported no differences in L*, a*, b* values between FLO and LED lights, but their reported case temperatures were much lower than those in this study which may have affected the objective color values reported.

Higher a/b ratios indicate a greater amount of redness and ultimately less discoloration (AMSA, 2012). Calculated a/b ratios show that DRK displayed patties have a higher a/b ratio than FLO patties ($P < 0.05$) on d 3 of the study, with LED showing no differences between either treatment (Table 3.1). By d 5 of the study, differences ($P < 0.05$) in a/b ratios occurred between all light treatments with DRK > LED > FLO. Indicating that by d 5 of retail display, LED displayed patties retained more redness than FLO displayed patties.

Saturation index (SI) values decreased over time for patties within all light treatments. Patties displayed with no light had higher SI values ($P < 0.05$) for all retail display days. Patties displayed with FLO and LED lights showed no differences ($P > 0.05$) in SI values until d 5 where LED displayed patties had a higher ($P < 0.05$) SI value

than patties displayed with FLO lights. Higher saturation indices along with higher a* values for LED displayed patties on d 5 further indicate a greater amount of retained redness within LED displayed patties over a longer retail display period. Five percent fat patties had a higher ($P < 0.05$) SI value than their 25% counterparts on retail display d 1. However, for the remaining retail display days there were no significant differences in SI values for both fat percentages as seen in Table 3.2.

Hue angle (HA) values for patties within all light treatments increased with an increase in retail display time. As seen in Table 3.1, there were no differences ($P > 0.05$) in hue angle values for all light treatments for retail display d 1 and 3. On d 5 differences were seen between light treatments ($P < 0.05$) where FLO > LED > DRK, respectively. Larger HA values indicate greater discoloration and loss of redness within the patties. Further indicating that light treatment plays a role in discoloration of ground patties within retail display. Values in Table 3.2 show that HA values increased for both 5 and 25% fat patties over time as would be expected, however there were no differences found for fat percentage HA values over retail display time ($P > 0.05$). Steele et al. (2016) found no differences in SI or HA values due to lighting source which support the findings in this study.

Myoglobin concentrations. Deoxymyoglobin percentage values decreased over time with retail display d 1 > 3 > 5 > 7. These values were to be expected due to increased oxidation because of oxygen exposure over time. On retail display d 3 and 5, average DMb values were higher ($P < 0.05$) for LED displayed patties than FLO displayed patties. Oxymyoglobin values decreased over time for patties within all light treatments

as seen in Table 3.1. For retail display d 1 and 3, there were no differences in MbO2 percentages within all light treatments ($P > 0.05$). Oxymyoglobin values for patties with no light treatment were superior to both LED and FLO displayed patties on retail display d 5 and 7. Higher MbO2 values over time indicate the retention of the bright cherry red desirable meat color.

Metmyoglobin concentrations increased over time for patties within each light treatment. As seen in Table 3.1, MMb concentrations were not different ($P > 0.05$) between treatments until retail display d 5. Retail display d 5 and 7 MMb percentage values were lower and superior for patties not exposed to light compared to LED and FLO displayed patties. Metmyoglobin concentrations were lower ($P < 0.05$) for LED displayed patties than MMb concentration values for FLO displayed patties on d 5 with means of 40.10 and 41.70, respectively. Data indicates that patty discoloration changes, as indicated by MMb concentrations, was greater for FLO displayed patties than those displayed with LED lights. McMillin (2008) reported that ground beef patties packaged in oxygen permeable over wrap had an overall display life of 2 to 7 d. Greater retention of redness by patties displayed with LED lights through d 5 indicates that the use of LED lights can contribute to a longer display life for ground product. Patties displayed with no light source had the lowest amount of MMb formation, further indicating that light exposure does have an effect of MMb formation and discoloration of ground beef.

Fat percentage impacted myoglobin concentration percentages over time. Table 3.2 shows that patties with 5% fat had higher MMb concentrations on retail display d 1 through 5 of the study when compared to patties with 25% fat. Oxymyoglobin concentrations decreased over display time for both fat percentages with differences ($P <$

0.05) on retail display d 7, where patties containing 5% fat had greater MbO_2 concentrations than patties containing 25% fat with means of 53.71 and 52.43, respectively. These findings were to be expected as patties with lower fat content have a greater amount of lean available for discoloration.

Table 3.2: Means of fat percentage and retail display day on objective color values, myoglobin concentrations and TBARS values of ground beef

Item	Retail Display Day									
	1		3		5		7			
	Fat Percentage									
	5	25	5	25	5	25	5	25	SEM	P-Value[1]
L*	43.14[d]	49.90[b]	42.44[e]	50.53[a]	42.33[e]	49.24[c]	42.48[e]	49.60[bc]	0.49	0.0161
a*	24.78[a]	22.97[b]	19.95[c]	18.93[d]	15.93[e]	15.40[e]	12.47[f]	10.98[g]	0.38	0.0081
b*	21.78[b]	22.41[a]	19.29[d]	20.50[c]	17.66[f]	18.55[e]	16.53[h]	17.05[g]	0.23	0.0199
DMb	4.61	4.56	4.08	4.22	3.13	2.89	2.15	2.29	0.17	0.4477
MbO_2	57.81[ab]	58.23[a]	57.40[ab]	57.79[ab]	56.41[c]	57.07[bc]	53.71[d]	52.43[e]	0.37	0.0042
MMb	37.59[e]	37.21[e]	38.52[d]	37.99[de]	40.47[c]	40.04[c]	44.14[b]	45.28[a]	0.40	0.0086
TBARS	0.61[f]	0.44[f]	1.50[d]	1.15[e]	2.28[b]	1.54[d]	2.73[a]	1.89[c]	0.21	<0.0001
SI	33.00[a]	32.10[b]	27.77[c]	27.92[c]	23.84[d]	24.16[d]	20.81[e]	20.36[e]	0.38	0.0056
HA	41.32	44.31	44.08	47.31	48.30	50.64	53.70	57.74	0.57	0.1180

[abcdefgh-] Means within a row lacking a common superscript differ ($P < 0.05$)

[1-]P-Value fat percentage x retail display day interactions

Lipid oxidation. Table 3.2 shows that TBARS values increased over time for each fat treatment. Patties with 5% fat content had greater TBARS values ($P < 0.05$) over retail display d 3 through 7, where d $3 < 5 < 7$ than patties with 25% fat content. Polyunsaturated fatty acids found in membrane phospholipids have greater susceptibility to oxidation than saturated fatty acids (Wood et al., 2003, Aberle et al., 2012; Jiang and Xiong, 2016). An increase in red muscle fibers increases the susceptibility of lipid oxidation due to increased iron and phospholipids (Wood et al., 2004; Faustman et al., 2010). Due to a greater amount of red muscle fibers in patties containing 5% fat compared to those of 25% fat, which contain a greater proportion of added adipose tissue to muscle fibers; the 5% fat patties have an increased opportunity for a greater amount of oxidation to occur within cell phospholipid membranes. Previous research has indicated a concurrent increase in the discoloration of meat and lipid oxidation (Greene et al., 1971, Renerre, 2000). Min et al. (2010) reported that metmyoglobin induced lipid oxidation and increased TBARS values linearly in a phospholipid liposome system. Polyunsaturated fatty acids are almost exclusively restricted to the phospholipid fraction of muscle and adipose tissue in ruminants (Wood et al., 2003). This supports our findings of 5% fat patties having higher TBARS values than their 25% counterparts. While all TBARS values increased over retail display period for all light treatments, there were no differences ($P > 0.05$) between treatments for TBARS values. Steele et al. (2016) reported similar findings for TBARS values in ground beef under LED and FLO retail display with no differences being found between light treatments.

3.4 Conclusion

Light treatment had an impact on the formation of MMb in fresh ground beef patties. Patties exposed to no light were superior in all aspects of the study compared to those displayed with both LED and FLO lights. Data indicates that introducing controlled temperature environments and the use of LED lighting changed the rate of discoloration as indicated by decreases in a* values and formation of MMb in ground beef patties over extended retail display. TBARS values were higher for patties containing 5% fat as compared to those containing 25% fat; indicating that lipids in the cell membrane play a larger role in lipid oxidation than added fat.

Chapter 4

Impact of light emitting diode (LED) lights on beef steaks produced from a color labile muscle: Triceps brachii

ABSTRACT: Color of fresh meat is one of the driving quality attributes and purchasing factors for consumers in a retail setting. The objectives of this study were to evaluate the impact of modern light sources on surface color and lipid oxidation of fresh beef steaks from the *Triceps brachii* (TB) over retail display time. Steaks from the TB [low oxidative and color stabilities] (n=20) were packaged on Styrofoam trays and overwrapped with oxygen permeable polyvinyl chloride. Steaks were then assigned to one of three lighting treatments (High UV fluorescent [HFLO], low UV fluorescent [FLO], and light emitting diode [LED]) within temperature controlled deli cases. Steaks were removed on retail display days 1, 3, 5, and 7 for objective color determination, myoglobin concentrations, metmyoglobin reducing activity (MRA), and lipid oxidation levels (TBARS). Objective color (L*, a*, and b*) values were determined utilizing a Hunter MiniScan. Objective color measurements for redness, as indicated by a* values decreased daily ($P < 0.05$) for steaks produced from the TB with values of 22.14, 17.73, 15.72, and 13.49 for days 1, 3, 5, and 7 respectively. Lighting type also impacted a* values for steaks with HFLO displayed steaks having higher ($P < 0.05$) a* values than steaks exposed to both FLO or LED light sources. Oxymyoglobin (MbO_2) concentrations were higher ($P < 0.05$) for steaks from the TB displayed with HFLO lights than those displayed under FLO (days 3 and 7) or LED (days 5 and 7) lights. Steaks displayed with HFLO lights had less ($P <$

0.05) metmyoglobin (MMb) concentrations than those exposed to both FLO or LED lights on retail display days 5 and 7. Lipid oxidation, as indicated by TBARS values, increased numerically over retail display time with day 1 < 3 < 5 < 7. On day 7 of retail display, steaks displayed with HFLO light sources had less (P< 0.05) TBARS values than those displayed with FLO or LED light sources. Data indicate that muscles that are color labile are impacted by modern lighting technologies.

Key words: Triceps brachii, oxidation, myoglobin, lighting, color

4.1 Introduction

Consumer purchasing decisions are influenced by fresh meat color more than any other quality parameter in a retail setting (Mancini and Hunt, 2005). Fresh meat quality is impacted by retail display in numerous ways such as, length of retail display (Jeremiah and Gibson, 2001; Martin et al., 2013;), temperature (Martin et al., 2013;), light source (Steele et al., 2016; Cooper et al., 2016). With multiple factors impacting meat color stability, it is imperative that we ensure that retail settings are implementing procedures to capitalize on product freshness and overall marketability of fresh meat products to maximize profit. Therefore, evaluating the impact of light sources during retail display on muscles dependent on their color stability is important to determine overall retail recommendations.

The Department of Energy (DOE) has stated that by 2035, over 85% of the installed lighting technologies in the United States will be light emitting diode (LED) sources compared to only 5% in 2016 (US DOE, 2016). These reports indicate that a transition in the retail setting from the common fluorescent lights to LED lights is underway. While the economic impact from an energy standpoint is beneficial to the retail industry, it is important to evaluate if this transition of retail display lights will have a quality impact on fresh meat products.

In a study conducted at the University of Missouri, ground beef displayed under LED, fluorescent, and no light (DRK) showed that LED light sources promoted redness retention in high and low fat ground beef patties during retail display (Cooper et al., 2016). Conflicting reports by Steele et al (2016) reported no differences in a* values between LED and fluorescent displayed ground beef but did find differences in steaks

from the *semimembranosus* (SM) a muscle with moderate color stability. It is known that beef color stability varies greatly from muscle to muscle within a carcass (McKenna et al., 2005; Canto et al., 2016). Therefore, evaluating the impact of varying light sources in a muscle determined color labile presented itself as the necessary next step for evaluation.

McKenna et al. (2005) reported that *Triceps brachii* (TB) is a "low color stability" muscle when evaluating biochemical and physical factors that impacted discoloration characteristics of beef muscles. Muscles were evaluated on color stability, myoglobin concentrations, metmyoglobin reducing activity, oxidation and other quality attributes (McKenna et al. 2005). The objectives of this study were to evaluate the impact of low-UV fluorescent (FLO), high-UV fluorescent (HFLO), and LED lighting sources on the surface color of beef steaks of a low color stability muscle during retail display and determine the contribution of myoglobin activity and lipid oxidation to surface color changes.

4.2 Materials and Methods

Triceps brachii steak manufacture. USDA Select clod hearts (n = 20, IMPS 114E) were purchased and delivered to the University of Missouri meat laboratory. Muscles aged for 20 days post packaging date and were ultimately processed to isolate the *Triceps brachii* (TB) for further processing. After isolation, 12 steaks, 1.9 cm thick were cut from each TB. Steaks were then packaged on Styrofoam® trays and overwrapped with oxygen permeable, polyvinyl chloride. Steaks were then assigned to one of three lighting treatments (HFLO, FLO, LED), day of retail display removal (1, 3, 5, 7), and were placed into the appropriate deli case (TDBD-72-4, True Food Service Equipment, O'Fallon,

MO) containing the appropriate lighting treatment. Each deli case had a temperature scale of 0 - 9, with 0 being the warmest and 9 being the coldest; each deli case was set to 7. Temperature was monitored by factory supplied thermometers within each deli case.

Fat percentage determination. Determination of fat percentage was done in triplicate utilizing the CEM method described in Dow et al. (2011) with a CEM SMART Trac rapid fat analysis system. Two CEM sample pads were heated and dried before 3.75 - 4.5 g of minced sample was smeared across one pad and topped with the remaining pad. Samples were dried using the CEM Moisture/Solids Analyzer; moisture was determined on a dry weight basis. Following determination of moisture, sample pads were wrapped in TRAC paper, inserted into a CEM TRAC tube and was placed into the CEM Rapid Fat Analyzer. Fat percentage of samples was then determined on a dry basis using NMR and was ultimately converted to a wet basis. Triplicate values were averaged to determine overall fat percentages for each muscle.

pH determination. Muscle pH was determined according to AMSA (2012). Duplicate, 10 g sample of each muscle was homogenized with 100 ml of distilled water. After homogenization, pH of the homogenate was measured using a benchtop probe.

Objective color determination. Instrumental measurements of surface steak color were utilized to monitor color change within light treatments over retail display time. Objective color measurements of L*, a*, and b* were taken using a HunterLab MiniScan 45/0 LAV (Hunter Associates Laboratory, Virginia, USA) with a D65 light source, 25

mm aperture and physical standard. Objective color was measured immediately after steaks were removed on their assigned retail display day of 1, 3, 5, or 7. Objective color measurements were taken on each steak surface in triplicate in order to obtain more accurate surface color values. Values were averaged to determine an overall surface color value. Objective color readings were also utilized to calculate a/b ratio, saturation index, and hue angle values using equations found in AMSA (2012).

Calculated myoglobin concentrations. Concentrations of myoglobin (deoxymyoglobin [DMb], oxymyoglobin [MbO_2], and metmyoglobin [MMb]) were determined using wavelengths described in AMSA (2012). Reflectance was measured at isobetic wavelengths of 470, 530, 570, and 700 nm which were reported by readings on a HunterLab MiniScan 45/0 LAV (Hunter Associates Laboratory, Virginia, USA). Values for each wavelength were obtained in triplicate on days 1, 3, 5, and 7 of retail display for steaks from each light treatment. Myoglobin concentrations were determined utilizing equations provided in AMSA (2012).

Measured myoglobin content. Duplicate 2.5 g minced steak surface samples were homogenized using a Polytron homogenizer (Polytron 10-35 GT, Kinematica, Bohemia, New York) in 22.5 ml of ice cold sodium phosphate buffer for 90 seconds. Homogenate was then filtered into clean tubes. Filtrate absorbance was read at 525 nm on a Genesys 20 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts). Myoglobin concentrations were calculated utilizing the equation provided in AMSA (2012) and values were averaged to determine total myoglobin concentration of each steak.

Metmyoglobin reducing activity. Duplicate cubes from the center of each steaks surface were removed on each day of retail display for all light treatments. Upon removal, samples were submerged in 0.3% sodium nitrite solution for 20 min to induce MMb formation. After 20 min, samples were removed from the solution, blotted dry, and vacuum sealed (Multivac, Chamber Machine P200, Kansas City, Missouri) in individual packages. Readings of each sample were taken immediately after packaging utilizing a HunterLab MiniScan in triplicate to obtain reflectance data. Samples were incubated at room temperature for 2 hours to induce MMb reduction. After incubation, samples were rescanned in triplicate with a HunterLab MiniScan. Surface MMb values were calculated using K/S ratios and formulas provided in AMSA (2012). Metmyoglobin reducing activity was calculated using the equation below.

$$\text{MRA} = \frac{100 \times (\text{Pre-incubation \% metmyoglobin} - \text{Post-incubation \% metmyoglobin})}{\text{Pre-incubation \% metmyoglobin}}$$

Lipid oxidation. Lipid oxidation was determined utilizing the distillation method to analyze thiobarbituric acid reactive substances (TBARS) as described in Tarlagdis et al. (1960) with modifications found in Fernando et al. (2013). Duplicate 5 g surface steak samples were minced, and homogenized (Polytron 10-35 GT, Kinematica, Bohemia, New York) with 25 ml of distilled water. Homogenate was then poured into a 250 ml Kjeldahl flask and blending tubes were rinsed with an additional 25 ml of distilled water and

transferred into the same flask. Two drops of antifoam solution along with 2.5 ml of 4N HCL to balance sample pH between 1.5 - 1.6 were added to the flask immediately before distillation. Flasks were placed into controlled heating elements (Fisher Scientific, Pittsburg, PA) and 25 ml of sample was distilled through a water-cooled distillation apparatus. After distillation, 5 ml of sample was pipetted into a glass tube containing 5 ml of thiobarbituric acid reagent (TBA) and vortexed individually. Tubes were then placed into a boiling water bath for 35 min. Immediately following removal from the water bath, tubes were submerged into an ice bath for 10 min. Color absorbance was measured at 538 nm using a Genesys 20 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts). Values for TBARS concentrations were obtained by obtaining the average absorption of the duplicate sample readings and mg/kg of malonaldehyde was determined using the K value of 7.8 (Tarladgis et al., 1960; AMSA, 2012).

Statistical analysis. Data were analyzed as a randomized complete block design with the model including fixed effects of light (HFLO, FLO, LED), length of retail display (1, 3, 5, or 7 days), and all possible interactions. Analysis for objective color values, calculated myoglobin concentrations, measured myoglobin concentrations, metmyoglobin reducing activity and lipid oxidation was done using the GLIMMIX function of SAS (SAS Version 9.4, SAS Inst. Inc. Cary, North Carolina) to obtain LS means and standard error estimates. Significance was determined at $P < 0.05$. The PROC CORR procedure of SAS was then used to generate correlations.

4.3 Results and Discussion

Deli case environment and steak characteristics. All three deli cases in this study had temperatures of 2°C ± 1°C and average light intensities for HFLO, FLO, and LED bulbs were 289.97, 168.44, and 757.44 flux, respectively (non-tabular data). As seen in Table 4.1, average pH values for steaks from the TB were 5.42. Von Seggren et al. (2005) reported similar pH values for the TB with a mean value of 5.66. Fat content had high amounts of variation between TB muscle samples, these values indicate the variation of fat deposition from animal to animal. Von Seggren et al. (2005) reported a mean fat percentage of 5.65% for TB muscles, further indicating variation in fat levels in available muscle.

Table 4.1: Chemical characterization of beef *Triceps brachii* steaks (n = 20)

Item	Average	Minimum	Maximum
pH	5.42	5.31	5.63
Fat (%)	6.04	2.85	11.18
Moisture (%)	75.10	68.87	79.21

Objective color. No differences ($P < 0.05$) were found in lightness (L*) values of TB steaks produced from the TB for light treatment (Table 4.2) or over the duration of retail display (Table 4.3). Data agrees with the findings of McKenna et al. (2005) and King et al. (2011) who reported that steaks produced from the TB had no changes in L* values after day 1 of retail display.

As seen in Table 4.2, steaks produced from the TB had higher a* values ($P < 0.05$) in steaks exposed to HFLO lights than those exposed to FLO and LED light

sources. Indicating that the use of HFLO lights promoted greater retention of redness than FLO and LED light treatments in retail display. This disagrees with findings in Steele et al. (2016), where no differences were reported for a* values in steaks between FLO and LED light treatments over retail display. Retail display time also impacted a* values in steaks produced from the TB with values decreasing over retail display length with day 1 > 3 > 5 > 7 (Table 4.3). These data agree with the literature (Hamling et al., 2008; Steele et al., 2016; Canto et al., 2016) which showed decreases in a* values and redness retention with increasing retail display time. Data from this study indicate that light treatment and retail display length impact a* value and red color retention in steaks with low color stability.

Values for b* mimicked trends in a* values for steaks produced from the TB, agreeing with findings in Canto et al. (2016). As seen in Table 4.2, mean b* values were higher ($P < 0.05$) for steaks displayed under HFLO lights than steaks displayed with both FLO and LED light sources (Table 4.3). Steele et al. (2016) found that LED displayed SM steaks had higher b* values under LED than fluorescent lights. Mean values for b* decreased as retail display time increased in steaks from the TB with day 1 > 3 > 5 > 7 indicating a loss of yellowness over retail display. This could be attributed to the formation of brown MMb. This data indicates that b* values were impacted by both light treatment and retail display time in color labile muscles.

Light source and retail display time impacted mean values for a/b ratios in steaks produced from the TB (Table 4.4). Mean a/b ratio values decreased for steaks displayed with all light treatments over each day of retail display indicating loss of red color over time (AMSA, 2012). Steaks displayed with HFLO light sources had higher ($P < 0.05$)

mean a/b ratio values than steaks displayed with FLO and LED light sources on retail display days 5 and 7. These values indicate that steaks displayed with HFLO light sources retained greater ($P < 0.05$) amounts of surface redness over retail display than steaks displayed under FLO or LED light sources.

Saturation index mean values were higher ($P < 0.05$) in steaks displayed with HFLO displayed lights than for steaks displayed with FLO or LED light sources as seen in Table 4.2. These values indicate greater levels of redness retention in HFLO steaks than those displayed with FLO or LED light sources (AMSA, 2012). Higher ($P < 0.05$) SI values for HFLO displayed steaks indicated greater amounts of surface redness as opposed to FLO and LED displayed steaks. Saturation index values decreased over duration of retail display with day 1 > 3 > 5 > 7 (Table 4.3). Data from this study indicated that SI values decreased over the duration of retail display agreeing with Steele et al. (2016) and Cooper et al. (2016), indicating a loss of redness on the meat surface over time.

Hue angle mean values increased for each light treatment over the duration of retail display, indicating a loss of redness and increased discoloration (AMSA, 2012). This agrees with findings in Steele et al. (2016). Steaks from the TB displayed under HFLO lights had lower ($P < 0.05$) HA mean values than steaks displayed with both FLO and LED lights (Table 4.4). These values indicate that steaks produced from the TB had greater amounts of discoloration and loss of redness at the end of retail display when displayed with FLO or LED lights when compared to those displayed with HFLO lights during retail

Table 4.2: Effect of light source on color of beef *Triceps brachii* steaks (n = 20)

	Retail Display Light[1]				
Item	HFLO	FLO	LED	SEM	P-Value[2]
L*	42.89	42.62	42.33	0.25	0.0848
a*	18.45[a]	16.72[b]	16.61[b]	0.25	<0.0001
b*	16.97[a]	16.12[b]	16.30[b]	0.14	<0.0001
SI[3]	25.13[a]	23.42[b]	23.22[b]	0.26	<0.0001
DMb[4]	4.56	4.28	4.32	0.18	0.2483
MYO[5]	5.41	5.2	5.22	0.17	0.4681
MRA[6]	14.48[b]	18.28[a]	14.52[b]	1.70	0.0389

[1]HFLO = high uv fluorescent, FLO = low uv fluorescent, LED = light emitting diode
[2] P-Value of LS Means
[3] SI- Saturation Index
[4] DMb- Deoxymyoglobin
[5] MYO- Measured myoglobin concentrations
[6] MRA- Metmyoglobin reducing activity
[ab] Data lacking a common superscript differ $P < 0.05$

display. Steele et al. (2016) found that beef steaks from the SM had higher HA values during retail display under LED lights than steaks under fluorescent light sources.

Calculated myoglobin concentrations. Table 4.2 indicates no differences ($P > 0.05$) were found between steaks in all light treatments for mean calculated DMb concentrations. Not surprisingly, retail display time did impact DMb values with decreases ($P < 0.05$) occurring with extended retail display time with day 1 > 3 > 5 > 7 as seen in Table 4.3. These results agree with those found in Djenane et al. (2001) as increased oxygen exposure results in the oxidation of myoglobin and the conversion of DMb to MbO_2 and potentially MMb.

Mean values for MbO_2 concentrations decreased ($P < 0.05$) in steaks under all light treatments over retail display (Table 4.4). Steaks exposed to HFLO lights had greater ($P < 0.05$) MbO_2 concentrations than FLO displayed steaks on retail display day 3. Mean values for MbO_2 on retail display day 5 showed that HFLO displayed steaks had greater ($P < 0.05$) concentrations of MbO_2 than steaks from the TB exposed to LED lights. By retail display day 7, HFLO displayed steaks had greater ($P < 0.05$) MbO_2 concentrations than both FLO and LED exposed steaks. Data from this study indicates that steaks with low oxidative and color stability retained more redness during retail display, as indicated by MbO_2 concentrations, when displayed with HFLO light sources as opposed to FLO or LED light sources.

As expected with decreasing MbO_2 values, MMb increased for each light display over retail display period which can be seen in Table 4.4. Steaks displayed with HFLO lights had less ($P < 0.05$) MMb concentrations on retail display days 5 and 7 than steaks

Table 4.3: Effect of retail display day on color of beef *Triceps brachii* steaks (n=20)

	Retail Display Day					
Item	1	3	5	7	SEM	P-Value[1]
L*	42.96	42.42	42.50	42.50	0.29	0.2377
a*	22.14[a]	17.73[b]	15.72[c]	13.49[d]	0.29	<0.0001
b*	18.61[a]	16.47[b]	15.69[c]	15.09[d]	0.16	<0.0001
SI[2]	28.94[a]	24.21[b]	22.24[c]	20.30[d]	0.30	<0.0001
DMb[3]	5.73[a]	4.75[b]	4.10[c]	2.96[d]	0.21	<0.0001
MYO[4]	5.72[a]	5.03[b]	5.39[a]	5.02[b]	0.19	0.0008
MRA[5]	29.97[a]	19.27[b]	10.58[c]	3.22[d]	1.96	<0.0001

[1] P-Value of LS Means
[2] SI- Saturation Index
[3] DMb- Deoxymyoglobin (%)
[4] MYO- Measured myoglobin concentrations (mg/g)
[5]MRA- Metmyoglobin reducing activity (%)
[abcd] Data lacking a common superscript differ $P < 0.05$

displayed with both FLO and LED light sources. McKenna et al. (2005) reported that discoloration and MMb formation occured over retail display time for steaks produced from the TB. Data from this study supports those findings and indicates that in low color and oxidative stability muscles, the use of HFLO lights can promote redness retention throughout extended retail display as opposed to FLO and LED light sources.

Measured myoglobin concentration. Light treatment had no impact ($P > 0.05$) on measured myoglobin concentrations in steaks produced from the TB (Table 4.2). Retail display time showed differences ($P < 0.05$) in myoglobin concentrations with retail display days 1 and 5 having greater mean myoglobin concentration values than retail display days 5 and 7, which can be found in Table 4.3. McKenna et al. (2005) found that variation in muscle led to variation of myoglobin concentrations during retail display; but no relationship between myoglobin concentration and color stability was determined. These results can potentially be attributed to the low oxidative and color stability of the TB muscle (McKenna et al., 2005). Increased oxidation could have led to the degradation of myoglobin within each muscle sample; potentially leading to changes in myoglobin concentrations over retail display time (McKenna et al., 2005).

Table 4.4: Impact of light source and retail display day on color and lipid oxidation of beef *Triceps brachii* steaks (n = 20)

Item	Retail Display Day													
	1			3			5			7				
	Lighting Treatment[1]													
	HFLO	FLO	LED	HFLO	FLO	LED	HFLO	FLO	LED	HFLO	FLO	LED	SEM	P-Value[1]
a/b[2]	1.20[a]	1.19[a]	1.18[a]	1.10[b]	1.07[bc]	1.05[c]	1.05[c]	0.98[d]	0.97[d]	0.97[d]	0.84[e]	0.86[e]	0.02	0.0198
HA[3]	39.79[d]	40.17[d]	40.39[d]	42.24[c]	43.06[c]	43.68[c]	43.69[c]	45.89[b]	46.16[b]	46.10[b]	50.43[a]	48.59[a]	0.75	0.0063
MbO_2[4]	57.03[a]	56.45[abcd]	56.64[abc]	56.76[ab]	56.15[cde]	56.37[bcd]	56.17[bcde]	55.57[ef]	55.36[f]	55.93[def]	54.18[g]	54.40[g]	0.31	0.0365
MMb[5]	37.38[f]	37.62[f]	37.68[ef]	38.55[de]	38.95[cd]	38.96[cd]	39.46[c]	40.54[b]	40.61[b]	40.48[b]	42.43[a]	42.69[a]	0.46	0.0008
TBARS[6]	1.16[e]	1.30[e]	1.25[e]	2.20[d]	2.25[d]	2.32[d]	2.82[c]	3.10[bc]	2.90[bc]	3.28[b]	4.33[a]	3.97[a]	0.21	0.0205

[1] HFLO = high uv fluorescent, FLO = low uv fluorescent, LED = light emitting diode
[2] P-Value of LS Means
[3] HA- Hue Angle
[4] MbO_2- Oxymyoglobin (%)
[5] MMb- Metmyoglobin (%)
[6] TBARS- Thiobarbituric acid reactive substances (mg/kg)
[abcdef] Data lacking a common superscript differ $P < 0.05$

Metmyoglobin reducing activity. Metmyoglobin reducing activity has been thought to increase color stability by reducing MMb to myoglobin (McKenna et al., 2005; King et al., 2011; Garner et al., 2014). Mean MRA values for steaks produced from the TB were higher ($P < 0.05$) for steaks displayed with FLO lights than those displayed with HFLO or LED light sources, as seen in Table 4.2. Table 4.3 shows that retail display time also impacted MRA values with values decreasing over retail display with day 1 > 3 > 5 > 7. These values indicate a loss in MRA and subsequent discoloration occurring over retail display, this agrees with the findings in Reddy and Carpenter (1991) and Canto et al. (2016) who found that higher MRA values resulted in greater color stability. These findings disagree with those of McKenna et al. (2005) who found that muscles with low color stability often had higher MRA values than their color stable counterparts. There is debate on the significance of MRA and color stability predictions; O'Keefe and Hood (1982) who found that MRA is of little importance in determining color stability. These findings agree with Atkinson and Follett (1973) and Bekhit et al. (2001) who found no relationship between MRA and color stability in muscles of multiple species. Low oxidative and color stability within the muscle of the TB lead to high variation in surface color of steaks over retail display time. This low stability led to high variation in MRA values between duplicate samples for each steak.

Lipid oxidation. Lipid oxidation, as indicated by TBARS values increased numerically over retail display for each light treatment. Research (McKenna et al. 2005; Faustman et al. 2010; Canto et al., 2016; Steele et al., 2016; Cooper et al., 2016) has reported that increased retail display time leads to increases in lipid oxidation in fresh beef products.

As these steaks were utilized due to their low oxidative stability, these results were anticipated, and agreed with findings in McKenna et al. (2005).

On each day of retail display, TBARS values for HFLO < FLO < LED exposed steaks occurred (Table 4.4). No differences ($P > 0.05$) occurred between light treatments on retail display days 1, 3, and 5. However, on retail display day 7 HFLO displayed steaks had lower ($P < 0.05$) TBARS values than steaks displayed with FLO or LED light displays. Steele et al., (2016) also found that beef steaks exposed to LED lights had higher ($P < 0.05$) TBARS values than steaks displayed with FLO light sources. Data from this study indicates that over retail display time, the use of HFLO light sources promotes less lipid oxidation in steaks with low color and oxidative stability.

Relationship between color measurements and lipid oxidation. Strong negative correlations exist between TBARS values and a* ($P < 0.0001$) and b* ($P < 0.0001$) color measurements. Weak, but highly significant ($P < 0.001$) negative correlations occurred between TBARS and MbO_2 concentrations. Conversely, strong ($P < 0.0001$) positive correlations occurred between TBARS and MMb concentrations. Strong relationships have been reported (Faustman and Cassens, 1990; Martin et al., 2013) between lipid oxidation and MMb formation in fresh meat products. Faustman et al. (2010) reported that greater amounts of iron and myoglobin are associated with lipid oxidation and resulting MMb accumulation on steak surfaces. Data from this study supports findings that lipid oxidation and the oxidation of MbO_2 to form MMb are related.
Moderate positive correlations between MRA and objective color measurements for a* ($P< 0.0001$) and b* ($P< 0.0001$), as well as DMb ($P< 0.0001$) occurred. Correlations

between MRA and MbO_2 concentrations were weak ($P< 0.0001$). Alternatively, MRA had moderate negative correlations between both TBARS ($P< 0.0001$) and MMb concentrations ($P< 0.0001$). MRA is an indicator of color stability in fresh meat products (McKenna et al., 2005; Bekhit and Faustman, 2005; Wu et al. 2015) therefore negative correlations between TBARS and MMb values are to be expected.

4.4 Conclusion

Light treatment changes discoloration and oxidation of steaks from the TB over retail display. HFLO light sources in retail display of steaks with low oxidative and color stability promote retention of redness over retail display time as indicated by a* values, MbO_2 concentrations and the formation of MMb on steak surface as well as less lipid oxidation represented by TBARS values in comparison to steaks displayed with FLO or LED light sources.

Table 4.5. Simple correlation coefficients for various traits among beef *Triceps brachii* steaks (n=20)

Item	L*	a*	b*	DMb	MbO_2	MMb	TBARS	MYO	MRA	pH
L*	1.00									
a*	0.03	1.00								
b*	0.26***	0.91***	1.00							
DMb[1]	-0.02	-0.86***	-0.73***	1.00						
MbO_2[2]	0.11	0.74***	0.60***	-0.77***	1.00					
MMb[3]	-0.08	0.53***	0.48***	-0.71***	- 0.67***	1.00				
TBARS[4]	-0.14*	-0.74***	-0.70***	0.71***	- 0.67***	- 0.37***	1.000			
MYO[5]	-0.17*	0.25***	0.18*	-0.18*	0.08	0.19*	-0.15*	1.00		
MRA[6]	-0.16*	0.57***	0.44***	-0.53***	0.45***	0.34***	-0.43***	0.09	1.00	
pH	-0.18*	0.10	0.04	-0.11	0.10	0.06	-0.17*	0.10	0.29***	1.00

[n.a.] – non-applicable data due to auto-correlated values
[1] DMb- Deoxymyoglobin (%)
[2] MbO_2- Oxymyoglobin (%)
[3] MMb- Metmyoglobin (%)
[4] TBARS- Thiobarbituric acid reactive substances (mg/kg)
[5] MYO- Measured myoglobin concentrations (mg/g)
[6] MRA- Metmyoglobin reducing activity (%)
*- $P < 0.05$
**- $P < 0.001$
***- $P < 0.0001$

Chapter 5

Impact of light emitting diode (LED) lights on beef steaks produced from a color stable muscle; Semimembranosus

ABSTRACT: Consumer purchasing decisions are heavily impacted by meat color as an indicator of fresh meat quality in a retail setting. The objectives of this study were to evaluate the impact of light emitting diodes (LED) on surface color and lipid oxidation of fresh beef steaks from the *Semimembranosus* (SM) over retail display time. Steaks from the SM [moderate oxidative and color stabilities] (n = 20) were packaged on Styrofoam® trays and overwrapped with oxygen permeable polyvinyl chloride. Steaks were then assigned to one of three lighting treatments (High UV fluorescent [HFLO], low UV fluorescent [FLO], and LED) within temperature controlled deli cases. Steaks were removed on retail display days 1, 3, 5, and 7 for objective color determination, myoglobin concentrations, metmyoglobin reducing activity, and lipid oxidation levels. Redness, as indicated by a* values differed ($P < 0.05$) for steaks displayed with all light sources with HFLO > FLO > LED with values of 20.85, 20.11, and 19.37, respectively. Mean a* values decreased ($P < 0.05$) over retail display day with 1 > 3 > 5 > 7 with values of 24.52, 20.34, 19.35, and 16.25, respectively. These values indicate that HFLO displayed steaks retained greater amounts of redness compared FLO and LED displayed steaks, and that loss of redness occurs over retail display. Steaks displayed with both HFLO and FLO light sources had higher ($P < 0.05$) oxymyoglobin values than those displayed with LED lights indicating that steaks displayed under LED lights had less desirable red color than its HFLO and FLO counterparts. Values for MbO_2 were lower ($P < 0.05$) on day 7 of

retail display indicating that steaks produced from the SM discolor as retail display time increased. Metmyoglobin (MMb) concentrations increased over retail display with LED exposed steaks having greater ($P < 0.05$) amounts of MMb than steaks displayed with HFLO and FLO light sources. By day 7 of retail display, HFLO exposed steaks had less ($P < 0.05$) MMb than both FLO and LED displayed steaks. Lighting display played no role in lipid oxidation ($P > 0.05$) in steaks produced from the SM. Over retail display, lipid oxidation as indicated by TBARS values increased ($P < 0.05$) daily with $1 < 3 < 5 < 7$ confirming that increased retail display time increases the occurrence of lipid oxidation. Data indicates there is no advantage in regard to color or lipid oxidation, for using LED lights in retail display for beef muscles with high color and oxidative stabilities.

Keywords: Semimembranosus, myoglobin, lipid oxidation, oxidation, color

5.1 Introduction

Consumer perception of fresh meat quality relies heavily on product color in retail display (Faustman and Cassens 1990; Bekhit et al, 2001; Holman et al. 2017). Numerous factors in retail display impact meat color stability and oxidation rates such as temperature (Jeremiah and Gibson, 2001) retail display length (Mancini and Ramanathan, 2014) and lighting source (Cooper et al., 2016; Steele et al., 2016). Therefore, the evaluation of fresh meat color in various retail settings is crucial for continued consumer satisfaction with products as new lighting technologies are developed.

Myoglobin is the heme protein responsible for meat color (Aberle et al., 2012). When oxygen is not present deoxymyoglobin (DMb) is the primary myoglobin state, resulting in a dark purple product color (Mancini and Ramanathan, 2014). As oxygen is introduced to the environment, myoglobin oxygenation occurs, and oxymyoglobin (MbO_2) produces the bright, cherry red desirable fresh beef color (Bekhit and Faustman, 2005). As oxygen exposure continues, MbO_2 oxidizes to ultimately become metmyoglobin (MMb) (Bekhit and Faustman, 2005). Metmyoglobin formation results in brown undesirable fresh meat color. Therefore, ensuring retention of MbO_2 in products in a retail setting is imperative.

Color and oxidative stability varies greatly between muscles in a beef carcass (McKenna et al. 2005; Von Seggern et al. 2005). McKenna et al. (2015) reported that the *Semimembranosus* (**SM**), a muscle isolated from the top round, is a muscle with moderate color and oxidative stability based on objective color, myoglobin concentrations, metmyoglobin reducing ability, lipid oxidation and other factors. Cooper et al. (2016) found that ground beef patties produced from the top round retained more

redness over retail display indicated by a* under light emitting diode (LED) lights in comparison to those displayed under fluorescent light sources. Thus, indicating more research was needed to understand the impact that lighting technologies have on quality aspects of fresh beef products.

With retail display conditions impacting consumer quality perception, monitoring impacts of condition changes is crucial. The US Department of Energy (US DOE, 2016) reported that by 2035, over 85% of lighting technologies will be LED sources compared to the 5% that are today. With findings in Cooper et al. (2016) indicating that ground beef produced from the SM retained redness longer under LED lights, little information is available regarding the impact of lighting technologies on whole muscle with increased color and oxidative stabilies. Therefore, the objectives of this study were to evaluate the impact of LED, high-UV fluorescent (HFLO), and low-UV fluorescent (FLO) light sources on beef steaks from the SM during the duration retail display on surface color and lipid oxidation.

5.2 Materials and Methods

Semimembranosus steak manufacture. USDA Select top rounds (n = 20, IMPS 168) were purchased and delivered to the University of Missouri meat laboratory. Muscles were aged for 20 days post packaging date and were processed to isolate the *Semimembranosus* SM for further processing. After isolation, 12 steaks 2.54 cm thick were cut from each SM. Steaks were then packaged on Styrofoam® trays and overwrapped with oxygen permeable, polyvinyl chloride. Steaks were then assigned to one of three lighting treatments (HFLO, FLO, LED), day of retail display removal (1, 3,

5, 7), and were placed into the appropriate deli case (TDBD-72-4, True Food Service Equipment, O'Fallon, MO) containing the appropriate lighting treatment. Each deli case had a temperature scale of 0 - 9, with 0 being the warmest and 9 being the coldest; each deli case was set to 7. Temperature in each case was monitored on a factory supplied case thermometer inside the case.

Fat percentage determination. Determination of fat percentage was done in triplicate utilizing the CEM method described in Dow et al. (2011) with a CEM SMART Trac rapid fat analysis system. Two CEM sample pads were heated and dried before 3.75 - 4.5 g of minced sample was smeared across one pad and topped with the remaining pad. Samples were dried using the CEM Moisture/Solids Analyzer; moisture was determined on a dry weight basis. Following determination of moisture, sample pads were wrapped in TRAC paper, inserted into a CEM TRAC tube and was placed into the CEM Rapid Fat Analyzer. Fat percentage of samples was then determined on a dry basis using NMR and was ultimately converted to a wet basis. Triplicate values were averaged to determine overall fat percentages for each muscle.

pH determination. Muscle pH was determined according to AMSA (2012). Duplicate, 10 g sample of each muscle was homogenized with 100 ml of distilled water. After homogenization, pH of the homogenate was measured using a benchtop probe.

Objective color determination. Instrumental measurements of surface steak color were utilized to monitor color change within light treatments over retail display time.

Objective color measurements of L*, a*, and b* were taken using a HunterLab MiniScan 45/0 LAV (Hunter Associates Laboratory, Virginia, USA) with a D65 light source, 25 mm aperture and physical standard. Objective color was measured immediately after steaks were removed on their assigned retail display day of 1, 3, 5, or 7. Objective color measurements were taken on each steak surface in triplicate in order to obtain more accurate surface color values. Values were averaged to determine an overall surface color value. Objective color readings were also utilized to calculate a/b ratio, saturation index, and hue angle values using equations found in AMSA (2012).

Calculated myoglobin concentrations. Concentrations of myoglobin (DMb, MbO_2, MMb) determined using wavelengths described in AMSA (2012). Reflectance was measured at isobetic wavelengths of 470, 530, 570, and 700 nm which were reported by readings on a HunterLab MiniScan 45/0 LAV (Hunter Associates Laboratory, Virginia, USA). Values for each wavelength were obtained in triplicate on days 1, 3, 5, and 7 of retail display for steaks from each light treatment. Myoglobin values were determined utilizing equations provided in AMSA (2012).

Measured myoglobin content. Duplicate 2.5 g minced steak surface samples were homogenized using a Polytron homogenizer (Polytron 10-35 GT, Kinematica, Bohemia, New York) in 22.5 ml of ice cold sodium phosphate buffer for 90 seconds. Homogenate was then filtered into clean tubes. Filtrate absorbance was read at 525 nm on a Genesys 20 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts). Myoglobin

concentrations were calculated utilizing the equation provided in AMSA (2012) and values were averaged to determine total myoglobin concentration of each steak.

Metmyoglobin reducing activity. Triplicate cubes from the center of each steaks surface were removed on each day of retail display for all light treatments. Upon removal, samples were submerged in 0.3% sodium nitrite solution for 20 min to induce MMb formation. After 20 min, samples were removed from the solution, blotted dry, and vacuum sealed (Multivac, Chamber Machine P200, Kansas City, Missouri) in individual packages. Readings of each sample were taken immediately after packaging utilizing a HunterLab MiniScan in triplicate to obtain reflectance data. Samples were incubated at room temperature for 2 hours to induce MMb reduction. After incubation, samples were rescanned in triplicate with a HunterLab MiniScan. Surface MMb values were calculated using K/S ratios and formulas provided in AMSA (2012). Metmyoglobin reducing activity (**MRA**) was calculated using the equation below.

$$\text{MRA} = \frac{100 \times (\text{Pre-incubation \% metmyoglobin} - \text{Post-incubation \% metmyoglobin})}{\text{Pre-incubation \% metmyoglobin}}$$

Lipid oxidation. Lipid oxidation was determined utilizing the distillation method to analyze thiobarbituric acid reactive substances (**TBARS**) as described in Tarlagdis et al. (1960) with modifications found in Fernando et al. (2013). Duplicate 5 g surface steak samples were minced, and homogenized (Polytron 10-35 GT, Kinematica, Bohemia, New York) with 25 ml of distilled water. Homogenate was then poured into a 250 ml Kjeldahl

flask and blending tubes were rinsed with an additional 25 ml of distilled water and transferred into the same flask. Two drops of antifoam solution along with 2.5 ml of 4N HCL to balance sample pH between 1.5 - 1.6 were added to the flask immediately before distillation. Flasks were placed into controlled heating elements (Fisher Scientific, Pittsburg, PA) and 25 ml of sample was distilled through a water-cooled distillation apparatus. After distillation, 5 ml of sample was pipetted into a glass tube containing 5 ml of thiobarbituric acid reagent (**TBA**) and vortexed individually. Tubes were then placed into a boiling water bath for 35 min. Immediately following removal from the water bath, tubes were submerged into an ice bath for 10 min. Color absorbance was measured at 538 nm using a Genesys 20 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts). Values for TBARS concentrations were obtained by obtaining the average absorption of the duplicate sample readings and mg/kg of malonaldehyde was determined using the K value of 7.8 (Tarladgis et al., 1960; AMSA, 2012).

Statistical analysis. Data was analyzed as a randomized complete block design with the model including fixed effects of light (HFLO, FLO, LED), length of retail display (1, 3, 5, or 7 days), and all possible interactions. Analysis for objective color values, calculated myoglobin concentrations, measured myoglobin concentrations, metmyoglobin reducing activity and lipid oxidation was done using the GLIMMIX function of SAS (SAS Version 9.4, SAS Inst. Inc. Cary, North Carolina) to obtain LS means and standard error estimates. Significance was determined at $P < 0.05$. The PROC CORR procedure of SAS was then used to generate correlations.

5.3 Results and Discussion

Deli case environment and steak characteristics. All three deli cases in this study had temperatures of 2°C ± 1°C and average light intensities for HFLO, FLO, and LED bulbs were 289.97, 168.44, and 757.44 flux, respectively (non-tabular data). As seen in table 5.1, average pH values of steaks produced from the SM were similar to those found by Von Seggern et al. (2005) and King et al. (2011). Von Seggren et al. (2005) reported average fat percentages of 4.36 for SM, indicating variation between muscles for fat content. These variations in fat and moisture concentrations of fresh beef products can impact objective color measurements as increased fat content can lead to a larger L* value.

Table 5.1: Chemical characterization of *Semimembranosus* steaks (n=20)

Item	Average	Minimum	Maximum
pH	5.41	5.02	6.24
Fat(%)	2.10	0.43	5.53
Moisture(%)	74.86	70.04	79.00

Objective color. Higher L* values indicate a lighter product; as seen in Table 5.2, mean L* values in steaks from the SM showed differences ($P < 0.05$) between light sources with LED displayed steaks having the highest L* value indicating a lighter steak surface. However, Steele et al. (2016) reported no differences in L* values for steaks from the SM under FLO and LED light sources. Retail display time played a role in L* values in steaks. Values decreased ($P < 0.05$) over retail display day with $1 > 3 > 5 > 7$ as seen in Table 5.3. However, variation in L* values could potentially be attributed to fat content

and connective tissue levels. This agrees with findings by King et al. (2011) who saw decreases in L* values from day 0 to 6 of retail display of steaks produced by the SM. Cooper et al. (2016) also reported decreases in L* values over the duration of retail display of ground beef patties.

Mean values for a* differed ($P < 0.05$) between light treatments, as seen in Table 5.2. Greater values for a* indicate a more red, desirable product. These values indicate that the use of HFLO light sources promoted greater redness retention than both FLO and LED light treatments. Steele et al. (2016) reported that on day 0 of retail display, mean a* values for steaks from the SM were higher for steaks kept under LED lights. Case temperatures in Steele et al. (2016) were quite lower than those in this study, which could impact rate of discoloration for fresh meat products. Decreases in a* is indicative of discoloration of fresh meat products. Retail display time showed decreases in a* values with increases in retail display time with day 1 > 3 > 5 > 7 which can be seen in Table 5.3. This data supports findings (McKenna, 2005; King et al. 2011; Colle et al. 2016; Steele et al., 2016) that a* values decreased over retail display time for fresh beef products. Variation in reported values for a* exists (Von Seggren et al., 2005; King et al. 2011; Garner et al., 2014; Colle et al., 2016) however, reported trends of decreasing values over time is consistent. Differences in a* values could be attributed to differences in color measuring devices and light source (AMSA, 2012), muscle location (McKenna et al., 2005), and aging time (English et al., 2016). Data from this study supports findings that increased retail display results in discoloration, as indicated by a* values, in steaks

Table 5.2: Effect of light source on color of beef *Semimembranosus* steaks (n=20)

	Retail Display Light[1]				
Item	HFLO	FLO	LED	SEM	P-Value[2]
L*	41.83[ab]	41.14[b]	42.36[a]	0.46	0.0335
a*	20.85[a]	20.11[ab]	19.37[b]	0.43	0.0031
b*	19.59	19.37	19.20	0.35	0.5434
a/b[3]	1.07[a]	1.04[a]	1.00[b]	0.02	0.0006
SI[4]	28.68[a]	28.02[ab]	27.37[b]	0.51	0.0400
HA[5]	43.48[b]	44.43[a]	45.30[a]	0.45	0.0004
DMb[6]	4.68[a]	4.20[b]	4.53[ab]	0.18	0.0229
MbO_2[7]	56.59[a]	56.45[a]	55.95[b]	0.16	0.0002
MYO[8]	4.39	4.29	4.24	0.11	0.3981
MRA[9]	18.64	19.04	15.53	2.11	0.1928
TBARS[10]	1.23	1.19	1.31	0.08	0.3197

[1] HFLO = high uv fluorescent, FLO = low uv fluorescent, LED = light emitting diode
[2] P-Value of LS Means
[3] a/b- a/b ratio
[4] SI- Saturation Index
[5] HA- Hue Angle
[6] DMb- Deoxymyoglobin (%)
[7] MbO_2- Oxymyoglobin (%)
[8] MYO- Measured myoglobin concentrations (mg/g)
[9] MRA- Metmyoglobin reducing activity (%)
[10] TBARS – Thiobarbituric acid reactive substances (mg/kg)
[ab] Data lacking a common superscript differ $P< 0.05$

with moderate oxidative and color stability. Holman et al. (2017) reported that an a* value of 14.5 is considered the acceptable threshold for consumer acceptability of fresh beef. According to that recommendation, steaks produced from the SM would be acceptable to consumers under all light treatments and for the duration of retail display.

No differences ($P > 0.05$) were found between mean b* values for steaks displayed with all three light sources as seen in Table 5.2. However, as seen in Table 5.3, over the duration of retail display mean b* values decreased with day 1 > 3 > 5 > 7 indicating thatsurface discoloration is occurring over extended retail display in steaks from the SM. King et al. (2011) reported the same trend in b* values for steaks produced from the SM over the duration of retail display.

Mean values for a/b ratios were smaller ($P < 0.05$) for steaks displayed with LED lights compared to those displayed with HFLO and FLO lights (Table 5.2). Decreases in a/b ratio values indicate a loss of redness on fresh beef surfaces (AMSA, 2012) These values indicate greater amounts of discoloration occurring in patties displayed with LED lights than both UV levels of fluorescent bulbs. Mean values decreased ($P < 0.05$) for a/b ratios over retail display duration as seen in Table 5.3.

Saturation index values differed ($P < 0.05$) between light sources with HFLO steaks having higher SI values than steaks displayed with both FLO and LED light sources as seen in Table 5.2. These values indicate less discoloration in steaks displayed with HFLO light sources than FLO and LED lights in retail display. Steele et al. (2016) reported higher SI values for steaks from the SM on retail display day 0; values were similar for fluorescent and LED for the remainder of retail display. Mean values for SI decreased ($P < 0.05$) over the duration of retail display (Table 5.3), agreeing with

multiple reports (King et al. 2011; Steele et al. 2016; Cooper et al. 2016) indicating discoloration over retail display.

Values for HA increased as product discoloration increased. Mean HA values for steaks displayed with HFLO lights were smaller ($P < 0.05$) than mean values for both FLO and LED displayed steaks (Table 5.2). These values indicate the use of HFLO bulbs promotes less product discoloration in retail display. Table 5.3 shows that length of retail display also impacted ($P < 0.05$) discoloration as indicated by HA values; with day 1 > 3 > 5 > 7 indicating product is discoloring with increasing retail display time. Data from this study agrees with reports (King et al. 2011; Cooper et al. 2016; Steele et al. 2016) of increased retail display results increased discoloration of fresh meat products as indicated by HA values.

In steaks produced from the SM, objective color values indicate that the use of HFLO lighting displays promoted greater amounts of redness retention as indicated by a*, a/b ratios, SI and HA values. However, according to Holman et al. (2017) steaks displayed under all light sources maintained acceptable a* values for the duration of retail display. Indicating that light display does not have the same impact on high color and oxidative stability muscles as it does on ground product (Cooper et al., 2016). These findings indicate that decisions about lighting technologies in regard to display of color stable muscles, could be made on other deciding factors, such as economic and accessibility impacts.

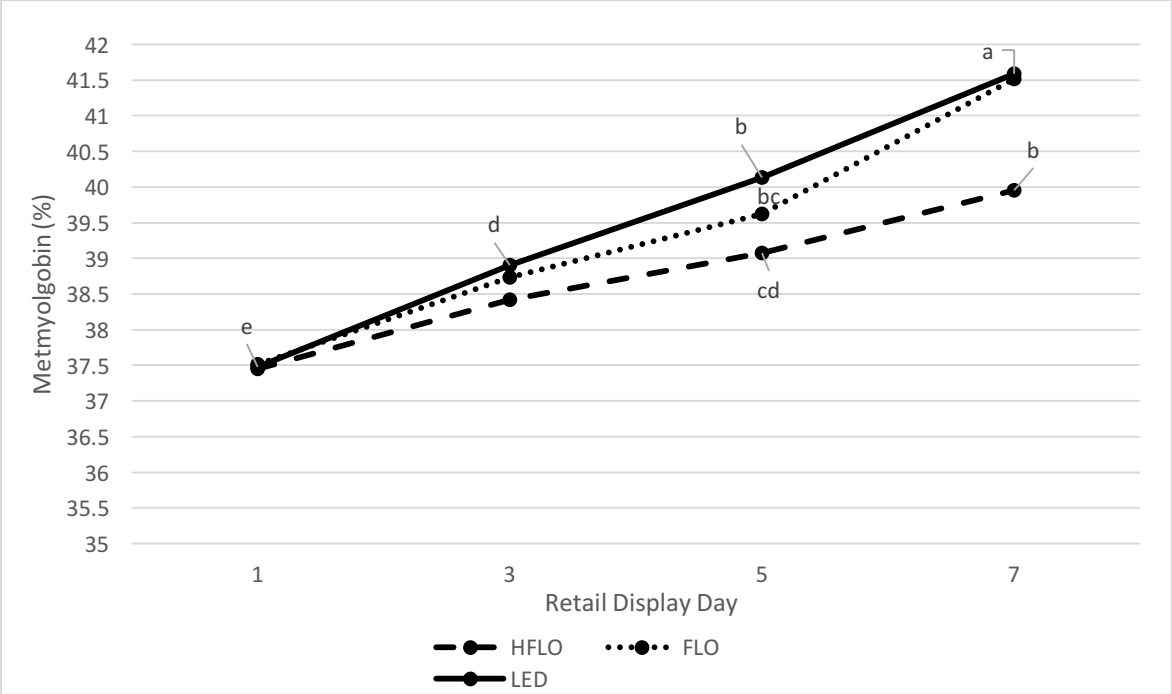
Calculated myoglobin concentrations. Differences ($P < 0.05$) in DMb concentration values occurred between light treatments as seen in Table 5.2. Over the duration of retail

display, DMb values decreased ($P < 0.05$) which can be seen in Table 5.3. These values were to be expected as oxygen exposure during retail display allows for the oxidation of DMb to MbO_2 and potentially MMb to occur (Faustman and Cassens, 1990). Oxymyoglobin is the bright, cherry red, desirable color in fresh beef products. As oxidation occurs, MbO_2 values decrease indicating that discoloration is occurring due to the formation of MMb on steak surface (Mancini and Ramanathan, 2014). Oxymyoglobin values differed between lighting treatments for moderately oxidative and color stable steaks (Table 5.2). Steaks displayed with both HFLO and FLO bulbs had greater ($P < 0.05$) mean MbO_2 values than steaks displayed with LED light sources. These values indicate that the use of LED light sources on moderately oxidative and color stable muscles promotes discoloration at greater levels than both high and low UV-fluorescent bulbs in retail display. Mean MbO_2 values decreased over the duration of retail display with differences ($P < 0.05$) occurring on day 7 on retail display which can be seen in Table 5.3. These values indicate that steaks produced from a muscle with moderate oxidative and color stability retained redness through day 5 of retail display before discoloration occurred.

Metmyoglobin is the product of the oxidation of MbO_2 which results in a brown, undesirable color on fresh beef products (Faustman and Cassens, 1990). As expected with decreases in MbO_2, values for MMb values increased for steaks under each light treatment over the duration of retail display which can be seen in Figure 5.1. By display day 5, LED displayed steaks had higher ($P < 0.05$) MMb concentrations than both HFLO and FLO displayed steaks. Indicating greater amounts of discoloration occurring in steaks displayed with LED lights. On day 7 of retail display, both LED and FLO displayed

steaks had higher ($P < 0.05$) MMb concentrations than those displayed with HFLO lights. Data indicates that the use of HFLO light sources on steaks with moderate color and oxidative stabilities promotes less oxidation and discoloration during retail display

Figure 5.1: Impact of light source[1] and retail display length on metmyoglobin concentrations of *Semimembranosus* steaks



[1] HFLO- High-UV fluorescent, FLO- Low-UV fluorescent, LED- light emitting diode
[abcd] Data lacking a common superscript differ $P < 0.05$

Measured myoglobin concentrations. Table 5.2 indicates no differences ($P > 0.05$) were found in myoglobin concentrations for steaks in all three light treatments. Retail display time also did not impact ($P > 0.05$) myoglobin concentrations as seen in Table 5.3. This could be attributed to the color and oxidative stability of the SM muscle (McKenna, 2005) as the amount of myoglobin in a product does not change data points to less myoglobin degradation occurring throughout retail display

Table 5.3: Effect of retail display day on color of beef *Semimembranosus* steaks (n=20)

	Retail Display Day					
Item	1	3	5	7	SEM	P-Value[1]
L*	42.91[a]	42.18[ab]	41.49[bc]	40.52[c]	0.57	0.0001
a*	24.52[a]	20.34[b]	19.35[c]	16.25[d]	0.50	<0.0001
b*	21.02[a]	19.73[b]	19.24[b]	17.57[c]	0.41	<0.0001
a/b[2]	1.18[a]	1.06[b]	0.98[c]	0.92[d]	0.02	<0.0001
SI[3]	32.33[a]	28.05[b]	27.68[b]	24.03[c]	0.60	<0.0001
HA[4]	40.55[d]	43.46[c]	45.85[b]	47.75[a]	0.52	<0.0001
DMb[5]	5.82[a]	4.85[b]	3.82[c]	3.39[d]	0.20	<0.0001
MbO_2[6]	56.70[a]	56.58[a]	56.46[a]	55.59[b]	0.19	<0.0001
MYO[7]	4.40	4.34	4.25	4.23	0.13	0.5078
MRA[8]	27.11[a]	15.39[b]	16.06[b]	12.38[b]	2.43	<0.0001
TBARS[9]	0.47[d]	1.06[c]	1.49[b]	1.95[a]	0.09	<0.0001

[1] P-Value of LS Means
[2] a/b- a/b ratio
[3] SI- Saturation Index
[4] HA- Hue Angle
[5] DMb- Deoxymyoglobin (%)
[6] MbO_2- Oxymyoglobin (%)
[7] MYO- Measured myoglobin concentrations (mg/g)
[8] MRA- Metmyoglobin reducing activity (%)
[9] TBARS – Thiobarbituric acid reactive substances (mg/kg)
[abcd] Data lacking a common superscript differ $P < 0.05$

Metmyoglobin reducing activity. Light treatment did not impact ($P > 0.05$) MRA values for steaks from the SM which can be found in Table 5.2. Metmyoglobin reducing activity was greater ($P < 0.05$) on day 1 of retail display than on days 3, 5, and 7 as seen in Table 5.3. Wu et al. (2015) reported that MRA values showed no changes in *Semitendinosus* steaks from retail display day 0 – 5; indicating color stability and redness retention. The use of SM as a muscle with moderate oxidative and color stability (McKenna et al., 2005) could attribute to the lack of variation of MRA values found between lighting treatment and during the duration of retail display.

Lipid oxidation. Light treatment played no role ($P > 0.05$) in lipid oxidation in steaks produced from the SM (Table 5.2). Since the SM was chosen for use due to its color and oxidative stability (McKenna, 2005), these results were expected. Cooper et al. (2016) found no differences in lipid oxidation as indicated by thiobarbituric acid reactive substances (TBARS) between ground beef under LED and fluorescent lighting, conflicting with results from Steele (2016) who found that patties displayed under LED lights had greater ($P < 0.05$) TBARS values than patties under fluorescent light sources. Steele et al., (2016) also found that steaks displayed under LED lights had greater amounts of lipid oxidation over the duration of retail display. Lipid oxidation increased with increases in retail display time ($P < 0.05$) with day $1 > 3 > 5 > 7$ which can be seen in Table 5.3. These results agree with findings (Martin et al. 2013; Colle et al. 2016; Cooper et al. 2016; Steele et al. 2016) who reported that increased retail display time results in increased lipid oxidation of fresh beef products. Data indicates that retail

display time has a greater impact on high color and oxidative stability muscles than light treatment does in comparison to color and oxidative labile cuts or as a ground product.

Relationship between color measurements and lipid oxidation. Strong negative correlations ($P < 0.0001$) occurred between TBARS and a* values indicating that increases in lipid oxidation results in product discoloration (Table 5.4). Moderate negative correlations occurred between TBARS and b* and DMb values ($P < 0.0001$). Conversely, a strong positive correlation ($P < 0.0001$) occurred between TBARS and MMb values. These correlations indicate that an increase in TBARS values correlates with a decrease in redness as indicated by a* values. Numerous reports of lipid oxidation and concurrent discoloration of fresh beef products have been made (Faustman and Cassens, 1990; Lynch et al., 1999; Renerre, 2000; Faustman et al., 2010;) As expected, increases in TBARS values positively correlated with MMb values indicating that increases in TBARS values and discoloration often occur simultaneously in fresh beef products.

Myoglobin reducing activity produced moderate negative correlations ($P < 0.05$) with L* and weak negative correlations ($P < 0.0001$) with both MMb and TBARS values. Moderately positive correlations ($P < 0.0001$) occurred between MRA and a* values. These values indicate that increases in MRA values correlate with decreases in TBARS and MMb concentrations as well as L* values indicating lightness of a product. As MRA is a measurement of color stability, positive correlations with a* are expected as increases in redness would be an indicator of color retention.

Strong correlations between objective color and calculated myoglobin values occurred, however, due to autocorrelation these values are not applicable.

5.4 Conclusion

The use of LED lights during retail display of steaks with high color and oxidative color stability promotes less red color retention and increased discoloration during retail display indicated by calculated a*, a/b ratios, SI and HA values; along with calculated MMb and MbO_2 concentrations than currently utilized lighting technologies. Light source did not impact lipid oxidation in steaks produced from the SM. Lipid oxidation as indicated by TBARS values increased over the duration of retail display indicating that lipid oxidation occurs with extended retail display time. With light source having limited impact on discoloration or oxidation rates of fresh meat products alternating light sources for meat quality aspects is not necessary.

Table 5.4. Simple correlation coefficients for various traits among beef *Semimembranosus* steaks (n=20)

Item	L*	a*	b*	DMb	MbO_2	MMb	TBARS	MYO	MRA	pH
L*	1.00									
a*	-0.28***	1.00								
b*	0.08	0.73***	1.00							
DMb[1]	-0.03	0.58[n.a.]	0.17[n.a.]	1.00						
MbO_2[2]	-0.13*	0.44[n.a.]	-0.45[n.a.]	-0.23**	1.00					
MMb[3]	0.12	-0.83[n.a.]	-0.45[n.a.]	-0.56***	-0.31	1.00				
TBARS[4]	0.15*	-0.72***	-0.45***	-0.56***	-0.31	0.72***	1.00			
MYO[5]	-0.13*	0.01	-0.08	-0.11	0.14*	0.01	0.05	1.00		
MRA[6]	-0.50***	0.51***	0.24***	0.31***	0.09	-0.34***	-0.26***	-0.001	1.00	
pH	-0.54***	0.20*	-0.11	0.27***	-0.10	-0.18*	-0.08	0.06	0.40***	1.00

[n.a.] – non-applicable data due to auto-correlated values
[1] DMb- Deoxymyoglobin (%)
[2] MbO_2- Oxymyoglobin (%)
[3] MMb- Metmyoglobin (%)
[4] TBARS- Thiobarbituric acid reactive substances (mg/kg)
[5] MYO- Measured myoglobin concentrations (mg/g)
[6] MRA- Metmyoglobin reducing activity (%)
*- $P < 0.05$
**- $P < 0.001$
***- $P < 0.0001$

Chapter 6

Conclusion

Grinding and total fat percentage of meat products has an increased impact on oxidative potential. Use of energy efficient light sources, such as LED bulbs, on ground beef products resulted in prolonged retail display life as indicated by greater a* values and MbO_2 concentrations. The use of these bulbs could prove to be useful both from an energy efficiency standpoint as well as retail display life of ground beef products.

Little to no benefit in retail display life was found in beef steaks produced from both a moderate and low color stability muscle displayed under LED lights. The use of HFLO light sources resulted in the greatest amount of redness retention indicated by a* values and MbO_2 concentrations in beef steaks of both moderate and low color stabilities. Retail display light sources had a greater impact on a low color stability muscle (TB), with lipid oxidation levels for all light sources being above rancidity thresholds by day three of retail display. There were no differences in lipid oxidation levels for steaks from a muscle with moderate color stability (SM).

APPENDIX A: MATERIALS AND METHODS

Appendix A. 1. Ground Beef Patty Preparation and Packaging

1.) USDA Select grade top rounds were purchased and brought to the University of Missouri Meat Laboratory.

2.) Rounds were aged for 20 days before processing.

3.) All external subcutaneous fat was trimmed from each round and placed aside for further use.

4.) Lean was coarse ground through a 10 mm kidney grinding plate.

5.) Fat was coarse ground through a 10 mm kidney grinding plate.

6.) Fat was added to lean on a weight basis to produce two batches of ground beef (5%, 25%) from each round.

7.) Fat and lean combinations were then mixed and reground through a 4.5 mm grinding plate.

8.) Patties were made using a patty press.

9.) Patties were packaged on white Styrofoam trays and overwrapped with oxygen permeable polyvinyl chloride.

10.) Patties were labeled according to light treatment (dark, fluorescent, LED) and retail display removal day (1, 3, 5, 7).

11.) Patties were immediately placed into their respective deli case immediately with all windows blacked out after packaging and labeling.

Grinder Info:

#8 Meat Grinder .35 H, LEM Products, West Chester, OH

Appendix A. 2. TB Steak Preparation and Packaging

1.) Clod hearts were purchased and brought to the University of Missouri Meat laboratory.

2.) Product was aged for 20 days post packaged date.

3.) After aging, clod hearts were removed from packaging and sliced, using a bacon slicer to produce 12, 1.9 cm steaks.

4.) Steaks were placed on black Styrofoam trays and overwrapped immediately with oxygen permeable polyvinyl chloride.

5.) Steaks were labeled according to light treatment (HFLO, FLO, LED) and retail display removal day (1, 3, 5, 7).

6.) Steaks were placed into their respective deli case immediately after packaging and labeling.

Appendix A. 3. SM Steak Preparation and Packaging

1.) Top rounds were purchased and brought to the University of Missouri Meat laboratory.

2.) Product was aged for 20 days post packaged date.

3.) After aging, top rounds were removed from packaging and sliced, using a bacon slicer to produce 12, 2.54 cm steaks.

4.) Steaks were placed on black Styrofoam trays and overwrapped immediately with oxygen permeable polyvinyl chloride.

5.) Steaks were labeled according to light treatment (HFLO, FLO, LED) and retail display removal day (1, 3, 5, 7).

6.) Steaks were placed into their respective deli case immediately after packaging and labeling.

Appendix A. 4. Objective Color Determination

1.) Fresh meat samples were removed from coolers and immediately transported to the research lab for analysis.

2.) Hunter MiniScan 45/0 colorimeter was equipped with a 25 mm aperture and a D65 light source.

3.) Standardize the colorimeter using the supplied black and white standardizing discs.

4.) Take product surface reading through polyvinyl chloride overwrap in three different locations.

5.) Using the colorimeter, save the calculated average of all three readings for objective color values (*L*, a*, b*).*

Appendix A. 5. Calculated Percentage Myoglobin Concentrations

1.) Equip the Hunter MiniScan 45/0 colorimeter with a 25 mm aperture.

2.) Standardize the colorimeter using the supplied black and white standardizing discs.

3.) Take product surface reading through polyvinyl chloride overwrap in three different locations

4.) Using the colorimeter, save the calculated average of all three readings for objective color values (*L*, a*, b*).*

5.) After average objective color readings are saved, use the following equations to calculate myoglobin concentrations via isobetic wavelengths.

a.) $A = \log 1/R$

b.) $\%MMb = \{1.395 - [(A572 - A730)/(A525 - A730)]\} \times 100$

c.) $\%DMb = \{2.375 \times [(A473 - A730)/(A525 - A730)]\} \times 100$

d.) $\%MbO_2 = 100 - (\%MMb + \%DMb)$

Appendix A. 6. pH Determination

1.) 5 g of fresh meat sample was homogenized with 50 ml of DI water for 30 seconds.

2.) After homogenization, pH of homogenate was determined using a benchtop pH probe.

3.) pH was measured in duplicate on each sample and values were averaged to determine overall sample pH.

Polytron Info:

Polytron 10-35 GT, Kinematica, Bohemia, New York

Appendix A. 7. Fat and Moisture Determination.

1.) Two CEM sample pads were dried inside a CEM SMART Trac rapid analysis system.

2.) After sample pads were dried, 3.75 – 4.5 g of fresh meat sample was diced and placed directly on top of one sample pad.

3.) Sample was smeared across pad and sandwiched between remaining pad.

4.) Moisture was determined via CEM Moisture/Solids analyzer on a weight basis.

5.) Following moisture determination, samples were wrapped in TRAC paper and placed inside a CEM TRAC tube.

6.) Tubes containing dried samples were placed into the CEM Rapid Fat Analyzer and fat percentage was determined on a dry basis using NMR and ultimately converted to a wet basis.

7.) Fat and moisture determinations were completed in triplicate for each sample. Values were averaged to determine overall moisture and fat percentage values for each sample.

Appendix A. 8. Measured Myoglobin Concentrations.

1.) 2.5 g of fresh meat sample was homogenized with 22.5 ml of ice cold sodium phosphate buffer.

2.) After homogenization, homogenate was filtered through Whatman #4 filter paper into a plastic tube.

3.) Filtrate was measured at 525 nm on a spectrophotometer and myoglobin concentrations using the equation below.

$$\textbf{Myoglobin (mg/g)}=[A_{525}/(7.6\ mM^{-1}\ cm^{-1}\times 1\ cm)]\ x\ [17{,}000/1000]\times 10$$

4.) Myoglobin concentrations were determined in duplicate, values were average to determine overall myoglobin concentrations per sample.

Solutions:

5.3 g of Sodium Phosphate Monobasic- Fisher Chemical S369-500

1 L Ice Cold Water

Polytron Info:

Polytron 10-35 GT, Kinematica, Bohemia, New York

Appendix A. 9. Metmyoglobin Reducing Activity

1.) Steaks surface were removed for evaluation.

2.) Two (TB) or three (SM) center portions were removed from steak surface.

3.) Samples were placed in individual weigh boats and submerged in sodium nitrite solution for 20 minutes.

4.) After incubation, samples were individually vacuum packaged and objective color was measured in triplicate on each sample with a HunterLab MiniScan 45/0.

5.) Samples were left at room temperature for 2 h.

6.) After 2 h, objective color was taken in triplicate on each sample.

7.) MRA was calculated using the following equation.

Solutions:

6 g of Sodium Nitrite- Fisher Chemical S347-500

2 L Water

Appendix A. 10. Lipid Oxidation.

1.) 5 g of sample was homogenized with 25 ml of DI water in a disposable tube.

2.) After homogenization, sample was transferred to a 250 ml Kjedhal flask.

3.) Disposable flask was rinsed with 25 ml of DI water and added to Kjedhal flask.

4.) 2.5 ml of 4M HCl was added to flask to balance sample pH.

5.) Two drops of antifoam solution was added to each flask before heating.

6.) Flasks were placed into heating mantles and samples were distilled through a water-cooled distillation apparatus until 25 ml of distillate was present in a graduated cylinder.

7.) 5 ml of distillate was combined with 5 ml of TBA reagent in a glass tube.

8.) Tubes were placed in a boiling water bath for 35 minutes.

9.) After removal from boiling water, samples were immediately placed in an ice bath for 10 minutes.

10.) Samples were read on a spectrophotometer at 538 nm.

11.) Values obtained from the spectrophotometer were multiplied by 7.8 to determine malonaldehyde (mg/kg) concentrations.

12.) Samples were evaluated in duplicate and averaged to determine total lipid oxidation levels.

Solutions:

900 mL Glacial Acetic Acid- Fisher Chemical A38C-212 added to 100 mL water

2.228 g/L 2- Thiobartbituric Acid- Sigma Aldrich T5500-100G

Polytron Info:

Polytron 10-35 GT, Kinematica, Bohemia, New York

APPENDIX B: SAS Programs

Appendix B. 1. Ground Beef SAS Editor

```
options ls=95 ps=70 pageno=1;
data one; infile 'e:\gbdatafinal.csv' dsd firstobs=2 missover;
input rnd light$ perf  d  l  a      b      mmb      dmb      omb      tba t h dew k ha si ab;
proc print;
proc corr;

proc glimmix; class rnd light perf d;
model l=light|perf|d;
random rnd;
lsmeans light|perf|d/pdiff lines;

proc glimmix; class rnd light perf d;
model a=light|perf|d;
random rnd;
lsmeans light|perf|d/pdiff lines;

proc glimmix; class rnd light perf d;
model b=light|perf|d;
random rnd;
lsmeans light|perf|d/pdiff lines;

proc glimmix; class rnd light perf d;
model mmb=light|perf|d;
random rnd;
lsmeans light|perf|d/pdiff lines;

 proc glimmix; class rnd light perf d;
model dmb=light|perf|d;
random rnd;
lsmeans light|perf|d/pdiff lines;

proc glimmix; class rnd light perf d;
model omb=light|perf|d;
random rnd;
lsmeans light|perf|d/pdiff lines;

proc glimmix; class rnd light perf d;
model tba=light|perf|d;
random rnd;
lsmeans light|perf|d/pdiff lines;

proc glimmix; class rnd light perf d;
model k=light|perf|d;
random rnd;
```

```
lsmeans light|perf|d/pdiff lines;

proc glimmix; class rnd light perf d;
model si=light|perf|d;
random rnd;
lsmeans light|perf|d/pdiff lines;

proc glimmix; class rnd light perf d;
model ha=light|perf|d;
random rnd;
lsmeans light|perf|d/pdiff lines;

proc glimmix; class rnd light perf d;
model ab=light|perf|d;
random rnd;
lsmeans light|perf|d/pdiff lines;

run;
```

Appendix B. 2. TB SAS Editor

```
options ls=95 ps=70 pageno=1;
data one; infile 'e:\tbdatacorr.csv' dsd firstobs=2 missover;
input tb light$  d  l  a      b      mmb      dmb      omb      tba myo mra ph f moist flu ab si
ha;
proc print;
proc corr;
VAR l  a      b      mmb      dmb      omb      tba myo mra ph f moist flu ab si ha;

proc glimmix; class tb light d;
model l=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model a=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model b=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model dmb=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model omb=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model mmb=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model tba=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
```

```
model myo=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model mra=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model ph=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model f=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model moist=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model flu=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model ab=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model si=light|d;
random tb;
lsmeans light|d/pdiff lines;

proc glimmix; class tb light d;
model ha=light|d;
random tb;
lsmeans light|d/pdiff lines;

run;
```

Appendix B. 3. SM SAS Editor

```
options ls=95 ps=70 pageno=1;
data one; infile 'e:\smdatacorr.csv' dsd firstobs=2 missover;
input sm light$  d  l  a      b      dmb      omb      mmb      tba myo mra ph f moist flu ab si
ha;
proc print;
proc corr;
VAR l  a      b      dmb      omb      mmb      tba myo mra ph f moist flu ab si ha;

proc glimmix; class sm light d;
model l=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model a=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model b=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model dmb=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model omb=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model mmb=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model tba=light|d;
random sm;
lsmeans light|d/pdiff lines;
```

```
proc glimmix; class sm light d;
model myo=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model mra=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model ph=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model f=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model moist=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model flu=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model ab=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model si=light|d;
random sm;
lsmeans light|d/pdiff lines;

proc glimmix; class sm light d;
model ha=light|d;
random sm;
lsmeans light|d/pdiff lines;

run;
```

LITERATURE CITED

Aberle, E. D., J. C. Forrest, D. E. Gerrard, and E. W. Mills. 2012. Principles of meat science. 5th rev. ed. Kendall Hunt Publishing. Dubuque, IA.

AMSA. 2012. Meat Color Measurement Guidelines. American Meat Science Association. Champaign, IL.

Atkinson, J. J., and M. J. Follett. 1973. Biochemical studies on the discoloration of fresh meat. Food Technology. 8:51-58.

Balentine, C. W., P. G. Crandall, C. A. O'Bryan, D. Q. Duong, and F. W. Pohlman. 2006. The pre- and post-grinding application of rosemary and its effects on lipid oxidation and color during storage of ground beef. Meat Sci. 73 (3):413-421. doi:10.1016/j.meatsci.20005.12.003

Barbut, S. 2001. Effect of illumination source on the appearance of fresh meat cuts. Meat Sci. 59:187-191.

Baron, C. P., and H. J. Anderson. 2002. Myoglobin-induced lipid oxidation. a review. Agric. Food Chem. 50:3887-3897. doi:10.1021/jf011394w

Bekhit, A. E. D., G. H. Geesink, J. D. Morton, and R. Bickerstaffe. 2001. Metmyoglobin reducing activity and colour stability of ovine longissimus muscle. Meat Sci. 57:427-435.

Bekhit, A. E. and C. Faustman. 2005. Metmyoglobin reducing activity. Meat Sci. 71(3):407-439. doi:10.1016/j.meatsci.2005.04.032

Beltran, E., Pla, R., Yuste, J., & Mor-Mur, M. 2003. Lipid oxidation of pressurized and cooked chicken: Role of sodium chloride and mechanical processing on TBARS and hexanal values. Meat Sci. 64:19–25. doi:10.1016/S0309-17(02)00132-8

Berčík, J., E. Horská, R. W. Y. Wang, and Y.C. Chen. 2016. The impact parameters of store illumination on food shopper response. Appetite. 106:101-109. doi:10.1016/j.appet.2016.04.010

Boccard, R. L., R. T. Naudé, D. E. Cronje, M. C. Smit, H. J. Venter, and E. J. Rossouw. 1979. The influence of age, sex and breed of cattle on their muscle characteristics. Meat Sci. 3(4):261-280. doi:10.1016/0309-1740(79)90003-2

Bruce, H. L., Stark, J. L., & Beilken, S. L (2004). The effects of finishing diet and postmortem ageing on the eating quality of the M. longissimus thoracis of electrically stimulated Brahman steer carcasses. Meat Science, 67(2), 261–268.

Canto, A. C. V. C. S., B. R. C. Costa-Lima, S. P. Suman, M. L. G. Monteiro, F. M. Viana, A. P. A. A. Salim, M. N. Nair, T. J. P. Silva, and C. A. Conte-Junior. 2016. Color attributes and oxidative stability of longissimus lumborum and psoas major muscles from nellore bulls. Meat Sci. 121: 19-26. doi:10.1016/j.meatsci.2016.05.015

Carpenter, C. E., D. P. Cornforth, and D. Whittier. 2001. Consumer preferences for beef color and packaging did not affect eating satisfaction. Meat Sci. 57: 359-363

Chappin, E. J. L., and M. R. Afman. 2013. An agent-based model of transitions in consumer lighting: policy impacts from the E.U. phase-out of incandescents. Env. Innovation and Societal Trans. 7:16-36. doi:10.1016/j.eist.2012.11.005

Colle, M. J., R. P. Richard, K. M. Killinger, J. C. Bohlscheid, A. R. Gray, W. I. Loucks, R. N. Day, A. S. Cochran, J. A. Nasados and M. E. Doumit. 2016. Influence of extended aging on beef quality characteristics and sensory perception of steaks

from the *biceps femoris* and *semimembranosus.* Meat Sci. 119:110-117. doi:10.1016/j.meatsci.2016.04.028

Cooper, J. V., B. R. Wiegand, A. B. Koc, L. Schumacher, I. Gruën, and C. L. Lorenzen. 2016. Impact of contemporary light sources on oxidation of fresh ground beef. Anim. Sci. 941-6. doi:10.2527./jas2016-0728

Cooper, J. V., B. R. Wiegand, A. B. Koc, L. Schumacher, and C. L. Lorenzen. 2015. Effect of different light sources on discoloration of fresh ground beef. J. Anim. Sci 98(Suppl.1):193.

Dean, R. W. and Ball, C. D. 1960. Analysis of the myoglobin fractions on the surfaces of beef cuts. Food Technol. 14:271.

Demos, B. P., and R. W. Mandigo. 1996. Color of fresh, frozen and cooked ground beef patties manufactured with mechanically recovered neck bone lean. Meat Sci. 42(4):415-429. doi:10.1016/0309-1740(95)00046-1

Descalzo, A. M., L. Rossetti, G. Grigioni, M. Irurueta, A. M. Sancho, J. Carrete, and N. A. Pensel. 2007. Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle. Meat Sci. 75(2):299-307. doi:10.1016/j.meatsci.2006.07.015

Diaz, P., M. B. Linares, M. Egea, S. M. Augui, and M. D. Garrido. 2014. TBARs distillation method: revision to minimize the interference from yellow pigments in meat products. Meat Sci. 98: 569-573. doi:10.1016/j.meatsci.2014.06.012

Djenane, D., A. Sánchez-Escalante, J. A. Beltrán and P. Roncalés. 2001. Extension of the Retail Display Life of Fresh Beef Packaged in Modified Atmosphere by Varying Lighting Conditions. J. Food Sci. 66(1): 181-186.

Dow D. L., B. R. Wiegand, M. R. Ellersieck, C. L. Lorenzen. 2011. Prediction of fat percentage within marbling score on beef longissimus muscle using 3 different fat determination methods. J. Anim. Sci. 89, 1173-1179. doi:10.2527/jas.2010-3382

Dunne, P. G., F. J. Monahan, F. P. O'mara, and A. P. Moloney. 2005. Colour stability, under simulated retail display conditions, of M. longissimus dorsi and M. semimembranosus from steers given long-term daily exercise and supplemented with vitamin E. Meat Sci. 71(3):480-489. doi:10.1016/j.meatsci.2005.04.025

Echevarne, C., M. Renerre, and R. Labas. 1990. Metmyoglobin reductase activity in bovine muscles. Meat Sci. 27(2):161-172. doi:10.1016/0309-1740(90)90063-C

English, A. E., G. G. Mafi, D. L. VanOverbeke, and R. Ramanaathan. 2016. Effects of extended aging and modified atmosphere packaging on beef top loin steak color. Anim. Sci. 94:1727-1737. doi:10.2527/jas2015-0149

Estévez, M. 2011. Protein carbonyls in meat systems: a review. Meat Sci. 89(3):259-279. doi:10.1016/j.meatsci.2011.04.025

Falowo, A. B., P. O. Fayemi, and V. Muchenje. 2014. Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: a review. Food Res. 64:171-181. doi:10.1016/j.foodres.2014.06.022

Faustman, C., and R. G. Cassens. 1990. The biochemical basis for discoloration in fresh meat: a review. Musc. Foods. 1:217-243.

Faustman, C., Q. Sun, R. Mancini, and S. Suman. 2010. Myoglobin and lipid oxidation interactions: mechanistic bases and control. J. Meat Sci. 86:86-94. doi: 10.1016/j.meatsci.2010.04.025

Ferguson, D. M., & Warner, R. D. (2008). Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? Meat Sci. 80(1):12–19. doi:10.1016/j.meatsci.2008.05.004.

Fernando, L. N., E. P. Berg, and I. U. Grün. 2013. Quantitation of hexanal by automated SPME for studying dietary influences on the oxidation of pork. J. Food Composition 16(2): 179- 88. doi:10.106/S0889-1575(02)00173-4

Ferioli, F., M. F. Caboni, and P. C. Dutta. 2008. Evaluation of cholesterol and lipid oxidation in raw and cooked minced beef stored under oxygen-enriched atmosphere. Meat Sci. 80(3):681-685. doi:10.1016/j.meatsci.2008.03.005

Garner, C. M., J. A. Unruh, M. C. Hunt, E. A. E. Boyle, and T. A. Houser. 2014. Effects of subprimal type, quality grade, and aging time on display color of ground beef patties. Meat Sci. 98:301-309. doi:10.1016/j.meatsci.2014.05.004

Girard, I., J. L. Aalhus, J. A. Basarab, .I. L. Larsen, and H. L. Bruce. 2012. Modification of beef quality through steer age at slaughter, breed cross and growth promotants. Can. J. Anim. Sci. 92:175-188. doi:10.4141/CJAS2012-001

Greene, B. E., I. M. Hsin, and M. Y. W Zipser. 1971. Retardation of oxidative color changes in raw ground beef. J. Food Sci. 36: 940-942.

Hamling, A. E., B. E. Jenschke, and C. R. Calkins. 2008. Effects of dark storage and retail display on beef chuck and round muscles enhanced with ammonium hydroxide, salt, and carbon monoxide. Anim. Sci. 86:972-981. doi:10.2527/jas.2007-0394

Hayes, N. S., C. A. Schwartz, K. J. Phelps, P. Borowicz, K. R. Maddock- Carlin, and R. J. Maddock. 2015. The relationship between pre-harvest stress and the carcass

characteristics of beef heifers that qualified for kosher designation. Meat Sci. 100:134-138. doi:10.1016/j.meatsci.2014.09.145

Holman, B. W. B., Y. Mao, C. E. O. Coombs, R. J. van de Ven, and D. L. Hopkins. 2016. Relationship between colorimetric (instrumental) evaluation and consumer-defined beef colour acceptability. Meat Sci. 121:104-106. doi:10.1016/j.meatsci.2016.05.002

Holman, B. W. B., R. J. van de Ven, Y. Mao, C. E. O. Coombs, and D. L. Hopkins. 2017. Using instrumental (CIE and reflectance) measures to predict consumers acceptance of beef colour. Meat Sci. 127: 57-62. doi:10.1016/ j.meatsci.2017.01.005

Jeremiah, L. E. and L. L. Gibson. 2001. The influence of storage temperature and storage time on color stability, retail properties, and case-life of retail-ready beef. J. Food Research Int. 34: 815-826. doi:10.1016/S0963-9969(01)00104-1

Jiang, J., and Y. L. Xiong. 2016. Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: a review. Meat Sci. 120:107-117. doi:10.1016/j.meatsci.2016.04.005

Joseph, P., S. P. Suman, G. Rentfrow, S. Li, and C. M. Beach. 2012. Proteomics of muscle-specific beef color stability. Agric. Food Chem. 60:3196-3203. doi:10.1021/jf204188vl

Juárez, M., M. E. R. Dungan, J. L. Aalhus, N. Aldai, J. A. Basarab, V. S. Baron, and T. A. McAllister. 2011. Effects of vitamin E and flaxseed on rumen-derived fatty acid intermediates in beef intramuscular fat. Meat Sci. 88(3):434-440. doi:10.1016/j.meatsci.2011.01.023

Kapper, C., Walukonis, C., Scheffler, T., Scheffler, J., Don, C., Morgan, M., & Gerrard, D. (2014). Moisture absorption early postmortem predicts ultimate drip loss in fresh pork. Meat Sci. 96(2):971–976. doi:10.1016/j.meatsci.2013.10.009

Kilic, B., A. Simsek, J. R. Claus, and E. Atilgan. 2014. Encapsulated phosphates reduce lipid oxidation in both ground chicken and ground beef during raw and cooked meat storage with some influence on color, pH and cooking loss. Meat Sci. 97(1):93-103. doi:10.1016/j.meatsci.2014.01.014

Kim, Y. H., E. Huff- Lonergran, J. G. Sebranek, and S. M. Lonergran. 2010. High-oxygen modified atmosphere packaging system induces lipid and myoglobin oxidation and protein polymerization. Meat Sci. 85(4):759-767. doi:10.1016/j.meatsci.2010.04.001

King, D. A., S. D. Shackelford, A. B. Rodriguez, and T. L. Wheeler. 2011. Effect of time measurement on the relationship between metmyoglobin reducing activity and oxygen consumption to instrumental measures of beef longissimus color stability. Meat Sci. 87:26-32. doi:10.1016/j.meatsci.2010.08.013

Kropf, D. H. 1980. Effects of retail display conditions on meat color. Reciprocal Meats Conference Proceedings. 33: 15-32.

Lanari, M. C. and R. G. Cassens. 1991. Mitochondrial activity and beef muscle color stability. Food Sci. 56(6):1476-1479. doi:10.111/j.1365-2621.1991.tb08619.x

Lavieri, N., and S. K. Williams. 2014. Effects of packaging systems and fat concentrations on microbiology, sensory and physical properties of ground beef stored at 4 +/- 1 ºC for 25 days. Meat Sci. 97(4):534-541.doi:10.1016 /j.meatsci.2014.02.014

Limbo, S., L. Torri, N. Sinelli, L. Franzetti, and E. Casiraghi. 2010. Evaluation and predictive modeling of shelf life of minced beef stored in high-oxygen modified atmosphere packaging at different temperatures. Meat Sci. 84(1):129-136. doi:10.1016/j.meatsci.2009.08.035

Liu, J., A. Arner, E. Puolanne, and P. Ertbjerg. 2016a. On the water-holding of myofibrils: effect of sarcoplasmic protein denaturation. Meat Sci. 119:32-40. doi:10.1016/ j.meatsci.2016.04.020

Liu, J., W. Zhang, X. Chu, and Y. Liu. 2016b. Fuzzy logic controlled for energy savings in a smart LED lighting system considering lighting comfort and daylight. Energy and Buildings. 127:95-104. doi:10.1016/j.enbuild.2016.05.066

Lund, M. N., R. Lametsch, M. S. Hvidd, O. N. Jensen, and L. H. Skibsted. 2007. High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine longissmus dorsi during chill storage. Meat Sci. 77(3):295-303. doi:10.1016/j.meastci.2007.03.016.

Lynch, M. P., J. P. Kerry, D. J. Buckley, C. Faustman, and P. A. Morrissey. 1999. Effect of dietary vitamin e supplementation on the colour and lipid stability of fresh, frozen and vacuum-packaged beef. Meat Sci. 52:95-99. doi:10.1016/S0309-1740(98)00153

Lyte, J. M., J. F. Legako, J. N. Martin, L. Thompson, K. Surowiec, and J. C. Brooks. 2016. Volatile compound characterization of modified atmosphere packaged ground beef held under temperature abuse. Food Control. 59:1-6. doi:10.1016./j.foodcont.2015.04.041

Martin, J. N., J. C. Brooks, T. A. Brooks, J. F. Legako, J. D. Starkey, S. P. Jackson, and M. F. Miller. 2013. Storage length, storage temperature, and lean formulation influence the shelf-life and stability of traditionally packaged ground beef. Meat Sci. 95:495-502. doi:10.1016/j.meatsci.2013.05.032

Martin, N., N. Carey, S. Murphy, D. Kent, J. Bang, T. Stubbs, M. Wiedmann, and R. Dando. 2016. Exposure of fluid milk to LED light negatively affects consumer perception and alters underlying sensory properties. Dairy Sci. 99:4309-4324. doi:10.3168/jds.2015-9603

Martinez, L. I. Cilla, J. A. Beltran, and P. Roncales. 2007. Effect of illumination on the display life of fresh pork sausages packaged in modified atmosphere. Influence of the addition of rosemary, ascorbic acid and black pepper. Meat Sci. 75:443-450. doi:10.1016/j.meatsci.2006.07.021

Mancini, R. A. and M. C. Hunt. 2005. Current research in meat color. Meat Sci. 71:100-121. doi:10.1016/j.meatsci.2005.03.003

Mancini, R. A. and R. Ramanathan. 2014. Effects of postmortem storage time on color and mitochondria in beef. Meat Sci.98:65-70. doi:10.1016/j.meatsci.2014.04.007

McKeith, R. O., D. A. King, A. L. Grayson, S. D. Shackelford, K. B. Gehring, J. W. Savell, and T. L. Wheeler. 2016. Mitochondrial abundance and ef ciency contribute to lean color of dark cutting beef. Meat Sci. 116:165–173. doi:10.1016/j.meat- sci.2016.01.016

McKenna, D. R., P. D. Mies, B. E. Baird, K. D. Pfeiffer, J. W. Ellebracht, J. W. Savell. 2005. Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. Meat Sci. 70:665-682. doi:10.1016/j.meatsci.2005.02.016

McMillin, K.W. 2008. Where is MAP Going? A review and future potential of modified atmosphere packaging for meat. Meat Science. 80(1):43-65. doi:10.1016/j.meatsci.2008.05.028

Min, B., K. C. Nam, and D. U. Ahn. 2010.Catalytic mechanisms of metmyoglobin on the oxidation of lipids in phospholipid liposome model system. J. Food Chem. 123: 231-236. doi:10.1016/j.foodchem.2010.04.013

Montgomery, J. l., F. C. Parrish Jr., D. G. Olson, J. S. Dickson, and S. Niebuhr. 2003. Storage and packaging effects on sensory and color characteristics of ground beef. Meat Sci. 64: 357-363.

Morrissey, P. A., P. J. A. Sheehy, K. Galvin, J. P. Kerry, and D. J. Buckley. 1998. Lipid stability in meat and meat products. Meat Sci.49(1):S73-S86. doi:10.1016/S0309-1740(8)90039-0

Nair, M. N., S. P. Suman, M. K. Chatil, S. Li, P. Joseph, C. M. Beach and G. Rentfrow. 2016. Proteome basis for intramuscular variation in color stability of beef semimembranosus. Meat Sci. 113:9-16. doi:10.1016/j.meastci.2015.11.003

O' Grady, M. N., F. J. Monahan, R. M. Burke, and P. Allen. 2000. The effect of oxygen level and exogenous α-tocopherol on the oxidative stability of minced beef in modified atmosphere packs. Meat Sci. 55: 39-45. doi:10.1016/S0309-1740(99)00123-0

O'Keefe, M. O., and D. E. Hood. 1982. Biochemical factors influencing metmyoglobin formation on beef from muscles of differing color stability. Meat Sci. 7:209-228.

Ordway, G. A. and D. J. Garry. 2004. Myoglobin: an essential hemoprotein in striated muscle. Experimental Bio. 207:3441-3446. doi:10.1242/jeb.01172

Paul, C. G., H. Paulette, L. Hyun-joo, K. Mihyun, C. Yeasun, L. Jerrold, and S. Lisa. 2014. Seeing the light: consumer perceptions of ground beef packages. J. Management 4(4): 77-89. doi: 10.5923/j.mm.20140404.01

Pearce, K. L., K. Rosevold, H. J. Anderson, and D. L. Hopkins. 2011. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes – a review. Meat Sci. 89:111-124. doi:10.1016/j.meatsci.2011.04.007

Pouzo, L. B., N. E. Zaritzky, E. Pavan, L. Rossetti, and A. M. Descalzo. 2016. Utilization of fluorescence spectroscopy as a novel approach to evaluate the oxidative stability in beef retail displayed. Meat Sci. 119:7-13. doi:10.1016 /j.meatsci.2016.03.027

Raines, C. R., M. C. Hunt, and J. A. Unruh. 2009. Cow biological type affects ground beef color stability. J. Meat Sci. 83: 752-758. doi:10.1016/j.meatsci.2009.08.032

Raines, C. R., Hunt, M. C., & Unruh, J. A. (2010). Contributions of muscles of various color stabilities to the overall color stability of ground beef. Food Science. 75: 85–89.

Ramanathan, R., R. A. Mancini, P. Joesph, and S. P. Suman. 2013. Bovine mitochondrial oxygen consumption effects on Oxymyoglobin in the presence of lactate as a substrate for respiration. Meat Sci. 93: 893-897. doi:10.1016/ witj.meatsci.2012.12.005

Reddy, I. M., and C. E. Carpenter. 1991. Determination of metmyoglobin reductase activity in bovine skeletal muscles. Food Sci. 56:1161-1164.

Renerre, M., & Labas, R. (1987). Biochemical factors influencing metmyoglobin formation in beef muscles. Meat Science. 19:151–165.

Renerre, M. 1990. Factors involved in the discoloration of beef meat. International J. Food Science & Technology 25(6): 613-630.

Renerre, M. 2000. Oxidative processes and myoglobin. In: Wood, J. G, R. I. Richardson, G. R. Nute, A. V. Fisher, M. M. Campo, E. Kasapidou, P. R. Sheard, and M. Enser. 2003. Effects of fatty acids on meat quality: a review. Meat Sci 66:21-32

Rogers, H. B., J. C. Brooks, J. N. Martin, A. Tittor, M. F. Miller, and M. M. Brashears. 2014. The impact of packaging system and temperature abuse on the shelf life characteristics of ground beef. Meat Sci. 97(1):1-10. doi:10.1016/ j.meatsci.2013.11.020

Romero, M. H., L. F. Uribe-Velásquez, J. A. Sanchez, and G. C. Miranda-de la Lama. 2013. Risk factors influencing brusising and high muscle pH in Colombian cattle carcasses due to transport and pre-slaughter operations. Meat Sci. 95(2):256-263. doi:10.1016/j.meatsci.2013.05.014

Schleich, J., B. Mills, and E. Dütschke. 2014. A brighter future? Quantifying the rebound effect in energy efficient lighting. Energy Policy. 72:35-42. doi:10.1016/ j.enpol.2014.04.028

Smith, G. C., K. E. Belk, J. N. Sofos, J. D. Tatum and S. N. Williams. 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: 397−426. Chichester, England: John Wilet and Sons, Inc.

Steele, K. S., M. J. Weber, E. A. E. Boyle, M.C. Hunt, A. S. Lobaton-Sulabo, C. Cundith, Y. H. Hiebert, K. A. Abrolat, J.M. Attey, S.D. Clark, D. E. Johnson, & T. L. Roenbaugh. 2016. Shelf life of fresh meat products under LED or fluorescent lighting. J. Meat Sci. 117: 75-84. doi:10.1016/j.meatsci.2016.02.032

Strydom, P. E. and M. Hope-Jones. 2014. Evaluation of three vacuum packaging methods for retail beef loin cuts. Meat Sci. 98(4):689-694. doi:10.1016/ j.meastsci.2014.05.030

Suman, S. P., M. N. Nair, M. K. Chatli, S. Li, P. Joseph, C. M. Beach, and G. Rentfrow. 2014. Differential abundance of sarcoplasmic proteome in beef inside and outside semimembranosus muscle. Meat Sci. 96(1):490-491. doi:10.1016/j.meatsci.2013.07.0147

Tarladgis, B. G., B. M. Watts, N. T. Younathan, and L. Dugan. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. American Oil Chemists Society 37: 44–48.

United States Department of Energy (US DOE). 2016. Energy savings forecast of solid-state lighting in general illumination applications. (Avaliable from: https://energy.gov/sites/prod/files/2016/10/f33/energysavingsforecast16_0.pdf)

Utera, M., D. Morcudende, and M. Estévez. 2014. Temperature of frozen storage affects the nature and consequences of protein oxidation in beef patties. Meat Sci. 96(3):1250-1257. doi:10.1016/j.meatsci.2013.10.032

van den Oord, A. H. A., and J. J. Wesdorp. 1971. Colour rating and pigment composition of beef: relation between colour measurement by reflectance spectrophotometry, subjective colour evaluation, and the relative concentration of oxymyoglobin and

ferric myoglobin in chilled retail-packed beef. Food Sci and Tech. 6(1):15-20. doi:10.111/j.1365-2621.1971.tb01588.x

Von Seggern, D. D., C. R. Calkins, D. D. Johnson, J. E. Brickler, and B. L. Gwartney. 2005. Muscle profiling: characterizing the muscles of the beef chuck and round. Meat Sci. 71:39-51. doi:1016/j.meatsci.2005.04.010

Wegner, J., E. Albrecht, I. Fiedler, F. Teuscher, H. J. Papstein, and K. Ender. 2000. Growth- and breed-related changes of muscle fiber characteristics in cattle. Anim. Sci. 78(6):1485-1496.

Wood, J. D., G. R. Nute, A. V. Fisher, M. M. Campo, E. Kasapidou, P. R. Sheard, and M. Enser. 2003. Effects of fatty acids on meat quality: A review. Meat Sci. 66:21-32. doi:10.1016/S0309-1740(03)00022-6

Wood, J., G. Nute, R. Richardson, F. Whittington, O. Southwood, G. Plastow. 2004. Effects of breed, diet and muscle on fat deposition and eating quality in pigs. Meat Sci. 67: 651-657.

Wu, W., X. G. Gao, Y. Dai, Y. Fu, X. M. Li, and R.T. Dai. 2015. Post-mortem changes in sarcoplasmic proteome and its relationship to meat color traits in m. *semitendinosus* of Chinese *luxi* yellow cattle. Food Res. 72:98-105. doi:10.1016/j.foodres.2015.03.030

Xiong, Y. L., O. E. Mullins, J. F. Stika, J. Chen, S. P. Blanchard, and W. G. Moody. 2007. Tenderness and oxidative stability of post-mortem muscles from mature cows of various ages. Meat Sci. 77:105-113. doi:10.1016/j.meatsci.2007.04.012

VITA

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While at Oklahoma State, Jade was under the guidance of Dr. Deb VanOverbeke, who introduced Jade to meat science. After discovering her passion for the industry, Jade decided to continue her education with a masters degree in animal science. Jade was introduced to Dr. Carol Lorenzen by Dr. VanOverbeke. After completing a summer internship at the University of Missouri in Dr. Lorenzen's lab, Jade was accepted to complete a M.S. program beginning the following Spring semester. Jade graduated from Oklahoma State in December of 2014 and began her M.S. at the University of Missouri in January of 2015 under Dr. Lorenzen.