

CHARACTERIZATION OF COLOR STABILITY IN THE LONGISSIMUS DORSI, TRICEPS
BRACHII, AND PSOAS MAJOR FROM FRESH PORK

BY

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DISSERTATION

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ABSTRACT

Consumers discriminate against meat that appears brown in color, perceiving it to be spoiled or not fresh. While color stability in beef is well understood, differences in color stability among and within, muscles have received limited attention in pork. Therefore, the overall objectives of this work were to characterize quality and color stability of pork loin chops that were categorized as stable or labile, and to determine differences in color and biochemical characteristics among the longissimus dorsi (LD), triceps brachii (TB), and psoas major (PM) muscles of pork during simulated retail display.

Three total experiments were conducted for this work. For the first experiment, boneless pork loins (N=484) were evaluated for quality traits at 11 or 14 d postmortem. One chop was cut from each loin near the 10th rib for retail display, overwrapped, and displayed under constant fluorescent lighting for 7 d. Objective color, myoglobin (Mb) forms, and subjective visual discoloration traits were evaluated daily. After display, loins were categorized based on final visual discoloration (d7) as: Very Color Stable (VCS; 0 – 5% discoloration), Color Stable (CS; 5 – 10% discoloration), Neutral (NT; 10 – 25% discoloration), Color Labile (CL; 25 – 30% discoloration), or Very Color Labile (VCL; >30% discoloration). Quality and color traits were analyzed using the GLIMMIX (visual discoloration) or MIXED (all other measures) procedure of SAS. Retail display data were analyzed as repeated measures. Loins classified as CS or VCS were darker, less yellow, had greater pH values, greater marbling scores, decreased color score, and decreased purge loss compared to CL and VCL loins ($P < 0.01$); N loins were intermediate for each of these measurements ($P < 0.01$). No differences in aged redness were observed between groups ($P \geq 0.16$). A group x day interaction was observed for all traits measured during

retail display ($P < 0.0001$). Stable loins generally had increased redness and R630/580 as well as decreased visual discoloration scores, lightness, yellowness, and % MMb values during display.

For the second and third experiments, the LD, TB, and PM from the left sides of 20 pigs were used in this study. Muscles were aged in vacuum packages for 21 d, then fabricated to yield 3 chops per muscle. The pH of the most anterior chop from each muscle was measured. All chops from a single muscle were overwrapped and displayed for 5 d under constant lighting. On each display day, packages were evaluated for instrumental color, myoglobin forms and subjective visual discoloration. Chops were removed on d1, 3, or 5, to measure oxygen consumption (OC), metmyoglobin reducing activity (MRA), and lipid oxidation. Day 1 chops were also evaluated for myoglobin content. Data were analyzed using the MIXED procedure of SAS as a repeated measures. Pearson correlation coefficients for changes in measurements between the LD and other muscles were determined using the CORR procedure. Pearson correlation coefficients between initial and final traits were calculated for each muscle using the CORR procedure of SAS. Correlations were compared between the LD, TB, and PM using a Fisher's z test. Stepwise regression models for prediction of final visual discoloration or 630/580 nm ratio for each muscle were developed using the REG procedure. Over the 5d display period, TB and PM chops had greater changes in a^* , b^* , chroma, 630/580 nm ratio, DMb, and OMb compared to LD chops (display time x muscle interaction $P \leq 0.01$), but the TB and PM did not differ from each other ($P \geq 0.07$). Conversely, the LD had decreased OC and increased MRA on each display day compared to the TB and PM ($P < 0.01$). However, no differences in rate of MMb accumulation during display were observed between muscles ($P = 0.10$). Using 20% discolored as a threshold for acceptability, 85% of LD samples were considered acceptable while only 5% and 20 % of TB and PM samples were considered acceptable, respectively, on d5 of

display. Myoglobin content and pH differed between muscles ($P < 0.0001$). The LD was correlated with the TB and PM for changes in redness ($0.51 \leq r \leq 0.55$; $P \leq 0.02$), and trending towards a correlation or correlated with changes in TB and PM 630/580 nm ratio and hue angle ($0.39 \leq r \leq 0.63$). All initial traits were correlated to final visual discoloration and 630/580 nm ratio in the TB and PM ($|0.47| \leq r \leq |0.72|$), except redness, d1 630/580 nm ratio, OC, and Mb content ($P \geq 0.07$). Initial redness and OC were also correlated to final 630/580 nm ratio ($|0.45| \leq r \leq |0.65|$), Final LD discoloration was correlated to initial LD L^* , but not any other trait ($P \geq 0.06$). Final LD 630/580 nm ratio was correlated to initial L^* , a^* , hue angle, DMb, and pH ($|0.48| \leq r \leq |0.75|$), but not any other traits ($P \geq 0.06$). Yellowness explained the majority of variation in visual discoloration for the TB and PM and instrumental 630/580 nm ratio for all muscles ($0.32 \leq \text{partial } R^2 \leq 0.85$). Initial lightness (partial $R^2 = 0.21$) and Mb (partial $R^2 = 0.20$) were most predictive of final LD visual discoloration (partial $R^2 = 0.33$).

Overall, loins classified as color stable were generally darker, redder, and had greater pH and marbling levels at 14 d postmortem compared to color labile loins. Chops from the TB and PM were less color stable during a 5d display period compared to LD chops, but had few color stability differences from each other. Furthermore, correlations were generally stronger in the TB and PM compared to the LD, but did not differ from each other. In all muscles, meat with improved color stability was initially darker, less yellow, and had lesser hue angles and greater chroma values.

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CHAPTER 1: REVIEW OF LITERATURE

Introduction

Meat color is well documented as an important trait used by consumers to make purchasing decisions (Mancini and Hunt, 2005, Suman and Joseph, 2013). Although meat color does not directly affect eating satisfaction (Carpenter et al., 2001; Wilson et al., 2016), consumers prefer meat products to be a bright red (beef and lamb) or reddish pink (pork) color, as they consider these colors to be indicators of meat freshness (Hood and Riordan, 1973; Schwimmer, 1981; Brewer and McKeith, 1999). Alternatively, meat with a purple or brown color is typically considered to be lower quality, as consumers interpret this to mean the meat is spoiled or not fresh (Carpenter et al., 2001; Font-i-Furnols and Guerrero, 2014). When fresh meat products discolor, they are either discounted, reworked into different products, or simply discarded, decreasing the value that can be attained from the original product (Zerby et al., 1999). Nearly 15% of beef is discounted in price as a result of surface discoloration, which corresponds to annual revenue losses of \$1 billion (Smith et al., 2000).

Research exists showing that using modified packaging and antioxidant inclusion can improve color stability in meat products. However, these methods incur additional costs, and consumer preference is shifting towards a desire for “natural” meat that does not contain additional ingredients (Font-i-Furnols and Guerrero, 2014). As a result, there is a current interest in developing methods for improving color stability that do not involve packaging changes or ingredient additions. Several factors exist that affect meat color stability, including muscle characteristics, genetics, sex, diet, and storage conditions (Mancini and Hunt, 2005). Even within a single animal, differences in color stability are present. McKenna et al. (2005) reported that beef muscles could be categorized as color labile, color stable, or intermediate based on rate of discoloration. One area of research that is lacking, however, is how this color stability changes

from animal to animal. It is not known if one animal with a more color stable longissimus will also have a more stable psoas major than another, or if muscle stability relative to other muscles changes between animals. Furthermore, the majority of research discussing these factors has been conducted in beef, whereas similar research in pork is much more limited. While knowledge of color stability in beef is useful when it comes to evaluating pork color, research directly evaluating pork is necessary to gain a better understanding of not only pork color stability, but meat color stability as a whole. Finally, much of the research conducted in meat is related to meat quality. Some commonly measured quality traits, including pH, water holding capacity, and color, are related to color stability. However, it is not known if these quality traits can be used to predict overall color stability. Developing a set of quality traits that are predictive of color stability would allow geneticists to select for these traits and improve the stability of future products. Therefore, the objective of this document is to review existing literature to define meat color on a chemical basis and highlight intrinsic and extrinsic factors affecting color stability in meat.

Meat Color Chemistry

Myoglobin

Meat color is primarily controlled by a sarcoplasmic protein called myoglobin (AMSA, 2012). Generally, meat containing more myoglobin appears redder in color. Myoglobin is water-soluble protein containing 8 α -helices with a prosthetic heme group. This heme ring contains a central iron atom that has 6 binding sites: 4 connect iron to the heme ring, one is attached to a proximal histidine-93 group, and the 6th site is able to reversibly bind ligands. Normally, the central iron atom is in the ferrous (Fe^{2+}) state; however, when no ligand is present iron can also be oxidized to the ferric (Fe^{3+}) state. The ligand that is reversibly bound to iron determines the

color of myoglobin, as the molecule will shift and reflect light differently depending on what ligand is present. In fresh meat, myoglobin typically exists in one of three forms: deoxymyoglobin (DMb), where there is no ligand, oxymyoglobin (OMb), where the ligand is oxygen, or metmyoglobin (MMb), where the central iron is oxidized and the ligand is water. Deoxymyoglobin formation results in meat that is dark purplish-red in color, and is typically present in freshly cut meat or meat that has been vacuum packaged. A low oxygen tension (< 1.4 mm Hg) is necessary to maintain DMb in meat (AMSA, 2012). The interior of meat naturally contains DMb due to the lack of oxygen after slaughter. When DMb comes into contact with the atmosphere, oxygen can reversibly bind to iron to form OMb, resulting in the bright red color typically associated with meat. This phenomenon is referred to as “blooming” in the meat industry. The rate of blooming is dependent on pH, temperature, oxygen partial pressure, and competition for oxygen with mitochondria (AMSA, 2012). At higher temperature and pH values, the rate of oxygenation is increased, whereas when more mitochondria are present, less oxygen is available for myoglobin binding and the oxygenation rate is slowed (Ramanathan and Mancini, 2018). Oxidation of the central iron atom can lead to formation of MMb, resulting in a brown or tan color that is associated with discolored meat. Metmyoglobin typically forms at low oxygen levels (1 – 2% oxygen); in fresh meat that is not vacuum sealed, MMb formation occurs below the meat surface where oxygen levels are lower. Although MMb forms below the surface initially, enough accumulation will result in MMb migrating towards the surface to the point that it becomes visible to consumers.

Reduction of Metmyoglobin

While on the meat surface it appears that OMb is directly converted to MMb, this reaction is not kinetically favorable. In reality, OMb is first converted to DMb, which is then

oxidized to form MMb. This step is necessary because iron cannot be oxidized if the available electron cannot be removed; when in the OMb state, this electron is bound to oxygen and unavailable for redox reactions. Oxidation of MMb can be initiated by reactive oxygen species derived from mitochondrial respiration or lipid oxidation products (Faustman et al., 2010; Ramanathan et al., 2018). Enzymatic systems in meat are able to reduce MMb back to DMb, preventing MMb from accumulating to the point that it can be perceived by consumers. Color stability of fresh meat is dependent on the rate of myoglobin oxidation and the capability for enzymes to reduce MMb (Faustman et al., 2010). The ability for a muscle to reduce MMb is referred to as metmyoglobin reducing activity (MRA). Several enzymatic systems have been implicated in MMb reduction, including NADH-cytochrome *b*₅ MetMb reductase, NADPH-quinone reductase, and NAD(P)H-quinone oxidoreductase (Bekhit and Faustman, 2005). Of these systems, NADH-cytochrome *b*₅ MetMb reductase is the best characterized enzyme involved in oxidized heme protein reduction (Bailey and Driedzic, 1992; Bekhit and Faustman, 2005). This enzyme functions by transferring two electrons from NADH to two molecules of cytochrome *b*₅; reduced cytochrome *b*₅ then transfers electrons to a variety of acceptors, including MMb (Shirabe et al., 1992). Because of its use as a cofactor in this reaction, NADH is commonly regarded as a rate-limiting factor in overall MRA (Echevarne et al., 1990; Renner, 1990; Mancini and Hunt, 2005). Metmyoglobin reduction can also occur non-enzymatically via the mitochondrial electron transport chain (Mancini et al., 2018). Electrons produced during this process can reduce metmyoglobin between complex III and complex IV of the mitochondria via cytochrome *c* (Tang et al., 2005).

Other Myoglobin Forms

Other, less common forms of myoglobin also exist, but require external factors to form. Carboxymyoglobin is formed when carbon monoxide attaches to the heme iron, and produces a bright red color similar to OMb. Because oxygen is more kinetically favorable as a ligand than carbon monoxide, carboxymyoglobin cannot occur unless the atmosphere surrounding the meat is entirely devoid of oxygen (AMSA 2012). Carbon monoxide is commonly utilized in modified atmosphere packaging (MAP) with a low oxygen environment because it produces a desirable color, without accelerating oxidation (AMSA, 2012). Sodium nitrite, which is included in curing solutions, is reduced to nitric oxide (NO) during curing. Nitric oxide is then able to bind to myoglobin forming nitric oxide myoglobin, resulting in the characteristic pink color associated with cured products. While it is commonly accepted that NO is the ligand in this situation, speculation exists that due to the lack of solubility of NO in curing solution, nitrate may be the actual ligand (AMSA, 2012). Bacterial contamination can affect the chemical state of myoglobin as well. When bacteria is present on meat, sulfmyoglobin or cholemyoglobin formation may occur resulting in green pigment formation (Lawrie, 2017). Nicol et al. (1970) reported that Hydrogen Sulfide produced by *Pseudomonas mephitica* contributed to sulfmyoglobin formation. Sulfmyoglobin can be further converted to Cholemyoglobin via oxidation of the porphyrin ring in the presence of reducing agents, such as ascorbate (deMan,1999).

Intrinsic Factors Affecting Meat Color Stability

Muscle Differences

Overall color stability differs between muscles (O’Keeffe and Hood, 1982). McKenna et al. (2005) reported that beef muscles could be categorized into one of four groups based on discoloration patterns: “high” stability (e.g. longissimus lumborum, semitendinosus),

“intermediate” stability (e.g. trapezius, gluteus medius), “low” stability (e.g. triceps brachii, biceps femoris), and “very low” stability (e.g. infraspinatus, psoas major). Other studies have reported similar results, particularly regarding stability of the longissimus and psoas major (Bendall and Taylor, 1972; Hood, 1980; Echevarne et al., 1990, Nair et al., 2018). These differences can partially be attributed to fiber type differences between muscles (Jeong et al., 2009). There are two primary fiber types in muscle: red, oxidative fibers, and white, glycolytic fibers (Aberle et al., 2012). Red fibers primarily utilize oxidative metabolism and are more prominent in load-bearing muscles, such as the bicep femoris and triceps brachii. Because of the constant need for energy to maintain a standing position, these muscles utilize aerobic metabolism as a means to produce the maximum amount of ATP for energy. Red fibers also possess a greater amount of myoglobin in order to store more oxygen, making the fibers appear more red in color (Aberle et al., 2012). Furthermore, red fibers contain a greater proportion of lipids for use in the TCA cycle when glucose is limited (Aberle et al., 2012). Alternatively, white fibers are more prominent in locomotive muscles such as the longissimus dorsi and gluteus medius, and utilize glycolytic metabolism for short bursts of energy. These fiber types contain greater levels of glycogen and glycolytic enzymes in order to produce sufficient energy with less mitochondria.

Typically, muscles with a greater proportion of red fibers discolor more quickly (Faustman et al., 2010). In order to produce more ATP through aerobic means, oxidative fibers possess greater amounts of mitochondria (Ramanathan et al., 2018). Mitochondria are still able to consume oxygen in postmortem muscle. Antioxidant systems in the muscle are compromised postmortem; as a result, reactive oxygen species produced by mitochondria can accumulate and place the muscle under greater oxidative stress, leading to increased myoglobin oxidation

(Mancini et al., 2018; Ramanathan et al., 2018). Mitochondrial damage has also been implicated in decreased color stability, particularly in the psoas major, due to loss of access to the electron transport chain (Ke et al. 2017; Mancini et al. 2018). Meat with a larger concentration of myoglobin also has a greater iron concentration, which can also promote myoglobin oxidation (Neethling et al., 2017). Furthermore, the increased proportion of lipids in red fibers also makes them more susceptible to lipid oxidation. When lipids are oxidized, primary and secondary products are generated that can further oxidize other substances, including myoglobin. Several studies have indicated that lipid oxidation accelerates meat discoloration (Hutchins et al., 1967; Zakrys et al., 2008; Faustman et al., 2010). As a result, muscles with greater susceptibility to lipid oxidation are also likely more prone to pigment oxidation.

Color stability differences between muscles may also be attributable to non-mitochondrial reducing enzyme content. Nair et al. (2018) evaluated various metabolite levels in 3 muscles considered to have different levels of color stability. This study reported that the longissimus lumborum and semitendinosus had greater levels of enzymes involved in anaerobic metabolism, including triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, and phosphoglucumutase-1, compared to the psoas major. They hypothesized that a greater concentration of glycolytic enzymes lead to increased NADH regeneration, resulting in improved MRA. Joseph et al. (2012) reported that proteins correlated with redness (aldose reductase, creatine kinase, and β -enolase) and color stability (peroxiredoxin-2, peptide methionine sulfoxide reductase, and heat shock protein-27 kDa) were more abundant in the longissimus lumborum than the psoas major. Overall, mitochondrial oxygen consumption and enzymatic reduction both play a role in meat color stability. McKenna et al. (2005) speculated that ultimate color stability within a muscle is not dependent on oxygen consumption or MRA alone, but rather a

combination of the two. In particular, the most stable muscles likely have a low oxygen consumption rate and high MRA.

While several studies have indicated differences in color stability between muscles, limited data exists identifying how color stability changes within a muscle. It has been well documented that color is not uniform across the muscle, and that these disparities can be partially attributed to muscle fiber differences (Hunt and Hedrick, 1977; Himm et al., 2006; Barkley et al., 2018; Nair et al. 2018; Picard and Gagaoua, 2020). Hunt and Hedrick (1977) reported that the interior of the semitendinosus had more glycolytic fibers on the interior of the muscle compared to the exterior while the interior of the semimembranosus was more oxidative than the exterior. Barkley et al. (2018) did not investigate differences in muscle fibers, but concluded that the anterior end of the pork longissimus was lighter and less red than the posterior end. Because of the differences in fiber types within a muscle, it is likely that color stability differs across a muscle as well; additional research is necessary to confirm this.

Genetics

Color stability differences between animals can also be attributed to genetics. King et al. (2010) investigated heritability of discoloration markers in longissimus thoracis steaks from steers of Angus, Charolais, Gelbvieh, Hereford, Limousin, Red Angus, and Simmental genetic lines. This study reported that myoglobin concentration was highly heritable (0.85), and that variation in metmyoglobin, and ΔE (overall change in color) were moderately explained by genetic factors (41% and 29% respectively). From this data, it was concluded that Charolais and Limousin carcasses produced steaks with greater color stability than Angus, Hereford, or Red Angus carcasses. Canto et al. (2015) also reported that animal variations contributed to beef longissimus color stability based on differences in sarcoplasmic proteomes. While breeds were

not reported in this study, three glycolytic enzymes (phosphoglucosmutase-1, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase M2) were over-abundant in color-stable steaks. These enzymes were also identified by Nair et al. (2018) as being indicators of color stability in the longissimus.

While limited research is available investigating pork color stability on a genetic basis, genetics have been implicated in initial pork color. Brewer et al. (2004) evaluated several pork sire lines including Duroc, Synthetic, Duroc/Landrace, Pietrain, Duroc/Hampshire, and Large White, and reported that loin chop color traits were affected by genetic line. Edwards et al. (2003) suggested that Duroc progeny had a higher pH and increased redness compared to Pietrain sired pigs. Lowell et al. (2018) also reported that loins from Duroc-sired pigs had greater pH values than loins from Pietrain-sired pigs, but did not observe differences in instrumental or subjective color. At higher pH values, enzymatic reducing activity may be increased, therefore color stability may be improved as well. Based on literature conducted in beef, it is likely that muscle fiber and proteome differences also impact pork color stability; however, further research is necessary to conclude this. The relationship between meat quality traits and color stability will be discussed further later in this review.

Sex

Limited research has been conducted investigating the effect of sex on color stability, and studies evaluating the effect of sex on color are inconclusive. Seidman et al. (1982) reported that intact male animals (bulls, boars, and rams) possessed darker and redder meat compared to female and castrated animals due to higher concentrations of myoglobin. As mentioned previously, meat with a higher quantity of myoglobin may exhibit decreased color stability due to increased iron levels. Contrastingly, Kim et al. (2003) observed that steers had greater

instrumental lightness and yellowness compared to bulls and cows, yet did not observe color differences between bulls and cows. In pork, Overholt et al. (2016) reported that loins from barrows were lighter, redder, and more yellow than loins from gilts while Lowell et al. (2017) observed no color differences between the two. Nold et al. (1999) reported that boars and barrows were lighter than gilts, while boars were less red than barrows or gilts. There were no differences in lightness between barrows and gilts and no redness differences between barrows and gilts in that study. Overall, the role of sex in overall color and color stability is not clear, and further research is necessary to determine what relationships are present.

Extrinsic Factors Affecting Color Stability

Diet

Several studies have been conducted showing dietary effects on color stability and overall shelf life. Wicks et al. (2019) reported that cattle fed high forage diets have more oxidative muscle than cattle fed a concentrate diet. Additionally, Vestergaard et.al. (2000) reported that feeding cattle forage-based diets may result in lower glycogen storage and increased oxidative metabolism, potentially decreasing color life. Lynch et al. (2002) reported that longissimus color stability was greater in cattle finished indoors on silage and concentrate compared to cattle finished on grass. Differences in stability between finishing methods may be attributable to differences in fatty acid profiles. Lynch et al. (2002) also reported that pastured cattle had greater levels of polyunsaturated fatty acids (in particular α -linolenic acid). Polyunsaturated acids (fatty acids with multiple double carbon bonds) are more prone to oxidation than monounsaturated (one double bond) and saturated (no double bonds) fatty acids, which would cause decreased lipid stability in meat from pasture fed cattle. Because lipid oxidation products can also accelerate myoglobin oxidation, resulting in decreased color stability as well. Alternatively,

O'Sullivan et al. (2003) reported that forage diets did not affect color stability of overwrapped longissimus steaks compared to concentrate diets. This was attributed to an accumulation of lipid-soluble antioxidants in meat from cattle fed forage. Cattle from the O'Sullivan et al. (2003) and Lynch et al. (2002) studies were likely fed forages with different fatty acid profiles, which may have caused discrepancies in results. Overall, feeding cattle diets containing higher levels of unsaturated acids, such as forage diets, may lead to decreased color stability in meat compared to feeding concentrate, whereas feeding forages with high antioxidant levels may improve color stability.

Swine diets commonly contain fat as an added ingredient for use as an energy source (Lin et al., 2013). The oxidative state of fats included in these diets may have implications for pork color, but inconsistent results have been reported in the literature. Buckley et al. (1989) and Monahan et al. (1992) reported that feeding corn oil with a high peroxide value (4.5. – 9.0 mEq/kg) resulted in greater lipid oxidation of pork loin chops during storage. While these studies did not directly measure changes in color, based on the relationship between lipid and color oxidation, chop discoloration likely occurred as well. However, more recent research has reported that feeding peroxidized corn oil does not affect lipid oxidation or color stability of pork loin chops (Boler et al., 2012; Overholt et al., 2017). Overholt et al. (2017) reported that differences in results may have occurred due to inconsistencies in oil processing or dosage between studies, as oils with higher peroxide levels had a greater impact on product stability. However, that study also used oil with peroxide values up to 12.4 mEq/kg, a value greater than what was used in any of the previous studies, and still did not observe differences in color or lipid stability. Based on more recent research, feeding peroxidized oil appears to have little effect on color or lipid stability in pork loin chops.

Dietary inclusion of antioxidants can be used to improve color stability in meat. Multiple studies have shown vitamin E to improve color stability in beef and pork (Lanari et al., 1995; Faustman et al., 1998; Phillips et al., 2001; Bloomberg et al., 2012; Harsh et al., 2017). Much of the oxidation that takes place in meat is caused by highly reactive free radicals. Vitamin E functions as a free radical scavenger, and sequesters radicals before further radical formation can occur. Mineral supplementation, particularly with selenium, has also been shown to improve color stability. Ryu et al. (2005) reported that supplementation of dietary selenium at 8 ppm reduced lipid oxidation and improved color stability of meat from broiler chickens. Similar results were reported by Wang et al. (2009) when feeding selenium at 0.3 or 0.6 mg/kg to Langshan breeding hens. Calvo et al. (2017) reported that feeding 0.4 ppm of Selenium in combination with Vitamin E improved oxidative stability in York pigs. Other studies have reported that dietary selenium supplementation does not affect color stability in beef or lamb (O'Grady et al., 2001; Jose et al., 2010); contrasting results may have been caused by differences in inclusion level. Other dietary supplements have also been shown to improve meat color stability, including β -carotene, turmeric, and flaxseed (Muramoto et al., 2003; Karami et al., 2011; Pouzo et al., 2016). Overall, dietary ingredients with antioxidant function can be useful tools for improving color stability in meat from several species.

Storage Conditions

Fresh whole muscle products are stored for varying amounts of time before retail display and consumer purchase. Several factors exist during storage that can influence color stability, including packaging type, storage duration, and temperature. Meat products are commonly packaged in one of three ways: overwrap packaging, vacuum packaging, or modified atmosphere packaging (MAP; Mancini, 2009). Products intended for short-term storage in a retail case are

often packaged in oxygen-permeable overwrap packaging (McMillin, 2017). With this type of packaging, oxygen in the atmosphere can pass through the overwrap and interact with myoglobin, resulting in development of oxymyoglobin and a resulting red color. Overwrap packaging is the most common packaging type for fresh meat in retail cases because consumers value meat redness when making purchasing decisions (McMillin, 2017). While overwrap packaging results in a more desirable color for consumers, it does not inhibit oxidation or microbial growth (Mancini, 2009). Alternatively, vacuum packaging is commonly used for products stored for longer periods of time (McMillin, 2017). Vacuum packaging extends shelf life by removing oxygen and other gases from the package, minimizing oxidation and bacterial growth. When properly sealed, vacuum packaged products contain less than 1% oxygen (v/v) and 10 – 20% CO₂ (v/v) produced by mitochondrial and microbial respiration (Lambert et al., 1991). Rather than for retail display, vacuum packaging is primarily used for export and wet-aging because of its shelf-life enhancing properties. Modified atmosphere packaging is a newer form of packaging involves the removal or replacement of atmosphere surrounding a product before sealing it in vapor-barrier materials (McMillin, 2008). This type of packaging allows the atmosphere in a package to be controlled in a way that limits spoilage while promoting bloom formation (McMillin, 2008). Products packaged in this manner typically contain very high (80%) or very low (< 1%) levels of oxygen (Mancini, 2009). High oxygen packaging promotes bloom development, but also increases lipid oxidation, so products may become rancid while color appears normal. Alternatively, low oxygen packaging minimizes lipid oxidation and microbial growth, but does not allow sufficient oxygen for OMb formation. To circumvent this, carbon monoxide is commonly used in MAP at low levels (0.4%) in place of oxygen because carboxymyoglobin produces a cherry-red color similar to OMb (Hunt et al., 2004).

As mentioned previously, meat intended for wet-aging is often vacuum packaged for storage. However, this can decrease color stability once the product is removed from vacuum packaging (Mancini, 2009). This is partially because when the atmosphere is removed from the package, MMb forms initially before final conversion to DMb (Mancini, 2009). This reaction depletes reducing substrates and results in a decreased MRA once the product is removed from vacuum packaging. Extended aging periods can also have a detrimental effect on color stability. King et al. (2012) evaluated color attributes of beef longissimus steaks that were fabricated immediately after grading or wet-aged for 14 or 35 d. When evaluated for retail display, redness decreased more rapidly in steaks aged 35 d compared to steaks aged 14 d or cut immediately. Furthermore, Vitale et al. (2014) aged longissimus steaks from Friesian cattle for 0, 3, 6, 8, 14, or 21 d, and observed that steaks aged for 14 – 21 d demonstrated lower color stability than steaks aged 0 – 8 d. Decreases in color stability during storage are likely attributable to a decrease in reducing capacity over time. Kim et al. (2006) reported that substrates required to generate reducing equivalents such as NADH are continuously depleted during storage, rather than only depleted at the beginning. Tang et al. (2005) reported that isolated beef mitochondria had a lower MRA after 60 d of aging compared to mitochondria evaluated 2 h postmortem. Overall, aging meat is a valuable tool for extending color stability while vacuum sealed, but longer periods of aging decrease color stability when the product is removed from vacuum packaging. To optimize color stability without sacrificing tenderness contributed by wet-aging, shorter aging periods should be used when possible.

Myoglobin oxidation is accelerated by increased temperatures during storage (Faustman, 1990). Gotoh and Shikama (1974) reported that oxymyoglobin oxidation had a temperature coefficient of 5.3 at temperatures ranging from -3°C to 30°C and pH values from 5 to 10. The

temperature coefficient of a reaction represents the factor by which a reaction rate increases given a 10°C temperature increase, where a higher coefficient indicates an increased response (Faustman and Cassens, 1990). Typically, enzymatic temperature coefficients range from 2 – 4 (Mortimer, 1977); the value of 5.3 associated with myoglobin oxidation indicates that the reaction is heavily temperature dependent. Multiple studies have also shown that color stability is enhanced at lower temperatures (Hood, 1980; Jeremiah and Gibson, 2001; Li et al., 2017). At lower temperatures, the distance from the surface where MMb occurs is increases, requiring a greater accumulation to be perceptible by consumers (Urbin and Wilson, 1958). At greater temperatures lipid oxidation and bacterial growth are also accelerated, resulting in increased reactive oxygen species formation (Mancini, 2009). Furthermore, dissociation of oxygen from OMb is more favorable at higher temperatures, leading to increased levels of oxidation-prone DMb (O’Keefe and Hood, 1982). Temperature during storage of meat should be carefully controlled to maximize color stability.

Quality Traits and Color Stability

Ultimate pH

The ultimate pH of muscle represents how acidic the muscle is on a scale of 1 to 14 where 1 is acidic, 14 is alkaline, and 7 is neutral. During the postmortem conversion of muscle to meat, the pH of muscle decreases from 7.2 to 5.4 – 6.3, depending on the extent of glycolysis that occurs (Aberle et al., 2012). During postmortem metabolism, glycogen in the muscle is broken down to yield glucose-1-phosphate, which is then converted to pyruvate via glycolysis. Because blood and oxygen are removed from the carcass during exsanguination, pyruvate is converted to lactic acid to generate ATP rather than used for oxidative metabolism. The accumulation of lactate in in muscle drives postmortem pH decline, as muscles with more

glycogen tend to have a lower ultimate pH (Aberle et al., 2012). Typically, muscles with greater levels of glycogen (white fibers) experience an increased pH decline.

Conflicting results exist demonstrating the effect of pH on color stability itself. Increased pH values can improve efficacy of reducing systems in the cytoplasm and mitochondria. Additionally, at lower pH values superoxide formation is accelerated, which can oxidize myoglobin to MMb (Faustman and Cassens, 1990). However, a high pH combined with a high temperature postmortem can also lead to mitochondrial damage, resulting in increased oxidative stress (Ramanathan et al., 2018). A high pH can also increase mitochondrial oxygen consumption, decreasing color stability further (Ramanathan et al., 2018). Ledward et al. (1986) reported that beef with an ultimate pH of 5.8 was more stable than beef with an ultimate pH of 5.6. O'Grady et al. (2001) reported that beef longissimus steaks were more stable at higher pH values (6.4 or 7.2) compared to lower pH values (5.5). By contrast, Hood (1980) reported that differences in rate of discoloration between muscles were not related to pH, while McKenna et al. (2005) reported that pH was not well correlated with measures of discoloration other than Metmyoglobin reductase activity ($r = -0.32$ for MRA, $r \leq -0.17$ for others). Furthermore, some the least color stable muscles in the study by McKenna et al. (2005) also possessed the highest pH values. The two previous studies reported that pH was not well related to color stability when comparing multiple muscles, which may have cause discrepancies compared to studies only using the longissimus. However, even within a single muscle, the impact of pH on color stability is not clear. King et al. (2011) and Canto et al. (2015) each reported that longissimus steaks categorized as color-stable did not differ in pH from color-labile steaks. Rather than directly affecting color stability, it is more likely that the effect of pH on color stability depends on the reducing ability or oxygen consumption of the muscle in question. A high pH may be beneficial

for a muscle with a high MRA, yet detrimental in muscles with high oxygen consumption. Nonetheless, pH still has a role in the overall color stability of meat, and should be considered when doing color stability evaluations.

Water Holding Capacity

The water holding capacity (WHC) of meat refers to the ability to retain moisture during the application of an external force (Aberle et al., 2012). Ultimate pH and WHC are directly related; as the pH of meat approaches its isoelectric point (typically 5.0 – 5.2), proteins develop neutral charge and cannot bind water as well (Aberle et al., 2012). Myoglobin is a water soluble molecule; therefore, as meat loses moisture it also loses myoglobin that is suspended in that water. As a result, meat with a higher pH is typically darker in color compared to low pH meat. Research directly evaluating the relationship between WHC and color stability is limited. However, meat with a greater myoglobin concentration may be less color stable due to increased iron levels (Neethling et al., 2017). Alternatively, meat with greater purge loss may have less sarcoplasmic reducing enzymes and a lower reducing capacity as a result. Because of its relationship to pH, it is likely that WHC has a similar relationship with color stability, in that the effect of WHC on discoloration is related to the proteome of the meat in question.

Color

Initial meat color is also related to overall color stability. As mentioned previously in this review, meat color is determined by the amount and state of myoglobin present. As a quality trait, initial color is typically measured either by visual evaluation or instrumental assessment (AMSA, 2012). Visual evaluation is determined by trained technicians or consumers using a scale to rate the product based on how light or red it is. One color scale commonly used for pork research is the NPPC color scale (NPPC, 1999), where pork is evaluated on a scale of 1(lightest)

to 6 (darkest) by a technician. While visual appraisal represents how a consumer perceives color, it can be difficult to reproduce due to its subjective nature. Instrumental color is measured using a machine rather than the human eye, and is thus more replicable. Two main machine types exist for meat color measurement: colorimeters and spectrophotometers. Colorimeters function by shining light on a sample, then passing light reflected by that sample through three colored filters: red, green, and blue. The light that passes through these filters is converted to XYZ values, which represent the color of a sample (AMSA, 2012). For meat science purposes, XYZ values are often converted to CIE $L^*a^*b^*$ values (CIE, 1976), as these values are more representative of visual color (AMSA, 2012). L^* represents lightness on a scale of 1(darkest) to 100(lightest), while a^* represents redness and greenness on a scale of -60(more green) to +60(more red), and b^* represents yellowness and blueness on a scale of -60(more blue) to +60(more yellow). Tristimulus $L^*a^*b^*$ values can also be used to calculate Chroma, hue angle, and ΔE values. Chroma is a measure of how saturated a sample is ($Chroma = \sqrt{(a^*)^2 + (b^*)^2}$), while hue angle is a measure of the perceived color of a sample ($h_{ab} = \arctangent (b^*/a^*)$), and ΔE is the change in color of a sample ($\Delta E_{ab}^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$). Spectrophotometers measure absorbance in 1 – 10 nm increments over several wavelengths in the visual color range (400 – 700 nm), then convert absorbances to tristimulus values. Both measurement systems are valuable when measuring surface color. However, a spectrophotometer may be more useful for measuring discoloration because it can measure multiple color at wavelengths, whereas colorimeters can only measure at 1 set wavelength.

Of the three tristimulus values, initial redness likely has the strongest relationship to color stability. Several studies have reported decreased color stability in meat that has greater initial redness (McKenna et al., 2005; King et al., 2011; Canto et al., 2015). Furthermore, Seyfert et al.

(2006) reported that instrumentally redness was strongly correlated to metmyoglobin reducing activity ($r = 0.68$). Meat that appears more red initially may have a greater proportion of oxidative fibers and myoglobin, making it more susceptible to oxidation (Faustman et al., 2010). Alternatively, initial instrumental lightness appears to have a minimal effect on color stability both within and between muscle types (McKenna et al., 2005; King et al., 2011; Canto et al. 2015). McKenna et al. (2005) also reported that initial L^* values were inconsistent across color stable and color labile muscles. Initial yellowness and Chroma were greater in unstable longissimus steaks in a study by King et al. (2011), while hue angle was not different between steaks classified as stable or unstable. While these studies provide evidence for relationships between initial color measurements and color stability, only redness was shown to be predictive of any measure of discoloration. Furthermore, MRA is only a measure of reducing potential, not actual discoloration. Therefore, further research is necessary to identify relationships between initial color and discoloration, and determine if color stability can be predicted using initial color measurements.

Marbling

The degree of marbling present in meat is used to evaluate eating quality (Cannata et al., 2010). However, little research exists investigating relationships between marbling and color stability. Wheeler et al. (2005) ranked breeds of cattle based on their relative color stability; these rankings were consistent with rankings based on carcass yield and inversely related to the ranking of breeds based on marbling score. The authors perceived this to mean that metabolic differences contributing to increased muscle growth and decreased fatness may contribute to increased color stability. Oxidative muscle fibers tend to contain greater proportions of lipid than oxidative fibers (Aberle et al., 2012). Because muscles with more oxidative fibers tend to

discolor faster, it may follow that muscles with greater levels of marbling discolor faster as well. Alternatively, animals fed for longer periods of time will produce meat with increased marbling regardless of muscle fiber type (Aberle et al., 2012); in this case, marbling may not be indicative of color stability. Because of the importance of marbling in meat products, especially beef, it is pertinent to determine if relationships between marbling and color stability exist.

Conclusions

Several studies have been conducted defining the mechanisms behind meat color development and discoloration, as well as identifying factors that influence meat color stability. Meat color is affected by intrinsic factors, such as muscle type, genetics, and sex, as well as extrinsic factors such as diet and storage conditions. However, as evidenced in this review, the majority of research regarding color stability has been conducted in beef while research regarding other species is lacking, including pork. Furthermore, limited research is available characterizing color stability in multiple pork muscles.

Overall, three studies were conducted to evaluate relationships between meat traits and color stability in pork. The objective of the study was to characterize color stability of boneless pork loins using fresh quality traits and instrumental measures of discoloration. The objectives of the second study were to evaluate differences in color stability between the *triceps brachii*, *psaos major*, and *longissimus dorsi* muscles and relate those differences to biochemical measures, and to determine if color changes in the LD were related to color changes in other muscle types. the objectives of the third study were to determine relationships between initial retail display traits and final discoloration in the longissimus dorsi (LD), triceps brachii (TB), and psaos major (PM), and to determine what combination of initial traits was most predictive of final discoloration in each muscle.

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CHAPTER 2

CHARACTERIZATION OF PORK LOIN CHOP COLOR STABILITY USING LOIN QUALITY TRAITS AND INSTRUMENTAL DISCOLORATION MEASURES

Abstract

The objective of this study was to characterize the color stability of pork loin chops using fresh quality traits and instrumental measures of discoloration. Boneless pork loins (N=484) were evaluated for quality traits at 11 or 14 d postmortem. One chop was cut from each loin near the 10th rib for retail display, overwrapped, and displayed under constant fluorescent lighting for 7 d. Objective color, myoglobin (Mb) forms, and subjective visual discoloration traits were evaluated daily. After display, loins were categorized based on final visual discoloration (d 7) as: Very Color Stable (VCS; 0 – 5% discoloration), Color Stable (CS; 5 – 10% discoloration), Neutral (NT; 10 – 25% discoloration), Color Labile (CL; 25 – 30% discoloration), or Very Color Labile (VCL; >30% discoloration). Quality and color traits were analyzed using the GLIMMIX (visual discoloration) or MIXED (all other measures) procedure of SAS. Retail display data were analyzed as repeated measures. Loins classified as CS or VCS were darker, less yellow, had greater pH values, greater marbling scores, decreased color score, and decreased purge loss compared to CL and VCL loins ($P < 0.01$); N loins were intermediate for each of these measurements ($P < 0.01$). No differences in aged redness were observed between groups ($P \geq 0.16$). A group x day interaction was observed for all traits measured during retail display ($P < 0.0001$). Stable loins generally had increased redness and R630/580 as well as decreased visual discoloration scores, lightness, yellowness, and % MMb values during display. Overall, loins classified as color stable were generally darker, redder, and had greater pH and marbling levels at 14 d postmortem compared to color labile loins.

Introduction

It is well-known consumers use color in their meat purchasing decisions, typically preferring bright, cherry-red beef or lamb (Hood and Riordan, 1973; Schwimmer, 1981) and reddish-pink pork (Brewer and McKeith, 1999). In contrast, consumers discriminate against brown or purple hue when purchasing meat, using color as an indicator of wholesomeness and freshness (Font-i-Furnols and Guerrero, 2014). Accordingly, retailers may discount or rework discolored meat products to increase the likelihood of consumer purchase; however, extensive discoloration may ultimately result in products being discarded. For these reasons, the premature discoloration of fresh meat poses enormous economic and food waste challenges. For reference, in 2019, fresh beef discoloration resulted in an estimated \$14 billion of lost revenue globally (Ramanathan et al., 2021). In order to minimize profit losses resulting from discolored products, it is important to understand discoloration in meat and the factors that contribute to it.

While fresh beef discoloration characteristics and mechanisms have been investigated extensively, similar research in fresh pork is limited. Zhu and Brewer (1998) and King et al. (2011a) evaluated differences in color traits between stable and labile pork, but did not investigate differences in other common quality traits such as pH or marbling. In fresh beef, a number of studies have demonstrated relationships between color stability and loin quality traits. For example, previous studies have reported that *Longissimus lumborum* (LL) steaks with less marbling (Wheeler et al., 2005) or decreased pH values (Ledward et al., 1986; O'Grady et al., 2001) were more color stable. Further, several studies have indicated redder steaks discolor more quickly (McKenna et al., 2005; Faustman et al., 2010; Canto et al., 2015). While multiple studies have characterized quality traits of the U. S. pork supply (Arkfeld et al., 2017; Price et al., 2018; Newman et al., 2019; Redifer et al., 2020), none have evaluated characterized color stability

using these traits. Further, substantial differences between beef and pork myoglobin, intramuscular fat, and fatty acid profiles (Vizcarrondo et al., 1998, Suman et al., 2006;) likely limit the ability of beef-specific quality and discoloration findings to accurately represent pork color stability. Therefore, the objective of this study was to characterize color stability of boneless pork loins using fresh quality traits and instrumental measures of discoloration. It was hypothesized that color stable pork chops would possess quality characteristics different from what has been observed in color stable beef.

Materials and Methods

In total, 484 loins representing four independent shelf life trials (designated A through D) were used. All pigs were slaughtered at processing facilities under the supervision of USDA Food Safety Inspection Service. Pigs used in this study represented different commercial sire lines, housing systems, and slaughter conditions. Handling details for each of the four studies are outlined in Table 1. Live animal interaction occurred for pigs from trial C; therefore, all protocols for that trial were approved by the University of Illinois Institutional Animal Care and Use Committee (Protocol #20095). All animals were held in lairage for approximately 16 h prior to slaughter with no access to feed and ad libitum access to water, then slaughtered and allowed to chill prior to fabrication. Pigs slaughtered at the University of Illinois were immobilized via head-to-heart electrical stunning and terminated via exsanguination, after which carcasses were allowed to chill at 4°C for approximately 22 h. Pigs slaughtered at other facilities were immobilized via CO₂ stunning and terminated by exsanguination, then blast-chilled for approximately 90 min and held in an equilibration cooler until fabrication at approximately 22 h. After chilling, all carcasses were fabricated into primal cuts, and loins were further fabricated into boneless Canadian back loins (NAMP # 414). Loins from pigs slaughtered at the university

were weighed, then vacuum packaged and aged until 14 d postmortem. Loins acquired from pigs slaughtered at commercial facilities were vacuum packaged then transported to the University of Illinois Meat Science Laboratory where they were stored at 4 °C until 11 d (trial D) or 14 d (trial C) postmortem.

Aged ventral loin quality traits were measured on all loins used in this study. Loins acquired from pigs slaughtered at the University of Illinois were removed from bags and weighed a second time to determine purge loss ($[(\text{Initial wt.} - \text{final wt.})/\text{initial wt.} \times 100]$). Loins fabricated in commercial production facilities were weighed in the vacuum bag, then removed from packaging and weighed a second time to determine purge loss using the previous calculation. The initial weight of these loins was determined by subtracting the average weight of bags used from the weight of the bagged loin. Loins were then allowed to oxygenate for 20 minutes prior to quality evaluations. All quality evaluations were conducted on the ventral face of the loin at the approximate location of the 10th rib. Instrumental CIE L^* (lightness), a^* (redness), and b^* (yellowness) measurements were collected using a Minolta CR-400 Chromameter (Minolta Camera Co., Ltd., Osaka, Japan) with a D65 illuminant, closed 8 mm aperture, 2° observer, and calibrated with a white tile specific to the machine. Ultimate pH was measured using a Hanna pH meter fitted with a glass tipped electrode (model HI98163, Hanna Instruments, Woonsocket, RI) calibrated using pH 4 and pH 7 buffer at 4 °C. Visual color, visual marbling (NPPC, 1999), and subjective firmness (NPPC, 1991) were evaluated by a single, trained technician. After ventral loin quality measurements were completed, a single 2.54-cm thick chop was cut from each loin at the area of the 10th rib for retail display. Individual chops were placed on polystyrene trays (27.3 × 14.9 cm, Dyne-a-pak, Laval, Quebec, Canada) and overwrapped using oxygen-permeable polyvinyl chloride film (O_2 transmission rate = 23,250

mL·m²·d⁻¹, 72 gauge; Resinite Packaging Films, Borden, Inc., North 70 Andover, MA).

Packaged chops were displayed under constant lighting (Ecolux with Starcoat, 3000 K, General Electric, Boston, MA for trials A, B, and C; Octron XP, 6500 K, Osram Sylvania, Wilmington, MA for trial D) at 4°C for 7 days. Chops were removed from simulated retail display after 7d.

Subjective discoloration estimates and objective color measurements were collected each day of analysis in 24 h intervals. Samples were evaluated for subjective discoloration by a trained technician using a 10 cm unstructured line scale anchored at 0%, 50%, and 100% discoloration.

Objective color measurements were collected using a HunterLab MiniScan EZ

spectrophotometer (HunterLab, Reston, VA) set to collect tristimulus CIE L^* (lightness), a^* (redness), and b^* (yellowness) and spectral data ranging from 400 – 700 nm (AMSA 2012). The

Hunter device was equipped with a D65 illuminant, 10° observer, 31.8 mm aperture, and

calibrated with a black and white tile covered with the same PVC film used to wrap the chops.

Instrumental reflectance values were used to calculate the ratio of reflectance at 630 nm to the reflectance at 580 nm (smaller ratio indicates greater discoloration; R630/580) and estimated

myoglobin (Mb) redox forms (metmyoglobin (MMb), deoxymyoglobin (DMb), and

oxymyoglobin (OMb)) using the method described by Krzywicki (1979). Using this method,

reflectances at 474, 525, and 572 nm were determined by interpolation using wavelength

reflectances determined by the Hunter device. These reflectances in addition to the reflectance at

700 nm were then converted to attenuance (A) values by the equation: $A = \log (1/R)$, where R =

reflectance. Myoglobin forms were calculated using the following equations:

$$\% \text{ Metmyoglobin (MMb)} = \left\{ 1.395 - \left(\frac{A_{572} - A_{700}}{A_{525} - A_{700}} \right) \right\} \times 100$$

$$\% \text{ Deoxymyoglobin (DMb)} = \left\{ 1.395 - \left(\frac{A_{474} - A_{700}}{A_{525} - A_{700}} \right) \right\} \times 100$$

$$\% \text{ Oxymyoglobin (OMb)} = 100 - (\% \text{MMb} + \% \text{DMb})$$

Statistical Analyses

Loins were sorted into five color stability categories based on chop visual discoloration on the final day of display (d7): Very Color Stable (0 – 5% discoloration; VCS), Color Stable (5 – 10% discoloration; CS), Neutral (10 – 25% discoloration; NT), Color Labile (25 – 30% discoloration; CL), and Very Color Labile (30% + discoloration, VCL). Data were analyzed using the MIXED procedure of SAS (SAS v9.4, SAS Inst. Inc., Cary, NC) with loin serving as the experimental unit. Postmortem quality traits and color stability measures at the initial (d1) and final (d7) point of display were analyzed as a 1-way ANOVA with stability group as a fixed effect and slaughter group as a random effect. A multivariate model was fit using the repeated statement because of heterogeneous variance due to differences in sample size between stability categories. Although a variety of conditions were represented in this study, Arkfeld et al (2017) reported that marketing group and production focus together contributed less than 10% of variation in pork loin L^* , a^* , and b^* . This variation was accounted for by including pig group as a blocking factor in the model. Visual discoloration data were determined to be non-normal and were thus analyzed using the GLIMMIX procedure of SAS, while quality data and other color stability traits (L^* , a^* , b^* , R630/580, and MMb) were analyzed using the MIXED procedure. Data from the 7d simulated retail display period were analyzed as repeated measures with stability group, day, and the interaction between the two as fixed effects, and slaughter group as a random effect. Visual discoloration data over display was analyzed using the GLIMMIX procedure with day as a random statement. Other color stability traits were analyzed using the MIXED procedure with a Toeplitz covariance structure chosen to minimize variance. Least-square means were separated using the PDIFF option for both the GLIMMIX and MIXED

procedures. Main effects and interactions were considered significantly different at $P \leq 0.05$ and trending at $0.05 \leq P \leq 0.10$.

Results

Summary statistics for initial (d1), final (d1), and Δ (d7 – d1) color stability traits are displayed in Table 2. Initial loin color averaged an L^* of 57.27 (46.84-68.60), a^* of 8.77 (4.44 – 14.03), and b^* of 16.71 (12.07 – 20.51). Initial DMb averaged 24.96% (0.32 – 68.19) and initial discoloration was minimal with a range from 0-3%. Initial MMb was 1.96-15.06%, initial DMb was 0.32 – 68.19%, and initial OMb was 24.83 – 86.40%. After 7d of display, loin chop color ranges were similar to those of d1 display with the exception of DMb, which decreased from an average of 24.96% on d1 to 7.57% on d7. Magnitudes of color change during display differed between loins, as ΔL^* ranged from -16.80 to 14.18, Δa^* ranged from -6.95 to 6.06, and Δb^* ranged from -4.06 to 6.06. The average final visual discoloration of loins used in this study was 19.6%, with a minimum final discoloration of 0% and a maximum final discoloration of 54.0%. Metmyoglobin ranged from 8.64 to 46.39% at d7 of display. Therefore, some loin chops did experience discoloration and metmyoglobin formation during retail display. Images representing final discoloration of chops from each of the 5 categories are displayed in Figure 1.

Aged Loin Quality and Color Stability

The percentage of loins from each trial in a category are displayed in Figure 2. A total of 17 loins were categorized as “very color stable,” 23 were categorized as “color stable,” 305 were categorized as “neutral,” 77 were categorized as “color labile,” and 62 were categorized as “very color labile.” All 4 trials were represented in 4 of the 5 categories, with the exception of VCS. However, the two groups represented in the VCS category represented both commercial and

University conditions for housing and slaughter. Proportions of chops in a stability category were similar across color groups.

Differences in retail display traits on d 7 of display are presented in Table 3. Visual discoloration scores were different between all color stability groups ($P < 0.0001$); this was expected as color stability categories were determined using final day visual discoloration. Unsurprisingly, chops in stable categories (CS and VCS) also had greater R630/580 ($P < 0.0001$) and decreased MMb ($P < 0.0001$) than chops in labile categories (CL and VCL) on d7. Additionally, on d7 no difference ($P = 0.14$) in MMb of CL and VCL was observed, but there was a tendency for greater MMb in chops from CS loins compared to chops from VCS loins ($P = 0.08$). No differences in chop redness, yellowness, DMb, or OMb were observed between stability categories on d7 ($P \geq 0.23$).

Effects of color stability categories on aged ventral loin quality traits and d1 color stability traits are shown in Table 4. Loins categorized as stable (CS and VCS) were at least 2.79 L^* units darker ($P < 0.01$), at least 0.81 b^* units less yellow ($P < 0.0001$), and had visual color scores at least 0.80 units greater ($P < 0.0001$), than loins categorized as labile (CL and VCL). Loins categorized as CS or VCS also had ultimate pH values at least 0.16 units greater ($P < 0.01$), marbling scores at least 0.42 units greater ($P < 0.0001$), and purge losses at least 1.18% lesser ($P < 0.0001$) than CL and VCL loins. Neutral loins were intermediate ($P \leq 0.04$) in ultimate pH ($P < 0.0001$), visual color score ($P < 0.0001$) and purge loss ($P < 0.0001$) to stable (CS and VCS) and labile (CL and VCL) loins. There were no differences in ultimate pH, visual color score, yellowness, or purge loss between CS and VCS loins ($P \geq 0.26$). Similarly, there were no differences in ultimate pH, visual color score, yellowness, or purge loss between CL and VCL loins ($P \geq 0.41$). There was a tendency for CL and VCL loins to have less visual marbling

($P = 0.01$) and greater yellowness ($P = 0.07$) after aging than NT, CS, or VCS loins. No differences in redness were observed between color stability categories ($P = 0.99$).

Similar to what was observed on aged ventral loins, on d1 of retail display CL and VCL chops were lighter ($P < 0.0001$) and yellower ($P < 0.0001$) compared with NT, CS, and VCS chops ($P \leq 0.02$). No differences in visual discoloration or MMb were observed between color stability categories ($P \geq 0.12$) on d1. However, chops categorized as CL and VCL did exhibit lesser R630/580 at d1 than chops in stable categories (CS and VCS; $P < 0.0001$). Chops categorized as CS and VCS were redder than VCL chops on d1 of display ($P = 0.04$), but not different from NT or CL chops ($P \geq 0.46$). Chops from the VCS category had the greatest DMb on d1 ($P < 0.01$). Chops from VL and VCL categories had increased OMb compared to VCS chops ($P < 0.01$).

Chop Color Stability During Retail Display

An interaction between color stability category and day of retail display was for all color stability traits ($P < 0.0001$). Chops from labile categories (CL and VCL) displayed increased visual discoloration compared to NT, CS, and VCS chops as early as d2 of display (Figure 3A; $P < 0.0001$). Chops from the VCL category did not begin to discolor until d5 of display. On all days of display, chops categorized as CS and VCS had greater R630/580 (Figure 3B) compared to chops categorized as CL and VCL ($P < 0.0001$). Over the entire 7 d display period, R630/580 did not differ between VCS and CS chops ($P \geq 0.08$). Furthermore, VCL and CL chops did not differ in R630/580 from d2 – d7 ($P \geq 0.09$). On d1 of display, chops categorized as NT had R630/580 values similar to CL chops ($P = 0.52$), but were intermediate to chops from stable (CS and VCS) and labile (CL and VCL) categories for all remaining days of display ($P < 0.0001$).

Although an interaction ($P < 0.0001$) between stability group and day was observed for chop lightness (Figure 4A), this interaction was likely the result of an increase in CS and NT chop lightness between d6 and d7 of display. In agreement with ventral loin quality observations, chops categorized as CL and VCL were lighter than CS and VCS chops throughout the entire display period ($P < 0.0001$), with chops categorized as NT intermediate to chops from stable (CS and VCS) and labile (CL and VCL) categories ($P < 0.0001$) during the same period. Instrumental redness also aligned with quality observations. Chops categorized as CS and VCS were redder than chops categorized as CL and VCL for all days of display except d7 ($P < 0.0001$; Figure 4B). Redness of all chops increased from d1 to d2 ($P < 0.0001$), regardless of stability category, before decreasing between d2 - d6 ($P < 0.0001$). Chops from CL and VCL loins had greater decreases in redness from d2 to 6 ($\Delta a^* = 1.63$ and 1.77 , respectively) compared to chops from NT, CS, and VCS loins ($\Delta a^* = 0.88$, 0.73 , and 1.17 , respectively). Minimal differences in yellowness during display were observed between chops from stable (CS and VCS) and labile (CL and VCL) categories (Figure 4C). Interestingly, differences in yellowness were driven by decreased yellowness values for chops from NT loins from d1 to d5 of display compared to other categories ($P < 0.0001$). Minimal differences in yellowness were observed between color stability categories during display.

Chops categorized as NT had similar MMb to CL and VCL chops until d4 of display ($P \geq 0.08$; Figure 5A), after which N chops were intermediate to either stable (CS and VCS) and labile (CL and VCL) categories ($P < 0.0001$). Furthermore, chops from VCS, CS, and NT loins had smaller numerical decreases in MMb from d1 to d7 of display ($\Delta \text{MMb} = 14.80$, 14.36 , and 13.60 , respectively) compared to CL and VCL loins ($\Delta \text{MMb} = 17.33$ and 17.55 , respectively). A day x stability category interaction was observed for both DMb and OMb ($P < 0.0001$; Figures

5B and 5C, respectively). Chops from VCS loins had the greatest DMb and least OMb on d1 of display ($P < 0.0001$). However, differences in DMb and OMb between groups were inconsistent from d2 – d7 of display.

Discussion

Despite substantial research into consumer perception of and biochemical mechanisms behind fresh beef discoloration, little is known regarding fresh pork color stability. Many of the quality traits commonly measured in both beef and pork, including color, marbling, and pH, are related to beef color stability (O'Grady et al., 2001; McKenna et al., 2005; Wheeler et al., 2005) and may likely affect pork color stability as well. However, while Zhu et al. (1998) and King et al. (2011a) have identified color traits associated color stable and color labile pork loins, limited research has related other commonly measured quality traits to pork color stability. This is problematic because substantial differences in pork and beef color make difficult drawing conclusions about pork color stability using beef-specific research. Myoglobin, the water-soluble protein primarily responsible for meat color, has structural differences between pork and beef that may influence discoloration patterns between the two (Suman et al., 2006). Oxidation byproducts preferentially bind to different sites on beef myoglobin compared to pork myoglobin, resulting in decreased oxidative stability for beef myoglobin (Suman et al., 2006). Additionally, myoglobin forms (deoxymyoglobin, oxymyoglobin, and metmyoglobin) in beef typically differ in lightness and redness on a unit for unit basis (Hunt et al., 1999), while differing in lightness but not redness in pork (Fernández-López et al., 2000). Therefore, further investigation into relationship between quality traits and pork color stability was necessary.

Pork loins in the present study represented a wide range of visual discoloration scores (final discoloration scores ranged from 0 – 54% discoloration). The majority of loins were

categorized as NT, with 63% of loins falling into this category. More loins were categorized as labile (CL or VCL) than stable (CS or VCS), as 3.51% and 4.75% of loins were VCS and CS, respectively, while 15.91% and 12.81% of loins were CL and VCL, respectively. As expected, chops from loins in different categories differed in their rate of discoloration and final discoloration score. Categories began to differ in discoloration score as early as d3, then from d4 to d7 no categories had the same average discoloration score. There was at least a 6.61% difference in visual discoloration between groups on d7 of display. Overall, categorizing loins based on final discoloration score successfully differentiated loins based on rate of discoloration as well.

In comparing quality traits, loins that were considered “stable” (CS and VCS) had greater pH values and decreased purge loss compared to neutral or “labile” (CL and VCL) loins. As the pH of meat increases, the polarity of meat increases as well and water holding capacity is improved, resulting in less purge loss (Huff-Lonergan and Lonergan, 2005). Thus, the observation of increased pH and reduced purge loss was expected. Purge loss *per se* is not expected to alter color stability. However, pH difference may directly impact color stability. Fresh meat contains enzymatic systems that are capable of reducing metmyoglobin to deoxymyoglobin (Mancini and Hunt, 2005). Activity of these enzymatic processes increase with increasing pH, ultimately reducing metmyoglobin accumulation (Bekhit and Faustman, 2005). Further prolonging color shelf life, myoglobin is oxidized at a slower rate when pH increases (Faustman and Cassens, 1990). Despite this, the impact of pH on LL color stability in beef is unclear. Ledward et al (1986) and O’Grady et al (2001) each reported greater color stability of higher pH LL steaks, whereas King et al (2011b) and Canto et al. (2015) observed no pH differences between LL steaks classified as color stable or color labile. The latter study,

however, had a small range of pH values (5.41 – 5.74), while O’Grady et al. (2001) reported pH ranges of 5.25 – 6.26 (O’Grady et al., 2001). Ledward et al. (1986) did not report ranges in pH values; however, they had low and high pH groups with average pH values of 5.60 and 5.94, respectively. Had there been a greater range in pH values in the studies by King et al. (2011b) and Canto et al. (2015), differences between stability groups may have been observed.

Loins categorized as VCS or CS were also initially darker than VCL and CL chops when evaluated subjectively and instrumentally, and remained darker during retail display. In contrast, minimal differences in lightness were observed between beef longissimus steaks classified as color stable or color labile, both initially and during retail display (McKenna et al., 2005; King et al., 2011a; Canto et al., 2015). Differences in lightness between stability categories in the current study may have been attributable to pH differences. Ledward et al (1986) observed that steaks with greater pH values tended to have decreased lightness compared to lower pH steaks. When more water is present, the surface of the meat absorbs more light and appears darker (Huff-Lonergan et al., 2005). In pork, instrumental lightness has been negatively correlated with ultimate pH and positively correlated with NPPC color score and water holding capacity in multiple studies (Huff-Lonergan et al., 2002; Boler et al., 2010). Despite differences in lightness between color stability categories, chop lightness (L^*) did not change over display for any individual stability category until d7. Zhu et al. (1998) also reported that pork chop lightness did not change during retail display. Initial lightness may be more important for pork color stability than changes in lightness during display. Overall, stable pork chops came from loins that were darker and had decreases pH values. These would typically be considered “higher quality” loins, and according to the present study, would also possess improved color stability.

There were no differences in instrumental redness between groups during early or aged quality analyses. However, over the course of retail display, chops from stable categories were generally redder than chops from neutral and labile categories. The lack of differences during quality analyses may be due to instrumentation used. Early postmortem and aged ventral loin color were measured using a Minolta colorimeter, while instrumental color during retail display was measured using a HunterLab spectrophotometer. Because the Minolta colorimeter has a smaller aperture than the HunterLab (8 mm vs 31.8 mm), the magnitude of difference between a^* redness values is reduced (AMSA, 2012). Greater redness values observed in color stable loin chops were unexpected, as greater redness in beef is typically associated with a lower overall color stability (Faustman et al., 2010). In beef, greater redness has been associated with a greater proportion of oxidative muscle fibers, containing a greater number of mitochondria and myoglobin for use in oxidative metabolism (Ramanathan et al., 2018). In postmortem muscle, mitochondria produce reactive oxygen species as a byproduct of aerobic respiration that can accumulate and exacerbate myoglobin oxidation (Ramanathan et al., 2018). Alternatively, glycolytic fibers contain greater concentration of glycolytic enzymes, which may generate NADH to be used in MMb reducing reactions (Nair et al., 2018). Beef cattle possess a greater proportion of oxidative fibers than pigs (30% in beef longissimus vs 10% in pork longissimus; Zhang et al., 2017). Because pork skeletal muscle is more glycolytic by nature, pork may contain a greater proportion of glycolytic enzymes capable of supplying NADH to reduce MMb than beef. As a result, the increased number of mitochondria associated with more oxidative muscle may not as pronounced an effect on pork loin color stability. Alternatively, oxidative muscle experiences a less intense decline in pH decline compared to glycolytic fibers due to a decreased glycogen content (Huff-Lonergan and Lonergan, 2005). This increased pH may promote

enzymatic MMb reduction and improve color stability in more oxidative pork. Further research is necessary to determine whether differences in redness is an important indicator of color stability in pork as it is in beef. However, results of the current study would indicate that redder pork loins have increased color stability.

Color stable and very color stable chops were less yellow during postmortem quality evaluations and at d1 of retail display. Stable loins also had increased DMb and decreased OMb on d1 of display, both of which are related to decreased yellowness in pork (Karamucki et al., 2013). Despite these differences, minimal changes in yellowness were observed within color stability categories over the retail display period. Zhu and Brewer (1998) and King et al. (2011b) also observed decreased yellowness in pork loins or loin chops that were considered stable. Alternatively, conflicting results have been observed in beef, as King et al (2011a) reported increased yellowness in color stable steaks whereas Canto et al. (2015) observed no differences in yellowness between steaks assigned to different color stability groups. Instrumental yellowness is not typically related to meat, partially because consumers have difficulty determining differences in meat yellowness during sensory evaluation (O'Sullivan et al., 2003; Mancini and Hunt, 2005). However, based on results of this study and previous research, initial yellowness may be more important for pork stability characterization than for standard color evaluations, and may be more relevant for color stability in pork than beef. Overall, decreased yellowness appears to be associated with increased color stability in pork loins.

Stable loins in the current study generally had greater amounts of marbling compared to labile loins. Limited research is available investigating the relationship between marbling and color stability, regardless of animal type. However, Wheeler et al (2005) speculated that metabolic differences contributing to increased muscle growth and decreased fatness may

contribute to increased color stability in beef. This would imply that animals with a greater level of marbling discolor at a faster rate, in contrast to what was observed in the current study. The present study involved pigs from different sire lines, including some considered meat quality lines. Typically, loins from quality-focused pigs are darker and redder in color, have an increased pH, and have a greater proportion of marbling than lean growth-focused pigs (NPPC, 1998; Arkfeld et al., 2017; Lowell et al., 2018). In the present study, stable loins possessed each of the traits typically associated with higher quality pork, and may indicate that selecting for meat quality traits already important in the industry, such as higher marbling, may result in greater color stability as well. Nonetheless, further research is necessary to determine the direct impact of marbling on pork color stability.

Stable loins had increased R630/580 and decreased MMb over the display period compared to labile loins. This was expected, as an increased 630/580 nm ratio is indicative of greater redness and less brownness on the surface of fresh meat (AMSA, 2012). For this study, the Krzywicki (1979) method was used to determine MMb content. While MMb estimates generated using this method are different compared to the K/S method of determination, which provides a more accurate assessment of true MMb content, both methods are highly correlated ($R^2 = 0.9390$) to each other and equivalent for statistical analysis to determine differences in pigment concentration between samples (Hernández et al., 2015). However, 20% MMb measured using the Krzywicki method is approximately equivalent to 20% measured using the K/S method (Hernández et al., 2015). This percentage is important since 20% is the amount of MMb commonly referenced, in beef, as the threshold where consumers begin to discriminate against discoloration (Renner and Labas, 1987; Gill and Jones, 1994). Assuming the threshold is similar in pork, instrumental MMb values in this study would indicate that and “labile” and “very

labile” chops reach a perceptible level of discoloration at d 4 of retail display, while “neutral” chops do not discolor to a meaningful degree until d 5 and “stable” or “very stable” chops are acceptable until d 6. These values do not necessarily align with visual discoloration scores, as VCL and CL chops did not reach 20% discoloration on average until d 6 and 7, respectively, while NT, VCS, and CS did not reach 20% discoloration during the study. However, both instrumental MMb determination and visual discoloration scores would indicate that VCL chops discolored at least two days earlier than loins from stable groups. While further research is needed to determine actual consumer discrimination thresholds for discoloration in pork, data from this study would indicate that selecting for “color stable” pork could improve retail display duration by as much as 2 days.

Conclusions

Differences in color stability were observed between boneless pork loins classified as “stable” and “labile” in this study based on instrumental and visual discoloration assessment. Stable loins were generally darker, redder, and had greater levels of pH and marbling compared to labile loins. This study would also indicate differences in characteristics of stable pork compared to beef, as stable loins in the current study were typically more red and had greater marbling, whereas in beef stable loins are typically less red and have been indicated by some research to contain less marbling. Data from this study would also indicate that retail display duration could be improved by as much as 2 days in pork chops if loins were selected for higher levels of color stability. However, further research into consumer discrimination of discolored pork is necessary to draw further conclusions.

Tables

Table 2.1. Handling and slaughtering conditions for pigs from trials used in this study

Trial	Pigs, n	Housing	Diet	Harvest	Stunning	Chilling ¹	Aging Time ²	Display Lighting
A	48	Commercial	Commercial	Commercial	CO ₂	Blast Chilled	14 d	3000 K
B	54	University	Commercial	University	Electric	Slow Chilled	14 d	3000 K
C	148	University	Commercial	University	Electric	Slow Chilled	14 d	3000 K
D	234	Commercial	Commercial	Commercial	CO ₂	Blast Chilled	11 d	6500 K

¹Blast chilled carcasses were chilled in a blast freezer for approximately 90 minutes, then held in an equilibration cooler at 4 °C for 22 h. Slow chilled pigs placed in a cooler at 4°C immediately after slaughter and held for approximately 22 h.

²All loins, regardless of age time, were aged at 4° C.

Table 2.2. Population summary statistics of initial (d1), final (d7), and overall change in (Δ , d7 – d1) display characteristics.

Variable	N	Mean	Minimum	Maximum	SD ¹	CV ²
<i>Initial, d1 retail display</i>						
Lightness, L^*	484	57.27	46.84	68.60	3.25	5.67
Redness, a^*	484	8.77	4.44	14.03	1.72	19.62
Yellowness, b^*	484	16.71	12.07	20.51	1.28	7.68
R630/580	484	2.61	2.06	3.56	0.25	9.50
Visual Discoloration, %	484	0.02	0.00	3.00	0.21	1287.31
Metmyoglobin, % ³	484	9.12	1.96	15.06	2.20	24.18
Deoxymyoglobin, % ⁴	483	24.96	0.32	68.19	12.93	51.80
Oxymyoglobin, % ⁵	483	65.93	24.83	86.40	12.08	18.32
<i>Final, d7 retail display</i>						
Lightness, L^*	484	58.02	43.29	70.38	3.50	6.02
Redness, a^*	484	8.63	4.56	14.58	1.54	17.89
Yellowness, b^*	484	17.05	12.51	24.18	1.05	6.14
R630/580	484	2.17	1.45	3.28	0.33	15.25
Visual Discoloration, %	484	19.63	0.00	54.00	8.11	41.31
Metmyoglobin, % ³	484	23.89	8.64	46.39	7.37	30.85
Deoxymyoglobin, % ⁴	484	7.57	-21.28	59.75	7.07	93.46
Oxymyoglobin, % ⁵	484	68.55	12.93	84.47	8.92	13.01
<i>Δ (d7 - d1)</i>						
Lightness, L^*	484	0.75	-16.80	14.18	4.31	576.06
Redness, a^*	484	-0.14	-6.95	6.06	2.40	-1693.45
Yellowness, b^*	484	0.34	-4.06	7.15	1.47	428.67
R630/580	484	-0.44	-1.85	0.35	0.39	-88.13
Visual Discoloration, %	484	19.61	0.00	54.00	8.09	41.29
Metmyoglobin, % ³	484	14.77	2.28	40.56	6.30	42.67
Deoxymyoglobin, % ⁴	483	-17.45	-85.52	24.21	12.36	-70.81
Oxymyoglobin, % ⁵	483	2.75	-47.64	72.38	15.08	547.49

¹Standard Deviation.

²Coefficient of Variation, calculated by $(SD/mean) \times 100\%$.

³Calculated as: MMb, % = $\{1.395 - \frac{(A_{572} - A_{700})}{(A_{525} - A_{700})}\} \times 100$, where A represents reflex attenuance at a given wavelength.

⁴Calculated as: % DMb = $\{2.375 \times (1 - \frac{A_{572} - A_{700}}{A_{525} - A_{700}})\} \times 100$. where A represents reflex attenuance at a given wavelength.

⁵Calculated as: % OMb = $100 - \% \text{MMb} - \% \text{DMb}$.

Table 2.3. Effect of color stability category on color stability of boneless pork loin chops at day 7 of retail display.

Item ¹	VCS	CS	NT	CL	VCL	SEM	P-Value
n	17	23	305	77	62		
Visual Discoloration, %	1.47 ^e	8.35 ^d	17.21 ^c	26.02 ^b	32.79 ^a	0.73	< 0.0001
Lightness, L^*	54.92 ^c	58.11 ^b	58.52 ^b	57.91 ^b	59.57 ^a	1.05	< 0.0001
Redness, a^*	9.02	8.24	8.28	8.20	8.03	0.52	0.23
Yellowness, b^*	16.82	17.06	17.24	17.12	17.36	0.31	0.30
R630/580	2.32 ^a	2.31 ^a	2.12 ^b	1.88 ^c	1.79 ^d	0.13	< 0.0001
Metmyoglobin, % ²	21.72 ^c	23.88 ^c	26.27 ^b	29.50 ^a	30.56 ^a	3.33	< 0.0001
Deoxymyoglobin, % ³	6.15	7.29	7.11	6.76	6.60	0.93	0.67
Oxymyoglobin, % ⁴	75.65	76.06	76.40	76.27	75.82	3.33	0.89

¹Treatments with a row lacking common superscripts are different ($P < 0.05$).

²Calculated as: MMb, % = $\{1.395 - \frac{(A_{572} - A_{700})}{A_{525} - A_{700}}\} \times 100$, where A represents reflex attenuance at a given wavelength.

³Calculated as: % DMb = $\{2.375 \times (1 - \frac{A_{572} - A_{700}}{A_{525} - A_{700}})\} \times 100$, where A represents reflex attenuance at a given wavelength.

⁴Calculated as: % OMb = $100 - \% \text{MMb} - \% \text{DMb}$.

Table 2.4. Effect of color stability category on postmortem loin quality traits and color stability of boneless pork loin chops at days 1 and 7 of retail display.

Item ¹	VCS	CS	NT	CL	VCL	SEM	P-Value
n	17	23	305	77	62		
<i>Aged ventral surface quality</i> ²							
Ultimate pH	5.70 ^a	5.72 ^a	5.64 ^b	5.54 ^c	5.52 ^c	0.05	< 0.0001
Color	3.85 ^a	3.96 ^a	3.49 ^b	3.05 ^c	3.02 ^c	0.11	< 0.0001
Marbling	2.82 ^a	2.46 ^{ab}	2.32 ^b	2.04 ^c	2.03 ^c	0.22	< 0.0001
Firmness	3.04 ^a	2.80 ^{ab}	2.94 ^a	2.76 ^b	2.90 ^{ab}	0.24	0.01
Lightness, <i>L</i> *	47.22 ^b	45.04 ^c	47.53 ^b	50.01 ^a	50.74 ^a	1.06	< 0.0001
Redness, <i>a</i> *	7.20	7.44	7.40	7.38	7.38	0.51	0.99
Yellowness, <i>b</i> *	4.55 ^b	4.22 ^b	4.73 ^b	5.36 ^a	5.63 ^a	0.40	< 0.0001
Purge Loss, % ³	1.34 ^c	1.68 ^c	2.26 ^b	3.05 ^a	2.86 ^a	0.66	< 0.0001
<i>Initial, d1 retail display</i>							
Visual Discoloration, %	0.00	0.00	0.00	0.03	0.05	0.04	0.25
Lightness, <i>L</i> *	54.70 ^d	54.29 ^d	57.01 ^c	59.63 ^b	60.71 ^a	0.87	< 0.0001
Redness, <i>a</i> *	9.51 ^a	9.54 ^a	9.21 ^{ab}	9.21 ^{ab}	8.77 ^b	0.67	0.04
Yellowness, <i>b</i> *	16.68 ^{bc}	16.34 ^c	16.98 ^b	17.51 ^a	17.44 ^a	0.51	< 0.0001
R630/580	2.79 ^a	2.79 ^a	2.67 ^b	2.57 ^c	2.50 ^c	0.08	< 0.0001
Metmyoglobin, % ⁴	10.02	9.20	9.53	10.01	9.97	0.75	0.12
Deoxymyoglobin, % ⁵	15.12 ^a	10.06 ^{bc}	9.00 ^c	10.22 ^b	9.89 ^{bc}	3.43	< 0.01
Oxymyoglobin, % ⁶	67.24 ^c	71.42 ^{bc}	73.09 ^{ab}	73.11 ^{ab}	74.03 ^a	2.39	0.01

¹Treatments with a row lacking common superscripts are different ($P < 0.05$).

²Aged quality measurements were collected at d11 or d14 postmortem on the ventral face of the loin at the approximate location of the 10th rib.

³Purge loss was calculated as $([\text{Initial wt.} - \text{final wt.}]/\text{initial wt.} \times 100)$.

²Calculated as: $\text{MMb, \%} = \{1.395 - \left(\frac{A_{572} - A_{700}}{A_{525} - A_{700}}\right)\} \times 100$, where *A* represents reflex attenuation at a given wavelength.

³Calculated as: $\% \text{DMb} = \left\{2.375 \times \left(1 - \frac{A_{572} - A_{700}}{A_{525} - A_{700}}\right)\right\} \times 100$, where *A* represents reflex attenuation at a given wavelength.

⁴Calculated as: $\% \text{OMb} = 100 - \% \text{MMb} - \% \text{DMb}$.

Figures

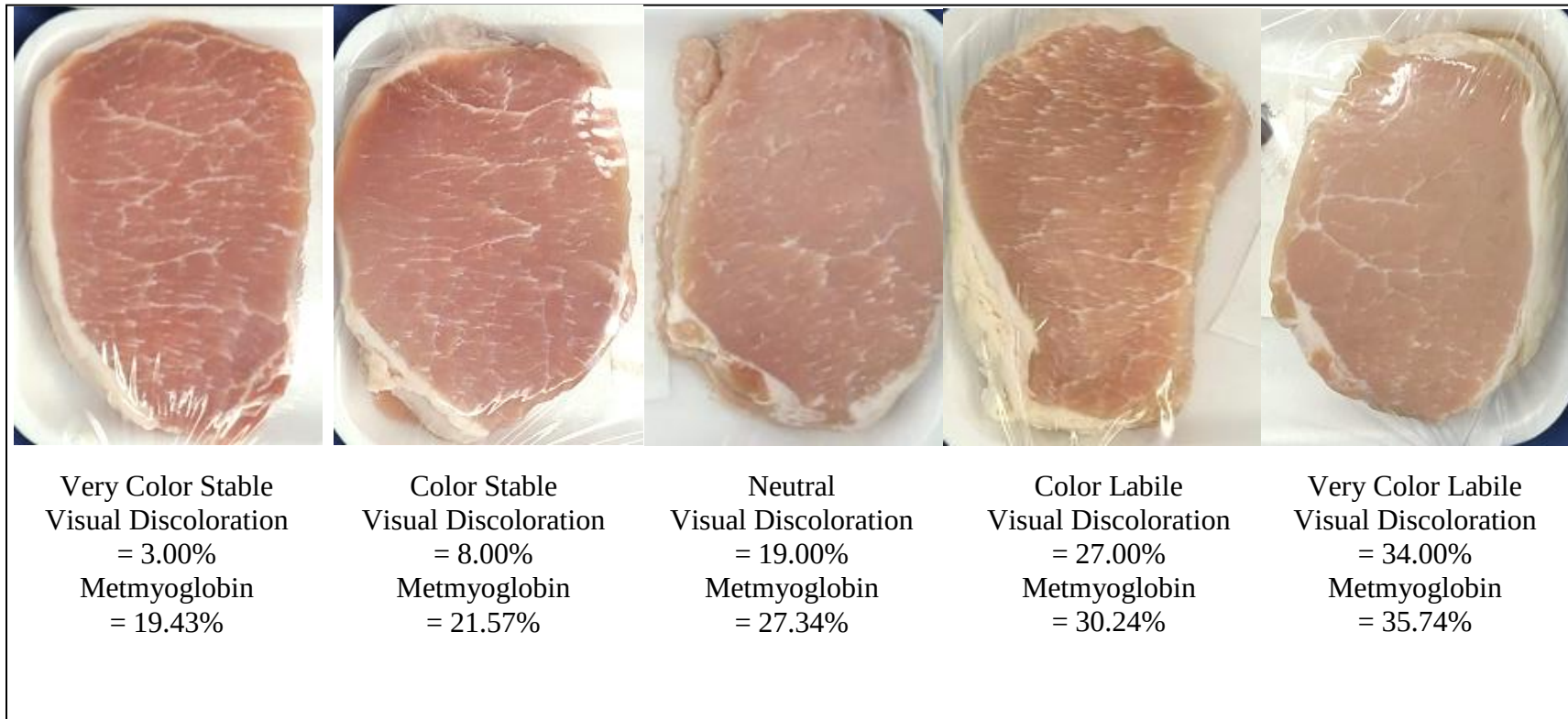


Figure 2.1. Final visual color stability of chops representing each color stability category.

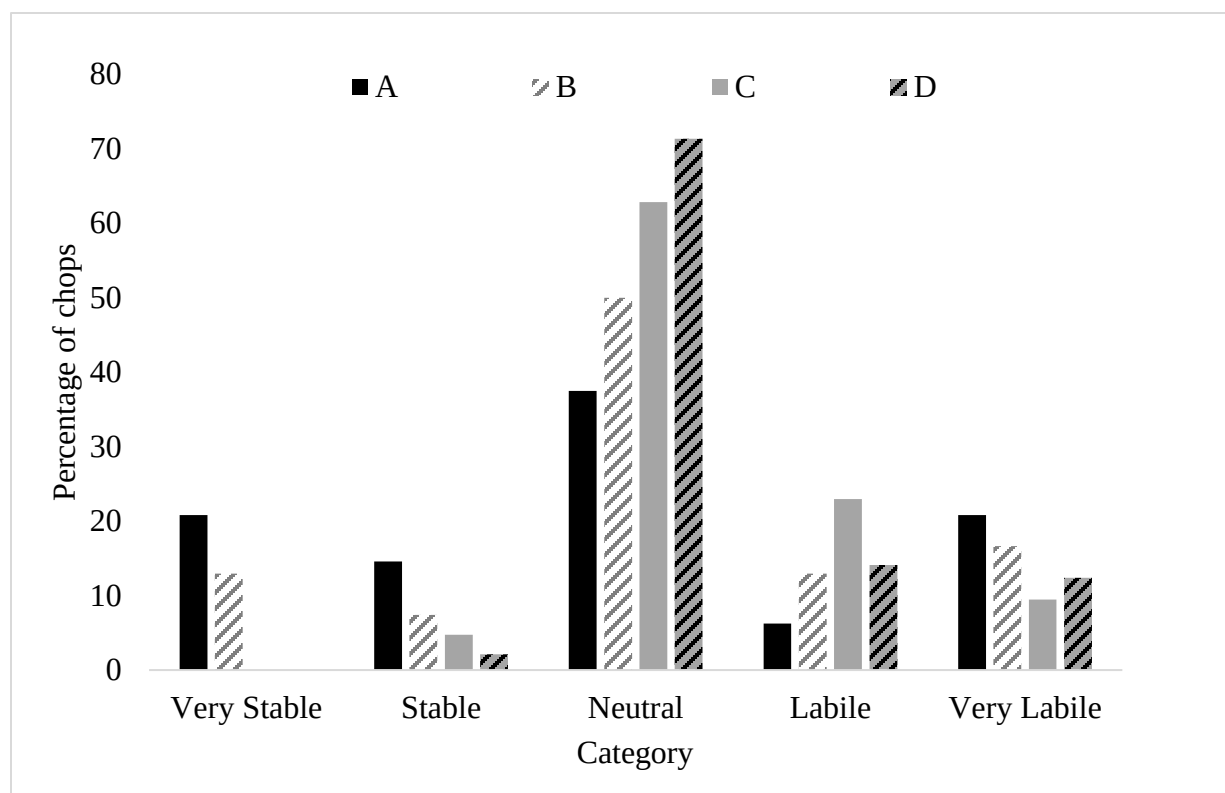


Figure 2.2. Percentage of chops from each trial represented in color stability categories.

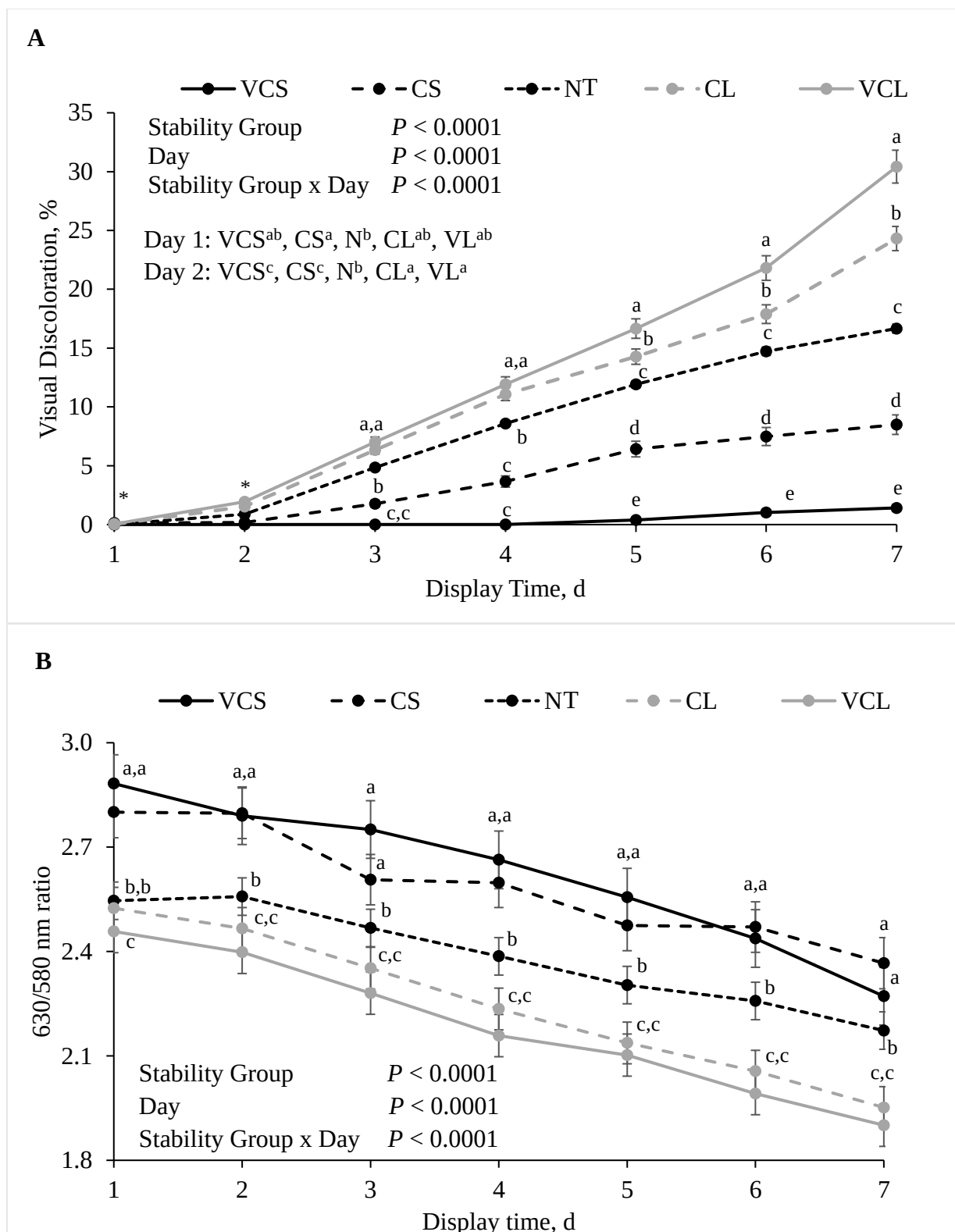


Figure 2.3. Effect of color stability group on visual discoloration (A) and R630/580 (B) of boneless pork chops across 7 d of retail display. Treatments within a day lacking common superscripts were different ($P < 0.05$).

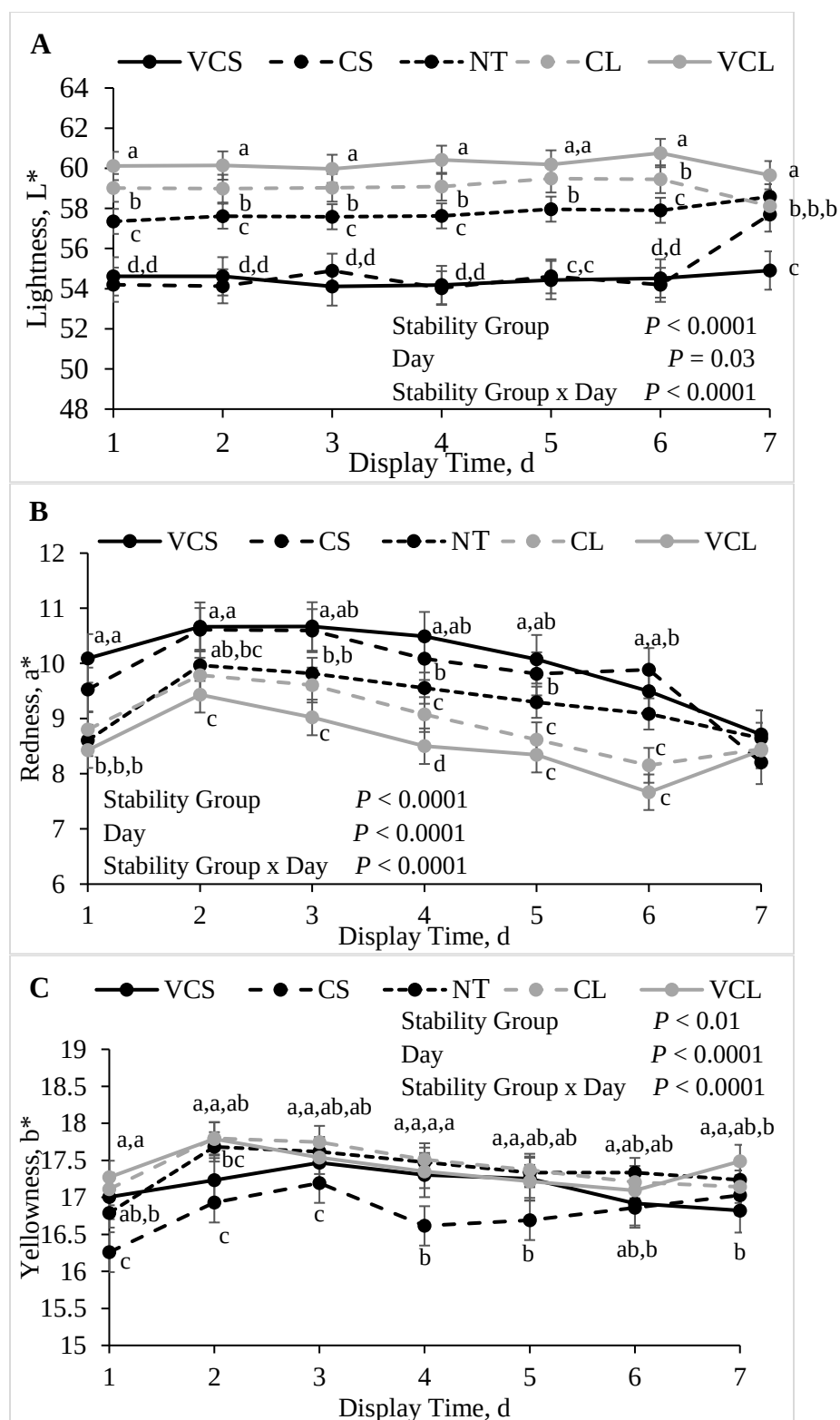


Figure 2.4. Effect of color stability group on instrumental lightness (A), redness (B), and yellowness (C) of boneless pork chops across 7 d of retail display. Treatments within a day lacking common superscripts were different ($P < 0.05$).

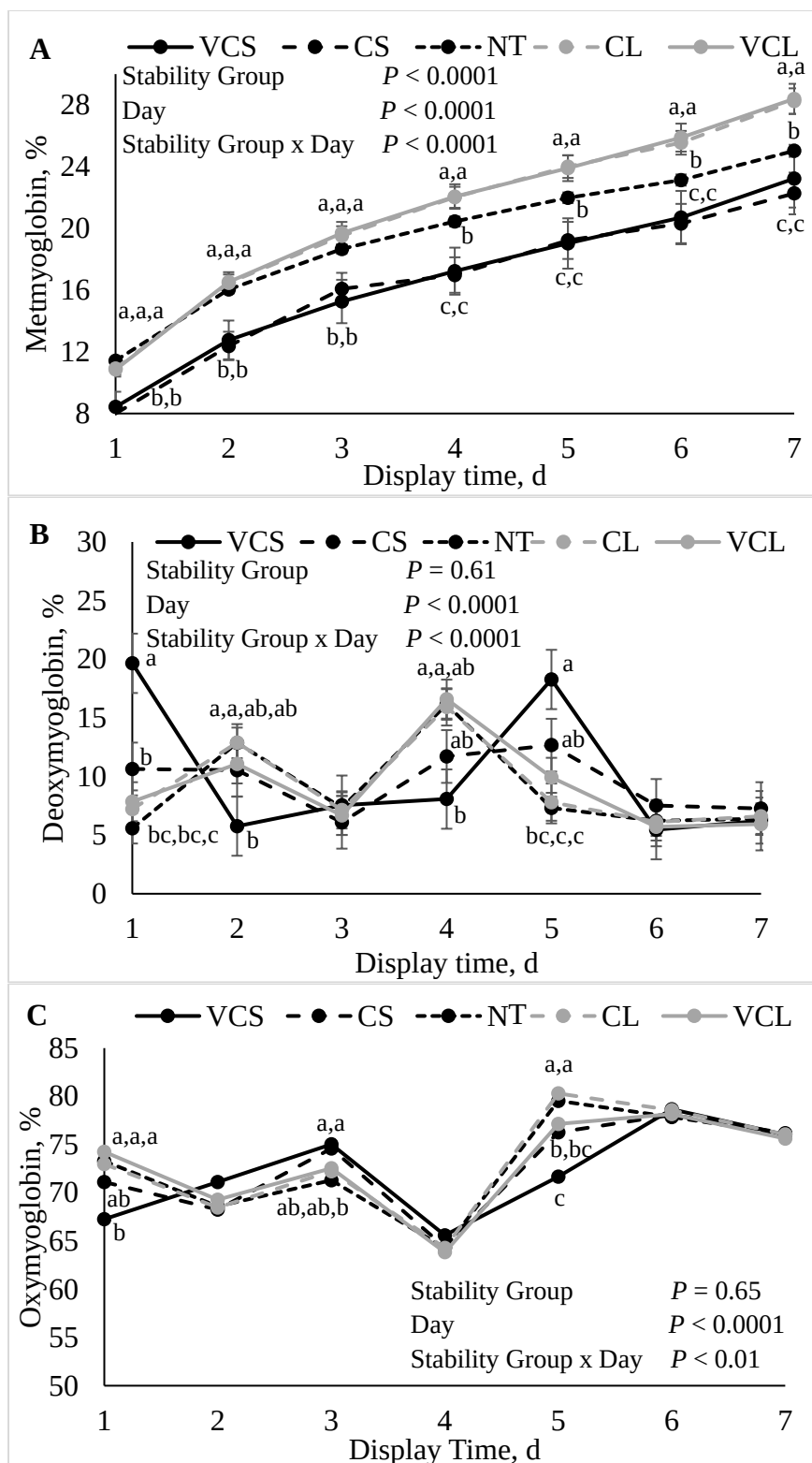


Figure 2.5. Effect of color stability group on metmyoglobin (A), deoxymyoglobin (B), and oxymyoglobin (C) content of boneless pork chops across 7 d of retail display. Treatments within a day lacking common superscripts were different ($P < 0.05$).

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CHAPTER 3

COLOR STABILITY DURING RETAIL DISPLAY DIFFERS BETWEEN MUSCLES IN PORK

Abstract

The objectives of this study were to determine differences in color stability among pork muscles and if changes in color measurements for *longissimus dorsi* (LD) were related with changes for *triceps brachii* (TB) or *psoas major* (PM). The LD, TB, and PM from 20 pigs were aged in vacuum packages for 21 d, then fabricated into 3 chops per muscle. All chops from a single muscle were overwrapped, displayed for 5 d under constant lighting, and evaluated daily for instrumental color, myoglobin forms and subjective visual discoloration. Chops were removed on d1, 3, or 5 to measure oxygen consumption (OC) and metmyoglobin reducing activity (MRA). Data were analyzed using the MIXED procedure of SAS as a repeated measures. Pearson correlation coefficients were determined using the CORR procedure. Over the 5d display period, TB and PM chops had greater changes in a^* , b^* , chroma, 630/580 nm ratio, deoxymyoglobin, and oxymyoglobin compared to LD chops (display time x muscle interaction $P \leq 0.01$), but TB and PM did not differ from each other ($P \geq 0.07$). Conversely, LD had decreased OC and increased MRA on each display day compared to TB and PM ($P < 0.01$). Using 20% discolored on d5 of display as a threshold for acceptability, 85% of LD samples were considered acceptable while only 5% and 20% of TB and PM samples were considered acceptable, respectively. Changes in LD were correlated with TB and PM changes for redness ($0.51 \leq r \leq 0.55$; $P \leq 0.02$), and trending towards a correlation or correlated with changes in TB and PM 630/580 nm ratio and hue angle ($0.39 \leq r \leq 0.63$). Chops from TB and PM were less color stable during display, but LD changes were not consistently correlated with changes in other muscles.

Introduction

Meat discoloration during retail display is a process characterized by changes in myoglobin (Mb), the main pigment in meat. Normally, Mb on the meat surface is bound to oxygen (oxymyoglobin; OMb) and appears red in color, with the color intensity depending on species (AMSA, 2012). However, Mb can be oxidized to form metmyoglobin (MMb), resulting in development of a brown or gray color. Consumers discriminate against meat that appears brown or gray in color, resulting in profit losses and potential discarding of product (Font-i-Furnols and Guerrero, 2014). When evaluating color stability of meat, MMb content is often used as an indicator of discoloration extent (Mancini and Hunt, 2005). Discoloration in pork is influenced by initial color traits, including lightness, redness, and hue angle (Zhu and Brewer, 1998; King et al., 2011a).

In beef, discoloration is muscle dependent. Multiple studies have reported that the *psoas major* (PM) and *triceps brachii* (TB) were more color labile than the longissimus lumborum (LL), and that those color-labile muscles had greater initial redness, myoglobin content, and consumed more oxygen during display (McKenna et al., 2005; Jeong et al., 2009; King et al., 2011b; Mancini et al., 2018). Muscle color stability differences can be partially attributed to muscle fiber differences, as color labile muscles possess predominantly red, oxidative fibers while color stable muscles primarily possess white, glycolytic fibers (Faustman et al., 2010). Despite differences between muscles, King et al. (2011b) observed relationships between the LL and other muscles for color stability, indicating that factors affecting the entire carcass like genetics, diet, or processing conditions may exert influence over color stability.

Studies regarding the color stability of different pork muscles are much more limited compared to beef. However, previous studies have reported color and muscle fiber type

differences between pork muscles. Cheng et al. (2021) observed that pork PM was darker, redder, and had a greater proportion of oxidative fibers compared to pork *longissimus dorsi* (LD). Souza et al. (2011) did not evaluate muscle fiber type, but observed that the triceps brachii was darker and redder than the LD. If observations from beef regarding differences in color stability hold true, redder, more oxidative muscles like the TB and PM would be expected to be less color stable than the LD. Furthermore, if factors controlling color stability in pork muscles are similar to beef, then color stability the LD in pork may be indicative of color stability for other muscles as well. Therefore, the objectives of this study were to evaluate differences in color stability between the TB, PM, and LD muscles and relate those differences to biochemical measures. Then, the goal was to determine if color changes in the LD were related to color changes in other muscle types. It was hypothesized that, similar to observations in beef, TB and PM would discolor more quickly than the LD, and that changes in LD color during display would be related to color changes in the TB and PM.

Materials and Methods

All protocols were approved by the University of Illinois Institutional Animal Care and Use Committee (Protocol #20095).

Sample Preparation

Pigs (n = 20) were transported to the University of Illinois Meat Science Laboratory and held in lairage for approximately 16 h with no feed and ad libitum access to water. Pigs were slaughtered under the supervision of the USDA Food and Safety Inspection Service. Pigs were immobilized by electrical stunning and terminated by exsanguination. Carcasses were allowed to chill for approximately 22 h at 4° C. Left carcass sides were fabricated to yield the picnic shoulder (NAMP #405) and bone in loin (NAMP #410). Picnic shoulders were further fabricated

to yield the TB and loins were fabricated to yield the LD and PM. The TB and PM were individually vacuum packaged and aged for 21 d at 4° C. The LD was cut into 2.54 cm chops using a push-feed slicer (Treif model 700 F, Oberlahr, Germany). The 3 chops cut immediately posterior to the 10th rib were vacuum packaged together and stored in the same manner as TB and PM chops. At the end of the aging period, TB and PM muscles were cut by hand to yield 3 chops measuring approximately 2.54 cm and 5.08 cm thick, respectively. Ultimate pH of the first chop for each muscle was measured using a Hanna pH meter fitted with a glass tipped electrode (model HI98163, Hanna Instruments. Woonsocket, RI) calibrated using pH 4 and pH 7 buffer at 4 °C.

Retail Display

All three chops from a pig representing a single muscle were placed on a 27.3 x 14.9 cm polystyrene tray (Dyne-a-pak, Laval, Quebec, Canada) with a soaker pad and overwrapped with polyvinyl chloride film (O^2 transmission = 23,250 mL·m² ·d⁻¹ , 72 gauge; Resinite Packaging Films, Borden, Inc., North 70 Andover, MA). The cut surface of chops were oriented to be visible through the film. Within a package, chops were randomly allotted to 1, 3, or 5 d of display in the package, and were removed from display at that point for biochemical analyses. Packages were arranged on 4 wire mesh shelves in a standing display cooler and displayed at 4 °C. Lighting was provided by 122 cm fluorescent bulbs (32 W, Octron XP, 6500 K, Osram Sylvania, Wilmington, MA) positioned at the front of the display case. Packages were rotated each day to minimize location effects on discoloration. Samples were allowed to oxygenate for at least 2 h prior to color evaluation on the first day of display. On each day of display, subjective and instrumental discoloration were evaluated on all chops in a package. Discoloration of chops within a single package were then averaged for statistical analyses.

Subjective visual discoloration was evaluated through the film by a panel of 8 trained technicians using an unstructured line 10-cm line scale anchored at 0%, 50%, and 100% discoloration. Instrumental surface color including CIE L^* (lightness), a^* (redness), b^* (yellowness; CIE, 1978) and reflectance data (400 – 700 nm) was evaluated using a HunterLab MiniScan EZ spectrophotometer (HunterLab, Reston, VA). The spectrophotometer was equipped with a D65 illuminant, 31.8 mm aperture, and calibrated with black and white tiles specific to the machine. Film was removed from packages prior to instrumental evaluation. Instrumental L^* , a^* , and b^* data were used to calculate chroma (measure of saturation: $Chroma = \sqrt{a^{*2} + b^{*2}}$) and hue angle (measure of color hue: $Hue\ angle = \left(\arctan \frac{b^*}{a^*} \right)$). Reflectance data was used to calculate the ratio of 630/580 nm (an indicator of surface brownness) and Mb forms using the attenuation method as described by Krzywicki (1979). Reflectances at 474, 525, 572, and 700 nm were converted to attenuation values (A) using the equation: $A = \log(1/R)$, where R = reflectance. Myoglobin forms were calculated using the following equations:

$$\% \text{ Metmyoglobin (MMb)} = \left\{ 1.395 - \left(\frac{A_{572} - A_{700}}{A_{525} - A_{700}} \right) \right\} \times 100$$

$$\% \text{ Deoxymyoglobin (DMb)} = \left\{ 1.395 - \left(\frac{A_{474} - A_{700}}{A_{525} - A_{700}} \right) \right\} \times 100$$

$$\% \text{ Oxy myoglobin (OMb)} = 100 - (\% \text{MMb} + \% \text{DMb})$$

Biochemical Analyses

On their respective day of analysis (d1, 3, or 5), chops for each muscle were removed from display and trimmed of all external fat. Chops from the LD and TB were cut in half perpendicular to the cut chop face surface, while PM chops were cut in half parallel to the cut surface. This was done to ensure muscle fibers ran in the same direction for all muscle samples. One half was frozen at -80° C to be used for Mb quantification (d1 only) and lipid oxidation,

while the remaining half was used to determine oxygen consumption (OC) and MMb reducing capacity (MRA). Sample portions reserved for OC and MRA were cut in half parallel to the muscle fiber direction, halves randomly allotted to each analysis.

Oxygen Consumption

Oxygen consumption was determined as described by AMSA (2012) with minor modifications. The freshly cut surface of the OC sample was covered with PVC film and allowed to oxygenate at 4°C for 2 hours. After oxygenation, samples were vacuum packaged and immediately scanned through the oxygen impermeable film with the same Hunter spectrophotometer used for instrumental color evaluations. After packaging, samples were allowed to incubate 20°C for 1h at, then scanned a second time with the Hunter. The proportion of OMb on oxygenated and deoxygenated samples was determined using the Krzywicki (1979) method as previously outlined. The % oxygen consumed was calculated using the initial and final OMb content of each sample as outlined by AMSA (2012):

$$\% \text{ Oxygen Consumption} = \frac{(\text{Initial \% OMb} - \text{Final \% OMb})}{\text{Initial \% OMb}} \times 100\%$$

Metmyoglobin Reducing Activity

Metmyoglobin reducing activity was determined as described by AMSA (2012) with minor modifications. The freshly cut surface of the sample designated for MRA was submerged in a solution of 0.3% sodium nitrite solution for 30 minutes at approximately 20°C. The sample was then removed from the solution, blotted dry, and vacuum packaged. Samples were immediately scanned with the Hunter spectrophotometer through the film after sealing, then incubated at 20°C for 3 h. Samples were scanned through the film a second time after the incubation period was complete. Proportions of MMb during initial and final measurements were

determined using the Krzywicki (1979). The % MRA was calculated using the initial and final MMb content of each sample as outlined by AMSA (2012):

$$\% \text{ Metmyoglobin Reducing Activity} = \frac{(\text{Initial \% MMb} - \text{Final \%MMb})}{\text{Initial \% MMb}} \times 100\%$$

Myoglobin Content

Myoglobin content was determined on samples removed on d1 of display according to the method outlined by Faustman and Phillips (2001). Samples were diced and pulverized into a fine powder using liquid nitrogen. Duplicate 5 g powdered samples were homogenized in 45 mL ice cold 40 mM potassium phosphate buffer (pH = 6.8) and filtered through Whatman no. 1 filter paper. After filtering, 150 μ L of the filtrate was pipetted into a 96-well plate with potassium phosphate buffer as a blank. The absorbance of the filtrate was measured at 525 nm using a plate reader (Synergy HT Multi-Model Microplate Reader, Bio-Tek, Winooski, VT). Myoglobin content was determined using the following equation:

$$\text{Myoglobin (mg/g)} = [A_{525} / 7.6 \text{mM}^{-1} \text{cm}^{-1} \times 1 \text{ cm}] \times [17,000/1,000] \times [(45 + \text{g sample})/\text{g sample}]$$

where A_{525} = absorbance at 525 nm, $7.6 \text{ mM}^{-1} \text{ cm}^{-1}$ = millimolar extinction coefficient of myoglobin at 525 nm; 1 cm = path length of cuvette; 17,000 Da = average molecular mass of myoglobin, and $[(45 + \text{g sample})/\text{g sample}]$ is the dilution factor based on how much initial sample was used.

Lipid Oxidation

Samples were prepared for lipid oxidation analysis in the same manner as for myoglobin content. Lipid oxidation was evaluated using the thiobarbituric acid reactive substances (TBARS) assay as outlined by Leick et al. (2010) with modifications. Duplicate 5 g powdered samples were homogenized with 1 mL of 0.2 mg/mL butylated hydroxytoluene and 45.5 mL of

10% trichloroacetic acid in 0.2 M phosphoric acid, then filtered through Whatman no. 1 filter paper. Two 5 mL aliquots of the filtrate were transferred into 15-mL conical tubes. Five mL of 0.02 M thiobarbituric acid was added to one tube while 5 mL of deionized water was added to the second to serve as a blank. A standard curve was also made to represent 0, 1.25, 2.5, 5, and 7.5 mg malondialdehyde (MDA)/mL using 25 μ M 1,1,3,3-tetraethoxypropane.

Samples were then allowed to incubate in the dark for approximately 17 h at 23°C. After, 150 μ L of the sample and blank were pipetted into 96-well flat bottomed plates. The absorbance of samples was read at 530 nm using a plate reader and compared to the standard curve to determine mg MDA content / kg meat.

Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (v 9.4; SAS Institute Inc., Cary, NC). Retail display data were analyzed as a repeated measure in time. The model included muscle and display day as fixed effects, as well as interactions between the two. A compound symmetry covariance structure was chosen using Akaike's information criterion to minimize variance. Overall changes in color and biochemical measurements during the 5d display period were calculated and analyzed as a 1-way ANOVA with muscle serving a fixed effect and carcass as a random effect. Least-square means were separated using the PDIFF option of the MIXED procedure. Ultimate pH and myoglobin content were analyzed as a 1-way ANOVA with muscle as a fixed effect and carcass as a random effect. For each day of display, the percentage of samples for each muscle type considered "acceptable" (< 20% visual discoloration score) was calculated. Differences in percentages of acceptable samples between muscles were determined using the FREQ procedure of SAS. Main effects and interactions were considered different at $P < 0.05$ and trending at $0.05 < P < 0.10$.

Pearson correlation coefficients were calculated using the CORR procedure of SAS to determine relationships between changes in LD measurements over time ($d5 - d1$) and changes in PM or TB measurements over time. Correlations were considered statistically significant at $P < 0.05$ and trending at $0.05 < P < 0.10$. Significant correlations were considered weak at $r < |0.35|$, moderate at $|0.36| \geq r < |0.67|$, and strong at $r \geq |0.68|$ (Taylor, 1990).

Results

On d1 of display, muscles differed for all color and biochemical traits except for visual discoloration and lipid oxidation ($P \geq 0.10$; Table 1). Chops from the LD were 11.69 – 14.59 L^* units lighter, 5.59 – 6.91 a^* units less red, 2.39 – 4.36 chroma units less saturated, had hue angles 11.8 – 13.17 unit greater, and had 630/580 nm ratios 1.48 – 1.53 units greater than TB and PM chops on d1 of retail display ($P < 0.0001$). Furthermore, on d1, LD chops had 5.14 – 7.42% less MMb, 7.63 – 17.23% greater OMb, 36.66 - 40.19% less OC, 8.38 – 13.37% greater MRA, 0.50 – 1.33 mg/g less myoglobin, and had pH values 0.23 – 0.29 less than TB and PM chops ($P < 0.0001$). Chops from the PM were 2.26 units more yellow than TB chops, with LD chops intermediate ($P < 0.0001$). Deoxymyoglobin in TB chops was 7.31 – 9.81% greater compared to LD and PM chops ($P < 0.0001$); however, DMb content did not differ between the LD and PM ($P = 0.16$). Chops from the PM were 2.90 L^* units lighter, 1.32 a^* units redder, 2.53 chroma units more saturated, and had 1.37 unit greater hue angles, 2.28% less MMb, 4.99% less MRA, and 0.83 mg/g less myoglobin than chops from the TB ($P \leq 0.04$). Alternatively, TB and PM chops did not differ in d1 630/580 nm ratio, OC, or pH ($P \geq 0.15$). Therefore, TB and PM chops were darker and redder during initial evaluations than LD chops, but not different from each other.

A muscle x day interaction was also observed for visual discoloration ($P < 0.0001$; Figure 1A). No differences in discoloration were observed between muscles on the first 3 days of display ($P \geq 0.30$). From d3 to d5, LD visual discoloration increased by 8.23 units compared with d1 observations while discoloration for PM and TB increased by 21.09 and 22.32 units, respectively ($P < 0.0001$). Therefore, over the entire display period, TB and PM had greater overall increases in visual discoloration (28.47 and 25.67, respectively) compared to LD (11.86; $P < 0.0001$), but did not differ from each other ($P = 0.29$; Table 1). In the current study, individual samples were considered “unacceptable” for display once average visual discoloration scores reached at least 20% (Figure 1B). On d1, d2, and d3, 100% of samples for each muscle were considered acceptable. However, on d4, TB had the smallest proportion of acceptable samples (70%), while 100 % of LD samples and 95% of PM samples were considered acceptable ($P < 0.0001$). On d5, LD had the greatest proportion of acceptable samples (85%), while only 5% of TB samples and 20% of PM samples were considered acceptable ($P < 0.0001$). Overall, TB and PM chops were less color stable than LD chops, especially from d3 – d5 of display.

Lightness decreased during display for all muscles evaluated ($P < 0.0001$) with no muscle x day interaction for instrumental lightness ($P = 0.69$; Figure 2A). On all days of display, LD chops were at least 11.69 L^* units lighter than PM and TD chops ($P < 0.0001$). Muscle x day interactions were observed for redness (Figure 2B) and yellowness (Figure 2C) during display ($P < 0.0001$). On d1 of display, PM chops were 1.32 a^* units redder than TB chops and 6.91 a^* units redder than LD chops ($P < 0.0001$). However, on d5 of display, PM and TB chops were only 2.31 and 3.42 a^* units redder than LD chops, respectively ($P < 0.0001$). Chops from the TB and PM had a greater decline in redness ($\Delta a^* = 5.41$ and 5.61, respectively) over the display period compared to LD chops ($\Delta a^* = 2.13$; $P < 0.0001$), but did not differ from each other ($P =$

0.64). There was also a greater decline in yellowness for chops from the TB and PM ($\Delta b^* = 3.01$ and 3.26 , respectively) compared to LD chops ($\Delta b^* = 1.09$; $P < 0.0001$).

A muscle x display day interaction ($P < 0.0001$) was observed for chroma (Figure 3A). Chops from the TB had intermediate chroma values to LD and PM chops on d 1 – 3 of display ($P < 0.0001$), but had the lowest chroma values by d 4 and 5 ($P < 0.0001$). Chops from the TB and PM had greater decreases in chroma over the display period ($\Delta \text{chroma} = 5.80$ and 6.07 , respectively; Table 1) compared with LD chops ($\Delta \text{Chroma} = 2.01$; $P < 0.0001$). No muscle x display day interaction was observed for hue angle ($P = 0.19$; Figure 3B). Despite the lack of interaction, LD chops had hue angles at least 10.14 units greater than PM and TB chops over the entire display period, indicative of shift toward a more yellow hue ($P < 0.0001$). A muscle x display day interaction ($P < 0.0001$) was also observed for 630/580 nm ratio (Figure 3B). On d1 of display, chops from the TB and PM had 630/580 nm ratios that were 1.48 and 1.53 units greater than ratios observed for LD chops, respectively ($P < 0.0001$; Figure 3C). However, on d5 of display 630/580 nm ratios were only 0.37 and 0.36 units greater in TB and PM chops, respectively, compared to LD 630/580 nm ratios ($P < 0.01$). Additionally, overall decreases in 630/580 nm ratio from d1 to d5 of display 1.85 units greater in the TB and 1.90 units greater in the PM compared to LD chops ($P < 0.0001$). Overall, TB and PM chops had greater changes in chroma and 630/580 nm ratio compared to LD chops, but changes in hue angle did not differ.

There was a tendency for TB and PM chops to have greater increases in MMb than LD chops during display ($P = 0.10$; Figure 4A). On all days of display, MMb was at least 4.94 % greater for TB and PM chops compared to LD chops ($P < 0.0001$), while TB and PM MMb values were not from each other ($P = 0.11$) except for at d4 ($P < 0.01$). Deoxymyoglobin decreased by 4.18 units from d1 to d3 for TB chops ($P = 0.02$), then increased by 14.12 % from

d3 to d5 ($P < 0.0001$; Figure 4B). Psoas major DMb increased by 8.95% during the display period, with the largest increase from d4 to d5 (6.10 units; $P < 0.01$). Alternatively, DMb for the LD did not change over the display period ($P \geq 0.12$). The TB had the greatest DMb on all days of display ($P < 0.0001$) except for d3, while the LD had the lowest DMb on all days of display ($P < 0.0001$), except d1. A muscle x display day interaction was also observed for OMb ($P < 0.0001$; Figure 4C). The LD had the greatest OMb on all days of display (≥ 7.64 units; $P < 0.0001$), while the TB had the lowest OMb on all days ($P \leq 0.01$) except d3. The TB and PM had greater increases in OMb from d1 to d5 (29.94% and 30.39%, respectively) compared to the LD (20.61%; $P < 0.0001$), but did not differ from each other ($P = 0.75$).

An interaction between muscle and display day was observed for OC ($P = 0.02$; Figure 5A), but not for MRA ($P = 0.11$; Figure 5B). Chops from the LD had decreased OC compared to chops from the TB and PM on d1, 3, and 5 of display ($P < 0.0001$). Oxygen consumption for chops from the LD decreased on each subsequent day of display ($P < 0.01$), with an overall decrease of 27.52 % from d1 to d5 ($P < 0.01$). Alternatively, chops from the TB and PM did not experience decreased OC during display ($P \geq 0.10$) and were not different from each other on any day of display ($P \geq 0.52$). Metmyoglobin reducing activity decreased by 5.63 – 12.57 % on each subsequent day of display for all muscles ($P < 0.0001$). Chops from the LD had at least 5.26% greater MRA than the PM and TB on each measurement day $P < 0.0001$). There was a tendency for the TB to have greater MRA than the PM on d3 (4.64 units; $P = 0.06$). No difference in MRA was observed between the TB and PM on d5 ($P = 0.34$). There was no muscle ($P = 0.80$) or muscle x day ($P = 0.39$) effect on lipid oxidation (Figure 5C). However, lipid oxidation had a slight numerical increase for all muscles from d1 to d3 (≥ 0.07 units; $P < 0.01$). Overall, TB and PM chops had increased OC and decreased MRA compared to LD chops,

but did not differ in lipid oxidation. This may have also contributed to increased DMb and decreased OMb on d1 in TB and PM chops compared to LD chops.

Correlation coefficients between overall changes in LD color measurements and overall changes in TB and PM measurements are presented in Table 2. Measurements for the LD were moderately correlated to TB measurements for redness ($r = 0.55$; $P = 0.01$) and Chroma ($r = 0.58$; $P = 0.01$), while LD OMb was strongly correlated to TB OMb ($r = 0.80$; $P < 0.0001$). There was a tendency for LD measurements to be correlated to TB measurements for hue angle ($r = 0.39$; $P = 0.09$) and 630/580 ratio ($r = 0.41$; $P = 0.07$). Measurements for the LD were moderately correlated to PM lightness ($r = 0.57$; $P = 0.01$), redness ($r = 0.51$; $P = 0.02$), hue angle ($r = 0.63$; $P < 0.01$), and 630/580 nm ratio ($r = 0.47$; $P = 0.04$). Yellowness, MMb, DMb, Visual discoloration, OC, MRA, and lipid oxidation measurements for the LD were not correlated to measurements from the TB or PM ($P \geq 0.15$).

Discussion

In beef, cuts containing the TB and PM are commonly displayed in overwrap packaging for retail sale, similar to LL chops (Lepper-Bililie et al., 2014). Therefore, the color stability of these muscles in beef has direct implications for retail display. In pork the TB and PM are less commonly overwrapped for sale. Thus, they were chosen for this study because they represent muscles that are biochemically different from the LD, rather than for economic implications. Understanding differences in color stability of among the TB, PM, and LD would improve understanding of pork discoloration as a whole, and provide additional insight into how muscle dependent discoloration differs between species. Furthermore, nearly 20% of pork in the U.S. is consumed as sausage (NPPC, 2011), which is produced using several muscles, including some that may have decreased color stability. Raines et al. (2010) reported that using muscles with

poor color stability reduced color stability of ground beef. As such, color stability of TB and PM may be indicative of color stability for other muscles used for further processed products like sausage. Therefore, there is also economic value in evaluating pork muscles traditionally considered to be color labile in beef, especially if muscle type has a similar impact among species.

Similar to what has been observed in beef, the TB and PM were more color labile during a 5 d display period than the LD. By d5 of display, 95% of TB chops and 80% of PM chops exceeded 20% visual discoloration, whereas only 15% of LD chops discolored in the same period of time. Furthermore, the TB and PM had greater decreases in redness, yellowness, and chroma, 630/580 nm ratio, and OMb during display than the LD, though minimal differences were observed between the TB and PM. The TB and PM also had higher pH and Mb contents on d1 or display compared to the LD. Results from the current study were generally in agreement with results observed in beef, as multiple studies have reported that the PM and TB were more color labile than the longissimus lumborum (LL; Seyfert et al., 2006; McKenna et al., 2005; Legako et al., 2018; Nair et al., 2018). Seyfert et al. (2006) and Legako et al. (2018) observed that visual discoloration in the PM and TB increased more quickly compared to the LL, while McKenna et al. (2005) and Nair et al. (2018) reported the TB and PM had greater decreases in color measurements during display than the LL, including redness, yellowness, chroma, hue angle, and 630/580 nm. Ultimate pH and Mb content, both of which were elevated in color labile beef muscles (McKenna et al., 2005; Jeong et al., 2009), were also greater in the TB and PM, compared to the LD in the current study. In beef, muscles considered “color labile” or “color stable” generally had similar characteristics, such as pH, redness, and OMb content, as other muscles in their stability class (McKenna et al., 2005; King et al., 2011b). Similarities between

the color labile TB and PM in the current study suggests that other traditionally color labile muscles in beef like the serratus ventralis, supraspinatus, and bicep femoris may also be color labile in pork.

These similarities between beef and pork discoloration were not unexpected as the rate of accumulation of MMb in meat, which is responsible for changes in meat surface color, is related to fiber types present within the muscle (Ramanathan et al., 2014). In both pork and beef, the PM and TB typically contain a greater proportion of red, oxidative muscle fibers, whereas the LD predominantly contains white, glycolytic fibers (Solomon et al., 1994; Picard and Gagaoua, 2020; Cheng et al., 2021). Oxidative muscle fibers primarily utilize aerobic metabolism to generate energy for movement, and thus contain greater proportions of mitochondria and myoglobin. The ability of mitochondria to consume oxygen continues postmortem, which can place the muscle tissue under additional oxidative stress and increase myoglobin oxidation (Ramanathan and Mancini, 2018). As a result, oxidative muscles also generally have an increased oxygen consumption rate, which is associated with decreased color stability in beef (O’Keefe and Hood, 1982; Renner and Labas, 1987). Oxygen consumption rate was increased in the TB and PM compared to the LL (Mckenna et al., 2005; Mancini et al., 2018) In the current study, the TB and PM both had greater OC than the LD, further supporting the hypothesis that pork color stability differences may be driven by differences in oxygen usage between muscles. Alternatively, glycolytic muscles have enzymes associated with anaerobic metabolism that are able to regenerate NADH, improving the capacity of a muscle to reduce MMb to DMb and mitigating brown pigment formation (Joseph et al., 2012; Nair et al., 2018). During display, MRA was reduced in all muscles, but was consistently greater in LD compared with PM and TB. This is similar to beef where MRA was increased in the LL compared to the TB and PM

(McKenna et al., 2005). Furthermore, while absolute MRA for muscle types differed slightly between the current study and McKenna et al. (2005), the pattern of difference between muscles was similar. The difference in MRA between the LL and TB (2.90% in beef, 8.36% in pork) was smaller than the difference between the LL and PM (17.9% in beef, 13.37% in pork). Similar to beef, differences in OC and MRA appear to underlie differences in color stability between muscles in pork.

While the TB and PM had greater overall MMb content compared to the LD in the current study, muscles did not differ in rate of MMb accumulation during display. Furthermore, no samples for any muscle reached 20% visual discoloration until d4, whereas the average MMb for the TB and PM exceeded 20% on d2 of display. This was unexpected, as studies in beef have reported that muscles with decreased color stability accumulate MMb more quickly. McKenna et al. (2005) reported that MMb content for the TB and PM in beef increased more rapidly during display than for the LL. Additionally, previous research in beef has hypothesized that consumers begin to discriminate against beef color when MMb reaches 20% (Renerre and Labas, 1987; Gill and Jones, 1994). Therefore, 20% visual discoloration was used as the threshold in this study for when a sample was no longer considered “acceptable” for consumers, as surface discoloration was used as an estimation for MMb formation (Boler et al., 2009; AMSA, 2012). However, no samples for any muscle reached 20% visual discoloration until d4, whereas the average MMb for the TB and PM exceeded 20% on d2 of display. This may indicate that MMb content alone is not sufficient for estimation of visual discoloration in pork, especially when evaluating multiple muscles. In the current study, however, both 630/580 nm ratio and OMb content had greater decreases during display in the TB and PM compared to the LD. Furthermore, large drops in 630/580 nm ratio and OMb were observed on d4 and d5 for the TB and PM, respectively,

corresponding to large increases in overall visual discoloration score for those muscles on the same days. Overall, it may be preferred to use additional measures of discoloration beyond MMb content, such as 630/580 nm ratio or OMb content, when evaluating color stability of multiple pork muscles.

Despite differences in color stability, muscles did not differ in lipid oxidation on any day of analysis in the current study. Lipid oxidation and discoloration are known to be related to each other in beef, with the two processes exacerbating each other (Faustman et al., 2010). However, multiple studies have indicated that myoglobin in pork behaves differently in the presence of lipid oxidation by-products than in beef (Suman et al., 2006). Lipid oxidation products like 4-hydroxy-2-nonenal preferentially adduct to the proximal histidine in beef myoglobin, destabilizing the molecule and making it more susceptible to oxidation. Alternatively, lipid oxidation products adduct to different sites on pork myoglobin than beef, causing pork myoglobin to remain more resistant to oxidation even in the presence of lipid oxidation (Suman et al., 2006). Therefore, differences in color stability in the current study were likely not related to lipid oxidation.

One of the objectives of the current study was to determine if changes in LD color measurements over the 5d display period were correlated to changes in PM or TB color measurements. If changes in LD display measurements were related to changes in measurements for other muscles, this could indicate that genetic selection of traits to improve color stability in the LD could improve color stability of other pork muscles as well. In the present study, changes in LD redness, hue angle, and 630/580 were moderately correlated or trending towards a moderate correlation with respective measurements in the PM or TB. Similarly, King et al (2011b) reported that beef LL color measurements on a given display day were correlated to

measurements in other muscles, including the TB. Redness and hue angle measurements for the LL were also correlated to TB measurements on the same display day in the study by King et al. (2011b). In that same study, LL lightness was correlated with TB lightness on all display days; however, in the current study, no lightness correlations were observed between muscles. Differences in lightness during display for the current study were relatively small for all muscles, which may explain the lack of correlations. In both the King et al. (2011b) study and the present study, no correlations for yellowness were observed between the LL or LD and TB. Other than a strong correlation between the LL and TB for OMb, no correlations were observed between muscles for changes in myoglobin forms in the current study. Hood (1980) reported that MMb content was correlated between the LL and PM in beef. However, that study was evaluated using a different species and calculated MMb content differently, which may explain the difference in results compared to the current study. Furthermore, both MMb and DMb were calculated using multiple reflectance values in the present study; this may have increased overall measurement variability and decreased correlations as a result. Changes in LD OMb were correlated to changes in OMb in the TB, but not the PM. This may indicate that changes in OMb content over time are more variable in the PM. Changes in LD visual discoloration were not predictive of changes in the TB or PM. However the LD discolored less overall compared to the TB in this study, making correlations between the LD and other muscles more difficult (Goodwin and Leach, 2006). Despite the lack of discoloration in the LD, however, correlations were still observed between the LD and other muscle for measures of instrumental redness and brownness. Overall, these data would indicate a moderate relationship between the LD muscles and other muscles for instrumental color measures, especially measures related to redness or brownness.

Therefore, changes in color during display for the LD may be indicative of color changes for other pork muscles as well.

Overall, muscle type affected the rate of discoloration in this study. Differences in discoloration between the pork LD, TB, and PM were similar to color stability differences for the same muscles in beef. The majority of TB and PM samples were discolored by the d5 of display, while 85% of LD samples were considered acceptable after the same time period. Furthermore, the TB and PM had greater changes in all traits during display except lightness, hue angle, MMb, OC., but the TB and PM had minimal differences from each other. Changes in instrumental color were related between the LD and muscles, especially measures of redness or brownness. Based on the relationships between these traits, color stability of the TB and PM could be improved by genetic selection for traits to improve color stability in the LD. While further research is necessary to evaluate color stability differences in other pork muscles, selecting for improved color stability in the LD could improve color stability of an entire carcass, and not just the TB or PM.

Tables

Table 3.1. Effect of muscle on initial (d1) color and biochemical traits of fresh pork during display.

Item	LD ¹	TB ¹	PM ¹	SEM	P-Value
Samples, n	20	20	20		
Lightness, L^*	59.10 ^a	44.51 ^c	47.41 ^b	0.7	< 0.0001
Redness, a^*	11.52 ^c	17.11 ^b	18.43 ^a	0.25	< 0.0001
Yellowness, b^*	18.57 ^b	17.17 ^c	19.43 ^a	0.31	< 0.0001
Chroma ²	21.87 ^c	24.26 ^b	26.79 ^a	0.35	< 0.0001
Hue Angle ³	58.24 ^a	45.07 ^c	46.44 ^b	0.44	< 0.0001
630/580 nm	2.83 ^b	4.31 ^a	4.36 ^a	0.06	< 0.0001
Metmyoglobin, % ⁴	11.86 ^c	19.28 ^a	17.00 ^b	0.31	< 0.0001
Deoxymyoglobin, % ⁵	3.83 ^b	13.64 ^a	6.33 ^b	1.9	< 0.0001
Oxymyoglobin, % ⁶	84.30 ^a	67.07 ^c	76.67 ^b	1.67	< 0.0001
Visual Discoloration, %	1.81	2.07	3.29	0.57	0.10
Oxygen Consumption, %	59.38 ^b	99.57 ^a	96.04 ^a	3.98	< 0.0001
Metmyoglobin Reducing Activity, %	48.99 ^a	40.61 ^b	35.62 ^c	2.34	< 0.0001
mg MDA / kg Meat	0.02	0.01	0.01	0.01	0.67
mg Myoglobin/g Meat	0.74 ^c	2.07 ^a	1.24 ^b	0.12	< 0.0001
pH	5.61 ^b	5.90 ^a	5.84 ^a	0.04	< 0.0001

^{a,b,c}Treatments within a row lacking common superscripts are different ($P < 0.05$)

¹Means for the *longissimus dorsi* (LD), *triceps brachii* (TB), and *psoas major* (PM)

²Chroma is a measure of color saturation, calculated by: $C = (b^{*2} + a^{*2})^{1/2}$.

³Hue angle is a measure of the overall color of a sample, calculated by: $H_{ab} = \arctangent(b^*/a^*)$.

⁴Calculated as: $\% \text{MMb} = \left\{ 1.395 - \left(\frac{A_{572} - A_{700}}{A_{525} - A_{700}} \right) \right\} \times 100$.

⁵Calculated as: $\% \text{DMb} = \left\{ 2.375 \times \left(1 - \frac{A_{572} - A_{700}}{A_{525} - A_{700}} \right) \right\} \times 100$.

⁶Calculated as: $\% \text{OMb} = 100 - \% \text{MMb} - \% \text{DMb}$.

Table 3.2. Correlation coefficients between overall changes during the 5 d display period in *longissimus dorsi* (LD) color measurements and changes in *triceps brachii* (TB) or *psoas major* (PM) color measurements.

LD Trait	TB		PM	
	Correlation, <i>r</i>	<i>P</i> -value ¹	Correlation, <i>r</i>	<i>P</i> -Value ¹
Δ Lightness, <i>L</i> *	-0.20	0.39	0.57	0.01
Δ Redness, <i>a</i> *	0.55	0.01	0.51	0.02
Δ Yellowness, <i>b</i> *	0.33	0.16	0.08	0.75
Δ Chroma ²	0.58	0.01	0.30	0.19
Δ Hue Angle ³	0.39	0.09	0.63	< 0.01
Δ 630/580 nm	0.41	0.07	0.47	0.04
Δ Metmyoglobin, % ⁴	0.26	0.27	0.34	0.15
Δ Deoxymyoglobin, % ⁵	-0.03	0.90	0.21	0.38
Δ Oxy myoglobin, % ⁶	0.80	<0.0001	0.31	0.18
Δ Visual Discoloration, %	-0.21	0.37	-0.24	0.31
Oxygen Consumption, %	-0.19	0.42	-0.03	0.88
Metmyoglobin Reducing Activity, %	-0.03	0.90	0.07	0.77
mg MDA / kg Meat	0.3	0.20	0.24	0.31

¹Highlighted *P*-values indicate significant correlations (*P* < 0.05)

²Chroma is a measure of color saturation, calculated by: $C = (b^{*2} + a^{*2})^{1/2}$.

³Hue angle is a measure of the overall color of a sample, calculated by: $H_{ab} = \arctangent(b^*/a^*)$.

⁴Calculated as: $\% \text{MMb} = \left\{ 1.395 - \left(\frac{A_{572} - A_{700}}{A_{525} - A_{700}} \right) \right\} \times 100$.

⁵Calculated as: $\% \text{DMb} = \left\{ 2.375 \times \left(1 - \frac{A_{572} - A_{700}}{A_{525} - A_{700}} \right) \right\} \times 100$.

⁶Calculated as: $\% \text{OMb} = 100 - \% \text{MMb} - \% \text{DMb}$.

Figures

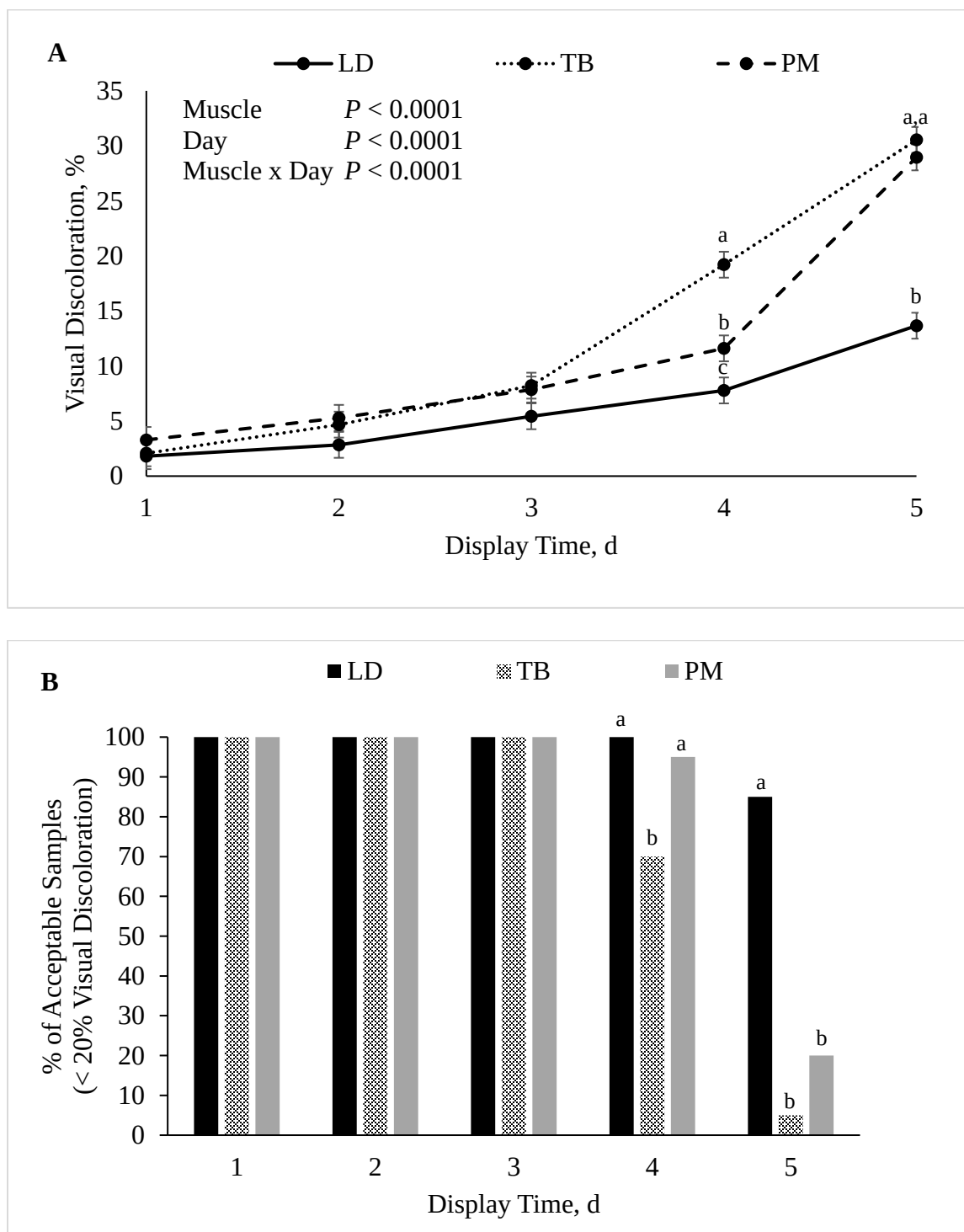


Figure 3.1. Effect of muscle on visual discoloration (A) of pork *longissimus dorsi* (LD), *triceps brachii* (TB), and *psoas major* (PM) chops over 5 d of retail display, and percentage of samples considered “acceptable” ($< 20\%$ visual discoloration) on a given display day (B). Treatments within a day lacking common superscripts are different ($P < 0.05$).

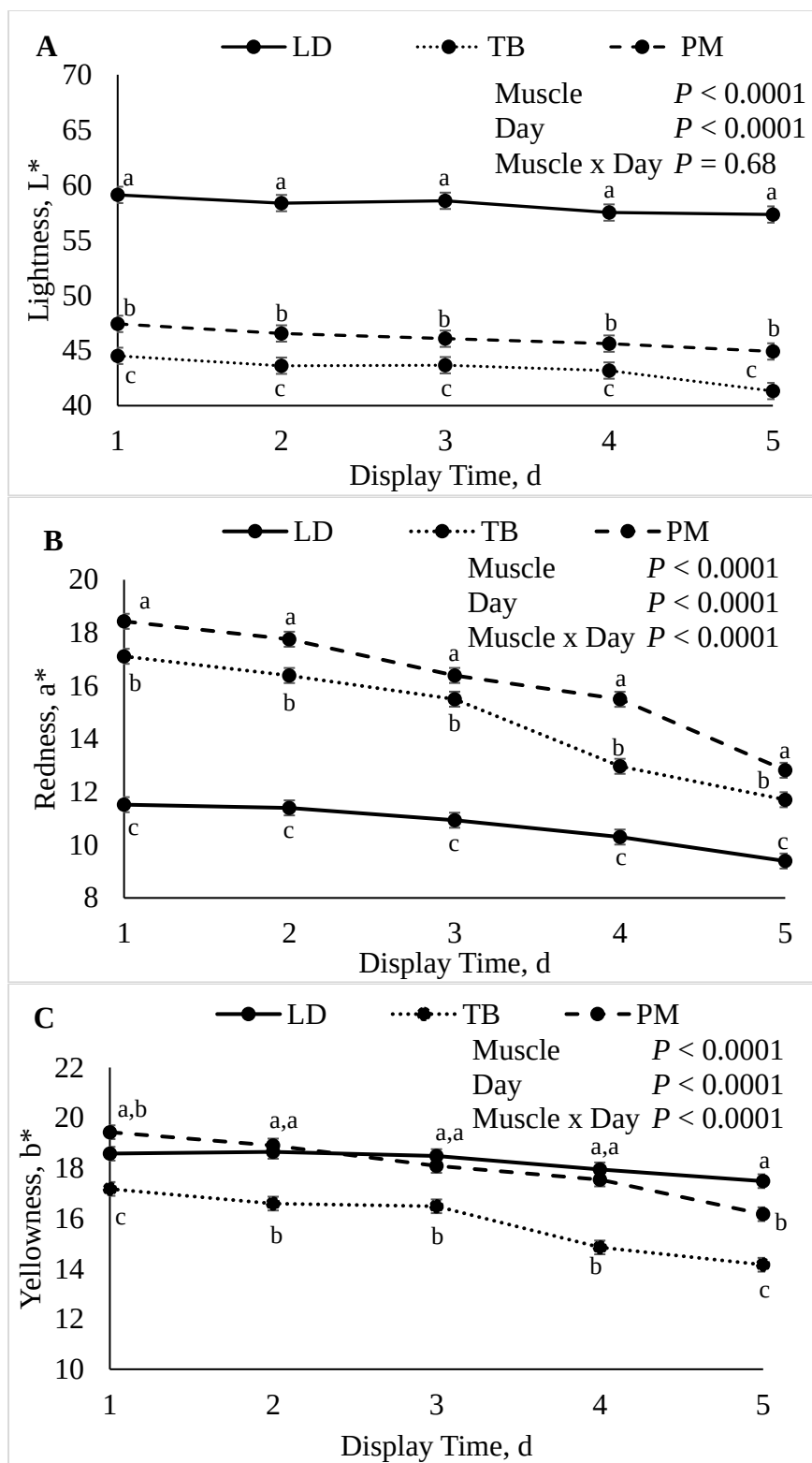


Figure 3.2. Effect of muscle on instrumental lightness (A), redness (B), and yellowness (C) of pork *longissimus dorsi* (LD), *triceps brachii* (TB), and *psoas major* (PM) chops over 5 d of retail display. Treatments within a day lacking common superscripts are different ($P < 0.05$).

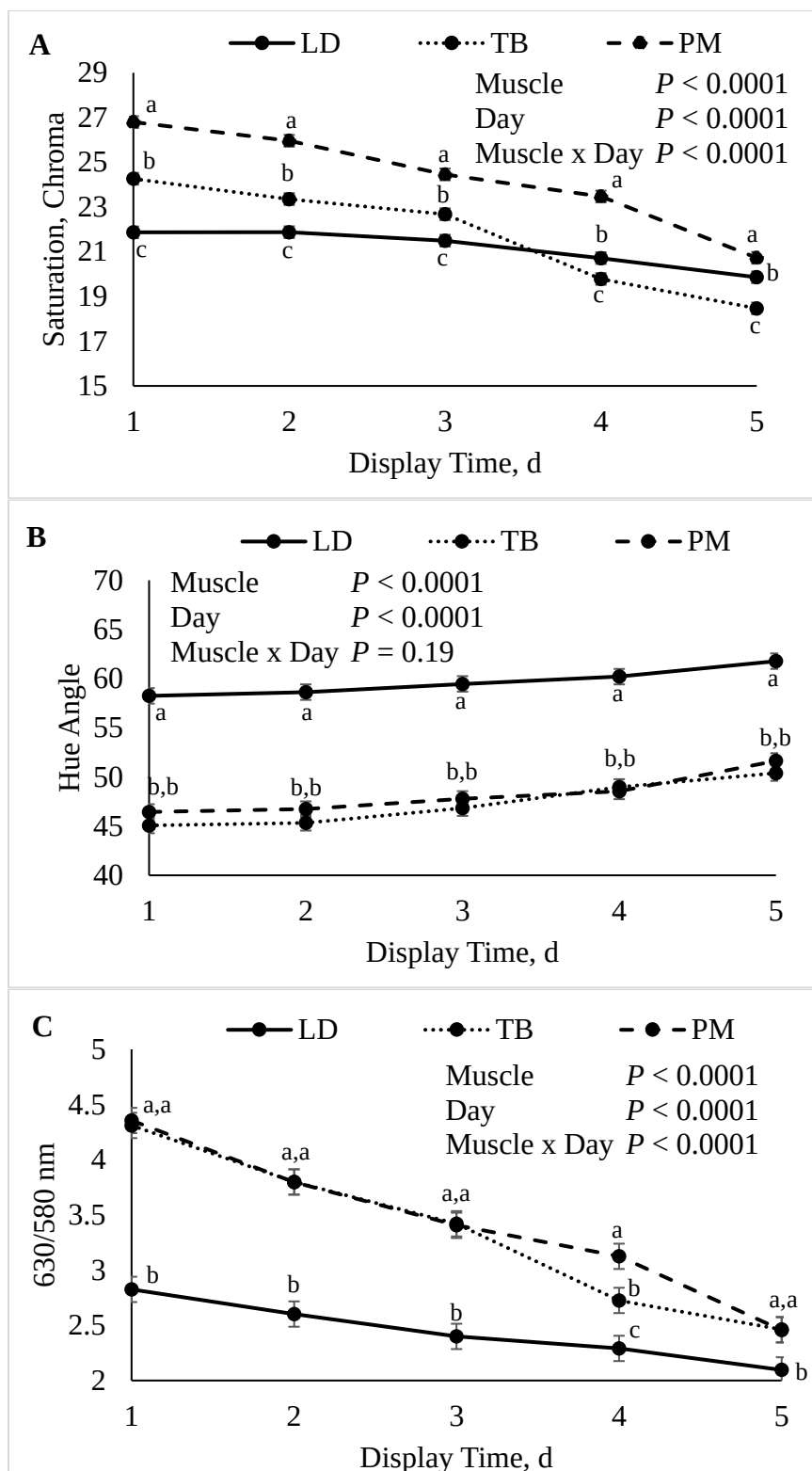


Figure 3.3. Effect of muscle on instrumental chroma (**A**), hue angle (**B**), and 630/580 nm ratio (**C**) of pork *longissimus dorsi* (LD), *triceps brachii* (TB), and *psoas major* (PM) chops over 5 d of retail display. Treatments within a day lacking common superscripts are different ($P < 0.05$).

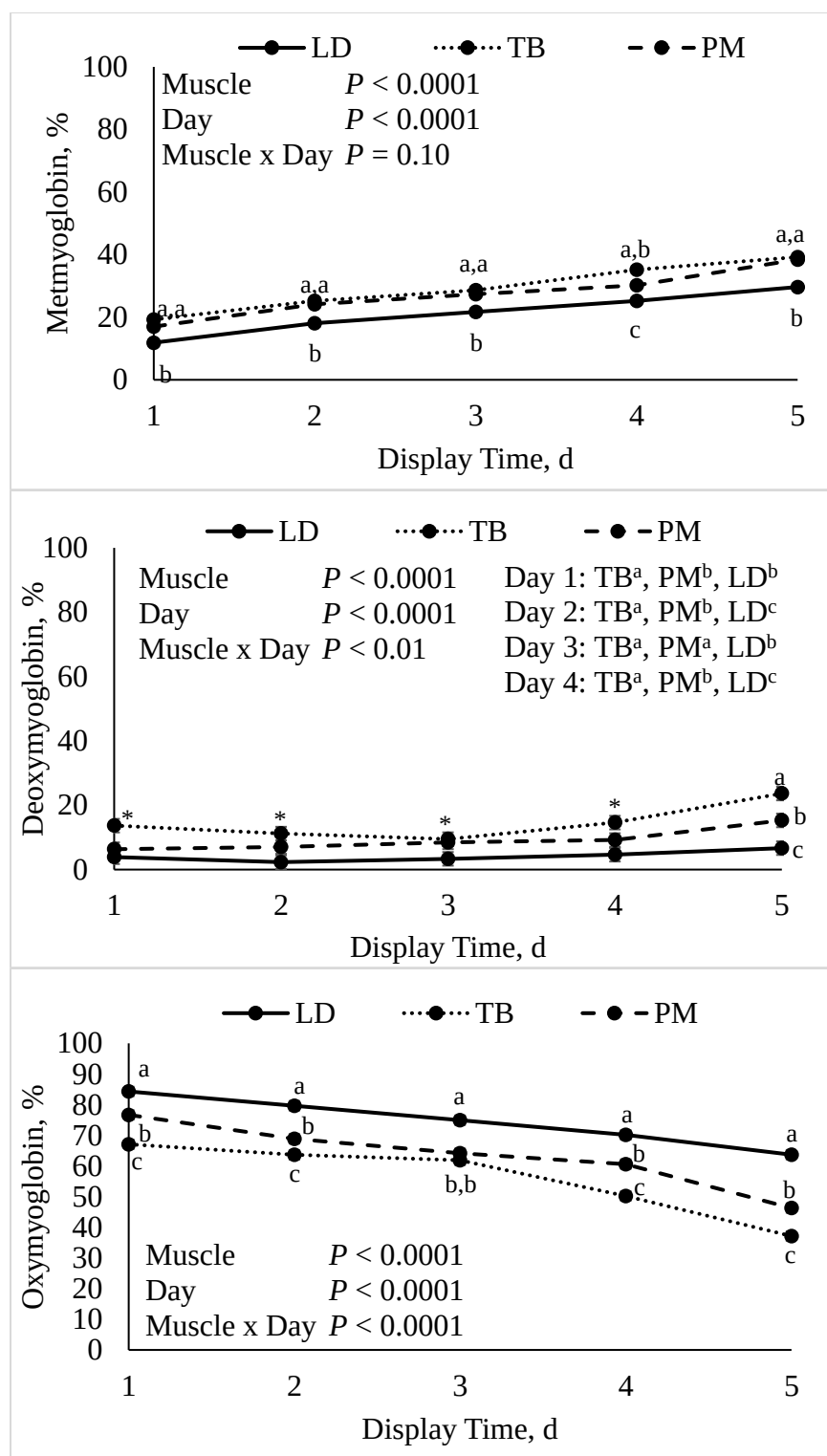


Figure 3.4. Effect of muscle type on Metmyoglobin (A), Deoxymyoglobin (B), and Oxymyoglobin (C) of pork *longissimus dorsi* (LD), *triceps brachii* (TB), and *psoas major* (PM) chops over 5 d of retail display. Treatments within a day lacking common superscripts are different ($P < 0.05$).

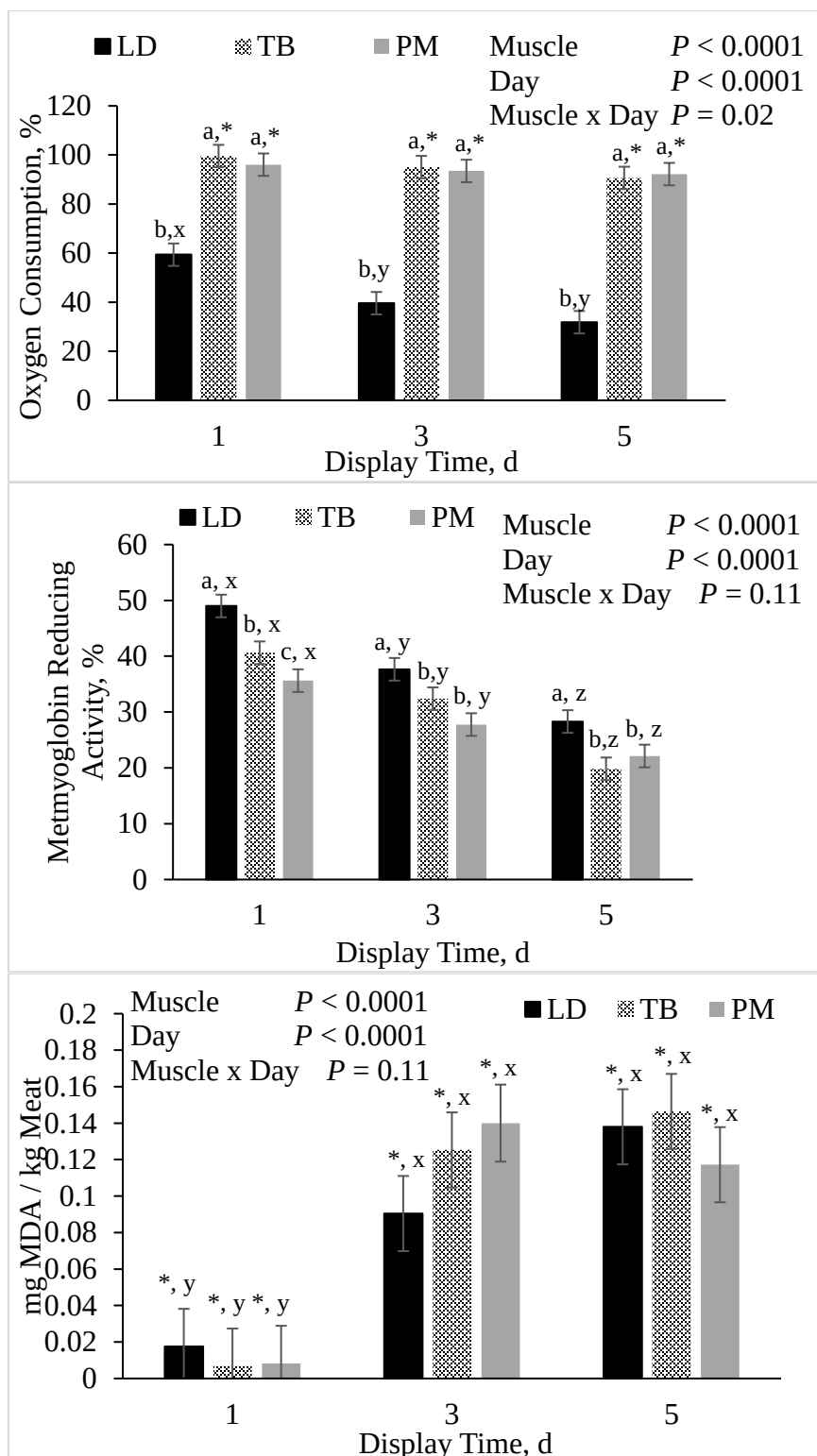


Figure 3.5. Effect of muscle type on oxygen consumption (A), metmyoglobin reducing activity (B), and lipid oxidation (C) of pork *longissimus dorsi* (LD), *triceps brachii* (TB), and *psoas major* (PM) chops over 5 d of retail display. Treatments within a day lacking common superscripts are different ($P < 0.05$).

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CHAPTER 4

**RELATIONSHIPS AMONG INITIAL COLOR OR BIOCHEMICAL TRAITS AND
FINAL DISCOLORATION IN THE PORK LONGISSIMUS DORSI, TRICEPS
BRACHII, AND PSOAS MAJOR**

Abstract

The goal was to determine relationships between color or biochemical traits at the beginning of retail display and final discoloration in the *longissimus dorsi* (LD), *triceps brachii* (TB), and *psoas major* (PM), and to determine what combination of initial traits was most predictive of final discoloration in each muscle. The LD, TB, and PM from 20 pigs were aged in vacuum packages then fabricated to yield 3 chops per muscle. Chops were overwrapped together and displayed under constant lighting for up to 5 days. On d1 all chops in a package were evaluated for visual discoloration, instrumental color, and myoglobin forms. One chop on d1 was evaluated for oxygen consumption (OC), metmyoglobin reducing activity (MRA), and myoglobin (Mb) content. Final visual discoloration and 630/580 nm ratio were evaluated on one chop from each muscle on d5. Pearson correlation coefficients were compared between the LD, TB, and PM using a Fisher's z test in SAS. Stepwise regression models were developed using the REG procedure. All initial traits were correlated to final visual discoloration and 630/580 nm ratio in the TB and PM ($|0.47| \leq r \leq |0.72|$), except redness, d1 630/580 nm ratio, OC, and Mb ($P \geq 0.07$). Final LD 630/580 nm ratio was correlated to initial L^* , a^* , hue angle, deoxymyoglobin, and pH ($|0.48| \leq r \leq |0.75|$). Initial lightness and Mb ($0.20 \leq \text{partial } R^2 \leq 0.21$) were most predictive of final LD visual discoloration ($R^2 = 0.33$). Yellowness was most predictive for TB and PM visual discoloration and 630/580 nm ratio for all muscles ($0.32 \leq \text{partial } R^2 \leq 0.85$). Correlations were generally stronger in the TB and PM compared to the LD, but did not differ

from each other. In all muscles, color stable meat was initially darker, less yellow, and had lesser hue angles.

Introduction

Consumers discriminate against meat that appears brown in color, as they perceive this to indicate that the product is spoiled or no longer fresh (Font-i-Furnols et al., 2014). This discoloration results in reduced willingness-to-purchase and greater food waste. Within the U.S. alone, beef discoloration results in an estimated \$3 billion lost value annually (Ramanathan et al., 2021), comprising nearly 2% of the \$123 billion total revenue from U.S. beef products in 2020 (USDA, 2020). While a similar economic analysis is not available for pork, fresh pork does discolor (Zhu and Brewer 1998a; King et al., 2011a). Although discoloration-related economic losses for fresh pork have not been quantified, if this same 2% reduction in total revenue were applied to the \$23.4 billion estimated for U.S. pork products (NPPC, 2020), pork discoloration could account for nearly \$500 million in lost revenue. Therefore, antemortem and postmortem interventions to improve fresh pork color stability would increase consumers' likelihood of purchase and maximize value.

Previous research has reported pork loins could be selected based on expected color stability at 1d postmortem with color stable loins being darker, less yellow, and having smaller hue angles than color labile loins (King et al., 2011a). However, published data evaluating discoloration of other pork muscles is limited. In beef, it is well-established that muscles differ in color stability (McKenna et al., 2005; King et al., 2011b; Suman et al., 2014). However, limited research has evaluated traits predictive of greater color stability, especially in muscles other than the longissimus. Based on differences in color stability between muscles, it is likely that traits

predictive of loin color stability may not be predictive of color stability in other pork muscles, especially muscles that are biochemically different.

While other pork muscles are not commonly sold in the U.S. using overwrap packaging as is typical of loin chops, muscle color stability likely has implications for other product types. For example, the use of color labile muscles in ground beef can have detrimental effects on color stability (Raines et al., 2010). With nearly 30% of pork in the U.S. sold as sausage or fresh cuts other than loin chops or ribs (Pork Checkoff, 2011), there is value in determining factors that can improve color stability of muscles other than the LD. Identifying traits on d1 of retail display that are indicative of improved color stability for multiple pork muscles could allow for genetic selection of these traits to produce pork with improved color stability, resulting in increased profits for the pork industry.

Therefore, the objectives of this study were to determine relationships between initial retail display traits and final discoloration in the longissimus dorsi (LD), triceps brachii (TB), and psoas major (PM), and to determine what combination of initial traits was most predictive of final discoloration in each muscle. It was hypothesized that initial traits would be related to final measures of discoloration and that the combination of traits that were most predictive of improved color stability would differ between muscles.

Materials and Methods

All protocols were approved by the University of Illinois Institutional Animal Care and Use Committee (Protocol #20095).

Pigs (n = 20) used in this study were slaughtered under the supervision of the USDA Food and Safety Inspection Service. Pigs (n = 20) were transported to the University of Illinois Meat Science Laboratory and held in lairage for approximately 16 h with no access to feed and

ad libitum access to water. Pigs (n = 20) used in this study were slaughtered under the supervision of the USDA Food and Safety Inspection Service. Animals were immobilized via electrical stunning and terminated via exsanguination, then allowed to chill at 4° C for approximately 22 h. After chilling, left sides of carcasses were fabricated to yield the picnic shoulder (NAMP #405) and bone-in loin (NAMP #410). Picnic shoulders were further fabricated to yield the triceps brachii long head (TB) and bone-in loins were fabricated to yield the longissimus dorsi (LD) and psoas major (PM). The TB and PM were vacuum packaged individually and aged for 21 d at 4°C. The LD was cut into 2.54 cm chops using a push-feed slicer (Treif model 700 F, Oberlahr, Germany). The three chops immediately posterior to the area of the 10th rib were vacuum packaged together and stored in the same manner as the TB and PM until 21 d postmortem. After aging, the TB and PM were hand cut to yield 3 chops measuring approximately 2.54 cm and 5.08 cm thick, respectively. Ultimate pH of the most anterior chop for each muscle was measured Hanna pH meter fitted with a glass tipped electrode (model HI98163, Hanna Instruments. Woonsocket, RI) calibrated using pH 4 and pH 7 buffer at 4 °C.

Retail Display

The three chops representing a single sample were placed with the cut side up on a 27.3 x 14.9 cm polystyrene tray (Dyne-a-pak, Laval, Quebec, Canada) with a soaker pad and overwrapped with polyvinyl chloride film (O^2 transmission = 23,250 mL·m² ·d⁻¹ , 72 gauge; Resinite Packaging Films, Borden, Inc., North 70 Andover, MA). Each of the three chops were randomly allocated to d1, d3, or d5 of display. Chops were removed from display after analysis on their respective days; d1 chops were used for biochemical analysis, while d3 and d5 chops were used for a separate study. Packages were displayed in a standing display cooler with four

shelves and rotated daily to minimize location effects on discoloration. Lighting was provided by 122 cm fluorescent bulbs (32 W, Octron XP, 6500 K, Osram Sylvania, Wilmington, MA) positioned at the front of the display case. Samples were allowed to oxygenate for at least 2 h prior to color evaluations on d1 of display. On d1, samples were evaluated for subjective visual discoloration and instrumental surface color. Subjective visual discoloration of an entire package was evaluated through the film by a panel of 8 trained technicians using an unstructured line 10-cm line scale anchored at 0%, 50%, and 100% discoloration. Then, packaging was removed from chops, and instrumental surface color was evaluated using a Hunter MiniScan EZ spectrophotometer (HunterLab, Reston, VA) and included CIE L^* (lightness), a^* (redness), b^* (yellowness; CIE, 1978) and reflectance data (400 – 700 nm). The spectrophotometer used a D65 illuminant, open 31.8 mm aperture, and was calibrated with a white and black tile specific to the machine. Instrumental $L^*a^*b^*$ data was used to calculate Chroma (measure of saturation: $Chroma = \sqrt{a^{*2} + b^{*2}}$) and hue angle (overall measure of color: $Hue\ angle = \left(\arctan \frac{b^*}{a^*} \right)$). Reflectance data was used to calculate the ratio of 630/580 nm (measure of brownness) and myoglobin (Mb) forms using the attenuation method as described by Krzywicki (1979). Reflectances at 474, 525, 572, and 700 nm were converted to attenuation values (A) using the equation: $A = \log(1/R)$, where R = reflectance. Myoglobin forms were calculated using the following equations:

$$\% \text{ Metmyoglobin (MMb)} = \left\{ 1.395 - \left(\frac{A_{572} - A_{700}}{A_{525} - A_{700}} \right) \right\} \times 100$$

$$\% \text{ Deoxymyoglobin (DMb)} = \left\{ 1.395 - \left(\frac{A_{474} - A_{700}}{A_{525} - A_{700}} \right) \right\} \times 100$$

$$\% \text{ Oxy myoglobin (OMb)} = 100 - (\% \text{MMb} + \% \text{DMb})$$

Packages were rewrapped and returned to display after color measurements. On d1 of display, subjective and instrumental discoloration were evaluated on all chops in a package.

Discoloration evaluations for chops within a single package were averaged for statistical analyses. On d5 of display, only one chop remained in each package and was evaluated for surface discoloration and 630/580 ratio in the same manner as on d1.

Biochemical Analyses

On d1 of display, designated chops from each package were removed from display and trimmed of all external fat. Chops from the LD and TB were cut in half perpendicular to the cut surface and chops from the PM were cut in half parallel to the cut surface. This was done to ensure muscle fibers were oriented in the same direction on the surface of each chop. One half of each sample was frozen at -80° C to be used for Mb quantification, while the remaining half was used for determination of oxygen consumption (OC) and MMb reducing activity (MRA). Sample portions reserved for OC and MRA were cut in half parallel to the muscle fibers, with the two halves randomly allotted to one analysis.

Oxygen Consumption

Oxygen consumption was determined using the method outlined by AMSA (2012) with modifications. The freshly cut surface of the sample designated for OC was covered with clear oxygen-permeable film and allowed to oxygenate for 2 h at 4°C. After oxygenation, samples were vacuum packaged and scanned immediately with the same Hunter spectrophotometer that was used for retail display evaluations. The Hunter was calibrated through the same material used for vacuum packaging. Samples were then allowed to incubate at 20° C for 1 h, then scanned a second time with the Hunter. The proportion of OMb from the initial and final scan was determined using the Krzywicki (1979) method as previously outlined. Percent OC was calculated using initial and final OMb content as outlined by AMSA (2012):

$$\% \text{ Oxygen Consumption} = \frac{(\text{Initial \% OMb} - \text{Final \% OMb})}{\text{Initial \% OMb}} \times 100\%$$

Metmyoglobin Reducing Activity

Metmyoglobin reducing activity was determined using the method outlined by AMSA (2012) with modifications. The freshly cut surface of the sample designated for MRA was submerged in a solution of 0.3% sodium nitrite for 30 minutes at approximately 20°C. Samples were then removed from solution, blotted dry, and individually vacuum packaged. Immediately after packaging, samples were scanned through the vacuum package with the same Hunter spectrophotometer that was used for oxygen consumption. After scanning, samples were allowed to incubate for 3 h at 20°C. Samples were scanned a second time through the packaging after incubation was complete. The proportion of MMb from the initial and final scan was determined using the Krzywicki (1979) method as previously outlined. Percent MRA was calculated using initial and final MMb content as outlined by AMSA (2012):

$$\% \text{ Metmyoglobin Reducing Activity} = \frac{(\text{Initial \% MMb} - \text{Final \% MMb})}{\text{Initial \% MMb}} \times 100\%$$

Myoglobin Content

Myoglobin content was determined according to the procedure outlined by Faustman and Phillips (2001) with modifications. Samples were diced and pulverized into a fine powder using liquid nitrogen. Duplicate 5g samples were homogenized with 45 mL ice cold mM potassium phosphate buffer (pH = 6.8) and filtered through Whatman no. 1 filter paper. After filtering, 150 µL of the filtrate was pipetted into a 96-well plate with potassium phosphate buffer as a blank. The absorbance of the filtrate was measured at 525 nm using a plate reader (Synergy HT Multi-Model Microplate Reader, Bio-Tek, Winooski, VT). Myoglobin content was determined using the following equation:

$$\text{Myoglobin (mg/g)} = [A_{525} / 7.6\text{mM}^{-1}\text{cm}^{-1} \times 1 \text{ cm}] \times [17,000/1,000] \times [(45 + \text{g sample})/\text{g sample}]$$

where A_{525} = absorbance at 525 nm, $7.6 \text{ mM}^{-1} \text{ cm}^{-1}$ = millimolar extinction coefficient of Mb at 525 nm; 1 cm = path length of cuvette; 17,000 Da = average molecular mass of Mb, and $[(45 + \text{g sample})/\text{g sample}]$ is the dilution factor based on how much initial sample was used.

Statistical Analyses

Summary statistics for initial (d1) color and biochemical measurements were determined using the MEANS procedure of SAS (v 9.4; SAS Institute Inc., Cary, NC). Initial measurement differences were evaluated using the MIXED procedure with muscle type serving as a fixed effect and individual animal serving as a random effect. Differences in color and biochemical traits between muscles were considered different at $P \leq 0.05$.

For each muscle, Pearson correlation coefficients (r) were determined between initial traits (color and biochemical) and final visual discoloration or 630/580 nm ratio using the CORR procedure. Comparisons of independent correlation coefficients between muscles were then achieved following the example of Kenny (1987) and Lowell et al. (2017) using z-tests for comparing 2 independent correlations. Two muscles were compared at a time: LD vs TB, LD vs PM, and TB vs PM. Data were grouped into three data sets by muscle (LD, TB, and PM). For each data set, correlation coefficients were transformed using the Fisher's r to z transformation with the FISHER option of the CORR procedure in SAS. The Fisher's r to z transformation was performed using the following equation:

$$z = \frac{1}{2} \left[\frac{1 + r}{1 - r} \right]$$

where r is the Pearson correlation coefficient and z is the transformed value of the correlation coefficient. This transformation was used to ensure that transformed coefficients were nearly normally distributed and that correlation variances were approximately the same regardless of the value of the population correlation (Kenny, 1987). If the z value was statistically significant,

then correlations between 2 populations were different (Kenny, 1987). Fischer's transformed z values were then merged into a single data set for each comparison (LD vs TB, LD vs PM, and TB vs PM) using the following equation:

$$z = \frac{z_{Muscle\ 1} - z_{Muscle\ 2}}{\sqrt{\frac{1}{n_{Muscle\ 1}^{-3}} + \frac{1}{n_{Muscle\ 2}^{-3}}}}$$

Correlations for an individual muscle were considered weak (in absolute value) at $r < 0.35$, moderate at $0.36 \leq r \leq 0.67$, and strong at $r > 0.68$. P-values for comparisons between correlations for two muscles were reported when both muscles had a significant correlation ($P > 0.05$). Figures displaying correlations between an initial trait and final trait were created when 2 of the 3 muscles had significant correlations between those traits.

Linear stepwise regression equations were developed using independent candidate variables to predict final visual discoloration and final 630/580 nm ratio for each muscle. Multicollinearity among independent variables was assessed using a variance inflation factor (VIF) statistic. Parameters that exceeded a VIF of 10 were excluded from respective models. Variables included in the model for LD were L^* , pH, a^* , b^* , OMb, OC, MRA, and Mb. Variables included in the model for TB were pH, a^* , b^* , OMb, OC, MRA, and Mb. Variables included in the model for PM were a^* , b^* , OC, MRA, and Mb. Influence of individual observations on estimation of regression parameters was evaluated using the difference of fit (DFFITS) statistic. Observations were considered to have excessive influence when $DFFITS \geq 2[(p/n)^{1/2}]$, where p = the number of parameters and n = the total number of observations. Two observations met this criteria for the models predicting LD traits, and were removed from the data set for those models. No other observations were removed. Using a stepwise selection

method, independent variables were required to have an SLENTY level = 0.50 to enter the model and SLSTAY level = 0.45 to remain in the model.

Results

Population summary statistics for color and biochemical measurements on d1 of display are reported in Table 1. The LD was the least variable muscle for several traits as indicated by lesser CV values, including ultimate pH, L^* , b^* , Chroma, Hue Angle, OMb, MRA, and OC. Visual Discoloration, DMb, and Mb content had larger CV values for all three muscles (>25); however, these were likely influenced by small overall means, meaning that this increased variability is likely not practically important (Liver, 1942). The LD had a greater CV for OC (CV = 48.69) compared to the TB (CV = 5.05) and PM (CV = 9.93), likely because the LD had a larger range in values (range = 61.26) than the TB (range = 9.32) or PM (range = 9.47).

Correlations of Initial Traits within Muscle with Final Discoloration

Despite averaging less than 2%, initial visual discoloration was correlated with final visual discoloration for the LD ($r = 0.76$), but not for any other muscle ($P \geq 0.08$; data not shown). Initial LD and TB lightness were moderately correlated to final LD and TB visual discoloration ($r = 0.46$ and $r = 0.65$, respectively) while initial PM lightness was strongly correlated to final PM visual discoloration ($r = 0.69$; Figure 1A). Correlation coefficients for initial L^* and final visual discoloration did not differ ($P \geq 0.31$) between muscles. Initial TB and PM yellowness were strongly correlated to final TB and PM visual discoloration ($r = 0.70$ and $r = 0.76$, respectively), but there was no correlation between initial LD yellowness and final LD visual discoloration ($P = 0.11$; Figure 1B). Initial TB chroma ($r = 0.62$; Figure 1C) and hue angle ($r = 0.49$; Figure 1D) were moderately correlated to final TB visual discoloration. Final PM visual discoloration was strongly correlated to initial PM chroma ($r = 0.69$; Figure 1C) and

moderately correlated to initial PM hue angle ($r = 0.62$; Figure 1D). No correlations were observed between final LD visual discoloration and initial LD chroma or hue angle ($P \geq 0.32$). Additionally, initial redness and 630/580 nm ratio were not correlated with final visual discoloration for any muscle ($P \geq 0.07$). Correlation coefficients (r) did not differ ($P \geq 0.59$) between the TB and PM for the relationships between final visual discoloration and initial lightness, yellowness, chroma, or hue angle. In other words, the relationships between final visual discoloration and these traits was similar for TB and PM.

Final TB visual discoloration was moderately correlated to initial TB DMb ($r = -0.66$; Figure 2A), OMb ($r = 0.67$; Figure 2B), MRA ($r = 0.52$; Figure 2C), and pH ($r = -0.59$; Figure 2D). Final PM visual discoloration was moderately correlated to initial PM MRA ($r = 0.47$; Figure 2C) and strongly correlated to PM DMb ($r = -0.72$; Figure 2A), OMb ($r = 0.70$; Figure 2B), and pH ($r = -0.70$; Figure 2D). Initial OC was moderately correlated to final visual discoloration for the TB ($r = -0.48$), but not correlated for the LD or PM ($P \geq 0.06$) data not shown). Final LD visual discoloration was not correlated to initial LD OMb, DMb, MRA, or pH ($P \geq 0.06$). No correlations were observed between final visual discoloration and initial Mb content for any muscle ($P \geq 0.09$). Correlation coefficients (r) between final visual discoloration and initial OMb, DMb, MRA, or pH did not differ between the TB and the PM ($P \geq 0.59$).

Final LD 630/580 nm ratio was moderately correlated to initial LD lightness ($r = -0.65$; Figure 3A) and redness ($r = 0.54$; Figure 3B), and strongly correlated to initial LD hue angle ($r = -0.75$; Figure 3E). Initial LD yellowness and chroma were not correlated to final LD 630/580 nm ratio ($P \geq 0.24$). Final TB 630/580 nm ratio was moderately correlated to initial TB redness ($r = -0.65$; Figure 3B) and hue angle ($r = -0.45$; Figure 3E), and strongly correlated to initial TB lightness ($r = -0.72$; Figure 3A), yellowness ($r = -0.87$; Figure 3C), and chroma ($r = -0.85$;

Figure 3D). Final PM 630/580 nm ratio was moderately correlated to initial PM redness ($r = -0.54$; Figure 3B) and strongly correlated to initial PM lightness ($r = -0.79$; Figure 3A), yellowness ($r = -0.92$; Figure 3C), chroma ($r = -0.85$; Figure 3D), and hue angle ($r = -0.73$; Figure 3E). Initial 630/580 nm ratio was strongly correlated to final visual discoloration for the LD ($r = 0.69$), but not correlated for the TB or PM ($P \geq 0.21$; data not shown). Correlation coefficients between final 630/580 nm ratio and initial lightness or hue angle did not differ between any muscles ($P \geq 0.15$). Correlations between final 630/580 nm ratio and was positively correlated to initial redness in the LD but negatively correlated to initial redness in the TB and PM ($P < 0.01$). However, this same correlation did not differ between the TB and PM ($P = 0.64$). Additionally, correlations between initial yellowness or chroma and final 630/580 nm ratio did not differ between the TB and PM ($P \geq 0.46$).

Final LD 630/580 nm ratio was moderately correlated to initial LD DMb ($r = 0.50$; Figure 4A) and pH ($r = 0.48$; Figure 4E). However, final LD 630/580 nm ratio was not correlated to initial LD OMb, OC, or MRA ($P \geq 0.32$). Final TB 630/580 nm ratio was moderately correlated to initial TB OC ($r = 0.47$; Figure 4C) and strongly correlated to initial TB DMb ($r = 0.91$; Figure 4A), OMb ($r = -0.91$; Figure 4B), MRA ($r = -0.77$; Figure 4D), and pH ($r = 0.83$; Figure 4E). Final PM 630/580 nm ratio was moderately correlated to initial PM OC ($r = 0.45$; Figure 4C) and MRA ($r = -0.65$; Figure 4D), and strongly correlated to initial PM DMb ($r = 0.89$; Figure 4A), OMb ($r = -0.88$; Figure 4B), and pH ($r = 0.94$; Figure 4E). Initial MMb and Mb content were not correlated to final 630/580 nm ratio for any muscle ($P \geq 0.15$). Correlations between final 630/580 ratio and initial DMb were stronger in the TB and PM compared to the LD ($P < 0.01$), but not different between the TB and PM ($P = 0.79$). Correlations between initial pH and final 630/580 nm ratio were weaker in the LD compared to the PM ($P < 0.01$), but not

different between the LD and TB ($P = 0.05$) or between the TB and the PM ($P = 0.10$). There were also no differences between the TB and PM for correlations between final 630/580 nm ratio and initial OMb, OC, or MRA ($P \geq 0.45$).

Stepwise Regression Models

Models developed for visual discoloration explained 33 – 53% of variation for individual muscles ($R^2 = 0.33$ to 0.53 ; $P < 0.01$; Table 2), while models developed for 630/580 nm ratio explained 60 – 85% of variation for individual muscle ($R^2 = 0.60$ to 0.85 ; $P < 0.01$; Table 3). For both visual discoloration and 630/580 nm ratio, models for the PM were most predictive ($R^2 = 0.53$ and 0.85 , respectively). Both final visual discoloration and final 630/580 nm ratio in the TB (partial $R^2 = 0.49$ and 0.76 , respectively) and PM (partial $R^2 = 0.57$ and 0.85 , respectively) were primarily explained by initial yellowness. Furthermore, final LD 630/580 nm ratio was primarily explained by initial yellowness (partial $R^2 = 0.32$) and redness (partial $R^2 = 0.29$), with yellowness contributing slightly more to the model. Alternatively, final LD visual discoloration was primarily explained by initial lightness (partial $R^2 = 0.21$) and Mb content (partial $R^2 = 0.20$), and did not contain yellowness in the model. Despite explaining the majority of variation in the other models, yellowness was not included in the model for LD visual discoloration. Redness was included in visual discoloration models for the LD and PM and 630/580 nm ratio models for the PM, but did not explain more than 2% of variation in any of those models. Ultimate pH, OC, MRA, and Mb content were also included in several of the models, but explained no more than 5% (partial $R^2 = 0.05$) of variation for any model, and thus may not be practically important. Due to the low contributions of these traits to their respective models, the variation accounted for by these traits may not be practically important and they may not be necessary in the models.

Discussion

Pork color traits on the first day of retail display were associated with improved color stability in the loin (Zhu et al., 1998a). However, limited research has evaluated traits related to pork color stability in muscles other than the LD. Nearly 30% of the pork in the U.S. is consumed as sausage or fresh pork from muscles other than the LD or ribs. (NPPC, 2011). While not all of this meat is overwrapped for retail sale, there is still potential for discounting or discarding of product due to discoloration. Therefore, there is value in identifying retail display traits related to discoloration in multiple pork muscles.

Correlations between initial traits and final measures of discoloration were different between muscles, but generally stronger for the TB and PM than the LD. When correlations between initial traits and final 630/580 nm ratio were observed for all three muscles, Correlations between initial traits and final 630/580 nm ratio for the LD were numerically weaker for lightness, redness, DMb, and pH compared to correlations for the TB and PM. For each of these initial traits except redness, LD traits were moderately correlated to final 630/580 nm ratio while TB and PM traits were strongly correlated. Furthermore, in several cases initial LD traits were not correlated with discoloration measurements when the same traits were correlated in other muscles. For example, final visual discoloration was correlated to lightness, yellowness, chroma, hue angle, DMb, OMb, MRA, and pH in TB and PM chops, but only correlated to lightness in LD chops. Similarly, final 630/580 nm ratio was correlated to all d1 traits except for initial 630/580 nm ratio, initial MMb, and Mb in the TB and PM. Alternatively, in the LD 630/580 nm ratio was only correlated to lightness, redness, hue angle, DMb, and pH.

Differences in correlation strength may be attributable to variability differences in muscles. Smaller ranges in values and CV were observed for several LD traits compared to the

same traits in the PM and TB. For example, LD chops had lesser ranges and CV for pH, yellowness, chroma, hue angle, OMb, MRA, and Mb compared to TB and PM chops. Furthermore, LD chops discolored less overall compared to TB and PM chops, as evidenced by reduced visual discoloration scores on d5 and smaller changes in 630/580 nm ratio from d1 to d5. When variability among observations used to develop correlation coefficients is low, then the strength of the correlation itself will be decreased (Goodwin and Leach, 2006). This decrease in correlation strength is more apparent when observations for both the x and y variables have low variability (Goodwin and Leach, 2006). Thus, the lack of variation in initial traits and the lower overall extent of discoloration likely both resulted in a lack of correlations for the LD. Reduced variability in loin traits could be expected. Pigs are commonly selected to minimize variation within the LD, as consumers prefer loin chops to be consistent when making purchasing decisions (King et al., 2011a). Alternatively, producers less commonly select pork to minimize variability in muscles other than the loin, resulting in more animal-to-animal variation for these muscles. For example, Barkley et al. (2021) reported that instrumental color was more variable in Boston butts than loins. Overall, the lack of variation in LD chops likely resulted in weaker relationships between initial retail display traits and final discoloration measures compared to TB and PM chops.

Despite differences in initial color and biochemical traits, minimal differences in correlation strengths were observed between the TB and PM. No differences in correlation strength were observed between the TB and PM when an initial trait was correlated to final visual discoloration or 630/580 nm ratio for both muscles. Studies evaluating discoloration differences between pork muscles are limited; however, multiple studies have characterized color stability in beef muscles. Both the TB and PM are primarily oxidative muscles and have similar

biochemical characteristics in beef (McKenna et al., 2005). In general, muscles characterized as “color labile” or “color stable” have similar characteristics to other muscles of the same category (Renerre and Labas, 1987; McKenna et al., 2005). Because of the similarities between the PM and TB in beef, it was not unexpected that relationships between initial traits and discoloration would be similar between these two muscles in pork. While further research is necessary to verify the color stability of other pork muscles, if relationships between the color labile PM and TB were similar, then characteristics of these muscles may be indicative of color stability for other pork muscles that are considered color labile in beef, such as the *supraspinatus*, *infraspinatus*, and *adductor* (McKenna et al., 2005).

Correlations with initial traits were also stronger for 630/580 nm ratio than visual discoloration for all muscles evaluated. Limited research has investigated differences in relationships between instrumental or visual measures of discoloration and color stability traits in pork or beef. However, instrumental measures are typically more precise than visual measures because they are evaluated using a machine (Barkley et al., 2018). Alternatively, because of the subjective nature of human sight, there is additional error associated with visual discoloration observations compared to instrumental assessment (Zhu and Brewer, 1999). Increased error for a set of measurements can also decrease correlation strength (Goodwin and Leach, 2006). As a result, relationships between initial traits and visual discoloration were weaker than relationships between the same initial traits and 630/580 nm ratio due to increased error associated with visual measurements. Therefore, measuring discoloration instrumentally may be more appropriate when evaluating relationships between discoloration and d1 retail display traits.

The relationship between redness and discoloration was weaker than expected based on previous research in beef. While redness was moderately correlated to final 630/580 ratio in all

muscles, it was not correlated to any muscles for visual discoloration. Furthermore, redness partially explained LD 630/580 nm ratio, but was either not included in other muscles or explained very little variability ($\leq 2\%$). This was unexpected, as discoloration in beef is commonly associated with increased redness (McKenna et al., 2005; King et al., 2011b; Joseph et al., 2012; Canto et al., 2015), whereas neither lightness nor yellowness are commonly associated with color stability differences (McKenna et al., 2005; Canto et al., 2015). Increased redness in beef muscles is associated with a greater proportion of oxidative muscle fibers (Ramanathan et al., 2020). Oxidative muscle fibers contain greater levels of myoglobin, which is responsible for the red color of meat (AMSA, 2012). However, in the current study, Mb content was not correlated to visual discoloration or 630/580 nm ratio for any muscle. Furthermore, while Mb content explained 20% of final LD visual discoloration variation in the current study, it did not explain more than 2% of variation in visual discoloration for TB or PM. Myoglobin content did not explain any variation in final 630/580 nm ratio for the LD or TB, and only explained 1% of variation for the PM.

The lack of relationship between Mb content and discoloration traits in pork may stem from pork having less Mb than beef. Myoglobin is present in smaller quantities in pork compared to beef.. This is partially because pork is less oxidative than beef, with pork LD containing 10% Type I (red, oxidative fibers) and beef LD containing 30% Type I fibers (Zhang et al., 2017). In the current study, Mb content ranged from 0.74 (LD) – 2.01 (TB) mg/g on average compared with 4.05 – 4.56 mg/g for the beef longissimus (Canto et al., 2015). The reduced Mb in pork may also contribute to the lack of relationship between redness and discoloration in this species.

Decreased lightness and yellowness were associated with more color stable pork for all muscles evaluated. Final visual discoloration and 630/580 nm ratio were moderately or strongly

correlated to lightness for all muscles, and strongly correlated to yellowness for the TB and PM. Furthermore, yellowness explained the majority of variation for visual and instrumental discoloration for all muscles evaluated in the study, with the exception of LD visual discoloration, which was explained by lightness and Mb. These results were in agreement with results from studies by King et al. (2011a) and Lindahl et al. (2006), who also reported that stable pork loins were darker and less yellow than labile loins. Decreases in both yellowness and lightness in pork are associated with increased DMb content and decreased OMb content (Karamucki et al., 2013), both of which were also observed for stable pork in the current study. Overall, yellowness and lightness were the traits most strongly related to discoloration in muscles evaluated in this study, while redness was less important compared to beef.

Several studies conducted in beef have observed that labile beef possesses high oxygen consumption rates while stable beef has high MMb reducing capacities (O'Keefe and Hood, 1982; Sammel et al., 2002; McKenna et al., 2005; Jeong et al., 2009), both across muscles and with a single muscle. In meat, mitochondria remain active postmortem and are able to consume oxygen (Mancini et al., 2018; Ramanathan et al., 2018). When mitochondria consume oxygen, they provide electrons or NADH for reduction of MMb, improving color stability (Ramanathan et al., 2018). On its face, greater mitochondrial content as evidenced by greater OC would seem to favor improved color stability. However, mitochondrial degradation and loss of mitochondrial functionality places muscles under additional oxidative stress (Ramanathan et al., 2018). Decreased color stability in the beef PM was partially attributed to greater mitochondrial degeneration compared to the loin (Mancini et al., 2018). Thus, greater OC in beef, though typically associated with higher mitochondria content, results in sharper OC declines than when OC (and by extension mitochondria content) is lower. Alternatively, MRA is typically increased

in beef that is considered color stable (Reddy and Carpenter, 1991; Suman et al., 2014). Muscles with more glycolytic fibers have enzymes involved in anaerobic metabolism that are able to provide substrates for MMb reduction, minimizing MMb accumulation (Nair et al., 2018).

However, this relationship of OC and MRA with color stability did not hold true in pork as samples with high visual discoloration and low 630/580 nm ratios displayed low OC and high MRA. For example, pork chops classified as dark, firm and dry (DFD) were more stable than normal and PSE pork chops over a 7d display period (Zhu et al., 1998b). Those DFD chops also displayed higher OC and lower MRA throughout display. In more color stable, DFD pork, while OC did decrease during storage, it decreased by a much smaller magnitude compared to normal and PSE pork, which had smaller initial OC values (Zhu and Brewer, 1998b). Thus, it is interesting to speculate that differences in OC and MRA between the two species may be related to mitochondrial functionality. Oxygen consumption of DFD pork may have also been favored by a higher pH compared to normal and PSE pork, as mitochondrial OC increases at higher pH values (Ramanathan et al., 2018). Increased pH was also related to decreased visual discoloration and increased 630/580 nm ratio in the current study. This could indicate that mitochondria are more stable in pork compared to beef, or that high pork pH favors OC enough to mitigate decreases in pork mitochondrial functionality. Furthermore, greater oxygen consumption by mitochondria also limits the availability of oxygen for myoglobin interaction, minimizing the need for MMb to be reduced, which may explain lower MRA values in stable pork in the study by Zhu et al. (1998) and the current study. This would also increase the proportion of DMb and decrease the proportion of OMb on the meat surface due to a lack of myoglobin oxygenation from lower oxygen levels, both of which were observed for stable samples in the current study. Further research is necessary to investigate differences in mechanisms behind OC and MRA

between species. Nonetheless, results from the current study would indicate that stable pork possesses a higher initial OC and DMb content and a decreased initial MRA and OMb content.

Conclusions

Color and biochemical traits at the beginning of retail display were more strongly correlated with final visual discoloration and 630/580 nm ratio in the PM and TB than the LD. However, correlations did not differ in strength between the PM and TB. Decreased discoloration and increased 630/580 nm ratio was associated with pork that was darker, less yellow, had decreased hue angles, and had increased DMb and pH values on d1 of display for all muscles. In the TB and PM, more color stable pork was also associated with higher OC and lower MRA on d1 of display. Initial yellowness was the best predictor of final visual discoloration for the TB and PM and the best predictor of 630/580 nm ratio for all three muscles, while initial lightness and Mb content were the best predictors for final LD visual discoloration. Therefore, selection for pork with decreased lightness or yellowness in the LD, TB, or PM could allow for increased pork color stability.

Tables

Table 4.1. Population summary statistics for d1 retail display color and biochemical traits for the *longissimus dorsi*, *triceps brachii*, and *psoas major*.

Variable	Samples, n	Mean	Minimum	Maximum	Range	SD ¹	CV ²
<i>Longissimus dorsi</i>							
pH	20	5.61	5.51	5.80	0.19	0.07	1.29
Lightness, <i>L</i> *	20	59.10	53.79	64.04	4.94	2.95	5.00
Redness, <i>a</i> *	20	11.52	9.33	13.22	1.70	0.93	8.11
Yellowness, <i>b</i> *	20	18.57	17.09	19.65	1.08	0.71	3.80
Chroma ³	20	21.87	19.48	23.11	1.24	0.92	4.23
Hue Angle ⁴	20	58.24	54.60	61.46	3.22	1.89	3.25
630/580 nm	20	2.83	2.47	3.24	0.41	0.23	8.19
Visual Discoloration, %	18	1.81	0.00	9.78	7.97	2.65	146.34
Metmyoglobin, % ⁵	20	11.86	7.13	15.11	3.25	1.96	16.51
Deoxymyoglobin, % ⁶	20	3.83	-4.50	15.94	12.11	5.10	133.02
Oxymyoglobin, % ⁷	20	84.30	73.60	91.12	6.82	4.23	5.02
Oxygen Consumption, %	20	59.38	17.09	120.64	61.26	28.91	48.69
Metmyoglobin Reducing Activity, %	20	48.99	27.68	59.41	10.42	7.79	15.89
mg Myoglobin/g Meat	20	0.74	0.38	1.26	0.52	0.20	27.29
<i>Triceps brachii</i>							
pH	20	5.90	5.59	6.57	0.67	0.22	3.76
Lightness, <i>L</i> *	20	44.51	40.15	48.83	4.32	2.65	5.96
Redness, <i>a</i> *	20	17.11	15.08	19.35	2.24	1.23	7.18
Yellowness, <i>b</i> *	20	17.17	14.25	19.86	2.69	1.48	8.61
Chroma ³	20	24.26	20.75	27.12	2.86	1.75	7.21
Hue Angle ⁴	20	45.07	41.66	48.75	3.68	1.90	4.23
630/580 nm	20	4.31	3.77	4.66	0.35	0.23	5.45
Visual Discoloration, %	20	2.07	0.00	6.00	3.93	1.89	91.27
Metmyoglobin, % ⁵	20	19.29	17.50	20.93	1.65	0.92	4.79
Deoxymyoglobin, % ⁶	20	13.64	0.81	36.26	22.62	8.99	65.90
Oxymyoglobin, % ⁷	20	67.07	43.88	80.22	13.15	9.02	13.45
Oxygen Consumption, %	20	99.57	86.95	108.89	9.32	5.02	5.05
Metmyoglobin Reducing Activity, %	20	40.61	15.30	51.74	11.13	8.64	21.27
mg Myoglobin/g Meat	20	2.07	1.02	3.83	1.76	0.79	37.87
<i>Psoas major</i>							
pH	20	5.84	5.58	6.40	0.56	0.24	4.08
Lightness, <i>L</i> *	20	47.41	42.01	55.34	7.93	3.67	7.75
Redness, <i>a</i> *	20	18.43	15.96	20.16	1.73	1.16	6.31
Yellowness, <i>b</i> *	20	19.43	15.12	21.18	1.75	1.76	9.07
Chroma ³	20	26.80	21.99	29.07	2.27	1.88	7.03
Hue Angle ⁴	20	46.44	43.24	50.37	3.93	2.06	4.43
630/580 nm	20	4.36	3.66	4.90	0.54	0.31	7.12
Visual Discoloration, %	20	3.29	0.22	9.11	5.82	2.97	90.38
Metmyoglobin, % ⁵	20	17.00	15.29	19.28	2.28	1.07	6.27
Deoxymyoglobin, % ⁶	20	6.33	-3.61	36.49	30.16	10.43	164.85

Table 4.1. (cont.)

Oxymyoglobin, % ⁷	20	76.67	45.55	87.58	10.91	10.58	13.79
Oxygen Consumption, %	20	96.04	72.19	105.51	9.47	9.54	9.93
Metmyoglobin Reducing Activity, %	20	35.62	15.73	46.29	10.66	8.40	23.59
mg Myoglobin/g Meat	20	1.24	0.67	2.46	1.22	0.42	33.50

¹Standard Deviation.

²Coefficient of Variation, calculated by $(SD/mean) \times 100\%$.

³Chroma is a measure of color saturation, calculated by: $C = (b^{*2} + a^{*2})^{1/2}$.

⁴Hue angle is a measure of the overall color of a sample, calculated by: $H_{ab} = \arctangent(b^*/a^*)$.

⁵Calculated as: $\% MMb = \{1.395 - \left(\frac{A_{572}-A_{700}}{A_{525}-A_{700}}\right)\} \times 100$.

⁶Calculated as: $\% DMb = \left\{2.375 \times \left(1 - \frac{A_{572}-A_{700}}{A_{525}-A_{700}}\right)\right\} \times 100$.

⁷Calculated as: $\% OMb = 100 - \% MMb - \% DMb$.

Table 4.2. Stepwise regression models predicting final (d5) visual discoloration (dependent variable) of the LD, TB, and PM using initial (d1) color and biochemical traits (independent variables).

Initial (d1) Independent Variables								
Item	Model R ²	Lightness, L*	Redness, a*	Yellowness, b*	Oxygen Consumption, %	Myoglobin, mg/g meat	Intercept	Model P-value
Longissimus dorsi								
R ²	0.33	0.21	-	-	-	0.20	-	< 0.01
β	-	0.77	-	-	-	9.53	-42.03	-
Triceps brachii								
R ²	0.48	-	0.02	0.49	0.02	-	-	< 0.01
β	-	-	-2.13	3.98	-0.54	-	52.75	-
Psoas major								
R ²	0.53	-	0.02	0.57	-	0.02	-	< 0.01
β	-	-	-1.30	4.21	-	3.53	--33.38	-

Table 4.3. Stepwise regression models predicting final (d5) 630/580 nm ratio (dependent variable) of the LD, TB, and PM using initial (d1) color and biochemical traits (independent variables).

Initial (d1) Independent Variables									
Item	Model R ²	pH	Redness, <i>a</i> *	Yellowness, <i>b</i> *	Oxygen Consumption, %	Metmyoglobin Reducing Activity, %	Myoglobin, mg/g meat	Intercept	Model <i>P</i> -value
<i>Longissimus dorsi</i>									
R ²	0.60	0.05	0.29	0.32	0.03	-	-	-	< 0.01
β	-	0.92	0.16	-0.13	-0.001	-	-	-2.29	-
<i>Triceps brachii</i>									
R ²	0.78	-	-	0.76	-	0.05	-	-	< 0.0001
β	-	-	-	-0.34	-	-0.03	-	9.30	-
<i>Psoas major</i>									
R ²	0.85	-	0.02	0.85	0.01	0.01	0.01	-	< 0.0001
β	-	-	0.17	-0.48	-0.01	-0.01	-0.39	10.88	-

Figures

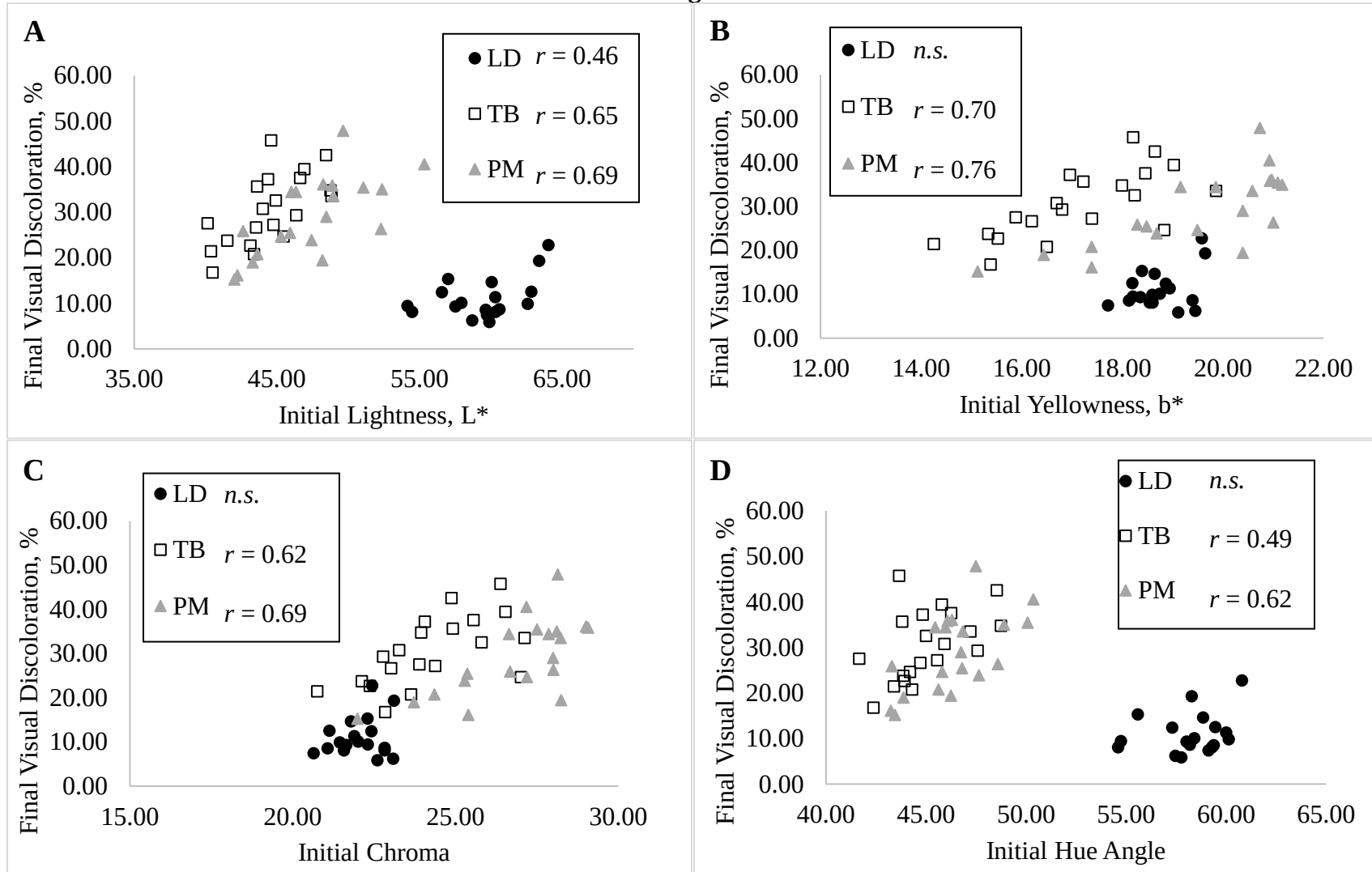


Figure 4.1. Pearson correlation coefficients between initial (d1) lightness (A), yellowness (B), Chroma (C), and hue angle (D) and final (d5) visual discoloration in the *longissimus dorsi* (LD), *triceps brachii* (TB), and *psoas major* (PM). Muscles with “n.s.” did not have significant correlations ($P < 0.05$). Correlations for muscles possessing different superscripts are different ($P < 0.05$).

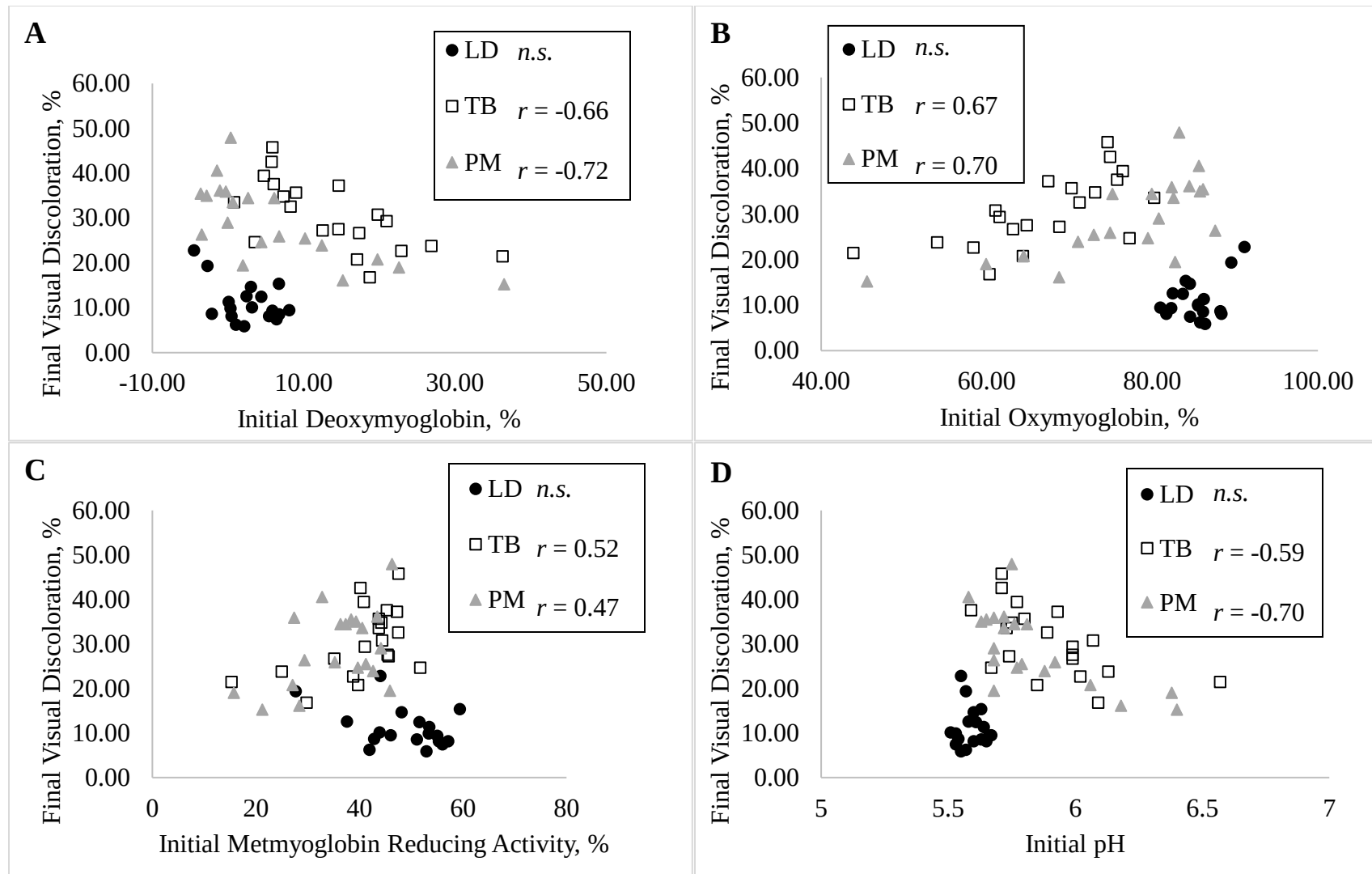


Figure 4.2. Pearson correlation coefficients between initial (d1) DMb (A), OMb (B), MRA (C), and pH (D) and final (d5) visual discoloration in the *longissimus dorsi* (LD), *triceps brachii* (TB), and *psaos major* (PM). Muscles with “n.s.” did not have significant correlations ($P < 0.05$). Correlations for muscles possessing different superscripts are different ($P < 0.05$).

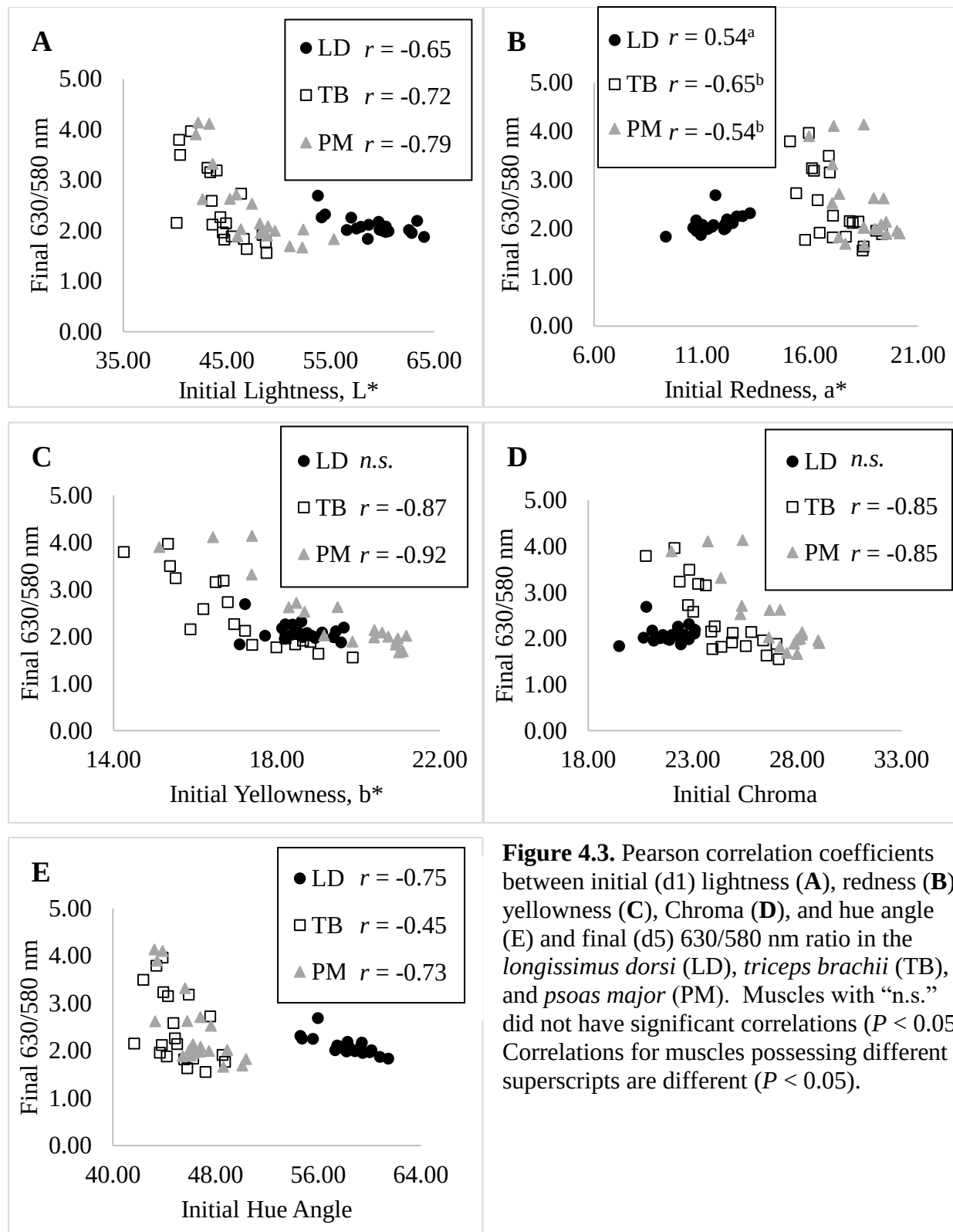


Figure 4.3. Pearson correlation coefficients between initial (d1) lightness (**A**), redness (**B**), yellowness (**C**), Chroma (**D**), and hue angle (**E**) and final (d5) 630/580 nm ratio in the *longissimus dorsi* (LD), *triceps brachii* (TB), and *psoas major* (PM). Muscles with “n.s.” did not have significant correlations ($P < 0.05$). Correlations for muscles possessing different superscripts are different ($P < 0.05$).

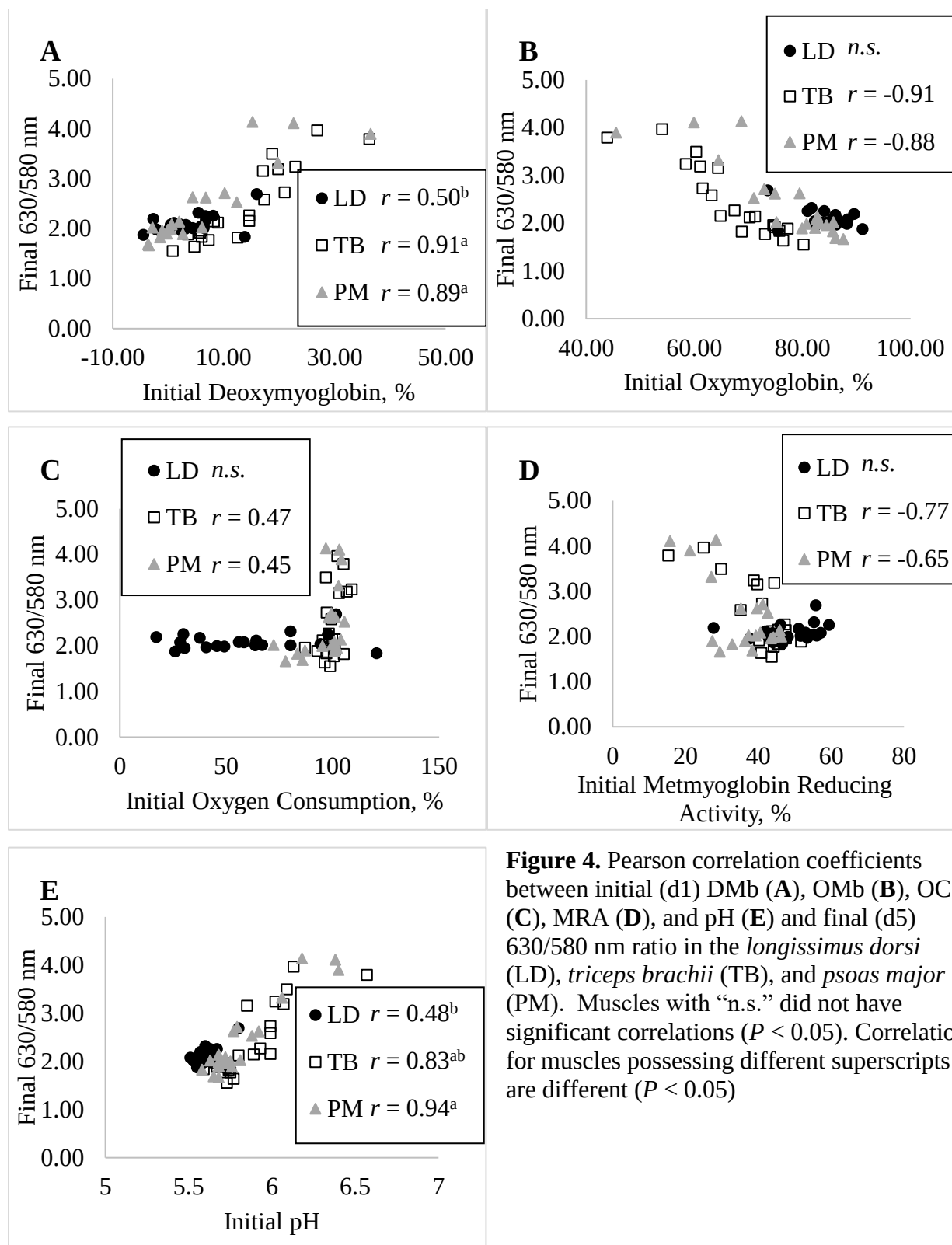


Figure 4. Pearson correlation coefficients between initial (d1) DMb (A), OMb (B), OC (C), MRA (D), and pH (E) and final (d5) 630/580 nm ratio in the *longissimus dorsi* (LD), *triceps brachii* (TB), and *psoas major* (PM). Muscles with “n.s.” did not have significant correlations ($P < 0.05$). Correlations for muscles possessing different superscripts are different ($P < 0.05$)

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