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IMPACT OF MYOGLOBIN OXYGENATION STATE ON COLOR STABILITY OF
BEEF STEAKS DURING FROZEN STORAGE AND THAWED RETAIL DISPLAY

by

Morgan L. Henriott

A THESIS

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Major: Animal Science

Under the Supervision of Professor Chris R. Calkins

Lincoln, Nebraska

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IMPACT OF MYOGLOBIN OXYGENATION STATE ON COLOR STABILITY OF BEEF STEAKS DURING FROZEN STORAGE AND THAWED RETAIL DISPLAY

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University of Nebraska, 2019

Advisor: Chris R. Calkins

Meat color is the most important product characteristic that impacts consumer purchasing decisions. Therefore, the major objectives of this thesis were to determine the impact of myoglobin oxygenation level and frozen storage duration on both frozen and thawed beef color. USDA Choice strip loins (n=36) were aged for 4 d or 20 d and cut into steaks. Steaks were randomly assigned to a myoglobin oxygenation level [deoxymyoglobin (DeOxy; immediately packaged), oxygenation (Oxy; oxygenated in air for 30 minutes), or high oxygenation (HiOxy; packaged for 24 h in 80% O₂)]. Steaks were then vacuum packaged in oxygen permeable or impermeable film and immediately frozen (-5° C). Following either 0, 2, 4, or 6 months of frozen storage, steaks were removed from the packaging and either immediately measured for frozen meat color (frozen steaks) or placed under simulated retail display (RD) conditions (thawed steaks). Steaks were analyzed for oxygen penetration, instrumental color (colorimeter and spectrophotometer), subjective discoloration, and lipid oxidation. The frozen steak study showed that the HiOxy steaks had greater oxygen penetration and the highest a* values compared to DeOxy and Oxy steaks, regardless of frozen storage packaging ($P < 0.0005$). Redness and percent oxymyoglobin were greatest for HiOxy steaks within each storage period ($P < 0.0002$). Conversely, HiOxy steaks that were frozen for 6 months and thawed

in RD had the lowest amounts of percent oxymyoglobin than all other treatments on days 4-7 of RD ($P < 0.05$). The HiOxy steaks had greater delta E values, discoloration, and lipid oxidation compared to Oxy and DeOxy for steaks thawed in RD ($P < 0.05$). Overall, frozen meat color for the HiOxy steaks was greater or similar when compared to Oxy steaks, but HiOxy steaks experienced more detrimental quality effects when frozen storage was extended out. For the steaks thawed in RD, the HiOxy steaks frozen for under 6 months and through the first few days of RD provided optimal meat color under RD, similar to that of DeOxy and Oxy. However, with extended frozen storage and RD, HiOxy steaks had worse color characteristics compared to the other myoglobin oxygenation levels.

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INTRODUCTION

Color is viewed by consumers as an indicator of freshness and it has been suggested that beef color is a major factor that is considered when purchasing beef (Hood, 1980; Kropf, 1980; Carpenter et al., 2001). Consumers are attracted to beef with a bright cherry red color; therefore, it is a common concern for the meat industry in maintaining this desired color. It has been reported that 15% of retail beef is discounted due to discoloration resulting in an economic loss of \$1 billion annually (Mancini and Hunt, 2005).

Hood and Riordan (1973) reported that 20% discoloration had a 50% reduction in beef sales. Even at lower levels of discoloration, while not resulting in discounted products, consumer discrimination against discolored meat was observed. Discoloration is caused by an accumulation of metmyoglobin on the surface of beef due to the oxidation of oxymyoglobin and deoxymyoglobin. Typically, metmyoglobin can be reduced back to deoxymyoglobin and oxygenated to oxymyoglobin (bright cherry red color), however, this is no longer possible when reducing components of the metmyoglobin reducing activity cycle are depleted. Meat color, specifically the reduction and oxidation of myoglobin, depends on many intrinsic (pH, species, breed, sex, animal maturity, muscle to muscle variation, oxygen consumption, metmyoglobin reducing activity, and lipid oxidation) and extrinsic factors (diet, electrical stimulation, postmortem aging, temperature, frozen storage, and packaging) (Hood, 1980; Renner, 1990; Mancini and Hunt, 2005; Suman and Joseph, 2013).

While beef is typically packaged and sold fresh, the market of frozen beef has started to grow. The meat export industry is worth over \$13 billion and freezing plays an essential role in that industry, ensuring the safety of meat being supplied all over the world (Leygonie et al., 2012). Freezing beef provides the opportunity to extend the product shelf life, but also has detrimental effects on meat quality (MacDougall, 1982; Kim et al., 2015).

One emerging market for frozen beef products has developed in recent years. Meal delivery service companies have grown. The meal-kit delivery service segment had a market of \$4.65 billion in 2017 and is expected to grow to \$11.6 billion by 2022 (Progressive Grocer, 2017). With this opportunity for increased product sales, it is essential that a better understanding is developed of how to retain meat with a bright red cherry color through frozen storage.

Therefore, the objectives of these studies were to:

1. Determine how myoglobin oxygenation levels affect color stability through frozen storage.
2. Determine the impact that frozen storage duration has on meat color during frozen storage and after thawing for retail display.
3. Determine how different oxygen permeability of the packaging film and aging impacts meat color through frozen storage.

These studies address the impact that different parameters have on the color of frozen beef and reveal how to freeze beef while obtaining a bright red cherry color.

LITERATURE REVIEW

Meat Color Chemistry

Meat color chemistry focuses on the heme pigments, myoglobin, and hemoglobin. Hemoglobin is a tetrameric, globular heme protein localized in erythrocytes that functions as an oxygen carrier in the bloodstream (Clydesdale and Francis, 1971; Livingston and Brown, 1981). Hemoglobin and cytochromes may contribute to meat color, but to a far lesser extent than myoglobin (Suman and Joseph, 2013). Myoglobin in live animals is responsible for storing and delivering oxygen in the cells, primarily in the heart and skeletal muscles (Clydesdale and Francis, 1971; Livingston and Brown, 1981). Myoglobin accounts for only 10% of the total iron in the live animal. However, during exsanguination, iron is removed as hemoglobin and as much as 95% of the remaining iron in beef skeletal muscle is accounted for as myoglobin (Clydesdale and Francis, 1971).

Myoglobin is a sarcoplasmic protein primarily responsible for meat color. Myoglobin is a water-soluble, monomeric, globular heme protein composed of approximately 153 amino acids (Yin et al., 2011). The globular or globin portion is also referred to as the protein moiety. The globin portion is folded around the prosthetic heme group iron atom in eight, alpha-helical segments, forming the total myoglobin molecule (Clydesdale and Francis, 1971). This globular structure protects the heme iron from external environments and from oxidation (Suman and Joseph, 2013). The heme portion is composed of an iron atom located in the center of the porphyrin ring, that is able to form six bonds. Four of these bonds are with the pyrrole nitrogens, while the fifth coordinates with the proximal histidine-93, leaving a sixth site available to reversibly

bind ligands (Mancini and Hunt, 2005). The various ligands that bind with the sixth binding site influence the oxidation state of iron and dictate muscle color.

Deoxymyoglobin, a reduced form of myoglobin, occurs when no ligand is present at the sixth coordination site and the heme iron is in the ferrous (Fe^{2+}) state (Mancini and Hunt, 2005). Typically, associated with freshly cut meat and vacuum packaged products, deoxymyoglobin appears as purplish-red or purplish-pink color. However, according to Carpenter et al. (2001), consumer acceptance of the purplish-red color of deoxymyoglobin is low. The deoxymyoglobin state can only be maintained under anoxic conditions where oxygen tensions are very low. Since nothing is bound to the sixth ligand site, deoxymyoglobin is very unstable and can easily react with oxygen or carbon monoxide (Suman and Joseph, 2013).

When deoxymyoglobin is exposed to oxygen it allows oxygen to bind to the sixth ligand, producing a bright red cherry color known as oxymyoglobin. This reaction occurs very quickly because myoglobin has a high affinity for oxygen and is typically referred to as “blooming” in the meat industry (Pirko and Ayres, 1957). When myoglobin becomes oxygenated, it still remains in a reduced state (Fe^{2+}) while producing a red color preferred by consumers. This binding of oxygen also makes myoglobin less susceptible to oxidation due to oxymyoglobin being more stable than deoxymyoglobin (Mancini and Hunt, 2005). With increasing exposure to oxygen, the depth of oxygen penetration in meat increases and therefore the thickness of the oxymyoglobin layer increases. However, the oxymyoglobin is not prevalent all the way through the meat since a purplish-red state of deoxymyoglobin exists within meat due to the lack of available oxygen in the muscle or the very low oxygen partial pressure typically present in the

center or bottom of the meat, respectively. The layer of oxymyoglobin depends upon several various factors including: temperature, oxygen partial pressure, pH and competition for oxygen by other metabolic processes (Mancini and Hunt, 2005).

The oxidation of both oxymyoglobin and deoxymyoglobin leads to metmyoglobin where the heme iron is in the oxidized ferric (Fe^{3+}) state and the ligand binding site is occupied by water (Suman and Joseph, 2013). Metmyoglobin is known for its brownish color that consumers find unattractive and relate to meat that is no longer fresh (Hood, 1980; Mancini and Hunt, 2005). Metmyoglobin is typically associated with discoloration on the surface, but metmyoglobin is also beneath the surface between the oxymyoglobin (outer layer) and deoxymyoglobin (inner layer). This layer of metmyoglobin is present due to the low levels of oxygen pressure typically found at 6 ± 3 mm Hg (Ledward, 1970). The metmyoglobin layer then continues to increase in thickness with warmer temperatures, diminishing reducing conditions, minimal oxygen partial pressure, and/or elevated microbial growth (Faustman and Cassens, 1990).

The reduction of metmyoglobin is crucial to meat color stability and depends on the muscle's oxygen scavenging enzymes, reducing enzyme system, and the amount of available NADH which depletes in the postmortem muscle (Mancini and Hunt, 2005). The reaction is typically a two-step process where oxymyoglobin is oxidized to metmyoglobin and then reduced back to deoxymyoglobin where it can be oxygenated again. However, this reduction reaction, typically called "MRA" or the metmyoglobin reducing activity, requires NADH to fuel the reaction and once NADH is depleted, the accumulation of metmyoglobin becomes more noticeable and meat discolors (Figure 1).

The reduction of metmyoglobin depends on many factors, but the ability to reduce ferric iron to the ferrous state is vital for meat color stability.

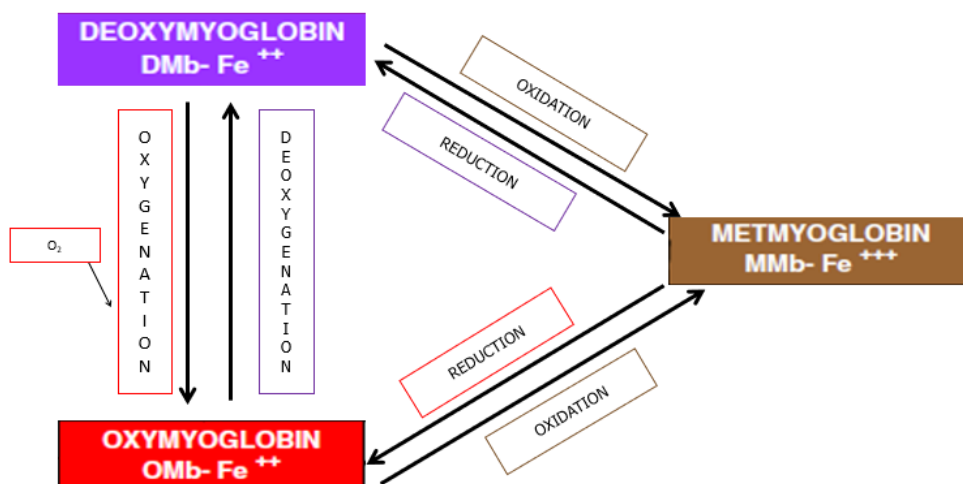


Figure 1. Metmyoglobin reducing activity cycle (adapted from Mancini and Hunt, 2005).

While deoxymyoglobin, oxymyoglobin, and metmyoglobin are the most common myoglobin forms, myoglobin can also interact with other ligands and form many different states (Livingston and Brown, 1981). Carboxymyoglobin is becoming a relevant chemical state of myoglobin due to the increased interest in packaging meat with carbon monoxide. Carboxymyoglobin is formed when carbon monoxide binds to the sixth ligand site producing a bright red color, similar to that of oxymyoglobin. Myoglobin has a greater affinity to carbon monoxide than to oxygen resulting in the increased stability of carboxymyoglobin (Suman and Joseph, 2013). While deoxymyoglobin is easily converted to carboxymyoglobin, carbon monoxide will begin to dissociate from myoglobin after it has been exposed to atmospheres without carbon monoxide (Mancini and Hunt, 2005). Carboxymyoglobin, while more stable than nitric oxide myoglobin, is susceptible to discoloration under retail lighting (Livingston and Brown, 1981).

Nitric oxide myoglobin or nitrosomyoglobin is generated from the reaction of deoxymyoglobin with nitrate/nitrite under anaerobic conditions, typically associated with the curing process. Under anaerobic conditions, this reaction produces a bright deep red color that is relatively unstable since oxygen is able to replace nitric oxide when it dissociates from the heme ring (Livingston and Brown, 1981). When the nitrosl myoglobin is heated it forms nitrosyl hemochrome which produces a pink color found in cured and cooked ham. However, if exposed to light and oxygen for extended periods of time the color can fade and turn gray.

Myoglobin can also interact with different derivatives such as hydrogen peroxide and hydrogen sulfide and produce a green appearance. Choleglobin, one of two myoglobin forms that produce a green appearance, is caused by the reaction of hydrogen peroxide on the heme ring (Seideman et al., 1984). The hydrogen peroxide is produced in one of three ways: from bacteria due to poor sanitary conditions, the interaction of ascorbic acid with oxymyoglobin, or from the muscle itself (Faustman and Cassens, 1990). Sulfmyoglobin is also known to produce a green color in meat. This myoglobin derivate is the effect of hydrogen sulfide and oxygen on myoglobin which can oxidize and produce red metsulfmyoglobin (Faustman and Cassens, 1990).

However, under normal retail display conditions in fresh meat, there is often a mixture of deoxymyoglobin, oxymyoglobin, and metmyoglobin. These three myoglobin states are continuously changing and depend on several intrinsic and extrinsic factors.

Color Measurements

Meat purchasing decisions are influenced by the appearance of the product more than any other quality factor (Hunt et al., 2012). Therefore, it is important to understand the different ways that color is measured in meat. Color has several different properties including hue, lightness, and saturation. Hue is described as the actual color of the object (red, green, blue, yellow, etc.). Hue is determined by the specific wavelengths reflected from a meat surface back to the detector whether that be the human eye or instrument (Hunt et al., 2012). Lightness, when referring to meat, describes the brightness or darkness of the color and the saturation of the color, refers to how vivid or dull it is. However, many different conditions can influence the way color is perceived including: light source (illuminant), observer differences, size differences in cut or object, smoothness of the surface, and background differences (Hunt et al., 2012). Color can be measured in two major ways: visual appraisal or instrumental measurement. Hunt (1980) and Hunt et al. (2012) believe that visual appraisals are the “fundamental standard” of color measurements because of their association with consumer evaluations and for setting the standard for instrumental measurement comparisons.

Color panels can be classified into trained visual color panels or consumer panels, both of which have advantages and disadvantages (Hunt et al., 2012). Strange et al. (1974) stated that the preferred method for measuring consumer acceptability is color evaluation by a panel of trained observers. However, they noted that for continuous evaluation of meat color, taking measurements can be time consuming, prone to subjective errors, and limited in the number of evaluations that can be made at one time (Strange et al., 1974). Setser (1984) had similar concerns in regards to the time and cost

of sensory analysis, adding that it is difficult to do in the time needed. She states that instruments are more likely to be available whenever needed compared to sensory panels (Setser, 1984). Therefore, while visual appraisal may be the preferred method for measuring color, instrumental color measurements have become common practice due to the ease, cost, and time associated with them.

Instrumental color measurements are an objective way to measure color that gives comparable, supporting results to visual color data. The main instruments to measure tristimulus values (CIE $L^*a^*b^*$ and XYZ) are colorimeters and spectrophotometers. The latter are more complex instruments that supply spectral analysis in intervals of 1 to 10 nm that can be used to calculate tristimulus values (Hunt et al., 2012). However, according to the American Meat Science Association Meat Color Measurement Guidelines, to estimate the percentage of surface myoglobin forms, a spectrophotometer must be used (Hunt et al., 2012). When measuring instrumental color, it is important to report different parameters used to measure color such as the type of instrument used, illuminant (light source), aperture size, observation angle, and the number of readings taken. However, according to Tapp et al. (2011), many articles fail to report the necessary information to replicate and interpret instrumental color results. Tapp et al. (2011) found that the majority of studies used a Minolta-branded instrument, preferred D_{65} illuminant and 10° observer, and repeated their measurements in triplicate.

Typically, fresh meat discoloration projects are carried out for three to seven days, with color analysis performed every day or every other day (Faustman and Phillips, 2001). However, time measurements for frozen storage samples vary with longer intervals in between and color analysis being performed less frequently (Faustman and

Phillips, 2001). Swatland (1989) described the sample area measured as approximately 0.5 cm² with reflectance attachments for spectrophotometers compared to several square centimeters for colorimeters with spectrophotometric capabilities.

The main instrument used to measure color seems to be the colorimeter with L*a*b* as one of the most popular color spaces for measuring objective color (Stancil and Jordan, 1985). The Commission Internationale de l'Eclairage (CIE) first developed the tristimulus values XYZ (Hunt et al., 2012). However, the CIE L*a*b* system was later developed since the XYZ colorimetric distances between the individual colors did not correspond to perceived color differences (Hunt et al., 2012). The development of the CIE L*a*b* color space allowed color to be expressed in a three dimensional space that measures a* values on the X axis, b* values on the Y axis, and L* values on the Z axis (Hunt et al., 2012). The L* value is a representation of lightness to darkness where 100 is white and 0 is black. The a* value represents redness to greenness with +60 being red and -60 being green. Finally, b* value correlates to yellowness and blueness with +60 being yellow and -60 being blue on the color scale. While all three values (L*a*b*) are typically reported, a* is most important for meat color since it describes the redness of the meat. Wulf and Wise (1999) stressed the importance of measuring L* values after 33 minutes of bloom compared to 78 minutes for a* and b* values because at these times they found little difference in the values. However, they urge to wait as long as possible if that time cannot be reached to allow the meat to bloom as long as possible (Wulf and Wise, 1999). Kirchofer et al. (2001) found that waiting just 12 min for bloom time reduced the variability in a* color measurements. There may be a large difference in instruments and color parameters used, however, Khliji et al. (2010) determined that in

lamb allowed to bloom for 30-40 mins consumers found acceptable a^* values that were equal to or greater than 9.5 and around 34 for acceptable L^* values. Khliji et al. (2010) used a Hunter Lab Miniscan colorimeter with an aperture size of 25 mm, D_{65} illuminant, and 10° observer to obtain those results.

Besides being able to measure lightness, redness, and yellowness, $L^*a^*b^*$ values also allow different calculations to determine various color measurements. Delta E is used to measure the total change in color space over a selected period of time. Delta E can be calculated as $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ and can be useful for showing the total color differences over time (Hunt et al., 2012). Ledward et al. (1986) concluded that Delta E values over 3.0 were classified as a color difference that was very obvious to most observers. Therefore, a larger Delta E indicates a greater change in color over time. Another common calculation that can be obtained from a^* and b^* values is hue angle. Hue angle is calculated by $HA = [\arctangent(b^*/a^*)]$ (Hunt et al., 2012). Tapp et al. (2011) found hue angle to be the change of color from red to yellow with large angles indicating less red meat and an increase in discoloration over time. The ratio of $a^*: b^*$ is another useful ratio that can be used as an indicator of redness. Setser (1984) and Hunt et al. (2012) indicate that larger ratios of $a^*: b^*$ indicate more redness and less discoloration.

Spectrophotometric instruments have become common practice to use when trying to measure individual myoglobin oxygenation forms. Two different approaches exist to directly measure myoglobin properties: reflectance spectrophotometry and transmission spectrophotometry. Dean and Ball (1960) were among the first to compare reflectance and transmission techniques for quantifying myoglobin forms. They found

that transmission data overestimated metmyoglobin and oxymyoglobin, but underestimated deoxymyoglobin (Dean and Ball, 1960). The method of transmission spectrophotometry is used to measure the oxygenation forms and total myoglobin concentrations in meat extracts (Krzywicki, 1982). However, according to Stewart et al. (1965) to follow the rapid change in fresh meat pigment it is best to measure directly on the sample without extracting the meat pigments. Stewart et al. (1965) also notes that the extraction procedure is cumbersome, possibly detrimental to the sample, and likely to result in changes in the relative proportions of the myoglobin pigments. Strange et al. (1974) notes that reflectance measurement is the preferred technique because it measures the color on the surface of the meat that the consumer is going to observe.

Reflectance spectrophotometry measurements involve mathematical manipulation of data from selected wavelengths (Hunt et al., 2012). The reflectance curves for deoxymyoglobin, oxymyoglobin, and metmyoglobin cross at various wavelengths, known as the isobestic wavelengths. The isobestic points are used to quantify myoglobin pigment forms (Hunt, 1980). The isobestic point of 525 nm is especially important because all three pigments cross at this point making it a denominator of many color calculations because of the relationship to total myoglobin concentration (Hunt, 1980). Hunt (1980) notes that other wavelengths are often selected because they are absorption maxima or minima for one of the myoglobin forms.

Two reflectance methods are typically used to measure the myoglobin redox forms. The first method is the K/S ratios method where K stands for the absorbance coefficient and S stands for the scattering coefficient. The K/S ratios method measures the surface reflectance at four isobestic wavelengths (474, 525, 572, and 610 nm) which

is then used to calculate the K/S ratios that relate with the pigment proportions (Hernández et al., 2015). For the K/S ratios method, K/S ratios from individual samples are compared to the pure K/S ratios for deoxymyoglobin, oxymyoglobin, and metmyoglobin that serve as reference values (Hernández et al., 2015). While determining K/S ratios can be more challenging, Stewart et al. (1965) stated that it was the most common method used to study the changes in meat pigments.

The other main method to measure reflectance spectrophotometry is the method presented by Krzywicki (1979). This method uses selected wavelengths and a correction factor to calculate the percentage of deoxymyoglobin and metmyoglobin which is then combined and subtracted from 100% to determine the amount of oxymyoglobin (Hunt et al., 2012). According to Hunt et al. (2012), this method is based on the concept of reflex attenuation (A) which is the logarithm of the reciprocal of reflectance. The reflectance values at the isobestic points 473, 525, 572, and 730 nm are then used to calculate the content of deoxymyoglobin, oxymyoglobin, and metmyoglobin on the surface of meat (Krzywicki, 1979; Hernández et al., 2015). The equation can be found below for calculating the different myoglobin forms (Figure 2). While this method is easier with the use of a reflectance spectrophotometer, Hernández et al. (2015) noted that this method provides pigment proportions that were not consistent with the expected values since the results conflicted with what was expected during the oxygenation/oxidation processes. Plus, they reported that several of the values obtained for oxymyoglobin appear negative when measuring a synthetic myoglobin mixture (Hernández et al., 2015). However, they also note that either method can be used in practice as an indicator of the deterioration of meat color (Hernández et al., 2015).

$$\text{Equation 1: } A = \log \frac{1}{R}$$

$$\text{Equation 2: } \%MMB = \left\{ 1.395 - \left[\frac{(A_{572} - A_{730})}{(A_{525} - A_{730})} \right] \right\} \times 100$$

$$\text{Equation 3: } \%DMB = \left\{ 2.375 \times \left[1 - \frac{(A_{473} - A_{730})}{A_{525} - A_{730}} \right] \right\} \times 100$$

$$\text{Equation 4: } \%OMB = 100 - (\%MMB + \%DMB)$$

Figure 2. Myoglobin Forms via selected wavelength calculation (Krzywicki, 1979; Hunt et al., 2012).

When measuring the surface of meat with reflectance spectrophotometry, it is important that the meat be sufficiently thick so that the reflectance value is not altered (Stewart et al., 1965b). Snyder (1965) stressed that the percent reflectance depends greatly on the concentration of pigment, the amount of intramuscular fat, the amount of moisture on the surface, and the oxidized state of the meat pigment. Regardless, Mohan et al. (2010) were able to find a high correlation between reflectance spectrophotometric data for myoglobin redox forms and colorimetric values. However, Morrissey et al. (2008) found a poor relationship between consumer scores and the ratio of oxymyoglobin to metmyoglobin.

Another value of reflectance spectrophotometry is the ability to calculate the ratio of 630/580 nm. The ratio of 630/580 nm was established to calculate the change in meat color due to the formation of metmyoglobin; therefore, it can be used to evaluate the surface color stability (Hunt, 1980; Joseph et al., 2012; Li et al., 2017). Larger ratios indicate more redness due to oxymyoglobin and therefore, a ratio of 1.0 would essentially be 100% metmyoglobin (Strange et al., 1974; Hunt et al., 2012; Joseph et al., 2012; Li et

al., 2017). Gatellier et al. (2005) found a high correlation between beef redness when comparing a^* values and the ratio of 630/580. However, Khlijji et al. (2010) found that a^* values were more strongly related to consumer perception than the 630/580 ratio. The exact ratio where consumers find the product to be unacceptable is not well established with previous research stating different thresholds for consumers. Morrissey et al. (2008) found that when the ratio fell below 3.5, consumers thought the color was more brown than red and unacceptable, but Khlijji et al. (2010) found the threshold to be 3.3 or lower and Jacob et al. (2007) found it to be below 3.0.

Another instrumental measurement that has been gaining popularity in recent years is the use of computer vision systems to measure color. Lu et al. (2000) stated that computer vision systems can provide consistent, efficient, and cost effective inspection. O'Sullivan et al. (2003) found that a digital camera derived a^* values and b^* values with greater correlation to the sensory terms than a Minolta colorimeter. The instrumental measurements taken with the digital camera were more highly correlated with both the trained and untrained sensory panels compared to the colorimeter which was attributed to the camera taking measure measurements over the entire surface representing a more uniform sample compared to the colorimeter (O'Sullivan et al., 2003). With the development of new technology, it is expected that new and improved ways to accurately measure meat color will be developed.

Intrinsic Factors

pH

Meat color is significantly affected by the ultimate pH of muscle and the rate of pH decline postmortem. Muscle pigment is highly influenced by various pH conditions.

During the conversion of muscle to meat, lactic acid begins to accumulate from anaerobic glycolysis, lowering the physiological pH from approximately 7.4 to an ultimate pH of 5.4-5.8 (Faustman and Cassens, 1990). Muscle pH has been continuously shown to be associated with meat color (Faustman and Cassens, 1990; Page et al., 2001; Neethling et al., 2017). Page et al. (2001) found that L^* , a^* , and b^* were negatively correlated to muscle pH, illustrating that as muscle pH increased, muscle color values decreased.

When muscle pH drops below the normal postmortem pH, it denatures the globin protein of the heme pocket, which can lead oxygen to disassociate from the heme molecule causing oxidation of the heme iron. Hence, a low pH favors myoglobin oxidation and a decrease in color stability (Ledward, 1985; Faustman and Cassens, 1990). Ledward et al. (1986) found that even beef with a pH of 5.8 was more color stable than beef with a pH of 5.6. Gutzke and Trout (2002) also found that the autoxidation rate of myoglobin was very pH dependent and decreased by approximately 50% for each 0.5 unit increase in pH. Also, lower pH can lead to a decrease in metmyoglobin reducing activity (Stewart et al., 1965a; Ledward, 1971).

Two extreme conditions associated with meat color and pH include pale, soft, and exudative (PSE), a condition typically found in pork, and dark, firm, and dry (DFD). Pale, soft, and exudative is typically caused by acute, short term stress prior to slaughter causing a rapid pH decline postmortem while carcass temperature is still relatively high. The low pH from the lactic acid build up causes protein denaturation, which reduces the water binding capacity of the meat. This not only impacts the texture of the meat, but the free water migrates to the surface of the meat and reflects the light, causing a paler color. Dark, firm, and dry is caused by a high ultimate pH in both beef and pork. The cause of

DFD is associated with long term stress that causes a depletion of glycogen. The lack of glycogen available for anaerobic glycolysis leads to a lack of lactic acid buildup and in turn a high ultimate pH. With the higher pH, muscles bind more strongly to water, preventing it from reflecting light and giving it a darker appearance. At higher pH water is more strongly bound due to less protein-protein interaction allowing for proteins to repel and for the binding of water to protein charges (Puolanne and Halonen, 2010). However, as the pH decreases or becomes closer to the isoelectric point, proteins interact with each other and become closer together, causing a shift of intracellular water into interfibrillar spaces (Neethling et al., 2017). When water is tightly bound in the cell the light is absorbed by the meat compared to meat with a lower pH that has more free water on the surface that easily reflects light making the meat appear lighter (Neethling et al., 2017).

Muscles at a higher pH can cause a decrease in the formation of oxymyoglobin due to an increase and prolonged activity of mitochondrial activity and increased oxygen consumption (Bendall and Taylor, 1972). According to Page et al. (2001), 91.7% of cattle sampled with a pH of 5.87 or greater fell into the dark cutter (high pH beef associated with DFD) category while only 0.6% of cattle with a pH below 5.87 were considered dark cutters, signifying the relationship between pH and dark cutters.

Species/Sex/Breed

The color of fresh meat is species dependent (Livingston and Brown, 1981; Faustman and Cassens, 1990; Mancini and Hunt, 2005). Pork typically is lighter appearing, with a greyish-pink color, whereas fresh beef and lamb are darker in color and a bright red cherry color is deemed desirable by consumers. The difference in species

color is largely attributed to the difference in myoglobin content (Suman and Joseph, 2013). Porcine muscle myoglobin has been shown to oxidize more slowly compared to beef and sheep which can most likely be attributed to pork having a lower amount of myoglobin and therefore a lower concentration of iron helping to delay oxidation (Gutzke and Trout, 2002). However, Suman et al. (2007) found that pork was slower to oxidize compared to beef due to a reduced number of histidine residues in myoglobin compared to pork leading to greater color stability. Yin et al. (2011) found no difference in myoglobin autoxidation among beef, sheep, and swine, but found that poultry had greater autoxidation than the other species. Bendall and Taylor (1972) also found that the oxygen consumption rate for species differed with beef having a higher oxygen consumption rate (OCR) than sheep. They attributed this difference in OCR to the difference in mitochondrial density and enzyme activity between species since pH, which also influences OCR, was the same for both species.

Sex also has an impact on the overall color and color stability of fresh meat. Page et al. (2001) found that steer carcasses had higher L*, a*, and b* values compared to heifers, regardless of pH. Seidemani et al. (1984) found that meat from intact males was darker than females and castrated males, most likely due to a higher myoglobin concentration which they believe was related to higher activity levels of bulls.

Beef color can also be affected by different breeds. Page et al. (2001) found that dairy type carcasses had lower L*, a*, and b* values and a higher pH than native (*Bos taurus*) or Brahman type cattle. Lanari and Cassens (1991) also found a difference in the color stability of Holstein and crossbred steers. They found that muscles from Holstein steers had greater oxygen consumption than crossbreed steers, attributing the variation to

the mitochondrial respiration activities among differing breeds. Mitochondrial respiration can be associated with the amount of oxygen the muscle requires based on the mitochondria activity. The difference in mitochondrial respiration activity among different breeds can most likely be attributed to the different muscle fiber types in muscle. Most likely the Holstein steers had more type I fibers than crossbreed steers causing a greater amount of oxygen required for the mitochondria and therefore greater oxygen consumption. However, based on the findings of Wegner et al. (2000), who compared German Angus, Galloway, Holstein, and Belgian Blue cattle, the only difference in muscle fiber total number and muscle fiber frequency was with the Belgian Blue cattle.

Animal Maturity

Research indicates that animal maturity affects heme iron concentration (Romans et al., 1965; Field et al., 1980). Several researchers demonstrated that with increased age, myoglobin and hemoglobin content tends to increase (Romans et al., 1965; Field et al., 1980; Kim et al., 2012). Field et al. (1980) reported that myoglobin and iron content of muscles from steers and cows was two to three times greater than the concentration of myoglobin pigment in veal. Typically, older animals have darker (lower L* values) and redder (higher a* values) meat. This can be attributed to greater myoglobin concentration in older animals appearing darker and redder. Kim et al. (2012) found that lambs harvested at three to four months of age were lighter in color and had lower a* values than the ten to eleven months of age lambs at the initial display time due to the difference in myoglobin content.

Muscle to Muscle Variation

Muscle to muscle variation has a large impact on meat color stability; therefore, a large amount of research has been conducted to investigate the cause of muscle variability effects on color and color stability (Hunt and Hedrick, 1977a; Hunt and Hedrick, 1977b; O’Keeffe and Hood, 1982; McKenna et al., 2005; Joseph et al., 2012). According to Hood (1980), 45.5% of the variation in discoloration was attributed to muscle variability. This variation among muscles having different color stability has been attributed to the differences in relative proportions of fiber types and the oxidative and reducing capacities of the muscle (Hunt and Hedrick, 1977a; Bekhit and Faustman, 2005). Type I (red) fibers are known for high myoglobin content, slow-twitch, oxidative fibers, with numerous mitochondria compared to type IIb (white) fibers that contain low myoglobin content, fast-twitch, glycolytic fibers, with low amounts of mitochondria. An intermediate (type IIa) also exists as a fast-twitch, oxidative and glycolytic fiber. The fiber type influences fresh meat color and attributes to the muscle to muscle variation. Muscles with a higher portion of type I fibers are a darker, deep red color compared to type IIb fibers due to the higher myoglobin content (Hunt and Hedrick, 1977a). The type of muscle fiber also impacts the oxygen consumption and blooming capabilities of the muscle. Type I fibers typically have a higher oxygen consumption rate (OCR) due to the increasing need for oxygen for the mitochondria compared to type IIb fibers that have a low OCR. Many researchers have characterized muscles as color stable and color liable based on the myoglobin content, fiber type, OCR, and MRA, however results continue to differ in causes of discoloration (Ledward, 1971; MacDougall and Taylor, 1975; Hunt

and Hedrick, 1977a; Hunt and Hedrick, 1977b; Hood, 1980; O’Keeffe and Hood, 1982; Claus et al., 2005; McKenna et al., 2005).

Research has consistently shown that the longissimus dorsi is a color stable muscle due to the low OCR and high MRA compared to the psoas major with a high OCR and low MRA. The difference in OCR and MRA can be attributed to the fact that the longissimus dorsi has higher proportion of type IIa fibers than type I fibers, opposite of the psoas major (Hunt and Hedrick, 1977a; O’Keeffe and Hood, 1982; McKenna et al., 2005; Neethling et al., 2017). O’Keeffe and Hood (1982) found that the psoas major had poor color stability and quickly discolored which was characterized by rapid oxygen consumption, susceptibility to oxidation, and a strong tendency to form metmyoglobin during the reduction reaction due to a low MRA, anaerobic reducing ability (ARA), and high OCR. However, the opposite was true for the longissimus dorsi which had good color stability and depth of oxygen penetration due to the low OCR that allows oxygen to bind to myoglobin and penetrate farther down into the muscle. Joseph et al. (2012) found similar results to O’Keeffe and Hood (1982) in that the longissimus had greater MRA than the psoas major throughout retail display; therefore, the longissimus had greater a^* values, greater 630/580 ratio for redness, and less lipid oxidation reiterating its color stability. Most research focuses on a^* values as it pertains to color, which supports McKenna et al. (2005) findings that L^* plays a minimal role in predicting color stability since muscles of high color stability were on opposite ends of the L^* spectrum.

Research has shown that both MRA and OCR vary within beef muscles. Some argue that MRA is the principal factor that influences muscle color stability (Ledward,

1971) while others argue that the reducing capability plays a limited role compared to OCR on muscle color stability (O’Keeffe and Hood, 1982; Renerre and Labas, 1987).

Oxygen Consumption Rate

The oxygen consumption rate (OCR) significantly impacts meat color and color stability. In meat, the oxidative capacity is generally measured as the oxygen consumption or OCR due to muscles with a high OCR more likely to oxidize (Neethling et al., 2017). The OCR is strongly related to the depth of oxygen penetration and varies among muscles (Bendall and Taylor, 1972; O’Keeffe and Hood, 1982; Renerre and Labas, 1987; Lanari and Cassens, 1991; McKenna et al., 2005). The OCR is determined by the mitochondrial respiration activity within the meat. Mitochondrial activity thrives in fresh meat at a high pH and temperature (Bendall and Taylor, 1972). In ideal conditions and with greater amounts of mitochondria, typically found in type I fibers, greater amounts of oxygen are required for ATP production. Therefore, the mitochondria are competing with the myoglobin for the available oxygen. When mitochondria outcompete the myoglobin, it is considered a high oxygen consumption rate for that muscle, and a decrease in oxygen penetration is noted since oxygen is not available to penetrate into the meat (Bendall and Taylor, 1972; O’Keeffe and Hood, 1982; Sammel et al., 2002). This decrease in oxygen penetration decreases the initial red color development and intensity of fresh meat (Mancini and Ramanathan, 2014).

With high OCR typically found in muscles such as the psoas major, color stability is poor (O’Keeffe and Hood, 1982; McKenna et al., 2005; Ramanathan and Mancini, 2018). Oxygen is more likely to be consumed by the mitochondria than binding to myoglobin. This leaves myoglobin in a less stable state and creates oxidative conditions

that favor the formation of metmyoglobin (O’Keeffe and Hood, 1982; Ledward, 1985; McKenna et al., 2005; King et al., 2011). Madhavi and Carpenter (1993) also attributed a high oxygen consumption rate with an increase in metmyoglobin. Since OCR limits the depth of oxygen penetration into the muscle, this creates ideal oxygen partial pressure for the development of a thin layer of metmyoglobin closer to the surface than a muscle with low OCR. The initial layer of metmyoglobin closer to the surface causes more rapid deterioration than a muscle with deep oxygen penetration typically seen in the longissimus (O’Keeffe and Hood, 1982).

Researchers have found that as meat is aged or stored, a decrease in OCR is observed (Bendall and Taylor, 1972; O’Keeffe and Hood, 1982; McKenna et al., 2005). Bendall and Taylor (1972) attribute this decrease due to the depletion of substrates and the degradation of enzymes involved with mitochondrial function. Ledward (1985) suggested that oxygen consumption has a large role on metmyoglobin formation initially, but as OCR decreases, the reducing activity becomes the predominant factor in color stability. However, poor color stability muscles with a high OCR typically have a lower MRA aiding in the low color stability (O’Keeffe and Hood, 1982; McKenna et al., 2005). Sammel et al. (2002) suggested that both very high and very low OCR have a negative impact on color stability. Speculating that a low OCR would result in low mitochondrial generation of NADH, thus limiting the reducing capacity compared to a high OCR. A high OCR would greatly reduce the partial oxygen pressure resulting in a thin oxymyoglobin layer and greater expression of the layer of metmyoglobin below the surface. Overall, the role of OCR on color stability and the importance of OCR compared to metmyoglobin reducing activity in discoloration still remains a topic of discussion

(Ledward, 1971; Bendall and Taylor, 1972; O’Keeffe and Hood, 1982; Lanari and Cassens, 1991; McKenna et al., 2005; Joseph et al., 2012).

Metmyoglobin Reducing Activity

Discoloration is the accumulation of metmyoglobin on the surface of meat. It has been estimated that meat departments lose 5.4% of sales each year due to fresh meat discoloration (Bekhit and Faustman, 2005). This accumulation of metmyoglobin comes from the loss of MRA. Metmyoglobin is typically not found in living systems because of an inherent metmyoglobin reducing system (Giddings and Solberg, 1977). However, in meat, Madhavi and Carpenter (1993) describe MRA as the inherent ability to reduce metmyoglobin back to the ferrous, deoxymyoglobin form, which can then be oxygenated to oxymyoglobin, sustaining the color stability. Scientists understand the importance of MRA in color stability, but its significance on color stability, how it changes over storage, how it should be measured, and the exact mechanisms that support MRA are not completely defined (Reddy and Carpenter, 1991; Bekhit et al., 2001; Bekhit and Faustman, 2005; McKenna et al., 2005; Ramanathan and Mancini, 2018; Mitacek et al., 2019). Some researchers argue that MRA within the muscle is the main factor in prolonging meat color (Ledward, 1971; Ledward, 1985). Therefore, muscles with higher reducing capacities would be more color stable (Reddy and Carpenter, 1991; Neethling et al., 2017). Others have reported that reducing ability has very limited importance in color stability compared to oxygen consumption (O’Keeffe and Hood, 1982; Renner and Labas, 1987; Bekhit et al., 2001). Bekhit et al. (2001) found that a large reduction in metmyoglobin reducing activity did not translate to a reduction in color stability. They believed that the lack of a relationship suggests that metmyoglobin reducing activity is

not the main factor in maintaining color stability. Yet, research has shown that both reducing activity and oxygen consumption have an influence on muscle color stability (Madhavi and Carpenter, 1993). The conflicting results between studies regarding the importance of MRA to meat color stability, could be partly attributed to the lack of uniformity in measuring MRA.

The techniques used to measure the reduction of metmyoglobin have continued to be a topic of research in color stability. Bekhit and Faustman (2005) compared different research techniques for measuring MRA. Several investigators have been criticized for using the ferricyanide method in determining MRA where ferricyanide serves as an oxidant since it facilitates electron transfer with myoglobin (Reddy and Carpenter, 1991; Bekhit and Faustman, 2005). Renerre and Labas (1987) and Lanari and Cassens (1991) have used methylene blue in the metmyoglobin reduction. However, Sammel et al. (2002) criticized that the assay may not actually represent the reduction of the muscle. Ledward (1972) developed an aerobic reducing ability method that involved the oxidation of meat and monitoring the pigment reduction. Sammel et al. (2002) found that ARA correlated best with color stability over display. Sammel et al. (2002) also supported the method of ARA because of the use of no chemicals as oxidizing agents in the reducing system, minimizing any altered enzymatic reduction (Faustman and Cassens, 1990; Sammel et al., 2002). O'Keeffe and Hood (1982) reported that the resistance to oxidation appeared to be a better indicator of color stability than percentage reductions since those were based on the initial level of metmyoglobin induced on the surface of samples. Measurement of metmyoglobin reduction without the use of artificial

indicators show a strong relationship between color stability and MRA (Reddy and Carpenter, 1991; Madhavi and Carpenter, 1993; Sammel et al., 2002).

The effect different measuring techniques have on MRA results can also be noted by the varying results that storage has on MRA. Lanari and Cassens (1991) reported that there was no significant change in MRA over seven days of storage in beef muscle. Bekhit et al. (2001) actually found an increase in MRA during storage when sampling in lamb muscle. However, most researchers observed a decrease in MRA with storage (O'Keeffe and Hood, 1982; Madhavi and Carpenter, 1993; Joseph et al., 2012; Mitacek et al., 2019). However, if MRA was consistent, then discoloration would be very difficult to explain with OCR decreases over time. Logically, it seems that the reducing ability of the muscle must decrease at a rate greater than OCR, otherwise, it would be difficult for metmyoglobin to accumulate and cause discoloration (McKenna et al., 2005). Research has also shown that both pH and temperature affect MRA (Stewart et al., 1965a; Bekhit et al., 2001; Bekhit and Faustman, 2005). Bekhit et al. (2001) noted that as pH increased the reducing activity also increased. Stewart et al. (1965) and Bekhit et al. (2001) found that MRA is lower at temperatures below 15°C and no reduction occurs at temperatures above 37°C, with reducing activity being destroyed by cooking at 70°C. However, it should be noted that increasing temperatures, while it increases enzymatic reduction, also accelerate the myoglobin oxidation rate.

Metmyoglobin reduction is thought to occur in two main ways: enzymatic and non-enzymatic pathways (Bekhit and Faustman, 2005; Mitacek et al., 2019). Regardless of what pathway, metmyoglobin reduction is dependent on nicotinamide adenine dinucleotide + hydrogen (NADH) (Giddings and Solberg, 1977; Livingston and Brown,

1981; Renerre and Labas, 1987; Tang et al., 2005; Kim et al., 2006). While NADH can be generated in live muscle, the lack of available substrates, such as NADH, is believed to limit the reducing capacity of meat rather than reduction of specific enzyme activity (Andrews et al., 1952; Bekhit et al., 2003; McKenna et al., 2005; Tang et al., 2005; King et al., 2011).

In the enzymatic pathway, an enzyme such as NADH-cytochrome b5 reductase (metmyoglobin reductase) is used to transfer an electron from NADH to metmyoglobin via a mediator. However, the role of NADH is both as a coenzyme and an electron carrier in the conversion of metmyoglobin to deoxymyoglobin (Renerre, 1999). Livingston et al. (1985) classified the enzyme as NADH-cytochrome b5 reductase based on the kinetic mechanism of the enzyme reduction. Bekhit et al. (2001) found that the enzyme was NADH-dependent for the reduction to occur. While it is generally accepted that metmyoglobin reduction in meat is primarily through an enzymatic pathway, Reddy and Carpenter (1991) reported no non-enzymatic reduction. Brown and Snyder (1969) were able to demonstrate the reduction through a non-enzymatic pathway. In the non-enzymatic pathway, an electron from NADH is carried by an artificial electron carrier to metmyoglobin (Mitacek et al., 2019). Brown and Snyder (1969) found that NADH or NADPH could reduce metmyoglobin if ethylenediaminetetraacetate (EDTA) was present. It was also determined that adding ATP, ADP, or AMP had no effect on the system, but if flavins were present with EDTA, they could catalyze the reduction of metmyoglobin. Overall, Brown and Snyder (1969) found that the addition of more NADH caused more oxymyoglobin to form and believed that the non-enzymatic system was more efficient at reducing metmyoglobin than the enzymatic system.

Lipid Oxidation

Lipid oxidation has a large role in limiting the quality and acceptability of meats (Morrissey et al., 1998). After slaughter, cells become damaged, promoting the oxidative process which can alter the overall quality of lipids, proteins, carbohydrates, and vitamins (Kanner, 1994). Quality deterioration can be noticed in flavor, color, texture, and nutritive value (Kanner, 1994). However, in fresh meat, the largest factors impacted by lipid oxidation are color and flavor (Faustman et al., 2010).

Lipid oxidation typically is explained as a three-step process: initiation, propagation, and termination (Kanner, 1994; Morrissey et al., 1998; Chaijan, 2008). During the initiation step, hydrogen is removed from a methylene carbon on a fatty acid chain (Morrissey et al., 1998). Kanner (1994) describes superoxide anion radicals, perhydroxyl radicals, hydrogen peroxides, and hydroxyl radicals as initiators of lipid oxidation that can cause the generation of free radicals that are very unstable. Following initiation, the free radical reacts rapidly with oxygen to form peroxy radicals which will oxidize other fatty acids and propagate the chain reaction (Morrissey et al., 1998). Finally, during the termination step, radicals react with one other resulting in non-radical products (Morrissey et al., 1998). Figure 3 displays the three phases of lipid oxidation (Chaijan, 2008). The primary oxidation products such as peroxy radicals can decompose into various secondary products including: aldehydes, ketones, alcohols, hydrocarbons, and other secondary products (Erickson, 2008). Secondary oxidation products are typically measured as an indicator of lipid oxidation, since secondary products of oxidation are generally odor-active, whereas primary oxidation products are colorless and flavorless (Erickson, 2008).

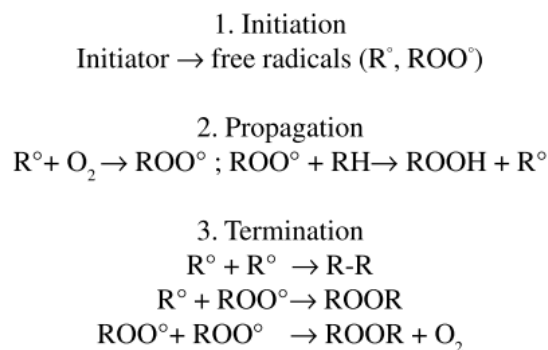


Figure 3. Steps and reactions involved in lipid oxidation (Chaijan, 2008).

In meat, lipid oxidation is often measured by thiobarbituric acid-reactive substances, TBARS, which measures the secondary oxidation product, malonaldehyde. Lipid oxidation is one of the main causes of quality loss in meat during storage and processing leading to rancidity and discoloration (Renner, 1999). Campo et al. (2006) showed that consumers were able to detect a rancid flavor that overpowered the beef flavor in beef steaks at 2.28 mg malonaldehyde/ kg tissue using the TBARS method. However, Greene and Cumuze (1981) determined this threshold to be even smaller with consumers being able to detect a rancid flavor in beef with TBARS values between 0.6 and 2.0 mg malonaldehyde/kg tissue. These rancid off-flavors and off-odors in meat are caused by oxidized lipids present in both raw and cooked meat (Greene, 1969; Kanner, 1994; Jakobsen and Bertelsen, 2000; Faustman et al., 2010).

Lipid oxidation can have negative impacts on color, flavor, texture, and nutritional value of meat (Kanner, 1994). The correlation between lipid oxidation and myoglobin oxidation has been well documented (Greene, 1969; Wanous et al., 1989; Akamittath et al., 1990; Faustman and Cassens, 1990; Faustman et al., 2010; Bonny et al., 2017). Lipid oxidation produces highly reactive products such as α , β -unsaturated

aldehydes that form covalent adducts with myoglobin and accelerate discoloration (Faustman et al., 1999; Faustman et al., 2010; Cooper et al., 2017; Neethling et al., 2017). McKenna et al. (2005) found that in 19 beef muscles, those with greatest color stability were also those that had the lowest lipid oxidation, reinforcing the relationship between lipid oxidation and color stability. However, Faustman et al. (2010) also noted that myoglobin can initiate lipid oxidation when oxygen is released from oxymyoglobin resulting in metmyoglobin and superoxide anion radicals. The diagram presented in Figure 4 was proposed by Faustman et al. (2010) to explain the interaction occurring between myoglobin and lipid oxidation.

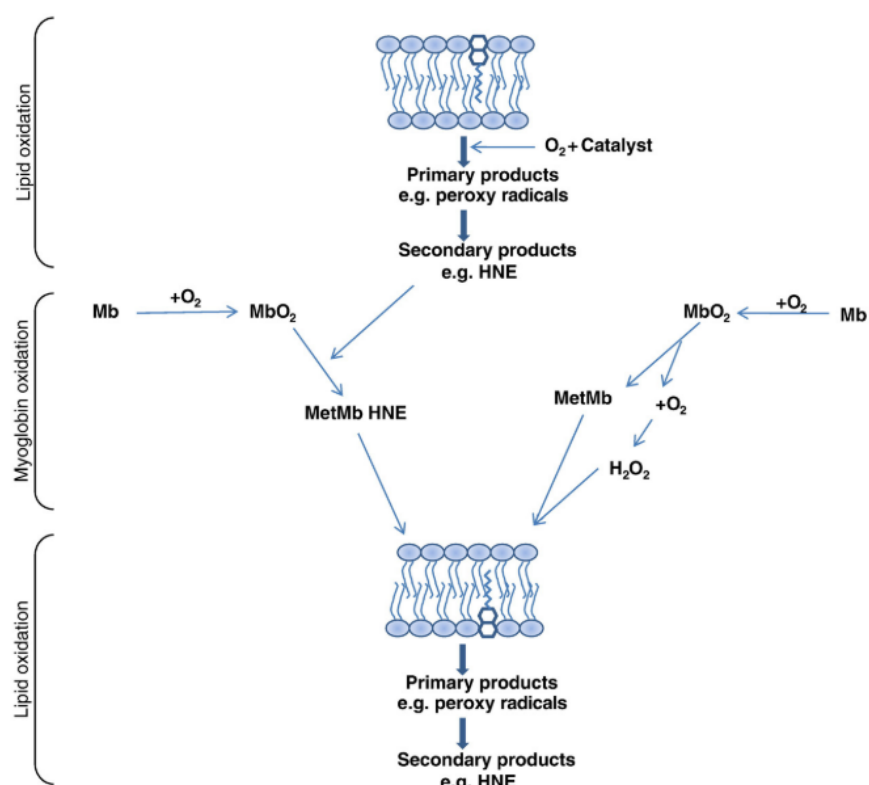


Figure 4. Interaction between lipid and myoglobin oxidation (Faustman et al., 2010).

Lipid oxidation can be impacted by species, anatomical location, diet, temperature, sex, age, phospholipid content, light, oxygen concentration, presence of anti- and pro-oxidants, presence of enzymes, composition and freshness of raw meat components, cooking or heating, processing steps, and added compounds (Kanner, 1994; Chaijan, 2008). In fresh meat, lipid oxidation research mainly focuses on different environmental impacts on lipid oxidation and other factors such as discoloration and flavor of the meat. Suman et al. (2007) showed that lipid oxidation and myoglobin stability is species dependent, with beef being more susceptible than pork to oxidize due to the location of the histidines that influence the susceptibility of myoglobin oxidation. Jakobsen and Bertelsen (2000) found that during storage an increase in temperature from 5 to 8 C in beef caused an increase in TBARS. Gutzke and Trout (2002) found similar results in beef and sheep myoglobin extracts noticing an increase in oxidation with a temperature increase. Postmortem aging and retail display time have also been documented to increase lipid oxidation (Faustman and Cassens, 1990; Jakobsen and Bertelsen, 2000; Joseph et al., 2012; Bonny et al., 2017). Faustman and Cassens (1990) contribute the increase in lipid oxidation from postmortem aging to a decrease in the endogenous antioxidant activity that aids in limiting oxidation. The addition of salt is also a known enhancer of lipid oxidation (Akamittath et al., 1990). Erickson (2008) found that unsaturated fatty acids and oxygen concentration are associated with an increase in lipid oxidation and the creation of secondary oxidation products that give an unpleasant flavor and aroma.

Most factors associated with impacting lipid oxidation have detrimental effects on meat quality, however, several aspects have been evaluated to have beneficial impacts.

Vitamin E has been proven to function as a strong and effective antioxidant in beef, limiting lipid oxidation and increasing color stability (Faustman et al., 1989; Arnold et al., 1992; Liu et al., 1995; Lynch et al., 1999). Antioxidants have also been proven to be effective in limiting lipid oxidation. Kanner (1994) discussed the importance that ascorbic acid, carotenoids, and carnosine play as antioxidants and limiting lipid oxidation. Nitrite, used in the curing process, has also been shown to act as an antioxidant due to its ability to be bound to ferrous iron and to work as an electron donor and scavenger of free radicals (Kanner, 1994). Faustman et al. (2010) document the increase in plant extracts as an antioxidant in meat produces in recent years.

Different packaging methods have had mixed results in regards to lipid oxidation (Greene, 1969; Bonny et al., 2017). Kim et al. (2010) examined meat in a high oxygen environment and found an increase in oxidation. Greene (1969) found that meat packaged anaerobically was effective at preventing metmyoglobin and rancidity associated with lipid oxidation, if sufficient MRA was present in the meat. She also noted that with the use of antioxidants, the value of anaerobic packaging was reduced.

Extrinsic Factors

Diet

Research has shown that diet can have both a negative and positive impact on beef color stability. Previous research has mixed results when comparing pasture-fed and grain-fed color in regards to a^* and b^* values. French et al. (2001) found no difference in any of the instrumental color values when comparing grass and concentrate diets.

Vestergaard et al. (2000) and Bruce et al. (2004) found that cattle-fed grain diets

compared to forage based diets had higher a^* and b^* values. Research has continuously shown that pasture-fed cattle have had lower L^* values representing a darker color lean compared to concentrate-fed cattle (McCaughey and Cliplef, 1996; Bruce et al., 2004; Realini et al., 2004). While the contribution of lower L^* values overall comes from a higher postmortem pH, the exact reasoning is still not understood. Vestergaard et al. (2000) believe that one reason for this difference could be that a forage-based restricted diet leads to lower glycogen storage and promotes oxidative metabolism compared to the ad libitum concentrate diet. Daly et al. (1999) also suggest that cattle on a forage diet may experience greater stress prior to harvest compared to cattle in a feedlot setting. Seidemani et al. (1984) believe that cattle on concentrate diets are less active and have less myoglobin in the muscle due to less locomotion contributing to a lighter color and lower L^* values.

Previous research has shown that cattle fed diets containing distiller grains at an inclusion rate at or above 30% can negatively affect beef color stability in retail display (Mello Jr et al., 2012; Domenech-Pérez et al., 2017; Ribeiro et al., 2018; Hart et al., 2019). Distiller grains alter the fatty acid profile by increasing the polyunsaturated fatty acids and linoleic acid content, resulting in increased lipid oxidation and decreased color stability. However, research regarding the inclusion of distiller grains has not been consistent, with some studies finding no difference in lipid oxidation and color stability (Gill et al., 2008; Domenech-Perez et al, 2017).

Conversely, it has been well documented that supplementing cattle with vitamin E in the diet aids in delaying the onset of discoloration in fresh and frozen beef (Faustman et al., 1989; Arnold et al., 1992; Lanari et al., 1993; Liu et al., 1995; Lynch et al., 1999).

Vitamin E delays discoloration by protecting the unsaturated fatty acid membrane, preventing early lipid oxidation that initiates in the cell membrane. In turn, this delay in lipid oxidation helps prevent myoglobin oxidation and discoloration.

Electrical Stimulation

Electrical stimulation is also associated with meat color due to the impact on meat pH. Electrical stimulation improves the appearance of fresh meat by producing a lighter, brighter-red color on meat surfaces (Sleper et al., 1983). Faustman and Cassens (1990) attribute this difference to the accelerated rate of postmortem pH decline seen in stimulated versus non-stimulated carcasses. Water binding myofibrillar proteins denature due to low pH and high temperature conditions, leading to less water binding. The free water increases the reflectance of light and results in a lighter appearance.

Sleper et al. (1983) found that electrically stimulated carcasses had brighter red visual color scores through day 5 of retail display and had no difference in metmyoglobin or oxymyoglobin throughout retail display when comparing electrically stimulated and non-stimulated carcasses. However, Ledward et al. (1986) found an increase in metmyoglobin formation in electrically stimulated carcasses compared to those that weren't. This contribution could be related to more structural damage to cells in electrically stimulated carcasses (Savell et al., 1978). The tissue disruption may result in a looser muscle structure that allows deeper oxygen penetration and result in a thicker oxymyoglobin layer and deeper metmyoglobin layer causing more light to scatter and consequently a lighter muscle appearance.

Postmortem Aging

Typically, beef is wet or dry aged to increase flavor and/or improve tenderness, but with that comes a decrease in weight loss, decreases in shelf life, increase in oxidation, and decrease in color stability (Epley, 1992). The main impact that postmortem aging has on meat color is that aged beef has a higher blooming capacity than non-aged beef and aged beef discolours at a faster rate than non-aged beef (Hood, 1980; MacDougall, 1982; Mancini and Ramanathan, 2014; Vitale et al., 2014; English et al., 2016). The high blooming capacity of aged meat can be attributed to the diminished mitochondrial activity and reduced oxygen consumption with longer storage times. This increase in storage time improves the initial color intensity by minimizing the competition for oxygen between myoglobin and mitochondria allowing the steaks to have a higher blooming capacity (MacDougall, 1982; Bekhit and Faustman, 2005). On the other hand, Hood (1980) showed that very fresh meat (up to three days post slaughter) forms metmyoglobin more rapidly than aged beef in early retail display. O'Keeffe and Hood (1982) argue that the high rate of oxygen consumption in fresh beef favors the deoxymyoglobin form of myoglobin compared to oxymyoglobin. This causes an increase in metmyoglobin due to the oxidation of deoxymyoglobin. Overall, meat that has been aged discolours at a quicker rate throughout retail display compared to fresh meat due to the decline of mitochondrial activity and depletion of metmyoglobin reducing activity (Hood, 1980).

While postmortem aging has been extensively studied the exact aging duration that causes differences in discoloration is not fully understood (Ledward, 1970; Ledward, 1971; Ledward et al., 1986; Vitale et al., 2014). Ledward et al. (1986) found no

difference in color when comparing 3 and 72 hours postmortem muscle. Ledward (1971) found no difference in metmyoglobin concentration between 2 and 4 weeks of postmortem storage and Ledward (1970) found no difference from 5 to 14 days. However, results continue to support that postmortem aging impacts initial color and color stability. English et al. (2016) found that steaks aged for 42 and 62 days had higher a^* values than 21-day steak at the start of retail display. They also found that aging decreased the metmyoglobin reducing activity, increased lipid oxidation by the end of retail display, and overall had a detrimental effect on color stability. Vitale et al. (2014) found similar results in meat aged for just 3 days, which had higher L^* , a^* , and b^* values than unaged meat at the initial start of retail display. However, after just three days of retail display, meat that had been aged for 14 d expressed lower a^* values, lower color stability, and an increase in lipid oxidation, with increasing postmortem aging (Vitale et al., 2014). MacDougall (1982) attributed the change in color stability, specifically the increase in lightness, to the structural changes in the muscle proteins during aging. Overall, postmortem aging has a large impact on many aspects of meat quality.

Temperature

Temperature is well known to have a large impact on meat quality. A colder temperature helps to limit microbial growth and preserve meat for extended periods of time. Storage temperature can affect enzymatic degradation with higher temperatures allowing for more enzyme activity and greater tenderness (Kim et al., 2018). Storage temperature can also minimize carcass shrinkage in low temperature environments (Savell et al., 2005). Research has consistently shown that lower temperatures improve color stability and delay meat discoloration across species (Bendall and Taylor, 1972;

Lanier et al., 1977; O’Keeffe and Hood, 1980; O’Keeffe and Hood, 1982; Jakobsen and Bertelsen, 2000; Neethling et al., 2017). Jakobsen and Bertelsen (2000) believe that the most important factor for retaining a stable meat color and minimizing lipid oxidation is temperature. They also found that as temperature increased a^* values decreased (Jakobsen and Bertelsen, 2000). Li et al. (2017) found similar results in that a^* , b^* , redness ratio, percent oxymyoglobin, MRA, and NADH content were higher in meat stored at lower temperatures than warmer temperatures. MacDougall and Taylor (1975) found that a 3°C to 5°C increase in temperature in a retail display case doubled the rate of discoloration. However, Hood (1980) discovered that an increase of 10°C caused a two to five times increase in discoloration.

The explanation for the decrease in color stability is that higher storage temperatures increase the activity of oxygen consumption enzymes, therefore, decreasing the ability of oxygen to bind with myoglobin (Bendall and Taylor, 1972). O’Keeffe and Hood (1982) attribute better color stability with lower temperatures because lower temperatures increase the depth of oxymyoglobin into the meat due to the increased solubility of oxygen in the meat. Higher storage temperatures also resulted in an increase in myoglobin and lipid oxidation (Gutzke and Trout, 2002; Faustman et al., 2010). Gutzke and Trout (2002) found that in beef and sheep the myoglobin oxidation rate increased approximately five times for every 10°C increased in temperature. However, the impact that storage temperature has on NADH has not been clearly established according to Kim et al. (2009).

Frozen Storage

Freezing meat is a common practice used in the meat industry to extend the shelf-life of meat for extended periods of time (Kim et al., 2015). Estévez (2011) argues that the optimum temperature for meat to be store frozen is -40°C since only a small amount of water is unfrozen at this point. Freezing meat provides many advantages and disadvantages in regard to the quality of the product. Ramsbottom and Koonz (1941) proposed that freezing meat can reduce enzyme activity, minimize the change in color, inhibit oxidation, and reduce drip loss. However, freezing is also known to cause several detrimental quality defects. Kiani and Sun (2011) attributed the quality defects to ice crystal formation in meat through the aggregation of water molecules that results in mechanical damage and weakening of the muscle structure, along with protein denaturation and oxidation. The structural changes that are induced by ice crystallization are suggested to increase meat susceptibility to oxidative damage due to ruptured cell membranes releasing prooxidant compounds into the muscle, accelerating oxidation (Soyer et al., 2010; Setyabrata and Kim, 2019).

Recent studies support similar findings in that freezing meat decreases the color and color stability of both frozen and thawed meat (Kim et al., 2018; Setyabrata and Kim, 2019). Setyabrata and Kim (2019) attributed the decrease in color stability caused by freezing to the denaturation of myoglobin and reduced myoglobin redox system caused by ice crystal damage. However, the color of frozen meat is influenced by several factors: freezing rate, storage temperature, light intensity, and packaging method (MacDougall, 1982).

When meat is frozen, ice crystals form within the meat. The size of the ice crystal formed is based on the rate of which the meat is frozen. Slow freezing rates cause the formation of large ice crystals within the muscle. These large ice crystals cause damage in the muscle proteins and cell membranes, decreasing the meat quality (Kim et al., 2015). On the other hand, fast freezing results in the formation of numerous small crystals that are distributed throughout the muscle (Kim et al., 2015). Meat that is slowly frozen is typically darker in color than fast frozen meat that appears pale, light, and more opaque (Kropf, 1971; MacDougall, 1982). Meat that is fast frozen, contains small crystals that scatter more light than large crystals making it appear lighter than meat that is slowly frozen (MacDougall, 1982). Ramsbottom and Koonz (1941) found that meat frozen at -12.2°C was considerably darker than meat frozen at -34.4°C .

Published research has provided mixed results when comparing freezing rates which can most likely be attributed to the different freezing methods used to obtain different rates. Muela et al. (2010) found no difference in redness with three different freezing methods (tunnel, blast freezer, and nitrogen chamber) compared to Wanous et al. (1989) who found lower redness values for their fastest freezing rate treatment compared to their slow freezing rate. Wanous et al. (1989) found no difference in lipid oxidation based on differing freezing rates, but Muela et al. (2010) found that meat frozen in the nitrogen chamber had the least amount of oxidation. Muela et al. (2010) concluded that a slow freezing rate with long duration was the worst option in terms of lipid oxidation.

Frozen storage duration can also impact the quality of the meat (Wanous et al., 1989; Brewer and Wu, 1993; Farouk and Swan, 1998; Vieira et al., 2009). Holman et al. (2018) found a decrease in myoglobin content associated with an increase in frozen

storage time. A decrease in myoglobin content would be expected to decrease redness, which Wanous et al. (1989), Brewer and Wu (1993), and Vieira et al. (2009) found as storage time increased. Farouk and Swan (1998 and Vieira et al. (2009) also found a decrease in lightness or L^* as frozen storage duration increased. With extended frozen storage, increases in lipid oxidation, odor intensity, tenderness, and decreases in metmyoglobin reducing enzymes have been noted (Wanous et al., 1989; Farouk and Swan, 1998; Vieira et al., 2009). Vieira et al. (2009) attributed the increase in tenderness for meat aged three days and frozen for more than 75 d to the breakdown of muscle cell structures caused by the ice crystal leading to an increase in enzyme activity.

The temperature that frozen meat is stored also has an impact on the color and overall quality of the meat. Research has shown that meat frozen at lower temperatures has better color stability and lower oxidation (Brown and Dolev, 1963; Ledward and MacFarlane, 1971; Gomes et al., 2019). Ledward and MacFarlane (1971) found that at -10°C the reduction of metmyoglobin was still able to occur, however, at -20°C , no reduction was apparent over a period of 27 days. Lower temperatures had similar impacts on myoglobin and lipid oxidation in that steaks frozen at lower temperatures were more slowly oxidized compared to steaks frozen at warmer temperatures (Brown and Dolev, 1963; Gomes et al., 2019).

Light and packaging also influence the color of frozen meat. MacDougall (1982) reported that the color of frozen beef remained attractive for three months when stored in the dark compared to meat stored in the light, which only lasted three days. The use of different packaging methods can also aid in the improvement of frozen meat color (discussed more fully in the packaging section on page 41). MacDougall (1982) and

Bhattacharya et al. (1988) found that oxygen impermeable packaging helped to maintain color and minimize discoloration, off-flavors, and lipid oxidation for frozen beef.

Meat quality and color stability are further altered once the meat is thawed after frozen storage. Abdallah et al. (1999) and Kim et al. (2011) believed that thawing caused disruption of the muscle cells leading to an increase in meat exudate that mainly included sarcoplasmic proteins and metabolites involved in the myoglobin redox system. During thawing, reducing enzymes and co-factors, such as NADH, can be lost to the environment through the exudate, which will contribute to lower color stability and accelerated oxidation (Abdallah et al., 1999). Benjakul and Bauer (2001) and Muela et al. (2010) found that freezing and thawing resulted in accelerated lipid and myoglobin oxidation compared to fresh meat that wasn't frozen. The accelerated oxidation can be attributed to the damaging of cell membranes by ice crystals that allow for pro-oxidants to react with the phospholipid cell membrane and heme iron (Benjakul and Bauer, 2001). While freezing and thawing are typically associated with detrimental meat quality effects, Kim et al. (2018) believed this could be minimized through postmortem aging prior to freezing. The exact mechanism in which postmortem aging prior to freezing could improve color stability still needs further research. Kim et al. (2015) believed it could be that meat that aged prior to freezing may sustain higher myoglobin stability and lower oxygen consumption rate through the aging process than meat that is only aged or frozen.

Besides color, freezing and thawing also have an impact on tenderness and the microbial environment of the meat. Wheeler et al. (1990) and Shanks et al. (2002) found an increase in tenderness that is correlated to the length of frozen storage and aging prior to freezing. Leygonie et al. (2012) attributed the increase in tenderness to the breakdown

of muscle fibers through proteolysis and the loss of muscle structure from the ice crystal formation. During the freezing and thawing process, Leygonie et al. (2012) found that neither process decreased the number of viable microbes in meat, but noted that microbial spoilage is basically halted during frozen storage as the microbes become dormant.

Light

Research has shown that lighting has an impact on meat color. Kropf (1980) attributes light impact on meat color to an increase in temperature on the surface of the meat, photochemical effect, and/or differences in light appearance due to different spectral views. Increasing temperature has detrimental effects on meat color. Therefore, an increase in temperature from light would decrease color stability. Santamaria (1970) found a temperature increase of about 6°-7°C on the surface of meat from incandescent and deluxe cool white fluorescent light bulbs compared to samples kept in the dark. Hood (1980) found similar results to Santamaria (1970) in that light storage always had greater discoloration compared to samples stored in the dark. However, Kraft and Ayres (1954) did not find a difference between fresh meat color exposed to light and meat stored in the dark. The intensity of the light also has an impact on the color of the meat. Kropf (1980) concludes that high-intensity lighting can reduce the display life of products and recommends 100-foot candles for lighting intensity or less in retail display.

The type of light source can also impact meat color. Kraft and Ayres (1954) and Hood (1980) found that ultraviolet (UV) light exhibited more drastic changes in color and caused higher discoloration than fluorescent lights. Steele et al. (2016) make some compelling arguments about the advantages of light emitting diode (LED) lights arguing they are most efficient, environmentally friendly, and cost saving. They also found that

the LED lights kept the retail display case cooler and extended retail beef cuts color life by 0.5-1 days under LED lighting compared to fluorescent bulbs. However, Callahan et al. (2019) found conflicting results about the superiority of LED lights. They found that by the end of retail display steaks exposed to LED lights had lower amounts of oxymyoglobin and higher amounts of metmyoglobin than the other treatments. Fluorescent lights and high-UV fluorescent lights were also found to have higher a^* , higher MRA, higher $a^*: b^*$ ratio, and lower TBARS values than LED lights (Cooper et al., 2016; Cooper et al., 2017; Callahan et al., 2019).

Packaging

The way meat is packaged can have a large impact on consumer perception and willingness to purchase. Different packaging methods can cause a different color appearance. Carpenter et al. (2001) found that the visual scores for appearance and likelihood to purchase were highly correlated and the scores decreased from red to purple to brown. However, the effects that color had on appearance and likelihood to purchase did not affect the taste scores (Carpenter et al., 2001). The packaging industry is continuously changing and constantly working to extend shelf life while still maintaining a bright red cherry color for consumers.

The atmosphere prior to packaging can have a large impact on meat color (MacDougall et al., 1986; Lanari et al., 1995). MacDougall et al. (1986) found that meat exposed to air prior to freezing caused an increase in initial redness in skin-packed frozen meat indicating that the rate of discoloration is impacted by the blooming time and oxygen availability prior to freezing. Elevated levels of oxygen can prolong color stability (Bartkowski et al., 1982; MacDougall et al., 1986; Asensio et al., 1988; Lanari et

al., 1995). This can be attributed to an oxygen-enriched atmosphere and longer blooming times enhancing the oxygen penetration and increasing the depth of the oxymyoglobin layer leading to a reduction in the rate of discoloration (O'Keeffe and Hood, 1982; MacDougall et al., 1986). Lanari et al. (1995) found similar results with exposure to oxygen prior to freezing causing delayed discoloration and improved a^* values. They found that with dark storage, steaks that bloomed for 6 h in oxygen resulted in more color stability and lower TBARS compared to steaks that were exposed to oxygen for 48 h (Lanari et al., 1995). Lanari et al. (1995) believed these results can be explained by considering that during blooming, reducing capacity is lost which, combined with freezing, increased the discoloration rate.

One of the main methods typically used in a retail setting is permeable films that allow oxygen to pass through. Films used for retail display require a high oxygen permeability to allow for bright red meat, typically having an oxygen transmission rate around 8,000-12,000 cc/m²/24 h/atm (Hood, 1984). Kropf (1971) found similar results in that packaging films must have some oxygen permeability or the muscles turn dark. He found this problem was more intense when meat was frozen after packaging and indicated that to obtain a bright red color, meat needs to be packaged in an oxygen permeable package (Kropf, 1971). Sandberg (1970) found similar results with lower permeability film causing a darker color during display compared to films that allowed more oxygen penetration. Using an oxygen permeable film leads to a more appealing meat product in display, but consumers are also more likely to purchase it based on the packaging (Carpenter et al., 2001). Carpenter et al. (2001) found that, based on the appearance of the packaging, consumers were more likely to purchase a package that was

overwrapped with oxygen permeable, polyvinyl chloride (PVC) compared to meat that was in an impermeable vacuum skin pack (VSP) or in a modified atmosphere package (MAP). However, as the permeability of the film decreases, low partial pressure is reached where oxidation is favored, forming metmyoglobin (Clydesdale and Francis, 1971).

Since low partial pressure of oxygen favors the formation of oxidation it is important to keep a low level of oxygen, hence impermeable packaging that prevents oxygen from entering and leaving the package. O’Keeffe and Hood (1980) found that oxygen levels below 0.1% help to prevent discoloration of meat. The use of a completely impermeable package leads to heme pigments that are able to reduce myoglobin back to deoxymyoglobin and then, once the package is open and exposed to air, become oxygenated (Clydesdale and Francis, 1971; Jeremiah, 2001). However, O’Keeffe and Hood (1982) noted that under anaerobic conditions, reduction of metmyoglobin does not take place. Anaerobic packaging, through the use of vacuum packaging (VP) with impermeable films, can prevent metmyoglobin formation and rancidity with sufficient MRA in the meat, as well as reducing the growth of aerobic organisms and therefore improving shelf life (Greene, 1969; Jeremiah, 2001; Li et al., 2012). Brewer and Wu (1993) evaluated VP, PVC, and saran wrap as packaging methods and found that the lowest amount of metmyoglobin throughout retail display occurred in samples that were vacuum packed. While VP and impermeable films can lead to less discoloration, they tend to have darker steaks and L* values compared to steaks in a high oxygen packaging (Mancini et al., 2009). Along with the darker color, consumers found the purple color of

vacuum packaged meat to be less desirable than red meat associated with oxygen permeable film (Carpenter et al., 2001).

Another option to the traditional VP is vacuum skin packaging (VSP). According to Li et al. (2012), VSP minimizes the formation of wrinkles and air pockets in the package. This occurs through the heating of the upper cover film and causing it to shrink tightly around the meat limiting the exudate found in the wrinkles of VP that can be more susceptible to bacterial growth (Li et al., 2012). As expected, VP and VSP have many similarities when comparing their properties such as higher content of deoxymyoglobin, lower metmyoglobin, and lower L^* values compared to MAP (Li et al., 2012). Li et al. (2012) also found no significant differences in NADH content of steaks packaged in VP, VSP, and MAP. However, results showed higher a^* values and better color stability for VSP samples than VP (Li et al., 2012). Li et al. (2012) attributed the difference to vacuum conditions during packaging. Vacuum skin packaging had a heated upper cover film that shrinks tightly around the meat, leading to the possibility of reduced residual oxygen to penetrating the surface (Li et al., 2012). The VSP may have its advantages for preserving meat, but it was also found to be a more appealing packaging method to consumers than MAP (Carpenter et al., 2001).

Meat can be packaged in several different atmosphere combinations that most commonly include oxygen, carbon dioxide, carbon monoxide, and nitrogen. The most common MAP method is the use of high-oxygen with carbon dioxide. According to Kim et al. (2010) and Jeremiah (2001), MAP systems with 80% oxygen (O_2) and 20% carbon dioxide (CO_2) are widely used by retail markets since MAP gives the beef a stable bright-red color and thick oxymyoglobin layer that masks the underlying metmyoglobin layer.

Previous research regarding high-oxygen MAP has shown that the technology has both advantages and disadvantages (Jayasingh et al., 2002; Grobbel et al., 2008a; Kim et al., 2010; Kim et al., 2011; Li et al., 2012; Bonny et al., 2017). Kim et al. (2010) noted that a high-oxygen MAP system creates oxidative conditions which can lead to myoglobin and lipid oxidation, and negatively affect tenderness and other sensory traits.

Jeremiah (2001) found that high-oxygen MAP paired with carbon dioxide inhibited the growth of aerobic organisms. Several researchers have found that high-oxygen MAP increased b^* values compared to VP (Li et al., 2012; Bonny et al., 2017). Research has well established that high oxygen MAP causes higher L^* values compared to samples that are VP or overwrapped with PVC (Kim et al., 2011; Kim et al., 2012; Li et al., 2012; Bonny et al., 2017). Kim et al. (2011) attributed the increase in lightness to elevated oxygenation of myoglobin under oxygen-rich conditions. Another aspect of high-oxygen MAP that has been well documented in the literature is that high-oxygen MAP produces initially higher a^* values and percent oxymyoglobin compared to PVC and VP (Kim et al., 2010; Kim et al., 2012; Li et al., 2012). However, steaks in a high-oxygen MAP had lower MRA and OCR compared to steaks packaged in oxygen permeable film (English et al., 2016; Ramanathan et al., 2019). This decrease in MRA and OCR can have detrimental effects on color stability.

Kim et al. (2010) found that myoglobin oxidation can occur in high-oxygen MAP with extended display time leading to the formation of metmyoglobin on the surface of the meat. Research has also demonstrated that high-oxygen MAP promotes oxidative changes in meat affecting both the color stability and discoloration more than atmospheric-oxygen MAP, VP, or carbon monoxide-MAP (Jayasingh et al., 2002;

Grobbel et al., 2008b; Grobbel et al., 2008a; Zakrys et al., 2008; Mancini et al., 2009; Kim et al., 2010). Greater discoloration and considerably lower a^* values are observed when comparing PVC with VP throughout retail display. Jakobsen and Bertelsen (2000) found that the level of oxygen in the headspace affected both color stability and lipid oxidation. Samples stored in just 20% oxygen were not able to retain bright red cherry color as samples stored in higher oxygen concentrations, but samples stored in lower concentrations had less lipid oxidation than those store at higher oxygen concentrations (Jakobsen and Bertelsen, 2000).

While high-oxygen MAP holds the potential of limiting the negative impacts of color stability of meat through freezing and thawing, it has a major drawback when oxygen inclusion reaches over 21% in MAP leading to increase in lipid and protein oxidation (Kim et al., 2010; Leygonie et al., 2012). The major drawback can most likely be seen over 21% oxygen exposure since 21% is the normal atmospheric level that meat is typically exposed to during oxygenation. Researchers have been able to show that high-oxygen MAP causes greater lipid oxidation compared to VP (Jayasingh et al., 2002; Zakrys et al., 2008; Kim et al., 2010; Bonny et al., 2017). The increase in oxidation from high-oxygen MAP can lead to the development of off-flavors and lower beef flavors that consumers find unappealing (Grobbel et al., 2008a; Zakrys et al., 2008; Kim et al., 2010). Along with off-flavors, high-oxygen MAP has also been associated with a decrease in tenderness (Grobbel et al., 2008a; Lund et al., 2008; Zakrys et al., 2008; Kim et al., 2010; Lagerstedt et al., 2011a; Lagerstedt et al., 2011b). Kim et al. (2010) associated this decrease in tenderness to protein oxidation and protein polymerization of cross-links

between myosin and titin. Finally, McMillin (2008) and Lagerstedt et al. (2011a) found that high-oxygen MAP had a negative effect on cooked meat color.

Nitrogen is another gas that can be used in MAP. O’Keeffe and Hood (1980) noted that meat packaged in 92% nitrogen and 8% hydrogen retained the capacity to bloom for storage periods of up to four weeks. They also found that when comparing nitrogen and carbon dioxide as storage atmospheres there was no significant difference in extending shelf-life (O’Keeffe and Hood, 1980).

Carbon dioxide is often paired with oxygen in MAP. Carbon dioxide inhibits microbial growth and therefore extends the shelf life of the product (Daun et al., 1971; Gill and Tan, 1980; Eilert, 2005). However, the use of carbon monoxide in packaging has become increasingly relevant in the U.S. since its approval by the FDA for use at a level of 0.4% in red meat MAP systems (Eilert, 2005; Mancini et al., 2009). Previous research has reported that carbon monoxide has a significant effect on improving the color stability of beef (Jayasingh et al., 2001; Hunt et al., 2004). Mancini et al. (2009) found that carbon monoxide MAP led to an increase and maintained redness during storage. They also found that steaks packaged in carbon monoxide MAP were redder and had higher reflectance ratios than steaks in high-oxygen MAP and VP (Mancini et al., 2009). The purpose of the Mancini et al. (2009) study was to see if carbon monoxide MAP could help improve the darkness of meat injected with lactate, an ingredient known to stabilize and minimize surface discoloration (Mancini and Hunt, 2005; Kim et al., 2006). While carbon monoxide packaging created the greatest a^* values of all packaging types, it was not able to counteract the darkening effects of 2.5% lactate (Mancini et al., 2009).

Conclusion

Meat color has a profound impact on consumer willingness to purchase product due to the belief that meat color is associated with freshness. Given the opportunity of expanding the market for fresh beef it is imperative to gain a better understanding and insight as to how meat color is impacted by frozen storage. While meat color research is extensive, it often provides varying results on different topics such as the importance of metmyoglobin reducing activity and oxygen consumption. In the past, research has shown that frozen storage and aging typically have more detrimental impacts on meat color. With varying parameters of aging, packaging, and storage, it is imperative to have a deeper understanding of how these impacts varying levels of myoglobin oxygenation during and after frozen storage. Greater knowledge of beef color may lead to the development of marketing a frozen bright cherry red beef product, consumers find appealing.

MATERIALS AND METHODS

Study 1: Impact of Myoglobin Oxygenation State on Color Stability of Frozen Beef Steaks

Sample Collection

Thirty-six USDA Choice strip loins in vacuum packaging were obtained 3 d post-harvest from Greater Omaha Packing, Omaha, NE (August, 2018). Loins were then transported to the Loeffel meat laboratory at the University of Nebraska-Lincoln. Strip loins were randomly assigned to aging for 4 or 20 d post-harvest ($2 \pm 5^{\circ}\text{C}$) under dark storage.

Sample Fabrication

For each aging period, 4 or 20 d, 18 loins were fabricated anterior to posterior where a 0.318 cm steak was removed at both anterior and posterior ends to expose fresh surfaces. Loins were split in half into loin segments and loin segments were randomly assigned to a frozen storage period of 0 (frozen for 1 d), 2, 4, or 6 months (nine loin segments for each frozen storage period) prior to fabrication. Each loin contained only two frozen storage periods forming the incomplete block design. Within each frozen storage period, six steaks were cut with a slicer into 1.27 cm steaks for each myoglobin oxygenation level and packaging film combination for a total of 432 steaks in the study. The steaks were randomly preassigned to a myoglobin oxygenation level and packaging film. Myoglobin oxygenation level included: deoxymyoglobin (DeOxy), oxygenation (Oxy), and high oxygenation (HiOxy). The DeOxy steaks were immediately measured for instrumental color, vacuum sealed, and placed in the freezer within 3 min after slicing. The Oxy steaks were sliced, allowed to bloom and oxygenated in air at

atmospheric pressure for 30 min, measured for instrumental color, vacuum sealed, and frozen. The HiOxy steaks were sliced and packaged for 24 h in a modified atmosphere packaging (MAP) mixture of 80% O₂ and 20% CO₂ before being measured for instrumental color, repackaged in the oxygen permeable or impermeable film, and frozen. A KOCH tray sealer with a MAP option (Koch Equipment LLC, Kansas City, MO) was used with the 80% O₂ and 20% CO₂ mixture. A 2.4 mil high barrier lidding film with an oxygen transmission rate (OTR) of 3 cc/m²/24 h at standard temperature and pressure (STP; 0°C and 10⁵ Pa; Ultra Source, Kansas City, MO) was used to cover 22.0 cm x 16.8 cm x 5.5 cm plastic food tray (Coextruded Plastic Technologies, Janesville, WI) (Calvert, 1990). Steaks were packaged into either oxygen permeable or impermeable film bags (20 cm x 30 cm) and vacuum sealed with a Multivac Packaging machine (Multivac C500, Multivac, Kansas City, MO). Packaging films consisted of oxygen permeable (Cryovac Shrink Bags, 10K OTR Fresh Fish Non-Barrier Straight End Seal, Sealed Air, Charlotte, NC) and oxygen impermeable film (3 mil STD barrier, Ultra Source, Kansas City, MO). The oxygen permeable film had an OTR of at least 10,000 cc/m²/24 h at (STP), classifying it as a high transmitter of oxygen. The oxygen impermeable film had an OTR of 6.77 cc/m²/24 h at STP, making it a high barrier film. Steaks were then frozen at -10 ± 3°C on flat trays in a single layer for 3 hours. After the initial freezing steaks were then transferred into boxes and stored in the dark at -5 ± 3°C for either 0, 2, 4, or 6 months. Following frozen storage, steaks were removed from the packaging and immediately analyzed (while frozen) for oxygen penetration, instrumental color, and subjective color. After color was measured all steaks were repacked (3 mil STD barrier, Ultra Source, Kansas City, MO), vacuum sealed, and frozen for further analysis (-80°C) of lipid

oxidation. Lipid oxidation was measured one month following the completion of the last frozen storage period. Later, samples were trimmed of subcutaneous fat, frozen in liquid nitrogen, and then powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples were stored at -80°C for approximately two weeks prior to measuring for lipid oxidation.

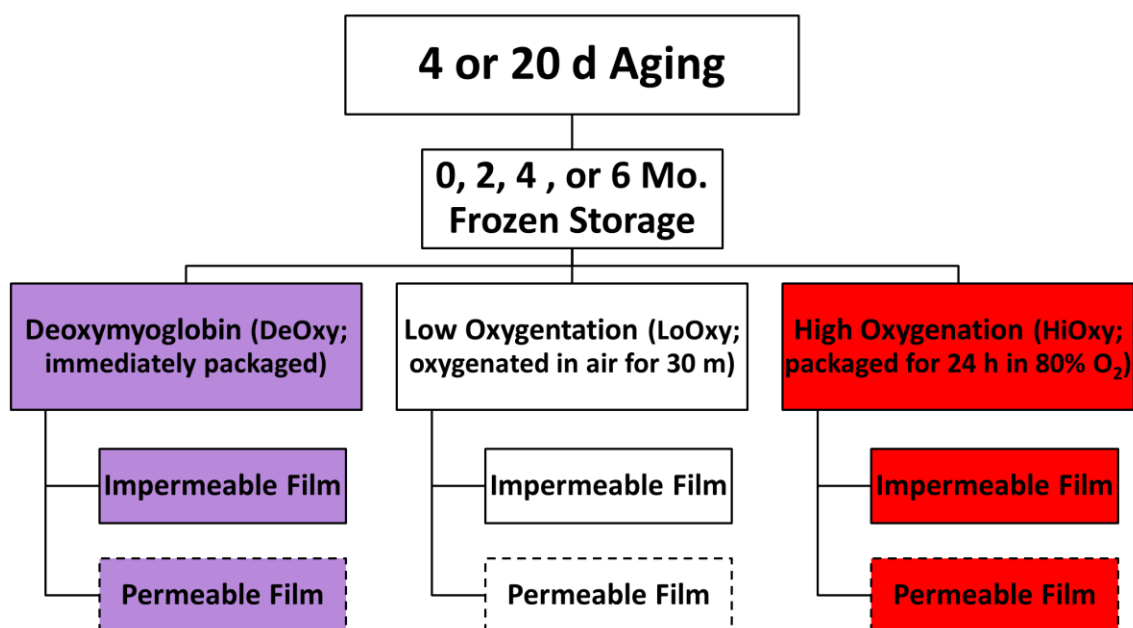


Figure 1. Treatment map

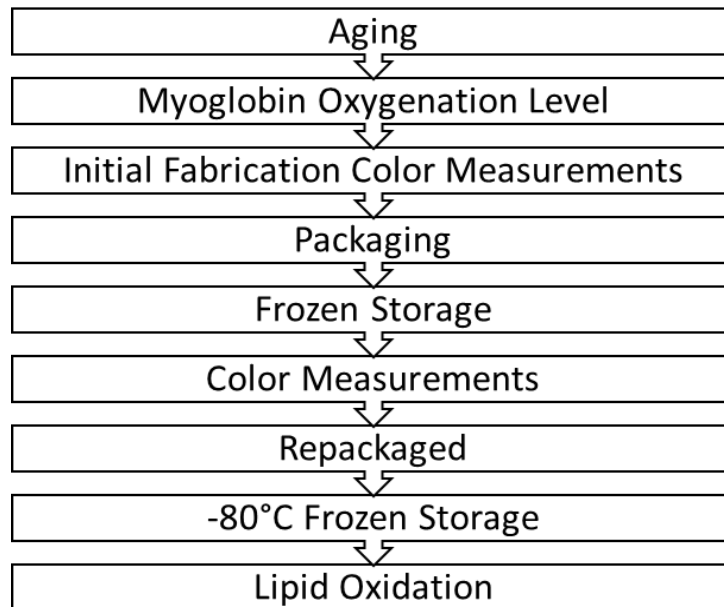


Figure 2. Frozen Storage Process Flow Map

Oxygen Penetration

After being removed from the freezer, steaks were removed from packaging film and a cut was made 2.54 cm from the lateral end of the steak. A Westward caliper (6"/150 mm Elec Value Caliper, Grainger International, Inc, Lake Forest, IL) was used to quickly measure the oxygen penetration depth (bright red band) of the sample. Two measurements were taken for each steak, 2.54 cm from ventral and dorsal end. The measurement was taken from the edge of the surface exposed to oxygen to the edge of the oxymyoglobin band within the steak. The mean of the two measurements was used for statistical analysis.

Instrumental Color (L^ , a^* , b^*)*

Instrumental color was assessed after each frozen storage period once steaks were removed from the packaging films, prior to oxygen penetration measurements.

Instrumental color measurements were collected using the L^* , a^* , b^* scales with a

Minolta CR-400 colorimeter (Minolta, Osaka, Japan) set with a D65 illuminant, 2° observer. The 8 mm opening of the handheld colorimeter was covered with an oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO). The colorimeter was set to record and print the mean of six readings per steak. Calibration was done with the oxygen permeable film prior to each day of frozen steak readings using a white ceramic tile (Calibration Plate, Serial No. 14933058, Konica Minolta, Japan) and the D65 settings were set as follows: $Y=93.13$, $x=0.3164$, and $y=0.330$. Once calibrated, the color space was selected to be the L^* , a^* , and b^* scale; where L^* is a measure of darkness to lightness and has a range from 0 (black) to 100 (white), a^* is a measure of greenness to redness and has a positive (red) to negative range (green), and b^* is a measure of blueness to yellowness with a positive (yellow) to negative (blue) range.

Instrumental Color (Percent Oxymyoglobin, Percent Deoxymyoglobin, and Percent Metmyoglobin)

Instrumental color was assessed after each frozen storage period and once steaks were removed from packaging film, prior to oxygen penetration measurements. Color was evaluated on steaks while frozen under an oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO). Six readings were taken on each steak using a portable Quality Spec[®] Trek (Malvern Panalytical, Longmont, CO, USA) with a spectral range of 350-2500 nm and a wavelength accuracy of ± 1.0 nm after calibration with a white tile and oxygen permeable film. Reflectance values at 473, 525, 572, and 730 nm were obtained and used to calculate percent oxymyoglobin, deoxymyoglobin, and metmyoglobin for each reading using the equations provided by the American Meat Science Association Meat Color Measurement

Guidelines on page 58 (Krzywicki, 1979; Hunt et al., 2012). Statistical analysis was calculated using the mean of the six myoglobin state calculations obtained from each reflectance values reading.

$$\text{Equation 1: } A = \log \frac{1}{R}$$

$$\text{Equation 2: } \%MMB = \left\{ 1.395 - \left[\frac{(A_{572} - A_{730})}{(A_{525} - A_{730})} \right] \right\} \times 100$$

$$\text{Equation 3: } \%DMB = \left\{ 2.375 \times \left[1 - \frac{(A_{473} - A_{730})}{A_{525} - A_{730}} \right] \right\} \times 100$$

$$\text{Equation 4: } \%OMB = 100 - (\%MMB + \%DMB)$$

Delta E

Delta E was measured as the magnitude of difference in L*, a*, and b* color space. The L*, a*, and b* values were obtained with the Minolta CR-400 colorimeter using the methods previously described. Delta E was calculated for all frozen storage periods using the initial color readings taken during fabrication for each individual steak (1 m for DeOxy, 30 m for Oxy, and 24 h for HiOxy) and the final readings when steaks were removed from the freezer in the frozen state. Delta E was then calculated using the equation $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Hunt et al., 2012).

Redness Ratio

Redness ratio was assessed after each frozen storage period and once steaks were removed from packaging film, prior to oxygen penetration measurements. Redness ratio was obtained using the Quality Spec[®] Trek (Malvern Panalytical, Longmont, CO, USA) with a spectral range of 350-2500 nm and a wavelength accuracy of +/- 1.0 nm to obtain reflectance values. Six spectral readings were taken on frozen steaks through a plastic

oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO). Reflectance values of 580 and 630 nm were used to calculate a redness ratio. The equation $630\text{nm} \div 580\text{ nm}$ was used to calculate the redness ratio (Hunt et al., 2012). Larger ratios or higher values indicate more redness whereas a ratio closer to 1.0 would indicate essentially 100% metmyoglobin (Strange et al., 1974). The mean of the six readings per steak was used for statistical analysis of redness ratio.

a: b* Ratio*

The a*: b* ratio was calculated using the equation provided by the American Meat Science Association Meat Color Measurement Guidelines (Hunt et al., 2012). The a* and b* values were obtained with the Minolta CR-400 colorimeter using the methods previously described. The a*: b* ratio was calculated using the a* and b* values from the final readings when steaks were removed from the freezer in the frozen state. The a*: b* ratio was calculated by using the equation $a^* \div b^*$. Larger ratios indicate more redness and less discoloration for the steaks (Setser, 1984).

Hue Angle

Hue angle was calculated based on the equation provided in the American Meat Science Association Meat Color Measurement Guidelines (Hunt et al., 2012). The a* and b* values were obtained with the Minolta CR-400 colorimeter using the methods previously described. Hue angle was calculated based on the a* and b* values, once steaks were removed from the freezer while still frozen. Hue angle (HA) was calculated as $HA = [\arctangent(b^*/a^*)]$ (Hunt et al., 2012). Tapp et al. (2011) found hue angle to be an indicator of redness and discoloration, with larger angles indicating less red meat and greater discoloration.

Subjective Color (Visual Discoloration)

Visual discoloration was assessed after each frozen storage period and once steaks were removed from packaging film, but prior to oxygen penetration measurements.

Steaks were assessed with a trained five-person panel composed of graduate students from the University of Nebraska-Lincoln. Students were trained prior to evaluating steaks with a color guide that was also used as a reference when evaluating individual steaks. A percentage scale was used where 0% meant no discoloration and 100% meant complete surface discoloration.

Lipid Oxidation

Lipid oxidation was determined using the 2-thiobarbituric acid reactive substances (TBARS) method for all steaks following all frozen storage periods, according to Ahn et al. (1998). Five grams of powdered meat were placed into a 50-mL conical tube to which 14 mL of distilled deionized water and 1 mL of butylated hydroxyanisole (BHA) solution (10% BHA: 90% ethanol) were added. Samples were homogenized using a Polytron (POLYTRON Kinimatica CH-6010, Switzerland) for 15 s at medium-to-high speed. The samples were centrifuged ($2,000 \times g$ for 5 min at 10 °C) and 1 mL of supernatant was transferred into a 15-mL conical tube with 2 mL of 2-Thiobarbuturic acid/Trichloroacetic acid solution (15% trichloroacetic acid and 20 mM 2-Thiobarbuturic acid in deionized distilled water). Tubes were then placed in a 70 °C water bath for 30 min. Tubes were cooled for at least 10 min in a water bath (22 °C) and centrifuged ($2,000 \times g$ for 15 min at 10 °C). Two hundred μ L of supernatant were transferred to a 96-well plate in duplicate. Absorbance values were then read at 540 nm using a microplate spectrophotometer (Model Epoch, Biotek, Winooski, VT). Results were expressed in mg of malonaldehyde per kg of tissue.

Statistical Analysis

Statistical analyses were conducted with SAS (version 9.4, Cary, NC). Data were analyzed as a split-split plot design with age as the whole-plot, frozen storage as the split-plot and a three by two factorial of oxygenation level and packaging film as the split-split plot. Frozen storage period was analyzed as an incomplete block design with each loin containing two random storage periods. Data were analyzed using the PROC GLIMMIX procedure of SAS and loin was the experimental unit. All means were separated with the LS MEANS statement with statistical significance determined at $P < 0.05$. Interactions with frozen storage as a significant term were analyzed using the slice function in SAS.

Study 2: Impact of Myoglobin Oxygenation State Prior to Frozen Storage on Color Stability of Thawed Beef Steaks through Retail Display

Sample Collection

The same thirty-six USDA Choice strip loins were used as explained in study one.

Sample Fabrication

For each aging period, 4 or 20 d, 18 loins were fabricated in the same way as described in study one. Loins were split in half into loin segments and loin segments were randomly assigned to a frozen storage period of 0 (frozen for 1 d), 2, 4, or 6 months (nine loin segments for each frozen storage period) prior to fabrication. Each loin contained only two frozen storage periods forming the incomplete block design. Within each frozen storage period, six steaks were cut with a slicer into 1.27 cm steaks for each myoglobin oxygenation level and packaging film combination for a total of 432 steaks in the study. The steaks were randomly preassigned to a myoglobin oxygenation level and packaging film. Myoglobin oxygenation level included: deoxymyoglobin (DeOxy), oxygenation (Oxy), and high oxygenation (HiOxy). Myoglobin oxygenation levels were induced the

same as explained in study one. However, no color measurements were made prior to packaging and freezing. Steaks were once again packaged into varying packaging film bags (20 cm x 30 cm) and vacuum sealed with a Multivac Packaging machine (Multivac C500, Multivac, Kansas City, MO). Packaging films consisted of oxygen permeable (Cryovac Shrink Bags, 10K OTR Fresh Fish Non-Barrier Straight End Seal, Sealed Air, Charlotte, NC) and oxygen impermeable film (3 mil STD barrier, Ultra Source, Kansas City, MO). The oxygen permeable film had an oxygen transmission rate (OTR) of at least 10,000 cc/m²/24 h at (STP), classifying it as a high transmitter of oxygen. The oxygen impermeable film had an OTR of 6.77 cc/m²/24 h at STP, making it a high barrier film. Following packaging, steaks were immediately frozen and stored at $-5 \pm 3^{\circ}\text{C}$ for either 0, 2, 4, or 6 months as explained in study one. Following frozen storage, steaks were removed from the assigned packaging and placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with a different oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO) and immediately set out for retail display (RD) where they were thawed for 24 h prior to any instrumental readings. Trays were placed under simulated RD conditions on tables in a meat cooler for 7 d ($3 \pm 1^{\circ}\text{C}$ under white fluorescence lighting at 1,000 to 1,800 lux; Lamp type: F32T8/TL741 700 Series 32 watt, Phillips, Inc., New Jersey) and randomly rotated daily. During retail display instrumental color and subjective color were measured every day after the initial 24 h thaw period. Following the last day of retail display oxygen penetration was also measured before steaks were repacked (3 mil STD barrier, Ultra Source, Kansas City, MO), vacuum sealed, and frozen for further analysis (-80°C) of lipid oxidation. Lipid oxidation was measured one month following the completion of

the last frozen storage period. Later, samples free of subcutaneous fat were frozen in liquid nitrogen and then powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples were stored at -80°C for approximately two weeks prior to measuring for lipid oxidation.

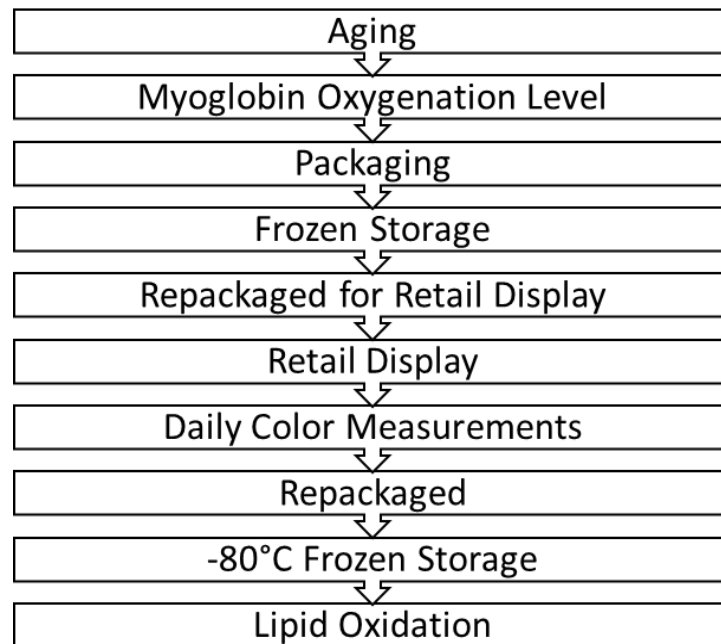


Figure 3. Thawed Retail Display Process Flow Map

Oxygen Penetration

After 7 days retail display, steaks were removed from retail display and a cut was made 2.54 cm from the lateral end of the steak. Oxygen penetration was measured with a Westward caliper as described in study one for only the final day of retail display.

Instrumental Color (L^ , a^* , b^*)*

Instrumental color was assessed every day of retail display starting on day 1 (24 hours after being placed in retail display) as explained in study one.

Instrumental Color (Percent Oxymyoglobin, Percent Deoxymyoglobin, and Percent Metmyoglobin)

Instrumental color was assessed each day of retail display starting on day 1 (24 hours after being placed in retail display) as described in study one.

Delta E

Delta E was measured as the magnitude of change in L*, a*, and b* color space. The L*, a*, and b* values were obtained with the method and equation described in study one. However, since no initial color measurements were taken, delta E was calculated using the initial color readings taken on day 1 of RD and the final readings taken on day 7 of RD.

Redness Ratio

Redness ratio was assessed every day of retail display that color measurements were taken. Redness ratio was calculated based on the description in study one.

a: b* Ratio*

The a*: b* ratio was calculated as described in study one for everyday color was measured during retail display.

Hue Angle

Hue angle was assessed throughout every day of retail display that color measurements were taken. Hue angle was calculated based on the explanation used in study one.

Subjective Color (Visual Discoloration)

Visual discoloration was assessed each day of retail display starting on day 1 (24 hours after being placed in retail display) by a trained panel as described in study one.

Lipid Oxidation

Lipid oxidation was determined with the TBARS protocol as described by Ahn et al. (1998) and is described in study one. Lipid oxidation was measured once on steaks that had been exposed to retail display for 7 days.

Statistical Analysis

Statistical analyses were conducted with SAS (version 9.4, Cary, NC). Data were analyzed as a split-split plot design with repeated measures. Age was analyzed as the whole-plot, frozen storage as the split-plot, a three by two factorial of oxygenation level and packaging film as the split-split plot, and retail display as repeated measures. Frozen storage period was analyzed as an incomplete block design with each loin containing two random storage periods. Oxygen penetration, delta E, and lipid oxidation were analyzed without repeated measures since they were measured only once throughout the study for each steak. Redness ratio, percent oxymyoglobin, percent deoxymyoglobin, and percent metmyoglobin were analyzed within aging basis and instead as a split-plot design with repeated measures. Frozen storage was the whole-plot and contained an incomplete block design, a three by two factorial of oxygenation level and packaging film as the split-plot, and retail display as repeated measures. The change in analysis for these variables is due to instrumental issues that lead to missing data for 20 d aging at 4 months of frozen storage. Data were analyzed using the PROC GLIMMIX procedure of SAS and loin was the experimental unit. All means were separated with the LS MEANS statement with statistical significance determined at $P < 0.05$. Interactions with frozen storage and/or retail display as significant terms were analyzed using the slice function in SAS.

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Impact of Myoglobin Oxygenation State on Color Stability of Frozen Beef Steaks

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Abstract

The emerging market of frozen meat emphasizes the need to better understand beef surface discoloration and the ideal parameters of freezing beef to retain an acceptable color. The objectives of this study were to determine the impacts of myoglobin oxygenation level prior to freezing and frozen storage duration on frozen beef color. USDA Choice strip loins ($n=36$) were aged for 4 d or 20 d. Steaks were randomly assigned to a myoglobin oxygenation level [deoxymyoglobin (DeOxy; immediately packaged after cutting), oxygenation (Oxy; oxygenated in air for 30 minutes), or high oxygenation (HiOxy; packaged for 24 h in 80% O_2)]. Steaks were then vacuum packaged in oxygen permeable or impermeable film and immediately frozen (-5°C). Following either 0, 2, 4, or 6 months of frozen storage, steaks were removed from the packaging and immediately analyzed for instrumental color (L^* , a^* , b^*), percent oxymyoglobin, metmyoglobin, and deoxymyoglobin, delta E, redness ratio, $a^*:b^*$ ratio, hue angle, subjective discoloration, and lipid oxidation. The HiOxy steaks had greater oxygen penetration and the greatest a^* values compared to DeOxy and Oxy steaks, regardless of packaging ($P < 0.0005$). With 4 d of aging, HiOxy steaks had greater a^* values than DeOxy and Oxy at all storage times ($P = 0.0118$). The HiOxy steaks aged for 20 d and frozen for 6 months had significantly higher delta E values than all other myoglobin oxygenation levels and postmortem aging periods ($P < 0.0001$). Redness and percent oxymyoglobin were highest for HiOxy steaks within each storage period ($P < 0.0002$). The HiOxy steaks had the highest percent oxymyoglobin and DeOxy had the lowest percent oxymyoglobin within each aging and storage period ($P < 0.01$). Conversely, DeOxy steaks had the highest percent metmyoglobin and HiOxy had the lowest percent

metmyoglobin when packaged in impermeable film ($P < 0.0001$). The HiOxy steaks from 20 d of aging had the highest discoloration compared to 4 d aging and more discoloration than all other myoglobin treatments at 6 months of storage ($P < 0.0001$). The HiOxy 20 d aged steaks exhibited the highest lipid oxidation values at 2, 4, and 6 months ($P = 0.0224$) and HiOxy steaks exhibited a brighter and deeper cherry red color compared to the DeOxy steaks. The HiOxy steaks were greater in redness or similar when compared to Oxy steaks, but experienced more detrimental effects when frozen storage was extended.

Keywords: oxymyoglobin; discoloration; frozen storage

Introduction

Color is often viewed as the number one factor consumers use when purchasing beef other than cost (Hood, 1980; Kropf, 1980; Carpenter et al., 2001). Therefore, maintaining a bright cherry red color is a common concern for the meat industry in attracting consumers. It has been reported that 15% of retail beef is discounted due to discoloration resulting in an economic loss of \$1 billion annually (Mancini and Hunt, 2005). Discoloration is caused by an accumulation of metmyoglobin on the surface of beef due to the oxidation of oxymyoglobin and deoxymyoglobin. Typically, metmyoglobin can be reduced back to deoxymyoglobin and oxygenated to oxymyoglobin (bright cherry red color), however, this is no longer possible when reducing components of the metmyoglobin reducing activity (MRA) are depleted. Meat color, specifically the reduction and oxidation of myoglobin and the bound ligand, depends on many intrinsic (pH, species, breed, sex, animal maturity, muscle to muscle variation, oxygen consumption, MRA, and lipid oxidation) and extrinsic factors (diet, electrical stimulation, postmortem aging, temperature, frozen storage, and packaging) (Hood, 1980; Renner, 1990; Mancini and Hunt, 2005; Suman and Joseph, 2013).

While beef is typically packaged and sold fresh, the market of frozen beef has started to grow. The global meat export industry is worth over \$13 billion and freezing plays an essential role in that industry, helping to ensure the safety of meat being supplied all over the world (Leygonie et al., 2012). The extended shelf-life of frozen beef allows the industry to export beef on an international basis when compared to fresh beef. One emerging market for frozen beef products is meal delivery service companies and the opportunity for increased revenue of beef products due to the increased shelf life.

Recently, meal-kit delivery has a market of \$4.65 billion in 2017 and is expected to grow to \$11.6 billion by 2022 (Progressive Grocer, 2017). With the opportunity for increased product sales, it is essential that a better understanding is developed of how different myoglobin oxygenation levels retain color through frozen storage. Therefore, the objectives of this study were to determine the impacts of myoglobin oxygenation level prior to frozen storage and frozen storage duration on frozen beef color, and to evaluate the effects of oxygen permeability of packaging and aging time on meat color through frozen storage.

Materials and Methods

Sample Collection

Thirty-six USDA Choice strip loins were vacuumed packaged and obtained from Greater Omaha Packaging, Omaha, NE prior to being transported to the Loeffel Meat Lab at the University of Nebraska-Lincoln. Prior to fabrication loins were aged for either 4 or 20 d post-harvest ($2 \pm 5^{\circ}\text{C}$) under dark storage.

Sample Fabrication

For each aging period, 4 or 20 d, 18 loins were fabricated anterior to posterior where a 0.318 cm steak was removed at both anterior and posterior ends to expose fresh surfaces. Loins were split in half into loin segments and loin segments were randomly assigned to a frozen storage period of 0 (frozen for 1 d), 2, 4, or 6 months (nine loin segments for each frozen storage period) prior to fabrication. Each loin contained only two frozen storage periods forming the incomplete block design. Within each frozen storage period, six steaks were cut with a slicer into 1.27 cm steaks for each myoglobin

oxygenation level and packaging film combination for a total of 432 steaks in the study. The steaks were randomly preassigned to a myoglobin oxygenation level and packaging film. Myoglobin oxygenation level was described as deoxymyoglobin (DeOxy), oxygenation (Oxy), and high oxygenation (HiOxy). The DeOxy steaks were immediately measured for instrumental color, vacuum sealed, and placed in the freezer within 3 min after slicing. The Oxy steaks were sliced, and oxygenated in air at atmospheric pressure for 30 min, measured for instrumental color, vacuum sealed, and frozen. The HiOxy steaks were sliced and packaged for 24 h in a modified atmosphere packaging (MAP) mixture of 80% O₂ and 20% CO₂ before being measured for instrumental color, repackaged in an oxygen permeable or impermeable film, and frozen. A KOCH tray sealer with a MAP option (Koch Equipment LLC, Kansas City, MO) was used with the 80% O₂ and 20% CO₂ mixture. Steaks were randomly assigned to a packaging film and vacuum sealed with a Multivac Packaging machine (Multivac C500, Multivac, Kansas City, MO). Packaging films consisted of oxygen permeable (Cryovac Shrink Bags, 10K OTR Fresh Fish Non-Barrier Straight End Seal, Sealed Air, Charlotte, NC) and oxygen impermeable film (3 mil STD barrier, Ultra Source, Kansas City, MO). The oxygen permeable film had an oxygen transmission rate (OTR) of at least 10,000 cc/m²/24 h at standard temperature and pressure (STP; 0°C and 10⁵ Pa), classifying it as a high transmitter of oxygen (Calvert, 1990). The oxygen impermeable film had an OTR of 6.77 cc/m²/24 h at STP, making it a high barrier film. Steaks were then frozen at $-10 \pm 3^{\circ}\text{C}$ on flat trays in a single layer for 3 hours. After the initial freezing steaks were then transferred into boxes and stored in the dark at $-5 \pm 3^{\circ}\text{C}$ for either 0, 2, 4, or 6 months. Following frozen storage, steaks were removed from the packaging and immediately

analyzed (while frozen) for oxygen penetration, instrumental color, and subjective color. All steaks were then repacked (3 mil STD barrier, Ultra Source, Kansas City, MO), vacuum sealed, and frozen for further analysis (-80°C) of lipid oxidation. Lipid oxidation was measured one month following the completion of the last frozen storage period. Later, samples were trimmed of subcutaneous fat, frozen in liquid nitrogen, and then powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples were stored at -80°C for approximately two weeks prior to lipid oxidation analysis.

Oxygen Penetration

After being removed from the freezer, steaks were removed from packaging film and a cut was made 2.54 cm from the lateral end of the steak. A Westward caliper (6"/150 mm Elec Value Caliper, Grainger International, Inc, Lake Forest, IL) was used to quickly measure the oxygen penetration depth (bright red band) of the sample. Two measurements were taken for each steak, 2.54 cm from ventral and dorsal end. The measurement was taken from the edge of the surface exposed to oxygen to the edge of the oxymyoglobin band within the steak. The mean of the two measurements was used for statistical analysis.

Instrumental Color (Colorimeter)

Instrumental color was assessed after each frozen storage period once steaks were removed from packaging film, prior to oxygen penetration measurements. Instrumental color measurements were collected using the L*, a*, b* scales with a Minolta CR-400 colorimeter (Minolta, Osaka, Japan) set with a D65 illuminant, 2° observer. The 8 mm

opening of the handheld colorimeter was covered with an oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO). The colorimeter was set to record and print the mean of six readings per steak. Calibration was done through the oxygen permeable film prior to each day of use and with the white ceramic tile (Calibration Plate, Serial No. 14933058, Konica Minolta, Japan) and the D65 settings were set as follows: Y=93.13, x=0.3164, and y=0.330. Once calibrated, the color space was selected to be the L*, a*, and b* scale; where L* is a measure of darkness to lightness, a* is a measure of greenness to redness, and b* is a measure of blueness to yellowness.

Delta E measures the magnitude of difference in L*, a*, and b* color space. Delta E was calculated for all frozen storage periods using the initial color readings taken during fabrication and the final readings when steaks were removed from the freezer in the frozen state for each individual steak. Delta E was then calculated using the equation $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ from the values obtained by the colorimeter (Hunt et al., 2012). The a*:b* ratio was calculated using the equation provided by the American Meat Science Association Meat Color Measurement Guidelines (Hunt et al., 2012) with the values obtained from the colorimeter. The a*:b* ratio was calculated using the a* and b* values from the final readings when steaks were removed from the freezer in the frozen state. The a*:b* ratio was calculated by using the equation $a^* \div b^*$. Hue angle was calculated based on the equation provided in the American Meat Science Association Meat Color Measurement Guidelines (Hunt et al., 2012) with the values obtained from the colorimeter. Hue angle was calculated based on the a* and b* values, once steaks

were removed from the freezer while still frozen. Hue angle (HA) was calculated as $HA = [\arctangent(b^*/a^*)]$ (Hunt et al., 2012).

Instrumental Color (Spectrophotometer)

Instrumental color for the spectrophotometer measurements were assessed after each frozen storage period and once steaks were removed from packaging film, prior to oxygen penetration measurements. Color was evaluated on steaks while frozen under a plastic oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO). Six readings were taken on each steak using a portable Quality Spec Trek (Malvern Panalytical, Longmont, CO, USA) with a spectral range of 350-2500 nm and a wavelength accuracy of ± 1.0 nm after calibration with a white tile and oxygen permeable film. Reflectance values at 473, 525, 572, and 730 nm were obtained and used to calculate percent oxymyoglobin, deoxymyoglobin, and metmyoglobin for each reading using the equations provided by the American Meat Science Association Meat Color Measurement Guidelines on page 58 (Krzywicki, 1979; Hunt et al., 2012). Statistical analyses were calculated using the mean of the six myoglobin state calculations obtained from each reflectance values reading.

$$\text{Equation 1: } A = \log \frac{1}{R}$$

$$\text{Equation 2: } \%MMB = \left\{ 1.395 - \left[\frac{(A_{572} - A_{730})}{(A_{525} - A_{730})} \right] \right\} \times 100$$

$$\text{Equation 3: } \%DMB = \left\{ 2.375 \times \left[1 - \frac{(A_{473} - A_{730})}{A_{525} - A_{730}} \right] \right\} \times 100$$

$$\text{Equation 4: } \%OMB = 100 - (\%MMB + \%DMB)$$

Redness ratio was assessed after each frozen storage period and once steaks were removed from packaging film, prior to oxygen penetration measurements. Redness ratio was obtained using the Quality Spec[®] Trek (Malvern Panalytical, Longmont, CO, USA) as previously described. Reflectance values of 580 and 630 nm were used to calculate a redness ratio. The equation $630\text{nm} \div 580\text{ nm}$ was used to calculate the redness ratio (Hunt et al., 2012). The mean of the six readings per steak was used for statistical analysis of redness ratio.

Subjective Color (Visual Discoloration)

Visual discoloration was assessed after each frozen storage period and once steaks were removed from packaging film, prior to oxygen penetration measurements. Steaks were assessed with a trained five-person panel composed of graduate students from the University of Nebraska-Lincoln. Students were trained prior to evaluating steaks with a color guide that was also used as a reference when evaluating individual steaks. A percentage scale was used where 0% meant no discoloration and 100% meant complete surface discoloration.

Lipid Oxidation

Lipid oxidation was determined using the 2-thiobarbituric acid reactive substances (TBARS) method for all steaks following all frozen storage periods, according to Ahn et al. (1998). Five grams of powdered meat were placed into a 50-mL conical tube to which 14 mL of distilled deionized water and 1 mL of butylated hydroxyanisole (BHA) solution (10% BHA: 90% ethanol) were added. Samples were homogenized using a Polytron homogenizer (POLYTRON Kinimatica CH-6010, Switzerland) for 15 s at

medium-to-high speed. The samples were centrifuged ($2,000 \times g$ for 5 min at 10 °C) and 1 mL of supernatant was transferred into a 15-mL conical tube with 2 mL of 2-Thiobarbuturic acid/Trichloroacetic acid solution (15% trichloroacetic acid and 20 mM 2-Thiobarbuturic acid in deionized distilled water). Tubes were then placed in a 70 °C water bath for 30 min. Tubes were cooled for at least 10 min in a water bath (22 °C) and centrifuged ($2,000 \times g$ for 15 min at 10 °C). Two hundred μL of supernatant were transferred to a 96-well plate in duplicate. Absorbance values were then read at 540 nm using a microplate spectrophotometer (Model Epoch, Biotek, Winooski, VT). Results were expressed in mg of malonaldehyde per kg of tissue.

Statistical Analysis

Statistical analyses were conducted with SAS (version 9.4, Cary, NC). Data were analyzed as a split-split plot design with aging time as the whole-plot, frozen storage as the split-plot and a three by two factorial of oxygenation level and packaging film as the split-split plot. Frozen storage period was analyzed as an incomplete block design with each loin containing two random storage periods. Data were analyzed using the PROC GLIMMIX procedure of SAS and loin was the experimental unit. All means were separated with the LS MEANS statement with statistical significance determined at $P < 0.05$. Interactions with frozen storage as a significant term were analyzed using the slice function in SAS.

Results and Discussion

Tables are presented with least square means based on statistical interactions. In Table 1, the ANOVA table for P -values are reported with all main effects and possible

interactions. Table 2 displays the means for the selected interactions of aging time, packaging films, and frozen storage duration for the different myoglobin oxygenation levels. Means for the interaction of aging time and packaging films are displayed in Table 3. Table 4 contains the means for the interactions of aging time, packaging films, and frozen storage periods. In table 5, the interaction of aging time, frozen storage, and myoglobin oxygenation level are displayed. Finally, Table 6 contains the interactions for myoglobin oxygenation level, packaging film, aging time, and frozen storage. Figures follow the order in which they are presented in the results.

Oxygen Penetration

Oxygen penetration had a packaging by myoglobin oxygenation level interaction ($P = 0.0004$; Table 2). Oxygen penetration or the depth of the bright red cherry color, was significantly greater for the HiOxy steaks regardless of packaging ($P < 0.0001$; Figure 1). As expected, HiOxy steaks had a higher oxygen penetration depth since they were packaged in a high-oxygen MAP prior to frozen storage. The higher oxygen pressure environment allowed oxygen to penetrate deeper into the meat leading to a thicker oxymyoglobin layer. It is well established in the literature that high-oxygen MAP produces a redder meat product than meat that is exposed to atmospheric oxygen (Kim et al., 2010; Kim et al., 2012; Li et al., 2012). The Oxy and DeOxy treatments packaged in the permeable film had higher amounts of oxygen penetration than steaks packaged in the impermeable film ($P < 0.005$). The increase in oxygen penetration between the two packaging films can be attributed to the oxygen permeable film allowing oxygen to penetrate into the meat during freezing and frozen storage, creating a greater depth of oxygen penetration.

A three-way interaction between myoglobin oxygenation level, aging time, and frozen storage period was also observed for oxygen penetration depth ($P < 0.0001$; Table 5). The HiOxy steaks, regardless of aging time, had the greatest oxygen penetration depth compared to Oxy and DeOxy at 0 and 4 months frozen storage ($P < 0.0001$). However, at 2 months the HiOxy 20 d steaks had the greatest depth of oxygen penetration and at 6 months the HiOxy steaks from beef aged 4 d had the greatest depth ($P < 0.001$). The DeOxy and Oxy steaks had lower amounts of oxygen penetration most likely due to the limited oxygen exposure prior to freezing. Researchers have reported that an increase in aging improves the initial color intensity by minimizing the competition for oxygen between myoglobin and mitochondria, allowing the steaks to have a higher blooming capacity (MacDougall, 1982; Bekhit and Faustman, 2005). The greater depth of oxygen penetration at 2 months of frozen storage for the HiOxy 20 d aged steaks, compared to HiOxy 4 d aged steaks, could likely be attributed to the meat being aged, leading to a larger capacity to bloom due to the decrease in oxygen consumption with postmortem aging. Based on our results the HiOxy steaks had greater oxygen penetration values and within myoglobin oxygenation levels the permeable packaging aided in obtaining a thick oxymyoglobin layer. It is important to note that oxygen penetration depth values were minimal and more precise methods may need to be developed for greater accuracy.

Instrumental Color (L^ , a^* , b^*)*

Significant interactions for L^* included aging time by myoglobin oxygenation level ($P = 0.0416$; Table 2) and aging time by frozen storage period ($P < 0.0001$; Table 4). For the interaction of aging time by myoglobin oxygenation level, the HiOxy steaks aged for 4 d were lighter than all other treatments ($P < 0.05$). The HiOxy steaks aged for

20 d were lighter than the DeOxy steaks aged for 20 d ($P = 0.0301$). There was an interaction between aging time and frozen storage period. Steaks aged for 4 d were lighter than steaks aged for 20 d after 0 and 6 months of frozen storage. However, at 2 months steaks aged for 20 d were lighter than steaks aged for 4 d. Typically, meat that has been aged expresses a higher L^* value which MacDougall (1982) attributed to the structural changes in the muscle proteins during aging. Vitale et al. (2014) found similar results in meat aged for just 3 days, which had higher L^* , a^* , and b^* values than unaged meat at the initial start of retail display. Research has well established that high oxygen MAP causes higher L^* values compared samples that are vacuum packed (Kim et al., 2011; Kim et al., 2012; Li et al., 2012; Bonny et al., 2017). Kim et al. (2011) attributed the increase in lightness to an elevated oxygenation of myoglobin under an oxygen-rich condition which was observed with our HiOxy 4 d steaks. According to McKenna et al. (2005), L^* plays a minimal role in predicting color stability since muscles of high color stability were on opposite ends of the L^* spectrum in their study.

When comparing the a^* values for different treatments, a significant interaction between packaging and aging time ($P = 0.0065$; Table 3) and myoglobin oxygenation level by packaging ($P < 0.0001$; Table 2) was observed. There was also a significant three-way interaction between myoglobin oxygenation level, aging time, and frozen storage ($P = 0.0118$; Table 5). The interaction of packaging film and aging time showed that steaks aged for 4 d and frozen in the permeable film had the highest a^* values while steaks aged for 4 d, frozen in impermeable film and 20 d and frozen in permeable film were intermediate and steaks that were packaged in the impermeable film and aged for 20 d had the lowest a^* values ($P < 0.05$). Postmortem aging has an impact on meat color,

such that aged beef has a higher blooming capacity than non-aged beef and that aged beef discolors at a faster rate than non-aged beef (Hood, 1980; MacDougall, 1982; Mancini and Ramanathan, 2014; Vitale et al., 2014; English et al., 2016). The high blooming capacity of aged meat can be attributed to the diminished mitochondrial activity and reduced oxygen consumption with longer aging times. Conflicting results have been documented in the literature with several researchers finding no color differences with varying postmortem aging periods (Ledward, 1970; Ledward, 1971). On the contrary, Vitale et al. (2014) found that the initial a^* values were higher for meat that was aged compared to unaged meat. English et al. (2016) found similar results with steaks aged for 42 and 62 days having higher a^* values than 21-day steak at the start of retail display. However, our results contradict their results, especially at 0 months of frozen storage where steaks aged for 4 d had greater a^* values than 20 d within each myoglobin oxygenation level ($P < 0.05$). With an increase in frozen storage though, the difference of a^* values between aging periods decreased. The difference in our results could be related to the postmortem aging time that was selected. Aging for 4 d postmortem could have provided enough of a reduction in oxygen consumption that allowed for optimal blooming conditions and high a^* values.

Regardless of packaging, for the myoglobin oxygenation level and packaging film interaction, the HiOxy steaks had higher a^* values than the other treatments, shown in Figure 2 ($P < 0.0001$). While packaging did not impact the a^* values for HiOxy steaks, the Oxy and DeOxy steaks exhibited higher a^* values when packaged in the permeable film ($P < 0.05$) when compared to the impermeable film. The DeOxy steaks that were packaged in the impermeable film had the lowest a^* values ($P < 0.0001$). This can be

attributed to the limited oxygen exposure prior to packaging and freezing along with the fact that the impermeable packaging prohibited oxygen from coming in contact with the steaks.

The significant three-way interaction of myoglobin oxygenation level, frozen storage duration, and aging time displayed that the HiOxy steaks that were aged for 4 d and frozen for only 0 months had higher a^* values compared to all other myoglobin oxygenation levels, post mortem aging times, and frozen storage duration ($P < 0.05$). The next highest a^* values were the HiOxy steaks aged for 20 d and frozen for 0 months which was higher than all other treatments besides the HiOxy 4 d steaks at 0 months ($P < 0.05$). Within 0, 2, and 4 mo of frozen storage, the HiOxy steaks, regardless of aging time, had significantly higher a^* values than the other myoglobin oxygenation levels ($P < 0.05$). However, following 6 months of frozen storage, the HiOxy steaks 4 d age were the reddest and the HiOxy steaks aged 20 d were similar to the other myoglobin oxygenation levels ($P < 0.001$). It was expected that the HiOxy steaks would have the highest a^* values. It has been reported that high-oxygen MAP produces initially higher a^* values compared to packaging films (Kim et al., 2010; Kim et al., 2012; Li et al., 2012). The HiOxy steaks were exposed to a large amount of oxygen prior to freezing compared to the other treatments, allowing the oxygen to bind, create oxymyoglobin, and produce the higher a^* value and bright red cherry color. Current studies support similar findings in that freezing meat decreases the redness and color stability of frozen meat (Kim et al., 2018; Setyabrata and Kim, 2019). Holman et al. (2018) found a decrease in myoglobin content across an increase in frozen storage. With a decrease in myoglobin content, it is expected that a decrease in redness would occur, which Wanous et al. (1989), Brewer and

Wu (1993), and Vieira et al. (2009) found as storage time increased, similar to our results. In summary, high a^* values were easily obtained in meat that had been packaged in a high oxygen environment prior to freezing, oxygen permeable film improved the a^* values for steaks with limited oxygen exposure prior to freezing, a^* values decreased with frozen storage, and 4 d of aging appeared to provide optimal a^* compared to 20 d of aging.

There were four significant two-way interactions for b^* values including myoglobin oxygenation level by packaging ($P = 0.0022$; Table 2), myoglobin oxygenation level by aging time ($P = 0.0299$; Table 2), myoglobin oxygenation level by frozen storage duration ($P < 0.0001$; Table 2), and packaging by aging time ($P = 0.0197$; Table 3). The b^* values interaction between myoglobin oxygenation level and packaging was very similar to the a^* values. The HiOxy steaks regardless of film had the highest b^* values ($P < 0.0001$). Several researchers have found similar results with high-oxygen MAP increasing b^* values compared to other packaging systems (Li et al., 2012; Bonny et al., 2017). The impermeable film had lower b^* values for both the DeOxy and Oxy states ($P < 0.0005$). For the interaction of myoglobin oxygenation level by aging time, the HiOxy steaks aged for 4 d had the highest b^* values compared to the other treatments ($P < 0.0001$). The DeOxy steaks aged for 20 d had the lowest b^* values compared to the other treatments ($P < 0.0001$). Myoglobin oxygenation level by frozen storage duration showed the HiOxy steaks frozen for only 0 months had the highest b^* values compared to all other myoglobin oxygenation levels and frozen storage periods ($P < 0.05$). Within the 0 months of frozen storage the HiOxy steaks had the highest b^* values followed by Oxy, and DeOxy ($P < 0.0001$). However, at both 2 and 4 months, Oxy and DeOxy were no

longer different and at 6 months of frozen storage the only difference was between HiOxy and DeOxy steaks ($P = 0.0049$). With a significant interaction of packaging by aging time ($P = 0.0197$), steaks that were aged for 4 d then packaged in the permeable film had the highest b^* values followed by 4 d impermeable film, 20 d permeable film, and 20d impermeable film ($P < 0.05$). While differences in b^* values were observed, little value has been placed on the importance of b^* values in relation to meat color.

Instrumental Color (Percent Oxymyoglobin, Percent Deoxymyoglobin, and Percent Metmyoglobin)

There were four significant two-way interactions for percent oxymyoglobin: myoglobin oxygenation level by packaging film ($P < 0.0001$; Table 2), packaging film by aging ($P = 0.0130$; Table 3), myoglobin oxygenation level by aging ($P = 0.0073$; Table 2), and myoglobin oxygenation level by frozen storage period ($P = 0.0002$; Table 2). In Figure 3, the DeOxy steaks that were packaged in impermeable film had the lowest percent oxymyoglobin ($P < 0.0001$). Within Oxy and DeOxy, steaks packaged in permeable film had significantly more percent oxymyoglobin than steaks in the impermeable film ($P < 0.0001$). However, when comparing the impermeable films, the HiOxy steaks had more percent oxymyoglobin than Oxy and DeOxy ($P < 0.0001$). The high concentration of oxygen prior to freezing for the HiOxy steaks, particularly in the impermeable packaging, allowed more oxygen to bind to myoglobin and contribute to a larger percent oxymyoglobin. The difference in packaging films demonstrates the importance of a permeable film allowing oxygen to pass through and bind to the myoglobin molecule, increasing the percentage of oxymyoglobin.

The interaction of aging time by packaging film showed that steaks aged for 4 d and frozen in the permeable film had the most oxymyoglobin followed by steaks aged for 20 d in the permeable film, and then steaks packaged in impermeable film, regardless of aging time ($P = 0.0130$). The same trend was observed for a^* values which supports the finding of Mohan et al. (2010) who reported a strong relationship between reflectance spectrophotometric data for myoglobin redox forms and colorimetric values. The use of a completely impermeable package leads to heme pigments that are able to reduce to the deoxymyoglobin state and then become oxygenated once the package is open and exposed to air (Clydesdale and Francis, 1971; Jeremiah, 2001). However, after frozen storage, the DeOxy steaks may not have been able to bloom as well with exposure to oxygen. This could be attributed to a reduction in MRA or depletion of reducing equivalents such as NADH following frozen storage.

The interaction of myoglobin oxygenation level and aging time was also significant ($P = 0.0073$). The HiOxy steaks aged for 4 d had the highest percent oxymyoglobin compared to the other treatments ($P < 0.05$). Regardless of aging the DeOxy steaks had the lowest percent oxymyoglobin with Oxy being intermediate of the treatments ($P = 0.0073$). Myoglobin oxygenation level by frozen storage duration showed that the HiOxy steaks, within each frozen storage period, had the highest percent oxymyoglobin followed by Oxy and then DeOxy as seen in Figure 4 ($P = 0.0002$). With an increase in frozen storage, a decrease in percent oxymyoglobin for all myoglobin oxygenation levels was observed ($P = 0.0002$). As expected, the HiOxy steaks had high levels of percent oxymyoglobin. The literature has shown that high-oxygen MAP produces initially higher percent oxymyoglobin compared to other packaging treatments

(Kim et al., 2010; Kim et al., 2012; Li et al., 2012). Overall, the percent oxymyoglobin values support the findings observed with the a^* values in that HiOxy steaks in permeable packaging, and shorter frozen storage periods enhanced the bright red cherry color consumers desire.

The percent deoxymyoglobin had a significant interaction between aging and frozen storage ($P = 0.0009$; Table 4) and myoglobin oxygenation level, packaging, and frozen storage ($P = 0.0025$; Table 6). The interaction of aging time and frozen storage showed that within 0 and 2 months of frozen storage there were no statistical differences between 4 and 20 d aged steaks, but at 4 and 6 months steaks aged for 20 d had significantly more percent deoxymyoglobin ($P = 0.0009$). A significant three-way interaction of myoglobin oxygenation level, packaging, and frozen storage ($P = 0.0025$) was also present. Regardless of frozen storage period, steaks packaged in impermeable film in the DeOxy myoglobin oxygenation level had significantly higher percent deoxymyoglobin ($P = 0.0025$). Since the DeOxy steaks had limited exposure to oxygen prior to packaging it was expected that they would have the largest percent deoxymyoglobin initially at frozen storage. Li et al. (2012) also found that vacuum packaging in impermeable films yields a higher content of deoxymyoglobin compared to packaging with oxygen. This is because metmyoglobin formed at low oxygen pressure in vacuum packaging can be reduced back to deoxymyoglobin when reducing components of the MRA cycle are present. While there were statistically significant differences for percent deoxymyoglobin, it is important to note that values were obtained based on the isobestic wavelength calculations that could lead to small fluctuations in values. Based on our knowledge, this is the first study to use a portable spectrophotometer and the

equations by Krzywicki (1979) on frozen steaks which may attribute to the negative values.

Myoglobin oxygenation level by packaging film ($P < 0.0001$; Table 2), packaging film by aging ($P = 0.0250$; Table 3), packaging film by frozen storage ($P < .0001$; Table 4), and myoglobin oxygenation level by aging by frozen storage ($P = 0.0188$) were all significant interactions for percent metmyoglobin. While packaged in impermeable film the DeOxy steaks had the highest amount of percent metmyoglobin followed by the Oxy steaks in the impermeable film ($P < 0.0001$) for the interaction of myoglobin oxygenation level by packaging film. There was no difference in metmyoglobin percent between packaging film for the HiOxy steaks as noted in Figure 5 ($P < 0.05$). The interaction of packaging film and aging time showed that the steaks packaged in the impermeable film had significantly higher percent metmyoglobin values, regardless of aging ($P = 0.0250$). Our findings contradict Greene (1969), Jeremiah (2001), and Li et al. (2012) who assert that anaerobic packaging through the use of vacuum packaging in impermeable films can prevent metmyoglobin formation and rancidity with sufficient MRA in fresh meat. However, as the permeability of the film decreases, a low partial pressure of oxygen is reached where oxidation is favored, forming metmyoglobin (Clydesdale and Francis, 1971). This may be why our percent metmyoglobin values are higher for impermeable films than permeable films in that we could have had ideal partial pressure in our packaging due to limited oxygen exposure prior to packaging and frozen storage.

At 0 months of frozen storage, no difference in the percent of metmyoglobin between packaging film was observed for frozen storage duration and packaging film. There was a significant difference between packaging films at 2, 4, and 6 months with

impermeable film having higher percent metmyoglobin values than permeable films ($P < 0.0001$). For the interaction of myoglobin oxygenation level, aging time, and frozen storage duration we found that at 0 months of frozen storage there were no significant differences among myoglobin oxygenation level and aging ($P < 0.05$). Yet, the difference in myoglobin oxygenation level and aging exacerbated metmyoglobin differences over time ($P = 0.0188$). Throughout frozen storage at 2, 4, and 6 months the HiOxy steaks aged for 4 d had significantly less metmyoglobin percent than the DeOxy steaks, regardless of aging time ($P = 0.0188$). The increase in percent metmyoglobin can most likely be attributed to the decrease in metmyoglobin reducing enzymes that have been well documented with extended frozen storage (Wanous et al., 1989; Farouk and Swan, 1998; Vieira et al., 2009). Discoloration is the accumulation of metmyoglobin on the surface of meat. It has been estimated that meat departments lose 5.4% of sales each year due to fresh meat discoloration (Bekhit and Faustman, 2005). Therefore, it is important to note that myoglobin oxygenation level, aging, and packaging impact the percent metmyoglobin; frozen storage duration is detrimental and leads to the accumulation of metmyoglobin.

Delta E

Delta E was measured as the change in color space over time therefore, a large delta E indicates a greater change in color over time. There was a significant interaction between packaging and frozen storage duration ($P = 0.0047$; Table 4), as well as myoglobin oxygenation level, aging time, and frozen storage duration ($P = 0.0057$; Table 5) for delta E. With increasing frozen storage, a larger delta E value was observed for steaks frozen in impermeable film and was similar for permeable film steaks

($P = 0.0047$). This can be attributed to steaks having a lower MRA as longer frozen storage causes more discoloration and greater change in color over time. The interaction of myoglobin oxygenation level, aging time, and frozen storage duration showed steaks that were packaged in impermeable film for 0 months had the lowest delta E values compared to all other frozen storage periods ($P < 0.005$). The main difference when comparing steaks at different frozen storage durations, aging periods, and myoglobin oxygenation level was that the HiOxy steaks aged for 20 d and frozen for 6 months had significantly higher delta E values than all other treatments ($P < 0.0001$). Ledward et al. (1986) concluded that delta E values over 3.0 were very obvious to most observers. Based on our results, regardless of myoglobin oxygenation level, aging time, packaging film, and frozen storage period, all of the mean values would be over the 3.0 value, especially the HiOxy 20 d aged steaks which had over a threefold value of what consumers observe as an obvious change. With extended frozen storage, it was expected that meat would change color over time largely due to the association of frozen storage with an increase in discoloration. Setyabrata and Kim (2019) attribute the decrease in color stability to the denaturation of myoglobin and reduced myoglobin redox system caused by ice crystal damage. While freezing meat has been noted to alter the color (Ramsbottom and Koonz, 1941; Kiani and Sun, 2011; Kim et al., 2018), it was not expected that freezing for a short duration would have such a profound effect on meat color. With such a short duration and large change in delta E, it is evident that packaging and freezing play a large role in meat color.

Redness Ratio

Redness ratio had two significant two-way interactions between myoglobin oxygenation level and frozen storage period ($P < 0.0001$; Table 2) and aging time and frozen storage period ($P < 0.0001$; Table 4). There was also a significant three-way interaction between myoglobin oxygenation level, packaging film, and aging time ($P = 0.0485$; Table 6). The ratio of 630/580 nm was established to indicate the change in meat color due to the formation of metmyoglobin. Therefore, it can be used to evaluate surface color stability (Hunt, 1980; Joseph et al., 2012; Li et al., 2017). Larger ratios indicate a larger amount redness due to oxymyoglobin and therefore, a ratio of 1.0 would essentially be 100% metmyoglobin (Strange et al., 1974; Hunt et al., 2012; Joseph et al., 2012; Li et al., 2017).

At 0 months of storage, there was a significant difference between all myoglobin oxygenation levels with HiOxy having the highest redness ratio followed by Oxy and then DeOxy ($P < 0.0001$). Nonetheless, at 2, 4, and 6 months, the redness ratio for HiOxy steaks was significantly higher than Oxy and DeOxy that were similar with extended frozen storage ($P < 0.05$). The results for redness ratio are similar to the results for percent oxymyoglobin and a^* values, reinforcing that HiOxy steaks have greater redness compared to steaks minimally exposed to oxygen throughout frozen storage. Gatellier et al. (2005) found a high correlation between beef redness when comparing a^* values and the ratio of 630/580. However, Khlijji et al. (2010) found that a^* values were more strongly related to consumer perception of redness than the 630/580 ratio.

The interaction of aging time and frozen storage duration showed steaks that were aged for 4 d had significantly higher redness ratios than the 20 d aged steaks for 0, 4, or 6

months ($P < 0.0001$). Yet, at 2 months of frozen storage, the steaks that were aged for 20 d had a higher redness ratio than 4 d ($P = 0.0358$). The significant three-way interaction between myoglobin oxygenation level, packaging film, and aging time demonstrated that steaks that were aged for 4 d and in the HiOxy myoglobin oxygenation level treatment had the highest redness ratio regardless of packaging ($P < 0.05$). The lowest redness ratio was the DeOxy steaks packaged in impermeable film and aged for 20 d prior to freezing ($P < 0.0001$). However, when comparing within an aging period, HiOxy steaks were superior (greater redness), regardless of packaging, for both 4 and 20 d of aging ($P < 0.0001$). Within 4 d of aging for both Oxy and DeOxy, the permeable packaging film gave higher redness ratios than the impermeable packaging ($P < 0.05$). Typically, impermeable films can lead to less discoloration, but they tend to yield darker steaks (Mancini et al., 2009) which is similar to our results where 4 d of aging and impermeable packaging had a lower redness ratio and a^* values. Morrissey et al. (2008) reported that when the ratio fell below 3.5, consumers thought the color was more brown than red and unacceptable, but Khliji et al. (2010) found the threshold to be 3.3 or lower and Jacob et al. (2007) found it to be below 3.0. Redness ratio is an indicator of brown and the discoloration values observed in our study were fairly low, suggesting that steaks have an acceptable red color even at a threshold of 3.0. However, the varying results may be attributed to our steaks being frozen compared to the studies conducted on fresh beef.

$a^:b^*$ Ratio*

Aging had a significant main effect on the $a^*:b^*$ ratio ($P = 0.0002$). The mean $a^*:b^*$ ratio for steaks aged 4 d was 1.97 compared to steaks aged 20 d at 2.09. A frozen storage by myoglobin oxygenation level by packaging interaction was also observed for

the $a^*:b^*$ ratio ($P = 0.0442$; Table 6). During frozen storage, DeOxy steaks that were stored for 0 months in the impermeable film had a higher $a^*:b^*$ ratio than all other treatments at 0 months ($P < 0.0001$). However, at 2, 4, and 6 months of frozen storage, there was little significant difference among treatments. Setser (1984) and Hunt et al. (2012) indicated that larger ratios of $a^*:b^*$ indicate more redness and less discoloration. This supports our findings of having higher $a^*:b^*$ values with short frozen storage because meat that has been stored for shorter periods of time will be redder and less discolored. Research has shown that storage duration can impact the quality of meat (Wanous et al., 1989; Brewer and Wu, 1993; Farouk and Swan, 1998; Vieira et al., 2009). However, it is interesting to note that a higher $a^*:b^*$ values were observed for meat aged for 20 d ($P = 0.0002$). While a^* values were greater for 4 d so were the b^* values resulting in a lower $a^*:b^*$ ratio. The slight decrease in $a^*:b^*$ value highlights that meat loses redness and started to discolor with extended storage.

Hue Angle

Hue angle had a significant aging main effect ($P = 0.0004$). The mean hue angle for 4 d aged steaks was 0.47 and 0.45 for the 20 d steaks, respectively. There was also a packaging by frozen storage duration interaction for hue angle ($P = 0.0001$; Table 4). Steaks packaged in impermeable film at 6 months had a greater hue angle than the permeable film packaged steaks ($P < 0.05$). Steaks that were frozen in the impermeable film for 0 months of frozen storage was lower than the permeable film ($P = 0.0001$). Tapp et al. (2011) found hue angle to represent the change of color from red to yellow with large angles indicating less red meat and an increase in discoloration over time. The increase in hue angle supports the notion that frozen storage duration leads to an increase

in discoloration and decrease in redness also evident in oxygen penetration, a^* values, percent oxymyoglobin, and redness ratio.

Subjective Color (Visual Discoloration)

Discoloration is caused by an accumulation of metmyoglobin on the surface of beef due to the oxidation of oxymyoglobin and deoxymyoglobin. Packaging film had no effect on discoloration for frozen steaks. However, a three-way interaction between myoglobin oxygenation level, aging, and storage period was found to be significant for discoloration ($P < 0.0001$; Table 5). Discoloration was low ($<1\%$) for all treatments throughout frozen storage except at 6 months of frozen storage for the HiOxy 20 d aged steaks exhibited a mean of 18.80% discoloration. According to Hood and Riordan (1973), a level of 20% discoloration during retail display, reduced beef sales by 50%. Even at lower levels of discoloration, consumer discrimination against discolored meat was observed. It has been reported that 15% of retail beef is discounted due to discoloration resulting in an economic loss of \$1 billion annually (Mancini and Hunt, 2005). Typically, metmyoglobin can be reduced back to deoxymyoglobin and oxygenated to oxymyoglobin (bright cherry red color), however, this is no longer possible when reducing components of the MRA are depleted. Many factors influence MRA, but in particular Hood (1980) found that aged beef discolours at a quicker rate compared to fresh beef due to the decay of mitochondrial activity and depletion of MRA. While this provides an explanation for our discoloration values in frozen steaks it does not support our values for percent metmyoglobin that do not follow as close to our discoloration results as one would expect. This could be attributed to very low discoloration values that were subjectively measured by the trained panel.

Kim et al. (2010) found that myoglobin oxidation can occur in high-oxygen MAP leading to the formation of metmyoglobin on the surface of the meat. Research has also demonstrated that high-oxygen MAP promotes oxidative changes in meat affecting both the color stability and discoloration more than other packaging methods (Jayasingh et al., 2002; Grobbel et al., 2008a; Grobbel et al., 2008b; Zakrys et al., 2008; Mancini et al., 2009; Kim et al., 2010). Lipid oxidation is one of the main causes of quality loss in meat during storage and processing leading to rancidity and discoloration (Renner, 1999).

Lipid Oxidation

Lipid oxidation had a significant three-way interaction between myoglobin oxygenation level, aging time, and frozen storage period ($P = 0.0224$; Table 5). At both 4 and 6 months of frozen storage, the HiOxy steaks aged for 20 d had significantly higher TBARS values than all other treatments ($P < 0.05$). At 2 months of storage, the HiOxy steaks aged for 20 d had higher TBARS values than all treatments except the HiOxy 4 d steaks ($P < 0.0001$). Over the 6 months of frozen storage, HiOxy steaks aged for 20 d had the greatest increase in malonaldehyde values (Figure 6). According to Campo et al. (2006), consumers were able to detect a rancid flavor that overpowered the beef flavor in beef steaks at 2.28 mg malonaldehyde/ kg tissue using the TBARS method which is higher than any treatment at any frozen storage period. Therefore, even with the larger TBARS value for HiOxy 20 d aged steaks, oxidation in this study would not be a large concern for consumer acceptance since the values fell under the 2.28 mg malonaldehyde/kg tissue threshold amount.

Lipid oxidation has been noted as one of the largest factors impacting color and flavor and is a main cause of quality loss in meat during storage and processing, leading

to rancidity and discoloration (Renner, 1999; Faustman et al., 2010). Postmortem aging has been documented to increase lipid oxidation (Faustman and Cassens, 1990; Epley, 1992; Jakobsen and Bertelsen, 2000; Joseph et al., 2012; Bonny et al., 2017). While not always significant, aging steaks tended to cause higher TBARS values within the myoglobin treatments within this study. Faustman and Cassens (1990) attribute the increase in lipid oxidation from postmortem aging to a decrease in the endogenous antioxidant activity that aids in limiting oxidation. English et al. (2016) found that aging decreased the MRA, increased lipid oxidation, and had a detrimental effect on color stability. A relationship between lipid oxidation and myoglobin oxidation has been well documented (Greene, 1969; Wanous et al., 1989; Akamittath et al., 1990; Faustman and Cassens, 1990; Faustman et al., 2010; Bonny et al., 2017). However, in this study while discoloration was higher at 6 months of frozen storage for the HiOxy steaks aged 20 d similar to lipid oxidation, the same trend was not evident at 2 and 4 months for discoloration as seen in lipid oxidation. The lipid oxidation results, while a bit more divergent than discoloration, showed that the HiOxy steaks aged for 20 d at 6 months of frozen storage exhibited the largest discoloration and lipid oxidation. With the similar patterns between discoloration and lipid oxidation, it reasserts the relationship between lipid and myoglobin oxidation.

As expected, an upward trend of TBARS values was noted with an increase in frozen storage duration. Other researchers have found that as extended frozen storage increases, an increase in lipid oxidation, odor intensity, tenderness, and a decrease in metmyoglobin reducing enzymes have been noted (Wanous et al., 1989; Farouk and Swan, 1998; Vieira et al., 2009). Soyer et al. (2010) and Setyabrata and Kim (2019)

attribute this to the structural changes that are induced by ice crystallization, which increases meat susceptibility to oxidative damage due to ruptured cell membranes releasing prooxidant compounds into the muscle, accelerating oxidation.

Steaks that have been packaged in a highly oxygenated environment have been found to have an increase in oxidation (Jayasingh et al., 2001; Zakrys et al., 2008; Kim et al., 2010; Bonny et al., 2017). Researchers have shown that high-oxygen MAP causes greater lipid oxidation compared to other packaging treatments (Jayasingh et al., 2002; Zakrys et al., 2008; Kim et al., 2010; Bonny et al., 2017). The increase in oxidation from high-oxygen MAP can lead to the development of off-flavors and beef flavors that consumers find unappealing (Grobbel et al., 2008b; Zakrys et al., 2008; Kim et al., 2010). Since the HiOxy steaks were packaged in a high-oxygen MAP for 24 h it is not surprising that they typically had higher TBARS values than the other myoglobin level treatments. Overall, there is little difference in lipid oxidation expect for the HiOxy steaks aged for 20 d with extended frozen storage.

Conclusion

The HiOxy steaks exhibited a brighter and deeper cherry red color compared to the DeOxy steaks. The HiOxy steaks were superior or similar when compared to Oxy steaks, but displayed more detrimental effects when frozen storage was extended beyond 4 months. Steaks aged for 4 d typically displayed a redder color than 20 d steaks. Permeable packaging allowed oxygen to pass through the packaging, leading to a bright cherry red color of the frozen meat compared to impermeable packaging. Extended frozen storage had a negative impact on meat color and meat quality. Based on the

results, HiOxy steaks aged for 4 d give a superior red color for frozen storage up to 4 months with few unfavorable color effects. However, it is not advised to freeze deoxygenated steaks and expect a cherry red color through frozen storage.

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Table 1. Analysis of variance *P*-Values for each analysis and possible interactions including: aging time (4 d or 20 d), packaging films (impermeable or permeable), frozen storage (0, 2, 4, or 6 months), and myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹).

Analysis	Oxygen Penetration	L*	a*	b*	Oxy-myoglobin, %	Deoxy-myoglobin, %	Met-myoglobin, %	Delta E	Redness Ratio [†]	a*: b* ratio	Hue Angle	Discoloration	Lipid Oxidation
Aging Time	0.2241	0.1887	<0.0001	<0.0001	0.0143	0.0059	0.5976	0.0001	<0.0001	0.0002	0.0004	0.0788	0.0183
Frozen Storage	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0240	0.0169
Aging Time*Frozen Storage	<0.0001	<0.0001	0.1695	0.3623	0.2296	0.0009	0.4598	0.0217	<0.0001	0.3692	0.5605	0.0267	0.3029
Myoglobin Oxygenation Level	<0.0001	0.0005	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0366	0.0918	0.0041	<0.0001
Aging Time*Myoglobin Oxygenation Level	0.9457	0.0416	0.0105	0.0299	0.0073	0.2136	0.0003	<0.0001	0.0188	0.2779	0.2257	0.0047	0.0164
Frozen Storage*Myoglobin Oxygenation Level	<0.0001	0.0684	<0.0001	<0.0001	0.0002	<0.0001	0.0095	<0.0001	<0.0001	0.0244	0.1436	<0.0001	0.0232
Aging Time*Frozen Storage*Myoglobin Oxygenation Level	<0.0001	0.5891	0.0118	0.0833	0.0686	0.4519	0.0188	0.0057	0.7083	0.3134	0.1427	<0.0001	0.0224
Packaging Film	<0.0001	0.1940	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.9919	<0.0001	0.8655	0.3633	0.2239	0.0809
Aging Time*Packaging Film	0.3755	0.1145	0.0065	0.0197	0.0130	0.0622	0.0250	0.0559	0.3045	0.7581	0.7983	0.2365	0.7441

¹Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

[†] Redness Ratio - Reflectance values for 630nm ÷ 580 nm

Table 1 (continued). Analysis of variance *P*-Values for each analysis and possible interactions including: aging time (4 d or 20 d), packaging films (impermeable or permeable), frozen storage (0, 2, 4, or 6 months), and myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹).

Analysis	Oxygen Penetration	L*	a*	b*	Oxy-myoglobin, %	Deoxy-myoglobin, %	Met-myoglobin, %	Delta E	Redness Ratio [†]	a*: b* ratio	Hue Angle	Discoloration	Lipid Oxidation
Frozen Storage* Packaging Film	0.0619	0.6141	0.6053	0.2105	0.9767	0.0012	<0.0001	0.0047	0.4721	<0.0001	0.0001	0.2177	0.5205
Aging Time*Frozen Storage* Packaging Film	0.9294	0.9719	0.7482	0.6373	0.6210	0.4275	0.6541	0.0718	0.8872	0.8537	0.7974	0.2399	0.9398
Myoglobin Oxygenation Level*Packaging Film	0.0004	0.1602	<0.0001	0.0022	<0.0001	<0.0001	<0.0001	0.1048	0.0280	0.0670	0.1359	0.2444	0.7800
Aging Time* Myoglobin Oxygenation Level*Packaging Film	0.6338	0.2556	0.5508	0.8639	0.1624	0.3489	0.0814	0.0787	0.0485	0.5761	0.5372	0.2636	0.9163
Frozen Storage* Myoglobin Oxygenation Level*Packaging Film	0.1899	0.4553	0.0882	0.0859	0.1104	0.0025	0.2899	0.5859	0.5406	0.0442	0.2592	0.2042	0.5899
Aging Time* Frozen Storage* Myoglobin Oxygenation Level*Packaging Film	0.5679	0.8708	0.8683	0.8675	0.6511	0.9515	0.3181	0.8133	0.6896	0.7802	0.7405	0.2351	0.5272

¹Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

[†] Redness Ratio - Reflectance values for 630nm ÷ 580nm

Table 2. The effects of aging (4 d or 20 d), packaging films (impermeable or permeable), and frozen storage (0, 2, 4, or 6 months) for different myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹) on color variables of steaks (n = 72; n = 72; n = 36).

Variable	Age	Myoglobin Oxygenation Level			P-Value	SEM	
		DeOxy	Oxy	HiOxy			
Oxymyoglobin, %	L*	4	46.43 ^{bc}	46.28 ^{bc}	47.31 ^a	0.0416	0.2848
		20	45.85 ^c	46.38 ^{bc}	46.45 ^b		
	b*	4	6.87 ^b	7.02 ^b	8.06 ^a	0.0299	0.1534
		20	5.40 ^d	6.09 ^c	7.04 ^b		
		4	65.17 ^d	69.15 ^{bc}	73.57 ^a	0.0073	0.5757
		20	63.72 ^d	68.88 ^c	70.45 ^b		
Packaging							
Oxygen Penetration (mm)	Impermeable	.04 ^d	.06 ^c	.11 ^a	0.0004	0.0027	
	Permeable	.07 ^b	.07 ^b	.11 ^a			
a*	Impermeable	11.45 ^e	12.38 ^d	15.28 ^a	<0.0001	0.1921	
	Permeable	13.21 ^c	13.74 ^b	15.48 ^a			
b*	Impermeable	5.68 ^d	6.27 ^c	7.47 ^a	0.0022	0.1320	
	Permeable	6.59 ^b	6.83 ^b	7.63 ^a			
Oxymyoglobin, %	Impermeable	58.58 ^d	66.20 ^c	71.48 ^{ab}	<0.0001	0.5184	
	Permeable	70.31 ^b	71.82 ^a	72.54 ^a			
Metmyoglobin, %	Impermeable	31.98 ^a	31.01 ^b	29.32 ^c	<0.0001	0.2650	
	Permeable	28.77 ^{cd}	28.56 ^d	28.80 ^{cd}			
Frozen Storage							
b* ³	0	6.37 ^c	7.51 ^b	9.01 ^a	<0.0001	0.1828	
	2	5.66 ^b	5.77 ^b	7.01 ^a			
	4	5.79 ^b	5.92 ^b	6.89 ^a			
	6	6.71 ^{bc}	7.01 ^{ab}	7.31 ^a			
Oxymyoglobin, % ³	0	68.12 ^c	75.47 ^b	78.89 ^a	0.0002	0.7482	
	2	65.29 ^c	69.43 ^b	73.47 ^a			
	4	62.53 ^c	66.54 ^b	68.95 ^a			
	6	61.83 ^c	64.60 ^b	66.73 ^a			
Redness Ratio ^{2,3}	0	2.52 ^c	2.82 ^b	3.63 ^a	<0.0001	0.0520	
	2	2.39 ^b	2.41 ^b	2.78 ^a			
	4	1.99 ^b	2.05 ^b	2.29 ^a			
	6	2.15 ^b	2.15 ^b	2.29 ^a			

¹ Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

² Redness Ratio - Reflectance values for 630nm ÷ 580 nm

³ Means within each row with different superscripts are different ($P < 0.05$).

^{a-c} Means within the same variable with different superscripts are different ($P < 0.05$).

Table 3. The effects of aging time (4 d or 20 d) for different packaging films (impermeable or permeable) on color variables of steaks (n = 108).

Variable	Age	Packaging Film		P-Value	SEM
		Impermeable	Permeable		
a*	4	13.59 ^b	15.06 ^a	0.0065	0.1914
	20	12.49 ^c	13.23 ^b		
b*	4	6.94 ^b	7.69 ^a	0.0197	0.1406
	20	6.01 ^d	6.35 ^c		
Oxymyoglobin, %	4	65.77 ^c	72.72 ^a	0.0130	0.5125
	20	65.08 ^c	70.28 ^b		
Metmyoglobin, %	4	30.90 ^a	28.42 ^b	0.0250	0.2633
	20	30.64 ^a	29.01 ^b		

^{a-d} Means within the same variable with different superscripts are different ($P < 0.05$).

Table 4. The effects of aging time (4 d or 20 d) and packaging films (impermeable or permeable), for different frozen storage periods (0, 2, 4, or 6 months) on color variables of steaks (n = 54).

Variable	Age	Frozen Storage				P-Value	SEM	
		0	2	4	6			
L*	4	46.29 ^a	46.35 ^b	46.78 ^a	47.27 ^a	<0.0001	0.3372	
	20	44.89 ^b	47.99 ^a	47.51 ^a	44.51 ^b			
	Deoxymyoglobin, %	4	.37 ^a	1.18 ^a	1.72 ^b	.93 ^b	0.0009	0.5102
		20	.31 ^a	1.81 ^a	3.71 ^a	4.13 ^a		
Redness Ratio ¹	4	3.41 ^a	2.45 ^b	2.36 ^a	2.46 ^a	<0.0001	0.0474	
	20	2.57 ^b	5.60 ^a	1.86 ^b	1.94 ^b			
Metmyoglobin, %	Packaging							
	Impermeable	25.53 ^a	30.06 ^a	32.59 ^a	34.91 ^a	<0.0001	0.3417	
	Permeable	25.16 ^a	28.16 ^b	30.18 ^b	31.34 ^b			
Delta E	Impermeable	4.06 ^b	5.92 ^a	6.44 ^a	6.90 ^a	0.0047	0.2993	
	Permeable	5.11 ^a	5.95 ^a	5.85 ^a	6.42 ^a			
Hue Angle	Impermeable	.42 ^b	.45 ^a	.47 ^a	.51 ^a	0.0001	0.0050	
	Permeable	.43 ^a	.45 ^a	.46 ^a	.50 ^b			

¹ Redness Ratio - Reflectance values for 630nm ÷ 580 nm

^{a-e} Means within each column for each variable with different superscripts are different (P < 0.05)

Table 5. The effects of aging time (4 d or 20 d) by frozen storage (0, 2, 4, or 6 months) by myoglobin oxygenation level (DeOxy, Oxy, or HiOxy¹) on color variables of steaks (n = 18).

Variable	Frozen Storage	4 d			20 d			P-Value	SEM
		DeOxy	Oxy	HiOxy	DeOxy	Oxy	HiOxy		
Oxygen Penetration (mm)	0	.05 ^{cd}	.07 ^b	.14 ^a	.05 ^d	.07 ^{bc}	.13 ^a	<0.0001	0.0037
	2	.05 ^c	.05 ^c	.08 ^b	.07 ^b	.07 ^b	.11 ^a		
	4	.08 ^b	.08 ^b	.13 ^a	.06 ^c	.07 ^{bc}	.13 ^a		
	6	.02 ^e	.04 ^{de}	.10 ^a	.05 ^{cd}	.06 ^{bc}	.07 ^b		
a*	0	15.38 ^d	17.17 ^c	20.40 ^a	13.11 ^e	15.15 ^d	18.79 ^b	0.0118	0.3939
	2	12.75 ^b	12.35 ^{bc}	14.92 ^a	10.37 ^d	11.30 ^c	14.20 ^a		
	4	12.12 ^b	12.12 ^b	13.80 ^a	10.57 ^c	11.04 ^{bc}	14.01 ^a		
	6	13.17 ^b	12.94 ^b	14.78 ^a	11.17 ^c	12.44 ^b	12.17 ^{bc}		
Met-myoglobin, %	0	24.94 ^a	24.86 ^a	24.92 ^a	25.80 ^a	25.37 ^a	26.17 ^a	0.0188	0.5520
	2	29.71 ^{ab}	30.04 ^a	28.12 ^c	29.79 ^a	28.81 ^{ab}	28.20 ^{bc}		
	4	32.06 ^{ab}	31.79 ^{ab}	30.20 ^c	32.90 ^a	31.10 ^{bc}	30.27 ^{bc}		
	6	34.11 ^a	34.16 ^a	30.97 ^b	33.71 ^a	32.18 ^b	33.64 ^a		
Delta E	0	5.40 ^a	3.77 ^c	3.88 ^{bc}	5.24 ^{ab}	4.32 ^{abc}	4.92 ^{abc}	0.0057	0.4923
	2	4.83 ^c	5.60 ^{bc}	6.45 ^{ab}	4.76 ^c	7.13 ^a	6.83 ^{ab}		
	4	4.95 ^b	4.73 ^b	7.10 ^a	4.79 ^b	7.30 ^a	8.01 ^a		
	6	5.42 ^{cd}	4.81 ^d	6.15 ^{bc}	5.75 ^{cd}	7.03 ^b	10.79 ^a		
Discoloration	0	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b	<0.0001	1.7994
	2	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b		
	4	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b		
	6	0 ^b	0 ^b	0 ^b	.13 ^b	.13 ^b	18.80 ^a		
Lipid Oxidation	0	.50 ^{ab}	.44 ^b	.76 ^a	.70 ^{ab}	.72 ^{ab}	.88 ^{ab}	0.0224	0.1643
	2	.50 ^c	.50 ^c	1.03 ^{ab}	.88 ^{bc}	.73 ^{bc}	1.49 ^a		
	4	.59 ^b	.55 ^b	.84 ^b	.96 ^b	.95 ^b	1.42 ^a		
	6	.59 ^b	.66 ^b	.87 ^b	.89 ^b	.87 ^b	2.00 ^a		

¹ Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

^{a-c} Means within each row for each variable with different superscripts are different ($P < 0.05$).

Table 6. The effects of myoglobin oxygenation level (DeOxy, Oxy, or HiOxy¹) by packaging films (impermeable or permeable) by either aging (4 d or 20 d) or frozen storage (0, 2, 4, or 6 months) on color variables of steaks (n = 72; n = 36).

Variable	Age	Impermeable			Permeable			P-Value	SEM
		DeOxy	Oxy	HiOxy	DeOxy	Oxy	HiOxy		
Redness Ratio ²	4	2.37 ^e	2.44 ^{de}	2.90 ^a	2.66 ^{bc}	2.74 ^b	2.92 ^a	0.0485	0.0520
	20	1.90 ^g	2.09 ^f	2.52 ^{cd}	2.13 ^f	2.16 ^f	2.65 ^{bc}		
Deoxymyoglobin, % ³	Storage							0.0025	0.6998
	0	12.62 ^a	1.59 ^b	-4.58 ^c	.09 ^b	-3.07 ^c	-4.60 ^c		
	2	9.33 ^a	2.99 ^b	-1.51 ^d	.60 ^c	-.70 ^{cd}	-1.74 ^d		
	4	8.40 ^a	3.63 ^b	1.39 ^c	1.79 ^c	.62 ^c	.47 ^c		
	6	7.39 ^a	2.94 ^b	1.50 ^{bc}	1.22 ^{bc}	1.62 ^{bc}	.53 ^c		
a*/b* ratio ³	0	2.42 ^a	2.21 ^b	2.22 ^b	2.17 ^b	2.17 ^b	2.18 ^b	0.0442	0.0352
	2	2.06 ^a	2.03 ^a	2.11 ^a	2.09 ^a	2.09 ^a	2.09 ^a		
	4	1.94 ^b	1.95 ^{ab}	2.02 ^{ab}	2.00 ^{ab}	1.99 ^{ab}	2.03 ^a		
	6	1.81 ^{ab}	1.77 ^b	1.84 ^{ab}	1.85 ^{ab}	1.87 ^a	1.86 ^a		

¹ Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

² Redness Ratio - Reflectance values for 630nm ÷ 580 nm

³ Means within each row with different superscripts are different ($P < 0.05$).

^{a-e} Means within the same variable with different superscripts are different ($P < 0.05$).

Figure Legends

Figure 1. Oxygen penetration values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and impermeable or permeable packaging.

^{a-d} Different superscripts indicated differences among treatments ($P < 0.05$).

Figure 2. Instrumental color values for a^* of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and impermeable or permeable packaging.

^{a-c} Different superscripts indicated differences among treatments ($P < 0.05$).

Figure 3. Instrumental color values for percent oxymyoglobin of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and impermeable or permeable packaging.

^{a-c} Different superscripts indicated differences among treatments ($P < 0.05$).

Figure 4. Instrumental color values for percent oxymyoglobin of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and frozen for either 0, 2, 4, or 6 months.

^{a-c} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Figure 5. Instrumental color values for percent metmyoglobin of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and impermeable or permeable packaging.

^{a-d} Different superscripts indicated differences among treatments ($P < 0.05$).

Figure 6. TBARS values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, aged for 4 or 20 d and frozen for 0, 2, 4, or 6 months.

^{a-c} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Figure 1.

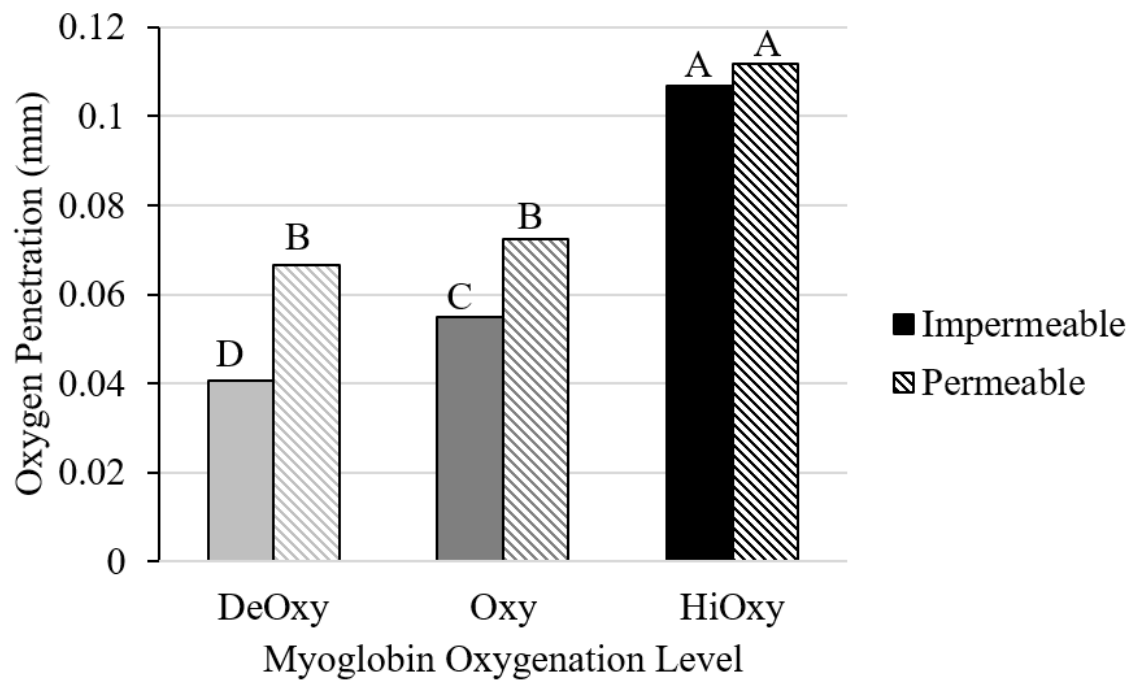


Figure 2.

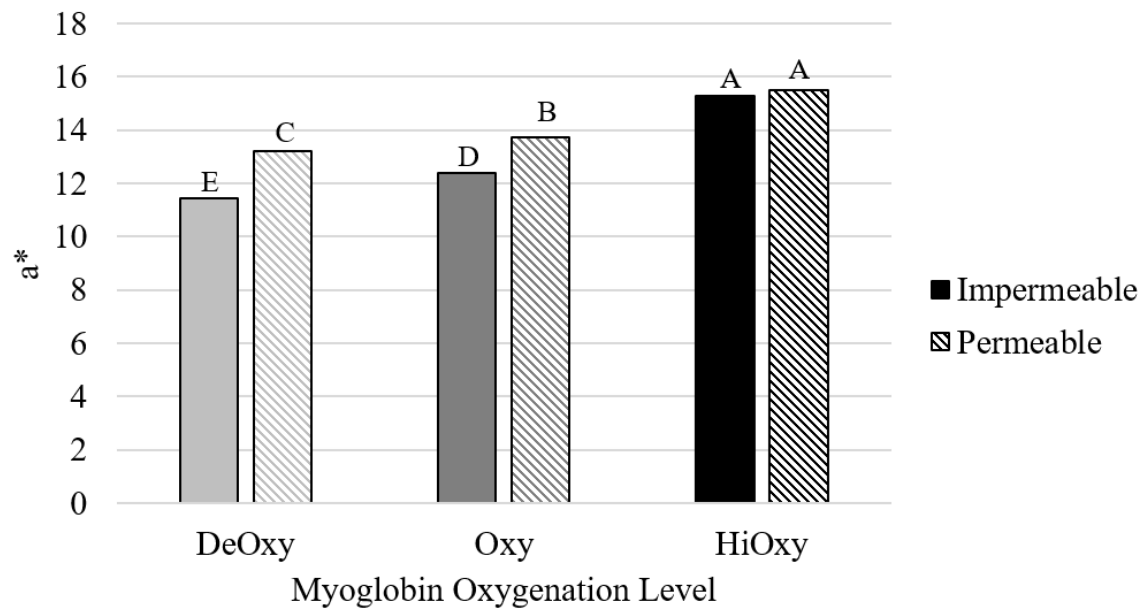


Figure 3.

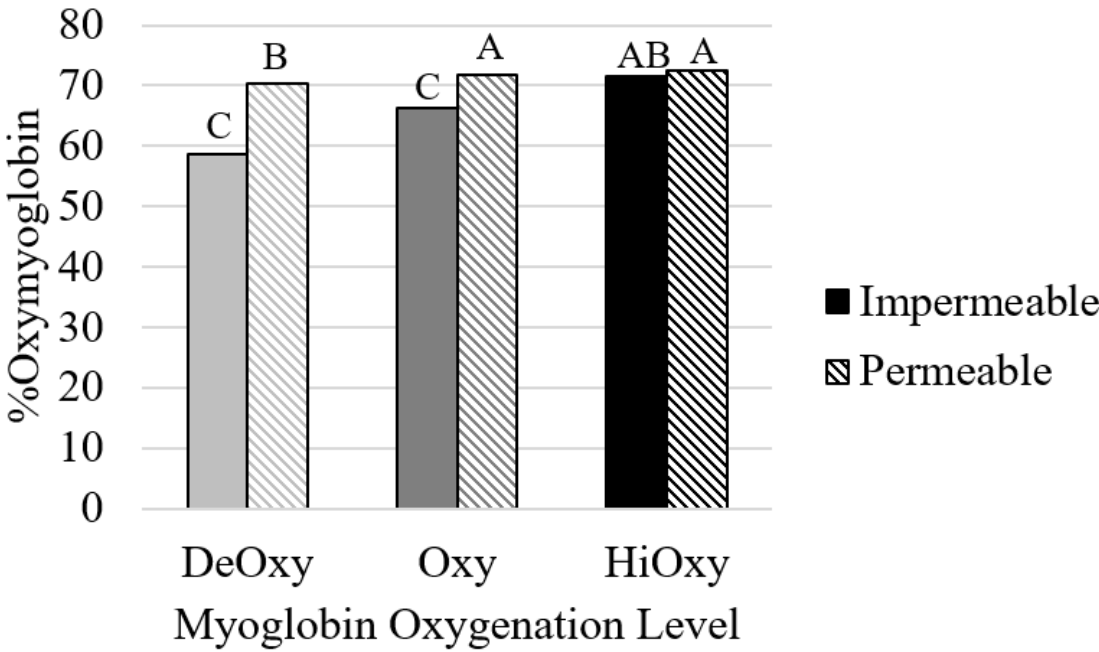


Figure 4.

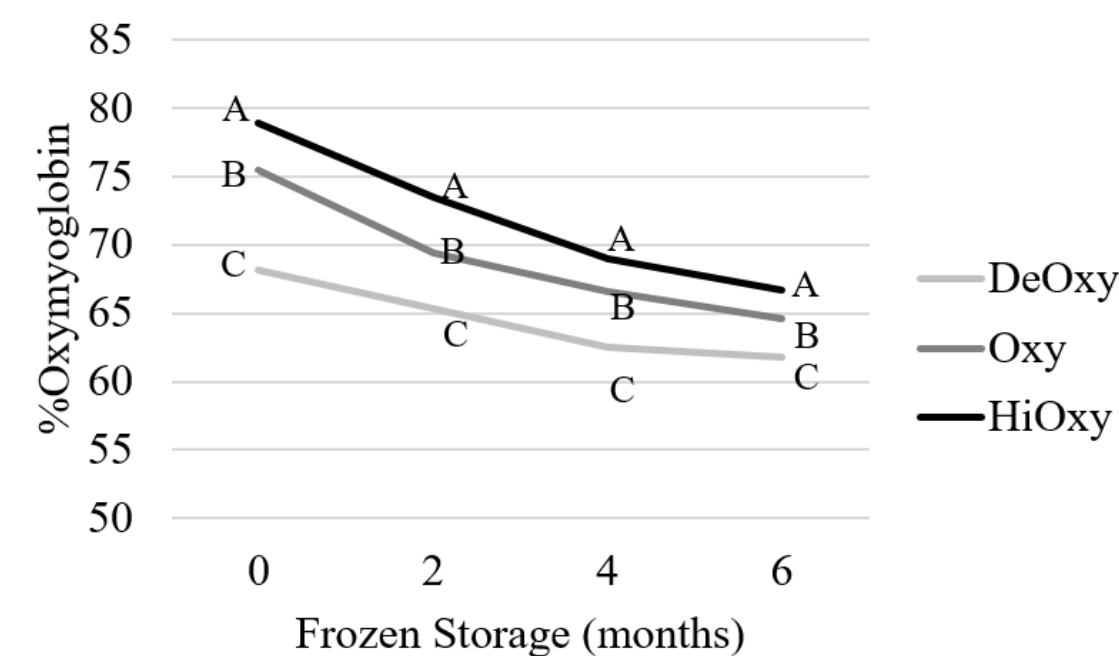


Figure 5.

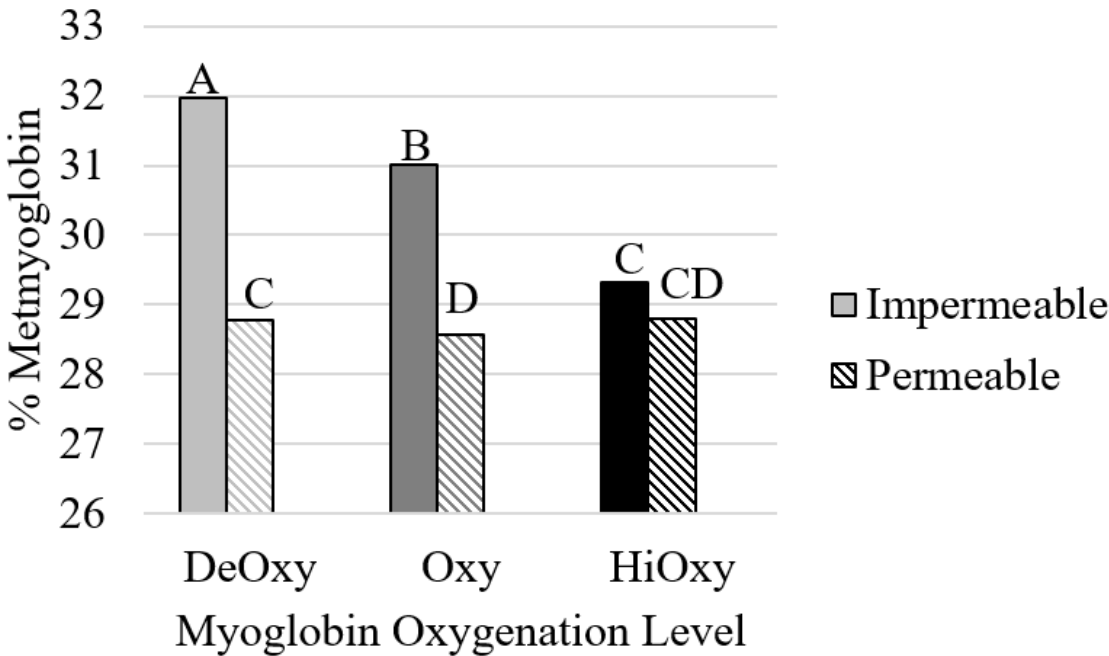
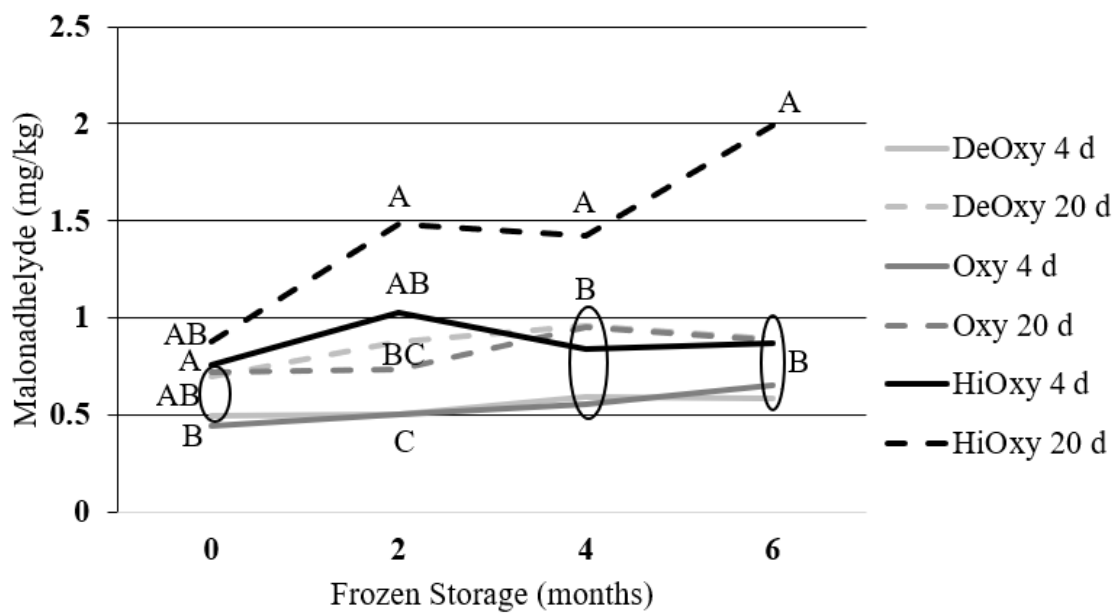


Figure 6.



Impact of Myoglobin Oxygenation State Prior to Frozen Storage on Color Stability of Thawed Beef Steaks through Retail Display

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Abstract

Consumers consider beef color to be an indicator of freshness and therefore is a major factor when purchasing beef. The ideal conditions for freezing beef and maintaining that color following frozen storage throughout retail display have yet to be established. Therefore, the objective of this study was to determine how myoglobin oxygenation levels during freezing affect color stability of thawed steaks during retail display (RD) and to determine the impact that frozen storage duration, aging, and packaging films have on meat color after thawing for RD. USDA Choice strip loins ($n=36$) were aged for 4 d or 20 d. Steaks were randomly assigned to a myoglobin oxygenation level [deoxymyoglobin (DeOxy; packaged within 2 m), oxygenation (Oxy; oxygenated in air for 30 minutes), or high oxygenation (HiOxy; packaged for 24 h in 80% O₂)]. Steaks were then vacuum packaged in oxygen permeable or impermeable film and immediately frozen (-5° C). Following either 0, 2, 4, or 6 months of frozen storage, steaks were removed from the packaging and immediately placed under simulated RD conditions. During RD instrumental color and subjective color were measured every day after the initial 24 h thaw period. Steaks were analyzed for instrumental color (L^* , a^* , b^*), percent oxymyoglobin, metmyoglobin, and deoxymyoglobin, delta E, redness ratio, $a^*:b^*$ ratio, hue angle, subjective discoloration, and lipid oxidation. For all days of RD, steaks that were frozen for 0 months had significantly higher a^* values (greater redness) than steaks frozen for 6 months which typically had the lowest a^* values ($P < 0.0001$). HiOxy steaks frozen for 6 months had the lowest amounts of percent oxymyoglobin than all other treatments on days 4 to 7 of RD ($P < 0.05$). The HiOxy steaks frozen for 4 and 6 months had significantly more percent metmyoglobin than DeOxy and Oxy, regardless of packaging ($P < 0.05$). Delta E, discoloration, and lipid oxidation were greatest for HiOxy

steaks compared to Oxy and DeOxy ($P < 0.05$). Extended storage brought about detrimental color effects for all differing levels of myoglobin oxygenation. The HiOxy steaks through the first few days of RD and frozen for under 6 months provided optimal meat color, similar to that of DeOxy and Oxy. However, with extended frozen storage and RD, HiOxy steaks had worse color characteristics than the other myoglobin oxygenation levels.

Keywords: oxymyoglobin; discoloration; frozen storage; retail display

Introduction

Consumers consider beef color to be an indicator of freshness and therefore it is a major factor consumers consider when purchasing beef (Hood, 1980; Kropf, 1980; Carpenter et al., 2001). Optimal beef color is a bright red cherry color, however that bright red cherry color is prone to discoloration. Hood and Riordan (1973) reported that 20% discoloration of steaks during retail display (RD), reduced beef sales by 50%. Even at lower levels of discoloration, consumer discrimination against discolored meat was observed. Under normal display conditions in fresh meat, there is often a mixture of deoxymyoglobin, oxymyoglobin, and metmyoglobin. An accumulation of metmyoglobin on the surface of beef due to the oxidation of oxymyoglobin and deoxymyoglobin leads to a greater amount of discoloration. Typically, metmyoglobin can be reduced back to deoxymyoglobin and oxygenated to oxymyoglobin (bright cherry red color), however, this is no longer possible when reducing components of the metmyoglobin reducing activity cycle are depleted. The three myoglobin states are continuously changing and depend on several intrinsic (pH, species, breed, sex, animal maturity, muscle variability, oxygen consumption, metmyoglobin reducing activity, and lipid oxidation) and extrinsic factors (diet, electrical stimulation, postmortem aging, temperature, frozen storage, and packaging) (Hood, 1980; Renner, 1990; Mancini and Hunt, 2005; Suman and Joseph, 2013).

The frozen beef market has recently started to grow since freezing beef provides the opportunity to extend the product shelf life, however, freezing has detrimental effects on meat quality (MacDougall, 1982; Kim et al., 2015). With the emerging market for frozen beef products having developed in recent years partly due to meal delivery service

companies with a market segment of \$4.65 billion in 2017 and is expected to grow to \$11.6 billion by 2022 (Progressive Grocer, 2017). Therefore, it is vital to have a better understanding of the factors that impact thawed meat color.

Retail display (RD) has detrimental effects on meat color due to the decrease in color stability of both frozen and thawed meat (Kim et al., 2018; Setyabrata and Kim, 2019). Setyabrata and Kim (2019) attribute the decrease in color stability caused by freezing to the denaturation of myoglobin and a reduction in the myoglobin redox system caused by ice crystal damage. To our knowledge, it appears that no prior research has focused on the myoglobin oxygenation levels prior to freezing and the impact that has on meat color once thawed in RD. Therefore, the objectives of this study were to determine how myoglobin oxygenation levels during freezing affect color stability of thawed steaks during RD and to determine the impact that frozen storage duration, length of aging, and packaging films have on meat color after thawing for RD.

Materials and Methods

Sample Collection

Thirty-six USDA Choice strip loins were vacuumed packaged and obtained from Greater Omaha Packaging, Omaha, NE prior to being transported to the Loeffel Meat Lab at the University of Nebraska-Lincoln. Prior to fabrication loins were aged for either 4 or 20 d post-harvest ($2 \pm 5^{\circ}\text{C}$) under dark storage.

Sample Fabrication

For each aging period, 4 or 20 d, 18 loins were fabricated anterior to posterior where a 0.318 cm steak was removed at both anterior and posterior ends to expose fresh

surfaces. Loins were split in half into loin segments and loin segments were randomly assigned to a frozen storage period of 0 (frozen for 1 d), 2, 4, or 6 months (nine loin segments for each frozen storage period) prior to fabrication. Each loin contained only two frozen storage periods forming the incomplete block design. Within each frozen storage period, six steaks were cut with a slicer into 1.27 cm steaks for each myoglobin oxygenation level and packaging film combination for a total of 432 steaks in the study. The steaks were randomly preassigned to a myoglobin oxygenation level and packaging film. Myoglobin oxygenation level was designated as deoxymyoglobin (DeOxy), oxygenation (Oxy), and high oxygenation (HiOxy). The DeOxy steaks were immediately vacuum sealed and placed in the freezer within 3 min after slicing. The Oxy steaks were sliced, and oxygenated in air at atmospheric pressure for 30 min, vacuum sealed, and frozen. The HiOxy steaks were sliced and packaged for 24 h in a modified atmosphere packaging (MAP) mixture of 80% O₂ and 20% CO₂ before being repackaged in oxygen permeable or impermeable film, and frozen. A KOCH tray sealer with a MAP option (Koch Equipment LLC, Kansas City, MO) was used with the 80% O₂ and 20% CO₂ mixture. Steaks were randomly assigned to a packaging film and vacuum sealed with a Multivac Packaging machine (Multivac C500, Multivac, Kansas City, MO). Packaging films consisted of oxygen permeable (Cryovac Shrink Bags, 10K OTR Fresh Fish Non-Barrier Straight End Seal, Sealed Air, Charlotte, NC) and oxygen impermeable film (3 mil STD barrier, Ultra Source, Kansas City, MO). The oxygen permeable film had an oxygen transmission rate (OTR) of at least 10,000 cc/m²/24 h at standard temperature and pressure (STP; 0°C and 10⁵ Pa), classifying it as a high transmitter of oxygen (Calvert, 1990). The oxygen impermeable film had an OTR of 6.77 cc/m²/24 h at STP, making it a

high barrier film. Steaks were then frozen at $-10 \pm 3^{\circ}\text{C}$ on flat trays in a single layer for 3 hours. After the initial freezing steaks were then transferred into boxes and stored in the dark at $-5 \pm 3^{\circ}\text{C}$ for either 0 (frozen for 1 d), 2, 4, or 6 months. Following frozen storage, steaks were removed from the packaging film treatment and placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with a different oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO) and immediately set out for retail display (RD) where they were thawed for 24 h prior to any instrumental readings. Trays were placed under simulated RD conditions on tables in a meat cooler for 7 d ($3 \pm 1^{\circ}\text{C}$ under white fluorescence lighting at 1,000 to 1,800 lux; Lamp type: F32T8/TL741 700 Series 32 watt, Phillips, Inc., New Jersey) and randomly rotated daily. During retail display, instrumental color and subjective color were measured every day after the initial 24 h thaw period. Following the last day of retail display, oxygen penetration was also measured before steaks were repacked (3 mil STD barrier, Ultra Source, Kansas City, MO), vacuum sealed, and frozen (-80°C) for further analysis of lipid oxidation. Lipid oxidation was measured one month following the completion of the last frozen storage period. Later, samples were trimmed of subcutaneous fat, frozen in liquid nitrogen, and then powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples were stored at -80°C for approximately two weeks prior to lipid oxidation analysis.

Oxygen Penetration

After 7 days of RD, steaks were removed from RD trays and a cut was made 2.54 cm from the lateral end of the steak. A Westward caliper (6"/150 mm Elec Value Caliper, Grainger International, Inc, Lake Forest, IL) was used to quickly measure the oxygen

penetration depth (bright red band) of the sample. Two measurements were taken for each steak, 2.54 cm from ventral and dorsal end. The measurement was taken from the edge of the surface exposed to oxygen to the edge of the oxymyoglobin band within the steak. The mean of the two measurements was used for statistical analysis.

Instrumental Color (Colorimeter)

Instrumental color was measured every day of retail display starting on day 1 (24 hours after being placed in retail display), prior to oxygen penetration measurements. Instrumental color measurements were collected using the L*, a*, b* scales with a Minolta CR-400 colorimeter (Minolta, Osaka, Japan) set with a D65 illuminant, 2° observer. The 8 mm opening of the handheld colorimeter was covered with an oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO). The colorimeter was set to record and print the mean of six readings per steak. The colorimeter was set to record and print the mean of six readings per steak. Calibration was done through the oxygen permeable film prior to each day of readings with the white ceramic tile (Calibration Plate, Serial No. 14933058, Konica Minolta, Japan) and the D65 settings were set as follows: Y=93.13, x=0.3164, and y=0.330. Once calibrated, the color space was selected to be the L*, a*, and b* scale; where L* is a measure of darkness to lightness, a* is a measure of greenness to redness, and b* is a measure of blueness to yellowness.

Delta E was measured as the magnitude of difference in L*, a*, and b* color space. Delta E was calculated using the initial color readings taken on day 1 of RD and the final readings taken on day 7 of RD for each individual steak. Delta E was then calculated using the equation $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Hunt et al., 2012). The

$a^*:b^*$ ratio ($a^* \div b^*$) was calculated using the equation provided by the American Meat Science Association Meat Color Measurement Guidelines (Hunt et al., 2012). The $a^*:b^*$ ratio was calculated using the a^* and b^* values from the instrumental color values that were collected daily during RD. Hue angle was calculated based on the equation provided in the American Meat Science Association Meat Color Measurement Guidelines (Hunt et al., 2012) with the values obtained from the colorimeter. Larger ratios indicate more redness and less discoloration for the steaks (Setser, 1984). Hue angle (HA) was calculated as $HA = [\arctangent(b^*/a^*)]$ (Hunt et al., 2012). Hue angle is an indicator of redness and discoloration, with larger angles indicating less red meat and greater discoloration (Tapp et al., 2011).

Instrumental Color (Spectrophotometer)

Instrumental color was assessed each day of retail display starting on day 1 (24 hours after being placed in retail display), prior to oxygen penetration measurements. Color was evaluated on steaks while in RD under a plastic oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO). Six readings were taken on each steak using a portable Quality Spec[®] Trek (Malvern Panalytical, Longmont, CO, USA) with a spectral range of 350-2500 nm and a wavelength accuracy of ± 1.0 nm after calibration with a white tile and oxygen permeable film. Reflectance values at 473, 525, 572, and 730 nm were obtained and used to calculate percent oxymyoglobin, deoxymyoglobin, and metmyoglobin for each reading using the equations provided by the American Meat Science Association Meat Color Measurement Guidelines on page 58 (Krzyszwicki, 1979; Hunt et al., 2012). Statistical

analyses were calculated using the mean of the six myoglobin state calculations obtained from each reflectance values reading.

$$\text{Equation 1: } A = \log \frac{1}{R}$$

$$\text{Equation 2: } \% \text{MMB} = \left\{ 1.395 - \left[\frac{(A_{572} - A_{730})}{(A_{525} - A_{730})} \right] \right\} \times 100$$

$$\text{Equation 3: } \% \text{DMB} = \left\{ 2.375 \times \left[1 - \frac{(A_{473} - A_{730})}{A_{525} - A_{730}} \right] \right\} \times 100$$

$$\text{Equation 4: } \% \text{OMB} = 100 - (\% \text{MMB} + \% \text{DMB})$$

Redness ratio was assessed every day of RD that instrumental color measurements were taken, prior to oxygen penetration measurements. Redness ratio was obtained using the Quality Spec[®] Trek as described above. Reflectance values of 580 and 630 nm were used to calculate a redness ratio of 630 nm ÷ 580 nm (Hunt et al., 2012). The mean of the six readings per steak was used for statistical analyses of redness ratio. Larger ratios or higher values indicate more redness whereas a ratio closer to 1.0 would indicate essentially 100% metmyoglobin (Strange et al., 1974).

Subjective Color (Visual Discoloration)

Visual discoloration was assessed each day of retail display starting on day 1 (24 hours after being placed in retail display), prior to oxygen penetration measurements. Steaks were assessed with a trained five-person panel composed of graduate students from the University of Nebraska-Lincoln. Students were trained prior to evaluating steaks with a color guide that was also used as a reference when evaluating individual steaks. A

percentage scale was used were 0% meant no discoloration and 100% meant complete surface discoloration.

Lipid Oxidation

Lipid oxidation was determined using the 2-thiobarbituric acid reactive substances (TBARS) method for all steaks following 7 d of RD, according to Ahn et al. (1998). Five grams of powdered meat were placed into a 50-mL conical tube to which 14 mL of distilled deionized water and 1 mL of butylated hydroxyanisole (BHA) solution (10% BHA: 90% ethanol) were added. Samples were homogenized using a Polytron (POLYTRON Kinimatica CH-6010, Switzerland) for 15 s at medium-to-high speed. The samples were centrifuged ($2,000 \times g$ for 5 min at 10 °C) and 1 mL of supernatant was transferred into a 15-mL conical tube with 2 mL of 2-Thiobarbuturic acid/Trichloroacetic acid solution (15% trichloroacetic acid and 20 mM 2-Thiobarbuturic acid in deionized distilled water). Tubes were then placed in a 70 °C water bath for 30 min. Tubes were cooled for at least 10 min in a water bath (22 °C) and centrifuged ($2,000 \times g$ for 15 min at 10 °C). Two hundred μL of supernatant were transferred to a 96-well plate in duplicate. Absorbance values were then read at 540 nm using a microplate spectrophotometer (Model Epoch, Biotek, Winooski, VT). Results were expressed in mg of malonaldehyde per kg of tissue.

Statistical Analysis

Statistical analyses were conducted with SAS (version 9.4, Cary, NC). Data were analyzed as a split-split plot design with repeated measures. Aging time was analyzed as the whole-plot, frozen storage as the split-plot, a three by two factorial of oxygenation

level and packaging film as the split-split plot, and retail display as repeated measures. Frozen storage period was analyzed as an incomplete block design with each loin containing two random storage periods. Oxygen penetration, delta E, and lipid oxidation were analyzed without repeated measures since they were measured only once throughout the study for each steak. Redness ratio, percent oxymyoglobin, percent deoxymyoglobin, and percent metmyoglobin were analyzed within aging time and instead as a split-plot design with repeated measures. Frozen storage was the whole-plot and contained an incomplete block design, a three by two factorial of oxygenation level and packaging film as the split-plot, and retail display as repeated measures. The change in analysis for these variables is due to instrumental issues that lead to missing data for 20 d aging at 4 months of frozen storage. Data were analyzed using the PROC GLIMMIX procedure of SAS and loin was the experimental unit. All means were separated with the LS MEANS statement with statistical significance determined at $P < 0.05$. Interactions with frozen storage and/or retail display as significant terms were analyzed using the slice function in SAS.

Results

Tables are presented with least square means based on statistical interactions. In Tables 1 and 2, the ANOVA table for P -values can be found with all possible main effects and interactions. Table 3 displays the means for the interactions of aging time, packaging film, and frozen storage with differing myoglobin oxygenation levels. Interaction of the least square means for myoglobin oxygenation levels, packaging films, and RD are displayed in Table 4. Table 5 shows the means for the interactions of packaging film and myoglobin oxygenation level by aging time or frozen storage. In

Table 6, the interaction of aging time and myoglobin oxygenation level by frozen storage and packaging film are displayed. Table 7 contains the interactions for myoglobin oxygenation level, frozen storage, aging time by RD. In Table 8, the interactions for packaging film aging time, and frozen storage are displayed. Finally, Table 9 presents the interactions for aging time, packaging film, and frozen storage by RD. Figures follow the order in which they are cited in the text.

Oxygen Penetration

Oxygen penetration had three significant two-way interactions: myoglobin oxygenation level by aging time ($P = 0.0002$; Table 3), frozen storage by myoglobin oxygenation level ($P < 0.0001$; Table 3), and frozen storage by packaging film ($P = 0.0108$; Table 8) when measured on steaks after 7 d of RD. The results for the interaction of myoglobin oxygenation level by aging time displayed that within myoglobin oxygenation level, Oxy 4 d steaks had deeper oxygen penetration depth compared to the Oxy steaks aged for 20 d ($P = 0.0193$). With 20 d of aging the DeOxy steaks had greater oxygen penetration depth than Oxy and HiOxy and within each aging period the HiOxy steaks had smaller levels of oxygen penetration depth compared to the other myoglobin oxygenation levels ($P < 0.05$). We attributed the lower levels of oxygen penetration in the HiOxy steaks to the decrease in available reducing equivalents, which were likely used up prior to freezing, and the high oxygen saturation of the meat leading to greater oxidizing conditions. The results for the interaction of frozen storage by myoglobin showed that oxygen penetration depth declined as frozen storage increased ($P < 0.0001$). At 0 and 4 months of frozen storage, HiOxy steaks had less oxygen penetration depth compared to DeOxy and Oxy ($P < 0.0001$). However, at 2 months of

frozen storage there was no significant difference in oxygen penetration among myoglobin oxygenation levels. During 6 months of frozen storage, the myoglobin oxygenation levels diverged, with DeOxy having greater oxygen penetration depth than Oxy, and Oxy having more than HiOxy ($P < 0.0001$). When comparing oxygen penetration depths for frozen storage and packaging film, the only difference between films occurred at 4 months of frozen storage with impermeable film being greater than permeable film ($P = 0.0184$). Overall, oxygen penetration depth following retail display was greater for DeOxy and Oxy steaks that were frozen for shorter periods of time. It should be noted that 41 out of the 432 steaks in the study had an oxygen penetration depth of 0 mm attributing to the lower least square means. Oxygen penetration depth values overall were minimal and more precise methods may need to be developed for greater accuracy.

Instrumental Color (L^ , a^* , b^*)*

Four significant three-way interactions were significant for L^* , including myoglobin oxygenation level by packaging film by aging time ($P = 0.0011$; Table 5), myoglobin oxygenation level by packaging film by frozen storage ($P < 0.0001$; Table 5), aging time by packaging film by frozen storage ($P = 0.0051$; Table 8), and aging time by frozen storage by RD ($P < 0.0001$; Table 9). There was an interaction of myoglobin oxygenation level by packaging film by aging time for L^* . Regardless of myoglobin oxygenation levels, steaks that were aged for 20 d and packaged in impermeable film during frozen storage had significantly higher L^* values than all steaks aged for 4 d ($P = 0.0011$). Within 20 d of aging, the DeOxy steaks packaged in permeable film had the lowest L^* values compared to all other steaks that were aged for 20 d ($P < 0.05$).

Myoglobin oxygenation level by packaging film by frozen storage duration was significant and presented in Table 5 ($P < 0.0001$). At 2 months of frozen storage, HiOxy steaks in the permeable films had the highest L^* values compared to all other treatments ($P < 0.0001$). However, within each storage period few differences were observed between treatments. Independent from myoglobin oxygenation level we found that regardless of packaging film the steaks were frozen in, at 0 and 6 months steaks aged for 20 d had significantly higher L^* values than steaks aged for 4 d ($P < 0.05$). The greater L^* values for steaks aged for 20 d could be attributed to the changes in the structure of the muscle proteins that occur during aging, allowing more water near the surface of the meat to reflect the light. During 2 and 4 months of storage, few differences between aging and packaging were observed. While statistical differences were observed for the interaction of aging time, frozen storage, and RD for L^* values, there was no discernible pattern for the differences. Although significant, the differences were of relatively small magnitude and not likely to impact consumer assessment of color. Overall, steaks that were aged for 20 d typically had a greater L^* value or were lighter than steaks aged 4 d.

The a^* values had significant two-way interactions between aging time by RD ($P = 0.0011$; Table 9), frozen storage by RD ($P < 0.0001$; Table 9), and myoglobin oxygenation level by RD ($P < 0.0001$; Table 4). Aging time by RD showed through days 1-3 of RD there were no significant differences between steaks that were aged for 4 or 20 d. However, throughout days 4-7 of RD, steaks aged for 4 d had significantly higher a^* values than 20 d aged steaks ($P < 0.02$). Throughout RD, a decrease in a^* values were observed, regardless of frozen storage period for the frozen storage by RD interaction ($P < 0.0001$). For all days of RD, steaks that were frozen for 0 months had significantly

higher a^* values than steaks frozen for 6 months which typically had the lowest a^* values, 2 and 4 months were intermediate ($P < 0.0001$). The interaction between myoglobin oxygenation level and RD showed that throughout days 4-7 of RD, DeOxy steaks had the highest a^* values followed by Oxy, and then HiOxy. However, at days 2 and 3 of RD, while DeOxy was still the highest, there was no statistical difference between Oxy and HiOxy ($P < 0.0001$).

There was also a significant four-way interaction displayed in Figure 1, between myoglobin oxygenation level, packaging film, aging time, and frozen storage ($P = 0.0096$; Table 6). Besides the DeOxy steaks at 4 d of aging and frozen in the impermeable packaging, HiOxy steaks, regardless of packaging or aging time, had greater a^* values than all other treatments at 0 months of frozen storage ($P < 0.05$). At 0 months of frozen storage the DeOxy and Oxy steaks aged for 4 d, regardless of packaging film type, had significantly higher a^* values than the 20 d steaks with the same myoglobin oxygenation levels ($P < 0.05$). At 4 months of frozen storage, HiOxy steaks aged for 4 d, regardless of packaging type, had significantly lower a^* values than DeOxy and Oxy ($P < 0.05$). However, at 6 months of frozen storage and regardless of age and packaging type, the HiOxy steaks had significantly lower a^* values than all other treatments except the Oxy permeable steaks aged for 20 d ($P = 0.0096$). Overall, extended frozen storage and longer postmortem aging prior to freezing and thawing for retail display lead to steaks with lower a^* values. The HiOxy steaks tended to have lower a^* values than the other myoglobin oxygenation levels when exposed to simulated RD conditions.

There was a significant interaction of myoglobin oxygenation level by frozen storage by RD ($P = 0.0242$; Table 7) and myoglobin oxygenation level by aging time by packaging film by frozen storage ($P = 0.0155$; Table 6) for b^* values. Myoglobin oxygenation level by frozen storage by RD interaction showed that throughout days 1-3 of RD, the HiOxy steaks frozen for 0 months had significantly higher b^* values followed by the DeOxy steaks frozen for 0 months ($P = 0.0242$). However, at 1 d of RD, the HiOxy steaks frozen for 6 months had the lowest b^* value compared to all other treatments ($P < 0.05$). When RD was extended the treatments began to assimilate together. The b^* values decreased for 2, 4, and 6 months of frozen storage, regardless of packaging or aging time ($P = 0.0155$). The significant four-way interaction in Table 6 displayed that the DeOxy steaks in impermeable packaging film aged for 4 d had significantly greater b^* values than all other treatments besides the HiOxy steaks in either packaging type and aged for 4 d at 0 months of frozen storage ($P < 0.05$). However, while significant, the differences for b^* values were of relatively small magnitude and are not likely to impact consumer perception of color.

Instrumental Color (Percent Oxymyoglobin, Percent Deoxymyoglobin, and Percent Metmyoglobin)

Steaks that were aged for 4 d had several significant interactions when measuring percent oxymyoglobin, frozen storage by RD ($P < 0.0001$; Table 9), including packaging film by RD ($P = 0.0118$; Table 9), myoglobin oxygenation level by RD ($P < 0.0001$; Table 4), and myoglobin oxygenation level by frozen storage by packaging ($P < 0.0001$; Table 5). Throughout RD, a decrease in percent oxymyoglobin was observed, regardless of frozen storage period for the frozen storage by RD interaction ($P < 0.0001$). Steaks

frozen for 0 months had significantly more percent oxymyoglobin for all days of RD and steaks frozen for 2 months had more through days 1-3 compared to 4 and 6 months of frozen storage ($P < 0.05$). At the end of RD (day 7), steaks frozen for 0 months had significantly more percent oxymyoglobin than all other frozen storage times ($P < 0.02$). Independent from myoglobin oxygenation level and frozen storage duration, steaks that were packaged in permeable film had more percent oxymyoglobin on days 1 and 5 of RD compared to steaks packaged in impermeable film ($P < 0.05$). Table 4 displays the interaction between myoglobin oxygenation level and RD where throughout RD, DeOxy and Oxy had significantly higher amounts of percent oxymyoglobin than HiOxy from days 2-7 ($P < 0.0001$). However, regardless of myoglobin oxygenation level, a decrease in percent oxymyoglobin was observed as days in RD increased ($P < 0.0001$). The interaction of myoglobin oxygenation level by frozen storage by packaging was significant for percent oxymyoglobin for steaks aged for 4 d ($P < 0.0001$). At 4 and 6 months of frozen storage DeOxy and Oxy steaks, regardless of packaging, had significantly more oxymyoglobin percent than HiOxy steaks ($P < 0.0001$). The HiOxy steaks in the permeable film had larger amounts of percent oxymyoglobin than the HiOxy steaks in the impermeable film at 4 and 6 months of frozen storage ($P < 0.0001$; Figure 2).

Percent oxymyoglobin for steaks aged 20 d had a significant interaction between myoglobin oxygenation level, packaging, and frozen storage ($P = 0.0039$; Table 5) and between myoglobin oxygenation level, frozen storage, and RD ($P < 0.0001$; Table 7). In Table 5, the interaction for myoglobin oxygenation level, packaging, and frozen storage is presented. During 0 and 2 months of frozen storage, regardless of packaging, the

HiOxy steaks had significantly more percent oxymyoglobin than Oxy steaks ($P < 0.05$). However, at 6 months of frozen storage, the DeOxy steaks had more percent oxymyoglobin than the HiOxy steaks, regardless of packaging, and the Oxy steaks were greater in percent oxymyoglobin than HiOxy steaks in impermeable packaging ($P < 0.05$). The interaction between myoglobin oxygenation level, frozen storage, and RD displayed that the HiOxy steaks frozen for 0 months had significantly more percent oxymyoglobin on days 1-3 of RD than all other treatments except DeOxy steaks frozen for 0 months ($P < 0.0001$). Conversely, HiOxy steaks frozen for 6 months had the lowest amounts of percent oxymyoglobin than all other treatments on days 4-7 of RD ($P < 0.05$; Figure 3). It is noteworthy that during every day of RD, the HiOxy steaks that were only frozen for 0 or 2 months had significantly greater amounts of percent oxymyoglobin than the HiOxy steaks frozen for 6 months ($P < 0.0001$). Overall, percent oxymyoglobin decreases during RD and percent oxymyoglobin is negatively impacted by extended frozen storage, especially for steaks exposed to a large amount of oxygen prior to freezing.

Myoglobin oxygenation level by packaging by frozen storage ($P < 0.0001$; Table 5) and myoglobin oxygenation level by frozen storage by RD were both significant interactions for percent deoxymyoglobin for steaks aged for 4 d ($P = 0.0028$; Table 7). Myoglobin oxygenation level by packaging by frozen storage was significant and presented in Table 5. Steaks that were frozen for 0 months as DeOxy in impermeable film had significantly greater percent deoxymyoglobin than the HiOxy/impermeable and DeOxy/permeable treatments ($P = 0.0174$). At 2 months of frozen storage, the HiOxy steaks frozen in impermeable film had significantly more percent deoxymyoglobin than

HiOxy steaks frozen in permeable film ($P = 0.0194$). Throughout frozen storage, an increase in percent deoxymyoglobin was observed at 4 and 6 months for all treatments ($P < 0.0001$). While statistical differences were observed for the interaction of myoglobin oxygenation level, frozen storage, and RD for percent deoxymyoglobin, there was no discernible pattern for the differences.

Percent deoxymyoglobin for steaks aged 20 d had a significant interaction between myoglobin oxygenation level, packaging film, and frozen storage ($P = 0.007$; Table 5) and myoglobin oxygenation level, frozen storage, and RD ($P = 0.0428$; Table 7). The DeOxy and Oxy steaks frozen for 6 months, regardless of packaging, had more percent deoxymyoglobin ($P < 0.05$). During 6 months of frozen storage, a significant decrease in percent deoxymyoglobin was observed for all myoglobin oxygenation levels over RD ($P = 0.0428$). However, while significant differences were observed for percent deoxymyoglobin, the differences were of relatively small magnitude and not likely to impact the overall steak color.

There were two significant three-way interactions for percent metmyoglobin for steaks aged for 4 d that included myoglobin oxygenation level by packaging by frozen storage ($P < 0.0001$; Table 5) and myoglobin oxygenation level by frozen storage by RD ($P < 0.0001$; Table 7). For 0 and 2 months of frozen storage, minimal differences were observed between myoglobin oxygenation level and packaging film. The HiOxy steaks frozen for 4 and 6 months had significantly more percent metmyoglobin than DeOxy and Oxy, regardless of packaging ($P < 0.05$). However, at both 4 and 6 months the HiOxy steaks in impermeable film had significantly more percent metmyoglobin than the HiOxy steaks in permeable film ($P < 0.0001$). Conversely, at 6 months of frozen storage the

DeOxy steaks frozen in permeable film had higher metmyoglobin percent than the DeOxy impermeable steaks, which had the least metmyoglobin compared to the other treatments at 6 months of frozen storage ($P < 0.0001$). With the interaction of myoglobin oxygenation level, frozen storage duration, and RD it displayed that during RD, steaks that were frozen for 0 months tended to have lower initial percent metmyoglobin values on day 1 of RD than steaks frozen for 4 or 6 months, regardless of myoglobin oxygenation levels ($P < 0.0001$). By the end of RD (days 5-7), the HiOxy steaks frozen for 4 or 6 months had the more percent metmyoglobin than all other treatments ($P < 0.05$).

Percent metmyoglobin for steaks aged 20 d had three significant three-way interactions: myoglobin oxygenation level by packaging by frozen storage ($P < 0.0001$; Table 5), myoglobin oxygenation level by packaging by RD ($P = 0.0324$; Table 4), and myoglobin oxygenation level by frozen storage by RD ($P < 0.0001$; Table 7). The significant interaction of myoglobin oxygenation level by packaging film by frozen storage least square means are displayed in Table 5. Following 0 and 2 months of frozen storage, the Oxy steaks frozen in impermeable film had significantly more percent metmyoglobin than HiOxy steaks, regardless of packaging ($P < 0.02$). After 6 months of frozen storage, the HiOxy in the impermeable packaging had the greatest amount of percent metmyoglobin followed by HiOxy steaks in the permeable packaging with DeOxy steaks in the impermeable packaging having the least percent metmyoglobin ($P < 0.0001$). Independent from frozen storage, during the first three days of RD, Oxy steaks, regardless of packaging film, had greater amounts of percent metmyoglobin than HiOxy steaks in either packaging film ($P < 0.05$). During days 3-5 of RD all packaging

and myoglobin oxygenation level treatment were similar. Then at days 6 and 7 of RD, HiOxy steaks had significantly more metmyoglobin percent than DeOxy steaks, regardless of packaging ($P < 0.05$). When comparing the effects of myoglobin oxygenation levels with frozen storage and RD, steaks frozen for 0 months, regardless of myoglobin oxygenation level, had the least amount of percent metmyoglobin on day 1 of RD compared to the other myoglobin oxygenation levels and frozen storage durations ($P < 0.0001$). Nevertheless, throughout days 4-7 of RD, HiOxy steaks frozen for 6 months had more percent metmyoglobin than all other steaks which can be seen in Figure 4 ($P < 0.05$). The amount of percent metmyoglobin seemed to be strongly influenced by frozen storage duration with extended storage of 4 and 6 months leading to larger amounts of metmyoglobin. Metmyoglobin percent increased with extended frozen storage and was increased by prolonged RD for HiOxy steaks.

Delta E

Delta E was significantly influenced by myoglobin oxygenation level ($P < 0.0001$; Table 3) and aging time by packaging film by frozen storage ($P = 0.0146$; Table 8). Delta E was measured as the change of color space over the start to the end of RD. The HiOxy steaks exhibited the greatest delta E values compared to DeOxy and Oxy ($P < 0.0001$). Independent from myoglobin oxygenation level, other than 20 d steaks frozen in the impermeable packaging for 0 months, delta E for steaks aged 4 d and frozen in impermeable packaging had the lowest delta E than other steaks frozen for 0 months ($P < 0.05$). After 2 months of frozen storage, steaks aged for 20 d in impermeable packaging had significantly higher delta E values than steaks in the same packaging, but

only aged for 4 d ($P < 0.01$). However, at 4 and 6 months of frozen storage, there were no differences between packaging and age.

Redness Ratio

The steaks that were aged for 4 d had two significant three-way interactions for redness ratio: myoglobin oxygenation level by packaging type by frozen storage time ($P < 0.0001$; Table 5) and myoglobin oxygenation level by frozen storage by RD ($P = 0.0066$; Table 7). Following 0 and 2 months of frozen storage, few differences in redness ratio were observed between myoglobin oxygenation level and packaging film. However, at both 4 and 6 months, the HiOxy steaks had significantly lower redness ratios than the other treatments, with HiOxy steaks in the impermeable packaging having the lowest redness ratio ($P < 0.0001$). Myoglobin oxygenation level by frozen storage by RD, showed that throughout RD, HiOxy steaks frozen for 0 months had significantly higher redness ratios than HiOxy steaks that were frozen for 2, 4, or 6 months ($P < 0.05$). In addition, the HiOxy steaks that were frozen for 4 and 6 months had the lowest redness ratio compared to all other steaks at days 1, 2, 3, 4, and 7 of RD ($P < 0.05$). Freezing 4 d aged HiOxy steaks for 0 or 2 months provided minimal differences between myoglobin oxygenation levels whereas 4 and 6 months of frozen storage had detrimental effects on redness ratio.

Redness ratio for steaks aged 20 d had two significant two-way interactions between frozen storage duration and RD ($P < 0.0001$; Table 9) and frozen storage and myoglobin oxygenation level ($P < 0.0001$; Table 3). Throughout RD, a decrease in redness ratio was observed, regardless of frozen storage duration ($P < 0.0001$). Steaks frozen for 0 months had significantly higher redness ratios on every day of RD than those

stored frozen for 6 months ($P < 0.0001$). Steaks frozen for 2 months had higher redness ratios on days 1, 3, 6, and 7 of RD ($P < 0.05$) than steaks frozen for 6 months. When comparing myoglobin oxygenation levels with frozen storage duration, it was observed that after 0 months of frozen storage the HiOxy steaks had significantly higher redness ratios than the Oxy and DeOxy steaks ($P < 0.005$). In contrast, at 6 months of frozen storage the reverse was seen with DeOxy steaks having greater redness ratios than Oxy and HiOxy steaks ($P < 0.04$). Extended storage had a detrimental impact on redness ratio, but more evident for HiOxy steaks compared to the other myoglobin oxygenation levels.

a:b* Ratio*

Four three-way interactions were significant for a*:b* ratio: myoglobin oxygenation level by packaging by frozen storage ($P < 0.0001$; Table 5), myoglobin oxygenation level by aging time by frozen storage ($P < 0.0001$; Table 6), myoglobin oxygenation level by frozen storage by RD ($P = 0.0012$; Table 7), and aging time by frozen storage by RD ($P = 0.0004$; Table 9). The myoglobin oxygenation level, packaging film, and frozen storage interaction showed that following 2 months of frozen storage, DeOxy steaks in impermeable film had significantly higher a*:b* ratios than all other treatments at that month besides DeOxy steaks frozen in permeable film ($P < 0.05$). The HiOxy steaks, regardless of packaging, had the lowest a*:b* ratios at both 4 and 6 months of frozen storage, with HiOxy steaks in impermeable film having the lowest values at both frozen storage periods ($P < 0.0001$). The interaction between myoglobin oxygenation level by aging time by frozen storage displayed that after 2 months of frozen storage, HiOxy steaks aged for 4 d had the lowest a*:b* ratios compared to all other treatments within the storage duration ($P < 0.05$). Except for the HiOxy steaks aged 4 d,

the HiOxy 20 d aged steaks had the lowest $a^*:b^*$ ratios at both 4 and 6 months of frozen storage ($P < 0.05$) compared to other myoglobin oxygenation levels at both aging times. Both DeOxy and Oxy steaks aged for 4 d had the highest $a^*:b^*$ ratios at 4 months, compared to 6 months, where DeOxy steaks aged for 4 d had the largest $a^*:b^*$ ratio ($P < 0.0001$). The three-way interaction for myoglobin oxygenation level by frozen storage duration by RD is displayed in Table 7. The $a^*:b^*$ ratios were higher at 0 months of frozen storage than at 4 and 6 months, regardless of myoglobin oxygenation level and day of RD ($P = 0.0012$). During days 5-7 of RD, the HiOxy steaks frozen for 4 and 6 months had the lowest $a^*:b^*$ ratios compared to all other treatments ($P < 0.05$). While statistical differences were observed for the interaction of aging, frozen storage, and RD for $a^*:b^*$ ratios, there was no discernible pattern for the differences. Similar to a^* and percent oxymyoglobin, $a^*:b^*$ values decreased over RD frozen storage duration. Out of all the myoglobin oxygenation levels, HiOxy steaks seemed to have the largest decrease in $a^*:b^*$ values with increasing frozen storage and RD.

Hue Angle

Myoglobin oxygenation level by packaging by frozen storage ($P < 0.0001$; Table 5), myoglobin oxygenation level by aging time by frozen storage ($P < 0.0001$; Table 6), myoglobin oxygenation level by frozen storage by RD ($P < 0.0001$; Table 7), and aging time by frozen storage by RD ($P = 0.0104$; Table 9) were all significant interactions for hue angle. The three-way interaction of myoglobin oxygenation level by packaging film by frozen storage duration showed that the HiOxy steaks frozen in permeable packaging for 2 months had significantly higher hue angle values than DeOxy steaks frozen in impermeable film for 2 months ($P < 0.0001$). The HiOxy steaks packaged in

impermeable film had the highest hue angle values followed by the HiOxy steaks in permeable film compared to all other treatments at 4 and 6 months of frozen storage ($P < 0.0001$). The interaction of myoglobin oxygenation level, aging time, and frozen storage duration is displayed in Table 6. Within both 4 and 20 d aged steaks the DeOxy steaks had the lowest hue angle values following 6 months of frozen storage ($P < 0.05$). However, at 6 months of frozen storage, the HiOxy steaks aged for 20 d had the largest hue angle values compared to all other treatments at 6 months of frozen storage ($P < 0.02$). With the interaction of myoglobin oxygenation level by frozen storage duration by RD, it is evident that throughout days 4-7 of RD the HiOxy steaks that were frozen for 6 months had the largest hue angle values compared to all other treatments ($P < 0.05$). Independent from myoglobin oxygenation level, steaks frozen for 4 and 6 months and aged for 20 d prior to freezing had the highest hue angle values for RD 5-7 except on day 7 where steaks aged for 4 d and frozen for 6 months were not statically different from steaks aged 20 d and frozen for 4 months ($P < 0.05$). Hue angle values generally increased over RD and with extended storage, similar to the percent metmyoglobin values.

Subjective Color (Visual Discoloration)

Myoglobin oxygenation level by packaging ($P = 0.0002$; Table 3) was a significant interaction for subjective discoloration scores. The HiOxy steaks in the impermeable packaging had the most discoloration compared to all other treatments followed by HiOxy steaks in the permeable packaging ($P < 0.05$). Within the permeable packaging during frozen storage, the DeOxy steaks had the least amount of discoloration ($P = 0.0002$). Myoglobin oxygenation level by aging time by frozen storage by RD was

another significant interaction for discoloration ($P < 0.0001$; Table 7). At days 4 and 5 of RD, the HiOxy steaks aged for 20 d and frozen for 6 months had the greatest discoloration ($P < 0.001$). However, at days 6 and 7 of RD, the HiOxy steaks, regardless of age, that were frozen for 4 or 6 months had significantly more discoloration than all other treatments ($P < 0.05$; Figure 5). Discoloration increased over RD and was increased further the longer the steaks were frozen prior to RD. The HiOxy steaks had more determinantal effects from increased RD and frozen storage than the other myoglobin oxygenation levels.

Lipid Oxidation

Lipid oxidation measured on steaks at the conclusion of 7 d of RD, had two significant two-way interactions of myoglobin oxygenation level by packaging type ($P = 0.0009$; Table 3) and myoglobin oxygenation level by frozen storage ($P < 0.0001$; Table 3). The myoglobin oxygenation level by frozen packaging film showed that regardless of packaging, HiOxy steaks had the largest amount of TBARS after 7 d of RD compared to Oxy and DeOxy ($P < 0.0001$). When comparing Oxy and DeOxy steaks, permeable film also led to greater TBARS values compared to the impermeable film ($P = 0.0009$). When looking at the interaction of myoglobin oxygenation level by frozen storage duration the results showed that during all frozen storage periods HiOxy had significantly greater TBARS values than Oxy and DeOxy ($P < 0.05$). Within each myoglobin oxygenation level, a significant increase in TBARS was observed with increasing frozen storage period except at 4 and 6 months where no statistical difference was observed ($P < 0.05$). The difference between the myoglobin oxygenation levels was greater with increasing frozen storage.

Discussion

The HiOxy steaks were negatively impacted by both extended frozen storage and throughout RD, exhibiting inferior color compared to the other myoglobin oxygenation levels. The HiOxy steaks had the lowest amount of oxygen penetration depth compared to the other myoglobin oxygenation level regardless of aging time. Delta E, discoloration, and lipid oxidation after 7 d of RD were greatest for HiOxy steaks compared to Oxy and DeOxy.

Following 4 and 6 months of frozen storage, HiOxy steaks had the lowest oxygen penetration compared to the other myoglobin oxygenation levels. After 6 months of frozen storage, a^* values for steaks aged for 4 d were significantly lower than all other myoglobin oxygenation levels in either packaging film. For steaks aged 4 d, regardless of packaging, the redness ratio, percent oxymyoglobin, and the $a^*:b^*$ ratio were lowest at 4 and 6 months of frozen storage for the HiOxy steaks compared to the other myoglobin oxygenation levels. Conversely, percent metmyoglobin and hue angle generally were highest at 4 and 6 months of frozen storage for the HiOxy steaks compared to the other frozen storage regardless of packaging and aging time. The HiOxy steaks also had the greatest lipid oxidation at all frozen storage periods. This can mostly be attributed to HiOxy steaks having greater oxygen saturation and having ideal conditions for oxidation. Kim et al. (2010) examined meat in a high oxygen environment and found an increase in oxidation, similar to our results.

With increasing RD, a decrease in red color was observed for HiOxy steaks. The HiOxy steaks had the lowest a^* values through days 4-7 of RD and lowest percent

oxymyoglobin for steaks aged 4 d through days 2-7 of RD compared to the other myoglobin oxygenation levels. On the other hand, percent metmyoglobin for steaks aged 20 d was greater for HiOxy steaks regardless of packaging than DeOxy steaks at days 6 and 7 of RD. Kim et al. (2010) found similar results in fresh steaks that were packaged in high oxygen modified atmosphere packaging with extended display time had high myoglobin oxidation and the formation of metmyoglobin on the surface of the meat.

Both extended frozen storage and RD in combination had detrimental effects on HiOxy steaks. Following 6 months of frozen storage, percent oxymyoglobin for 20 d aged steaks was lowest for HiOxy steaks through 4-7 days of RD. Similarly, HiOxy steaks had the lowest $a^*:b^*$ ratio from days 5-7 of RD at 4 and 6 months of frozen storage compared to the other myoglobin oxygenation levels. The Oxy and DeOxy steaks had greater oxymyoglobin percent due to less severe oxidizing conditions prior to frozen storage compared to HiOxy steaks. Less severe oxidizing conditions allow the muscle to save reducing capacity that can later be used through RD and allow the muscle to have a more stable, brighter red color. In contrast, at 4 and 6 months of frozen storage through days 4-7 of RD, 4 d aged HiOxy steaks had greater values than the other myoglobin oxygenation levels for hue angle and percent metmyoglobin. Generally, towards the end of RD, HiOxy steaks had the greatest discoloration compared to DeOxy and Oxy at 2, 4, and 6 months of frozen storage.

With extended frozen storage, detrimental effects on meat color were observed. Oxygen penetration depth declined as frozen storage increased. Throughout RD, it was observed that steaks frozen for 0 months had greater a^* values and redness ratios for the 20 d aged steaks than steaks frozen for 6 months. At the beginning of RD, steaks that

were aged for 4 d prior to freezing for 0 months had significantly higher percent oxymyoglobin than 4 and 6 months and by the end of RD, steaks frozen 0 months had more percent oxymyoglobin than all other frozen storage times. Wanous et al. (1989), Brewer and Wu (1993), and Vieira et al. (2009) found similar results in that a decrease in redness was observed when storage duration increased. During RD, HiOxy steaks frozen for 0 and 2 months had significantly higher amounts of percent oxymyoglobin than the HiOxy steaks frozen for 6 months ($P < 0.0001$). Redness ratios showed a similar pattern with HiOxy steaks frozen for 0 months having greater redness ratios than HiOxy steaks with extended frozen storage (2, 4, and 6 mo) ($P < 0.05$). Generally, steaks frozen for 6 months had higher hue angle values for RD than steaks only frozen for 0 or 2 months.

Benjakul and Bauer (2001) and Muela et al. (2010) found that freezing and thawing resulted in accelerated lipid and myoglobin oxidation compared to fresh meat that wasn't frozen. Therefore, the increase in TBARS and discoloration that we observed at 4 and 6 months of frozen storage, regardless of myoglobin oxygenation level, is supported by the previous literature. In addition, TBARS and discoloration values had greater increases for HiOxy steaks and towards the end of RD, compared to the other myoglobin oxygenation levels.

Previous research has shown that during thawing, reducing enzymes can be lost to the environment through the exudate, which will contribute to lower color stability and accelerated oxidation (Abdallah et al., 1999). However, even at the beginning of RD, steaks frozen for 0 months tended to have lower percent metmyoglobin values than the initial RD of steaks frozen 4 or 6 months, regardless of myoglobin oxygenation levels. This supports the concept that extended frozen storage has detrimental effects on meat

color, most likely attributed to the loss of myoglobin content and depletion of reducing equivalents in frozen storage and RD.

Steaks aged for an extended period of time prior to freezing have less color stability. This was observed with the Oxy steaks that were aged for 4 d having deeper oxygen penetration than those aged for 20 d ($P = 0.0193$). While several differences were observed for aging time, it is important to note that aging time was not always significant, which is supported by Ledward (1970) who found no difference from 5 to 14 d of aging in color characteristics. In support of the literature, we found higher L^* values for 20 d aged compared to 4 d aged steaks in impermeable film compared to permeable film ($P = 0.0011$). Steaks frozen for 0 and 6 months also had higher L^* values for 20 d aged steaks than 4 d aged steaks ($P < 0.05$). MacDougall (1982) attributed the change in color stability, specifically the increase in lightness, to the structural changes in the muscle proteins during aging.

It was also noted that higher a^* values occurred for 4 d aged steaks than 20 d steaks for DeOxy and Oxy steaks frozen for 0 months ($P < 0.05$). English et al. (2016) found similar results in fresh steaks, aged for 42 and 62 d having higher a^* values at the start of retail display than steaks aged 21 d. They also found that aging decreased the metmyoglobin reducing activity, increased lipid oxidation by the end of retail display, and overall had detrimental effects on color stability. After 2 months of frozen storage in impermeable packaging, higher delta E values were observed for 20 d aged steaks than 4 d aged steaks ($P < 0.01$). Having a larger change in color space or delta E for steaks aged 20 d verse 4 d supports the previous understanding that postmortem aging causes less color stability and discoloration.

Generally, within each myoglobin oxygenation level, 4 d aged steaks had higher $a^*:b^*$ ratios. With extended aging and RD, typically higher hue angle values were observed for 20 d aged steaks than 4 d. This supports the findings of Hood (1980) since higher hue angles represent greater discoloration. He found that aged meat discolors at a quicker rate throughout retail display compared to fresh meat due to the decay of mitochondrial activity and depletion of metmyoglobin reducing activity (Hood, 1980). Greater discoloration at days 4 and 5 of RD was observed for the HiOxy steaks aged for 20 d and frozen for 6 months than 4 d ($P < 0.001$). This could be attributed to the 20 d aged steaks having a greater depletion of NADH and reducing equivalents. Mancini and Hunt (2005) noted that the reduction of metmyoglobin is crucial to meat color stability and depends on the muscle's oxygen scavenging enzymes, reducing enzyme system, and the amount of available NADH which depletes in the postmortem muscle. Therefore, meat that has been aged longer will not be able to reduce back to deoxymyoglobin and remains as metmyoglobin, leading to an unappealing brown color. While freezing and thawing are typically associated with detrimental meat quality effects, Kim et al. (2018) believed this could be minimized through postmortem aging prior to freezing. The exact mechanism by which postmortem aging prior to freezing could improve color stability still needs further research. Kim et al. (2015) hypothesized that meat aged prior to freezing may sustain higher myoglobin stability and a lower oxygen consumption rate through the aging process than meat that was only aged or only frozen. However, our results with extended postmortem aging contradict this hypothesis. With lower oxygen penetration, lower a^* values, higher ΔE values, lower $a^*:b^*$ values, higher hue

angles, and higher discoloration scores, extended postmortem aging seemed to have a negative impact on steak color.

It is apparent that the color was impacted by the oxygen permeability of the packaging film in which the meat was frozen. Throughout days 1-5 of RD, the steaks frozen in permeable film had higher percent oxymyoglobin than impermeable film ($P < 0.05$). When comparing HiOxy steaks at extended frozen storage (4 and 6 mo), the impermeable film led to less cherry red color than the permeable film. This was evident by HiOxy permeable film having more percent oxymyoglobin ($P < 0.0001$), higher $a^*:b^*$ values ($P < 0.0001$), higher redness ratios ($P < 0.0001$), lower percent metmyoglobin ($P < 0.0001$), lower hue angle values ($P < 0.0001$), and less discoloration ($P < 0.05$) than the impermeable packaging film.

While permeable packaging provided better color than impermeable packaging for the HiOxy steaks, the opposite was seen for DeOxy and Oxy steaks. After 6 months of frozen storage, the DeOxy steaks frozen in the permeable packaging had more percent metmyoglobin than the impermeable ones ($P < 0.0001$). This is in support of Brewer and Wu (1993) who evaluated vacuum packaging, polyvinyl chloride film, and saran wrap as packaging methods and found that the lowest amount of metmyoglobin throughout retail display occurred in samples that were vacuum packed and thus were in the deoxymyoglobin state.

Steaks frozen in the permeable film had greater TBARS values than those frozen in impermeable film for both the Oxy and DeOxy steaks ($P = 0.0009$). MacDougall (1982) and Bhattacharya et al. (1988) found that oxygen impermeable packaging helped to maintain color and minimize discoloration, off-flavors, and lipid oxidation for frozen

beef. Contrary to what was expected, the HiOxy steaks had greater percent oxymyoglobin, $a^*:b^*$ values, redness ratios and lower percent metmyoglobin, hue angle values, discoloration in the permeable packaging than the impermeable packaging. Perhaps the exposure to a highly oxygenated environment prior to freezing caused more metmyoglobin reducing activity to occur, depleting reducing equivalent reservoirs. The depletion of reservoirs paired with ideal oxidizing conditions caused by high oxygen saturation and low oxygen partial pressure from impermeable packaging led to discolored meat that was not able to bloom throughout RD.

The various color measurements that were conducted provided many of the same results. The measurements conducted broadly measured discoloration through different instruments and calculations. It should be noted that to our understanding percent oxymyoglobin, deoxymyoglobin, and metmyoglobin have not been measured with a portable spectrophotometer and the equations used by Krzywicki (1979). Based on percent oxymyoglobin, a^* , and $a^*:b^*$ ratio providing very similar trends and patterns in the results it seems the portable spectrophotometer and calculation were an accurate measurement for percent oxymyoglobin and metmyoglobin. However, it appears to percent oxymyoglobin was more sensitive to slight changes in color. Oxygen penetration followed a similar decreasing pattern with an increase in RD or frozen storage, but was not as indicative of the change in color as the other methods. Conversely, percent metmyoglobin and hue angle often showed the opposite of the percent oxymyoglobin, a^* , and $a^*:b^*$ ratio and typically displayed a positive trend with increasing frozen storage and RD. Discoloration and lipid oxidation also tend to increase with frozen storage and RD, but do not follow the same patterns as percent metmyoglobin and hue angle. This

suggests that various measurements may provide the same information about the change in color.

Conclusion

The HiOxy steaks through the first few days of RD and frozen for under 6 months provided optimal meat color, similar to that of DeOxy and Oxy. However, with extended RD and frozen storage, HiOxy steaks were inferior in color characteristics to the other myoglobin oxygenation levels. Extended storage brought about detrimental color effects for all differing levels of myoglobin oxygenation. Aging beef for 20 d mainly led to less color stability and worse overall color or no difference compared to steaks aged for 4 d. The effects of packaging film greatly depended on the myoglobin oxygenation level with HiOxy steaks in permeable packaging having superior color to HiOxy steaks in impermeable packaging. Various color measurements provided similar results for color characteristics and can be used to describe variation in meat color. Myoglobin oxygenation level, frozen storage duration, postmortem aging time, and packaging permeability had a profound effect on meat color.

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Table 1. Analysis of variance *P*-Values for oxygen penetration, L*, a*, b*, percent oxymyoglobin, deoxymyoglobin, and metmyoglobin and possible interactions including: aging time (4 d or 20 d), packaging films (impermeable or permeable), frozen storage (0, 2, 4, or 6 months), myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹), and retail display (RD; 1-7 days).

Analysis	Oxygen Penetration	L*	a*	b*	Oxy-myoglobin 4 d, %	Oxy-myoglobin 20 d, %	Deoxy-myoglobin 4 d, %	Deoxy-myoglobin 20 d, %	Met-myoglobin 4 d, %	Met-myoglobin 20 d, %
Aging Time	0.1390	0.0138	0.0252	0.6126	N/A	N/A	N/A	N/A	N/A	N/A
Frozen Storage	<0.0001	<0.0001	<0.0001	<0.0001	0.0012	<0.0001	<0.0001	0.0225	0.0090	<0.0001
Aging Time*Frozen Storage	0.0800	<0.0001	0.0230	0.1677	N/A	N/A	N/A	N/A	N/A	N/A
Myoglobin Oxygenation Level	<0.0001	0.1692	<0.0001	<0.0001	<0.0001	0.0155	0.2110	0.0055	<0.0001	0.0146
Aging Time*Myoglobin Oxygenation Level	0.0002	0.0005	<0.0001	<0.0001	N/A	N/A	N/A	N/A	N/A	N/A
Frozen Storage*Myoglobin Oxygenation Level	<0.0001	0.0792	<0.0001	<0.0001	<0.0001	0.0001	0.0136	0.9702	<0.0001	<0.0001
Aging Time*Frozen Storage*Myoglobin Oxygenation Level	0.0903	0.2787	<0.0001	<0.0001	N/A	N/A	N/A	N/A	N/A	N/A
Packaging Film	0.0761	0.0882	<0.0001	0.0001	<0.0001	0.3602	0.0116	0.3982	0.0012	0.0859
Aging Time*Packaging Film	0.9246	0.0055	0.4552	0.4758	N/A	N/A	N/A	N/A	N/A	N/A
Frozen Storage*Packaging Film	0.0108	0.3523	0.0031	0.4788	0.6514	0.0052	0.5065	0.0207	0.5193	0.0036
Aging Time*Frozen Storage*Packaging Film	0.2991	0.0051	0.0010	0.0298	N/A	N/A	N/A	N/A	N/A	N/A
Myoglobin Oxygenation Level *Packaging Film	0.4928	0.1273	<0.0001	<0.0001	<0.0001	<0.0001	0.0015	0.3579	<0.0001	<0.0001

¹Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

Table 1 (continued). Analysis of variance *P*-Values for oxygen penetration, L*, a*, b*, percent oxymyoglobin, deoxymyoglobin, and metmyoglobin and possible interactions including: aging time (4 d or 20 d), packaging films (impermeable or permeable), frozen storage (0, 2, 4, or 6 months), myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹), and retail display (RD; 1-7 days).

Analysis	Oxygen Penetration	L*	a*	b*	Oxy-myoglobin 4 d, %	Oxy-myoglobin 20 d, %	Deoxy-myoglobin 4 d, %	Deoxy-myoglobin 20 d, %	Met-myoglobin 4 d, %	Met-myoglobin 20 d, %
Aging Time*Myoglobin Oxygenation Level *Packaging Film										
Frozen										
Storage*Myoglobin Oxygenation Level *Packaging Film	0.6166	<.0001	0.0490	0.1766	<0.0001	0.0039	<0.0001	0.0070	<0.0001	<0.0001
Aging Time*Frozen Storage*Myoglobin Oxygenation Level *Packaging Film	0.5944	0.2515	0.0096	0.0155	N/A	N/A	N/A	N/A	N/A	N/A
RD	N/A	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Aging Time*RD	N/A	0.0003	0.0011	<0.0001	N/A	N/A	N/A	N/A	N/A	N/A
Frozen Storage*RD	N/A	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0064	<0.0001
Aging Time*Frozen Storage*RD	N/A	<0.0001	0.9978	0.2837	N/A	N/A	N/A	N/A	N/A	N/A
Myoglobin Oxygenation Level *RD	N/A	0.3583	<0.0001	0.3405	<0.0001	<0.0001	0.0008	0.5588	<0.0001	<0.0001
Aging Time*Myoglobin Oxygenation Level *RD	N/A	0.2393	0.9639	0.7312	N/A	N/A	N/A	N/A	N/A	N/A
Frozen Storage*Myoglobin Oxygenation Level *RD	N/A	0.1886	0.7220	0.0242	0.1083	<0.0001	0.0028	0.0428	<0.0001	<0.0001
Aging Time*Frozen Storage*Myoglobin Oxygenation Level *RD	N/A	0.4944	0.9878	0.9703	N/A	N/A	N/A	N/A	N/A	N/A

¹Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

Table 1 (continued). Analysis of variance *P*-Values for oxygen penetration, L*, a*, b*, percent oxymyoglobin, deoxymyoglobin, and metmyoglobin and possible interactions including: aging time (4 d or 20 d), packaging films (impermeable or permeable), frozen storage (0, 2, 4, or 6 months), myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹), and retail display (RD; 1-7 days).

Analysis	Oxygen Penetration	L*	a*	b*	Oxy-myoglobin 4 d, %	Oxy-myoglobin 20 d, %	Deoxy-myoglobin 4 d, %	Deoxy-myoglobin 20 d, %	Met-myoglobin 4 d, %	Met-myoglobin 20 d, %
Packaging Film*RD	N/A	0.4914	0.6944	0.4850	0.0118	0.0843	0.0706	0.4983	0.0612	0.1530
Aging Time*Packaging Film*RD	N/A	0.6827	0.1779	0.2953	N/A	N/A	N/A	N/A	N/A	N/A
Frozen Storage*Packaging Film*RD	N/A	0.1655	0.7665	0.8097	0.7902	0.3975	0.8995	0.3708	0.9485	0.3116
Aging Time*Frozen Storage*Packaging Film*RD	N/A	0.7590	0.4752	0.6802	N/A	N/A	N/A	N/A	N/A	N/A
Myoglobin Oxygenation Level *Packaging Film*RD	N/A	0.5755	0.7203	0.5819	0.8216	0.0904	0.8553	0.9776	0.4728	0.0324
Aging Time*Myoglobin Oxygenation Level *Packaging Film*RD	N/A	0.8800	0.8895	0.9987	N/A	N/A	N/A	N/A	N/A	N/A
Frozen Storage*Myoglobin Oxygenation Level *Packaging Film*RD	N/A	0.5249	0.8583	0.8417	0.9813	0.9591	0.8879	0.9935	0.9999	0.9597
Aging Time*Frozen Storage*Myoglobin Oxygenation Level *Packaging Film*RD	N/A	1.0000	1.0000	0.9977	N/A	N/A	N/A	N/A	N/A	N/A

¹Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

Table 2. Analysis of variance *P*-Values for delta E, redness ratio, a*:b* ratio, hue angle, discoloration, and lipid oxidation and possible interactions including: aging time (4 d or 20 d), packaging films (impermeable or permeable), frozen storage (0, 2, 4, or 6 months), myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹), and retail display (RD; 1-7 days).

Analysis	Delta E	Redness Ratio 4d [†]	Redness Ratio 20d [†]	a*:b* Ratio	Hue Angle	Discoloration	Lipid Oxidation
Aging Time	0.0829	N/A	N/A	0.0488	0.0618	0.3966	0.1617
Frozen Storage	0.0018	<0.0001	<0.0001	<0.0001	<0.0001	0.0020	<0.0001
Aging Time*Frozen Storage	0.8683	N/A	N/A	0.1510	0.1375	0.3169	0.7695
Myoglobin Oxygenation Level	<0.0001	<0.0001	0.0032	<0.0001	<0.0001	<0.0001	<0.0001
Aging Time*Myoglobin Oxygenation Level	0.8340	N/A	N/A	<0.0001	<0.0001	0.2646	0.4933
Frozen Storage*Myoglobin Oxygenation Level	0.3650	<0.0001	<0.0001	<0.0001	<0.0001	<.00001	<0.0001
Aging Time*Frozen Storage*Myoglobin Oxygenation Level	0.6205	N/A	N/A	<0.0001	<0.0001	<0.0001	0.1309
Packaging Film	0.2421	0.5069	0.7279	0.6459	0.8919	0.2874	0.0073
Aging Time*Packaging Film	0.2085	N/A	N/A	0.0385	0.0189	0.2432	0.3934
Frozen Storage*Packaging Film	0.0169	0.3181	0.0636	0.0190	0.0480	0.8136	0.7413
Aging Time*Frozen Storage*Packaging Film	0.0146	N/A	N/A	0.8924	0.3129	0.4434	0.6615
Myoglobin Oxygenation Level *Packaging Film	0.3024	<0.0001	0.2803	0.0741	0.0121	0.0002	0.0009
Aging Time*Myoglobin Oxygenation Level *Packaging Film	0.5055	N/A	N/A	0.7048	0.4127	0.8159	0.5309
Frozen Storage*Myoglobin Oxygenation Level *Packaging Film	0.4213	<0.0001	0.6449	<0.0001	<0.0001	0.0934	0.8244
Aging Time*Frozen Storage*Myoglobin Oxygenation Level *Packaging Film	0.6506	N/A	N/A	0.0733	0.2407	0.8928	0.9472
RD	N/A	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	N/A
Aging Time*RD	N/A	N/A	N/A	0.3908	0.4109	0.9492	N/A

¹Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation – HiOxy

[†]Redness Ratio - Reflectance values for 630nm ÷ 580 nm

Table 2 (continued). Analysis of variance *P*-Values for delta E, redness ratio, a*:b* ratio, hue angle, discoloration, and lipid oxidation and possible interactions including: aging time (4 d or 20 d), packaging films (impermeable or permeable), frozen storage (0, 2, 4, or 6 months), myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹), and retail display (RD; 1-7 days).

Analysis	Delta E	Redness Ratio 4d [†]	Redness Ratio 20d [†]	a*:b* Ratio	Hue Angle	Discoloration	Lipid Oxidation
Frozen Storage*RD	N/A	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	N/A
Aging Time*Frozen Storage*RD	N/A	N/A	N/A	0.0004	0.0104	0.0874	N/A
Myoglobin Oxygenation Level *RD	N/A	<0.0001	0.2758	<0.0001	<0.0001	<0.0001	N/A
Aging Time*Myoglobin Oxygenation Level *RD	N/A	N/A	N/A	0.8682	0.8443	0.0001	N/A
Frozen Storage*Myoglobin Oxygenation Level *RD	N/A	0.0066	0.6995	0.0012	<0.0001	<0.0001	N/A
Aging Time*Frozen Storage*Myoglobin Oxygenation Level *RD	N/A	N/A	N/A	0.9963	0.9982	<0.0001	N/A
Packaging Film*RD	N/A	0.8587	0.8470	0.4428	0.8908	0.9863	N/A
Aging Time*Packaging Film*RD	N/A	N/A	N/A	0.9577	0.9989	0.9266	N/A
Frozen Storage*Packaging Film*RD	N/A	0.1675	0.9769	0.7564	0.8555	0.9998	N/A
Aging Time*Frozen Storage*Packaging Film*RD	N/A	N/A	N/A	0.9242	0.9818	0.8426	N/A
Myoglobin Oxygenation Level *Packaging Film*RD	N/A	0.8620	0.4035	0.9783	0.9454	0.5026	N/A
Aging Time*Myoglobin Oxygenation Level *Packaging Film*RD	N/A	N/A	N/A	0.6321	0.7914	0.9644	N/A
Frozen Storage*Myoglobin Oxygenation Level *Packaging Film*RD	N/A	0.8454	0.4077	0.9902	0.9997	1.0000	N/A
Aging Time*Frozen Storage*Myoglobin Oxygenation Level *Packaging Film*RD	N/A	N/A	N/A	0.9993	1.0000	1.0000	N/A

¹Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation – HiOxy

[†]Redness Ratio - Reflectance values for 630nm ÷ 580 nm

Table 3. The effects of aging (4 d or 20 d), packaging films (impermeable or permeable), and frozen storage (0, 2, 4, or 6 months) for different myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹) on color variables of steaks (n = 144; n = 72; n = 72; n = 36).

Variable	Treatment	Myoglobin Oxygenation Level			P-Value	SEM
		DeOxy	Oxy	HiOxy		
Delta E	N/A	3.80 ^b	3.47 ^b	4.69 ^a	<0.0001	0.2372
Oxygen Penetration (mm)	Age					
	4	.08 ^a	.08 ^a	.06 ^{bc}	0.0002	0.0047
	20	.07 ^a	.06 ^b	.06 ^c		
Discoloration	Packaging					
	Impermeable	1.74 ^d	3.28 ^c	10.13 ^a	0.0002	1.2105
	Permeable	2.47 ^{cd}	3.37 ^c	8.56 ^b		
Lipid Oxidation	Impermeable	4.00 ^c	4.23 ^c	7.01 ^a	0.0009	0.4805
	Permeable	4.71 ^b	4.67 ^b	6.74 ^a		
Lipid Oxidation ³	Frozen Storage					
	0	3.44 ^b	3.20 ^b	3.97 ^a	<0.0001	0.5223
	2	4.24 ^b	4.06 ^b	6.82 ^a		
	4	4.98 ^b	5.28 ^b	8.04 ^a		
	6	4.75 ^b	5.26 ^b	8.67 ^a		
Oxygen Penetration (mm) ³	0	.100 ^a	.103 ^a	.087 ^b	<0.0001	0.0049
	2	.077 ^a	.069 ^a	.071 ^a		
	4	.066 ^a	.060 ^a	.042 ^b		
	6	.068 ^a	.056 ^b	.026 ^c		
Redness Ratio 20d ^{2,3,4}	0	3.16 ^b	3.01 ^b	3.52 ^a	<0.0001	0.1029
	2	2.84 ^{ab}	2.69 ^b	3.01 ^a		
	4	N/A	N/A	N/A		
	6	2.68 ^a	2.44 ^b	2.23 ^b		

¹ Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

² Redness Ratio - Reflectance values for 630nm ÷ 580 nm

³ Means within each row with different superscripts are different ($P < 0.05$).

⁴ N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-d} Means within the same variable with different superscripts are different ($P < 0.05$).

Table 4. The effects of different myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹) and packaging films (impermeable or permeable) throughout retail display on color variables of steaks (n = 144; n = 72).

Variable	Myoglobin Oxygenation Level	Retail Display (days)							P-Value	SEM
		1	2	3	4	5	6	7		
a*	DeOxy	16.20 ^a	16.36 ^a	16.15 ^a	15.69 ^a	15.16 ^a	14.56 ^a	13.76 ^a	<0.0001	0.2107
	Oxy	15.29 ^b	15.65 ^b	15.55 ^b	15.03 ^b	14.67 ^b	14.17 ^b	13.24 ^b		
	HiOxy	15.87 ^a	15.80 ^b	15.33 ^b	14.55 ^c	13.85 ^c	13.03 ^c	12.20 ^c		
Oxy-myoglobin 4 d, %	DeOxy	74.22 ^a	74.10 ^a	73.06 ^a	71.70 ^a	70.73 ^a	69.55 ^a	65.70 ^a	<0.0001	0.8786
	Oxy	72.40 ^b	72.18 ^b	71.83 ^b	70.73 ^a	69.91 ^a	68.60 ^a	64.69 ^a		
	HiOxy	73.40 ^{ab}	70.70 ^c	69.96 ^c	68.32 ^b	66.53 ^b	63.42 ^b	59.80 ^b		
Met-myoglobin 20 d, %	Myoglobin Oxygenation Level	Packaging Film							0.0324	0.9732
	DeOxy	Impermeable	20.23 ^{cd}	20.84 ^d	21.83 ^c	23.12 ^b	24.57 ^c	26.66 ^d		
	DeOxy	Permeable	20.90 ^{bc}	22.21 ^{bc}	22.68 ^{bc}	24.20 ^a	25.22 ^{bc}	27.19 ^{cd}		
	Oxy	Impermeable	24.20 ^a	24.23 ^a	24.43 ^a	25.41 ^a	26.61 ^{ab}	28.02 ^{bcd}		
	Oxy	Permeable	22.31 ^b	23.50 ^{ab}	24.17 ^{ab}	25.37 ^a	26.77 ^a	28.69 ^{abc}		
	HiOxy	Impermeable	19.00 ^d	21.19 ^{cd}	22.76 ^{bc}	24.61 ^{ab}	26.39 ^{ab}	29.74 ^a		
	HiOxy	Permeable	18.72 ^d	20.90 ^{cd}	22.61 ^c	24.17 ^{ab}	26.75 ^{ab}	29.51 ^{ab}		

¹Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation – HiOxy

^{a-d} Means within the same column for each variable with different superscripts are different ($P < 0.05$).

Table 5. The effects of packaging films (impermeable or permeable) and myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹) on aging (4 d or 20 d) and frozen storage (0, 2, 4, or 6 months) on color variables of steaks (n = 72; n = 36).

Variable	Age	Impermeable			Permeable			P-Value	SEM	
		Deoxy	Oxy	HiOxy	Deoxy	Oxy	HiOxy			
L*	4	44.88 ^{cd}	44.79 ^d	44.74 ^{cde}	44.97 ^{cd}	44.50 ^e	45.11 ^{bc}	0.0011	0.3434	
	20	46.14 ^a	46.16 ^a	46.23 ^a	45.67 ^{cd}	46.14 ^a	45.98 ^{ab}			
L*±	Frozen Storage									
	0	45.61 ^{ab}	45.62 ^{ab}	45.97 ^a	45.72 ^{ab}	45.54 ^b	45.76 ^{ab}			
	2	46.38 ^b	46.39 ^b	45.89 ^c	46.06 ^{bc}	45.97 ^c	46.80 ^a	<0.0001	0.3189	
	4	44.65 ^a	44.38 ^{ab}	44.58 ^{ab}	44.48 ^{ab}	44.27 ^b	44.32 ^{ab}			
	6	45.39 ^{ab}	45.52 ^a	45.49 ^a	45.02 ^b	45.51 ^a	45.31 ^{ab}			
	0	74.25 ^{ab}	73.10 ^b	74.95 ^{ab}	74.58 ^{ab}	73.89 ^a	74.59 ^{ab}			
	Oxy-myoglobin d, % ³	2	72.73 ^a	69.66 ^{de}	70.23 ^{ce}	71.65 ^b	71.02 ^{abc}	71.25 ^{abd}	<0.0001	1.6041
		4	70.82 ^a	68.91 ^a	62.48 ^c	70.19 ^a	69.53 ^a	64.22 ^b		
		6	68.36 ^a	66.99 ^a	59.90 ^c	67.78 ^a	67.29 ^a	61.97 ^b		
		0	73.79 ^{abc}	72.31 ^c	75.88 ^a	74.21 ^{abc}	73.15 ^b	75.86 ^a		
Oxy-myoglobin 20 d, % ^{3,4}	2	68.55 ^{ab}	65.99 ^{bc}	70.55 ^a	67.44 ^c	66.24 ^c	70.54 ^a	0.0039	1.6901	
	4	N/A	N/A	N/A	N/A	N/A	N/A			
	6	64.54 ^a	60.98 ^{bc}	58.30 ^d	62.73 ^b	61.00 ^{bc}	58.76 ^{cd}			
	0	6.92 ^a	6.81 ^{ab}	6.15 ^c	6.19 ^{bc}	6.94 ^a	6.62 ^{ab}			
Deoxy-myoglobin d, % ³	2	5.93 ^{ab}	6.25 ^{ab}	6.16 ^a	5.94 ^{ab}	5.85 ^{ab}	5.66 ^b	<0.0001	0.4476	
	4	7.25 ^{bc}	7.52 ^{abc}	7.95 ^a	7.07 ^c	7.53 ^{abc}	7.80 ^{ab}			
	6	9.55 ^{ab}	8.96 ^{bc}	9.26 ^{bc}	8.79 ^{cd}	8.46 ^d	9.98 ^a			
	0	6.14 ^a	5.78 ^{ab}	5.34 ^{ab}	5.77 ^{ab}	5.69 ^{ab}	5.07 ^b			
Deoxy-myoglobin 20 d, % ^{3,4}	2	7.50 ^b	7.74 ^{ab}	7.15 ^b	8.12 ^a	7.90 ^{ab}	7.06 ^b	0.0070	0.6436	
	4	N/A	N/A	N/A	N/A	N/A	N/A			
	6	8.17 ^{ab}	8.63 ^a	7.29 ^c	8.03 ^{abc}	7.92 ^{bc}	7.62 ^{bc}			

¹ Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation – HiOxy

² Redness Ratio - Reflectance values for 630nm ÷ 580 nm

³ Means within each row of the same variable with different superscripts are different ($P < 0.05$).

⁴ N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-d} Means within the same variable with different superscripts are different ($P < 0.05$).

Table 5 (continued). The effects of packaging films (impermeable or permeable) and myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹) on aging (4 d or 20 d) and frozen storage (0, 2, 4, or 6 months) on color variables of steaks (n = 18).

Variable	Storage	Impermeable			Permeable			P-Value	SEM
		Deoxy	Oxy	HiOxy	Deoxy	Oxy	HiOxy		
Met-myoglobin 4 d, %³	0	18.83 ^{ab}	20.09 ^a	18.89 ^{ab}	19.24 ^{ab}	19.17 ^b	18.78 ^{ab}	<0.0001	1.4218
	2	21.33 ^b	24.09 ^a	23.61 ^a	22.41 ^a	23.14 ^a	23.09 ^{ab}		
	4	21.93 ^d	23.58 ^{cd}	29.58 ^a	22.74 ^{cd}	22.94 ^{cd}	27.99 ^b		
	6	22.10 ^d	24.06 ^c	30.84 ^a	23.43 ^c	24.25 ^c	28.05 ^b		
Met-myoglobin 20 d, %^{3,4}	0	20.07 ^{abc}	21.92 ^a	18.78 ^c	20.02 ^{abc}	21.16 ^b	19.07 ^{bc}	<0.0001	1.5119
	2	23.95 ^{bc}	26.27 ^a	22.31 ^c	24.44 ^{abc}	25.86 ^{ab}	22.40 ^c		
	4	N/A	N/A	N/A	N/A	N/A	N/A		
	6	27.29 ^e	30.39 ^d	34.41 ^a	29.26 ^{cd}	31.09 ^c	33.62 ^b		
Redness Ratio 4 d^{2,3}	0	3.49 ^{ab}	3.44 ^b	3.60 ^a	3.48 ^{ab}	3.44 ^b	3.56 ^{ab}	<0.0001	0.0832
	2	3.07 ^a	2.85 ^c	2.88 ^{bc}	2.97 ^{bc}	2.99 ^b	2.88 ^{bc}		
	4	2.97 ^a	2.87 ^a	2.32 ^c	2.90 ^a	2.85 ^a	2.44 ^b		
	6	3.21 ^a	2.99 ^b	2.36 ^e	3.03 ^{bc}	2.92 ^c	2.51 ^d		
a*: b* ratio³	0	2.01 ^a	1.98 ^b	2.04 ^a	2.04 ^a	2.01 ^a	2.02 ^a	<0.0001	0.0277
	2	1.95 ^a	1.90 ^{bc}	1.91 ^b	1.93 ^{ab}	1.92 ^b	1.88 ^c		
	4	1.88 ^a	1.83 ^b	1.70 ^d	1.87 ^a	1.84 ^b	1.73 ^c		
	6	1.88 ^a	1.82 ^{bc}	1.61 ^e	1.83 ^b	1.79 ^c	1.66 ^d		
Hue Angle³	0	.46 ^{ab}	.47 ^a	.46 ^b	.46 ^b	.46 ^b	.46 ^b	<0.0001	0.0076
	2	.48 ^c	.49 ^b	.49 ^b	.48 ^{bc}	.48 ^{bc}	.50 ^a		
	4	.49 ^e	.51 ^c	.54 ^a	.50 ^{de}	.50 ^{cd}	.53 ^b		
	6	.49 ^e	.50 ^d	.57 ^a	.50 ^e	.52 ^c	.56 ^b		

¹ Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation – HiOxy

² Redness Ratio - Reflectance values for 630nm ÷ 580 nm

³ Means within each row of the same variable with different superscripts are different ($P < 0.05$).

⁴ N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-d} Means within the same variable with different superscripts are different ($P < 0.05$).

Table 6. The effects of aging (4 d or 20 d) and myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹) by packaging films (impermeable or permeable) and frozen storage (0, 2, 4, or 6 months) on color variables of steaks (n = 18; n = 9).

Variable	Frozen Storage		4 d			20 d			P-Value	SEM			
			Deoxy	Oxy	HiOxy	Deoxy	Oxy	HiOxy					
a*: b* Ratio	0		2.05 ^a	2.05 ^a	2.05 ^a	1.99 ^a	1.94 ^b	2.01 ^a	<0.0001	0.0377			
	2		1.93 ^{ab}	1.94 ^a	1.85 ^c	1.95 ^a	1.88 ^b	1.94 ^{ab}					
	4		1.94 ^a	1.92 ^a	1.72 ^{bcd}	1.81 ^b	1.75 ^c	1.71 ^d					
	6		1.92 ^a	1.87 ^b	1.68 ^{cd}	1.80 ^b	1.74 ^c	1.58 ^d					
Hue Angle	0		.46 ^{ab}	.46 ^{ab}	.45 ^{ab}	.47 ^b	.48 ^a	.46 ^b	<0.0001	0.0105			
	2		.48 ^c	.48 ^c	.50 ^{ab}	.48 ^{bc}	.49 ^a	.48 ^{bc}					
	4		.48 ^e	.48 ^{de}	.53 ^{abc}	.51 ^{cd}	.53 ^b	.54 ^a					
	6		.48 ^d	.49 ^c	.54 ^b	.51 ^c	.53 ^b	.58 ^a					
a*	0	Frozen Storage							0.0096	0.3282			
		Packaging Film											
	2	Impermeable	18.18 ^a	17.31 ^b	18.14 ^a	16.21 ^{cd}	15.61 ^e	17.18 ^b					
		Permeable	17.35 ^b	17.30 ^b	18.29 ^a	16.36 ^c	15.88 ^{de}	17.43 ^{ab}					
	4	Impermeable	15.47 ^a	14.56 ^{cde}	13.78 ^f	14.93 ^{abcde}	14.45 ^{cdef}	15.44 ^{ab}					
		Permeable	14.63 ^{bcd}	14.51 ^{cdef}	14.19 ^{def}	14.43 ^{cdef}	14.05 ^{ef}	15.11 ^{abcd}					
	6	Impermeable	15.58 ^a	14.97 ^b	12.74 ^e	14.75 ^{ab}	13.42 ^{de}	13.08 ^{de}					
		Permeable	15.48 ^a	15.02 ^b	13.17 ^{cd}	13.87 ^c	13.40 ^{de}	13.21 ^{de}					
	0	Impermeable	15.62 ^a	15.30 ^a	11.85 ^g	14.72 ^{abc}	13.53 ^d	12.01 ^{fg}					
		Permeable	14.90 ^b	14.42 ^{cd}	12.48 ^{ef}	14.14 ^c	13.08 ^e	11.90 ^{fg}					
	b*	2	Impermeable	9.02 ^a	8.49 ^{cdef}	8.84 ^{abc}	8.14 ^f	8.20 ^{ef}			8.47 ^{cde}	0.0155	0.1666
			Permeable	8.56 ^{cdef}	8.42 ^{def}	8.95 ^{ab}	8.23 ^{ef}	8.11 ^f			8.67 ^{bcd}		
4		Impermeable	7.95 ^{ab}	7.58 ^{bcd}	7.36 ^e	7.67 ^{ae}	7.67 ^{ae}	7.88 ^{bcd}					
		Permeable	7.64 ^{cd}	7.40 ^e	7.71 ^{cd}	7.47 ^e	7.50 ^e	7.92 ^{bc}					
6		Impermeable	8.05 ^{ab}	7.93 ^{abcd}	7.47 ^d	8.17 ^a	7.66 ^{bcd}	7.77 ^{abcd}					
		Permeable	7.98 ^{abc}	7.80 ^{abcd}	7.57 ^{cd}	7.74 ^{abcd}	7.69 ^{bcd}	7.67 ^{bcd}					

¹Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

^{a-g} Means within each row of the same variable with different superscripts are different ($P < 0.05$).

Table 7. The effects of different myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹), frozen storage (0, 2, 4, or 6 months), and aging time (4 or 20 d) throughout retail display on color variables of steaks (n = 36).

Variable	Retail Display (RD)	Myoglobin Oxygenation Levels												P-Value	SEM
		DeOxy				Oxy				HiOxy					
		0 mo	2 mo	4 mo	6 mo	0 mo	2 mo	4 mo	6 mo	0 mo	2 mo	4 mo	6 mo		
b*	1	8.85 ^b	7.80 ^{de}	8.25 ^c	7.44 ^{ef}	8.48 ^c	7.28 ^f	7.86 ^d	7.25 ^f	9.33 ^a	7.76 ^{de}	7.66 ^{de}	6.91 ^g	0.0242	0.1498
	2	8.58 ^b	7.94 ^{defg}	8.09 ^{cd}	8.07 ^{cde}	8.44 ^{bc}	7.70 ^{efg}	7.87 ^{ef}	7.88 ^{defg}	9.16 ^a	7.95 ^{def}	7.64 ^{fg}	7.46 ^g		
	3	8.68 ^b	7.70 ^{ef}	8.23 ^c	8.14 ^{cd}	8.28 ^c	7.71 ^{ef}	7.92 ^{def}	8.04 ^{cde}	9.01 ^a	7.79 ^{def}	7.81 ^{def}	7.58 ^f		
	4	8.59 ^a	7.76 ^{cd}	7.90 ^{bcd}	8.26 ^{ab}	8.49 ^a	7.43 ^e	7.69 ^{cde}	7.99 ^{bc}	8.60 ^a	7.58 ^{de}	7.72 ^{cde}	7.61 ^{de}		
	5	8.20 ^b	7.66 ^{cde}	7.87 ^{bcd}	8.19 ^b	8.18 ^b	7.65 ^{cde}	7.64 ^{de}	7.96 ^{bc}	8.60 ^a	7.66 ^{cde}	7.50 ^e	7.45 ^e		
	6	8.34 ^a	7.40 ^d	7.74 ^{bcd}	8.11 ^{ab}	8.20 ^a	7.53 ^{cd}	7.68 ^{cd}	7.78 ^c	8.37 ^a	7.49 ^{cd}	7.45 ^{cd}	7.55 ^{cd}		
	7	8.18 ^a	7.52 ^{cd}	7.83 ^{abcd}	8.01 ^{ab}	8.05 ^{ab}	7.46 ^d	7.74 ^{bcd}	7.88 ^{abc}	8.08 ^{ab}	7.77 ^{bc}	7.57 ^{cd}	7.79 ^{bcd}		
Oxymyoglobin 20 d, % ³	1	82.01 ^{ab}	69.86 ^d	N/A	63.84 ^e	80.20 ^{bd}	67.17 ^{de}	N/A	60.19 ^f	83.95 ^a	75.20 ^c	N/A	63.54 ^e	<0.0001	1.7898
	2	77.43 ^{ab}	69.63 ^d	N/A	64.66 ^{ef}	75.13 ^{bd}	66.89 ^e	N/A	62.01 ^f	80.05 ^a	72.86 ^{bc}	N/A	62.07 ^f		
	3	75.38 ^b	69.32 ^{cd}	N/A	65.03 ^d	74.29 ^b	67.43 ^{de}	N/A	62.22 ^f	77.65 ^a	71.97 ^{bc}	N/A	60.88 ^f		
	4	73.41 ^a	68.15 ^{cd}	N/A	65.57 ^d	72.56 ^{abc}	66.79 ^d	N/A	62.55 ^e	74.93 ^a	71.20 ^{ab}	N/A	59.64 ^f		
	5	71.43 ^b	68.07 ^{bcde}	N/A	64.40 ^{ef}	70.38 ^{bcd}	65.73 ^{df}	N/A	61.60 ^g	74.39 ^a	69.41 ^{abce}	N/A	57.45 ^h		
	6	70.34 ^{ab}	65.74 ^{cde}	N/A	62.26 ^{def}	69.79 ^{abc}	64.78 ^{cdef}	N/A	60.36 ^f	71.90 ^a	67.20 ^{bcd}	N/A	54.31 ^g		
	7	68.01 ^a	65.22 ^a	N/A	59.68 ^{bc}	66.76 ^a	64.03 ^{ab}	N/A	57.97 ^c	68.22 ^a	65.95 ^a	N/A	51.82 ^d		

¹ Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

² Redness Ratio - Reflectance values for 630nm ÷ 580 nm

³N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-j} Means within each day of RD with different superscripts are different ($P < 0.05$).

Table 7 (continued). The effects of different myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹), frozen storage (0, 2, 4, or 6 months), and aging time (4 or 20 d) throughout retail display on color variables of steaks (n = 36).

Variable	Retail Display (RD)	Myoglobin Oxygenation Levels												P-Value	SEM
		DeOxy				Oxy				HiOxy					
		0 mo	2 mo	4 mo	6 mo	0 mo	2 mo	4 mo	6 mo	0 mo	2 mo	4 mo	6 mo		
Deoxymyoglobin 4 d, %	1	6.79 ^e _f	5.68 ^f _g	7.27 ^d _e	11.27 ^a	7.32 ^c _d e	6.51 ^e _f g	7.90 ^c _d	10.25 ^b	5.14 ^g	5.66 ^f _g	8.36 ^c	11.13 ^a _b	0.0028	0.5155
	2	6.00 ^e	6.48 ^d _e	7.56 ^c _d	9.24 ^b	6.22 ^d _e	7.19 ^c _d e	8.17 ^b _c	9.12 ^b	6.14 ^d _e	6.93 ^c _d e	9.01 ^b	10.60 ^a		
	3	6.50 ^c _d e	5.30 ^e	7.89 ^b _c	8.90 ^b	6.85 ^c _d	5.62 ^d _e	7.77 ^b _c	8.38 ^b	6.81 ^c _d	5.64 ^d _e	8.65 ^b	10.15 ^a		
	4	6.83 ^b _c	6.65 ^b _c	6.80 ^b _c	9.66 ^a	7.39 ^b	6.27 ^c	7.09 ^b _c	9.45 ^a	6.46 ^c	6.55 ^b _c	7.28 ^b _c	10.23 ^a		
	5	6.81 ^c _d e	6.60 ^d _e	7.02 ^b _c d	8.32 ^a _b	7.18 ^b _c d	6.11 ^d _e	7.35 ^b _c d	8.18 ^a _b c	6.04 ^e	6.31 ^d _e	7.51 ^a _b c	8.85 ^a		
	6	6.24 ^d _e f	5.08 ^f _g h	6.18 ^d _e f	8.56 ^a _b	5.85 ^e _f g	4.76 ^g _h	6.79 ^c _d e	8.04 ^b _c	7.09 ^c _d	4.54 ^h	6.84 ^c _d e	8.96 ^a		
	7	6.70 ^b _c	5.77 ^c	7.39 ^a _b	8.21 ^a	7.33 ^a _b	5.88 ^c	7.58 ^a _b	7.54 ^a _b	7.04 ^a _b c	5.76 ^c	7.45 ^a _b	7.42 ^a _b		
Deoxymyoglobin 20 d, % ³	1	4.98 ^b _c	8.30 ^a	N/A	9.31 ^a	4.68 ^b _c	8.20 ^a	N/A	9.80 ^a	4.57 ^c	6.77 ^b	N/A	9.38 ^a	0.0428	0.7444
	2	6.11 ^c _d	8.21 ^a _c	N/A	9.40 ^a _b	6.40 ^c	8.73 ^a	N/A	9.25 ^a _b	5.09 ^d	7.57 ^b _c	N/A	9.22 ^a _b		
	3	6.29 ^b _c d	8.14 ^a _b	N/A	9.07 ^a	5.73 ^c _d	7.93 ^a _b	N/A	9.50 ^a	5.23 ^d	7.74 ^a _b c	N/A	8.47 ^a		
	4	6.18 ^c	8.06 ^a _b	N/A	7.65 ^a _b c	5.84 ^c	7.77 ^a _b c	N/A	8.32 ^a	6.27 ^b _c	7.06 ^a _b c	N/A	7.74 ^a _b c		
	5	6.46 ^a _b	7.43 ^a _b	N/A	7.53 ^a _b	6.43 ^a _b	8.00 ^a _b	N/A	7.79 ^a	5.02 ^c	7.36 ^a _b	N/A	6.67 ^a _b c		
	6	5.79 ^b _c d	7.97 ^a	N/A	7.12 ^a _b c	5.31 ^c _d e	7.65 ^a _b	N/A	7.04 ^a _b c	4.87 ^e	7.30 ^a _b c	N/A	5.55 ^a _b d		
	7	5.87 ^a _b	6.56 ^a _b	N/A	6.58 ^a	5.72 ^a _b	6.44 ^a _b	N/A	6.21 ^a _b	5.38 ^a _b	5.93 ^a _b	N/A	5.16 ^a		

¹ Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

² Redness Ratio - Reflectance values for 630nm ÷ 580 nm

³N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-j} Means within each day of RD with different superscripts are different ($P < 0.05$).

Table 7 (continued). The effects of different myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹), frozen storage (0, 2, 4, or 6 months), and aging time (4 or 20 d) throughout retail display on color variables of steaks (n = 36).

Variable	Retail Display (RD)	Myoglobin Oxygenation Levels												P-Value	SEM
		DeOxy				Oxy				HiOxy					
		0 mo	2 mo	4 mo	6 mo	0 mo	2 mo	4 mo	6 mo	0 mo	2 mo	4 mo	6 mo		
Metmyoglobin 4 d, %	1	12.59 ^g	17.91 ^{def}	20.18 ^{acde}	21.44 ^{bcd}	13.90 ^{fg}	20.42 ^{abcd}	21.49 ^{abcd}	22.63 ^{abc}	12.27 ^g	16.47 ^{efg}	23.52 ^{ab}	23.82 ^a	<0.0001	
	2	15.51 ^{hi}	18.15 ^{eghi}	19.64 ^{defgh}	21.03 ^{bcde}	16.34 ^{fghi}	20.29 ^{bcdf}	21.08 ^{cde}	22.86 ^{bcd}	15.50 ⁱ	18.41 ^{defghi}	24.64 ^{ab}	25.95 ^a		
	3	17.85 ^{de}	19.55 ^{cde}	20.33 ^{cde}	21.42 ^{bcd}	18.59 ^{cd}	21.28 ^{bcd}	21.25 ^{cd}	22.95 ^{bc}	16.38 ^e	20.04 ^{cde}	25.60 ^{ab}	26.90 ^a		
	4	18.79 ^{cd}	21.16 ^{cd}	21.27 ^{cd}	22.04 ^{cd}	19.48 ^{cd}	22.28 ^{cd}	21.98 ^{cd}	23.13 ^{bc}	18.74 ^d	22.04 ^{cd}	27.29 ^{ab}	28.16 ^a		
	5	20.83 ^{bcd}	22.10 ^{cd}	22.36 ^{bcd}	23.02 ^{bcd}	21.20 ^{bcd}	23.35 ^{bcd}	22.89 ^{bcd}	24.08 ^{bcd}	20.27 ^d	24.85 ^b	29.71 ^a	30.33 ^a		
	6	22.33 ^d	24.70 ^{cd}	24.41 ^{bcd}	24.31 ^{bcd}	22.79 ^d	26.43 ^{cd}	25.32 ^{bcd}	25.65 ^{bcd}	23.65 ^d	28.55 ^b	33.36 ^a	33.34 ^a		
	7	25.32 ^{cde}	29.54 ^{de}	28.16 ^{de}	26.10 ^{de}	25.12 ^{cde}	31.24 ^{bc}	28.79 ^{cde}	27.76 ^{de}	25.06 ^e	33.11 ^a	37.36 ^a	37.59 ^a		
Metmyoglobin 20 d, % ³	1	13.00 ^{ef}	21.84 ^c	N/A	26.85 ^b	15.13 ^{de}	24.63 ^b	N/A	30.01 ^a	11.48 ^f	18.03 ^d	N/A	27.08 ^{ab}	<0.0001	1.5925
	2	16.47 ^{ef}	22.16 ^{bd}	N/A	25.94 ^b	18.47 ^{bcd}	24.38 ^{ab}	N/A	28.74 ^a	14.86 ^f	19.56 ^{ce}	N/A	28.70 ^a		
	3	18.33 ^{de}	22.54 ^{cd}	N/A	25.90 ^{bc}	19.98 ^d	24.64 ^{bc}	N/A	28.28 ^{ab}	17.12 ^e	20.28 ^{de}	N/A	30.65 ^a		
	4	20.41 ^{fg}	23.79 ^{cdef}	N/A	26.78 ^{bc}	21.60 ^{efg}	25.44 ^{bce}	N/A	29.13 ^b	18.80 ^g	21.74 ^{dffg}	N/A	32.62 ^a		
	5	22.12 ^{efg}	24.51 ^{defg}	N/A	28.07 ^{cd}	23.18 ^{ef}	26.27 ^{bcde}	N/A	30.61 ^b	20.59 ^g	23.23 ^{fg}	N/A	35.89 ^a		
	6	23.87 ^d	26.29 ^{cd}	N/A	30.62 ^{bc}	24.90 ^d	27.57 ^{cd}	N/A	32.59 ^b	23.23 ^d	25.50 ^d	N/A	40.15 ^a		
	7	26.11 ^d	28.22 ^d	N/A	33.76 ^{bc}	27.52 ^d	29.53 ^{cd}	N/A	35.82 ^b	26.40 ^d	28.12 ^d	N/A	43.02 ^a		

¹ Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

² Redness Ratio - Reflectance values for 630nm ÷ 580 nm

³N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-j} Means within each day of RD with different superscripts are different ($P < 0.05$).

Table 7 (continued). The effects of different myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹), frozen storage (0, 2, 4, or 6 months), and aging time (4 or 20 d) throughout retail display on color variables of steaks (n = 36).

Variable	Retail Display (RD)	Myoglobin Oxygenation Levels												P-Value	SEM
		DeOxy				Oxy				HiOxy					
		0 mo	2 mo	4 mo	6 mo	0 mo	2 mo	4 mo	6 mo	0 mo	2 mo	4 mo	6 mo		
Redness Ratio 4 d ²	1	4.31 ^b	3.53 ^{cd}	3.24 ^{ef}	3.37 ^{cde}	4.43 ^b	3.41 ^{de}	3.15 ^{ef}	3.08 ^{fg}	4.76 ^a	3.60 ^c	2.70 ^h	2.76 ^h	0.0066	0.1007
	2	4.09 ^a	3.42 ^c	3.21 ^{cde}	3.34 ^{cde}	3.89 ^b	3.32 ^{cde}	3.14 ^{de}	3.15 ^{cde}	4.11 ^a	3.39 ^{cd}	2.68 ^f	2.67 ^f		
	3	3.71 ^a	3.23 ^c	3.15 ^c	3.16 ^c	3.52 ^b	3.08 ^c	2.98 ^c	3.07 ^c	3.78 ^a	3.12 ^c	2.56 ^d	2.57 ^d		
	4	3.44 ^a	2.99 ^c	2.91 ^c	3.16 ^{bc}	3.40 ^{ab}	2.94 ^c	2.92 ^c	3.00 ^c	3.48 ^a	2.84 ^c	2.39 ^d	2.47 ^d		
	5	3.21 ^a	2.86 ^{cd}	2.86 ^{cde}	3.03 ^{abc}	3.17 ^{ab}	2.79 ^{cde}	2.79 ^{cde}	2.89 ^{bcd}	3.25 ^a	2.62 ^{ef}	2.29 ^g	2.38 ^{fg}		
	6	3.06 ^a	2.72 ^{bc}	2.69 ^{cd}	2.98 ^{ab}	3.02 ^a	2.61 ^{cd}	2.64 ^{cd}	2.81 ^{abc}	2.83 ^{bc}	2.44 ^{de}	2.12 ^f	2.19 ^{ef}		
	7	2.59 ^{bcd}	2.39 ^{de}	2.50 ^{cde}	2.79 ^{ab}	2.66 ^{bcd}	2.30 ^{ef}	2.38 ^{def}	2.68 ^{abc}	2.85 ^a	2.15 ^{fg}	1.93 ^g	1.99 ^g		
a*: b* Ratio	1	2.12 ^a	2.03 ^{bc}	1.93 ^{de}	1.97 ^{cd}	2.10 ^{ab}	2.03 ^{bc}	1.90 ^{de}	1.91 ^{de}	2.14 ^a	2.07 ^{ab}	1.90 ^{de}	1.88 ^e	0.0012	0.0351
	2	2.10 ^{ab}	2.05 ^{abc}	1.97 ^{ef}	1.93 ^{fg}	2.07 ^{abcd}	1.99 ^{def}	1.93 ^{fg}	1.87 ^g	2.12 ^a	2.03 ^{bcd}	1.88 ^g	1.79 ^h		
	3	2.14 ^a	2.03 ^{bc}	1.92 ^{de}	1.91 ^e	2.08 ^b	1.98 ^{cd}	1.88 ^e	1.86 ^{ef}	2.07 ^{bc}	2.00 ^{bcd}	1.79 ^{fg}	1.74 ^g		
	4	1.99 ^{ab}	1.95 ^{bc}	1.94 ^{bc}	1.88 ^{cde}	1.96 ^{bc}	1.95 ^{bc}	1.90 ^{bcd}	1.82 ^{def}	2.04 ^a	1.93 ^{bc}	1.78 ^{fg}	1.65 ^g		
	5	1.99 ^{ab}	1.93 ^{abcd}	1.87 ^{def}	1.84 ^{def}	1.97 ^{abc}	1.90 ^{cde}	1.82 ^{ef}	1.79 ^f	2.01 ^a	1.85 ^{ef}	1.65 ^g	1.58 ^g		
	6	1.94 ^a	1.85 ^b	1.82 ^{bc}	1.78 ^{bc}	1.94 ^a	1.81 ^{bc}	1.77 ^{bc}	1.76 ^c	1.95 ^a	1.77 ^c	1.58 ^d	1.45 ^e		
	7	1.87 ^a	1.74 ^b	1.68 ^{bc}	1.70 ^{bc}	1.84 ^a	1.72 ^b	1.62 ^{cd}	1.63 ^{bd}	1.87 ^a	1.62 ^{cd}	1.43 ^e	1.33 ^f		

¹ Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

² Redness Ratio - Reflectance values for 630nm ÷ 580 nm

³N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-j} Means within each day of RD with different superscripts are different ($P < 0.05$).

Table 7 (continued). The effects of different myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹), frozen storage (0, 2, 4, or 6 months), and aging time (4 or 20 d) throughout retail display on color variables of steaks (n = 36).

Variable	Retail Display (RD)	Myoglobin Oxygenation Levels												P-Value	SEM
		DeOxy				Oxy				HiOxy					
		0 mo	2 mo	4 mo	6 mo	0 mo	2 mo	4 mo	6 mo	0 mo	2 mo	4 mo	6 mo		
Hue Angle	1	0.44 ^e	0.46 ^{cde}	0.48 ^{abc}	0.47 ^{bcd}	0.45 ^e	0.46 ^{cde}	0.48 ^{ab}	0.48 ^{abc}	0.44 ^j	0.45 ^{de}	0.49 ^{ab}	0.49 ^a	<0.0001	0.0097
	2	0.45 ^{ij}	0.45 ^{hij}	0.47 ^{efgh}	0.48 ^{cdefg}	0.45 ^{hij}	0.47 ^{fghi}	0.48 ^{cdefg}	0.49 ^{bcd}	0.44 ^f	0.46 ^{ghij}	0.49 ^{ab}	0.51 ^a		
	3	0.45 ^f	0.46 ^{ef}	0.48 ^{cde}	0.48 ^{cde}	0.45 ^f	0.47 ^{def}	0.49 ^{cd}	0.49 ^{bc}	0.45 ^f	0.46 ^{ef}	0.51 ^{ab}	0.53 ^a		
	4	0.47 ^{ef}	0.48 ^{def}	0.48 ^{def}	0.49 ^{cd}	0.47 ^{def}	0.48 ^{def}	0.49 ^{cde}	0.50 ^{bc}	0.46 ^f	0.48 ^{cde}	0.52 ^b	0.55 ^a		
	5	0.47 ^{fg}	0.48 ^{efg}	0.49 ^{cde}	0.50 ^{cd}	0.47 ^{efg}	0.49 ^{def}	0.51 ^{cd}	0.51 ^c	0.46 ^g	0.50 ^{cd}	0.55 ^b	0.58 ^a		
	6	0.48 ^{ef}	0.50 ^{de}	0.51 ^{cd}	0.52 ^{cd}	0.48 ^{ef}	0.51 ^{cd}	0.52 ^{cd}	0.52 ^c	0.47 ^f	0.52 ^c	0.58 ^b	0.62 ^a		
	7	0.49 ^g	0.53 ^f	0.55 ^{def}	0.54 ^{ef}	0.50 ^g	0.53 ^f	0.56 ^{ce}	0.56 ^{cd}	0.49 ^g	0.57 ^{cd}	0.63 ^b	0.67 ^a		

¹ Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

² Redness Ratio - Reflectance values for 630nm ÷ 580 nm

³ N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-j} Means within each day of RD with different superscripts are different ($P < 0.05$).

Table 7 (continued). The effects of different myoglobin oxygenation levels (DeOxy, Oxy, or HiOxy¹), frozen storage (0, 2, 4, or 6 months), and aging time (4 or 20 d) throughout retail display on color variables of steaks (n = 18).

Variable	Retail Display (RD)	Aging	Myoglobin Oxygenation Levels												P-Value	SEM
			DeOxy				Oxy				HiOxy					
			0 mo	2 mo	4 mo	6 mo	0 mo	2 mo	4 mo	6 mo	0 mo	2 mo	4 mo	6 mo		
Discoloration	1	4 d	0.15 ^a	0.09 ^a	0.15 ^a	-0.30 ^a	0.14 ^a	0.09 ^a	0.24 ^a	-0.27 ^a	-0.05 ^a	0.32 ^a	0.67 ^a	-0.15 ^a	<0.0001	3.6349
		20 d	0.48 ^a	-0.01 ^a	1.09 ^a	-0.28 ^a	1.37 ^a	0.04 ^a	0.51 ^a	1.33 ^a	-0.88 ^a	0.20 ^a	3.69 ^a	5.50 ^a		
	2	4 d	0.13 ^{ab}	0.09 ^{ab}	0.15 ^{ab}	-0.29 ^{ab}	0.14 ^{ab}	0.14 ^{ab}	0.17 ^{ab}	-0.31 ^{ab}	0.02 ^{ab}	0.44 ^{ab}	1.50 ^{ab}	0.01 ^{ab}		
		20 d	0.32 ^{ab}	-0.18 ^{ab}	0.37 ^{ab}	0.10 ^b	1.01 ^{ab}	-0.54 ^{ab}	2.91 ^{ab}	1.28 ^b	-0.45 ^{ab}	0.14 ^{ab}	5.25 ^{ab}	7.68 ^a		
	3	4 d	0.14 ^{bc}	0.22 ^{bc}	0.22 ^{bc}	-0.26 ^{bc}	0.13 ^{bc}	0.22 ^{bc}	0.34 ^{bc}	-0.16 ^{bc}	-0.63 ^{bc}	1.28 ^{bc}	5.56 ^{ab}	0.95 ^{bc}		
		20 d	0.36 ^{bc}	-0.05 ^{bc}	0.26 ^c	0.22 ^{bc}	1.84 ^{bc}	-0.51 ^c	3.27 ^{bc}	1.24 ^{bc}	-1.49 ^c	0.72 ^{bc}	8.69 ^{ab}	15.07 ^a		
	4	4 d	0.15 ^{de}	0.20 ^{de}	0.16 ^{de}	-0.23 ^{de}	0.27 ^{de}	0.56 ^{bcd^e}	0.01 ^{de}	-0.13 ^{de}	-0.88 ^{de}	1.83 ^{bcd^e}	9.54 ^{bc}	2.16 ^{bcd^e}		
		20 d	0.30 ^{bcd^e}	-0.25 ^{bcd^e}	2.27 ^{bde}	2.21 ^{bcde}	0.73 ^{bcde}	0.34 ^{bc}	5.26 ^{bce}	7.02 ^{bcd}	-2.91 ^e	2.26 ^{bcde}	8.57 ^{cd}	27.45 ^a		
	5	4 d	0.03 ^{def}	0.57 ^{def}	0.90 ^{def}	0.03 ^{def}	0.52 ^{def}	1.30 ^{def}	1.06 ^{def}	0.21 ^f	-2.00 ^{ef}	5.21 ^{cdef}	14.73 ^{bc}	6.86 ^{bcd^e}		
		20 d	0.55 ^{def}	0.45 ^{def}	2.83 ^{def}	4.67 ^{cdef}	2.31 ^{def}	0.60 ^{ef}	6.95 ^{bcd^{ef}}	9.56 ^{bcd}	-2.47 ^f	3.32 ^{def}	12.35 ^{bc}	34.00 ^a		
	6	4 d	-0.19 ^{ef}	2.67 ^{cef}	3.77 ^{cdef}	1.53 ^{def}	0.50 ^{ef}	3.28 ^{ef}	6.52 ^{bcd^{ef}}	2.50 ^{cdef}	0.19 ^{ef}	10.36 ^{bd}	32.75 ^a	35.60 ^a		
		20 d	4.56 ^{cdef}	3.68 ^{cdef}	6.45 ^{cdef}	6.08 ^{cdef}	4.96 ^{cdef}	4.35 ^{cdef}	9.65 ^{cde}	11.72 ^{bce}	-0.62 ^f	6.13 ^c	15.83 ^b	35.38 ^a		
	7	4 d	0.73 ^j	7.63 ^{ghi}	10.18 ^{eghi}	5.26 ^j	1.74 ^j	9.98 ^{ghi}	15.63 ^{defghi}	6.84 ^{hi}	4.38 ^j	20.10 ^{df}	42.13 ^b	52.51 ^a		
		20 d	8.47 ^{hi}	5.94 ⁱ	17.01 ^{defg}	16.28 ^{defgh}	10.39 ^{fghi}	6.30 ^j	20.16 ^{de}	20.45 ^d	7.26 ^{hi}	9.39 ^{ghi}	30.18 ^c	47.77 ^{ab}		

¹ Deoxymyoglobin - DeOxy, Oxygenation - Oxy, and High oxygenation - HiOxy

² Redness Ratio - Reflectance values for 630nm ÷ 580 nm

³N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-j} Means within each day of RD with different superscripts are different ($P < 0.05$).

Table 8. The effects of aging (4 d or 20 d), packaging film (impermeable or permeable), and frozen storage (0, 2, 4, or 6 months) on color variables of steaks ($n = 27$; $n = 54$).

Variable	Frozen Storage	Packaging Film				P-Value	SEM
		Impermeable		Permeable			
		Aging					
		4 d	20 d	4 d	20 d		
L*	0	44.41 ^c	47.06 ^a	44.66 ^c	46.69 ^b	0.0051	0.4233
	2	45.95 ^a	46.49 ^a	46.16 ^a	46.39 ^a		
	4	44.84 ^a	44.23 ^{ab}	44.49 ^b	44.22 ^{ab}		
	6	44.01 ^c	46.92 ^a	44.14 ^c	46.42 ^b		
Delta E	0	3.72 ^c	4.74 ^{abc}	4.51 ^{ab}	5.51 ^a	0.0146	0.4360
	2	3.26 ^b	4.95 ^a	3.91 ^{ab}	3.79 ^b		
	4	3.73 ^a	4.38 ^a	3.57 ^a	4.01 ^a		
	6	3.02 ^a	3.44 ^a	3.07 ^a	4.16 ^a		
Oxygen Penetration (mm)	Frozen Storage	Packaging Film		0.0108	0.0045		
		Impermeable	Permeable				
		0	0.09 ^a			0.10 ^a	
		2	0.08 ^a			0.07 ^a	
		4	0.06 ^a			0.05 ^b	

^{a-c} Means within each row with different superscripts are different ($P < 0.05$).

Table 9. The effects of aging (4 d or 20 d), packaging film (impermeable or permeable), and frozen storage (0, 2, 4, or 6 months) throughout retail display (RD) on color variables of steaks (n = 216; n = 216; n = 108).

Variable	Treatment	Retail Display (days)							P-Value	SEM
	Aging	1	2	3	4	5	6	7		
a*	4 d	15.75 ^a	16.09 ^a	15.97 ^a	15.54 ^a	15.07 ^a	14.48 ^a	13.63 ^a	0.0011	0.2637
	20 d	15.82 ^a	15.79 ^a	15.38 ^a	14.65 ^b	14.05 ^b	13.36 ^b	12.50 ^b		
Oxymyoglobin 4 d, %	Packaging								0.0118	0.8268
	Impermeable	72.59 ^b	72.27 ^a	71.51 ^a	70.13 ^a	68.77 ^b	67.06 ^a	63.23 ^a		
	Permeable	74.09 ^a	72.39 ^a	71.72 ^a	70.37 ^a	69.35 ^a	67.32 ^a	63.57 ^a		
a*	Frozen Storage								<0.0001	0.2577
	0	18.84 ^a	18.29 ^a	17.86 ^a	17.12 ^a	16.48 ^a	16.10 ^a	15.03 ^a		
	2	15.48 ^b	15.89 ^b	15.40 ^b	14.67 ^b	14.45 ^b	13.56 ^b	12.96 ^b		
	4	15.07 ^b	15.12 ^c	14.89 ^{bc}	14.48 ^b	13.63 ^c	13.08 ^{bc}	12.13 ^c		
	6	13.76 ^c	14.46 ^d	14.56 ^c	14.09 ^b	13.66 ^c	12.95 ^c	12.15 ^c		
Oxymyoglobin 4 d, %	0	80.66 ^a	78.10 ^a	75.67 ^a	74.10 ^a	72.56 ^a	70.68 ^a	67.81 ^a	<0.0001	1.6281
	2	75.78 ^b	74.19 ^{ab}	74.19 ^a	71.68 ^{ab}	70.23 ^{ab}	68.65 ^{ab}	62.90 ^b		
	4	70.43 ^c	69.96 ^{bc}	69.50 ^b	69.43 ^{bc}	67.72 ^b	65.70 ^{bc}	61.09 ^b		
	6	66.49 ^c	67.07 ^c	67.10 ^b	65.78 ^c	65.74 ^b	63.72 ^c	61.79 ^b		

¹ Redness Ratio - Reflectance values for 630nm ÷ 580 nm

²N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-f} Means within each day of RD with different superscripts are different ($P < 0.05$).

Table 9 (continued). The effects of aging (4 d or 20 d), packaging film (impermeable or permeable), and frozen storage (0, 2, 4, or 6 months) throughout retail display (RD) on color variables of steaks (n = 108; n = 54).

Variable	Treatment		Retail Display (days)							P-Value	SEM
	Frozen Storage		1	2	3	4	5	6	7		
Redness Ratio, 20 d ^{1,2}	0		4.16 ^a	3.67 ^a	3.32 ^a	3.03 ^a	2.84 ^a	3.12 ^a	2.47 ^a	<0.0001	0.1301
	2		3.23 ^b	3.02 ^b	2.98 ^a	2.85 ^{ab}	2.77 ^{ab}	2.62 ^b	2.46 ^a		
	4		N/A	N/A	N/A	N/A	N/A	N/A	N/A		
	6		2.67 ^c	2.69 ^b	2.60 ^b	2.52 ^b	2.40 ^b	2.21 ^c	2.06 ^b		
L*	0	Frozen Storage								<0.0001	0.4577
		Aging									
	2	4 d	43.51 ^e	44.58 ^{bc}	44.55 ^{cd}	44.36 ^d	44.69 ^c	44.70 ^{de}	45.34 ^b		
		20 d	46.51 ^{ab}	46.91 ^a	46.85 ^a	46.68 ^b	46.74 ^{ab}	47.12 ^a	47.30 ^a		
	4	4 d	46.02 ^{ab}	45.12 ^{bc}	45.62 ^{ab}	45.62 ^{bc}	45.92 ^b	46.15 ^{abcd}	47.95 ^a		
		20 d	46.61 ^a	46.59 ^a	45.82 ^{bc}	46.20 ^b	46.33 ^b	46.83 ^{ab}	46.71 ^a		
	6	4 d	44.60 ^{cd}	44.76 ^{bc}	43.81 ^d	45.04 ^{cd}	44.00 ^c	45.67 ^{bcd}	44.76 ^{bc}		
		20 d	44.23 ^{de}	44.14 ^{cd}	43.93 ^d	44.43 ^{cd}	44.09 ^c	44.34 ^e	44.44 ^{bc}		
	6	4 d	43.72 ^{de}	43.42 ^d	43.68 ^d	43.85 ^d	44.05 ^c	45.49 ^{cde}	44.32 ^c		
		20 d	45.56 ^{bc}	45.51 ^b	45.82 ^{bc}	47.77 ^a	47.67 ^a	46.61 ^{abc}	47.77 ^a		

¹ Redness Ratio - Reflectance values for 630nm ÷ 580 nm

²N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-f} Means within each day of RD with different superscripts are different ($P < 0.05$).

Table 9 (continued). The effects of aging (4 d or 20 d), packaging film (impermeable or permeable), and frozen storage (0, 2, 4, or 6 months) throughout retail display (RD) on color variables of steaks (n = 54).

Variable	Treatment		Retail Display (days)							P-Value	SEM
	Frozen Storage	Aging	1	2	3	4	5	6	7		
a*: b* Ratio	0	4 d	2.12 ^{ab}	2.11 ^a	2.15 ^a	2.03 ^a	2.01 ^a	2.00 ^a	1.93 ^a	0.0004	0.0427
		20 d	2.11 ^a	2.09 ^{ab}	2.04 ^{ab}	1.96 ^{abc}	1.97 ^{ab}	1.88 ^{ab}	1.80 ^b		
	2	4 d	2.08 ^{abc}	2.00 ^{bcd}	1.98 ^{bc}	1.95 ^{ab}	1.88 ^{bc}	1.80 ^{bc}	1.65 ^{cd}		
		20 d	2.01 ^{bcd}	2.05 ^{abc}	2.03 ^b	1.93 ^{abcd}	1.90 ^{abc}	1.81 ^{bc}	1.73 ^{bc}		
	4	4 d	1.94 ^{de}	1.97 ^{cd}	1.90 ^{cd}	1.94 ^{abcd}	1.85 ^{bc}	1.81 ^{bc}	1.63 ^{cde}		
		20 d	1.88 ^e	1.89 ^{de}	1.83 ^d	1.81 ^e	1.71 ^d	1.64 ^{de}	1.52 ^{ef}		
	6	4 d	1.98 ^{cde}	1.91 ^{de}	1.88 ^d	1.84 ^{de}	1.78 ^{cd}	1.76 ^d	1.61 ^{def}		
		20 d	1.86 ^e	1.82 ^e	1.79 ^d	1.72 ^e	1.69 ^d	1.57 ^e	1.50 ^f		

¹ Redness Ratio - Reflectance values for 630nm ÷ 580 nm

²N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-f} Means within each day of RD with different superscripts are different ($P < 0.05$).

Table 9 (continued). The effects of aging (4 d or 20 d), packaging film (impermeable or permeable), and frozen storage (0, 2, 4, or 6 months) throughout retail display (RD) on color variables of steaks (n = 54).

Variable	Treatment		Retail Display (days)							P-Value	SEM
	Frozen Storage	Aging	1	2	3	4	5	6	7		
Hue Angle	0	4 d	0.44 ^d	0.45 ^d	0.44 ^d	0.46 ^d	0.46 ^d	0.46 ^d	0.48 ^f	0.0104	0.0121
		20 d	0.44 ^{cd}	0.45 ^d	0.46 ^{cd}	0.47 ^{cd}	0.47 ^{cd}	0.48 ^{cd}	0.51 ^{ef}		
	2	4 d	0.45 ^{cd}	0.47 ^{bcd}	0.47 ^{bcd}	0.48 ^{cd}	0.49 ^{bcd}	0.51 ^{bc}	0.55 ^{cd}		
		20 d	0.46 ^{bcd}	0.46 ^{cd}	0.46 ^{cd}	0.48 ^{bcd}	0.49 ^{bcd}	0.51 ^{bc}	0.53 ^{de}		
	4	4 d	0.48 ^{abc}	0.47 ^{bcd}	0.49 ^{abc}	0.48 ^{bcd}	0.50 ^{bc}	0.51 ^{bc}	0.56 ^{cd}		
		20 d	0.49 ^{ab}	0.49 ^{ab}	0.50 ^{ab}	0.51 ^{ab}	0.54 ^a	0.56 ^a	0.60 ^{ab}		
	6	4 d	0.47 ^{abcd}	0.48 ^{abc}	0.49 ^{abc}	0.50 ^{bc}	0.51 ^{ab}	0.52 ^b	0.57 ^{bc}		
		20 d	0.50 ^a	0.51 ^a	0.51 ^a	0.53 ^a	0.55 ^a	0.58 ^a	0.61 ^a		

¹ Redness Ratio - Reflectance values for 630nm ÷ 580 nm

²N/A since the spectrophotometer was not functional during RD at 4 mo of frozen storage.

^{a-f} Means within each day of RD with different superscripts are different ($P < 0.05$).

Figure Legends

Figure 1. Instrumental color values for a^* of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging, aged 4 or 20 d, and frozen for 0, 2, 4, or 6 months.

Superscripts for mean separation may be found in Table 6 (page 192).

Figure 2. Instrumental color values for percent oxymyoglobin of steaks aged 4 d in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging, and frozen for 0, 2, 4, or 6 months.

Superscripts for mean separation may be found in Table 5 (page 190).

Figure 3. Instrumental color values for percent oxymyoglobin of steaks aged 20 d in either a deoxymyoglobin, oxygenated, or high oxygenated state and frozen for 0, 2, or 6 months throughout 7 days of retail display.

Superscripts for mean separation may be found in Table 7 (page 193).

Figure 4. Instrumental color values for percent metmyoglobin of steaks aged 20 d in either a deoxymyoglobin, oxygenated, or high oxygenated state and frozen for 0, 2, or 6 months throughout 7 days of retail display.

Superscripts for mean separation may be found in Table 7 (page 195).

Figure 5. Discoloration values for steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, aged 4 or 20 d and frozen for 0, 2, 4, or 6 months throughout 7 days of retail display.

Superscripts for mean separation may be found in Table 7 (page 198).

Figure 1.

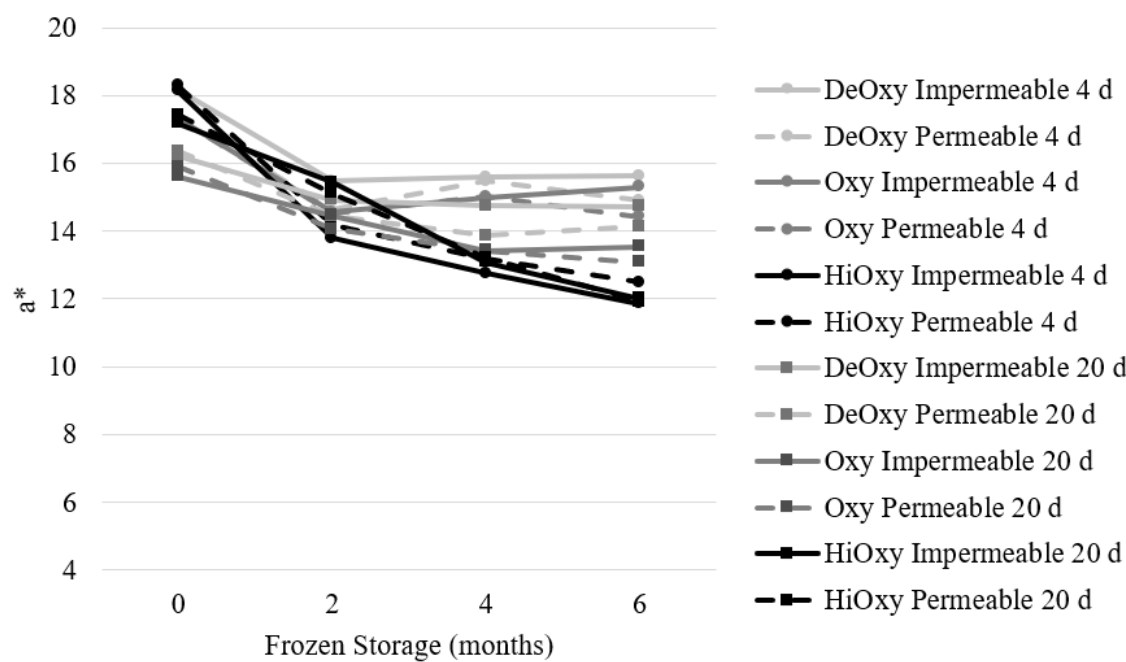


Figure 2.

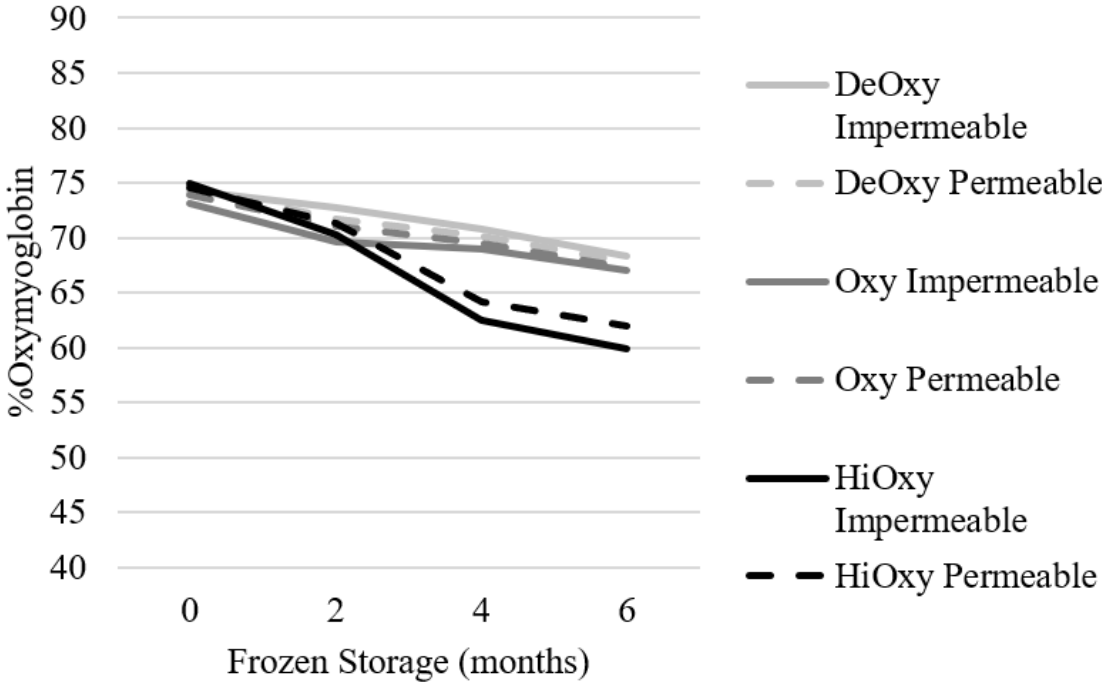


Figure 3.

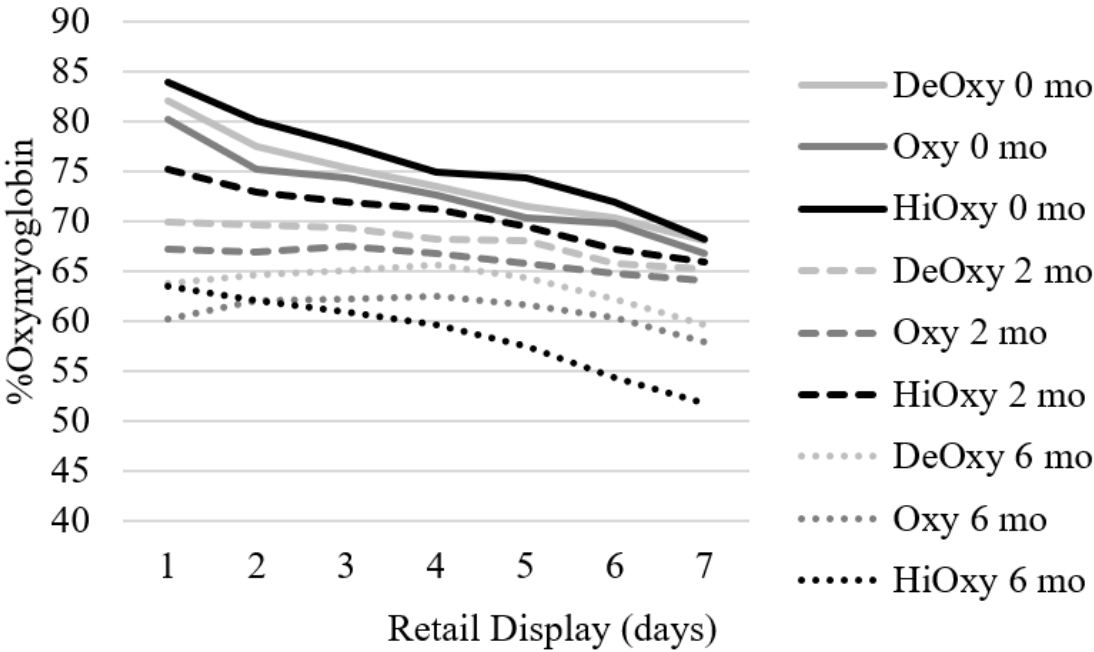


Figure 4.

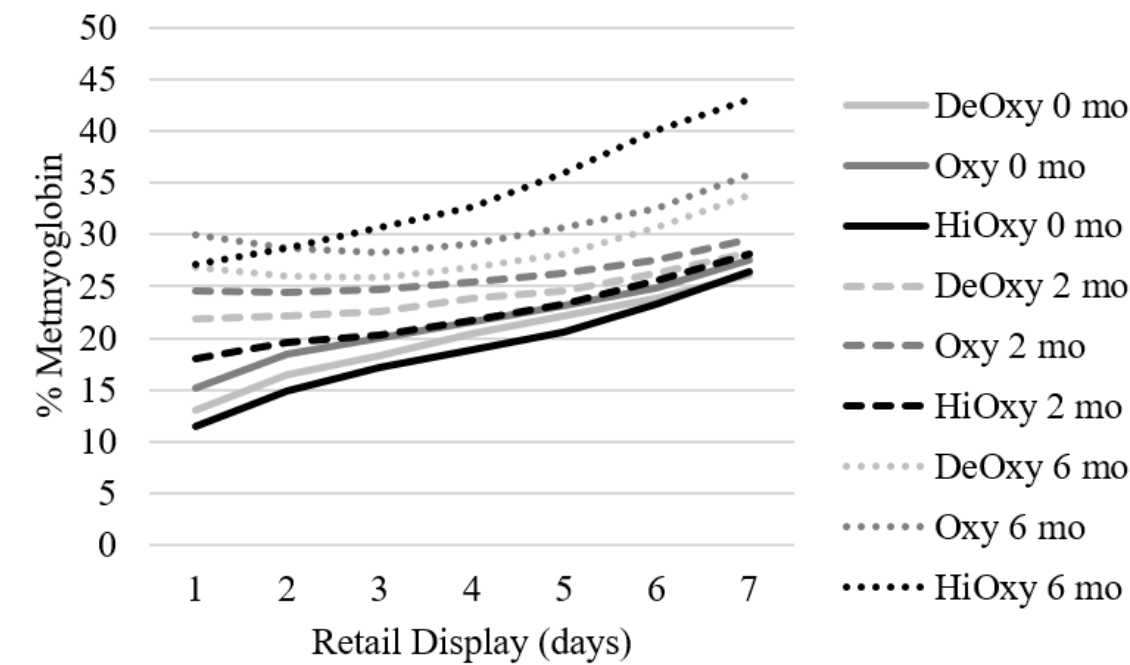
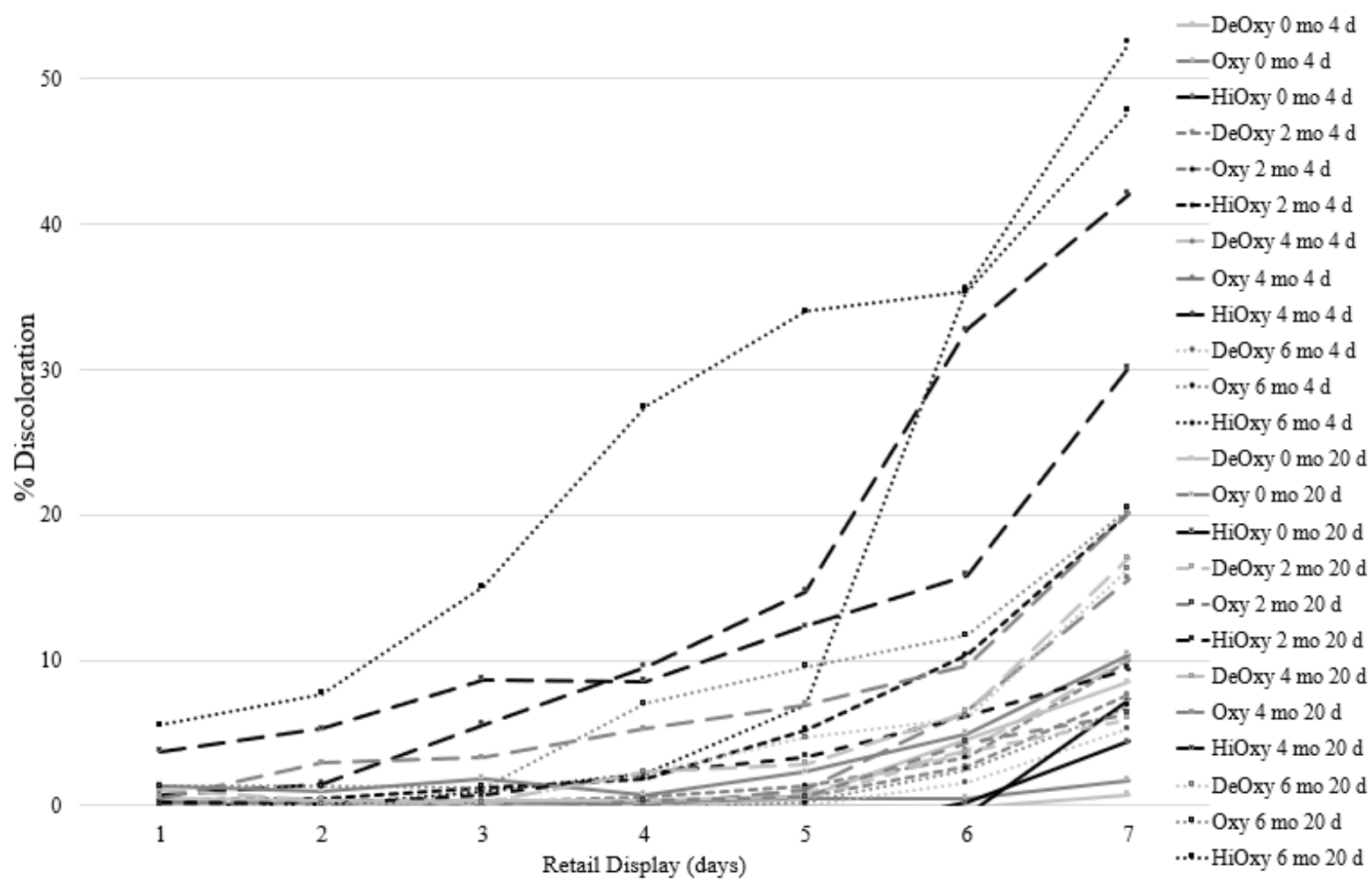


Figure 5.



RECOMMENDATIONS FOR FUTURE RESEARCH

Based on our results, I would highly recommend a consumer perception study related to color. Understanding there is difference among instrumental color instruments, I still believe it important to identify different measurements consumers relate to ideal/superior color. With our study we were able to identify differences in color, but finding more consumer data about what is acceptable for discoloration would aid processors and retailers by allowing them to measure color and determine how consumers will perceive it.

With the results from our study, it was difficult to explain what was occurring with L*, b*, and deoxymyoglobin percent. Therefore, more research is needed in regards to how these variables are altered through frozen storage. More research pertaining to the importance of these variables and the impact they have on meat color is needed. Another interesting result seen throughout the study was that highly oxygenated steaks in impermeable packaging, typically had worse color throughout retail display. However, the opposite was found for steaks exposed to little or no oxygen in impermeable packaging, having better color than those in the permeable packaging. Further research looking at the interaction of packaging and oxygenation levels through frozen storage may be beneficial for the industry to try and reach optimal red meat color.

Based on the complexity of our study, I would also recommend conducting a similar study, but with fewer treatment variables to truly identify what variables have the largest impact on frozen meat color. Throughout this series of studies, I would make sure to measure different levels of oxygenation times prior to freezing to try and identify ideal

oxygen exposure that would be most realistic with industry standards, but still provide a superior color. Along with differing oxygenation levels, I would advise more frozen storage increments. Based on our study, it appears that most detrimental effects take place between 2 and 4 months or 4 and 6 months. Therefore, I would recommend further evaluating frozen meat color on monthly increments to try and pinpoint the exact point color takes a drastic change.

Throughout these supplement studies, I would also recommend evaluating pH, NADH content, metmyoglobin reducing activity, and oxygen consumption rate. All of these variables greatly influence meat color. Hence, I believe it would be important to evaluate to see how they are impacted by freezing and to see how certain variables pertain to frozen meat color. Additionally, conducting analysis on tenderness, microbiology, and sensory analysis I believe could greatly contribute to the literature by determining how these factors are impacted by different freezing conditions.

In summary, based on our research I believe examining these different aspects of meat color will greatly help the industry:

1. Evaluate consumer perception of color in relationship to instrumental color.
2. Investigate the impact that L*, b*, and percent deoxymyoglobin have on meat color.
3. Examine the relationship between oxygenation level and packaging film permeability throughout frozen storage and retail display.
4. Conduct studies to evaluate specific parameters including varying oxygen levels and frozen storage durations.

5. Assess different underlying mechanisms that could be impacting meat color and how they are impacted by frozen storage.

6. Measure further meat quality variables to assess the impact that freezing and different oxygen levels have on varying meat quality characteristics.

Appendix 1.

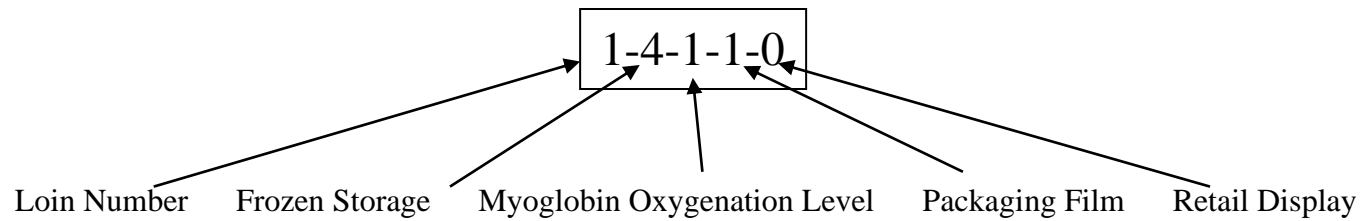
Storage, fabrication, and retail display dates

Storage and Fabrication Dates		
Storage Increment	28 days (1 Month)	
Aging	4 days	20 days
Fabrication Date	8/1/2018	8/17/2018
1 Month Storage	8/29/2018	9/14/2018
2 Month Storage	9/26/2018	10/12/2018
3 Month Storage	10/24/2018	11/9/2018
4 Month Storage	11/21/2018	12/7/2018
5 Month Storage	12/19/2018	1/4/2019
6 Month Storage	1/16/2019	2/1/2019

Retail Display Dates		
Aging	4 days	20 days
Fabrication Date	8/2/18-8/10/18	8/18/18-8/26/18
1 Month Storage	N/A	N/A
2 Month Storage	9/26/18-10/3/18	10/12/18-10/19/18
3 Month Storage	N/A	N/A
4 Month Storage	11/21/18-11/28/18	12/7/18-12/14/18
5 Month Storage	N/A	N/A
6 Month Storage	1/16/19-1/23/19	2/1/19-2/8/19

Appendix 2.

Randomization Key



Loin Number	Key
1-18	4 days of aging
19-36	20 days of aging

Frozen Storage Number	Key
0	0 Months
2	2 Months
4	4 Months
6	6 Months

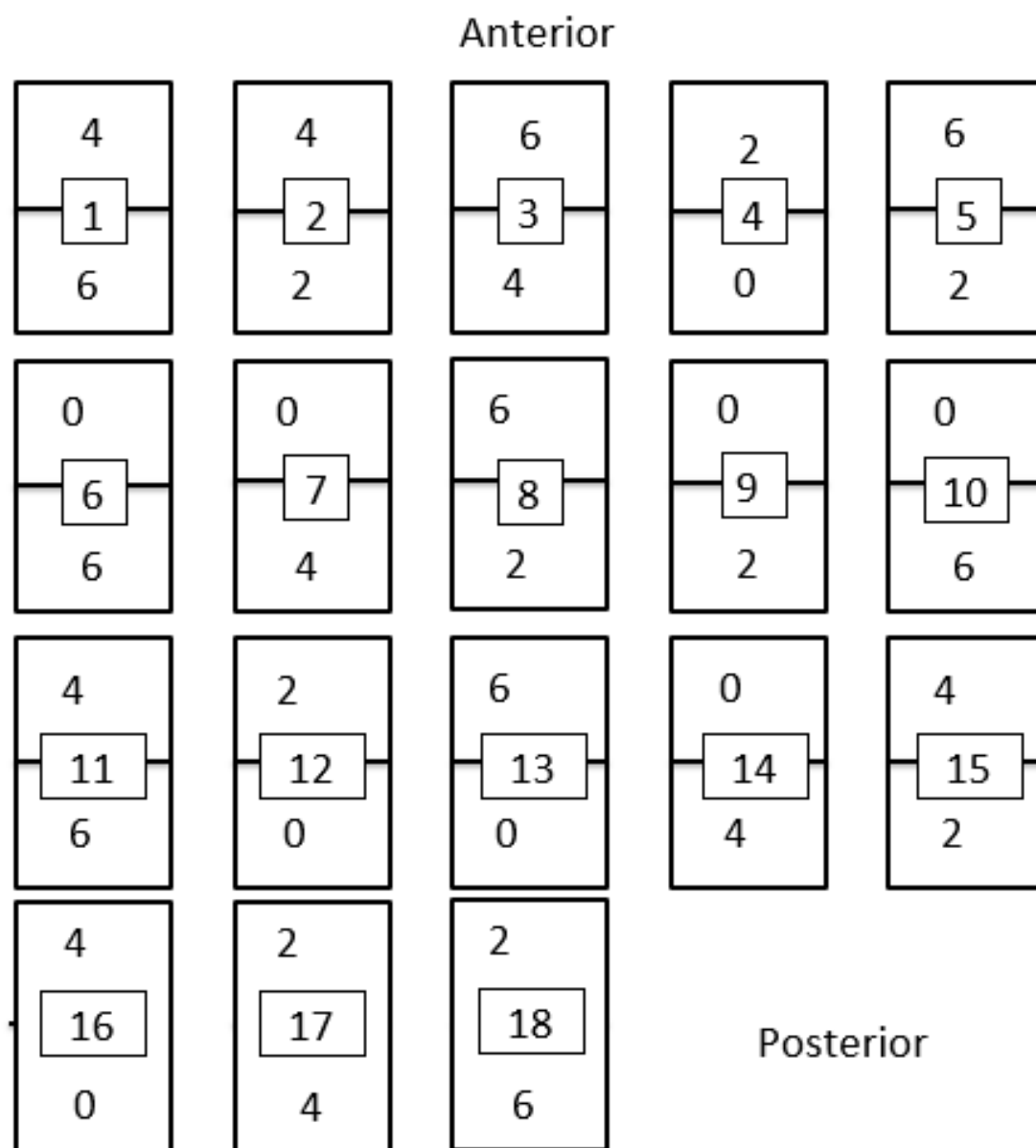
Myoglobin Oxygenation Level Number	Key
1	DeOxy
2	LoOxy
3	HiOxy

Packaging Film Number	Key
1	Permeable
2	Impermeable

Retail Display Number	Key
0	Frozen Steak Measurements
7	Thawed for Retail Display with Daily Color Measurements

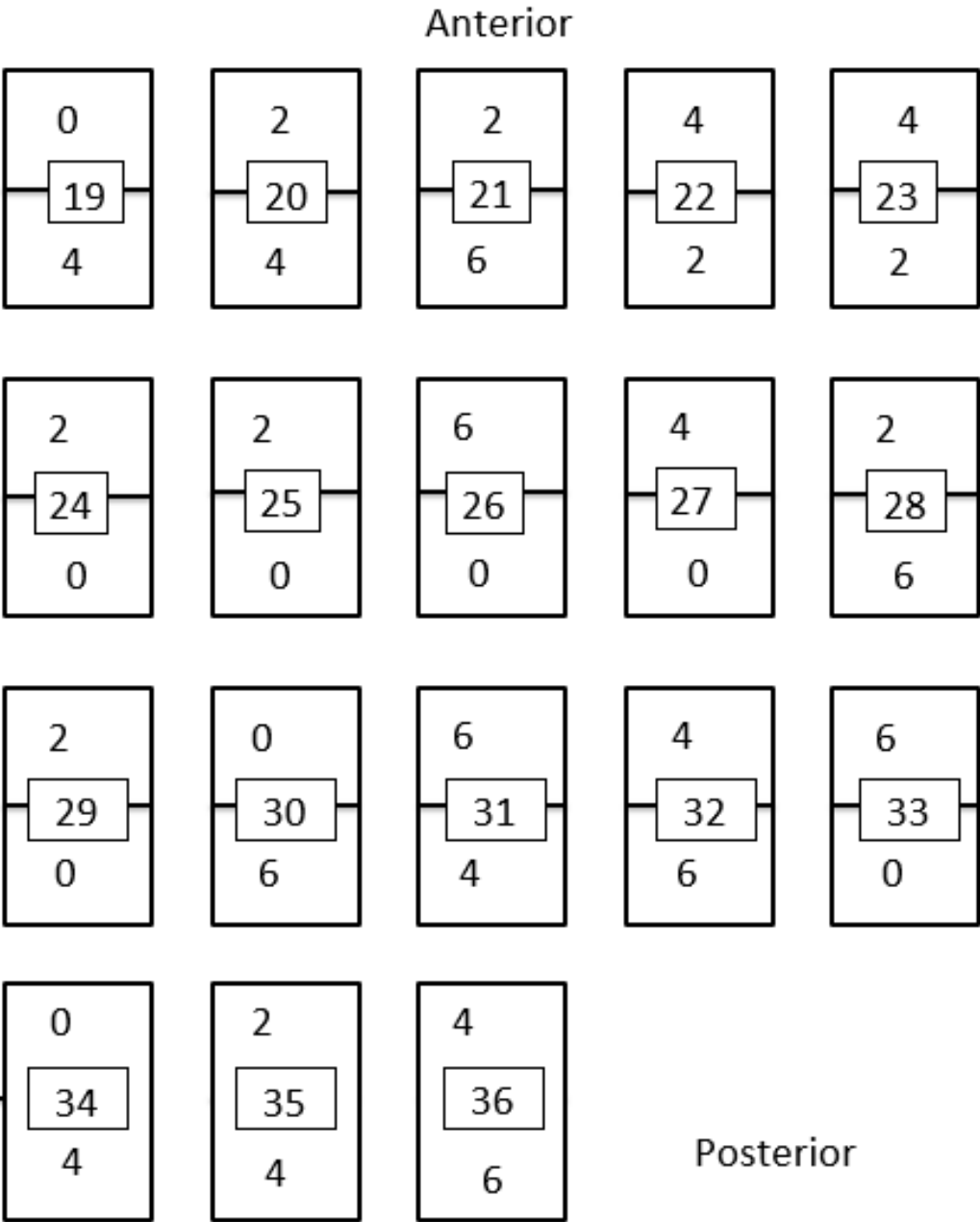
Appendix 3.

Frozen storage fabrication map for loins aged 4 d.



Appendix 4.

Frozen storage fabrication map for loins aged 20 d.



Appendix 5.

Spreadsheets for fabrication layout including the order steaks were fabricated.

Sample #	Order	Sample #	Order	Sample #	Order
1-4.	3-2-0 (HiOxy-Imperm-0)	3-6.	1-1-7 (DeOxy-Perm-7)	5-6.	3-2-7 (HiOxy-Imperm-7)
	3-2-7 (HiOxy-Imperm-7)		3-2-0 (HiOxy-Imperm-0)		3-2-0 (HiOxy-Imperm-0)
	1-2-7 (DeOxy-Imperm-7)		1-2-7 (DeOxy-Imperm-7)		2-2-7 (Oxy-Imperm-7)
	2-1-0 (Oxy-Perm-0)		2-1-0 (Oxy-Perm-0)		3-1-0 (HiOxy-Perm-0)
	2-1-7 (Oxy-Perm-7)		2-2-7 (Oxy-Imperm-7)		1-1-7 (DeOxy-Perm-7)
	3-1-0 (HiOxy-Perm-0)		2-1-7 (Oxy-Perm-7)		3-1-7 (HiOxy-Perm-7)
	2-2-7 (Oxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)		1-2-0 (DeOxy-Imperm-0)
	1-1-7 (DeOxy-Perm-7)		1-2-0 (DeOxy-Imperm-0)		2-1-0 (Oxy-Perm-0)
	2-2-0 (Oxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)		2-2-0 (Oxy-Imperm-0)
	1-1-0 (DeOxy-Perm-0)		3-2-7 (HiOxy-Imperm-7)		1-2-7 (DeOxy-Imperm-7)
	3-1-7 (HiOxy-Perm-7)		3-1-0 (HiOxy-Perm-0)		1-1-0 (DeOxy-Perm-0)
	1-2-0 (DeOxy-Imperm-0)		2-2-0 (Oxy-Imperm-0)		2-1-7 (Oxy-Perm-7)
1-6.	3-1-7 (HiOxy-Perm-7)	3-4.	1-1-0 (DeOxy-Perm-0)	5-2.	3-2-7 (HiOxy-Imperm-7)
	1-1-0 (DeOxy-Perm-0)		2-2-7 (Oxy-Imperm-7)		2-1-7 (Oxy-Perm-7)
	3-1-0 (HiOxy-Perm-0)		2-1-7 (Oxy-Perm-7)		3-1-0 (HiOxy-Perm-0)
	3-2-0 (HiOxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)		2-1-0 (Oxy-Perm-0)
	2-1-0 (Oxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)
	2-1-7 (Oxy-Perm-7)		3-1-7 (HiOxy-Perm-7)		3-1-7 (HiOxy-Perm-7)
	1-2-0 (DeOxy-Imperm-0)		2-2-0 (Oxy-Imperm-0)		2-2-0 (Oxy-Imperm-0)
	3-2-7 (HiOxy-Imperm-7)		1-2-0 (DeOxy-Imperm-0)		1-2-0 (DeOxy-Imperm-0)
	2-2-0 (Oxy-Imperm-0)		1-2-7 (DeOxy-Imperm-7)		1-2-7 (DeOxy-Imperm-7)
	1-1-7 (DeOxy-Perm-7)		1-1-7 (DeOxy-Perm-7)		2-2-7 (Oxy-Imperm-7)
	2-2-7 (Oxy-Imperm-7)		2-1-0 (Oxy-Perm-0)		1-1-7 (DeOxy-Perm-7)
	1-2-7 (DeOxy-Imperm-7)		3-1-0 (HiOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)
2-4.	3-2-0 (HiOxy-Imperm-0)	4-2.	1-2-7 (DeOxy-Imperm-7)	6-0.	2-1-0 (Oxy-Perm-0)
	2-2-0 (Oxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)		2-2-0 (Oxy-Imperm-0)
	3-1-0 (HiOxy-Perm-0)		1-1-7 (DeOxy-Perm-7)		1-1-7 (DeOxy-Perm-7)
	3-1-7 (HiOxy-Perm-7)		3-1-0 (HiOxy-Perm-0)		2-2-7 (Oxy-Imperm-7)
	2-1-0 (Oxy-Perm-0)		2-1-0 (Oxy-Perm-0)		3-1-7 (HiOxy-Perm-7)
	2-2-7 (Oxy-Imperm-7)		2-1-7 (Oxy-Perm-7)		2-1-7 (Oxy-Perm-7)
	2-1-7 (Oxy-Perm-7)		3-1-7 (HiOxy-Perm-7)		1-2-7 (DeOxy-Imperm-7)
	3-2-7 (HiOxy-Imperm-7)		2-2-7 (Oxy-Imperm-7)		1-2-0 (DeOxy-Imperm-0)
	1-1-7 (DeOxy-Perm-7)		3-2-0 (HiOxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)
	1-2-7 (DeOxy-Imperm-7)		2-2-0 (Oxy-Imperm-0)		3-2-0 (HiOxy-Imperm-0)
	1-1-0 (DeOxy-Perm-0)		1-1-0 (DeOxy-Perm-0)		3-1-0 (HiOxy-Perm-0)
	1-2-0 (DeOxy-Imperm-0)		1-2-0 (DeOxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)
2-2.	3-2-0 (HiOxy-Imperm-0)	4-0.	1-1-0 (DeOxy-Perm-0)	6-6.	3-2-7 (HiOxy-Imperm-7)
	1-1-7 (DeOxy-Perm-7)		1-2-0 (DeOxy-Imperm-0)		1-2-0 (DeOxy-Imperm-0)
	2-1-0 (Oxy-Perm-0)		2-1-7 (Oxy-Perm-7)		1-1-7 (DeOxy-Perm-7)
	3-1-0 (HiOxy-Perm-0)		2-2-7 (Oxy-Imperm-7)		2-1-0 (Oxy-Perm-0)
	1-2-7 (DeOxy-Imperm-7)		3-1-0 (HiOxy-Perm-0)		1-1-0 (DeOxy-Perm-0)
	2-2-0 (Oxy-Imperm-0)		1-2-7 (DeOxy-Imperm-7)		2-2-0 (Oxy-Imperm-0)
	2-1-7 (Oxy-Perm-7)		1-1-7 (DeOxy-Perm-7)		3-1-7 (HiOxy-Perm-7)
	3-2-7 (HiOxy-Imperm-7)		3-2-7 (HiOxy-Imperm-7)		3-1-0 (HiOxy-Perm-0)
	1-1-0 (DeOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)		2-1-7 (Oxy-Perm-7)
	1-2-0 (DeOxy-Imperm-0)		3-1-7 (HiOxy-Perm-7)		3-2-0 (HiOxy-Imperm-0)
	3-1-7 (HiOxy-Perm-7)		2-2-0 (Oxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)
	2-2-7 (Oxy-Imperm-7)		2-1-0 (Oxy-Perm-0)		1-2-7 (DeOxy-Imperm-7)

Sample #	Order	Sample #	Order	Sample #	Order
7-0.	1-1-0 (DeOxy-Perm-0)	9-0.	1-2-0 (DeOxy-Imperm-0)	11-4.	2-2-7 (Oxy-Imperm-7)
	1-1-7 (DeOxy-Perm-7)		3-1-0 (HiOxy-Perm-0)		2-2-0 (Oxy-Imperm-0)
	1-2-7 (DeOxy-Imperm-7)		3-2-0 (HiOxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)
	3-1-7 (HiOxy-Perm-7)		2-1-0 (Oxy-Perm-0)		2-1-7 (Oxy-Perm-7)
	1-2-0 (DeOxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)
	3-2-0 (HiOxy-Imperm-0)		2-2-0 (Oxy-Imperm-0)		2-1-0 (Oxy-Perm-0)
	2-2-0 (Oxy-Imperm-0)		3-1-7 (HiOxy-Perm-7)		3-2-0 (HiOxy-Imperm-0)
	2-1-0 (Oxy-Perm-0)		1-2-7 (DeOxy-Imperm-7)		1-2-7 (DeOxy-Imperm-7)
	3-1-0 (HiOxy-Perm-0)		1-1-0 (DeOxy-Perm-0)		1-2-0 (DeOxy-Imperm-0)
	3-2-7 (HiOxy-Imperm-7)		2-2-7 (Oxy-Imperm-7)		1-1-7 (DeOxy-Perm-7)
	2-2-7 (Oxy-Imperm-7)		2-1-7 (Oxy-Perm-7)		3-1-0 (HiOxy-Perm-0)
	2-1-7 (Oxy-Perm-7)		1-1-7 (DeOxy-Perm-7)		3-2-7 (HiOxy-Imperm-7)
7-4.	3-2-0 (HiOxy-Imperm-0)	9-2.	1-1-0 (DeOxy-Perm-0)	11-6.	3-2-0 (HiOxy-Imperm-0)
	3-1-0 (HiOxy-Perm-0)		2-1-7 (Oxy-Perm-7)		2-2-7 (Oxy-Imperm-7)
	3-1-7 (HiOxy-Perm-7)		1-1-7 (DeOxy-Perm-7)		3-1-0 (HiOxy-Perm-0)
	2-2-0 (Oxy-Imperm-0)		1-2-0 (DeOxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)
	1-1-0 (DeOxy-Perm-0)		2-2-7 (Oxy-Imperm-7)		1-2-0 (DeOxy-Imperm-0)
	2-2-7 (Oxy-Imperm-7)		3-2-0 (HiOxy-Imperm-0)		2-1-0 (Oxy-Perm-0)
	1-2-0 (DeOxy-Imperm-0)		2-2-0 (Oxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)
	1-2-7 (DeOxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)		1-1-7 (DeOxy-Perm-7)
	2-1-7 (Oxy-Perm-7)		3-1-0 (HiOxy-Perm-0)		3-1-7 (HiOxy-Perm-7)
	1-1-7 (DeOxy-Perm-7)		1-2-7 (DeOxy-Imperm-7)		1-2-7 (DeOxy-Imperm-7)
	3-2-7 (HiOxy-Imperm-7)		3-2-7 (HiOxy-Imperm-7)		2-2-0 (Oxy-Imperm-0)
	2-1-0 (Oxy-Perm-0)		2-1-0 (Oxy-Perm-0)		2-1-7 (Oxy-Perm-7)
8-6.	2-2-7 (Oxy-Imperm-7)	10-0.	2-2-7 (Oxy-Imperm-7)	12-2.	1-2-0 (DeOxy-Imperm-0)
	2-2-0 (Oxy-Imperm-0)		1-1-7 (DeOxy-Perm-7)		2-1-7 (Oxy-Perm-7)
	2-1-0 (Oxy-Perm-0)		1-2-7 (DeOxy-Imperm-7)		2-2-0 (Oxy-Imperm-0)
	1-2-7 (DeOxy-Imperm-7)		2-1-7 (Oxy-Perm-7)		3-1-0 (HiOxy-Perm-0)
	1-1-7 (DeOxy-Perm-7)		3-2-0 (HiOxy-Imperm-0)		2-1-0 (Oxy-Perm-0)
	3-2-0 (HiOxy-Imperm-0)		2-2-0 (Oxy-Imperm-0)		1-2-7 (DeOxy-Imperm-7)
	3-2-7 (HiOxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)		2-2-7 (Oxy-Imperm-7)
	2-1-7 (Oxy-Perm-7)		1-1-0 (DeOxy-Perm-0)		3-2-7 (HiOxy-Imperm-7)
	1-2-0 (DeOxy-Imperm-0)		1-2-0 (DeOxy-Imperm-0)		3-1-7 (HiOxy-Perm-7)
	3-1-0 (HiOxy-Perm-0)		2-1-0 (Oxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)
	1-1-0 (DeOxy-Perm-0)		3-2-7 (HiOxy-Imperm-7)		1-1-0 (DeOxy-Perm-0)
	3-1-7 (HiOxy-Perm-7)		3-1-0 (HiOxy-Perm-0)		1-1-7 (DeOxy-Perm-7)
8-2.	2-2-0 (Oxy-Imperm-0)	10-6.	3-1-7 (HiOxy-Perm-7)	12-0.	1-2-0 (DeOxy-Imperm-0)
	3-1-7 (HiOxy-Perm-7)		1-1-7 (DeOxy-Perm-7)		3-2-0 (HiOxy-Imperm-0)
	2-1-7 (Oxy-Perm-7)		2-2-0 (Oxy-Imperm-0)		3-1-7 (HiOxy-Perm-7)
	2-2-7 (Oxy-Imperm-7)		3-1-0 (HiOxy-Perm-0)		1-2-7 (DeOxy-Imperm-7)
	1-1-0 (DeOxy-Perm-0)		2-1-7 (Oxy-Perm-7)		3-1-0 (HiOxy-Perm-0)
	1-1-7 (DeOxy-Perm-7)		1-2-7 (DeOxy-Imperm-7)		1-1-0 (DeOxy-Perm-0)
	3-2-7 (HiOxy-Imperm-7)		1-2-0 (DeOxy-Imperm-0)		2-2-0 (Oxy-Imperm-0)
	3-2-0 (HiOxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)		2-2-7 (Oxy-Imperm-7)
	2-1-0 (Oxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)		2-1-0 (Oxy-Perm-0)
	3-1-0 (HiOxy-Perm-0)		3-2-7 (HiOxy-Imperm-7)		2-1-7 (Oxy-Perm-7)
	1-2-7 (DeOxy-Imperm-7)		2-1-0 (Oxy-Perm-0)		3-2-7 (HiOxy-Imperm-7)
	1-2-0 (DeOxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)		1-1-7 (DeOxy-Perm-7)

Sample #	Order	Sample #	Order	Sample #	Order
13-6	2-1-0 (Oxy-Perm-0)	15-4	2-2-7 (Oxy-Imperm-7)	17-2	1-1-7 (DeOxy-Perm-7)
	1-1-7 (DeOxy-Perm-7)		1-1-7 (DeOxy-Perm-7)		3-1-0 (HiOxy-Perm-0)
	2-1-7 (Oxy-Perm-7)		3-1-7 (HiOxy-Perm-7)		2-1-0 (Oxy-Perm-0)
	1-1-0 (DeOxy-Perm-0)		1-2-0 (DeOxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)
	2-2-0 (Oxy-Imperm-0)		3-1-0 (HiOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)
	2-2-7 (Oxy-Imperm-7)		1-2-7 (DeOxy-Imperm-7)		1-1-0 (DeOxy-Perm-0)
	3-2-7 (HiOxy-Imperm-7)		2-1-7 (Oxy-Perm-7)		2-2-0 (Oxy-Imperm-0)
	3-1-0 (HiOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)		1-2-7 (DeOxy-Imperm-7)
	3-1-7 (HiOxy-Perm-7)		2-1-0 (Oxy-Perm-0)		2-2-7 (Oxy-Imperm-7)
	1-2-0 (DeOxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)
	1-2-7 (DeOxy-Imperm-7)		1-1-0 (DeOxy-Perm-0)		2-1-7 (Oxy-Perm-7)
	3-2-0 (HiOxy-Imperm-0)		2-2-0 (Oxy-Imperm-0)		1-2-0 (DeOxy-Imperm-0)
13-0	2-1-0 (Oxy-Perm-0)	15-2	3-2-7 (HiOxy-Imperm-7)	17-4	3-2-0 (HiOxy-Imperm-0)
	1-1-0 (DeOxy-Perm-0)		3-1-0 (HiOxy-Perm-0)		1-2-0 (DeOxy-Imperm-0)
	2-2-7 (Oxy-Imperm-7)		1-1-7 (DeOxy-Perm-7)		2-1-0 (Oxy-Perm-0)
	3-1-0 (HiOxy-Perm-0)		1-2-7 (DeOxy-Imperm-7)		2-2-7 (Oxy-Imperm-7)
	3-2-7 (HiOxy-Imperm-7)		2-1-7 (Oxy-Perm-7)		3-1-0 (HiOxy-Perm-0)
	1-1-7 (DeOxy-Perm-7)		3-2-0 (HiOxy-Imperm-0)		1-2-7 (DeOxy-Imperm-7)
	1-2-0 (DeOxy-Imperm-0)		2-1-0 (Oxy-Perm-0)		1-1-0 (DeOxy-Perm-0)
	2-2-0 (Oxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)		2-2-0 (Oxy-Imperm-0)
	1-2-7 (DeOxy-Imperm-7)		1-2-0 (DeOxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)
	2-1-7 (Oxy-Perm-7)		2-2-0 (Oxy-Imperm-0)		3-1-7 (HiOxy-Perm-7)
	3-1-7 (HiOxy-Perm-7)		3-1-7 (HiOxy-Perm-7)		2-1-7 (Oxy-Perm-7)
	3-2-0 (HiOxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)		1-1-7 (DeOxy-Perm-7)
14-0	1-1-7 (DeOxy-Perm-7)	16-4	3-1-0 (HiOxy-Perm-0)	18-2	2-1-7 (Oxy-Perm-7)
	2-1-0 (Oxy-Perm-0)		2-1-7 (Oxy-Perm-7)		3-1-0 (HiOxy-Perm-0)
	3-2-0 (HiOxy-Imperm-0)		3-2-0 (HiOxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)
	1-1-0 (DeOxy-Perm-0)		2-1-0 (Oxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)
	2-1-7 (Oxy-Perm-7)		1-2-0 (DeOxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)
	2-2-0 (Oxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)
	2-2-7 (Oxy-Imperm-7)		1-2-7 (DeOxy-Imperm-7)		1-2-0 (DeOxy-Imperm-0)
	3-1-0 (HiOxy-Perm-0)		1-1-7 (DeOxy-Perm-7)		2-1-0 (Oxy-Perm-0)
	1-2-7 (DeOxy-Imperm-7)		2-2-0 (Oxy-Imperm-0)		1-2-7 (DeOxy-Imperm-7)
	3-1-7 (HiOxy-Perm-7)		3-1-7 (HiOxy-Perm-7)		2-2-0 (Oxy-Imperm-0)
	3-2-7 (HiOxy-Imperm-7)		1-1-0 (DeOxy-Perm-0)		1-1-0 (DeOxy-Perm-0)
	1-2-0 (DeOxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)		1-1-7 (DeOxy-Perm-7)
14-4	2-2-0 (Oxy-Imperm-0)	16-0	3-1-7 (HiOxy-Perm-7)	18-6	1-1-0 (DeOxy-Perm-0)
	1-2-7 (DeOxy-Imperm-7)		1-2-7 (DeOxy-Imperm-7)		2-1-7 (Oxy-Perm-7)
	3-2-7 (HiOxy-Imperm-7)		1-1-0 (DeOxy-Perm-0)		2-2-0 (Oxy-Imperm-0)
	3-2-0 (HiOxy-Imperm-0)		2-1-7 (Oxy-Perm-7)		3-1-0 (HiOxy-Perm-0)
	3-1-7 (HiOxy-Perm-7)		3-2-7 (HiOxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)
	2-1-7 (Oxy-Perm-7)		2-2-0 (Oxy-Imperm-0)		3-2-0 (HiOxy-Imperm-0)
	1-1-7 (DeOxy-Perm-7)		3-2-0 (HiOxy-Imperm-0)		1-2-7 (DeOxy-Imperm-7)
	1-2-0 (DeOxy-Imperm-0)		1-1-7 (DeOxy-Perm-7)		2-2-7 (Oxy-Imperm-7)
	2-1-0 (Oxy-Perm-0)		1-2-0 (DeOxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)
	1-1-0 (DeOxy-Perm-0)		2-2-7 (Oxy-Imperm-7)		2-1-0 (Oxy-Perm-0)
	3-1-0 (HiOxy-Perm-0)		2-1-0 (Oxy-Perm-0)		1-1-7 (DeOxy-Perm-7)
	2-2-7 (Oxy-Imperm-7)		3-1-0 (HiOxy-Perm-0)		1-2-0 (DeOxy-Imperm-0)

Sample #	Order	Sample #	Order	Sample #	Order
19-0	3-2-7 (HiOxy-Imperm-7)	21-2	2-2-0 (Oxy-Imperm-0)	23-4	3-2-7 (HiOxy-Imperm-7)
	1-2-0 (DeOxy-Imperm-0)		3-2-0 (HiOxy-Imperm-0)		1-1-7 (DeOxy-Perm-7)
	2-1-0 (Oxy-Perm-0)		2-1-7 (Oxy-Perm-7)		3-2-0 (HiOxy-Imperm-0)
	1-1-0 (DeOxy-Perm-0)		2-1-0 (Oxy-Perm-0)		2-2-0 (Oxy-Imperm-0)
	3-1-7 (HiOxy-Perm-7)		1-2-0 (DeOxy-Imperm-0)		1-2-7 (DeOxy-Imperm-7)
	3-2-0 (HiOxy-Imperm-0)		3-1-7 (HiOxy-Perm-7)		3-1-7 (HiOxy-Perm-7)
	1-1-7 (DeOxy-Perm-7)		2-2-7 (Oxy-Imperm-7)		3-1-0 (HiOxy-Perm-0)
	2-2-7 (Oxy-Imperm-7)		3-2-7 (HiOxy-Imperm-7)		2-1-7 (Oxy-Perm-7)
	1-2-7 (DeOxy-Imperm-7)		1-1-7 (DeOxy-Perm-7)		1-1-0 (DeOxy-Perm-0)
	3-1-0 (HiOxy-Perm-0)		1-1-0 (DeOxy-Perm-0)		1-2-0 (DeOxy-Imperm-0)
	2-1-7 (Oxy-Perm-7)		1-2-7 (DeOxy-Imperm-7)		2-1-0 (Oxy-Perm-0)
	2-2-0 (Oxy-Imperm-0)		3-1-0 (HiOxy-Perm-0)		2-2-7 (Oxy-Imperm-7)
19-4	2-2-0 (Oxy-Imperm-0)	21-6	2-2-0 (Oxy-Imperm-0)	23-2	1-1-7 (DeOxy-Perm-7)
	3-1-7 (HiOxy-Perm-7)		3-1-7 (HiOxy-Perm-7)		2-2-0 (Oxy-Imperm-0)
	2-1-7 (Oxy-Perm-7)		1-2-0 (DeOxy-Imperm-0)		3-1-0 (HiOxy-Perm-0)
	1-2-7 (DeOxy-Imperm-7)		2-2-7 (Oxy-Imperm-7)		2-2-7 (Oxy-Imperm-7)
	3-2-7 (HiOxy-Imperm-7)		3-2-7 (HiOxy-Imperm-7)		3-2-0 (HiOxy-Imperm-0)
	1-1-7 (DeOxy-Perm-7)		3-1-0 (HiOxy-Perm-0)		1-2-7 (DeOxy-Imperm-7)
	3-1-0 (HiOxy-Perm-0)		2-1-7 (Oxy-Perm-7)		1-1-0 (DeOxy-Perm-0)
	3-2-0 (HiOxy-Imperm-0)		3-2-0 (HiOxy-Imperm-0)		2-1-7 (Oxy-Perm-7)
	2-1-0 (Oxy-Perm-0)		1-1-7 (DeOxy-Perm-7)		2-1-0 (Oxy-Perm-0)
	2-2-7 (Oxy-Imperm-7)		1-2-7 (DeOxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)
	1-2-0 (DeOxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)		3-2-7 (HiOxy-Imperm-7)
	1-1-0 (DeOxy-Perm-0)		2-1-0 (Oxy-Perm-0)		1-2-0 (DeOxy-Imperm-0)
20-2	3-2-7 (HiOxy-Imperm-7)	22-6	3-2-7 (HiOxy-Imperm-7)	24-2	2-2-7 (Oxy-Imperm-7)
	3-1-7 (HiOxy-Perm-7)		1-2-7 (DeOxy-Imperm-7)		1-1-7 (DeOxy-Perm-7)
	1-1-0 (DeOxy-Perm-0)		1-1-0 (DeOxy-Perm-0)		2-1-7 (Oxy-Perm-7)
	2-1-7 (Oxy-Perm-7)		2-1-7 (Oxy-Perm-7)		2-2-0 (Oxy-Imperm-0)
	1-2-7 (DeOxy-Imperm-7)		2-2-0 (Oxy-Imperm-0)		3-1-7 (HiOxy-Perm-7)
	2-2-0 (Oxy-Imperm-0)		3-1-0 (HiOxy-Perm-0)		2-1-0 (Oxy-Perm-0)
	3-2-0 (HiOxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)		3-2-0 (HiOxy-Imperm-0)
	1-1-7 (DeOxy-Perm-7)		3-2-0 (HiOxy-Imperm-0)		1-2-7 (DeOxy-Imperm-7)
	3-1-0 (HiOxy-Perm-0)		1-1-7 (DeOxy-Perm-7)		3-1-0 (HiOxy-Perm-0)
	2-1-0 (Oxy-Perm-0)		2-1-0 (Oxy-Perm-0)		1-2-0 (DeOxy-Imperm-0)
	1-2-0 (DeOxy-Imperm-0)		3-1-7 (HiOxy-Perm-7)		3-2-7 (HiOxy-Imperm-7)
	2-2-7 (Oxy-Imperm-7)		1-2-0 (DeOxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)
20-4	3-1-7 (HiOxy-Perm-7)	22-2	1-1-7 (DeOxy-Perm-7)	24-0	3-2-0 (HiOxy-Imperm-0)
	1-1-7 (DeOxy-Perm-7)		3-1-0 (HiOxy-Perm-0)		2-2-0 (Oxy-Imperm-0)
	2-1-7 (Oxy-Perm-7)		1-2-7 (DeOxy-Imperm-7)		1-2-0 (DeOxy-Imperm-0)
	3-2-0 (HiOxy-Imperm-0)		1-2-0 (DeOxy-Imperm-0)		2-1-7 (Oxy-Perm-7)
	1-2-7 (DeOxy-Imperm-7)		2-2-0 (Oxy-Imperm-0)		1-1-7 (DeOxy-Perm-7)
	3-2-7 (HiOxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)		1-2-7 (DeOxy-Imperm-7)
	1-1-0 (DeOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)		2-1-0 (Oxy-Perm-0)
	2-2-7 (Oxy-Imperm-7)		2-1-7 (Oxy-Perm-7)		3-1-7 (HiOxy-Perm-7)
	2-2-0 (Oxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)		1-1-0 (DeOxy-Perm-0)
	3-1-0 (HiOxy-Perm-0)		2-1-0 (Oxy-Perm-0)		3-1-0 (HiOxy-Perm-0)
	1-2-0 (DeOxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)		2-2-7 (Oxy-Imperm-7)
	2-1-0 (Oxy-Perm-0)		3-2-7 (HiOxy-Imperm-7)		3-2-7 (HiOxy-Imperm-7)

Sample #	Order	Sample #	Order	Sample #	Order
25-2	2-2-0 (Oxy-Imperm-0)	27-4	1-2-7 (DeOxy-Imperm-7)	29-2	3-2-7 (HiOxy-Imperm-7)
	2-1-7 (Oxy-Perm-7)		3-1-0 (HiOxy-Perm-0)		3-1-0 (HiOxy-Perm-0)
	3-2-7 (HiOxy-Imperm-7)		2-2-0 (Oxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)
	1-1-0 (DeOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)		1-2-7 (DeOxy-Imperm-7)
	2-2-7 (Oxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)		1-2-0 (DeOxy-Imperm-0)
	3-2-0 (HiOxy-Imperm-0)		2-1-0 (Oxy-Perm-0)		1-1-7 (DeOxy-Perm-7)
	1-2-0 (DeOxy-Imperm-0)		1-2-0 (DeOxy-Imperm-0)		3-1-7 (HiOxy-Perm-7)
	1-2-7 (DeOxy-Imperm-7)		1-1-0 (DeOxy-Perm-0)		2-2-7 (Oxy-Imperm-7)
	3-1-0 (HiOxy-Perm-0)		1-1-7 (DeOxy-Perm-7)		2-2-0 (Oxy-Imperm-0)
	1-1-7 (DeOxy-Perm-7)		2-2-7 (Oxy-Imperm-7)		2-1-0 (Oxy-Perm-0)
	2-1-0 (Oxy-Perm-0)		3-2-7 (HiOxy-Imperm-7)		3-2-0 (HiOxy-Imperm-0)
	3-1-7 (HiOxy-Perm-7)		2-1-7 (Oxy-Perm-7)		2-1-7 (Oxy-Perm-7)
25-0	3-2-0 (HiOxy-Imperm-0)	27-0	1-1-0 (DeOxy-Perm-0)	29-0	1-1-0 (DeOxy-Perm-0)
	2-2-0 (Oxy-Imperm-0)		2-1-0 (Oxy-Perm-0)		2-2-0 (Oxy-Imperm-0)
	1-1-7 (DeOxy-Perm-7)		1-1-7 (DeOxy-Perm-7)		1-2-7 (DeOxy-Imperm-7)
	2-1-0 (Oxy-Perm-0)		1-2-0 (DeOxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)
	2-2-7 (Oxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)		1-2-0 (DeOxy-Imperm-0)
	3-1-0 (HiOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)
	3-1-7 (HiOxy-Perm-7)		2-2-7 (Oxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)
	1-2-0 (DeOxy-Imperm-0)		3-1-0 (HiOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)
	1-1-0 (DeOxy-Perm-0)		1-2-7 (DeOxy-Imperm-7)		1-1-7 (DeOxy-Perm-7)
	1-2-7 (DeOxy-Imperm-7)		2-1-7 (Oxy-Perm-7)		3-1-0 (HiOxy-Perm-0)
	3-2-7 (HiOxy-Imperm-7)		2-2-0 (Oxy-Imperm-0)		2-1-0 (Oxy-Perm-0)
	2-1-7 (Oxy-Perm-7)		3-2-7 (HiOxy-Imperm-7)		2-1-7 (Oxy-Perm-7)
26-6	1-1-7 (DeOxy-Perm-7)	28-2	2-1-0 (Oxy-Perm-0)	30-0	1-2-0 (DeOxy-Imperm-0)
	2-1-0 (Oxy-Perm-0)		1-1-7 (DeOxy-Perm-7)		3-1-0 (HiOxy-Perm-0)
	1-2-7 (DeOxy-Imperm-7)		3-1-0 (HiOxy-Perm-0)		1-2-7 (DeOxy-Imperm-7)
	1-1-0 (DeOxy-Perm-0)		2-2-0 (Oxy-Imperm-0)		1-1-7 (DeOxy-Perm-7)
	2-2-7 (Oxy-Imperm-7)		2-1-7 (Oxy-Perm-7)		3-1-7 (HiOxy-Perm-7)
	1-2-0 (DeOxy-Imperm-0)		3-2-0 (HiOxy-Imperm-0)		2-1-7 (Oxy-Perm-7)
	3-1-7 (HiOxy-Perm-7)		1-2-0 (DeOxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)
	2-1-7 (Oxy-Perm-7)		3-2-7 (HiOxy-Imperm-7)		2-1-0 (Oxy-Perm-0)
	3-2-0 (HiOxy-Imperm-0)		1-2-7 (DeOxy-Imperm-7)		2-2-7 (Oxy-Imperm-7)
	2-2-0 (Oxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)		1-1-0 (DeOxy-Perm-0)
	3-2-7 (HiOxy-Imperm-7)		1-1-0 (DeOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)
	3-1-0 (HiOxy-Perm-0)		3-1-7 (HiOxy-Perm-7)		2-2-0 (Oxy-Imperm-0)
26-0	1-1-7 (DeOxy-Perm-7)	28-6	1-2-7 (DeOxy-Imperm-7)	30-6	3-1-7 (HiOxy-Perm-7)
	2-1-0 (Oxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)		2-1-0 (Oxy-Perm-0)
	1-2-7 (DeOxy-Imperm-7)		1-1-0 (DeOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)
	1-2-0 (DeOxy-Imperm-0)		2-2-0 (Oxy-Imperm-0)		1-2-0 (DeOxy-Imperm-0)
	3-2-0 (HiOxy-Imperm-0)		1-2-0 (DeOxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)
	3-1-0 (HiOxy-Perm-0)		1-1-7 (DeOxy-Perm-7)		3-2-7 (HiOxy-Imperm-7)
	2-1-7 (Oxy-Perm-7)		3-1-0 (HiOxy-Perm-0)		2-2-0 (Oxy-Imperm-0)
	2-2-0 (Oxy-Imperm-0)		2-1-7 (Oxy-Perm-7)		1-1-0 (DeOxy-Perm-0)
	3-1-7 (HiOxy-Perm-7)		2-2-7 (Oxy-Imperm-7)		1-2-7 (DeOxy-Imperm-7)
	2-2-7 (Oxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)		1-1-7 (DeOxy-Perm-7)
	3-2-7 (HiOxy-Imperm-7)		3-2-7 (HiOxy-Imperm-7)		3-1-0 (HiOxy-Perm-0)
	1-1-0 (DeOxy-Perm-0)		2-1-0 (Oxy-Perm-0)		2-1-7 (Oxy-Perm-7)

Sample #	Order	Sample #	Order	Sample #	Order
31-6	2-2-7 (Oxy-Imperm-7)	33-6	3-1-7 (HiOxy-Perm-7)	35-2	3-2-7 (HiOxy-Imperm-7)
	2-1-7 (Oxy-Perm-7)		2-1-0 (Oxy-Perm-0)		3-1-7 (HiOxy-Perm-7)
	1-2-7 (DeOxy-Imperm-7)		1-2-0 (DeOxy-Imperm-0)		1-2-7 (DeOxy-Imperm-7)
	3-1-0 (HiOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)		2-2-0 (Oxy-Imperm-0)
	1-1-7 (DeOxy-Perm-7)		1-2-7 (DeOxy-Imperm-7)		3-1-0 (HiOxy-Perm-0)
	2-2-0 (Oxy-Imperm-0)		3-1-0 (HiOxy-Perm-0)		1-1-7 (DeOxy-Perm-7)
	3-2-7 (HiOxy-Imperm-7)		2-2-0 (Oxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)
	3-1-7 (HiOxy-Perm-7)		3-2-7 (HiOxy-Imperm-7)		2-1-0 (Oxy-Perm-0)
	1-1-0 (DeOxy-Perm-0)		2-1-7 (Oxy-Perm-7)		2-1-7 (Oxy-Perm-7)
	1-2-0 (DeOxy-Imperm-0)		1-1-0 (DeOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)
	2-1-0 (Oxy-Perm-0)		1-1-7 (DeOxy-Perm-7)		1-2-0 (DeOxy-Imperm-0)
	3-2-0 (HiOxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)		2-2-7 (Oxy-Imperm-7)
31-4	2-1-7 (Oxy-Perm-7)	33-0	3-2-7 (HiOxy-Imperm-7)	35-4	1-1-0 (DeOxy-Perm-0)
	3-2-0 (HiOxy-Imperm-0)		1-1-7 (DeOxy-Perm-7)		1-2-7 (DeOxy-Imperm-7)
	2-2-0 (Oxy-Imperm-0)		3-2-0 (HiOxy-Imperm-0)		1-1-7 (DeOxy-Perm-7)
	3-1-7 (HiOxy-Perm-7)		1-2-0 (DeOxy-Imperm-0)		2-2-0 (Oxy-Imperm-0)
	2-1-0 (Oxy-Perm-0)		2-1-0 (Oxy-Perm-0)		3-2-7 (HiOxy-Imperm-7)
	2-2-7 (Oxy-Imperm-7)		3-1-0 (HiOxy-Perm-0)		3-2-0 (HiOxy-Imperm-0)
	1-1-0 (DeOxy-Perm-0)		1-2-7 (DeOxy-Imperm-7)		3-1-0 (HiOxy-Perm-0)
	1-2-7 (DeOxy-Imperm-7)		1-1-0 (DeOxy-Perm-0)		2-2-7 (Oxy-Imperm-7)
	1-2-0 (DeOxy-Imperm-0)		2-1-7 (Oxy-Perm-7)		2-1-7 (Oxy-Perm-7)
	1-1-7 (DeOxy-Perm-7)		2-2-0 (Oxy-Imperm-0)		3-1-7 (HiOxy-Perm-7)
	3-1-0 (HiOxy-Perm-0)		2-2-7 (Oxy-Imperm-7)		1-2-0 (DeOxy-Imperm-0)
	3-2-7 (HiOxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)		2-1-0 (Oxy-Perm-0)
32-4	2-1-0 (Oxy-Perm-0)	34-0	1-1-0 (DeOxy-Perm-0)	36-4	1-1-7 (DeOxy-Perm-7)
	1-1-7 (DeOxy-Perm-7)		1-1-7 (DeOxy-Perm-7)		2-1-7 (Oxy-Perm-7)
	3-2-0 (HiOxy-Imperm-0)		3-1-0 (HiOxy-Perm-0)		1-1-0 (DeOxy-Perm-0)
	3-1-0 (HiOxy-Perm-0)		2-2-0 (Oxy-Imperm-0)		3-1-0 (HiOxy-Perm-0)
	2-1-7 (Oxy-Perm-7)		1-2-0 (DeOxy-Imperm-0)		3-1-7 (HiOxy-Perm-7)
	1-2-7 (DeOxy-Imperm-7)		2-1-7 (Oxy-Perm-7)		2-2-0 (Oxy-Imperm-0)
	3-1-7 (HiOxy-Perm-7)		1-2-7 (DeOxy-Imperm-7)		1-2-7 (DeOxy-Imperm-7)
	2-2-7 (Oxy-Imperm-7)		3-2-7 (HiOxy-Imperm-7)		3-2-0 (HiOxy-Imperm-0)
	1-2-0 (DeOxy-Imperm-0)		3-1-7 (HiOxy-Perm-7)		2-1-0 (Oxy-Perm-0)
	2-2-0 (Oxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)		1-2-0 (DeOxy-Imperm-0)
	1-1-0 (DeOxy-Perm-0)		2-1-0 (Oxy-Perm-0)		2-2-7 (Oxy-Imperm-7)
	3-2-7 (HiOxy-Imperm-7)		3-2-0 (HiOxy-Imperm-0)		3-2-7 (HiOxy-Imperm-7)
32-6	3-2-0 (HiOxy-Imperm-0)	34-4	1-2-7 (DeOxy-Imperm-7)	36-6	1-2-0 (DeOxy-Imperm-0)
	2-1-7 (Oxy-Perm-7)		2-2-7 (Oxy-Imperm-7)		3-1-7 (HiOxy-Perm-7)
	1-1-7 (DeOxy-Perm-7)		3-1-7 (HiOxy-Perm-7)		1-1-7 (DeOxy-Perm-7)
	1-2-7 (DeOxy-Imperm-7)		1-1-7 (DeOxy-Perm-7)		1-1-0 (DeOxy-Perm-0)
	1-2-0 (DeOxy-Imperm-0)		3-2-0 (HiOxy-Imperm-0)		2-2-7 (Oxy-Imperm-7)
	3-1-0 (HiOxy-Perm-0)		2-1-0 (Oxy-Perm-0)		1-2-7 (DeOxy-Imperm-7)
	1-1-0 (DeOxy-Perm-0)		1-2-0 (DeOxy-Imperm-0)		3-1-0 (HiOxy-Perm-0)
	2-1-0 (Oxy-Perm-0)		2-2-0 (Oxy-Imperm-0)		2-1-0 (Oxy-Perm-0)
	3-1-7 (HiOxy-Perm-7)		1-1-0 (DeOxy-Perm-0)		2-1-7 (Oxy-Perm-7)
	2-2-7 (Oxy-Imperm-7)		3-2-7 (HiOxy-Imperm-7)		3-2-0 (HiOxy-Imperm-0)
	2-2-0 (Oxy-Imperm-0)		2-1-7 (Oxy-Perm-7)		2-2-0 (Oxy-Imperm-0)
	3-2-7 (HiOxy-Imperm-7)		3-1-0 (HiOxy-Perm-0)		3-2-7 (HiOxy-Imperm-7)

Appendix 6.

Objective color (L^* , a^* , b^*) calibration instructions and helpful tips

Minolta Calibration Procedures

1. Before Calibration:

Calibrate Minolta in the same temperature conditions as the measurements being taken.

- Place the Minolta in the environment where samples will be measured about 5 or 10 minutes before calibrating so it can become equilibrated with the temperature.

Calibrate with the same materials as you will be taking measurements.

- If the measurements will not be taken directly on the meat surface, you must calibrate the Minolta with the same material it will be measuring through. For example, if you want to take readings from samples that are wrapped in overwrap, you must put some overwrap around the measuring head “eye” while calibrating using the white tile.

2. Turn the power to the measuring head *ON*.

3. Turn the power to the data processor *ON* while holding down the [DELETE/UNDO] key at the same time.

- Release the [DELETE/UNDO] key when you hear a BEEP. (This deletes any previous data that might still be stored in the data processor)

4. When the screen turns on, the question “Initial set ok?” appears, press the [Measure Enter] key.

5. Once you get to the measurement screen, press the [Index Set] key.

- Use the *arrows and the [Measure Enter] key* to adjust all the following settings:
 - Printer: On
 - Color space: Off
 - Protect: On
 - Auto Average: However many readings wanted per sample (1-30)
 - Illuminant: D65
 - Back light: Off
 - Buzzer: On
 - Disp. Limit
- Press the [Esc] key to return to the measurement screen.

6. Press the [Calibrate] key while in the measurement screen.
7. Enter in the numbers listed on the calibrating white tile for the D65 setting using the following:
 - [< >] keys and the numeric pad
 - (The [< >] keys move the cursor)
 - D65 settings:
 - Y: 93.13 x: 0.3164 y: 0.3330
8. Set up the measuring head so that it is resting on the LCD screen and the “eye” is facing up.
 - Place the white calibration tile on the measuring head, near the middle of the tile.
9. Press either the measurement button on the measuring head OR the [Measure Enter] key on the data processor after making sure the ready lamp is ON.
 - Make sure the white tile is completely on the measuring head “eye”.
 - The Calibration is complete after the lamp flashes 3 times and the screen returns to the measurement screen.
 - Do not move the measuring head during calibration.
10. Press the [Color Space] key until the L*, a*, b* screen shows up.
11. Calibration is finished and the Minolta is ready
 - To save battery life, turn both the measurer and data collector off after calibration is finished until you need it for measuring. The calibration and settings will not be erased.
 - When turning back on for measurements, ONLY turn on the power buttons. DO NOT hold down the [DELETE/UNDO] key at the same time. This will delete the calibration and settings and all of the steps will have to be repeated.

Cleaning

- Wipe machine down with a soft, clean dry cloth. Never use solvents such as thinner or benzene.
- If the white calibration tile becomes dirty, wipe it gently with a soft, clean dry cloth. If dirt is difficult to remove, wipe it with lens cleaner and cloth, then dry.

Minolta Helpful Tips

1. Make a separate datasheet

- The Minolta prints out data with sample numbers 1 to 2000. In order to correlate it back to a sample, you must make a separate data sheet that has a place to record meat sample i.d. and its corresponding Minolta number.

2. Batteries

- The measuring head requires 4 AAA batteries and the data processor requires 4 AA batteries

3. The auto protect setting

- The Minolta can only record and store up to 2000 readings, once you go past 2000 readings it will start deleting older readings.
- When the auto protect is on it will automatically prevent the 2001st reading from being taken so you cannot accidentally overwrite other data.

4. Auto Average Function

- During calibration, if you set the Auto Average function to a reading number above 1, for example 5, you only have to hit the measure button once and it will automatically take all 5 readings then print out the average.
- It only allows a second or two between readings so make sure you are paying attention and move the measuring head to where you want it before it automatically takes the next reading.

5. Recalibrate regularly

- If using the Minolta all day, or for long periods of time, make sure to recalibrate it regularly (approximately every 4 hours).

6. DELETE/UNDO KEY

- If you accidentally take a reading, hitting the [DELETE/UNDO] key will delete the last reading.
- If you accidentally delete a reading by hitting the [DELETE/UNDO] key, hitting the [DELETE/UNDO] key again will restore the previous reading.

7. Printer Paper

- The paper that the data is printed on is sensitive to heat and light. The printed data should be kept in a dark cool place, like a desk drawer. In order to prevent losing any data, you must make a photocopy of the printout in order to preserve it for long-term storage.

8. More than One Color Space on Print Out

If you want to print more than 1 color space (Example: L*a*b* AND XYZ) on the print out slip:

1. Press the [Index Set] key. Use the arrows and the [Measure Enter] key to adjust all the following settings:
 - o Color space: On
 - o Disp. Limit: press the [Measure Enter] key to select this option
 - o Once inside the Disp. Limit option, go through the list and change all the color spaces that you DO NOT want to OFF.
2. Press the [Esc] key until you return to the measurement screen.

9. Change Measurements to a Different Color Space

If you get done measuring and realize that you meant to measure in a different color space (For example: measured everything using Yxy and meant to use L*a*b*), you can correct it using these steps:

1. While in the measurement screen, press the [Color Space] key until your desired color space (in this example: L*a*b*) appears.
2. Press the [Data List] key while in the measurement screen.
3. Select the desired page using the up and down arrows.
 - o If you only have one page it will show up as P00, select this one.
4. Once you have the desired page selected, press the [Measure Enter] key.
5. Press the [Print/Feed] key
6. Select “All Meas. Data” using the up and down arrows.
7. Press the [Measure Enter] key
 - o This will reprint all the stored data in your newly selected color space (L*a*b* in this example).
8. Press the [Esc] key to return to the measurement screen.

Appendix 7.

Visual guide for percent surface discoloration



0%



5%



10%



20%



30%



40%



50%



60%



70%



80%



90%



100%

Appendix 8.

Thiobarbituric Acid Reactive Substances Assay

(Buege and Aust, 1978), Modified by Ahn et al., (1998)

TEP solution (1, 1, 3, 3-Tetraethoxypropane) (Make new weekly)

Stock Solution: Dilute 99 μ l TEP (97%) bring volume to 100 mL double distilled water (ddH₂O)

Working Solution: Dilute stock solution to 1:3 (TEP Solution:ddH₂O) (1×10^{-3} M)

TBA/TCA (2-Thiobarbituric Acid/Trichloroacetic Acid) Stock Solution: 1L

15% TCA (w/v) and 20 mM TBA (MW 144.5) reagent in ddH₂O

Dissolve 2.88 g TBA in warm ddH₂O first, then add TCA (150g) and ddH₂O to 1L

BHA (Butylated HydroxyAnisole) Stock Solution:

Make 10% stock solution by dissolving in 90% ethanol

10g BHA dissolved in 90mL ethanol (90%) + 5mL ddH₂O

Standards: In Duplicate

Blank:	1 ml ddH ₂ O	<u>Moles of TEP</u>
Standard 5:	100 μ l working TEP + 1.90 mL ddH ₂ O	(5×10^{-5} M)
Standard 4:	1 mL Std. 5 + 1 mL ddH ₂ O	(2.5×10^{-5} M)
Standard 3:	1 mL Std. 4 + 1 mL ddH ₂ O	(1.25×10^{-5} M)
Standard 2:	1 mL Std. 3 + 1 mL ddH ₂ O	(0.625×10^{-5} M)
Standard 1:	1 mL Std. 2 + 1 mL ddH ₂ O	(0.3125×10^{-5} M)

Remove 1 mL of Standard 1 and discard it, leaving 1 mL behind.

TBA Procedure:

1. Mix all reagents and standards before beginning.
2. Transfer 5 g powdered sample into a 50 mL conical tube; add 14 mL of ddH₂O and 1.0 mL of BHA.
3. Homogenize for 15 sec with a polytron.
4. Centrifuge for 2000 x g for 5 min.
5. Transfer 1 mL of homogenate or standard to 15 mL conical tube.
6. Add 2 mL of TBA/TCA solution, vortex.
7. Incubate in a 70°C water bath for 30 min. to develop color.
8. Cool samples in a cold water bath for 10 min.
9. Centrifuge tubes at 2000 x g for 15 min.
10. Transfer duplicate aliquots of 200 µl from each tube into wells on a 96-well plate.
11. Read absorbance at 540 nm.

Calculations: mg of malonaldehyde/kg of tissue

$$K (\text{extraction}) = (S/A) \times MW \times (10^6/E) \times 100$$

Where:

S = Standard concentration (1 x 10⁻⁸ moles 1, 1, 3,3-tetraethoxypropane)/5ml

A= Absorbance of standard

MW = MW of malonaldehyde

E = sample equivalent

P = percent recovery

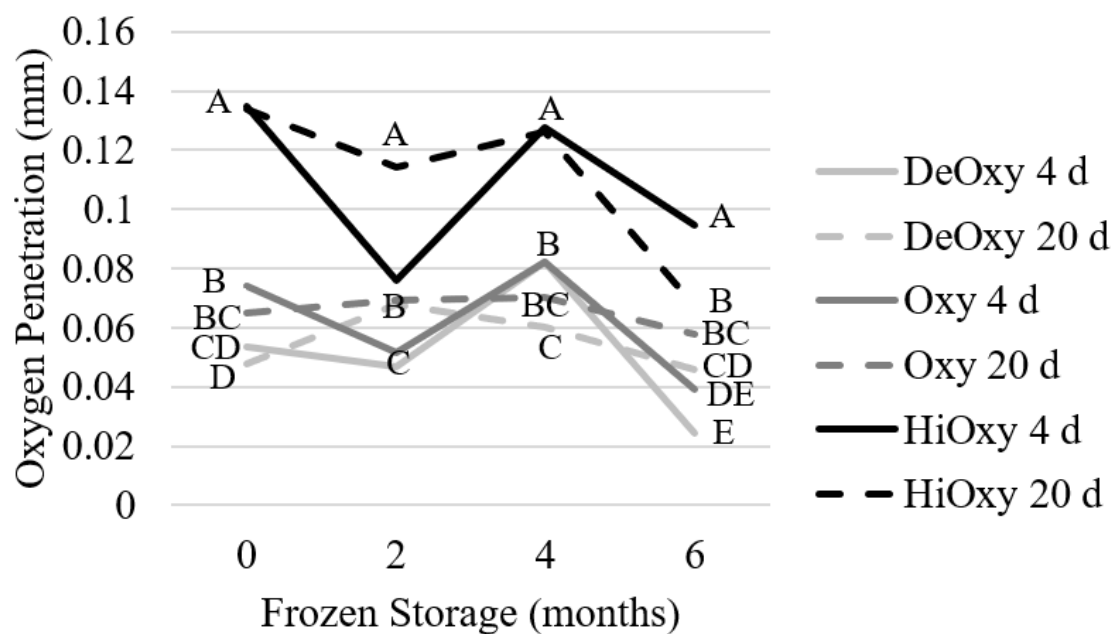
Final Calculation: .012 x concentration x 72.063 x 10⁻⁶ = mg of Malonaldehyde/kg tissue

Reagents (Sigma): TBA – T5500; TCA – T9159; TEP – T9889; BHA – B1253

Ahn, D. U., D. G. Olsen, C. Jo, X. Chen, C. Wu, and J. I. Lee. 1998. Effect of muscle type, packaging, and irradiation on lipid oxidation, volatile production, and color in raw pork patties. *Meat Sci.* 49:27-39.

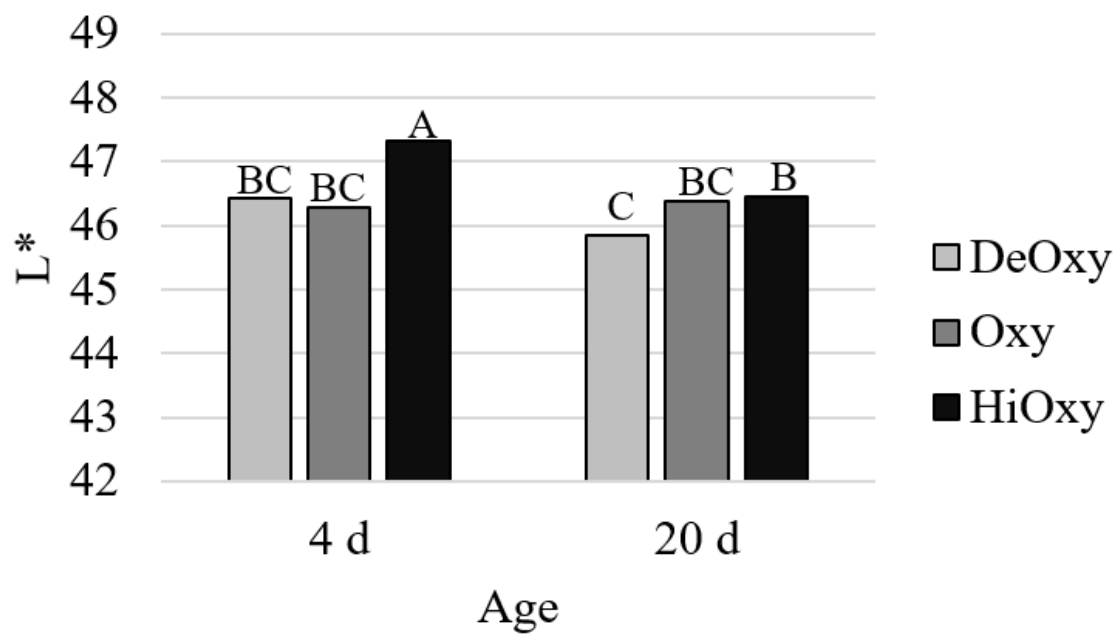
Buege, J. A. and Aust, S. D. 1978. Microsomal Lipid Peroxidation. *Methods in Enzymology.* 52:302-310.

Appendix 9. Oxygen penetration values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, aged for 4 or 20 d, and frozen for 0, 2, 4, or 6 months.



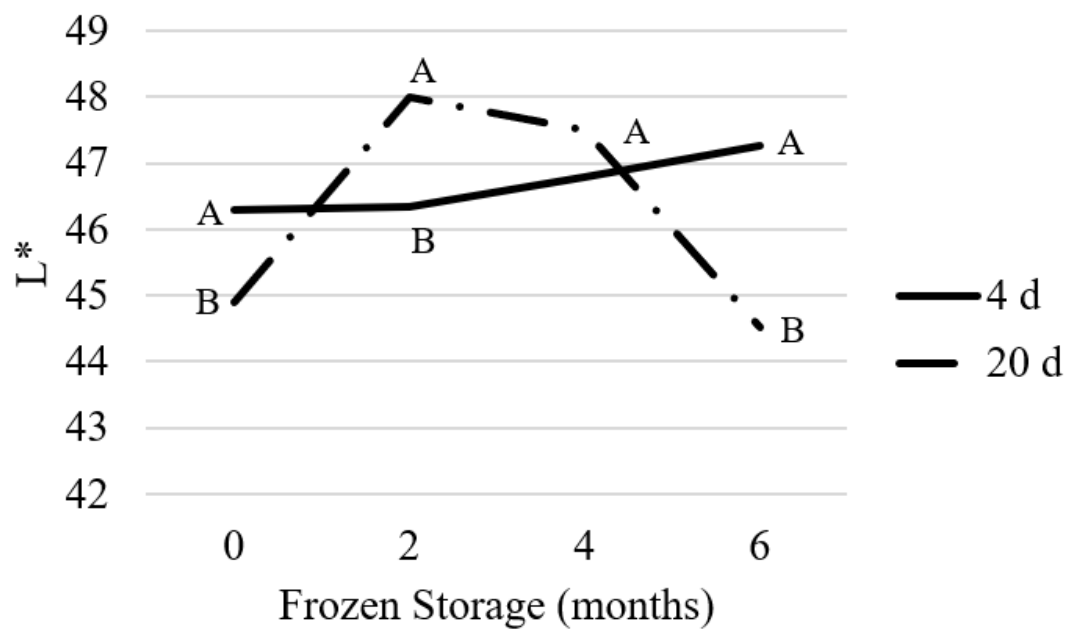
^{a-c} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 10. Instrumental color values for L^* of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and aged 4 or 20 d.



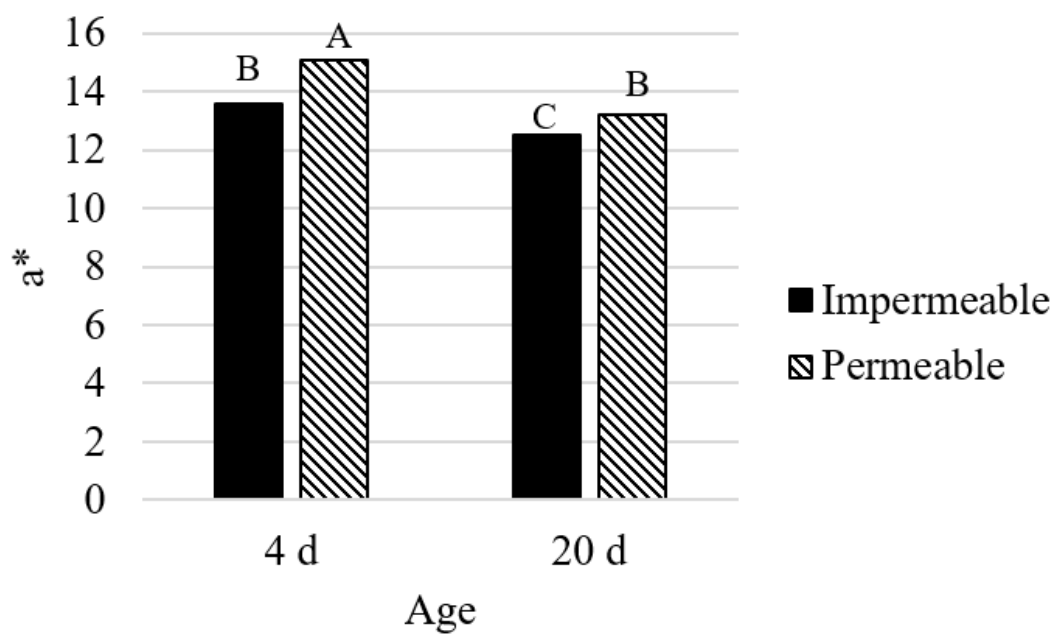
^{a-c} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 11. Instrumental color values for L^* of steaks aged for 4 or 20 d and frozen for either 0, 2, 4, or 6 months.



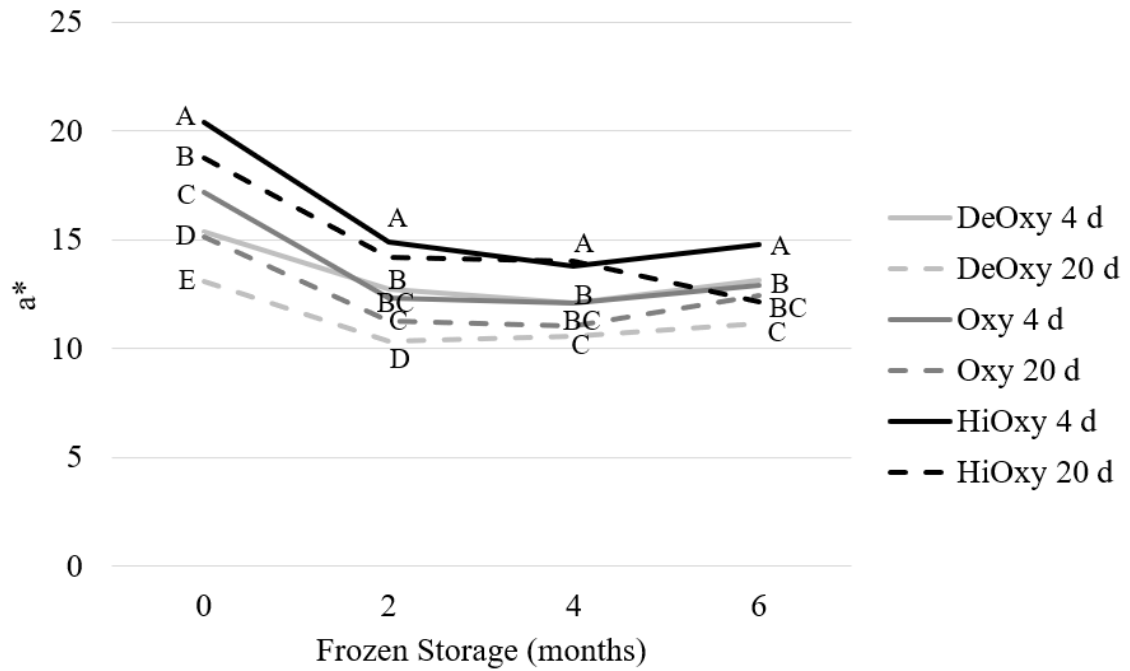
^{a-b} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 12. Instrumental color values for a^* of steaks aged 4 or 20 d and impermeable or permeable packaging.



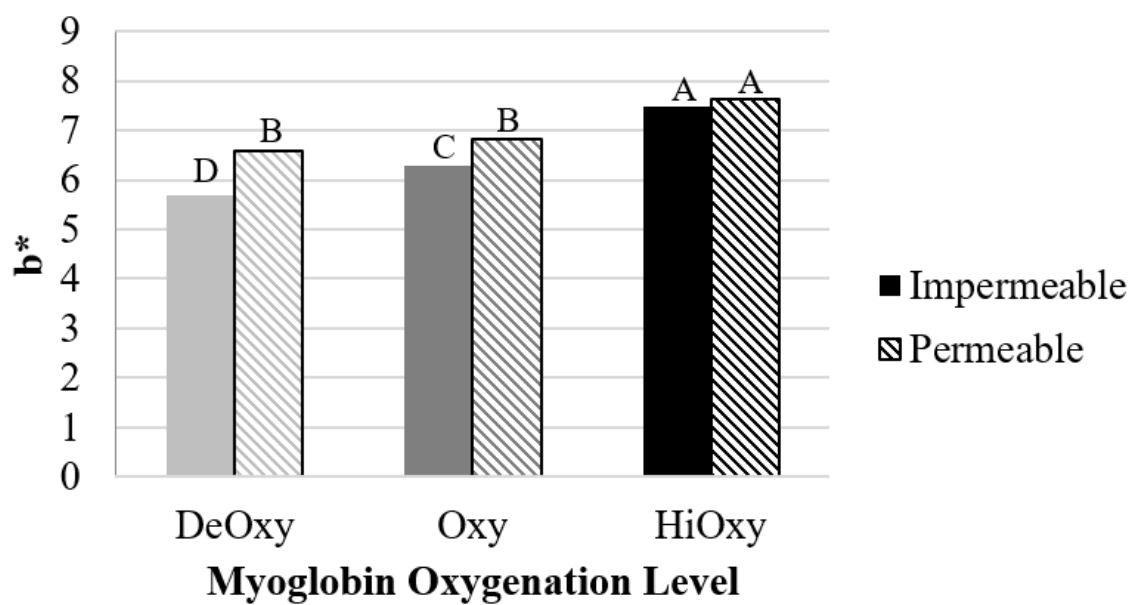
^{a-c} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 13. Instrumental color values for a^* of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, aged for either 4 or 20 d, and frozen for 0, 2, 4, or 6 months.



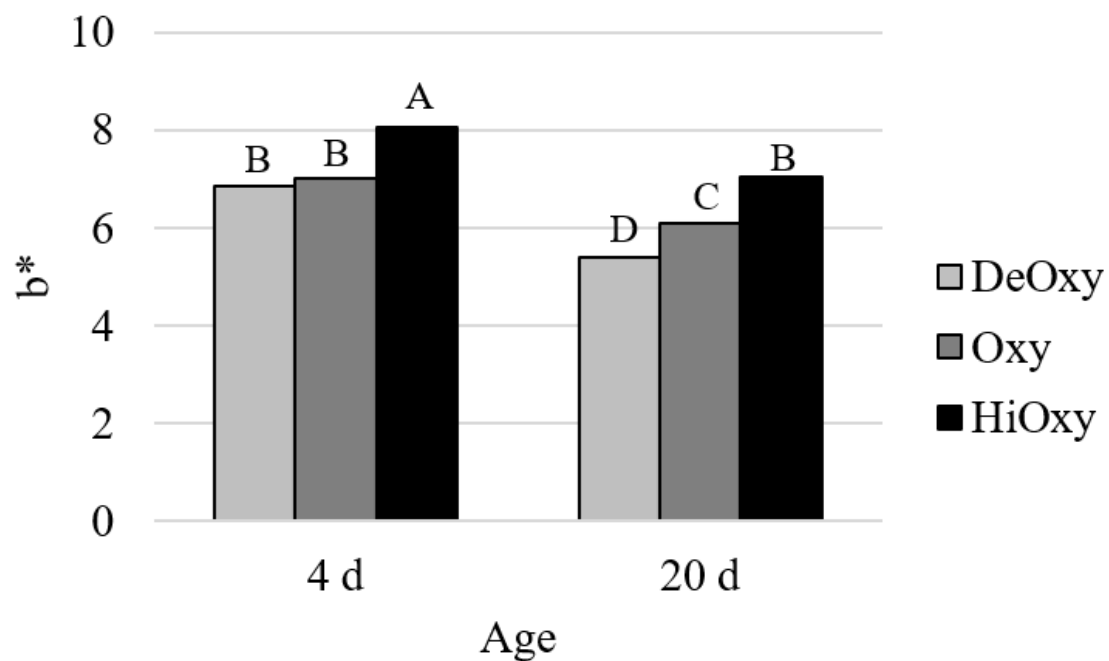
^{a-e} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 14. Instrumental color values for b^* of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and impermeable or permeable packaging.



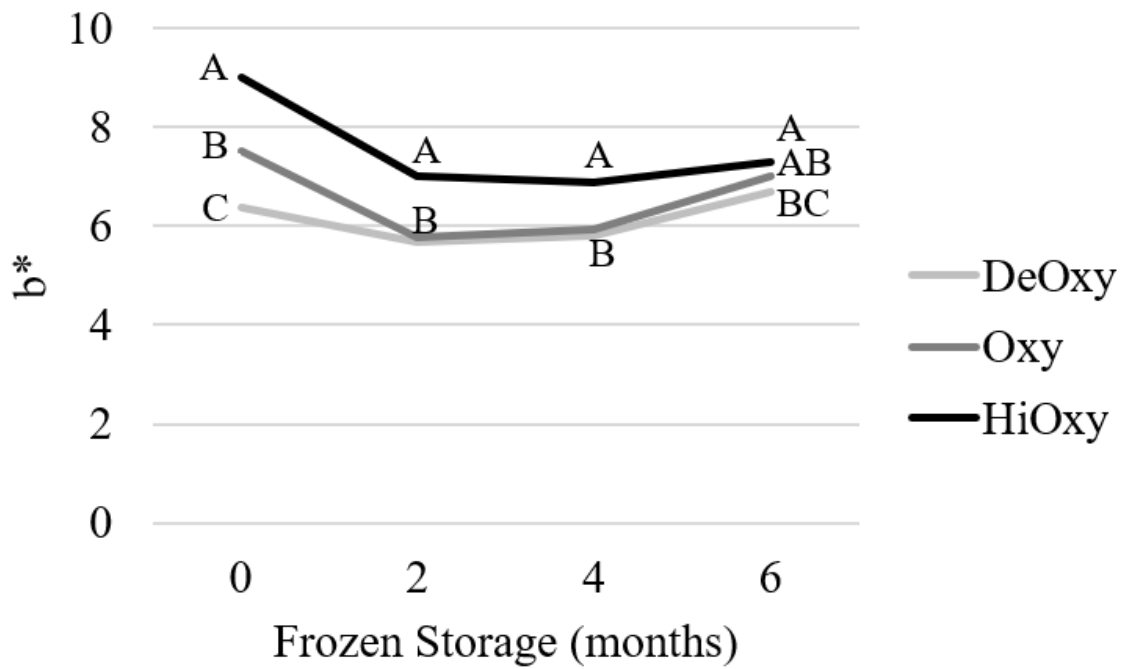
^{a-d} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 15. Instrumental color values for b^* of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and aged for 4 or 20 d.



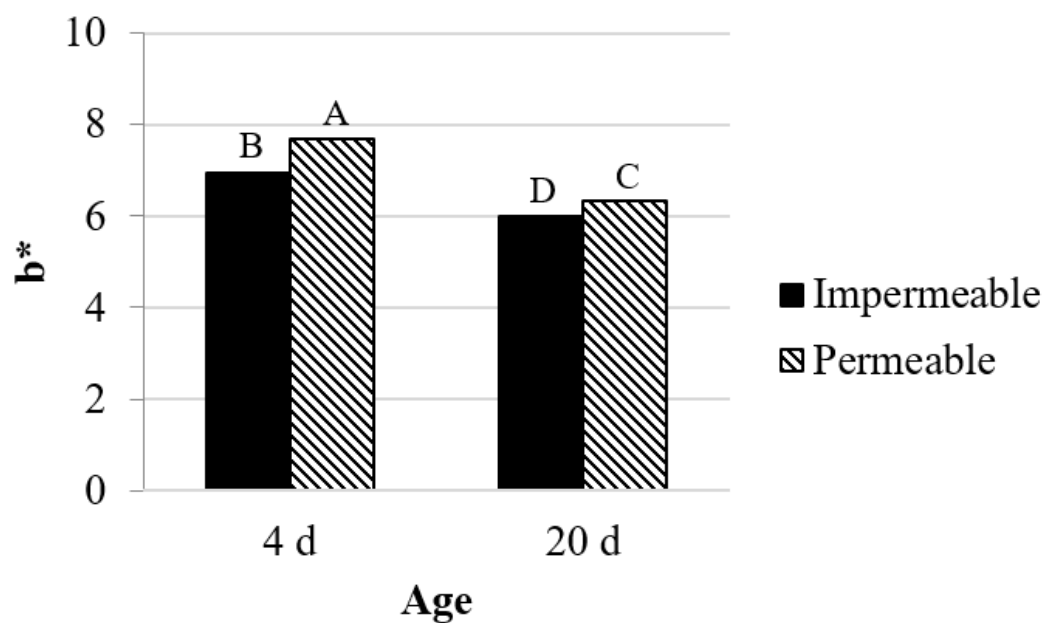
^{a-d} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 16. Instrumental color values for b^* of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and frozen for 0, 2, 4, or 6 months.



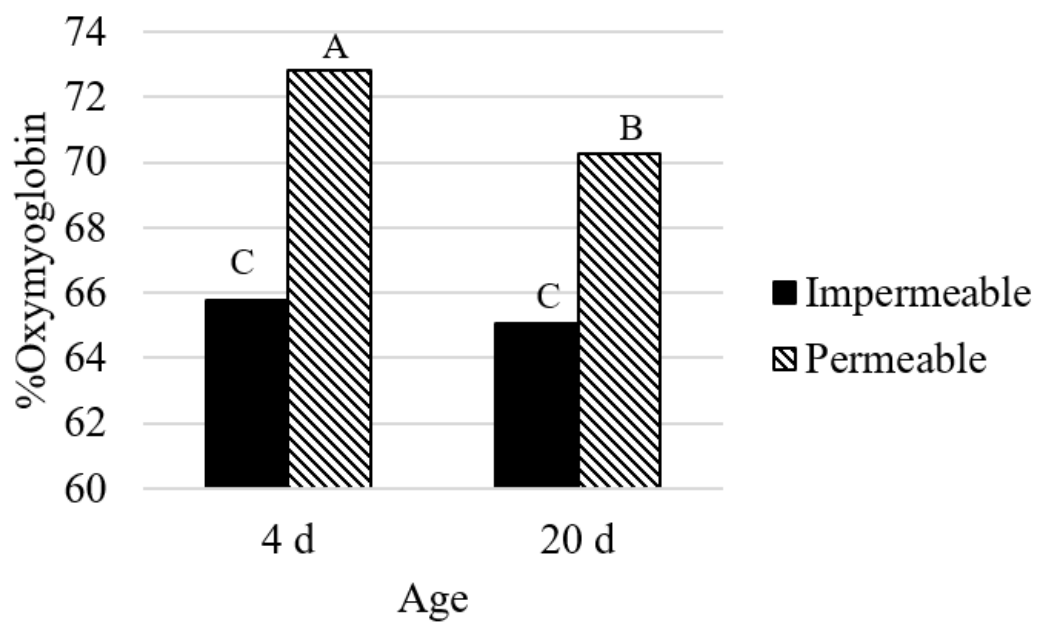
^{a-c} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 17. Instrumental color values for b^* of steaks aged 4 or 20 d and impermeable or permeable packaging.



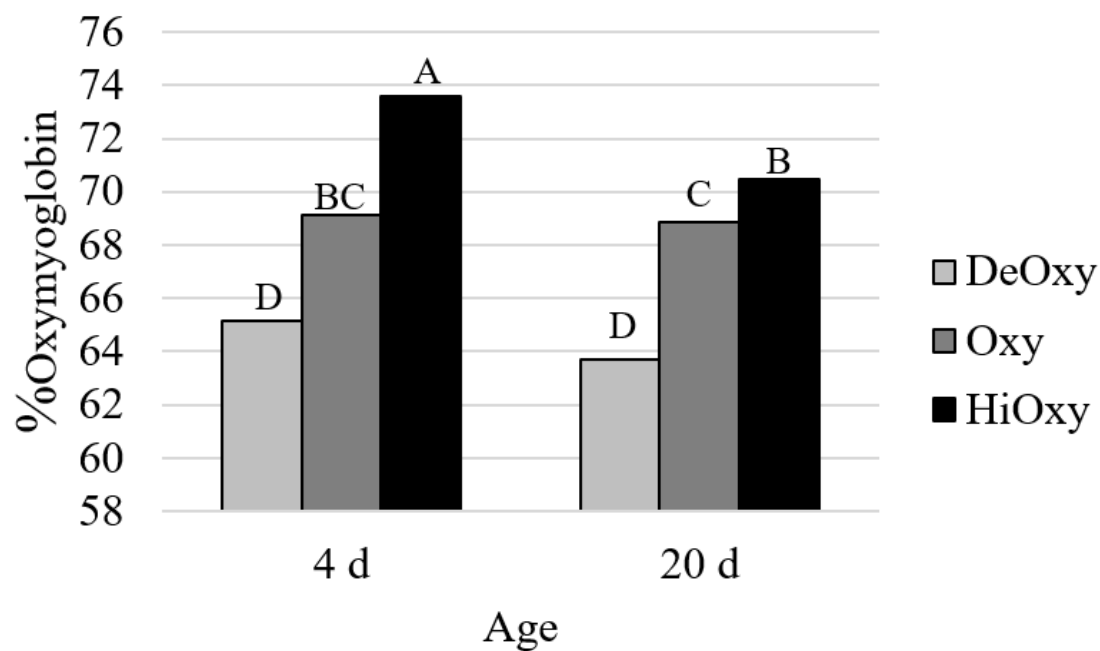
^{a-d} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 18. Instrumental color values for percent oxymyoglobin of steaks aged 4 or 20 d and impermeable or permeable packaging.



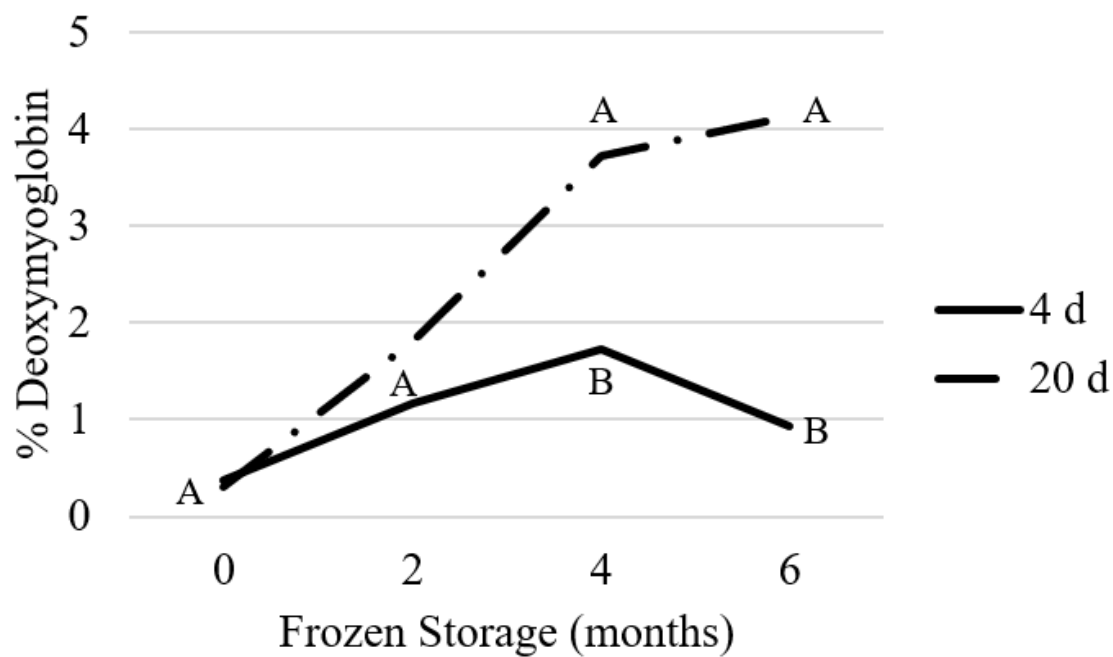
^{a-c} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 19. Instrumental color values for percent oxymyoglobin of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and aged 4 or 20 d.



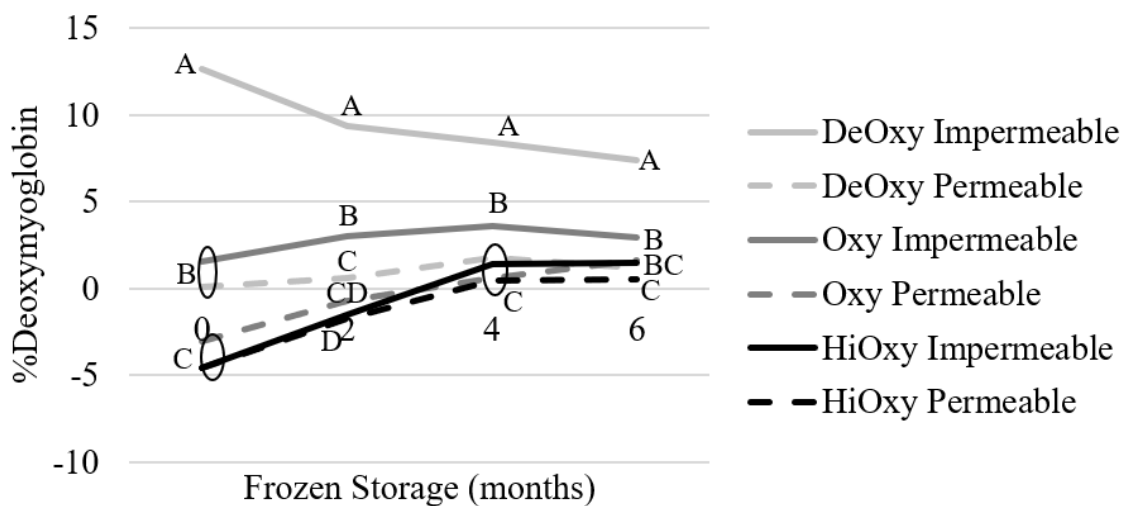
^{a-d} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 20. Instrumental color values for percent deoxymyoglobin of steaks aged 4 or 20 d and frozen for 0, 2, 4, or 6 months.



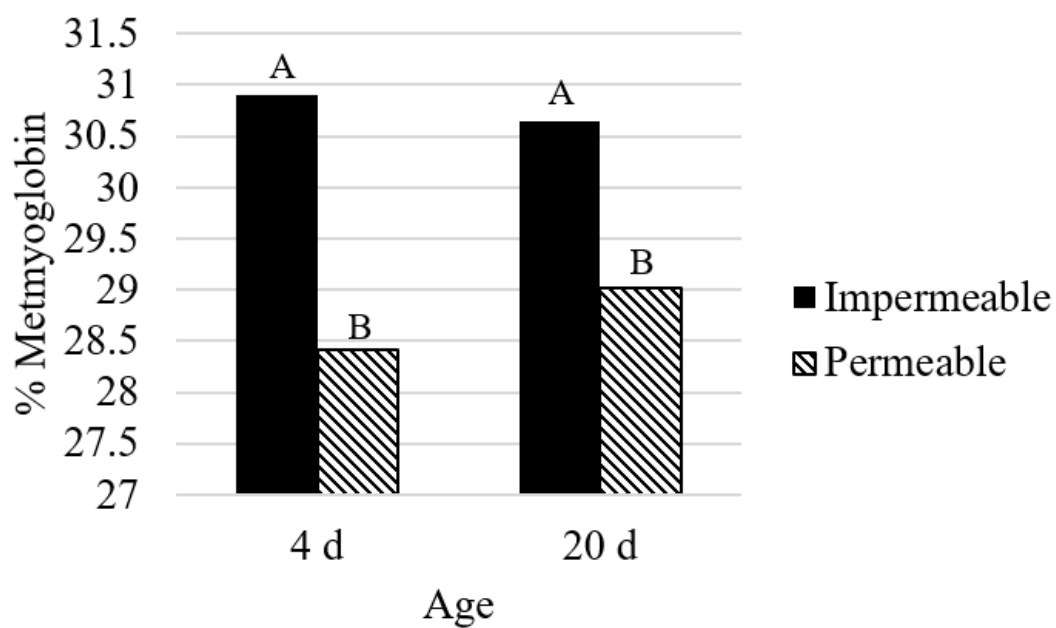
^{a-b} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 21. Instrumental color values for percent deoxymyoglobin of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging, and frozen for 0, 2, 4, or 6 months.



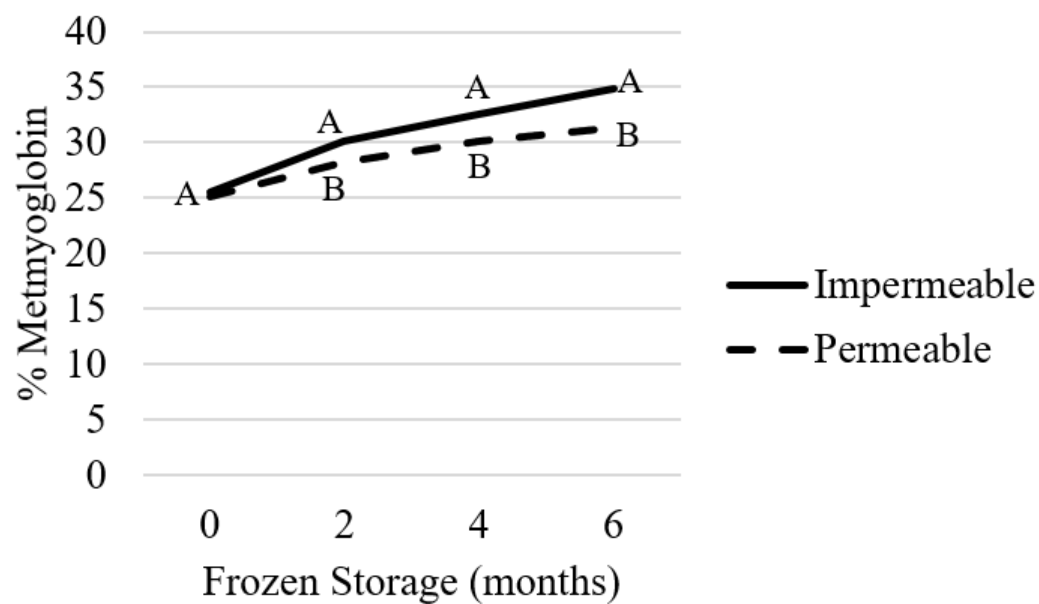
^{a-d} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 22. Instrumental color values for percent metmyoglobin of steaks aged 4 or 20 d and impermeable or permeable packaging.



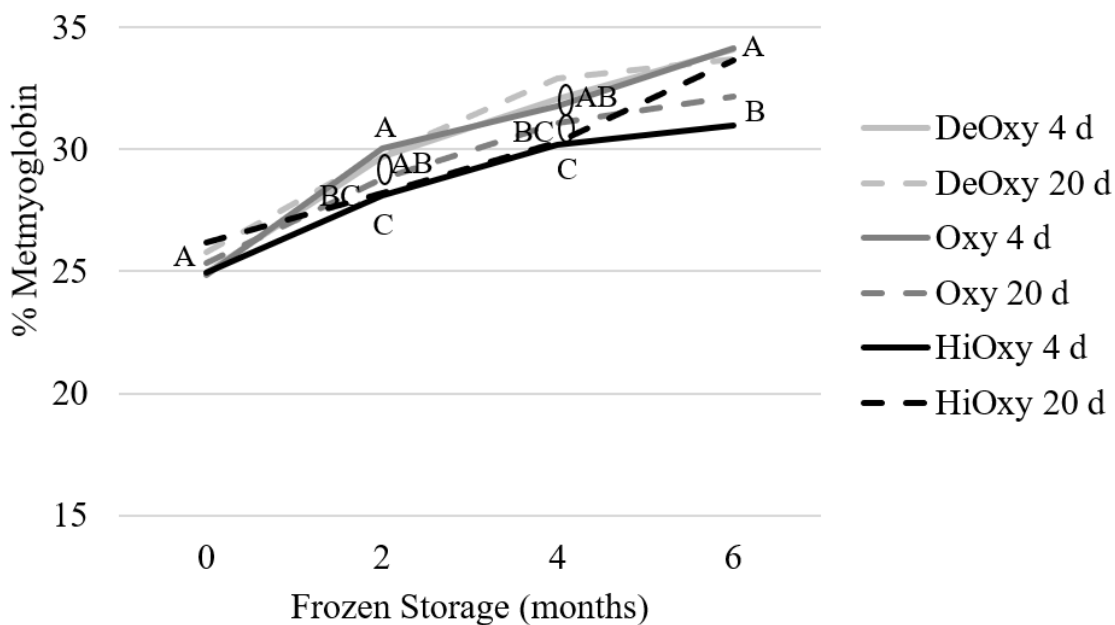
^{a-b} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 23. Instrumental color values for percent metmyoglobin of steaks in impermeable or permeable packaging and frozen for 0, 2, 4, or 6 months.



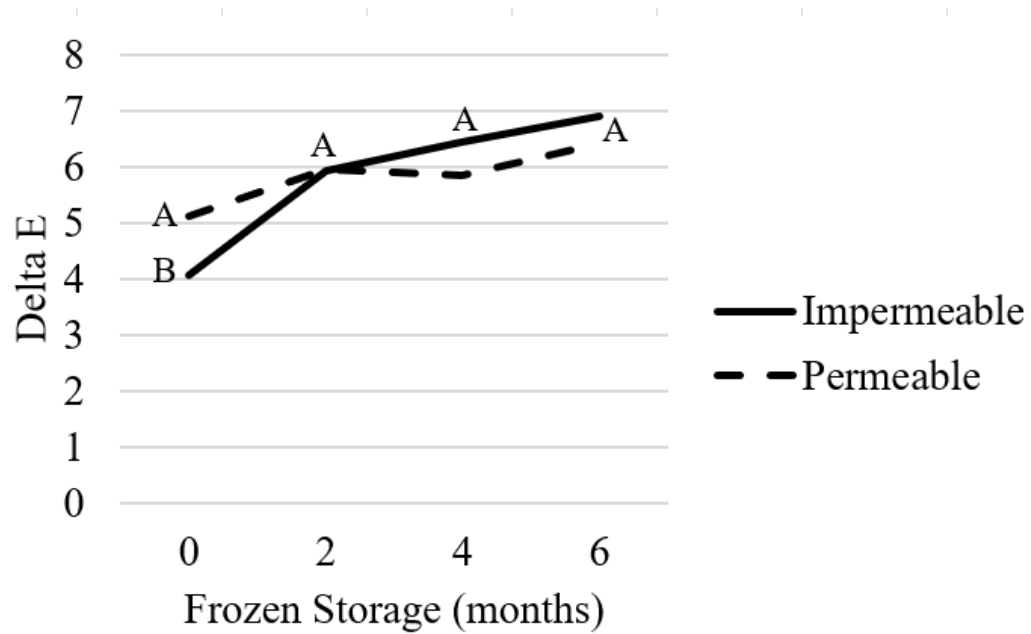
^{a-b} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 24. Instrumental color values for percent metmyoglobin of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, aged 4 or 20 d and frozen for 0, 2, 4, or 6 months.



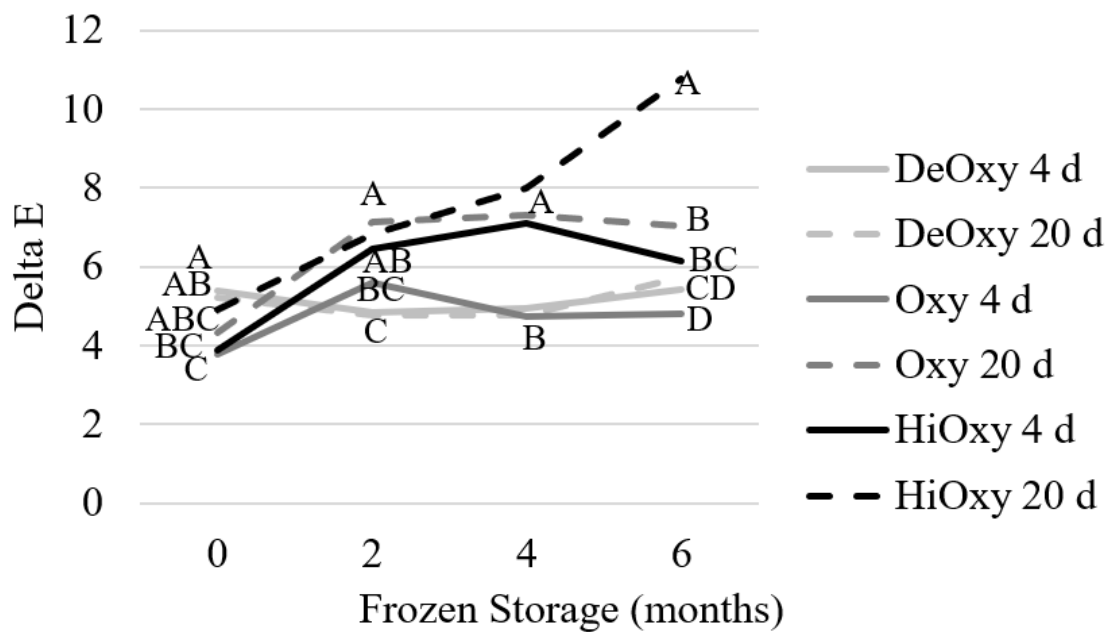
^{a-c} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 25. Delta E values of steaks frozen in impermeable or permeable packaging for 0, 2, 4, or 6 months.



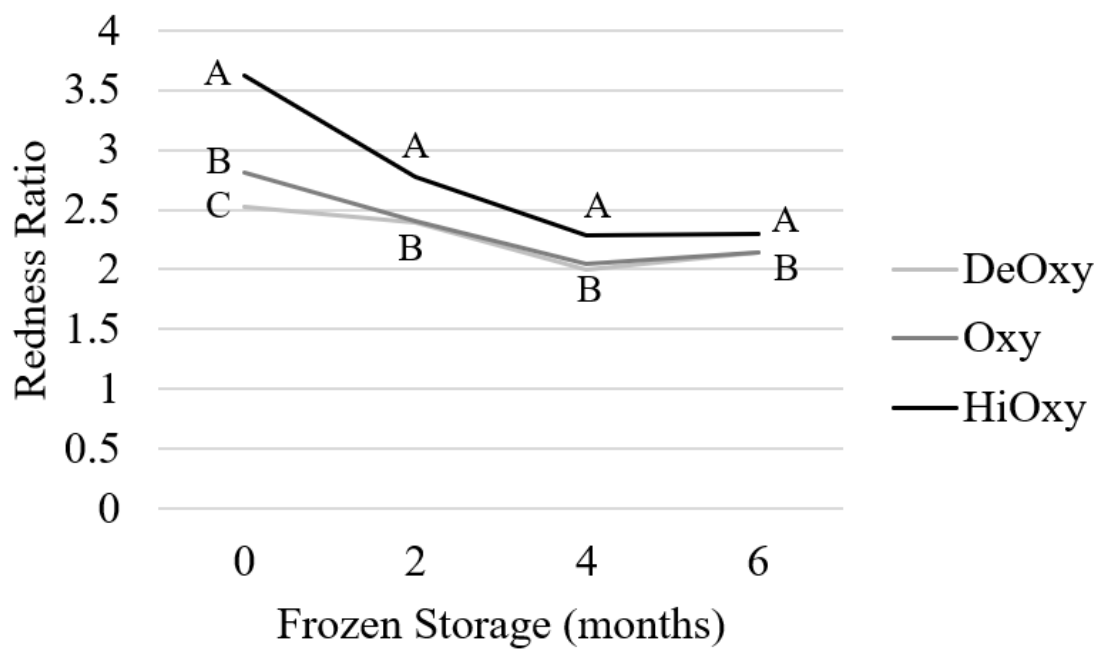
^{a-b} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 26. Delta E values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, aged 4 or 20 d and frozen for 0, 2, 4, or 6 months.



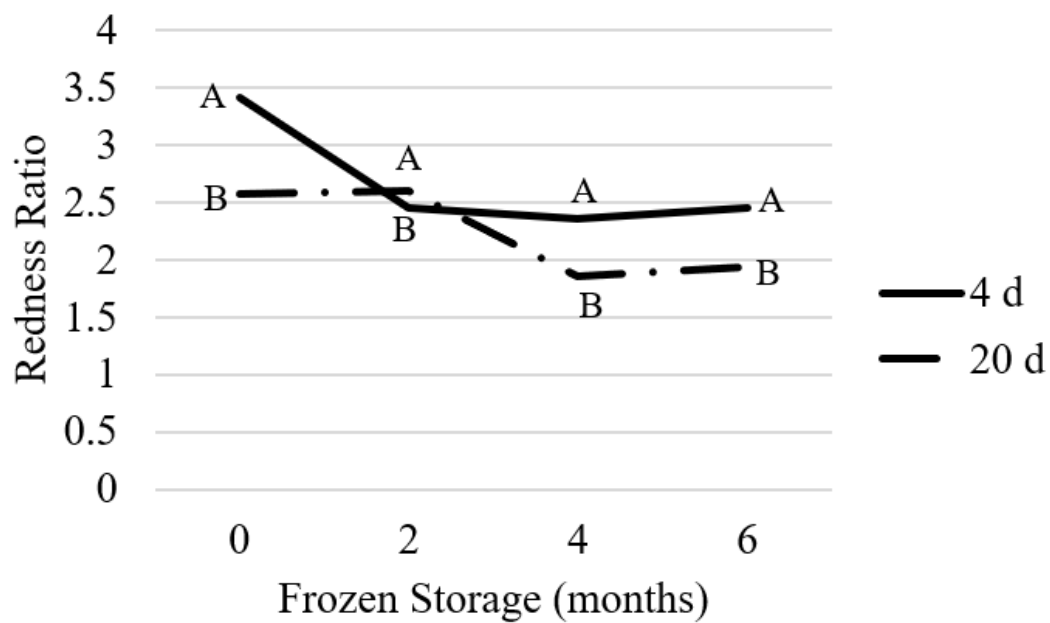
^{a-d} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 27. Redness ratio values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and frozen for 0, 2, 4, or 6 months.



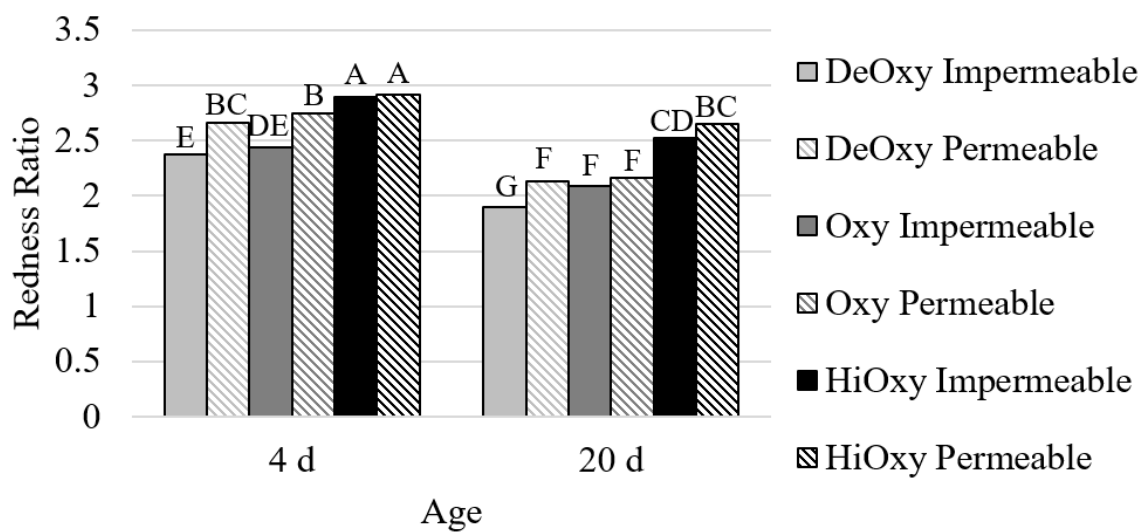
^{a-c} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 28. Redness ratio values of steaks aged 4 or 20 d and frozen for 0, 2, 4, or 6 months.



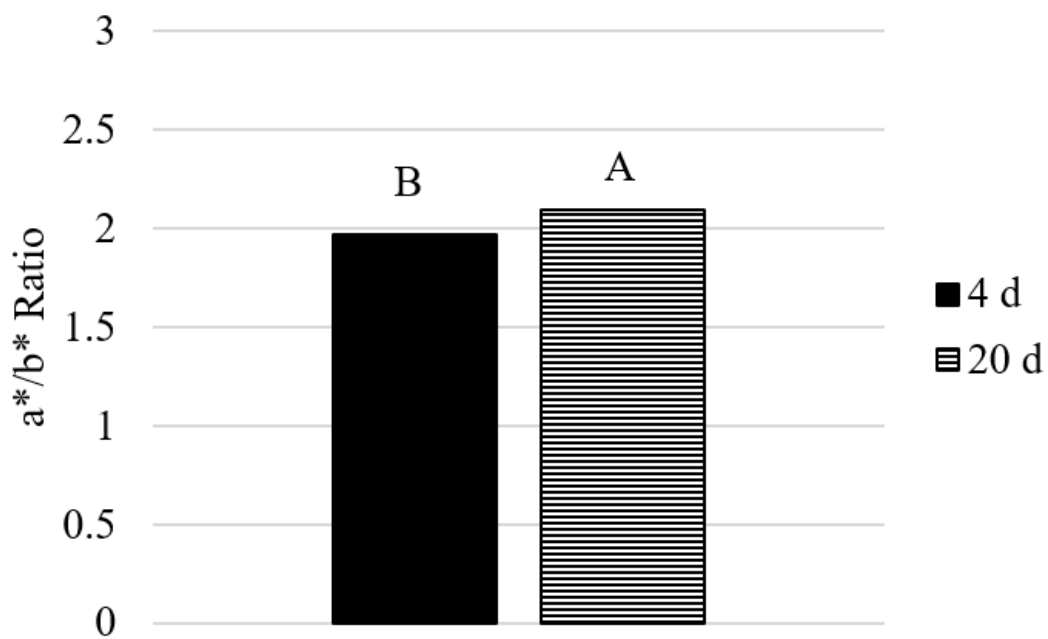
^{a-b} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 29. Redness ratio values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, aged for 4 or 20 d, and frozen in impermeable or permeable packaging.



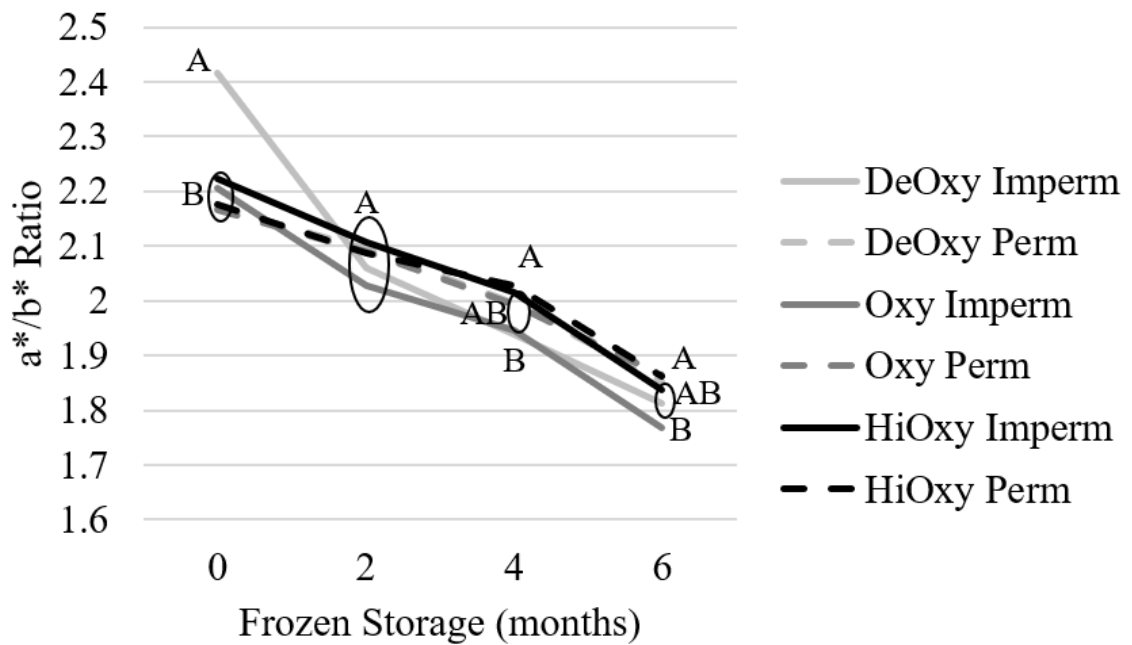
^{a-g} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 30. The $a^*:b^*$ ratios of steaks aged for 4 or 20 d.



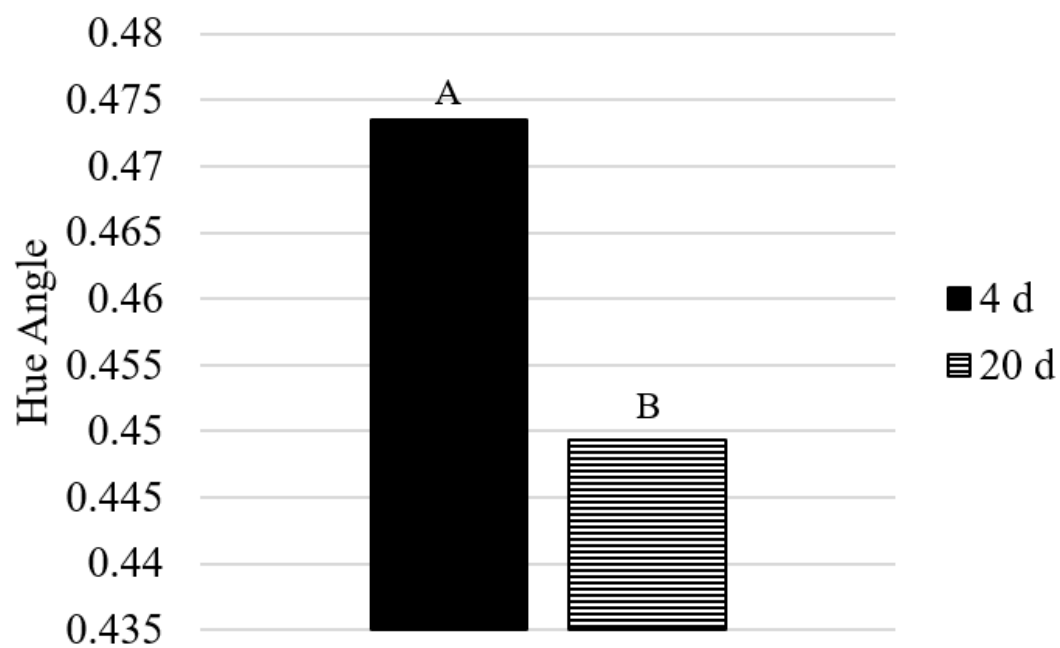
^{a-b} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 31. The $a^*:b^*$ ratios of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging, and frozen for 0, 2, 4, or 6 months.



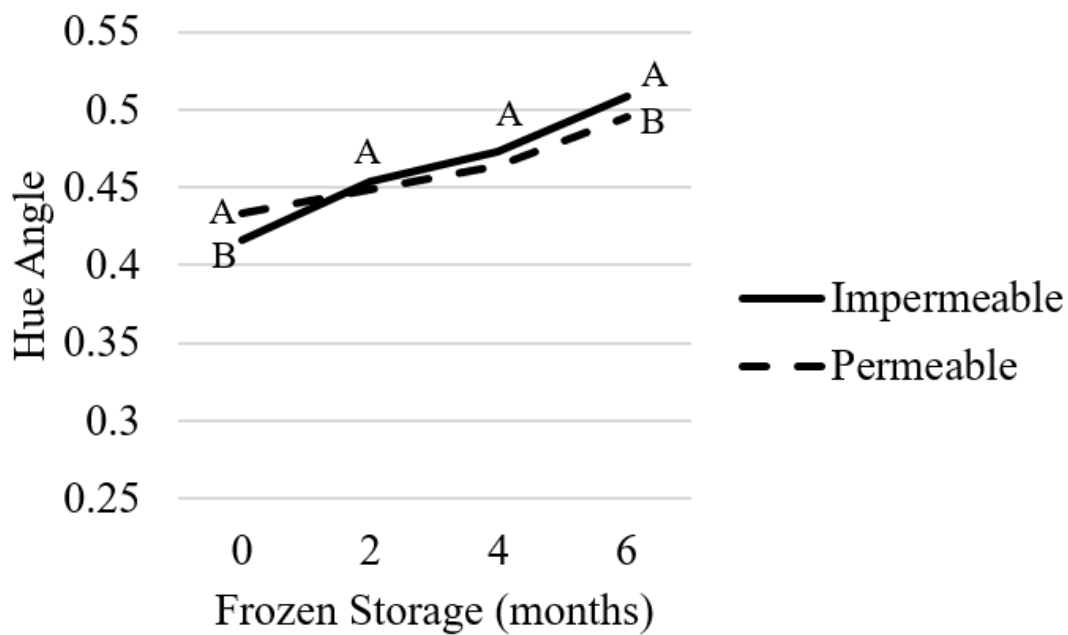
^{a-b} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 32. Hue angle values of steaks aged 4 or 20 d.



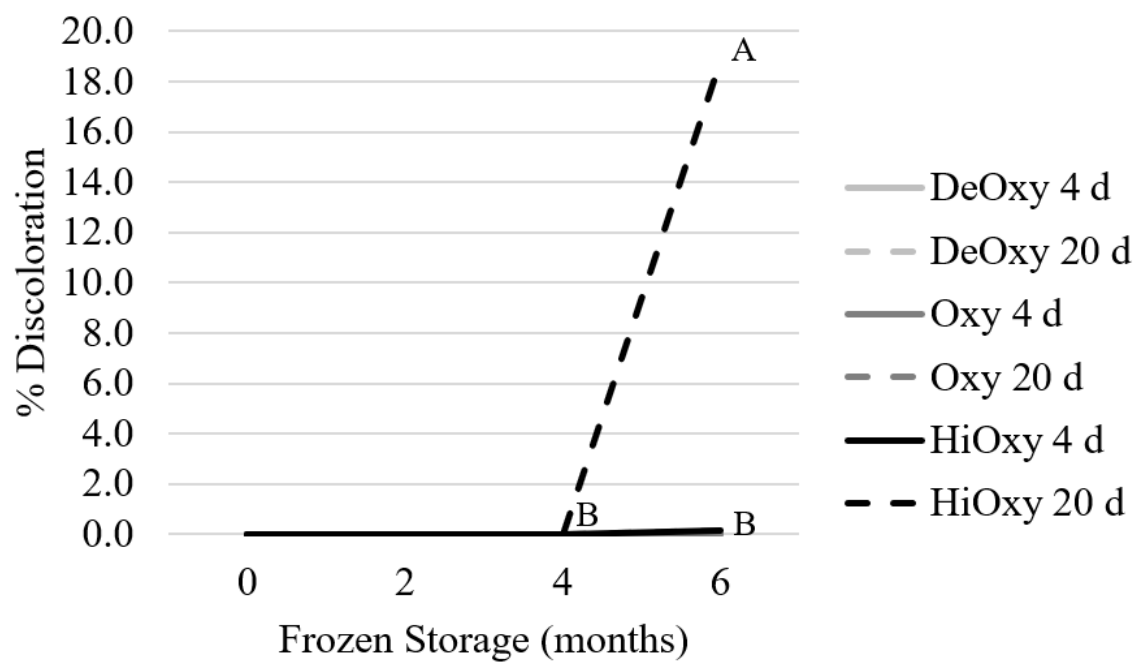
Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 33. Hue angle values of steaks in either impermeable or permeable packaging and frozen for 0, 2, 4, or 6 months.



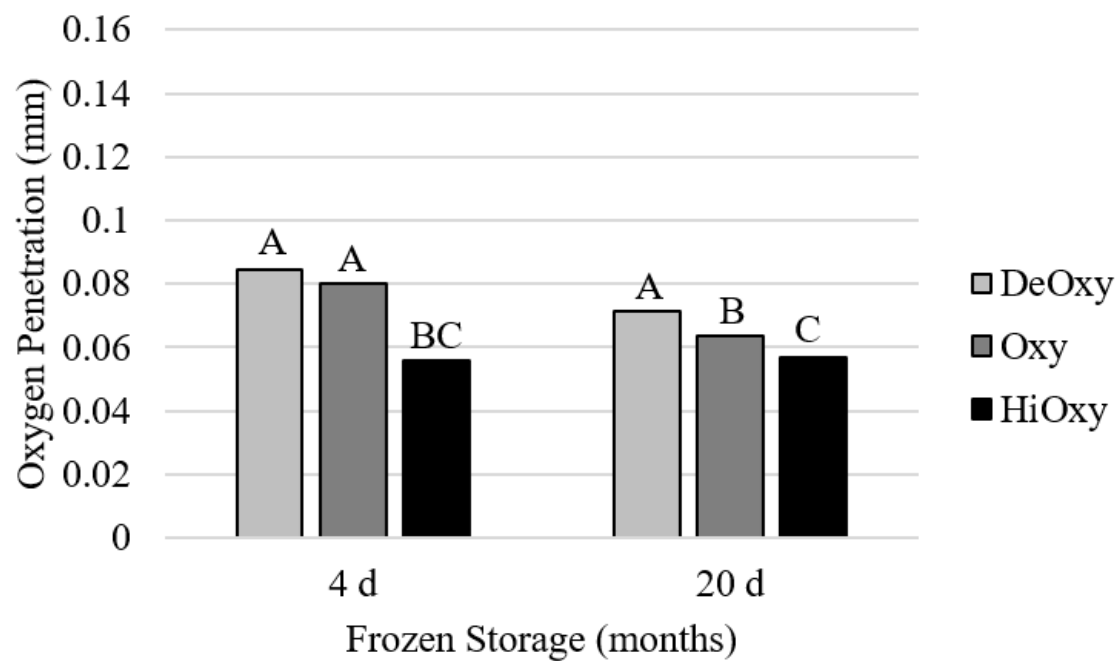
^{a-b} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 34. Discoloration values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, aged 4 or 20 d, and frozen for 0, 2, 4, or 6 months.



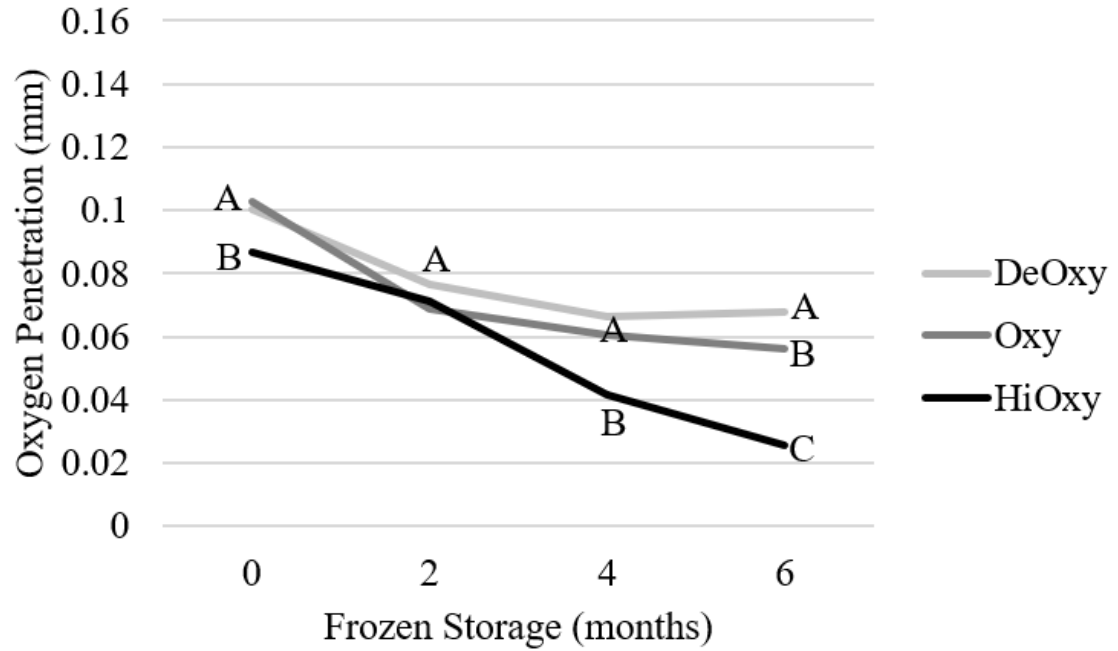
^{a-b} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 35. Oxygen penetration values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and aged 4 or 20 d.



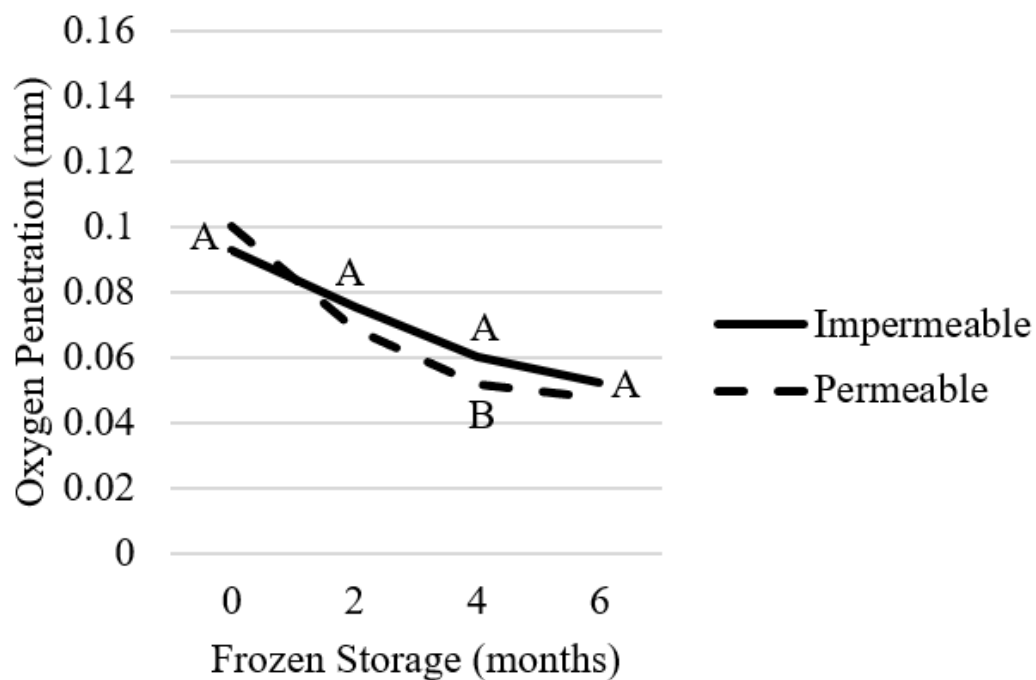
^{a-c} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 36. Oxygen penetration values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and frozen for 0, 2, 4, or 6 months.



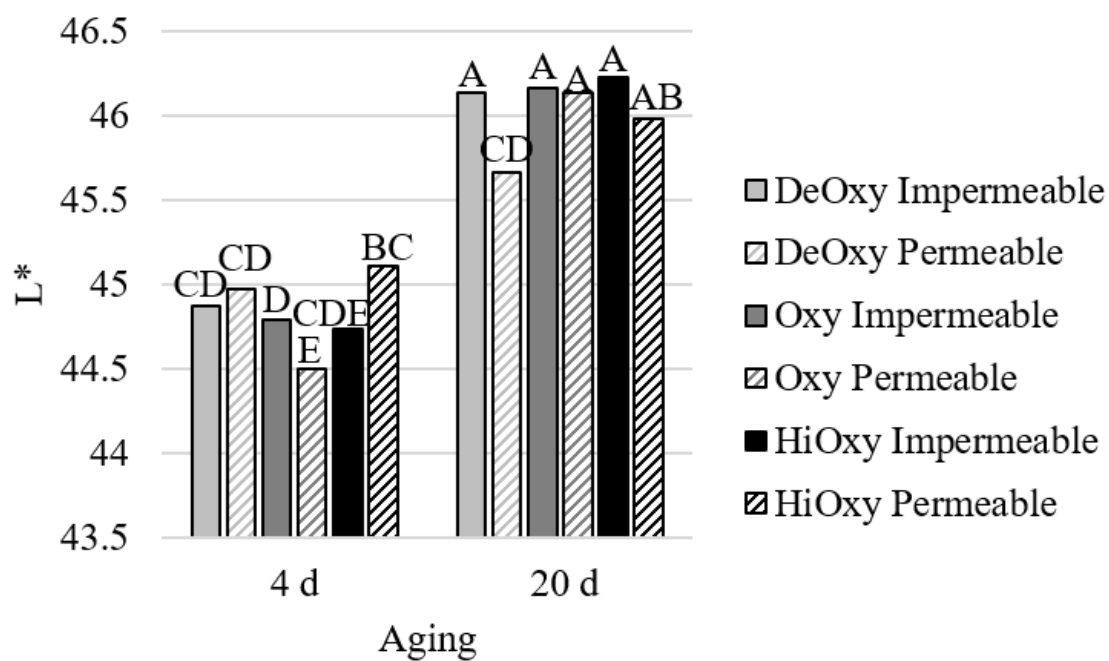
^{a-c} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 37. Oxygen penetration values of steaks frozen in impermeable or permeable packaging for either 0, 2, 4, or 6 months.



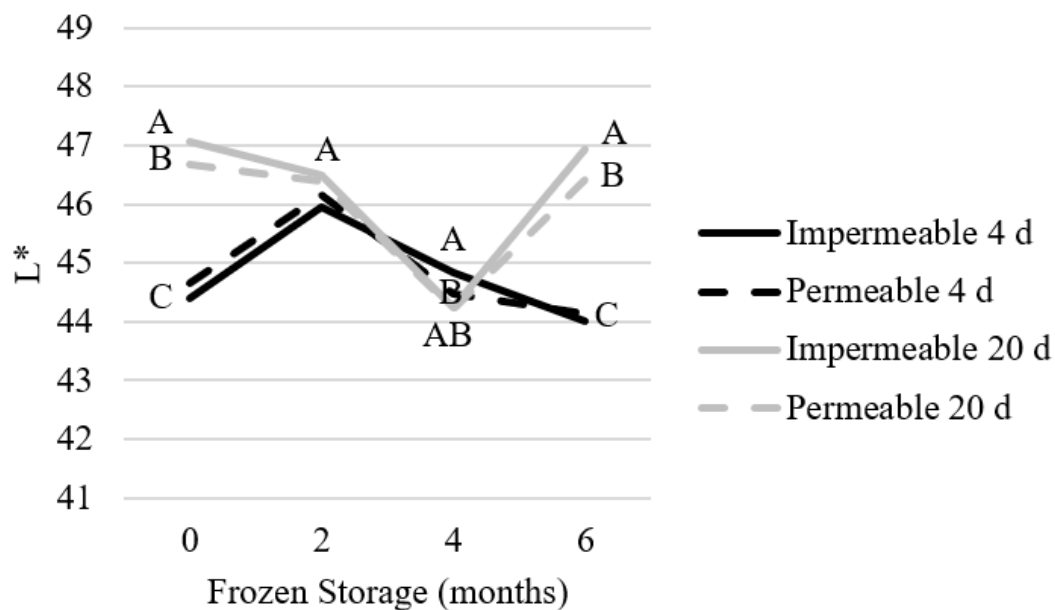
^{a-b} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 38. Instrumental color values for L* of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, aged 4 or 20 d, and packaged in impermeable or permeable film.



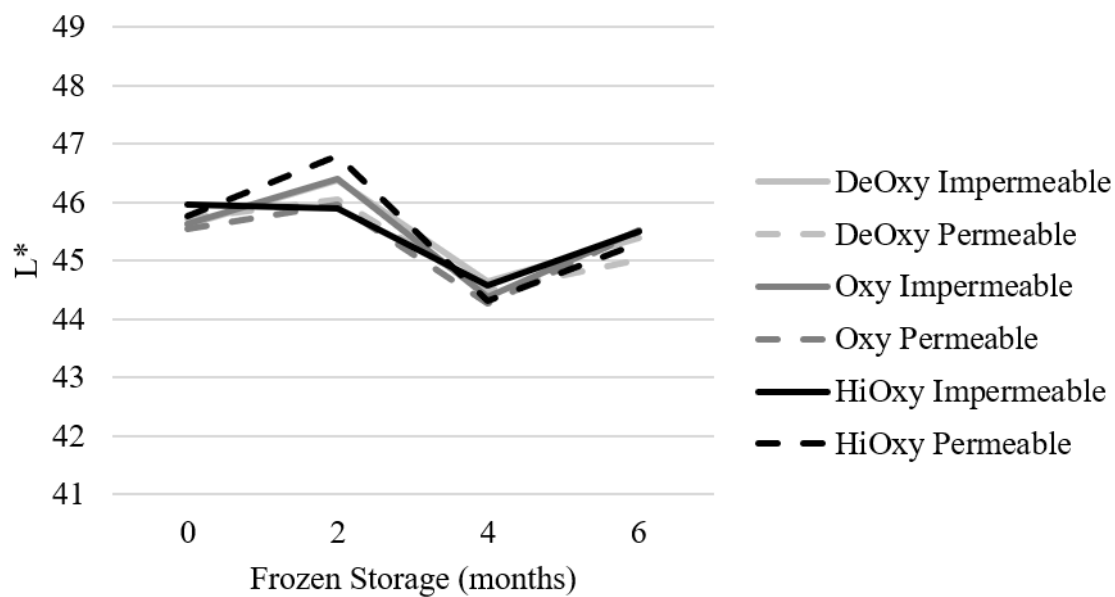
^{a-e} Different superscripts indicated differences among treatments ($P < 0.05$).

Appendix 39. Instrumental color values for L^* of steaks packaged in impermeable or permeable film, aged 4 or 20 d and frozen for 0, 2, 4, or 6 months.



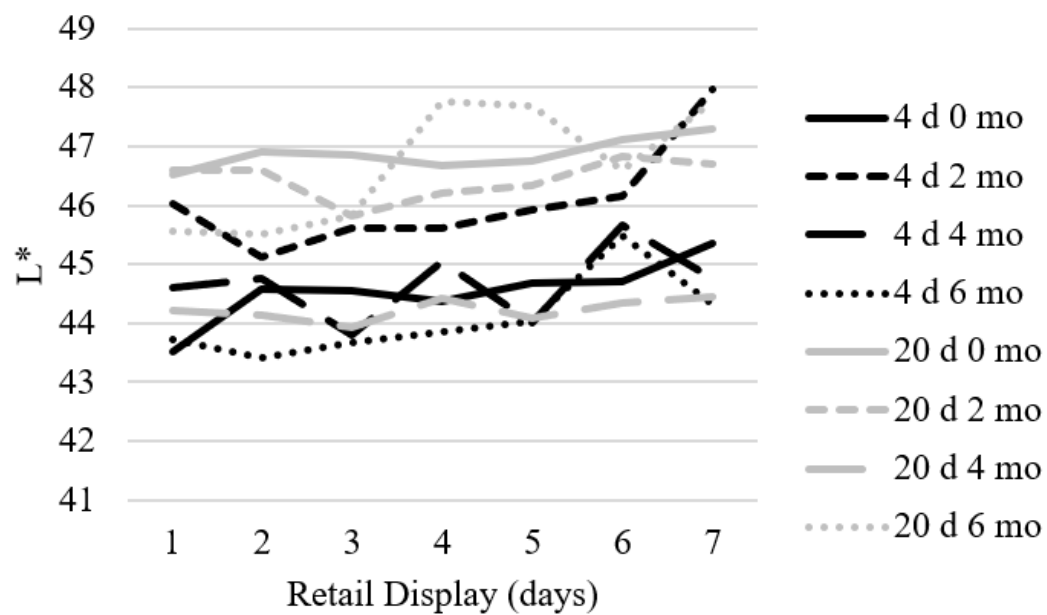
^{a-c} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 40. Instrumental color values for L* of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, packaged in impermeable or permeable film and frozen for 0, 2, 4, or 6 months.



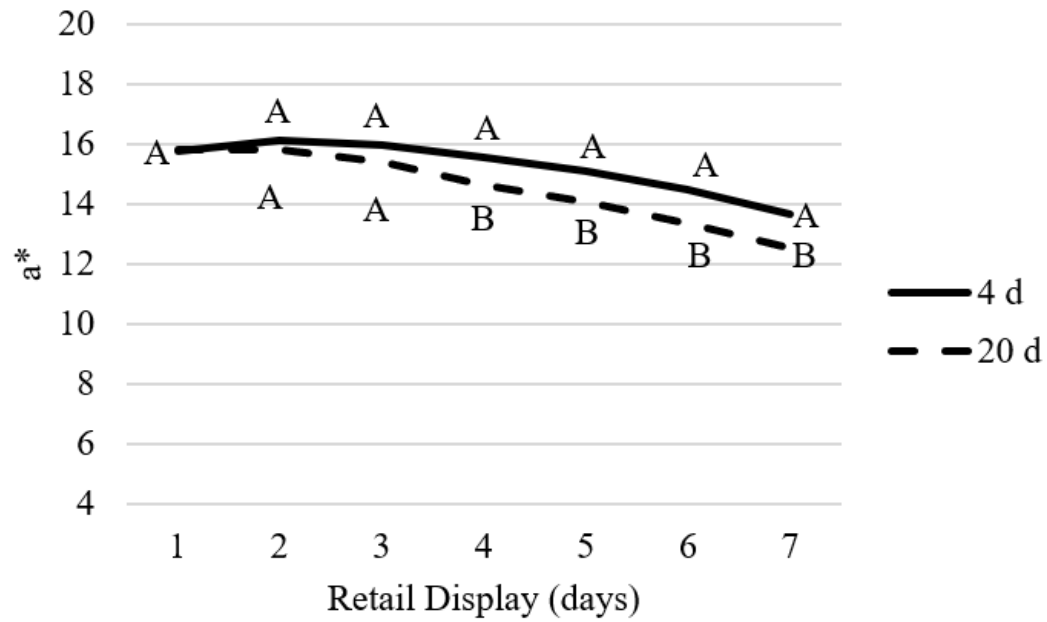
Superscripts for mean separation may be found in Table 5 (page 190).

Appendix 41. Instrumental color values for L^* of steaks aged 4 or 20 d and frozen for 0, 2, 4, or 6 months throughout 7 days of retail display.



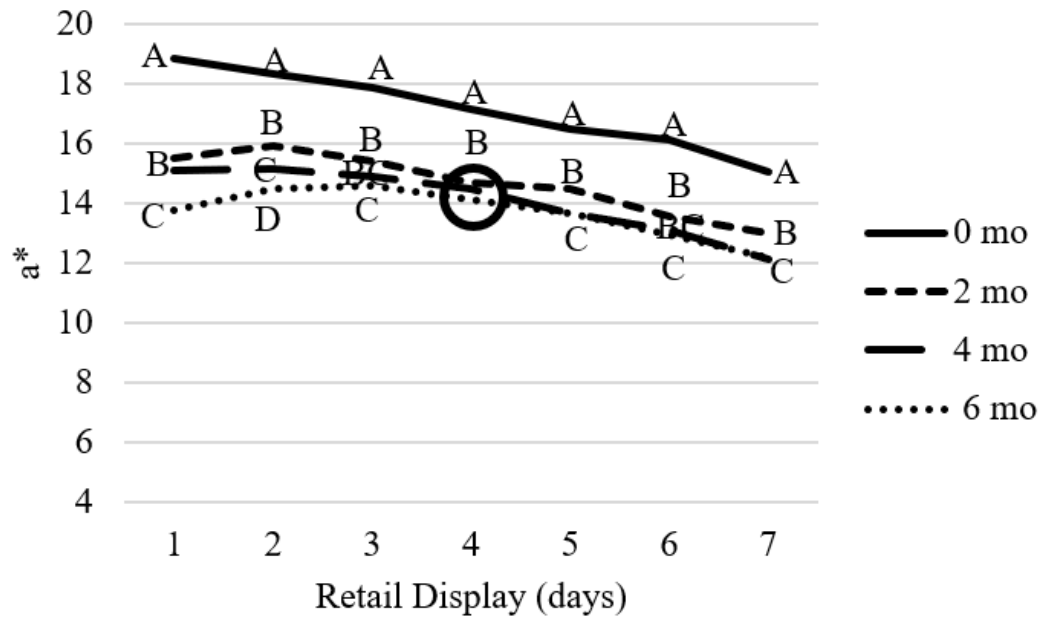
Superscripts for mean separation may be found in Table 9 (page 201).

Appendix 42. Instrumental color values for a^* of steaks aged 4 or 20 d throughout 7 days of retail display.



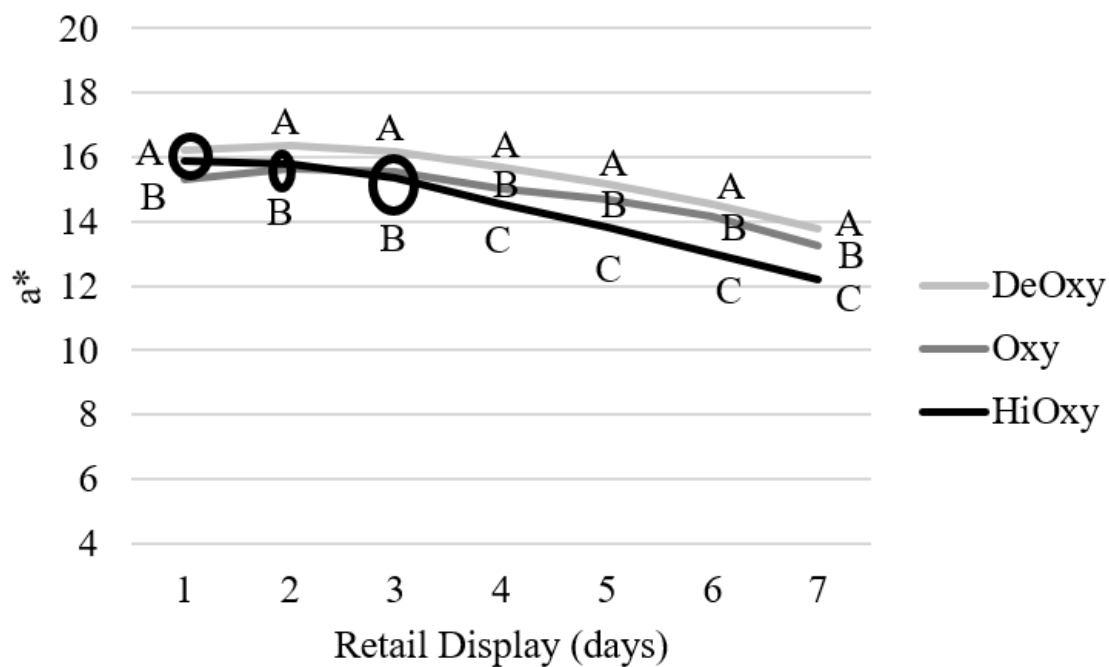
^{a-b} Different superscripts indicated differences within retail display day ($P < 0.05$).

Appendix 43. Instrumental color values for a^* of steaks frozen 0, 2, 4, or 6 months throughout 7 days of retail display.



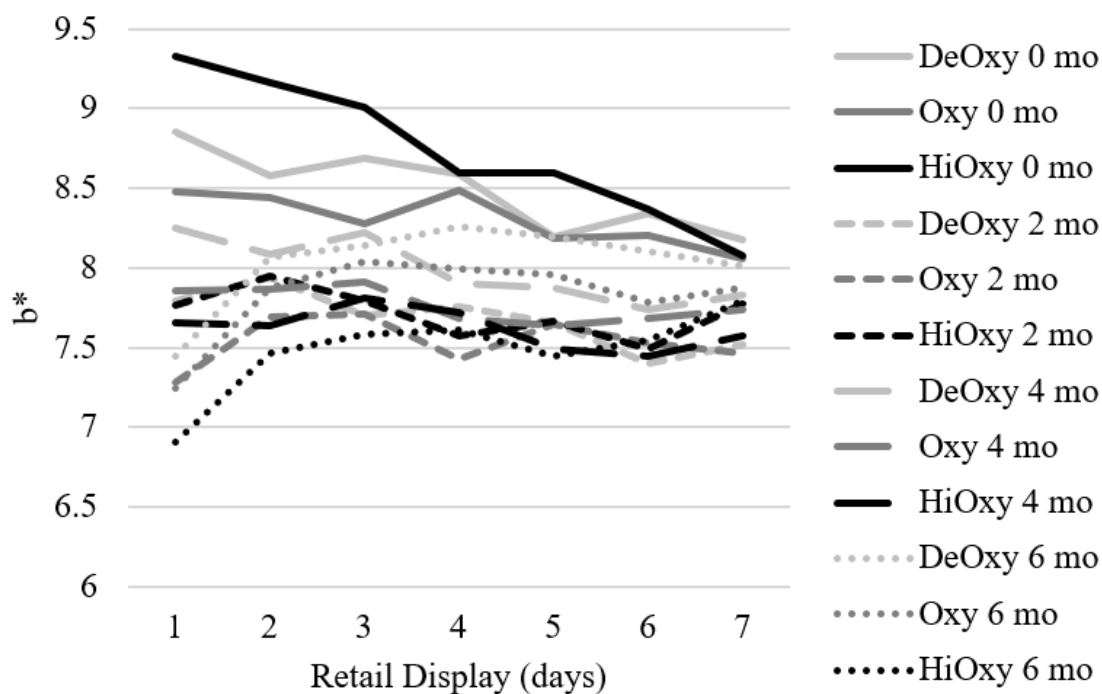
^{a-d} Different superscripts indicated differences within retail display day ($P < 0.05$).

Appendix 44. Instrumental color values for a^* of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state throughout 7 days of retail display.



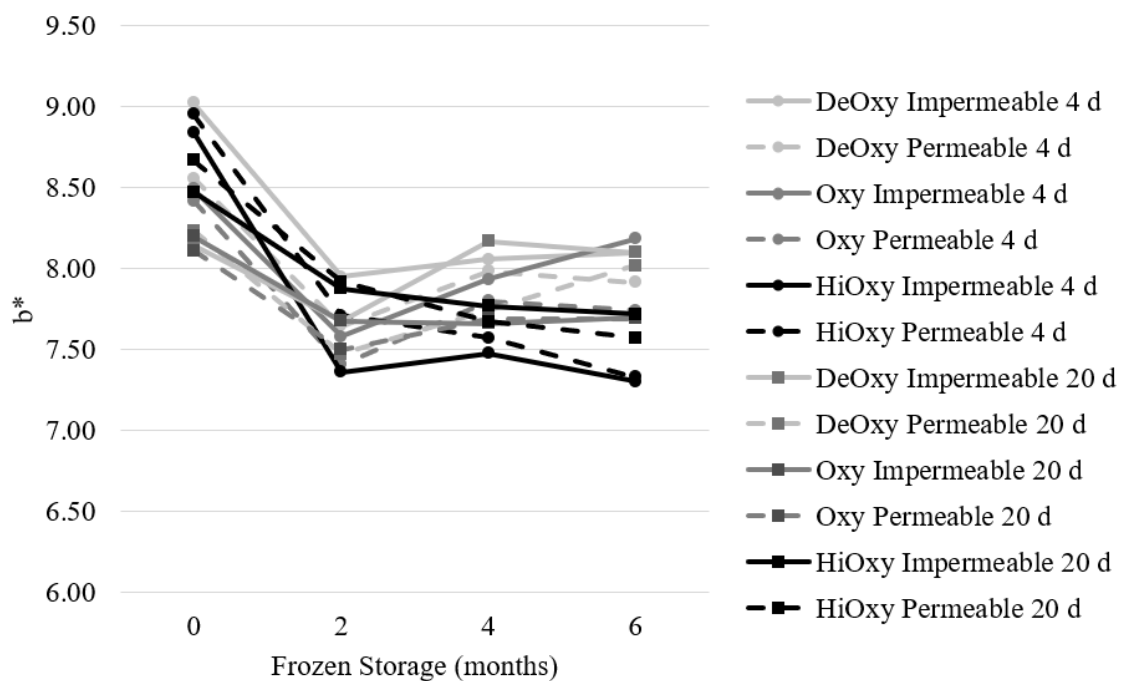
^{a-c} Different superscripts indicated differences within retail display day ($P < 0.05$).

Appendix 45. Instrumental color values for b^* of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and frozen for 0, 2, 4, or 6 months throughout 7 days of retail display.



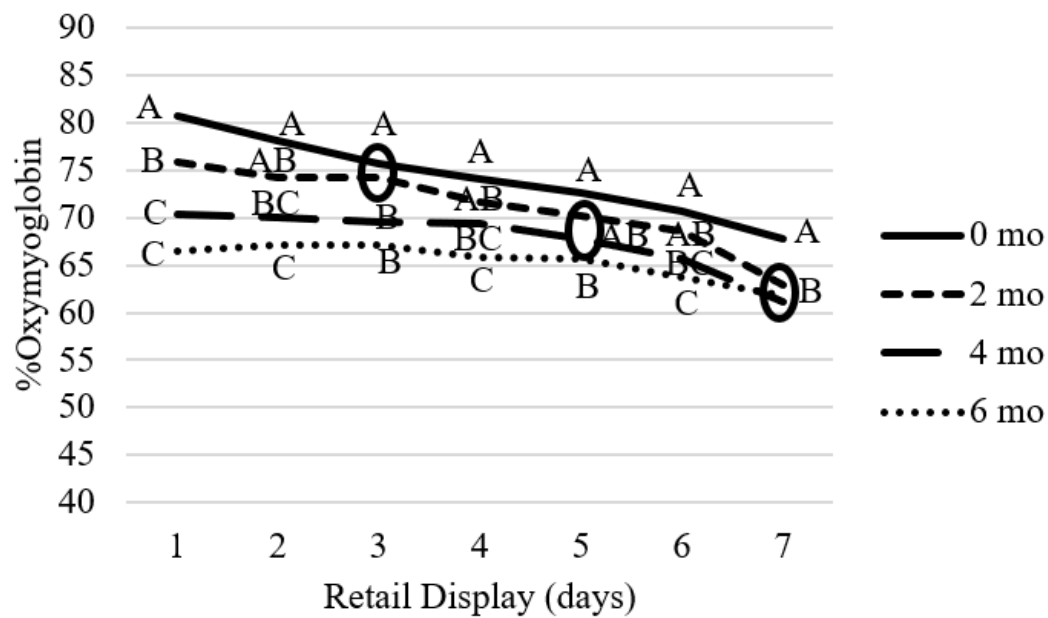
Superscripts for mean separation may be found in Table 7 (page 193).

Appendix 46. Instrumental color values for b^* of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging, aged 4 or 20 d, and frozen for 0, 2, 4, or 6 months.



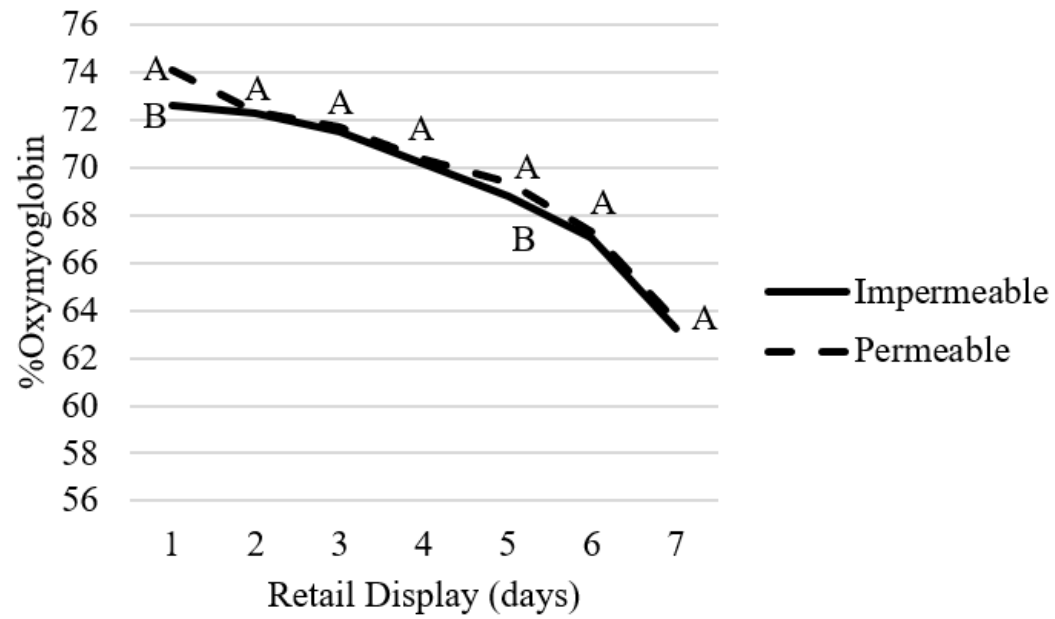
Superscripts for mean separation may be found in Table 6 (page 192).

Appendix 47. Instrumental color values for percent oxymyoglobin of steaks aged 4 d, frozen for 0, 2, 4, or 6 months throughout 7 days of retail display.



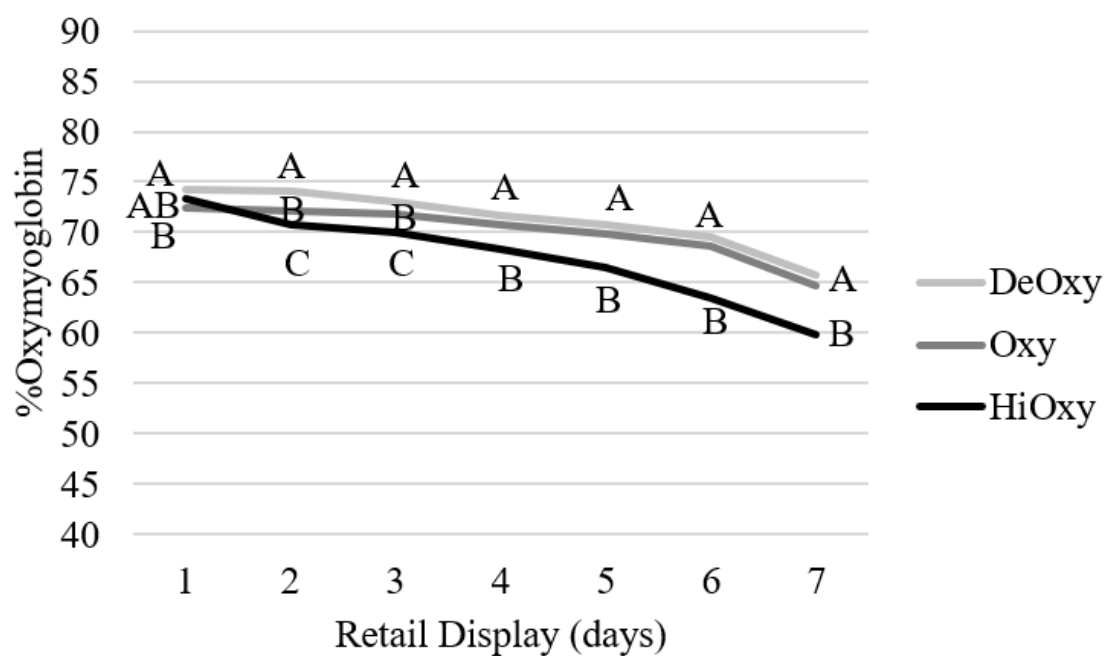
^{a-c} Different superscripts indicated differences within retail display day ($P < 0.05$).

Appendix 48. Instrumental color values for percent oxymyoglobin of steaks aged 4 d, frozen in impermeable or permeable packaging throughout 7 days of retail display.



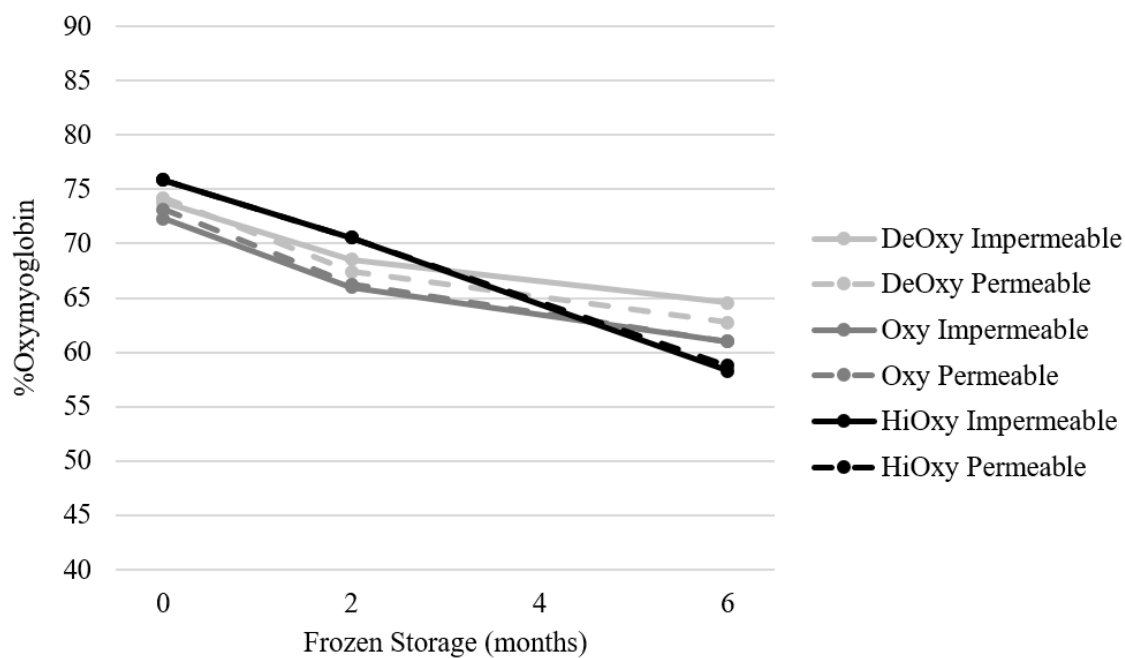
^{a-b} Different superscripts indicated differences within retail display day ($P < 0.05$).

Appendix 49. Instrumental color values for percent oxymyoglobin of steaks aged 4 d, in either a deoxymyoglobin, oxygenated, or high oxygenated state throughout 7 days of retail display.



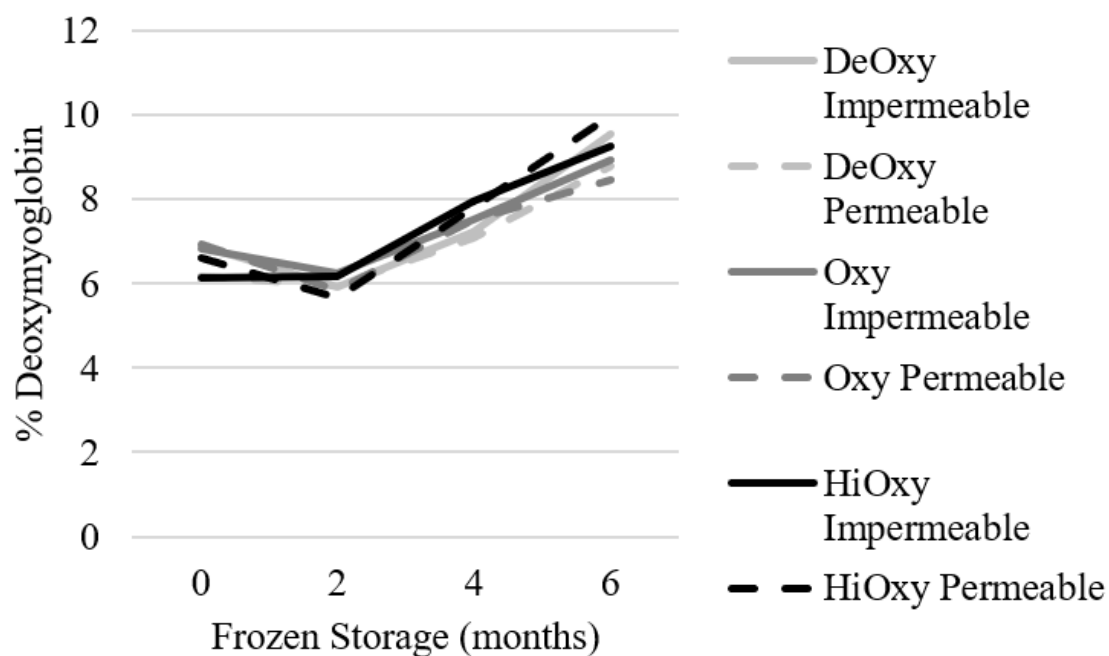
^{a-c} Different superscripts indicated differences within retail display day ($P < 0.05$).

Appendix 50. Instrumental color values for percent oxymyoglobin of steaks aged 20 d, in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging and frozen for 0, 2, or 6 months.



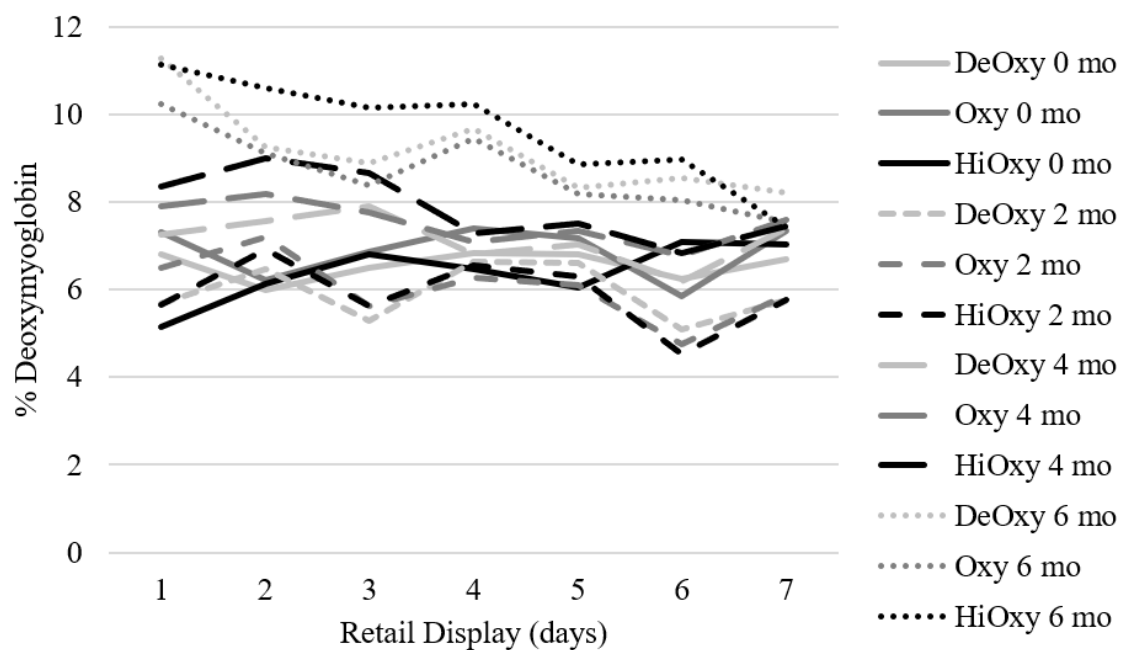
Superscripts for mean separation may be found in Table 5 (page 190).

Appendix 51. Instrumental color values for percent deoxymyoglobin of steaks aged 4 d, in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging and frozen for 0, 2, 4, or 6 months.



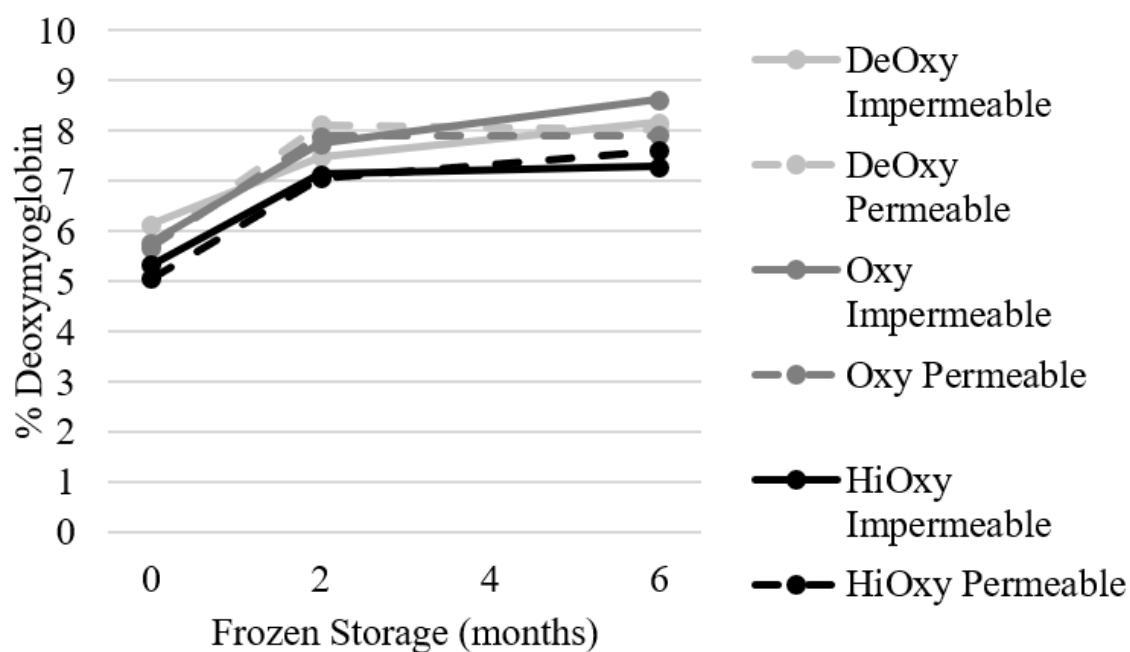
Superscripts for mean separation may be found in Table 5 (page 190).

Appendix 52. Instrumental color values for percent deoxymyoglobin of steaks aged 4 d, in either a deoxymyoglobin, oxygenated, or high oxygenated state, and frozen for 0, 2, 4, or 6 months throughout 7 days of retail display.



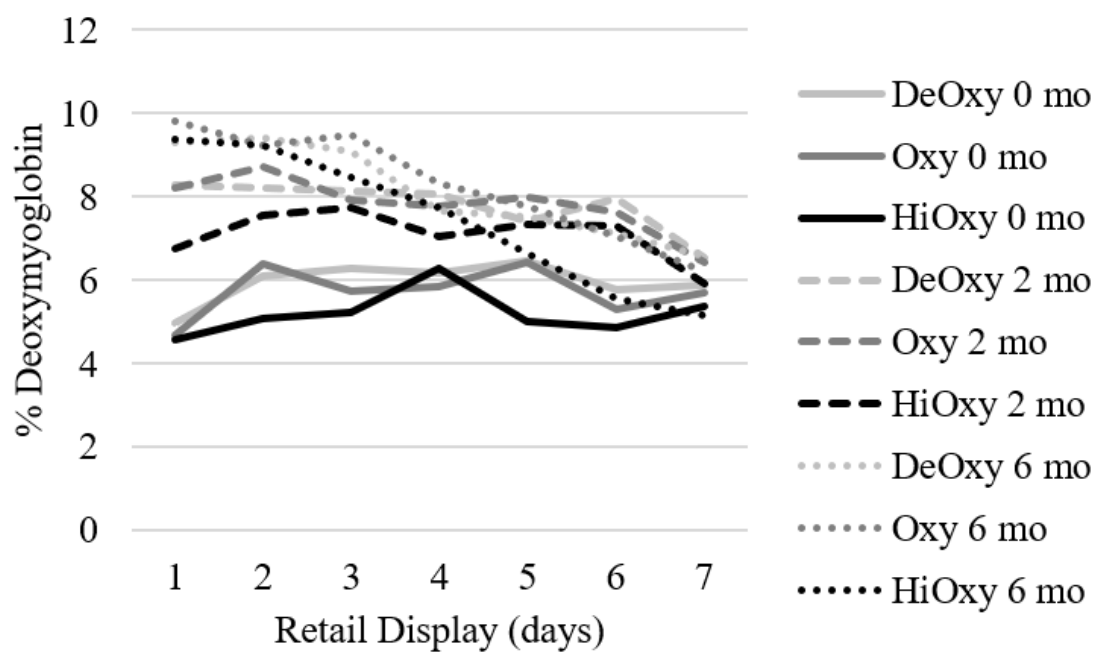
Superscripts for mean separation may be found in Table 7 (page 194).

Appendix 53. Instrumental color values for percent deoxymyoglobin of steaks aged 20 d, in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging and frozen for 0, 2, or 6 months.



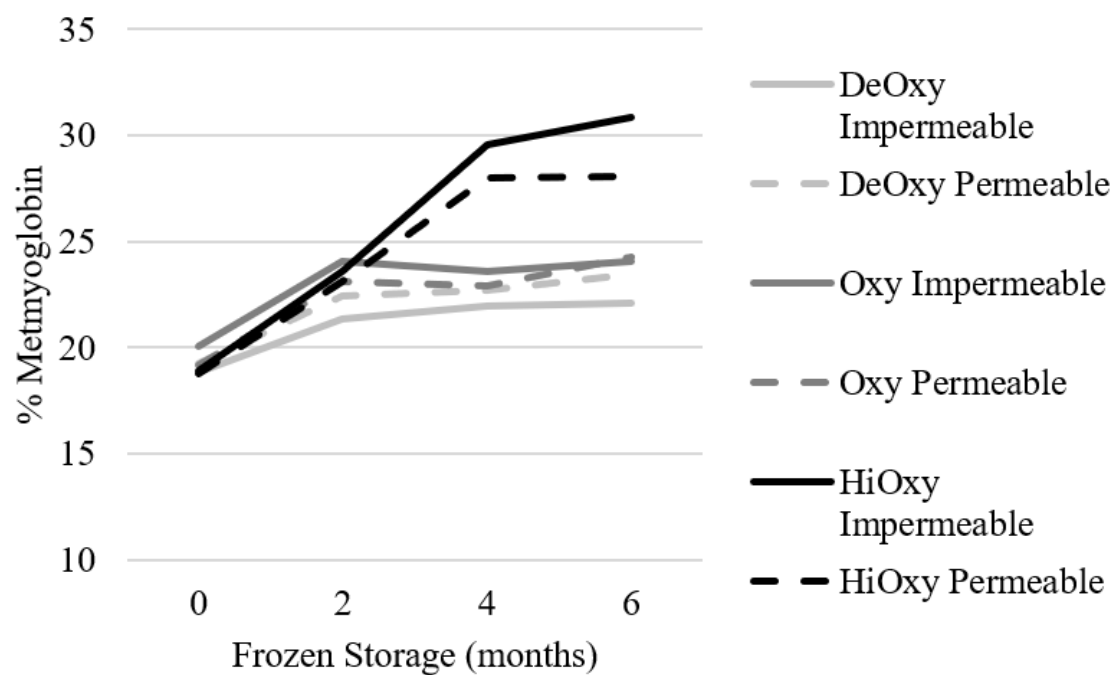
Superscripts for mean separation may be found in Table 5 (page 190).

Appendix 54. Instrumental color values for percent deoxymyoglobin of steaks aged 20 d, in either a deoxymyoglobin, oxygenated, or high oxygenated state, and frozen for 0, 2, or 6 months throughout 7 days of retail display.



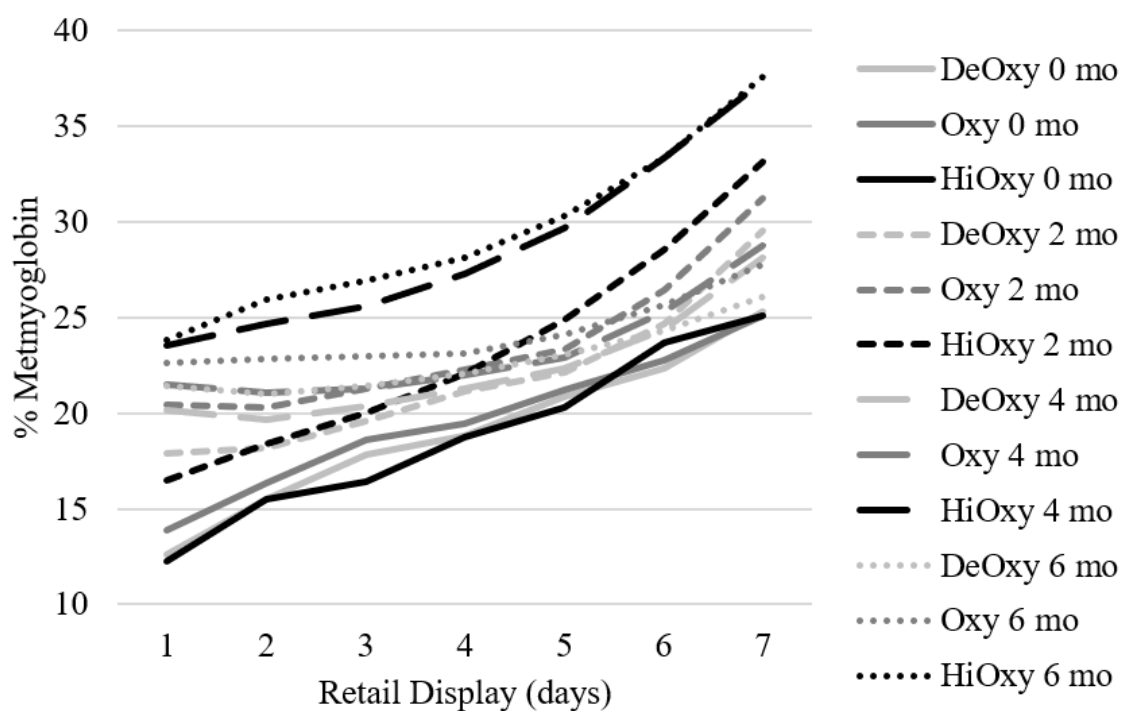
Superscripts for mean separation may be found in Table 7 (page 194).

Appendix 55. Instrumental color values for percent metmyoglobin of steaks aged 4 d, in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging and frozen for 0, 2, 4, or 6 months.



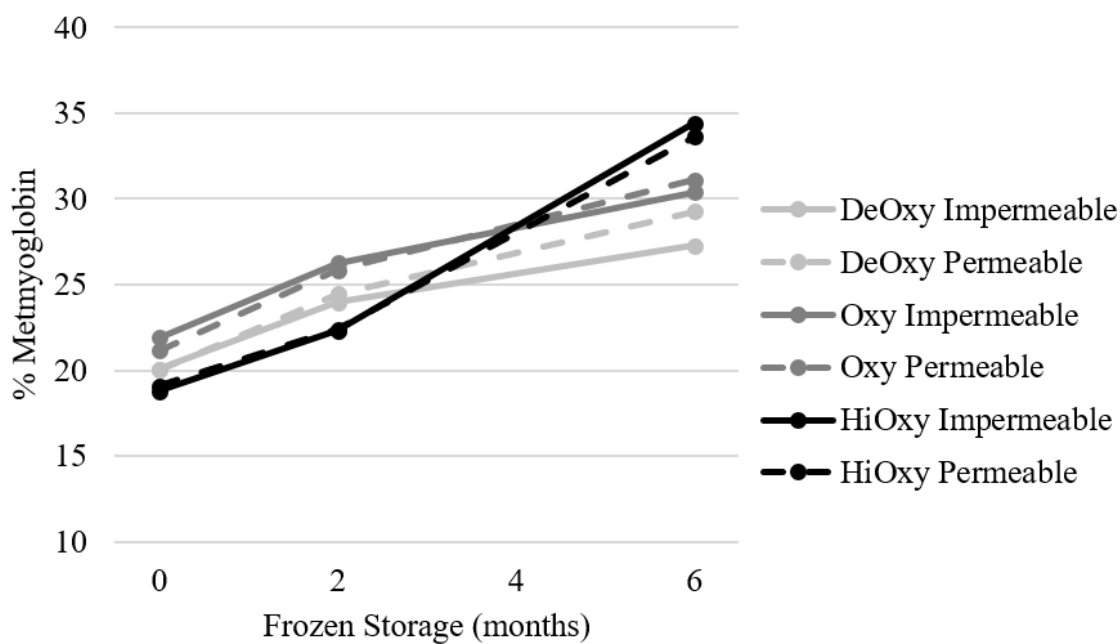
Superscripts for mean separation may be found in Table 5 (page 191)

Appendix 56. Instrumental color values for percent metmyoglobin of steaks aged 4 d, in either a deoxymyoglobin, oxygenated, or high oxygenated state, and frozen for 0, 2, 4, or 6 months throughout 7 days of retail display.



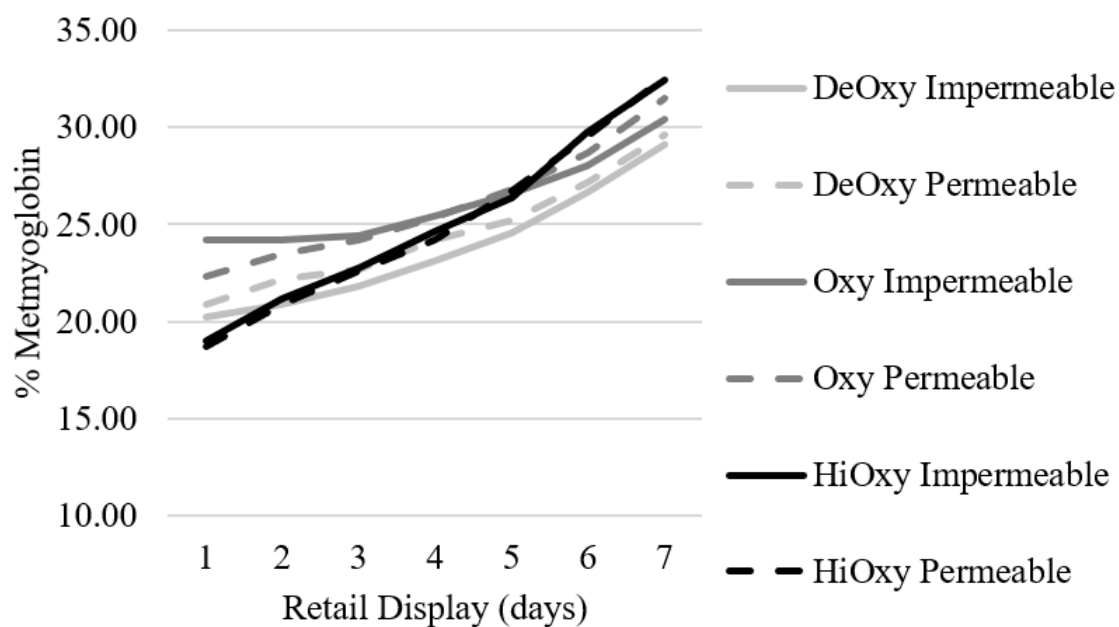
Superscripts for mean separation may be found in Table 7 (page 195).

Appendix 57. Instrumental color values for percent metmyoglobin of steaks aged 20 d, in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging, and frozen for 0, 2, or 6 months.



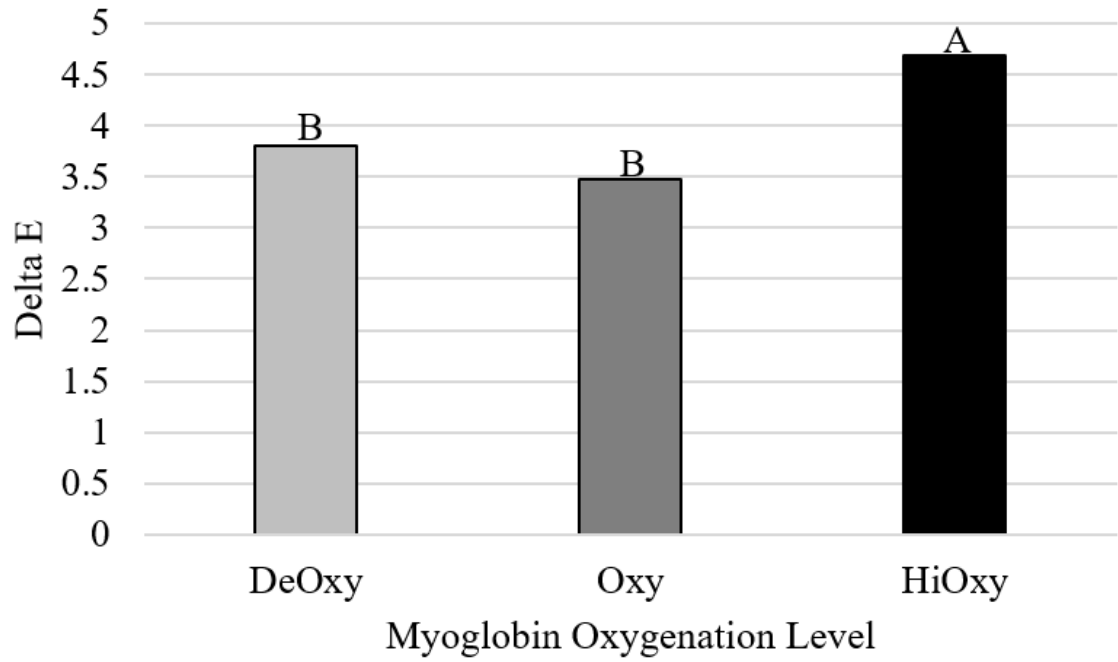
Superscripts for mean separation may be found in Table 5 (page 191).

Appendix 58. Instrumental color values for percent metmyoglobin of steaks aged 20 d, in either a deoxymyoglobin, oxygenated, or high oxygenated state and impermeable or permeable packaging throughout 7 days of retail display.



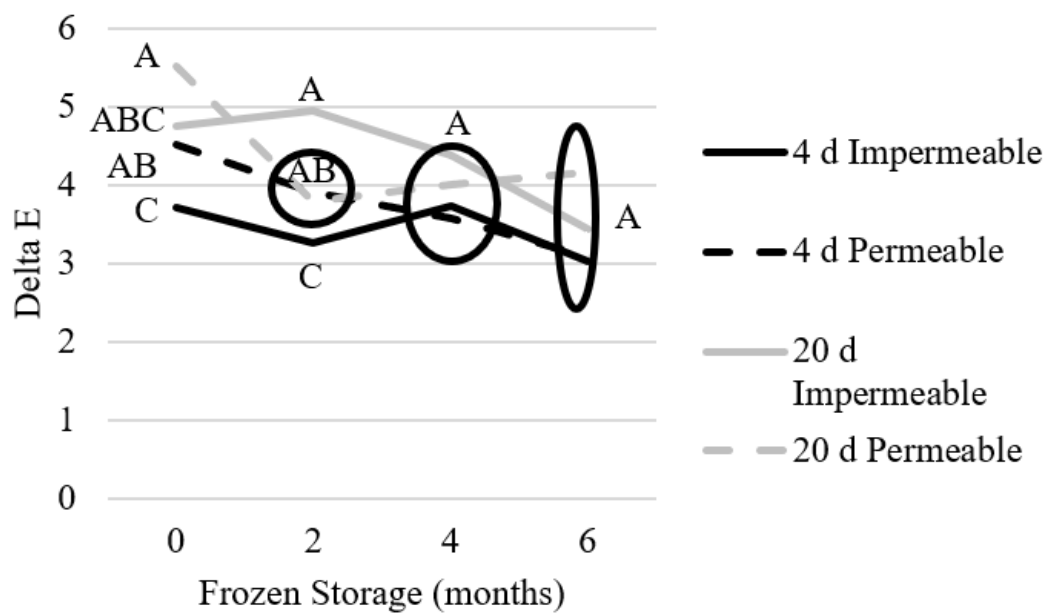
Superscripts for mean separation may be found in Table 4 (page 189).

Appendix 59. Delta E values for steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state.



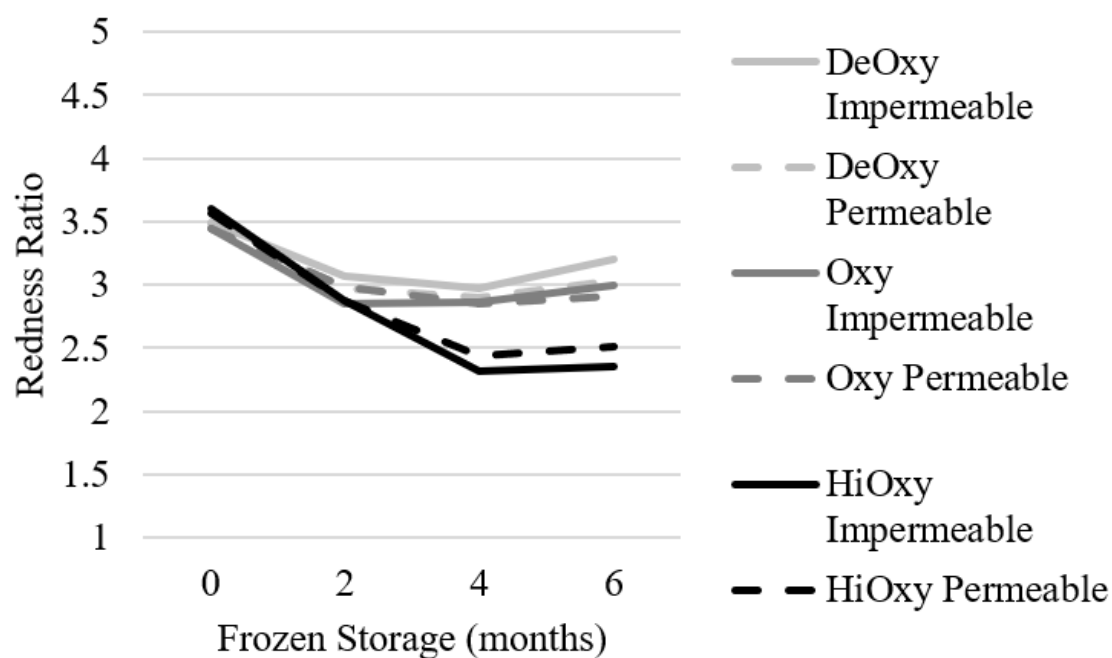
^{a-b} Different superscripts indicated differences within treatments ($P < 0.05$).

Appendix 60. Delta E values for steaks aged 4 or 20 d, packaged in impermeable or permeable film, and frozen for 0, 2, 4, or 6 months.



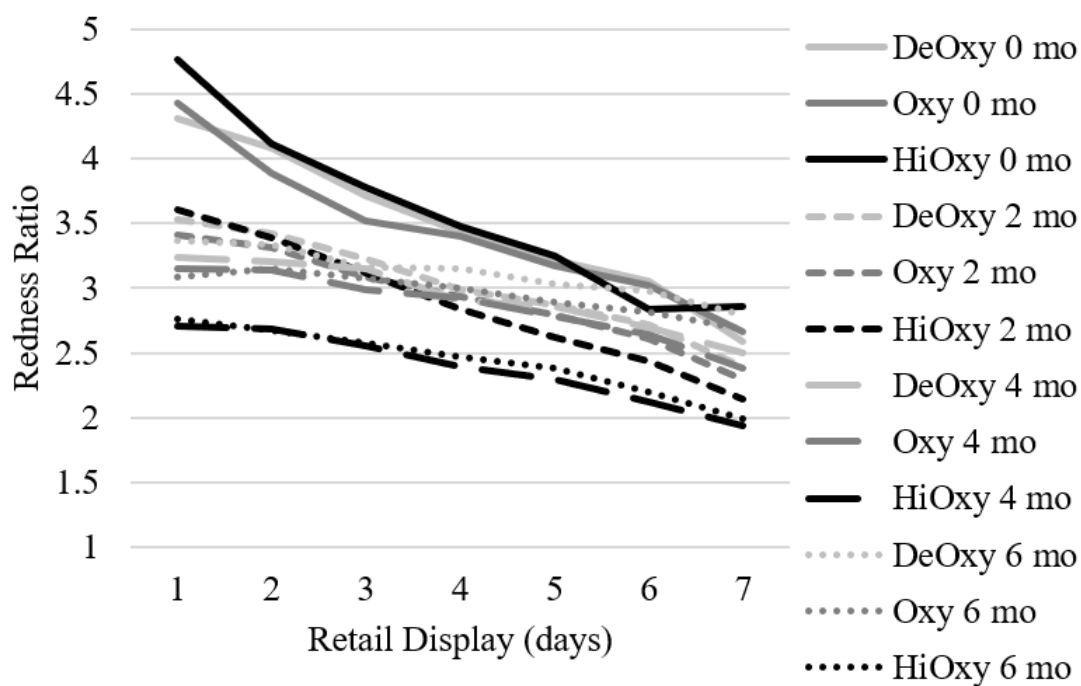
^{a-c} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 61. Redness ratio values of steaks aged 4 d, in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging, and frozen for 0, 2, 4, or 6 months.



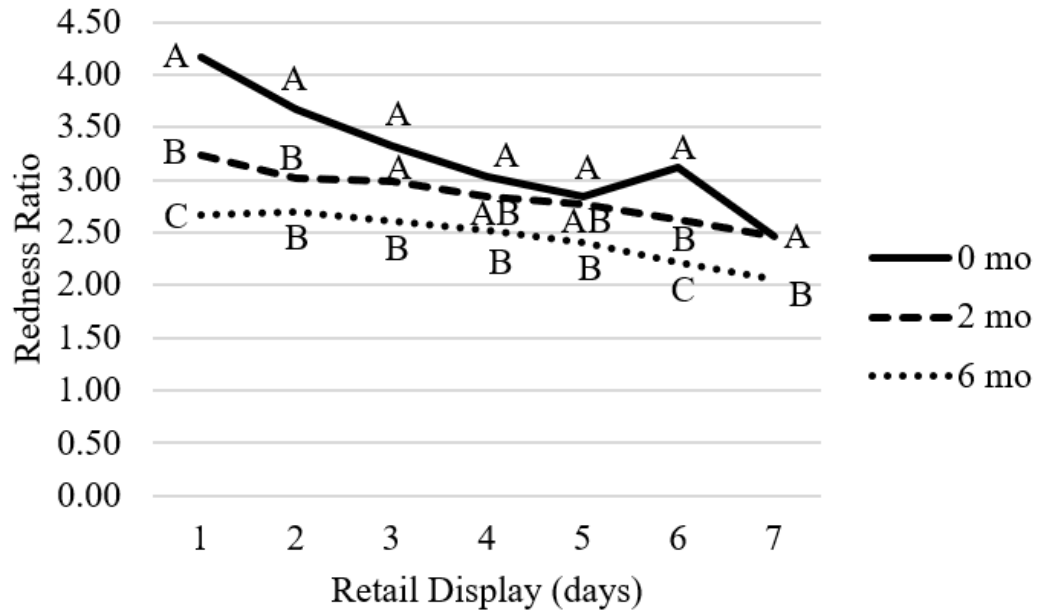
Superscripts for mean separation may be found in Table 5 (page 191).

Appendix 62. Redness ratio values of steaks aged 4 d, in either a deoxymyoglobin, oxygenated, or high oxygenated state, and frozen for 0, 2, 4, or 6 months throughout 7 days of retail display.



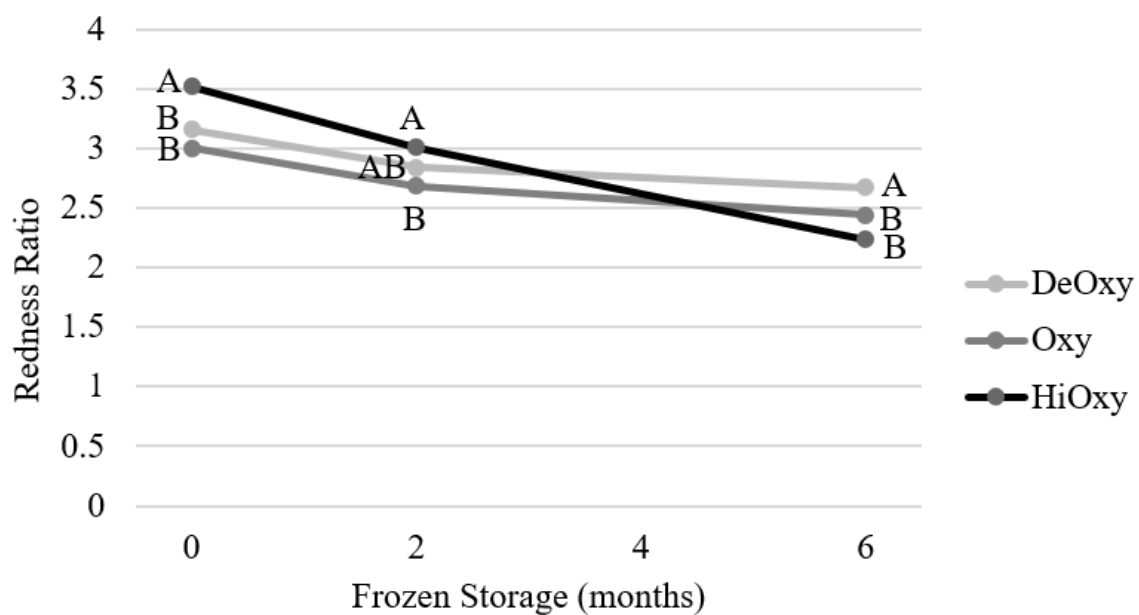
Superscripts for mean separation may be found in Table 7 (page 196).

Appendix 63. Redness ratio values of steaks aged 20 d, frozen for 0, 2, or 6 months throughout 7 days of retail display.



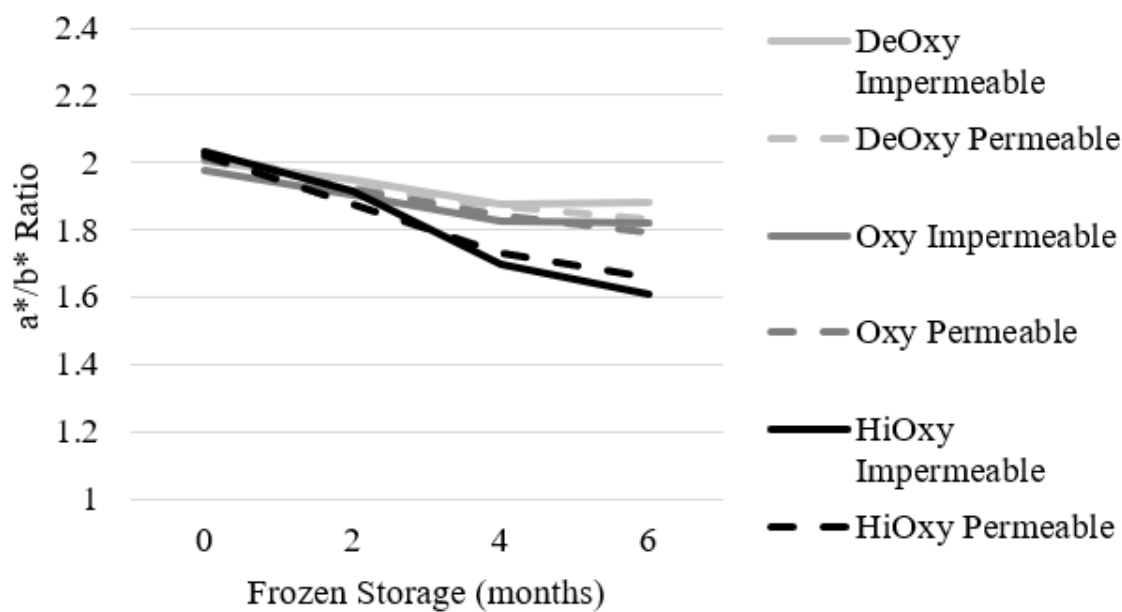
^{a-c} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 64. Redness ratio values of steaks aged 20 d in either a deoxymyoglobin, oxygenated, or high oxygenated state, and frozen for 0, 2, or 6 months.



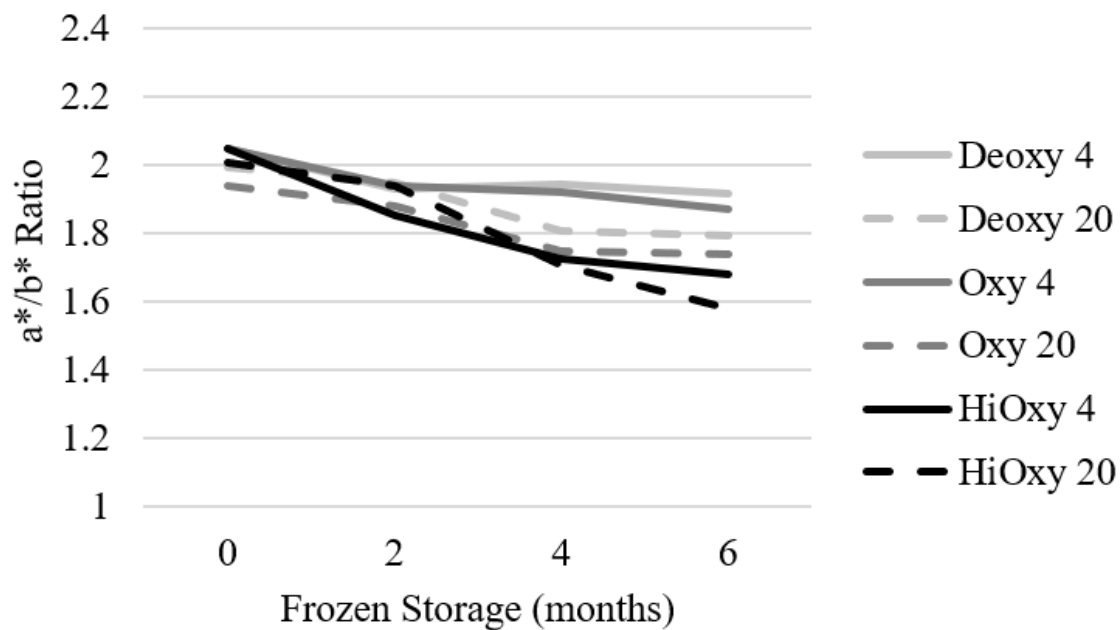
^{a-b} Different superscripts indicated differences within frozen storage period ($P < 0.05$).

Appendix 65. The $a^*:b^*$ ratios of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging, and frozen for 0, 2, 4, or 6 months.



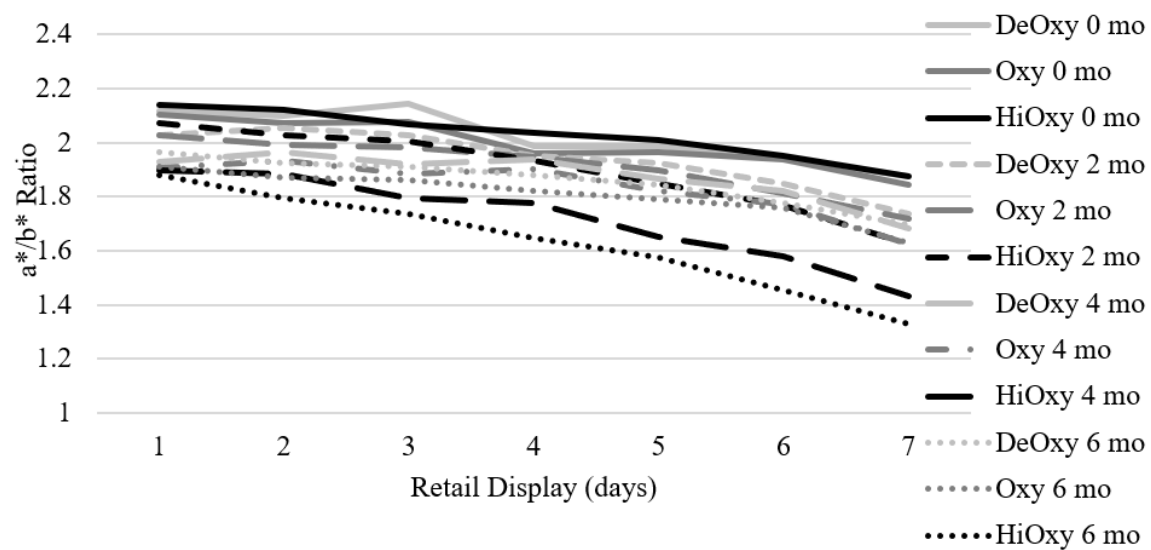
Superscripts for mean separation may be found in Table 5 (page 191).

Appendix 66. The $a^*:b^*$ ratios of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, aged 4 or 20 d, and frozen for 0, 2, 4, or 6 months.



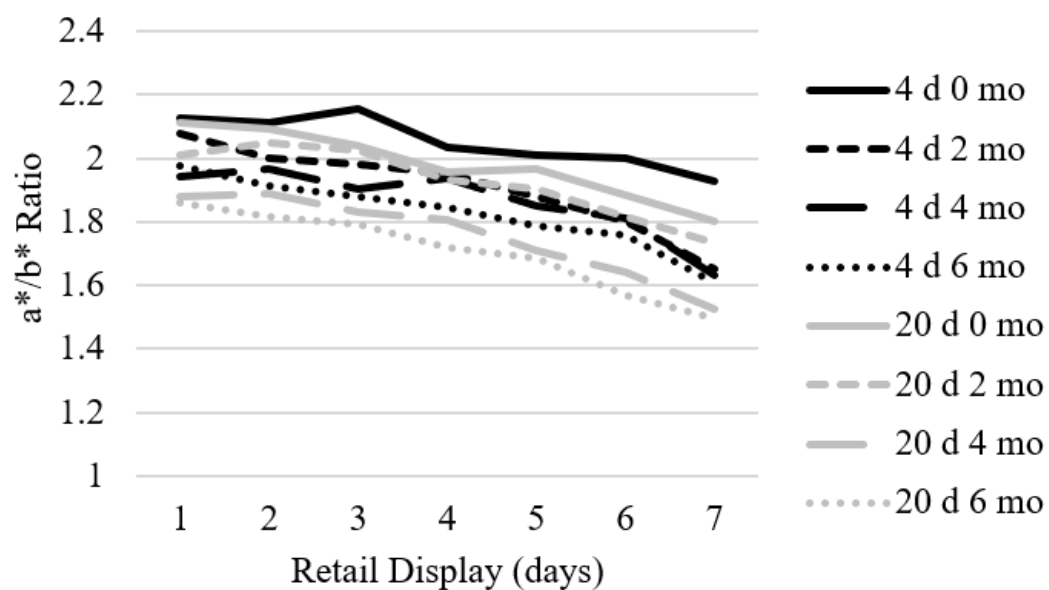
Superscripts for mean separation may be found in Table 6 (page 192).

Appendix 67. The $a^*:b^*$ ratios of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, and frozen for 0, 2, 4, or 6 months throughout 7 days of retail display.



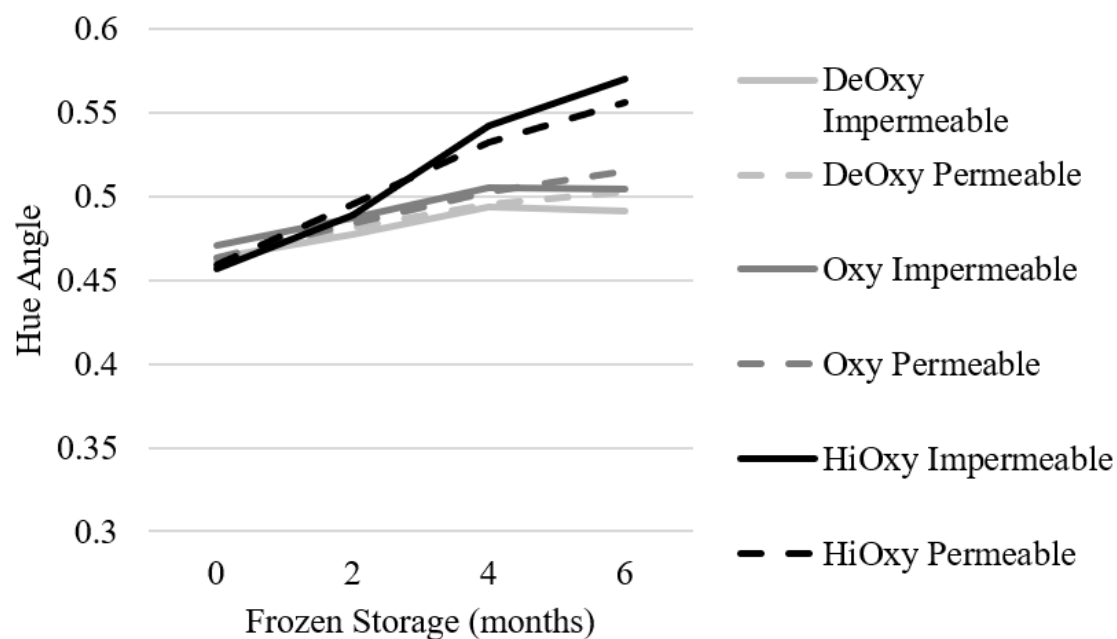
Superscripts for mean separation may be found in Table 7 (page 196).

Appendix 68. The $a^*:b^*$ ratios of steaks aged 4 or 20 d and frozen for 0, 2, 4, or 6 months throughout 7 days of retail display.



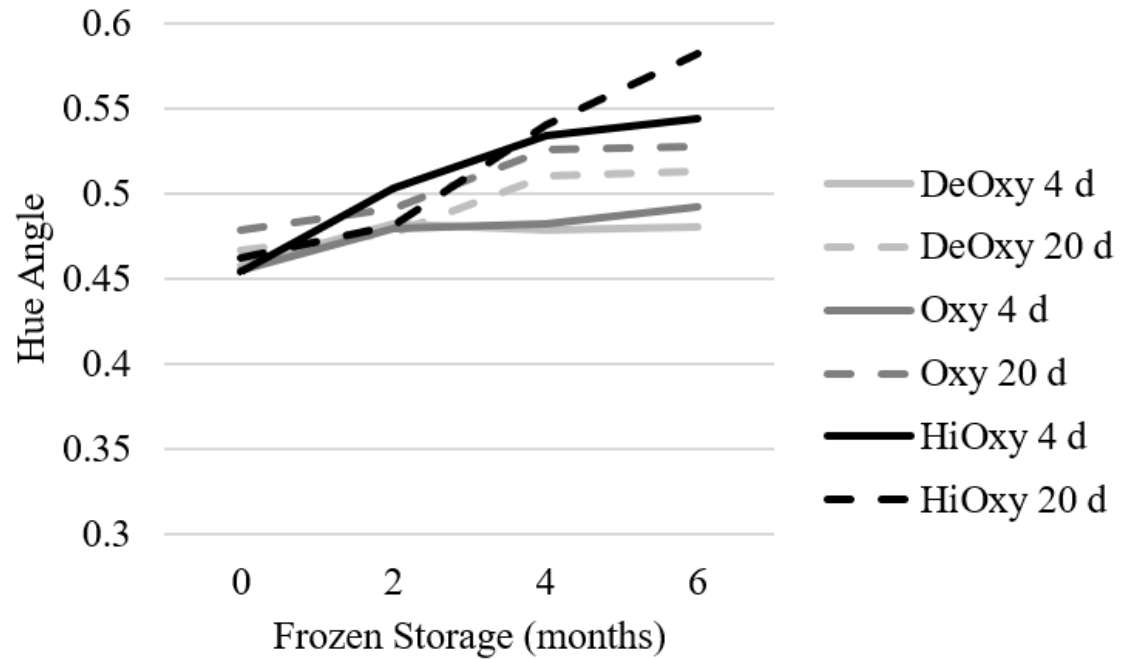
Superscripts for mean separation may be found in Table 9 (page 202).

Appendix 69. Hue angle values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, impermeable or permeable packaging, and frozen for 0, 2, 4, or 6 months.



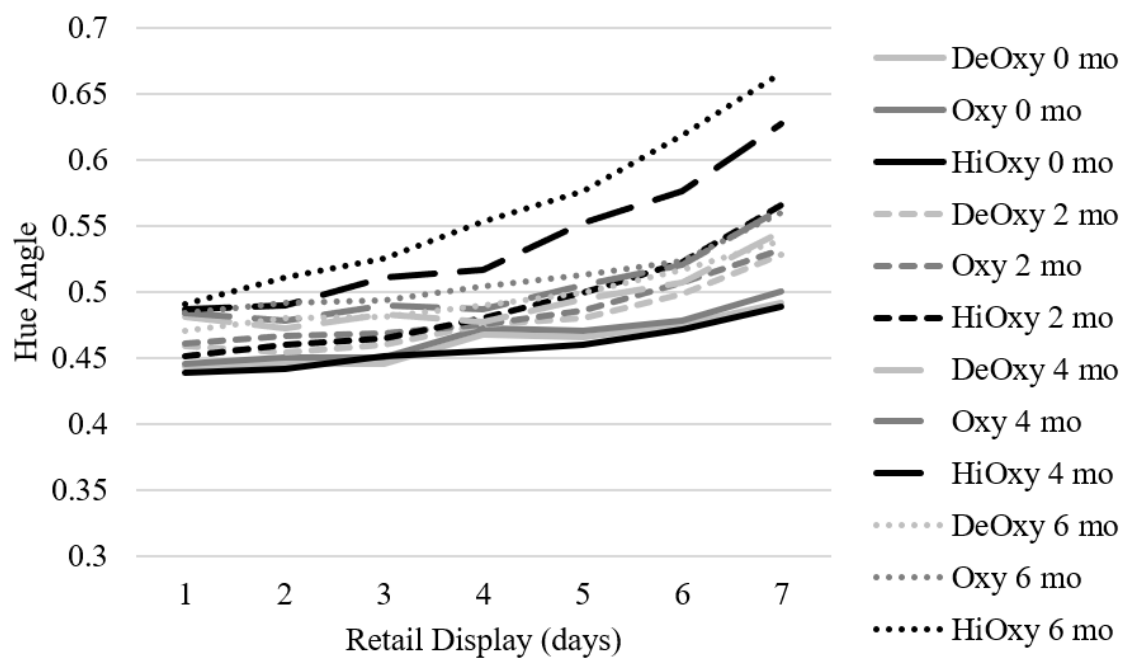
Superscripts for mean separation may be found in Table 5 (page 191).

Appendix 70. Hue angle values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, aged 4 or 20 d, and frozen for 0, 2, 4, or 6 months.



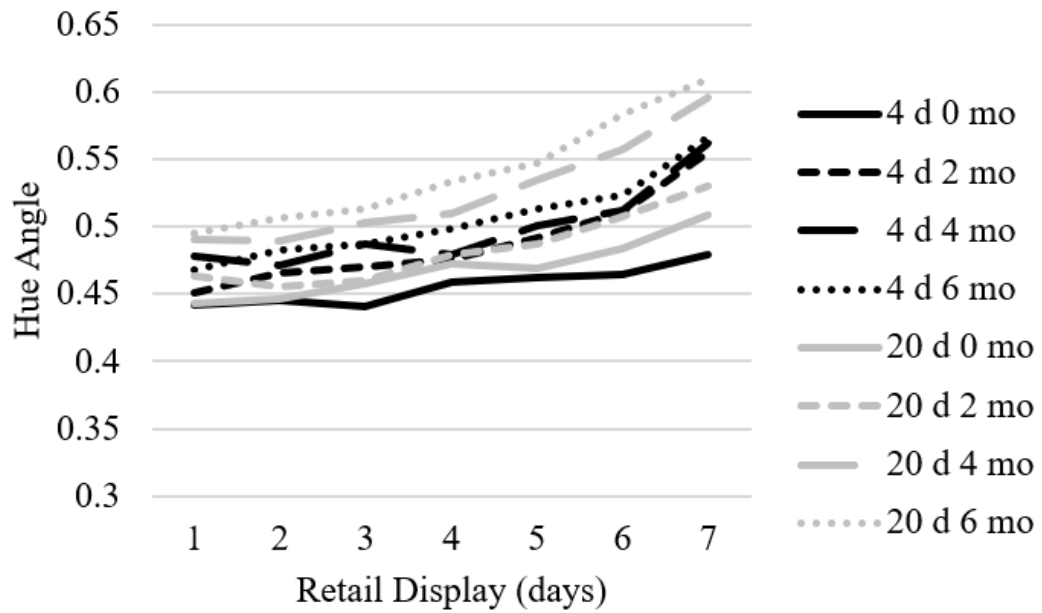
Superscripts for mean separation may be found in Table 6 (page 192).

Appendix 71. Hue angle values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state, and frozen for 0, 2, 4, or 6 months throughout 7 days of retail display.



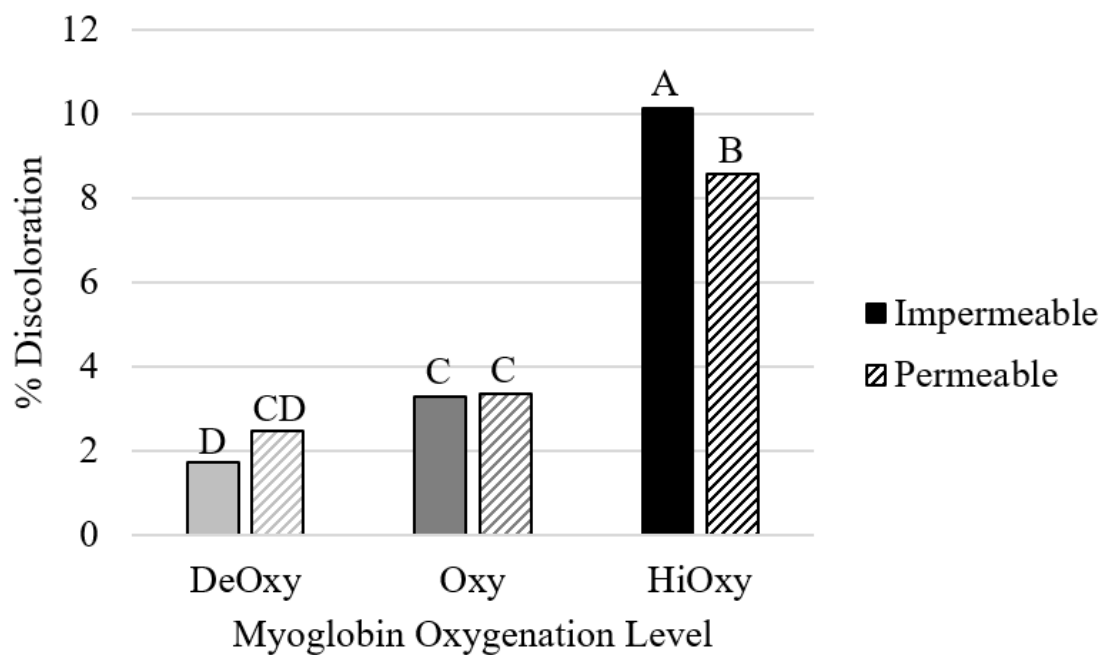
Superscripts for mean separation may be found in Table 7 (page 197).

Appendix 72. Hue angle values of steaks aged 4 or 20 d and frozen for 0, 2, 4, or 6 months throughout 7 days of retail display.



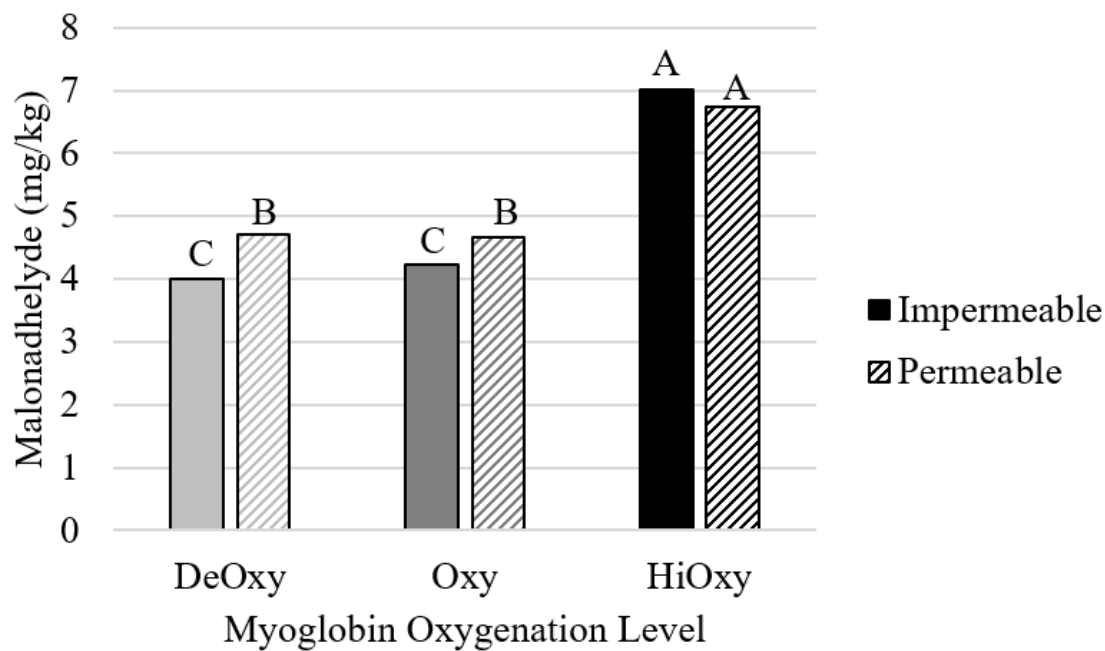
Superscripts for mean separation may be found in Table 9 (page 203).

Appendix 73. Discoloration values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and impermeable or permeable packaging.



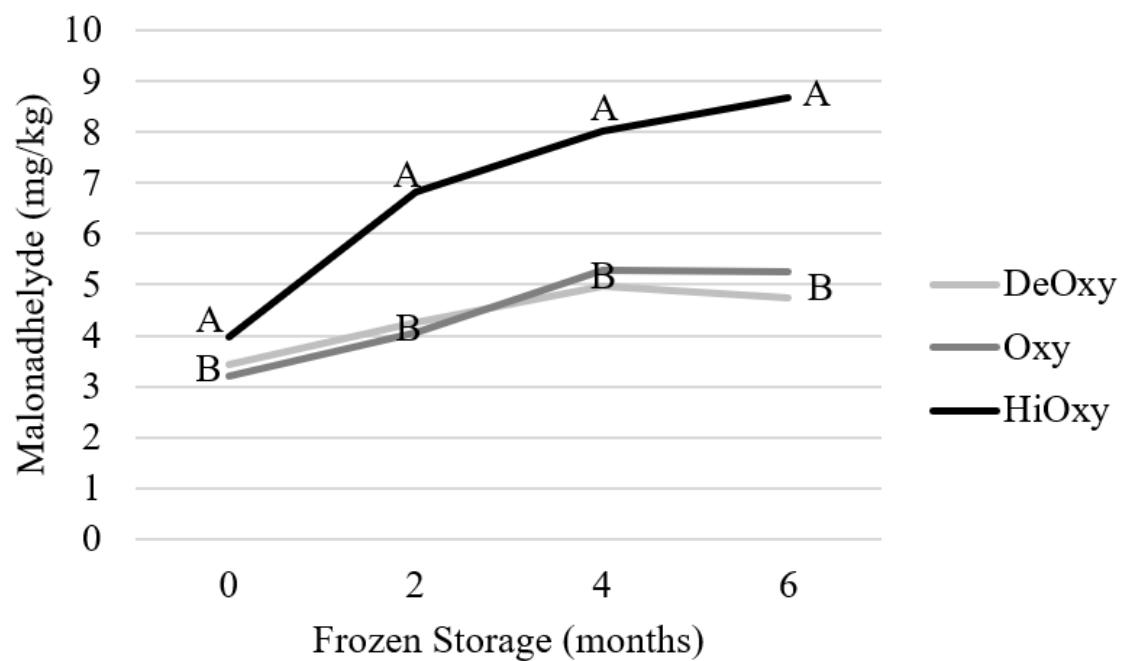
^{a-d} Different superscripts indicated differences in treatments ($P < 0.05$).

Appendix 74. Lipid oxidation values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and impermeable or permeable packaging.



^{a-c} Different superscripts indicated differences in treatments ($P < 0.05$).

Appendix 75. Lipid oxidation values of steaks in either a deoxymyoglobin, oxygenated, or high oxygenated state and frozen for 0, 2, 4, or 6 months.



^{a-b} Different superscripts indicated differences within frozen storage period ($P < 0.05$).