EFFECTS OF ENHANCEMENT AND NITRITE-EMBEDDED PACKAGING ON DARK-CUTTING BEEF COLOR

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EFFECTS OF ENHANCEMENT AND NITRITE-EMBEDDED PACKAGING ON DARK-CUTTING BEEF COLOR

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Abstract: The objective of this study was to determine the effects of novel nitrite-embedded packaging (NEP) and enhancement on retail and cooked color and palatability of dark-cutting beef. Selection of dark-cutting beef strip loins (n = 10; pH > 6.0) and USDA Choice beef strip loins (normal-pH, n = 10) occurred at a commercial packing plant. Dark-cutting loins were divided into 2 sections and randomly selected as non-enhanced dark-cutting (DCN) and enhanced dark-cutting (DCE) treatments with 10% injection of the green weight. A final concentration of 0.5% glucono delta-lactone and 0.1% rosemary in the loin. Steaks (1.91 cm) were removed from nonenhanced normal-pH, DCN, and DCE loins. One steak from the normal-pH and DCN loins was packaged in trays overwrapped with poly-vinyl chloride, and one steak from the DCE loins was packaged in NEP. Steaks were kept in simulated retail display for 6 d with instrumental color measurements and trained color panel (n = 6) evaluation every 24 h. Remaining steaks were randomly assigned to cooked color, sensory, and Warner-Bratzler shear force. Steaks for cooked color were placed in dark storage for 72 h after packaging the DCE steak in NEP and the DCN and normal-pH steaks in vacuum packaging. A trained sensory panel (n= 6) evaluated sensory steaks for beef palatability. By d 6 of retail display, DCE steaks had greater ($P < 0.05$) a* values than DCN and normal-pH steaks. There was significantly more surface discoloration on normal-pH steaks than DCE and DCN steaks by d 6 of retail display. The cooked DCE steaks had similar ($P > 0.05$) internal a* values to cooked normal-pH steaks. A similar decrease in redness was observed by panelists evaluating the internal cooked color of DCE steaks. However, external cooked color of DCE was determined to have significantly more pink appearance than normal-pH and DCN steaks. The trained sensory panel determined there was no difference in tenderness and juiciness between the normal-pH and DCE steaks, paralleling with the Warner-Bratzler shear force results. In conclusion, NEP improved surface redness of DCE steaks during retail display, and the enhancement decreased internal redness upon cooking.

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CHAPTER I

INTRODUCTION

Meat color plays a critical role in consumer purchasing decisions with preferences for a bright-cherry-red appearance, and deviations from a bright-cherry red result in decreased purchases (Carpenter et al., 2001). Myoglobin is the primary protein contributing to meat color containing a heme ring with six binding sites (Aberle et al., 2012). Specifically, the ligand bond to the sixth binding site and the redox state of the heme ring impacts the meat color (Aberle et al., 2012). Binding of oxygen to the reduced heme results in the bright-cherry-red color of oxymyoglobin (Aberle et al., 2012). Anaerobic conditions result in the formation of the purple color of deoxymyoglobin (Aberle et al., 2012). Dark-cutting (DC) beef appears darker due to a greater postmortem pH (Hughes et al., 2017). The lack of pH decline results in more mitochondrial oxygen consumption (Ashmore et al., 1972). With less oxygen available to bind to myoglobin, the bloom is decreased. More deoxymyoglobin is formed (English et al., 2016, Hughes et al., 2017) and an undesirable color for consumers. The high pH results from stress prior to harvest leaving a lower glycogen content in the muscle and a smaller decline in pH postmortem (Lawrie, 1958; Scanga et al., 1998). Additionally, a greater pH contributes to increased microbial growth, pinkish red cooked color and tender meat. The pH of DC beef shifts

the microbial growth allowing for increased microbial count of some spoilage bacteria compared to normal-pH meat (Patterson and Gibbs, 1977; Gill and Newton, 1979). At a greater pH, myoglobin does not denature at typical cooking temperature (70□) (Hunt et al., 1999). This results in an internal pink color when a grey appearance is expected (Hunt et al., 1999). As mentioned, pH plays a role in the structural characteristics of DC beef, and these characteristics have also been shown to impact palatability of DC beef. Tenderness research of DC steaks has determined a curvilinear relationship between pH and tenderness (Wulf et al., 2002; Grayson et al., 2016). In relation to pH, tenderness is greatest in a high pH range of 6.7-6.9 and lowest in a range from 5.8-6.4 (Wulf et al., 2002; Grayson et al., 2016). Typically, normal-pH (5.6) beef falls in the middle (Wulf et al., 2002; Grayson et al., 2016).

Enhancement of DC beef has led to improvements in fresh and cooked color. Lactic acid enhancement increased the L* values of DC steaks and led to a greyer internal cooked appearance compared to nonenhanced DC steaks (Sawyer et al., 2009; Apple et al., 2011). However, surface discoloration increased with lactic acid enhancement of DC beef (Sawyer et al., 2009; Apple et al., 2011). Previously, glucono delta-lactone (GDL) has shown to improve fresh color of DC beef (Dolezal et al., 2013). In lieu of enhancement, nitrite-embedded packaging (NEP) has been reported to improve surface redness of DC beef, anaerobically, and redness was further improved through a combination with a 0.2% rosemary dip (Ramanathan et al., 2018). The NEP increases redness by the formation of nitric oxide myoglobin. Nitrite forms nitric oxide by the reducing ability of meat (Siegel, 2011). The NEP forms anaerobic conditions decreasing the oxygen partial pressure. At a low oxygen partial pressure (6-7 mmHg), metmyoglobin

is formed (Ledward, 1970; Brantley et al., 1993). Metmyoglobin binds with nitric oxide to form nitric oxide metmyoglobin, which is reduced to nitric oxide myoglobin, causing a bright-red color (Fox Jr. and Ackerman, 1968; Siegel, 2011). In NEP normal-pH steaks, an increase in surface redness was seen in 5 d (Yang et al., 2016); however, in DC steaks, redness was improved in 24 h (Ramanathan et al., 2018). Upon removing from NEP, redness of normal-pH steaks significantly decreased by 24 h (Claus and Du, 2013). Repackaging steaks after variable storage periods and the effects of NEP in combination with enhancement on cooked color has not been investigated.

Limiting the microbial growth of the DC beef is important to extend shelf life. Beyond the effects of anaerobic conditions, no significant change in microbial growth from the use of NEP with DC steaks has occurred (Ramanathan et al., 2018). Although lactic acid is used as an antimicrobial in the industry, limited research has presented the effects of organic acid enhancement on microbial growth of DC beef. To our knowledge, an evaluation of an acid enhancement, specifically GDL, combined with NEP on the impact of meat color has not occurred. The research has not indicated microbial effects of GDL on DC beef. The objective of this study was to evaluate the effects of GDL and NEP on the fresh and cooked color of DC beef. The impact of GDL on DC beef palatability and tenderness will be determined as well. We hypothesized the use of GDL and rosemary would improve the retail and cooked color and decrease microbial growth of DC beef in NEP.

CHAPTER II

REVIEW OF LITERATURE

Meat Color

Meat color is predominantly due to the sarcoplasmic protein myoglobin. Myoglobin is a monomer with an iron-containing heme ring that can form six bonds. The iron is bound to four nitrogen pyrrole rings; while at the fifth position, histidine stabilizes the heme ring. The sixth coordinate position remains open to reversibly bind ligands. Meat color depends on the redox state of heme iron and the ligand bound to myoglobin at the sixth binding site (Aberle et al., 2012). Deoxymyoglobin forms in low oxygen conditions (< 1.4 mmHg) when the heme iron is in the reduced state and the sixth position remains unbound (Mancini and Hunt, 2005). Oxygen exposure forms oxymyoglobin through the binding of oxygen to the ferrous iron in a process commonly known as "bloom" or oxygenation (Aberle et al., 2012). Bloom produces the consumer-preferred bright cherry-red color known as oxymyoglobin (Carpenter et al., 2001). Meat color plays an important role in consumer purchases due to consumers relating freshness with a cherry-red color of beef lean (Carpenter et al., 2001). Consumers are more likely to purchase beef products with a bright red appearance over products with deviations in red appearance (Carpenter et al., 2001). Carcasses deviating from the bright red lean color can be discounted at the plant due to consumer preferences.

Dark-cutting Beef

Dark-cutting (DC) beef or dark, firm and dry (DFD) beef is a color deviation from the characteristic bright-red appearance of beef. Compared to normal beef, dark-cutting beef has increased darkness and decreased redness in appearance (Wulf et al., 2002; Galloway et al., 2005; Hughes et al., 2017). When consumers were asked to choose between bright-cherry-red beef and DC beef, consumers discriminated against the DC beef because of its darker appearance (Viljoen et al., 2002). Due to consumer perception of DC beef, packers discount DC beef increasing the economic losses to the beef industry. According to the 2016 National Beef Quality Audit, approximately 1.9% of carcasses are considered DC carcasses (Boykin et al., 2017), and it is accepted that DC meat results from pre-harvest stress. The stress leads to increased use of glycogen reserves prior to harvest decreasing lactic acid formation postmortem creating an ultimate pH greater than normal-pH muscle (Lawrie, 1958; Newton and Gill, 1981; Scanga et al., 1998). Pre-harvest stress can be caused by a variety of factors including, but not limited to, weather, management practices, and seasonality (Scanga et al., 1998). The high pH of DC beef influences the mitochondrial activity, water-holding capacity, fresh and cooked color, sensory characteristics, and microbial growth of the meat.

Previous research has shown the number of mitochondria increases as the pH of DC beef increases and the electron loss in the mitochondria was greater for DC beef compared to normal-pH beef (McKeith et al., 2016). The increase in electron loss indicates the mitochondria of DC beef were not as efficient as the normal-pH mitochondria (McKeith et al., 2016). Ashmore et al. (1972) determined mitochondrial activity remained elevated in DC meat due to the high pH. Mitochondria utilize oxygen

postmortem leaving very little oxygen to oxygenate myoglobin increasing deoxymyoglobin formation in DC beef based on research from McKeith et al. (2016) and Ashmore et al. (1972). An increase in deoxymyoglobin formation influences the meat color providing a darker surface appearance than normal-pH beef (Hughes et al., 2017). Compared to DC beef, normal pH beef has a lower and shorter period of mitochondria respiration decreasing incidence of deoxymyoglobin as stated by Ashmore et al. (1972). Along with the elevated mitochondria respiration, the high pH of DC beef influences the muscle fibers and water holding capacity of the muscle impacting the appearance of the meat surface.

Dark-cutting meat has a greater water holding capacity than normal-pH beef (Sawyer et al., 2008; Sawyer et al., 2009; Apple et al., 2011; Wills et al., 2017). A greater pH of DC beef promotes larger muscle fiber diameter due to repulsion of the myofilaments increasing space for water binding (Hughes et al., 2017). In dark muscles, Hughes et al. (2017) determined a larger muscle fiber diameter increased the space between the scattering elements in the muscle. This decreases the light scattering and reflectance and was indicative of lower lightness of the muscle and darker appearance to the eye (Hughes et al., 2017). In agreement, Lawrie (1958) determined the muscle swelling of DC beef results in a ‘closed’ structure; therefore, less oxygen penetration occurred with less bloom and more deoxymyoglobin present (English et al., 2016; Hughes et al., 2017). In support, as the pH of DC beef increased from 5.8 to 6.6-6.9, the L* values increased and a* values decreased (McKeith et al., 2016). Reducing the pH of DC beef saw a reduction in fiber width increasing the lightness of the muscle (Hughes et

al., 2017), which indicates acidification of dark cutters could impact the surface appearance.

The high ultimate pH also negatively impacts the cooked color of beef products. Typically in normal-pH meat, the heating of meat products results in the denaturation of deoxymyoglobin, oxymyoglobin, or metmyoglobin leaving a grey cooked pigment called denatured globin ferrihemochrome (Fox, 1966). However, in high pH beef, the pH stabilizes myoglobin causing less myoglobin denaturation upon cooking (Trout, 1989; Hunt et al., 1999). A decline in denaturation results in a persistent pink internal color at temperatures that should be sufficient to result in a grey cooked appearance (Mendenhall, 1989; Trout, 1989). Consumers expect a grey color for cooked fresh meats, and any indication of internal red or pink will be interpreted as undercooked (Cornforth et al., 1991). Therefore, the persistent pink color of DC beef negatively impacts consumers' perception of cooked beef. Previous research in high-pH ground beef patties noted the internal cooked color was an intense, stable pink at 71°C, and the pink intensity increased with greater myoglobin concentration (Mendenhall, 1989). Recent research has established myoglobin content is greater in high-pH beef than normal-pH beef (Moiseev and Cornforth, 1999; Sawyer et al., 2009; English et al., 2016; Hughes et al., 2017). The high pH and increased myoglobin content of DC beef negatively influenced the cooked color, resulting in persistent pinking.

Tenderness characteristics of DC beef compared to normal-pH beef have been conflicting. Warner-Bratzler shear force (WBSF) is utilized to evaluate tenderness by coring meat products parallel to muscle fiber orientation and shearing cores to measure the force per kilogram to cut through the core. A greater force means a tougher product.

Evaluation of WBSF for different muscles of DC carcasses noted muscles with pH between 5.7-6.0 were tougher than the normal pH (5.6) muscles evaluated except the *semitendinosus* (Wulf et al., 2002). In agreement, a trained sensory panel indicated DC beef was tougher than normal-pH beef (Wulf et al., 2002). When comparing dark cutters in the pH range of 6.1-6.9, slice shear force was highest for an ultimate pH between 6.1-6.4, indicating increased toughness (Grayson et al., 2016). A trained sensory panel evaluated DC steaks for tenderness and reported the tenderness to be as followed highest pH (6.7-6.9) DC steaks > normal pH steaks (5.7) > lower pH DC steaks (6.1-6.4) (Grayson et al., 2016). A curvilinear relationship between pH and tenderness was determined based on the results from WBSF and the trained sensory panel (Grayson et al., 2016). A similar curvilinear relationship was observed in research by Watanabe et al. (1996). Yu and Lee (1986) reported an intermediate pH (5.8-63) was less tender than muscle from low-pH (< 5.8) and high-pH (> 6.3) meat. They indicated the pH-dependent proteolytic activity played a role in this shift in tenderness. In low-pH muscle, they speculated acidic proteases from the lysosome were responsible for the increase in tenderness. High-pH muscle was speculated to have more active neutral-pH calcium-dependent proteases based on the degradation pattern. In support, Koohmaraie (1992) observed more calpain activity at a neutral pH compared to an acidic pH. However, Yu and Lee (1986) mentioned acidic and neutral-pH proteases would not be as effective in the intermediate pH muscles. Galloway et al. (2005) determined the WBSF was lower in DC beef from stressed Holstein calves. Hughes et al. (2017) demonstrated increased muscle swelling at a greater pH supporting an increase in water holding capacity. This supports an increase in tenderness of DC beef by creating more space in the muscle.

Although, Apple et al. (2011) reported no difference in WBSF between normal pH steaks and DC steaks. Consumer acceptability research has been more limited. When comparing fried DC beef steaks and fried normal-pH steaks, acceptability for tenderness and flavor was not significantly different between the fried DC and normal-pH steaks for the consumer panel; although, female consumers commented on off-flavors in the DC steaks (Viljoen et al., 2002). More "off-flavors" have been associated with DC steaks than normal steaks in trained panels (Wulf et al., 2002; Grayson et al., 2016). Tenderness of DC beef has been highly pH-dependent indicating a potential for further research when enhancing DC beef.

Microbial growth in DC beef is a concern due to the greater ultimate pH. Bacterial growth occurs best at pH between 6.6-7.5, (Jay et al., 2005), and bacterial growth of some spoilage bacteria has occurred more readily in DC beef compared to normal-pH beef (Patterson and Gibbs, 1977; Gill and Newton, 1979), indicating bacteria grows more readily in high pH beef. The amount of glycogen stored in the muscle postmortem also influences microbial growth. In normal-pH meat, the remaining glycogen can be utilized as glucose by the bacteria limiting the spoilage (Newton and Gill, 1981). When no glucose remains, bacteria begin to use amino acids causing spoilage in the meat. As previously mentioned, DC beef has low amounts of glycogen, which is the storage form of glucose in the muscle (Lawrie, 1958; Scanga et al., 1998). Thus, the spoilage rate of DC meat is increased because amino acids are used sooner (Gill and Newton, 1979). Therefore, the combination of low glycogen content and high pH in DC beef leads to more microbial growth.

Acidification of Meat Products

Organic acids can be utilized in meat products to decrease microbial growth and impact meat color by shifting the pH. Applying 5% lactic acid through tumbling normal-pH beef trimmings resulted in a decrease in pH and reduction of *Escherichia coli*, coliforms, and aerobic psychotropic bacterial growth (Stivarius et al., 2002). However, the lactic acid addition provided a lighter, less red appearance, and more discoloration compared to the control in a retail display (Stivarius et al., 2002). Dipping fresh beef in 3% and 5% lactic acid, acetic acid, and citric acid reduced growth of E*scherichia coli, Salmonella typhimurium, Listeria monocytogenes,* and total viable bacteria, but acetic acid and citric acid had negative implications on beef color in 24 hours (Kassem et al., 2017). Organic acid addition to normal-pH beef has resulted in microbial reduction but negative implications on the fresh color.

Acid enhancement has been shown to improve the fresh and cooked color of DC beef. Research has indicated acetic acid injection of DC strip loins increased the lightness of the DC steaks (Tapp et al., 2017). Tapp et al. (2017) determined DC steaks injected with 1.2% and 1.6% acetic acid were redder than the non-injected DC steaks. However, the cooked color of DC beef was not improved through the addition of acetic acid (Tapp et al., 2017). Tenderness of DC steaks by WBSF and trained panel was not improved by acetic acid enhancement (Tapp et al., 2017). Acetic acid has been shown to improve the fresh color of DC beef, but the cooked palatability of DC steaks was not affected.

Previous research on lactic acid injection of DC beef determined the fresh color, cooked color, and tenderness can be impacted. Lactic acid enhancement from 0.25-0.75% in DC beef increased the lightness compared to nonenhanced DC steaks, and the L*

values were not significantly different from normal-pH steaks (Sawyer et al., 2009; Apple et al., 2011). Visual color scores of 0.25-0.75% lactic acid-enhanced DC steaks indicated a brighter redness than nonenhanced DC steaks (Sawyer et al., 2009). Surface discoloration increased with lactic acid enhancement resulting in more discoloration than normal-pH steaks and nonenhanced DC steaks (Sawyer et al., 2009; Apple et al., 2011). Panelists commented on the grey and black surface discoloration of enhanced DC steaks with lactic acid enhancement above 0.5% (Sawyer et al., 2009). However, 0.25% lactic acid DC steaks were no more discolored than normal-pH steaks (Sawyer et al., 2009).

Upon cooking, 0.5% lactic acid-enhanced DC steaks with and without salts resulted in similar L* values to normal-pH steaks, and lactic acid above 0.75% resulted in darker cooked internal appearance than normal-pH steaks (Sawyer et al., 2008; Sawyer et al., 2009). The cooked internal a* values of 0.25% and 0.35% lactic acid-enhanced DC steaks were not different from the normal-pH steaks (Sawyer et al., 2008; Apple et al., 2011). Dark-cutting steaks enhanced with 0.25% lactic acid had similar cooked color to normal-pH steaks and appeared more done than nonenhanced DC steaks by a trained color panel (Sawyer et al., 2009). Lactic acid enhancement at 0.5% and 1.0% with salt had a decrease in internal redness comparable to normal-pH steaks (Sawyer et al., 2008). Without the presence of salt, lactic acid at 0.5% and 1.0% decreased a* values lower than the a* values of normal-pH steaks when cooked to 71°C (Sawyer et al., 2008; Sawyer et al., 2009). Internal doneness scores, cooked color scores and a* values of normal-pH steaks were similar to DC steaks enhanced with 0.5% lactic acid (Apple et al., 2011). Degree of doneness of DC steaks was improved with lactic acid enhancement with DC steaks enhanced with 0.5% lactic acid and salt most closely aligned to normal pH steaks

internal doneness (Sawyer et al., 2008). Lactic acid at 1.0% in DC steaks resulted in the grey-brown internal cooked appearance at 71°C (Sawyer et al., 2008; Sawyer et al., 2009). This indicated premature browning when compared to normal-pH steaks.

Low lactic acid (0.15-0.35%) enhancement did not impact the WBSF of DC steaks compared to normal-pH steaks and nonenhanced DC steaks (Apple et al., 2011). Trained sensory panel noted that the normal-pH steaks had a greater first-bite hardness than 0.15% and 0.35% enhanced and nonenhanced DC steaks (Apple et al., 2011). Increasing the lactic acid enhancement concentration (0.50%) resulted in no difference in first-bite hardness compared to normal-pH (Apple et al., 2011). At the same lactic acid concentration (0.50%), the enhanced DC steaks had greater WBSF than normal-pH and DC steaks, with DC steaks indicated as the most tender in agreement to the first-bite hardness results from the panel (Apple et al., 2011). Moisture release was decreased with the addition of lactic acid enhancement at 0.50% in DC steaks compared to normal-pH and nonenhanced DC steaks (Apple et al., 2011). However, moisture release was not impacted by the lactic acid enhancement at 0.35% and 0.15% in DC steaks with no difference between normal-pH, enhanced DC, nonenhanced DC steaks. Overall, lactic acid enhancement can eliminate the persistent pinking and negatively affect palatability in DC beef; nonetheless, lactic acid enhancement has negative impacts on the fresh color of DC beef at higher concentrations.

Research on the implications of organic acid use in DC beef to impact microbial growth has been limited. However, previous research with organic acid enhancement has indicated buffered acetic acid, buffered citric acid, and lactic acid injections led to a decrease in pH in the meat (Sawyer et al., 2008; Sawyer et al., 2009; Apple et al., 2011;

Stackhouse et al., 2016; Tapp et al., 2017). The decline in pH could have microbial implications since bacteria grow more readily at a high pH (Jay et al., 2005).

Glucono delta-lactone (GDL) is a food additive typically used to decrease the pH of dry sausages by replacing the starter culture of lactic acid bacteria and acting as an acidulant. Glucono delta-lactone is a lactone of gluconic acid; therefore, in solutions, GDL hydrolyzes into gluconic acid (Combes and Birch, 1988; Parke et al., 1997) and decreases the pH of the solution (Maijala et al., 1993). Beyond pH reduction in dry sausages, GDL has been used in meat products as an antimicrobial. Glucono delta-lactone at 0.5% in ready-to-fry meat products called "tenderloin rolls" reduced total viable bacteria growth below the untreated "tenderloin rolls" (Farkas and Andrássy, 1993). In minced meat, GDL was effective in decreasing fecal streptococci, total aerobic bacteria, and coliforms (Maijala et al., 1993). The use of GDL in DC beef for microbial reduction has not been reported, but a recent patent indicated that GDL could influence fresh DC beef color. Injection of DC steaks with 0.4% GDL and phosphate resulted in a lighter appearance compared to the DC control (Dolezal et al., 2013). Redness was increased for the GDL-enhanced DC steaks (Dolezal et. al., 2013). However, cubed DC meat soaked with 0.5% GDL without phosphate were shown to have a decrease in a* values over time (Dolezal et al., 2013). Glucono delta-lactone has an impact on the fresh color of DC steaks, but limited research has been conducted on the impact on microbial growth and cooked color of DC meat.

Antioxidants

Natural extracts and powders are blossoming as the new source for antioxidants

for meat processors (Ali et al., 2018). Antioxidants are compounds that extend shelf life through prevention oxidation of lipids and proteins by limiting rancidity and color changes in meat products (Karre et al., 2013). The products of lipid oxidation can accelerate the oxidation of oxymyoglobin forming metmyoglobin (Chan et al., 1997). Therefore, limiting the oxidation of lipids can decrease the oxidation myoglobin and increase color stability. A variety of natural antioxidants have been determined to be effective in controlling lipid oxidation and color stability in ground products. Red grape extract slowed discoloration of ground lamb patties in retail display (Andrés et al., 2017). Epazote extract in raw ground pork patties decreased discoloration and increased redness compared to untreated patties (Villalobos-Delgado et al., 2017). Gaillac red wine powder, prune flesh extract, and grape seed extract increased redness of ground beef patties compared to control patties after 12 d of storage (Bouarab-Chibane et al., 2017). Green tea leaves, pomegranate peel extract, grape seed extract, Gaillac red wine powder, epazote extract, and red grape extract limited lipid oxidation in ground meat products throughout storage (Andrés et al., 2017; Bouarab-Chibane et al., 2017; Villalobos-Delgado et al., 2017). Natural antioxidants have been effective in improving meat quality, and rosemary has been shown to be very effective and has popularity in the industry.

Rosemary has been shown to be a very effective natural antioxidant in ground and whole muscle beef, including DC beef. Rosemary enhancement in ground beef patties resulted in the highest numerical L* values throughout display with the L* values after 3 d of retail display significantly greater than L* values of the patties without rosemary (Balentine et al., 2006). Rosemary enhancement in ground beef increased the a* values compared to untreated ground beef (Sánchez-Escalante et al., 2003; Balentine et al.,

2006). Evaluation by a trained sensory panel indicated lower discoloration in ground beef treated with rosemary (Sánchez-Escalante et al., 2003). Rosemary has been recognized to decrease lipid oxidation of ground beef patties (Sánchez-Escalante et al., 2003; Balentine et al., 2006). Previous research has indicated the effectiveness of rosemary and vitamin C spray on beef steaks to stabilize color and limit lipid oxidation in various light and dark storage (Djenane et al., 2002, 2003). Wills et al. (2017) determined 0.1% and 0.2% rosemary-injected DC steaks in PVC increased redness and lightness compared to unenhanced DC steaks in PVC. Rosemary-dipped DC steaks packaged in nitrite packaging had larger numerical a* values than DC steaks packaged in PVC and nitrite (Ramanathan et al., 2018). Rosemary-enhanced DC steaks had less discoloration than normal-pH steaks after retail display in PVC (Wills et al., 2017). However, rosemary at 0.2% in DC steaks resulted in more discolored than nonenhanced DC steaks in PVC (Wills et al., 2017). Lipid oxidation was reduced by rosemary-enhancement of DC steaks aged 14 and 21 d compared to nonenhanced DC steaks (Wills et al., 2017). Rosemary enhancement can improve the color of beef products, including DC beef, while inhibiting lipid oxidation.

Rosemary has had inconclusive impacts on microbial growth in beef products. *Escherichia coli* inoculated ground beef patties containing rosemary did not have significantly different microbial growth compared to inoculated control ground beef patties in 6 d of display (Balentine et al., 2006). However, in uninoculated ground beef patties, lower numerical counts of bacteria were reported in rosemary-treated patties when stored in the dark for 24 d (Sánchez-Escalante et al., 2003). To impact microbial growth in beef products, other means may be necessary beyond rosemary enhancement.

Nitrite-embedded Packaging

In order to meet consumer demands for cherry-red color in retail meat, a variety of methodologies and technologies have been implemented in the industry. One such emerging technology is the Freshcase® nitrite film packaging or nitrite-embedded packaging (NEP). The packaging creates an anaerobic environment where the sealant layer of the package is embedded with nitrite, allowing the nitrite to interact with the meat surface (Siegel, 2011). The anaerobic condition of the packaging causes limited oxygen available to bind to myoglobin (Siegel, 2011), and due to low oxygen partial pressure, there is an increase in the formation of metmyoglobin (Mancini and Hunt, 2005). The innate reducing ability of meat reduces nitrite to nitric oxide which binds to metmyoglobin leading to nitric oxide metmyoglobin. The nitric oxide metmyoglobin is reduced to nitric oxide myoglobin (Fox Jr. and Ackerman, 1968), appearing as bright-red on the surface of meat (Siegel, 2011; Claus and Du, 2013). Freshcase® packaging contains 113 mg of sodium nitrite per m^3 with approximately less than 2 ppm of nitrite going into beef products with residual nitrite remaining undetectable (Siegel, 2011). However, residual nitrite has been a concern on the surface of fresh steaks due to research finding a greater average concentration of nitrite on the surface of fresh steaks, but considering the total steak weight, the residual nitrite concentration would not be a concern (Claus and Du, 2013). A similarly high residual surface nitrate concern was noted on cooked steaks (Claus and Du, 2013). Overall, NEP provides a route to impact fresh meat color.

The NEP has been used in numerous studies to improve color of retail cuts. In bison steaks, NEP has been shown to reduce discoloration (Roberts et al., 2017) and

increase color stability (Narváez-Bravo et al., 2017). Fresh beef color stability was extended in NEP, and there was an increase in a* values indicating a bright-red appearance (Yang et al., 2016), which is not typically associated with anaerobic package conditions (Siegel, 2011). However, lightness was not improved through NEP of beef steaks and ground beef patties in 25 d of dark storage (Yang et al., 2016). Similar improvements in redness were observed in previous research in fresh beef steaks in retail display as well as improvements in appearance of color labile muscles packaged in NEP (Claus and Du, 2013). Nitrite-embedded packaged DC beef in retail display saw an increase in a* values compared to PVC packaged DC and normal-pH beef, but L* values were not significantly changed in the DC steaks in NEP (Ramanathan et al., 2018). The appearance of red color for the steaks did take variable periods of time. In fresh normal-pH beef, the NEP increased red appearance in 5 d (Yang et al., 2016); while, Claus and Do (2013) saw a redder appearance after a combination of 4 d in dark storage and display. Previous research on DC steaks determined nitric oxide myoglobin was formed in 24 h due to the greater pH of the meat leading to a greater reducing capacity for metmyoglobin (Ramanathan et al., 2018). When repackaging NEP steaks into PVC, a decline in redness was noted for both 2-d aged and 9-d aged normal-pH beef steaks after 24 h (Claus and Du, 2013). Overall, NEP can improve color in retail display, which is key to improving the color of beef for consumer acceptability.

Nitrite-embedded film has low impacts on microbial growth. Past research determined NEP did not affect the aerobic psychotropic bacteria and lactic acid bacteria growth in pork and beef when compared to vacuum packaging (Smith et al., 2016; Yang et al., 2016). Previous research on bacterial growth of bison steaks resulted in no

significant differences in lactic acid bacteria and psychrophilic bacteria growth between NEP and vacuum packaging, but PVC-packaged bison steaks had more growth than bison steaks packaged in NEP (Narváez-Bravo et al., 2017). Based on these findings, Narváez-Bravo et al. (2017) commented that microbial reduction in NEP was due to the anaerobic conditions leading naturally to 1-log reduction. Similar conclusions were drawn in research by Ramanathan et al. (2018) where DC steaks packaged in NEP had lower microbial growth than PVC-packaged DC steaks. The low microbial growth reduction in NEP indicates further antimicrobial enhancement could be key to reducing microbial concerns of DC beef.

Conclusion

The 2016 National Beef Quality Audit determined 1.9% of carcasses are considered DC beef (Boykin et al., 2017), but consumers are less willing to purchase it due to its darker appearance. Therefore, DC beef can lead to losses for the beef industry. The myoglobin in DC beef is more thermally stable due to the greater pH (Hunt et al., 1999). This stability leads to a more internal red cooked color, which negatively impacts consumer perceptions. Acidification of DC beef has been shown to improve cooked color but can lead to issues with fresh color which can be impacted through antioxidant enhancement and NEP. However, limited research has been done on the effect of NEP in combination with acidification on fresh and cooked color. Beyond the appearance, DC beef is concerning from a microbial and sensory stand point due to its increased rate of spoilage and variable sensory characteristics. Research on the effects of GDL enhancement of DC beef on meat color and sensory characteristics is limited to our knowledge.

Hypothesis and objective

We hypothesize the addition of rosemary and GDL will improve the retail and cooked color and reduce microbial growth of DC steaks in NEP. The objective of this research was to determine the effects of acidification of DC beef with GDL and rosemary on sensory, retail color, cooked color, and microbial in combination with NEP.

CHAPTER III

EXTENDED DARK STORAGE OF ENHANCED DARK-CUTTING BEEF IN NITRITE-EMBEDDED PACKAGING DECREASED SURFACE REDNESS UPON REPACKAGING

Abstract

This study evaluated the effects of novel nitrite-embedded packaging (NEP) and enhancement on the color of dark-cutting beef in dark storage and after repackaging into polyvinyl chloride (PVC) for display. Dark-cutting beef strip loins (n = 8; pH = 6.39) and USDA Choice beef strip loins (normal-pH, n = 6) were selected at a commercial packing plant. Dark-cutting loins were bisected and randomly assigned to nonenhanced dark-cutting (DCN) and enhanced dark-cutting (DCE) treatments with 10% injection of the green weight. The final concentration was 0.5% glucono delta-lactone and 0.1% rosemary in the loin. Steaks (1.91 cm) were removed from nonenhanced normal-pH, DCN, and DCE loins with steaks randomly assigned to 3, 6, or 9 d in dark storage. Normal-pH and DCN steaks were vacuum packaged while DCE steaks were packed in nitrite-embedded packaging (NEP). All steaks were stored in dark storage for 3, 6, or 9 d then repackaged in PVC and displayed for 6 d. On d 6 of display, microbial growth was evaluated for all steaks. During dark storage, instrumental color was evaluated every

24 h, and upon repackaging, instrumental and visual color (n = 6) was evaluated every 12 h. The DCE steaks had an increase in a* value in 24 h of dark storage, paralleling with an increase ($P < 0.05$) in nitric oxide myoglobin. Enhanced dark-cutting steaks packaged in NEP had significantly greater a* and L* values than DCN steaks during dark storage. Upon repackaging DCE steaks, nitric oxide myoglobin decreased ($P < 0.05$) during the first 12 h of display. The loss of nitric oxide myoglobin corresponds with a darker red appearance, increased surface discoloration and decreased a* values. Storage time had limited impact on the stability of the color of DCE steaks in display. Normal-pH steaks had brighter red color ($P < 0.05$, greater a*) than DCE steaks on d 6 for steaks packaged 3-d and 6-d. The enhancement did not decrease ($P > 0.05$) microbial growth of DC steaks compared to DCN steaks. In conclusion, repackaging DCE steaks decreased color stability and redness of steaks within 12 h of display.

Introduction

Consumers prefer to purchase meat products with a bright cherry-red color due to their interpretation of the red appearance as an indication of freshness (Carpenter et al., 2001). Dark-cutting (DC) beef deviates from this bright cherry-red color appearing as a dark red color due to an increased postmortem pH (Lawrie, 1958; Scanga et al., 1998). Although the mechanism for dark-cutting beef is not fully understood, stress prior to harvest leads to lower glycogen content in the muscle reducing lactic acid formation through glycolysis resulting in a greater postmortem pH (Lawrie, 1958; Scanga et al., 1998). The resulting pH changes the characteristics of the beef, with dark cutters typically having more mitochondrial oxygen consumption and more muscle swelling (Ashmore et al., 1972; Hughes et al., 2017). A darker appearance occurs due to expansion

of muscle fibers which increases water holding capacity and decreases light scattering and reflectance (Hughes et al., 2017). Mitochondrial oxygen consumption and muscle swelling decreases the binding of oxygen to myoglobin and decreases the bloom and increases deoxymyoglobin (Hughes et al., 2017; English et al., 2016). Due to the high pH and low glycogen content, DC beef also has a faster rate of microbial growth and spoilage than normal-pH beef (Gill and Newton, 1979).

Characteristics of DC beef have been improved through enhancement and packaging. High oxygen and carbon monoxide modified atmospheric packaging improved fresh color of DC (Wills et al., 2017); although, lipid oxidation increased in high-oxygen packaging systems (Cornforth and Hunt, 2008). Injection of DC beef with lactic acid improved the lightness of fresh steaks (Apple et al., 2011), but lactic acid enhancement has increased the surface discoloration (Sawyer et al., 2009; Apple et al., 2011). Nitrite-embedded packaging (NEP) has shown promise in improving the redness of DC beef, anaerobically (Ramanathan et al., 2018). Nitric oxide is formed by the reduction of nitrite in the film, and the nitric oxide binds to the metmyoglobin formed by the low oxygen partial pressure (6-7 mmHg) (Ledward, 1970; Brantley et al., 1993) and oxidizing activity of nitrite. Reduction of nitric oxide metmyoglobin by the inherent reducing ability of meat forms nitric oxide myoglobin (Siegel, 2011; Fox Jr. and Ackerman, 1968). The formation of red color from nitric oxide myoglobin occurred within 24 h for DC beef (Ramanathan et al., 2018) because DC beef has greater reducing ability due to greater pH shortening the time to achieve desired color (English et al., 2016; McKeith et al., 2016). Removing normal-pH steaks from the NEP into polyvinyl

chloride (PVC) decreased a* values in a 24 h period (Claus and Du, 2013). Past research has not evaluated color stability of repackaged enhanced DC beef for a 6-d display.

Although, NEP has had limited impacts on microbial growth. Ramanathan et al. (2018) reported 1-log reduction in DC steaks pacakged in NEP, which was attributed to the anaerobic conditions. Acidification of DC beef with nitrite-embedded packaging has not been reported. However, a patent has indicated that glucono delta-lactone (GDL) can improve the fresh color DC beef (Dolezal et al., 2013). The objective of this study is to evaluate combination of GDL and NEP on fresh color of DC beef during extended periods of dark storage. After dark storage, an evaluation of the color stability of repackaged steaks in PVC will occur for a 6-d retail display.

Materials and Methods

Raw materials and processing

Eight DC strip loins (pH = 6.21-6.77) and six USDA Low-Choice strip loins (normal-pH: pH = 5.53-5.59less than 7-d aged were collected from a local purveyor, Creekstone Farms (Arkansas City, KS). Strip loins were transported on ice to the Robert M. Kerr Food and Agricultural Products Center at Oklahoma State University campus in Stillwater. Upon arrival, normal-pH and DC loins were bisected, vacuum packaged (Walton's Vacuum Pouch; 12 × 22 pouches; 3 mil thickness; 1.3 – 1.6 oxygen transmission rate $cm^3/100\ in^2$) using Multivac C5000 vacuum packager and stored in the dark at 2°C until use. Each half of dark-cutting strip loins was randomly assigned to enhancement with GDL and rosemary or nonenhanced. Loins were injected to 110% of the green weight with a combination of rosemary (Herbalox oleoresin rosemary; Kalsec) and GDL (Glucono Delta-Lactone; PMP Fermentation Products, Inc) resulting in a final

concentration of 0.1% and 0.5%, respectively. Loins were allowed to equilibrated for 2 h after enhancement. Enhanced dark-cutting (DCE) loins, nonenhanced dark-cutting (DCN) loins, and USDA Choice loins were sliced into 1.91 cm thick steaks from the anterior end using a meat slicer (Bizerba USA INC., Piscataway, NJ).

pH and proximate composition analysis

Initial pH on normal and DCN strip loins was measured in three random locations using Hanna Instruments pH probe (Handheld HI 99163; probe FC232; Hanna Instruments). Post-enhancement and equilibration, pH was measured for DCE strip loins at three locations. On d 6 of display, steaks were removed from the display, and pH was measured in triplicate for each steak. Percent protein, fat, and moisture were determined of each strip loin using two steaks from the posterior. Steaks were ground using a table top grinder (Big Bite Grinder, 4.5 mm, fine grind, LEM) and pressed into 140-mm sample cup. The samples were analyzed using AOAC-approved near-infrared spectrophotometer (FoodScan Lab Analyzer, Serial No. 91753206; Foss, NIRsystem Inc.; Slangerupgrade, Denmark).

Packaging and simulated retail display

Steaks of the DCE, DCN, and normal-pH loins were randomly selected to be stored for 3, 6 or 9 d in dark storage. Enhanced DC steaks were packaged in NEP (FreshCase; Curlon Grade A5106 Protective Packaging Film; approximately 115 mg/m^2 nitrite, 6 × 12 pouches; 7 mil thickness; <0.15 oxygen transmission rate cm^3/100 in^2/24 h @ 73°F, 0% FH, 1 atm; <0.5 water vapor transmission rate g/100 in^2/24 h @ 100°F, 90% RH, 1 atm; Bemis Innovation Center in Neenah, WI), and DCN and normal-pH steaks were vacuum packaged (FlairPak 500 Vacuum Pouch; 10 × 14 pouches; 4.7 mil

thickness; 1.3 – 1.6 oxygen transmission rate $cm^3/100\ in^2$) using a Multivac C500 vacuum packager. Nonenhanced DC steaks were packaged in vacuum packaging to serve as a control. Steaks were held in dark storage at 2 ± 1°C for the selected storage period. After 3, 6, or 9 d in dark storage, steaks were removed from the anaerobic packaging systems and placed in Styrofoam trays and overwrapped with PVC (15,500-16,275 cm^3 $O_2/m^2/24$ h at 23°C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film; Koch Supplies; Kansas City, MO) using a film wrap machine (Winholt WHSS-1, 115V; Woodbury, NY). Packaged steaks were placed in a white coffin-style display case and stored under continuous LED lighting (Philips LED lamps; 12 watts, 48 inches, color temperature = 3,500°K; Phillips, China) at 2 ± 1°C for 6 d.

Color analysis

During dark storage, instrumental color of steaks was measured every 24 h using a HunterLab 4500L MiniScan EZ Spectrophotometer (2.5-cm aperture, illuminant A, and 10° standard observer angle; HunterLab Associates, Reston, VA). The surface of each steak was read twice providing the CIE L*, a* and b* values. The dark storage days 0-3 were evaluated to determine the shift to nitric oxide myoglobin in NEP and deoxymyoglobin in vacuum packaging. Derived variables were determined from the dark storage period. Initial (d 0) L*, a*, and b* values for respective storage days 3, 6 and 9 were subtracted from final values on d 3, 6, 9 to represent the change in color during dark storage. Upon repackaging, steaks bloomed for thirty minutes prior to color evaluation. Instrumental color utilized the HunterLab 4500L MiniScan EZ Spectrophotometer and was read in duplicate every 12 hours for 6 d. The CIE L*, a* and b* values and spectral

readings from 400 to 700 nm determined the surface color. Chroma $\left[\sqrt{(a^{*^2} + b^{*^2}})\right]$ was determined using CIE a* and b* values, representing the red intensity of the color (AMSA, 2012). The nitric oxide myoglobin formation was determined for DCE steaks and indicated by the ratio of reflectance at 650 and 570 nm with a greater number indicating a greater amount of nitric oxide myoglobin (AMSA, 2012). The CIE a* and b* were used to determine the hue angle $(\tan^{-1}(\frac{b*}{a*}))$, representing the color present (Aberle et al., 2012).

Visual color was evaluated by a trained panel (n = 6) for all repackaged steaks during 6-d display period. All panelists passed the Farnsworth Munsell 100-hue test. Panelists determined muscle color (MC) using a 7-point scale (1 = Extremely bright cherry-red, 7 = Dark red) and surface discoloration (SD) using a 7-point scale (1 = No discoloration (0%)), 7 = Extensive discoloration (81-100%)) for 6 d every 12 h.

Microbiology

On d 6 of display, total plate count of normal-pH, DCE, and DCN steaks was determined. The surface of steaks was swabbed using a sterile 2.54 × 2.54 cm^2 grid and an environmental swab (Puritan® Environmental Sampling Kit HP007-BPW Puritan Medical Products Co LLC, Guildford Maine). One mL from the swab container was serially diluted into 9 mL of 0.1% sterile peptone water (Bacto™ Peptone Ref 211677 Becton; Dickinson and Company,;Sparks, MD). On 3M Petriflim Rapid Aerobic Count plates (3M Health Care; St. Paul, MN), one mL from each dilution was aseptically plated in duplicate. Plates were incubated at 37°C for 48 h in a VWR Forced Air General Incubator (5.4 ft^3; VWR, Radnor, PA). After 48 h, plates were removed and counted to

determine the total plate count per cm^2 using an Interscience Scan 100 pressure sensitive pad (Interscience; Woburn, MA).

Statistical analysis

The experimental design was a split-split plot. In the whole plot each DC loin section was randomly assigned to enhanced DC (DCE; n = 8) and non-enhanced DC (DCN; n = 8). In the sub-plot (split-factor), DCE and DCN sections were cut into steaks and assigned to 3, 6, or 9-d in dark storage. In the sub-sub plot, steaks assigned to 3, 6, or 9-d of dark storage is repackaged in PVC. The fixed effects include enhancement treatment, storage time in dark storage, display time in PVC and their interactions. Loin was a random effect, and hour was a repeated measure. The covariance-variance structure for the repeated measures was determined by evaluating the AIC output, and the compound symmetry structure was used based on the AIC value.

Derived variables were calculated for the instrumental color parameters including L*, a*, b*, hue, chroma, and nitric oxide myoglobin content. To determine derived variables, dark storage day 0 values for all parameters were subtracted from the final day (3, 6, 9 d) dark storage values for all parameters. The loin was a random effect for all parameters, and the fixed effects were enhancement, storage time, and storage time × enhancement for L*, a*, b*, hue and chroma. The fixed effects for nitric oxide myoglobin content was storage time because nitric oxide myoglobin was only evaluated for DCE steaks.

Least squares means were determined using the MIXED procedure of SAS (SAS 9.4; SAS Inst.; Cary, NC) and considered significant at $P < 0.05$. Using the PDIFF options, least squares means were separated and significant at $P < 0.05$.

Results and Discussion

Proximate composition and pH

Dark-cutting loins had a greater pH ($P < 0.05$) than normal-pH loins (Table 3.1) in agreement with previous findings from Sawyer et al. (2009) and Mitacek et al. (2018). There was no difference ($P > 0.05$) in moisture and fat content between the normal-pH and DCN beef. Stackhouse et al. (2016) noted similar levels of moisture between normal-pH beef and DCN beef. Once enhanced, pH of the DCE loins decreased significantly (Table 3.2), and after six days of display, the pH of normal-pH steaks and DCE steaks were similar (Table 3.3, $P > 0.05$). Lactic acid enhancement of DC loins has been shown to decrease the pH (Sawyer et al., 2009). Sawyer et al. (2009) determined lactic acid enhancement decreased the pH of DC beef below the pH of normal-pH loins. In combination with salt, lactic acid enhanced DC loins at 0.5% and 1.0% had similar pH to normal-pH loins (Sawyer et al., 2008). Glucono delta-lactone has been observed to reduce pH of various meat products (Acton and Dick, 1977; Dolezal et al., 2013; Chun et al., 2014) because of the formation of gluconic acid in the presence of water (Combes and Birch, 1988; Parke et al., 1997).

Dark storage in nitrite packaging

L values*

There was a significant dark storage day × enhancement on the L* values, a* values, hue and chroma (Figure 3.2-3.3). The normal-pH steaks were lighter ($P < 0.05$, greater L*) compared to DCE steaks and remained stable in the first 3 d of dark storage. The L* values of DCN steaks did not change and remained lower than normal-pH and DCE steaks throughout the first three days of storage. There was a significant

enhancement effect on the derived L* values (Table 3.5). The derived variables were determined by the change in the parameter from the initial to the final day in dark storage (ΔL* value = [L* value d 3, d 6, or d 9] – [L* value d 0]). With an enhancement effect, a positive number would indicate an increase in the parameter throughout all storage periods since storage time main effect did not significantly impact color; while a negative number indicates a decrease in the parameter and zero means no change during storage time. All enhancements had increased L* values throughout dark storage; however, the normal-pH and DCN steaks had smaller positive changes. Thus, there was a limited increase in lightness from d 0 to the last d of storage for both. Dark-cutting beef has been shown to have more myoglobin than normal-pH (McKeith et al., 2016; Hughes et al., 2017), and upon blooming, deoxymyoglobin formation is greater (English et al., 2016; McKeith et al., 2016;Hughes et al., 2017). The appearance is darker in DC beef compared to normal-pH beef (English et al., 2016; McKeith et al., 2016; Stackhouse et al., 2016; Hughes et al., 2017; Wills et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018). In an anaerobic system, as presented here, DCN and normal-pH beef would both be shifting to deoxymyoglobin. Zhang et al. (2018) noted in vacuum packaged systems DC beef has smaller L* values compared to normal-pH beef, and the L* values did not significantly change with storage time for DC steaks and normal-pH steaks stored in vacuum packaging. A similar low change in L* values of DCN steaks and normal-pH steaks was noted.

From d 0 to 1, lightness significantly increased in the DCE steaks and remained stable through 3 d of dark storage (Figure 3.2A). Additionally, the derived variable for the DCE steaks demonstrated a significantly greater change in L* value compared to the

normal-pH and DCN steaks. This demonstrates the lightness increased more throughout storage for the DCE steaks. Nitrite-embedded packaging did not impact the L* values of normal-pH steaks (Yang et al., 2016), DC steaks, (Ramanathan et al., 2018) and bison steaks (Roberts et al., 2017). This indicates the enhancement effect on the lightness of DCE steaks in NEP. Rosemary has had limited impacts on the L* values of DC beef packaged in NEP (Ramanathan et al., 2018); however, rosemary has been shown to increase lightness of aerobically packaged DC steaks (Wills et al., 2017). Wills et al. (2017) attributed the impact of rosemary on initial L* values on the influence of free water on meat color. Additionally, free water by enhancement has been show to increase L* values of normal-pH beef (Ramanathan et al., 2010). Enhancement of DC beef with weak organic acids has been shown to improve lightness of DC beef (Sawyer et al., 2009; Apple et al., 2011; Stackhouse et al., 2016; Tapp et al., 2017). Glucono delta-lactone increased the L* values of DC beef at 0.5% in a patent by Dolezal et al. (2013). Therefore, the shift in pH by the acidification of DC beef by GDL and increased free water by enhancement improved the L* values. In support, Hughes et al. (2017) determined pH change in DC beef was an acceptable avenue to influence lightness by affecting the scattering elements in meat.

a* values and chroma

Within 24 h, DCE steaks significantly increased in a* values (Figure 3.2B). Although normal-pH steaks were the reddest initially, DCE steaks had greater ($P < 0.05$) a* values from d 1-3. Also, the DCE steaks had an increase in redness from d 0 to the last day of storage as shown by the positive derived variable for a* values. Normal-pH steaks had significantly lower a* values from d 1-3 of dark storage compared to the initial a*

values of the normal-pH steaks. The derived change in a* values was negative for normal-pH steaks indicating the redness decreased with storage time. Nonenhanced DC steaks had minimal changes in redness throughout the first 3 d of dark storage and had a derived a* value close to zero. Additionally, DCN steaks had no change ($P < 0.05$) in chroma in the first 3 d of dark storage (Figure 3.3A) and a derived chroma value of -0.24. Therefore, the redness and red intensity did not change during dark storage for DCN steaks. However, DCE steaks had significantly greater red intensity than other enhancements after d 0 of dark storage, and the derived chroma value demonstrates DCE steaks had an increase ($P < 0.05$) in red intensity throughout dark storage compared to normal-pH and DCN steaks. Normal-pH steaks had the most ($P < 0.05$) red intensity on d 0 of dark storage, but the intensity decreased ($P < 0.05$) by dark storage d 1, and the negative derived chroma value shows the normal-pH steaks decreased in red intensity during the dark storage.

Enhanced DC steaks in NEP shifted from metmyoglobin to nitric oxide myoglobin in the first day of dark storage as indicated by the increase in a* values and chroma and remained in the form of nitric oxide myoglobin during dark storage as shown by the derived variables. This parallels with previous results from Ramanathan et al. (2018) where DC steaks in NEP had greater a* values and chroma than DC steaks in PVC by d 1 of retail display, and the NEP DC steaks had increased color stability. The rosemary addition has been shown to increase stability of DC steaks in NEP by reducing nitric oxide myoglobin oxidation and increasing nitrite diffusion by water (Ramanathan et al., 2018). Dolezal et al. (2013) saw a rapid decrease in a* values by the addition of GDL to DC beef. In the transition to nitric oxide myoglobin, metmyoglobin is formed in

NEP (Fox Jr. and Ackerman, 1968). The combination of metmyoglobin and enhancement using GDL could explain the decreased redness at d 0 in the current study. By the final day of dark storage, the myoglobin of DCE steaks was in the form of nitric oxide myoglobin, which was indicated by the positive values for derived a* and chroma.

Normal-pH steaks have been shown to have larger a* values and chroma compared to nonenhanced DC steaks in display settings and aerobic packaging (Apple et al., 2011; Stackhouse et al., 2016; Wills et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018). Similar results were reported here in anaerobic packaging and dark storage. Normal-pH steaks had a decrease in chroma and a* values from d 0 to 1. The decrease in red appearance can be explained by the formation of deoxymyoglobin. Brewer et al. (1994) and Zhang et al. (2018) determined normal-pH steaks had a decrease in a* values when stored in vacuum packaging systems. In support, deoxymyoglobin in ground beef patties was noted to have lower a* values compared to oxymyoglobin in ground beef patties (Hunt et al., 1999), and high-oxygen modified atmospheric packaging displayed greater a* values in comparison to vacuum packaging for normal-pH steaks (John et al., 2005; Lagerstedt et al., 2011). Previous research has indicated nonenhanced DC steaks has more myoglobin content and deoxymyoglobin compared to normal-pH steaks (McKeith et al., 2016; Hughes et al., 2017). Therefore, in anaerobic environments, the amount of pigment shifting from oxymyoglobin to deoxymyoglobin is not as great for nonenhanced DC steaks, limiting the change in redness throughout storage. Also, Zhang et al. (2018) noted no change in a* values of DC beef in vacuum packaging as seen in the current study. Therefore, the myoglobin state influenced the a* values and redness of the present study.

Hue angle

The hue angle indicates the true red axis; a greater number indicates a change from red to yellow axis and increased discoloration. Nonenhanced DC steaks did not change ($P > 0.05$) in hue throughout the 3 d of dark storage (Figure 3.3B), and there was no change ($P > 0.05$) in derived hue during storage with a number close to zero. Normal-pH steaks decreased ($P < 0.05$) in hue from d 0 to d 1 after remaining constant ($P > 0.05$) from dark storage d 1 to 3. This aligns with the shift to deoxymyoglobin from oxymyoglobin for normal-pH steaks. Normal-pH steaks had greater ($P < 0.05$) hue than DCN steaks for the 3 d of dark storage. Research has established DC steaks have lower hue values compared to normal-pH. Thus, normal-pH steaks are more yellow and further from the true red axis, initially (Sawyer et al., 2009; Apple et al., 2011; McKeith et al., 2016; Stackhouse et al., 2016). The DCE steaks paralleled in hue changes with the normal-pH steaks: the highest hue at d 0 and significantly decreasing by d 1of dark storage. By d 2, normal-pH steaks had similar ($P > 0.05$) hue to DCE steaks. There was no difference ($P > 0.05$) in derived hue during dark storage between normal-pH and DCE steaks with both being a negative number. Therefore, the hue decreased during storage for DCE and normal-pH steaks, and the color shifted closer to the true red axis. The shift in hue occurs in the first day of dark storage and aligns with the shift of the DCE steaks to nitric oxide myoglobin. Additionally, researchers have shown rosemary decreased hue values of ground beef patties and extends color shelf life (Sánchez-Escalante et al., 2003), and acidification of DC steaks with lactic acid increased hue angles to be similar to normal-pH steaks (Apple et al., 2011). Therefore, the combination of enhancement and nitric oxide formation resulted in decrease hue angles in the DCE steaks.

Nitric oxide myoglobin

There was a dark storage day effect ($P < 0.05$) on the nitric oxide myoglobin formed for DCE steaks (Table 3.4). By d 1 of dark storage, there was an increase ($P < 0.05$) in the ratio of R650 ÷ R570 nm, indicating an increase in nitric oxide myoglobin. Ramanathan et al. (2018) reported an increase in nitric oxide myoglobin formation in DC steaks during retail display in 24 h. This parallels with the nitric oxide myoglobin increase seen in this study. Nitric oxide myoglobin is formed through the reduction of nitric oxide metmyoglobin (Fox Jr. and Ackerman, 1968), and meat has an inherent ability to reduce metmyoglobin. Dark-cutting beef has more metmyoglobin reducing activity compared to normal-pH (English et al., 2016; McKeith et al., 2016). The more activity allows for faster reduction and quicker nitric oxide myoglobin formation as presented. In the first 3 d of dark storage, the amount of nitric oxide myoglobin formation increased.

The derived ratio of R650 ÷ R570 nm was not impacted ($P > 0.05$, data not included) by the time spent in dark storage. All of the derived variables were positive; a positive number indicates the nitric oxide myoglobin was greater on the last day of dark storage compared to d 0; however, since the change from d 0 and the respected dark storage times were not significantly different, the amount of nitric oxide formed was not impacted by dark storage time. This parallels with the lack of significance of the main effect of storage time on the a* values and chroma. Therefore, increasing the time in the NEP did not lead to a change in amount nitric oxide myoglobin formed as seen in the first three days of dark storage. From the Freshcase® specifications, the level of nitrite going into beef is less than 2 ppm (Siegel, 2011). Claus and Du (2013) determined residual

nitrite concentration of normal-pH beef steaks ranged from 1.44 - 2.14 ppm after 19 d of display. Their results aligned with the Freshcase® specifications. The lack of change in nitric oxide myoglobin content through dark storage time in the current study matches the expected level of penetration of the nitrite in beef from Claus and Du (2013) and Siegel (2011).

Retail display color

There was dark storage time in nitrite packaging × retail display hour in PVC and enhancement × hour of retail display in PVC interactions for the L* values. When evaluating the dark storage time effect on retail display of all steaks, display hour did not impact ($P > 0.05$) the steaks stored in dark storage for 3 d or 6 d (Table 3.6). As display time increased, the 9-d stored steaks significantly decreased in L* values by h 132 indicating a dark appearance for all steaks. However, 9-d stored steaks were not darker than 3-d or 6-d steaks at h 144 ($P > 0.05$). When comparing the enhancements during retail display, the DCN steaks had no change ($P > 0.05$) and resulted in a darker appearance compared to the DCE and normal-pH steaks (Table 3.7). Normal-pH steaks had greater ($P < 0.05$) L* values than DCE and DCN steaks throughout display; however, L* values of normal-pH steaks did not significantly change during the display period. Enhanced DC steaks were lighter ($P < 0.05$) than the DCN steaks at all times except h 72 of display and had lower ($P < 0.05$) L* values than normal-pH steaks for the entire display period.

There was a significant dark storage time in nitrite-embedded packaging × enhancement × hour of retail display in PVC effect on a* values, chroma and hue (Table 3.8 and Table 3.9). Nonenhanced DC steaks did not change ($P > 0.05$) in a* values for 3-

d dark storage time (Table 3.8); however, for 6-d and 9-d dark storage time, the a* values of DCN steaks decreased significantly by h 132 compared to h 0. Normal-pH steaks decreased numerically in a* values during retail display for all dark storage times. Although for 3-d and 6-d dark storage, the a* values at h 144 were not significantly different from a* values at h 0. Enhanced DC steaks had a significant decrease in a* value from h 0 to h 12. After, the a* values numerically continued to decline for 3-d and 9-d dark storage times, but there were no significant differences from h 12. After 12 h, the DCE steaks had lower ($P < 0.05$) a* values compared to normal-pH steak for all dark storage times. Steaks from DCE and DCN were not different at 12 h. By the end of retail display, DCN steaks and DCE steaks had no difference ($P > 0.05$) in a* values for all dark storage times, and normal-pH steaks were redder ($P < 0.05$) than DCE and DCN steaks stored 3-d and 6-d. Normal-pH steaks decreased numerically in chroma during retail display; however, normal-pH steaks stored for 9-d showed a lower ($P < 0.05$) chroma value at h 144 than h 0. The chroma of DCN steaks stored for 3 d was not impacted ($P > 0.05$); however, dark storage of DCN steaks for 6 d and 9 d saw a significant decrease in chroma from h 0 to h 144 indicating less red intensity. At h 0, chroma of normal-pH and DCE steaks were not significantly different. Enhanced DC steaks decreased ($P < 0.05$) in chroma from h 0 to h 12 for all dark storage times; by h 12, DCE steaks decreased ($P < 0.05$) intensity compared to normal-pH steaks, but DCE steaks were comparable to DCN steaks for all dark storage times. From h 12 to h 144, there was no change ($P < 0.05$) in chroma for DCE steaks for all dark storage times. By h 144, DCE steaks had similar ($P < 0.05$) chroma to DCN steaks for stored 3-d, 6-d and 9-d. Normal-pH steaks had greater ($P < 0.05$) chroma than DCN and DCE steaks for 3-d

and 6-d dark storage at h 144. The more chroma supports the greater redness seen in the a* values. The hue of normal-pH steaks did not change ($P > 0.05$) for steaks stored 3-d and 6-d in dark storage. The dark storage for 9 d resulted in a significant increase in hue for normal-pH steaks during retail display. With an increase in hue, this indicates an increase in discoloration for normal-pH steaks. Nonenhanced DC steaks had no change ($P > 0.05$) in hue during display for all dark storage times. The hue of DCE steaks numerically increased as display time increased. For 3-d, 6-d, and 9-d dark storage time, a significant hue increase for DCE steaks was seen in 24 h, 12 h, and 144 h, respectively. Enhanced DC steaks were not significantly different from normal-pH steaks in hue throughout display for every dark storage time. Nonenhanced DC steaks had lower ($P < 0.05$) hue than DCE steaks at 144 h of display for all dark storage days. Overall, dark storage time had no impact on the DCE steaks color stability in retail display. However, normal-pH steaks had more stability with 3-d in dark storage compared to 9-d in dark storage. Nonenhanced DC steaks were not strongly impacted by dark storage time.

The ratio of R650 ÷ R570 nm was significantly impacted by the hour of retail display (Table 3.10). With the highest ratio at h 0, the highest amount of nitric oxide myoglobin was formed. From h 0 to h 12, there was a decline ($P < 0.05$) in the nitric oxide myoglobin. As a red pigment, the decline in nitric oxide myoglobin in DCE steaks aligned with a* and chroma results. Throughout the display period, the nitric oxide myoglobin continued to decline as shown with the decline in a* values for the DCE steaks. Nitric oxide myoglobin forms a bright-red color; therefore, a decrease in a* values and chroma supports a decline in the nitric oxide myoglobin content.

For muscle color and surface discoloration, there was a significant dark storage time in nitrite-embedded packaging × enhancement × hour of retail display in PVC interaction (Table 3.11). Dark storage time had minimal effects on the muscle color and surface discoloration for h 0 of display within each enhancement. Normal-pH steaks stored for 3-d and 6-d had similar changes in muscle color during the display period with the muscle color becoming darker red as display time increased. Increasing dark storage time to 9-d increased the rate of darkening during display for the normal-pH steaks compared to 3-d and 6-d of dark storage. However, normal-pH steaks remained significantly brighter red in appearance compared to DCE and DCN steaks for all dark storage times and during display. Nonenhanced DC steaks had the greatest ($P < 0.05$) muscle color score with a dark red appearance at h 0 of display for all dark storage times. Additionally, DCN steaks did not significantly change color as display h increased for all dark storage times; therefore, the dark storage time had no effect on the muscle color of DCN steaks. Enhanced DC steaks had brighter ($P < 0.05$) red color than DCN steaks at h 0 but decreased in redness within 12 h of display for all dark storage times with a significant decrease for steaks stored 3-d and 9-d. This parallels with the significant decrease in nitric oxide myoglobin, a* values, and chroma in the first 12 h of display. The muscle color of DCE steaks was equivalent ($P > 0.05$) to DCN steaks by 24 h of display for steaks stored 3-d and 6-d. Enhanced DC steaks stored for 9-d were not as dark ($P < 0.05$) as DCN steaks during display h; however, there was a significant increase in muscle color score from h 132 to 144 for DCE steaks stored 9-d. Although 9-d of dark storage for DCE steaks did not result in similar muscle color to DCN steaks during retail

display, the dark storage time overall did not impact the initial or change in muscle color over display time for DCE steaks.

Nonenhanced DC steaks, DCE steaks and normal-pH steaks had no discoloration ($P > 0.05$) at h 0 of display. Nonenhanced DC steaks stored for 3-d had no significant change in surface discoloration during retail display; however, for steaks stored 6-d and 9-d, DCN steaks discolored more rapidly. A decrease in color stability was determined with increased dark storage for DCN steaks and normal-pH steaks. Aligning with hue results, normal-pH steaks have similar levels of discoloration to DCN steaks for 3-d and 6-d dark storage times with no change ($P > 0.05$) during display, and at 9-d of dark storage, discoloration of normal-pH steaks increased significantly throughout the display period. This resulted in greater ($P < 0.05$) discoloration compared to DCN steaks and equivalent ($P > 0.05$) level of discoloration to DCE steaks at the end of display. Enhanced DC steaks increased ($P < 0.05$) in surface discoloration after 12 h of repackaging. The level of discoloration of DCE steaks was more ($P < 0.05$) than normal-pH and DCN steaks. For 3-d and 6-d of dark storage, the DCE steaks did not increase ($P > 0.05$) in surface discoloration after the 12 h of display. However, the surface discoloration of DCE steaks was greater than normal-pH and DCN steaks at the end of the display period for steaks stored 3-d and 6-d ($P < 0.05$). With an increased dark storage time to 9 d, the discoloration increased significantly with increased display h for DCE steaks, and by the end of the retail display, the DCE steaks had a similar ($P > 0.05$) level of discoloration to normal-pH steaks.

Use of NEP has resulted in greater a* values and increased color stability in normal-pH (Claus and Du, 2013; Yang et al., 2016) and DC steaks (Ramanathan et al.,

2018). This is due to the formation of nitric oxide myoglobin from the nitrite packaging (Siegel, 2011). However, limited studies have evaluated the change in color upon repackaging steaks in aerobic condition for 6 d of retail display. Claus and Du (2013) determined L* and a* values significantly decreased for repackaged normal-pH steaks in 6 h of retail display. A similar decline in a* values was seen in this study at 12 h. At a pH of 6.7 *in vitro,* nitric oxide myoglobin oxidized rapidly in light and aerobic conditions (Walsh and Rose, 1956). Degradation of nitric oxide myoglobin has been shown to increase at a greater pH value (Walsh and Rose, 1956; Munk et al., 2010). With the presence of nitrite, the oxidation at a pH below 6.3 was increased (Walsh and Rose, 1956); however, at a pH of 5.6, low concentrations (less than 100 ppm) of nitrite reduced the photooxidation rate (Walsh and Rose, 1956). Siegel (2011) has commented the nitrite in beef is less than 2 ppm using NEP while Claus and Du (2013) determined the residual nitrite in the NEP packaged beef to be 1.44 ppm in the *longissimus* of normal-pH steaks. Therefore, the low concentration of nitrite may have helped to reduce the degradation of nitric oxide upon air and light exposure. Research has determined metmyoglobin is formed upon the degradation of nitric oxide myoglobin (Andersen and Skibsted, 1992; Munk et al., 2010). In support, Claus and Du (2013) noted an increase in metmyoglobin with an increased display time after repackaging steaks into PVC. Munk et al. (2010) determined upon degradation of nitric oxide myoglobin there was a formation of a radical causing the oxidation and formation of metmyoglobin. While in the present study, there was an increase in surface discoloration and decrease in redness within the first 12 h. After the 12 h mark, there were limited changes. This may be due to the presence of the antioxidant rosemary. Rosemary can bind to free radicals and thereby, slow the oxidation

of the pigment. Rosemary has been shown to increase the color stability of DC steaks in NEP previously (Ramanathan et al., 2018).

It is important to note some of the negative implications of acidification of DC beef on fresh meat color, which could have also contributed to the increased surface discoloration and decreased red color presented in this study. Use of weak organic acids has improved L* values of DC steaks (Sawyer et al., 2009; Apple et al., 2011; Stackhouse et al., 2016; Tapp et al., 2017). Specifically, the use of GDL has increased L* of DC strip loins (Dolezal et al., 2013) and was seen in the present study during display. Acidification also resulted in improved a* values during retail display of DC steaks (Sawyer et al., 2009; Apple et al., 2011; Stackhouse et al., 2016; Tapp et al., 2017). Lactic acid has also been shown to numerically improve red intensity (Sawyer et al., 2009; Apple et al., 2011); however, at greater concentrations (0.5%-1.00%) lactic acid had detrimental effects on the red appearance of DCE steaks (Sawyer et al., 2009; Apple et al., 2011). Additionally, lactic acid has been shown to increase surface discoloration and hue angle of DCE steaks (Sawyer et al., 2009; Apple et al., 2011), and GDL enhancement has decreased a* values of DC beef (Dolezal et al., 2013). The addition of GDL could explain some of the discoloration and loss of red appearance in decrease in a* values and chroma present in this study.

Research has indicated normal-pH steaks have increased a* values (Sawyer et al., 2009; Apple et al., 2011; McKeith et al., 2016; Wills et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018), greater red intensity (Apple et al., 2011; McKeith et al., 2016; Wills et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018), brighter red visual color (Sawyer et al., 2009; Apple et al., 2011; Wills et al., 2017), increased L* values

(Sawyer et al., 2009; Apple et al., 2011; McKeith et al., 2016; Wills et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018), greater hue angles (Sawyer et al., 2009; Apple et al., 2011; McKeith et al., 2016), and increased discoloration (Sawyer et al., 2009; Apple et al., 2011; Wills et al., 2017) than nonenhanced DC steaks. Previous research on aging of DC beef has shown limited impacts on a* and chroma when aged for 7 and 14 d (Wills et al., 2017). The limited effects of dark storage time on the nonenhanced DC steaks of this study aligns with the results of Wills et al. (2017). While the dark storage time of this study was not extended wet aging, dark storage time did appear to have an effect on normal-pH steaks. Mitacek et al. (2019) determined L* values decreased with display time for both 3-d and 7-d aged normal-pH steaks as seen with the results presented. They also observed there was no effect on the initial a* values of steaks 3-d, 7-d, and 14-d aged steaks, and both 3-d and 7-d aged steaks decreased in a* values with increased display time. The 7-d aged steaks were less red than 3-d aged steaks on d 5 of display. They determined there was a decreased color stability with an increased aging period. This is consistent with the decreased stability seen in the current study of normal-pH steaks 3-d versus 9-d in dark storage. Mitacek et al. (2019) also determined oxygen consumption and mitochondrial oxygen consumption rate were decreased with increased aging from 3 to 7-d and metmyoglobin reducing activity was not impacted, which are important to have in balance for color stability. Therefore, the dark storage time of this study could have decreased the color stability of normal-pH steaks.

Microbial growth

There was a significant dark storage time × enhancement effect of microbial growth (Table 3.12). The normal-pH steaks had lower ($P < 0.05$) microbial growth than

the DCE and DCN steaks for all dark storage periods. Gill and Newton (1979) determined DC beef has more microbial growth due to an increased pH. Acidification by GDL in the past has resulted in a decrease in microbial growth for various meat products (Farkas and Andrássy, 1993; Maijala et al., 1993); however, the enhancement did not provide a significant decline in microbial growth compared to nonenhanced DC steaks. This may be due to the enhancement process increasing microbial contamination. Nitrite-embedded packaging typically results in a 1-log reduction of microbial growth due to anaerobic conditions (Yang et al., 2016; Narváez-Bravo et al., 2017; Ramanathan et al., 2018); however, the reduction was not seen in the current study. This may be due to the repackaging of steaks into PVC and aerobic conditions shifting the microbial growth.

Conclusion

Nitrite-embedded packaging has been shown to improve color of DC steaks in retail and dark storage. The use of GDL in combination with NEP improved color of DC steaks. However, repackaging enhanced NEP steaks in PVC resulted in a decline in red appearance and increase in discoloration with various dark storage periods. In conclusion, NEP in combination with acidification can improve retail color of DC steaks but exposure to air reduced color stability. Therefore, further research should evaluate the extension of color stability after repackaging NEP steaks.

Table 3.1. pH and proximate composition (%) of normal-pH and dark-cutting strip loins (Normal-pH n = 6, Dark cutting n = 8)

Component	Normal-pH	Dark-cutting beef	SEM
pH	5.56[a]	6.39[b]	0.04
Moisture	69.13[a]	71.08[a]	1.15
Fat	6.03[a]	5.91[a]	1.41
Protein	23.47[b]	21.39[a]	0.29

[ab]Least squares means with different letters are significantly different (P < 0.05).

Table 3.2. Initial pH of dark-cutting loins prior to enhancement and 2-hr after enhancement (n = 8)

Time	pH	SEM
Initial	6.39[b]	0.06
2-hr	5.65[a]	0.06

[ab]Least squares means with different letters are significantly different (P < 0.05).

Table 3.3. Effect of enhancement[1] on d 6 pH of steaks in retail display (Normal pH n = 6, DCN/DCE n = 8)

Enhancement	Final pH	SEM
Normal-pH	5.54[a]	0.09
DCN	6.42[b]	0.09
DCE	5.88[a]	0.09

[ab]Least squares means with different letters are significantly different (P < 0.05).
[1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Table 3.4. Effects of dark storage day[1] on the nitric oxide myoglobin formation[2] in the enhanced dark-cutting steaks packaged in nitrite-embedded packaging (n = 8)

Dark storage day (d)	Nitric oxide myoglobin formation
0	2.37[a]
1	5.60[b]
2	5.27[b]
3	6.01[c]
SEM = 0.08	

[a-d]Least squares means with different letters are significantly different (P < 0.05).
[1]Specific day during dark storage the parameter was measured.
[2]Nitric oxide myoglobin formation was calculated as the ratio of R650 ÷ R570 nm. A greater number indicates more nitric oxide formation.

Table 3.5. Effects of enhancement[1] on derived dark storage color[2] determined by the change in attribute from d 0 of dark storage to final d of dark storage (Δa* value = (a* value d 3, d 6, or d 9) – (a* value d 0)) (Normal-pH n = 6, DCN/DCE n = 8)

	Enhancement		
Attribute	Normal-pH	DC	DCE
ΔL* values SEM = 0.55	1.05[a]	2.50[a]	5.27[b]
Δa* values SEM = 0.36	-4.81[a]	-0.24[b]	13.07[c]
ΔHue SEM = 0.67	-2.69[a]	0.18[b]	-5.15[a]
ΔChroma SEM = 0.36	-6.42[a]	-0.24[b]	14.26[c]

[a-c]Least squares means with different letters within an attribute are significantly different ($P < 0.05$).

[1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

[2]Derived variable determined for each parameter by the following equation: Δa* value = (a* value d 3, d 6, or d 9) – (a* value d 0).

Table 3.6. Least squares means for L* (dark storage time[1] × hour of retail display) of steaks displayed for 144 h (Normal-pH n = 6, DCN/DCE n = 8)

Retail display hour	Days in dark storage	L* values
0	3 d	39.47[c]
	6 d	38.99[c]
	9 d	40.13[c]
12	3 d	40.00[c]
	6 d	39.06[c]
	9 d	39.92[c]
24	3 d	39.34[c]
	6 d	39.34[c]
	9 d	39.71[c]
72	3 d	40.02[c]
	6 d	39.14[c]
	9 d	38.29[bc]
84	3 d	39.36[c]
	6 d	39.33[c]
	9 d	37.88[abc]
132	3 d	38.70[bc]
	6 d	37.82[abc]
	9 d	35.65[a]
144	3 d	38.10[abc]
	6 d	37.93[abc]
	9 d	36.15[ab]
SEM = 0.64		

[a-c]Least squares means with different letters are significantly different (P < 0.05).
[1]Total time the steaks spent in dark storage prior to retail display.

Table 3.7. Least squares means for L* (enhancement[1] × hour of retail display) of steaks displayed for 144 h (Normal-pH n = 6, DCN/DCE n = 8)

Retail display hour	Enhancement	L* values
0	Normal-pH	45.70^{i}
	DCN	33.54^{abc}
	DCE	39.34^{fgh}
12	Normal-pH	46.56^{i}
	DCN	33.90^{abcd}
	DCE	38.52^{fg}
24	Normal-pH	46.35^{i}
	DCN	33.86^{abc}
	DCE	38.18^{def}
72	Normal-pH	45.58^{i}
	DCN	34.06^{abcde}
	DCE	37.81^{cdef}
84	Normal-pH	45.06^{i}
	DCN	33.22^{ab}
	DCE	38.30^{ef}
132	Normal-pH	43.31^{hi}
	DCN	31.59^{a}
	DCE	37.26^{bcdef}
144	Normal-pH	42.63^{ghi}
	DCN	32.20^{a}
	DCE	37.36^{bcdef}
	SEM = 1.07	

[a-i]Least squares means with different letters are significantly different ($P < 0.05$).
[1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and n nitrite-embedded packaging (DCE).

Table 3.8. Least squares means for a* and chroma (dark storage time[1] × enhancement[2] × hour of retail display) of steaks for displayed 144 h (Normal-pH n = 6, DCN/DCE n = 8)

			Retail display hour						
Parameter	Dark storage Time	Enhancement	0	12	24	72	84	132	144
a* values	3 d	Normal-pH	$28.04^{ab,wx}$	$31.69^{b,w}$	$31.12^{ab,z}$	$29.65^{ab,y}$	$29.45^{ab,y}$	$28.10^{ab,y}$	$26.70^{a,y}$
		DCN	$22.69^{a,v}$	$23.67^{a,v}$	$24.62^{a,xy}$	$24.32^{a,wx}$	$24.38^{a,vwx}$	$22.32^{a,wx}$	$22.11^{a,wx}$
		DCE	$27.84^{b,wx}$	$21.12^{a,v}$	$19.97^{a,vw}$	$20.46^{a,vw}$	$20.69^{a,v}$	$17.79^{a,vw}$	$18.65^{a,vw}$
	6 d	Normal-pH	$27.34^{ab,wx}$	$30.49^{b,w}$	$30.29^{b,z}$	$29.24^{ab,y}$	$28.83^{ab,xy}$	$26.32^{ab,xy}$	$24.97^{a,xy}$
		DCN	$23.89^{b,vw}$	$23.57^{b,v}$	$23.93^{b,wx}$	$24.01^{b,vwx}$	$23.59^{b,vw}$	$18.57^{a,vw}$	$18.42^{a,vw}$
		DCE	$28.77^{b,x}$	$20.02^{a,v}$	$18.40^{a,v}$	$19.52^{a,v}$	$20.20^{a,v}$	$20.27^{a,vw}$	$20.10^{a,vw}$
SEM = 1.14	9 d	Normal-pH	$25.46^{b,vwx}$	$29.45^{b,w}$	$29.00^{b,yz}$	$27.65^{b,xy}$	$27.61^{b,wxy}$	$19.60^{a,vw}$	$17.75^{a,vw}$
		DC	$23.66^{b,vw}$	$23.82^{b,v}$	$24.12^{b,wx}$	$22.13^{b,vw}$	$20.61^{ab,v}$	$16.75^{a,v}$	$16.40^{a,v}$
		DCE	$27.48^{c,wx}$	$20.26^{ab,v}$	$21.06^{ab,vwx}$	$21.51^{b,vw}$	$19.90^{ab,v}$	$17.08^{ab,v}$	$16.82^{a,v}$
Chroma	3 d	Normal-pH	$35.25^{ab,z}$	$39.92^{b,x}$	$39.18^{b,y}$	$37.16^{ab,y}$	$37.13^{ab,y}$	$35.31^{ab,y}$	$33.63^{a,z}$
		DCN	$26.93^{a,w}$	$28.83^{a,w}$	$30.23^{a,x}$	$29.89^{a,wx}$	$30.04^{a,xy}$	$27.41^{a,x}$	$27.16^{a,xy}$
		DCE	$33.84^{b,z}$	$26.78^{a,w}$	$25.81^{a,wx}$	$26.34^{a,w}$	$26.64^{a,wx}$	$23.27^{a,wx}$	$24.21^{a,wx}$
	6 d	Normal-pH	$34.71^{abc,z}$	$38.60^{c,x}$	$38.04^{bc,y}$	$36.76^{bc,y}$	$36.27^{abc,y}$	$33.21^{ab,y}$	$31.68^{a,yz}$
		DCN	$28.54^{b,wxy}$	$28.49^{b,w}$	$29.07^{b,wx}$	$29.22^{b,w}$	$28.65^{b,wx}$	$22.53^{a,wx}$	$22.27^{a,wx}$
		DCE	$35.22^{b,z}$	$25.99^{a,w}$	$24.50^{a,w}$	$25.66^{a,w}$	$26.29^{a,wx}$	$26.01^{a,x}$	$25.62^{a,x}$
SEM = 1.25	9 d	Normal-pH	$32.57^{b,wyz}$	$37.13^{b,x}$	$36.86^{b,y}$	$34.89^{b,xy}$	$34.58^{b,y}$	$26.15^{a,x}$	$23.97^{a,wx}$
		DCN	$28.23^{c,wx}$	$28.72^{c,w}$	$29.18^{c,wx}$	$26.53^{c,w}$	$24.47^{bc,w}$	$19.89^{ab,w}$	$19.36^{a,w}$
		DCE	$33.49^{b,yz}$	$25.65^{a,w}$	$26.55^{a,wx}$	$27.28^{a,w}$	$25.55^{a,wx}$	$22.58^{a,wx}$	$22.66^{a,wx}$

[a-c]Least squares means within a row with different letters are significantly different ($P < 0.05$).

[v-z]Least squares means within a column with different letters are significantly different ($P < 0.05$).

[1]Total time the steaks spent in dark storage prior to retail display.

[2]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Table 3.9. Least squares means for hue (dark storage time[1] × enhancement[2] × hour of retail display) of steaks displayed for 144 h (Normal-pH n = 6, DCN/DCE n = 8)

			Retail display hour						
Parameter	Dark storage Time	Enhancement	0	12	24	72	84	132	144
Hue	3 d	Normal-pH	37.27^{a,xy}	37.47^{a,wx}	37.46^{a,wxy}	37.10^{a,wxyz}	37.58^{a,xyz}	37.41^{a,wxy}	37.58^{a,vwx}
		DCN	32.38^{a,w}	34.59^{a,w}	35.35^{a,wx}	35.43^{a,wxy}	35.66^{a,wxy}	35.34^{a,vwx}	35.34^{a,uvw}
		DCE	34.63^{a,wxy}	38.15^{ab,wx}	39.71^{b,xy}	39.60^{b,yz}	39.72^{b,yz}	40.31^{b,yz}	39.81^{b,xyz}
	6 d	Normal-pH	38.06^{a,y}	37.89^{a,wx}	37.29^{a,wxy}	37.37^{a,wxyz}	37.42^{a,xyz}	37.64^{a,wxy}	38.06^{a,vwz}
		DCN	32.94^{a,wx}	34.07^{a,w}	34.46^{a,w}	34.57^{a,wx}	34.44^{a,wx}	34.29^{a,vw}	34.04^{a,uv}
		DCE	35.20^{a,wxy}	39.94^{b,x}	41.57^{b,y}	41.09^{b,z}	40.28^{b,z}	38.89^{ab,xyz}	38.66^{ab,wxy}
SEM = 1.10	9 d	Normal-pH	38.70^{abc,y}	37.59^{a,wx}	38.24^{ab,wxy}	37.66^{a,wxyz}	37.07^{a,xyz}	42.09^{bc,z}	42.94^{c,yz}
		DCN	32.75^{a,w}	33.82^{a,w}	34.08^{a,w}	33.27^{a,w}	32.26^{a,w}	32.36^{a,v}	31.89^{a,u}
		DCE	34.80^{a,wxy}	38.13^{a,wx}	37.76^{a,wxy}	38.03^{a,xyz}	39.08^{ab,yz}	41.50^{ab,yz}	43.11^{b,z}

$^{a-c}$Least squares means within a row with different letters are significantly different ($P < 0.05$).
$^{w-z}$Least squares means within a column with different letters are significantly different ($P < 0.05$).
[1]Total time the steaks spent in dark storage prior to retail display.
[2]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Table 3.10. Effects of hour of retail display on nitric oxide formation[1] in the enhanced dark-cutting steaks packaged in nitrite-embedded packaging (n = 8)

Retail display hour	Nitric oxide myoglobin formation
0	5.49[d]
12	4.70[c]
24	4.65[c]
36	4.58[bc]
48	4.54[bc]
60	4.51[bc]
72	4.44[abc]
84	4.40[abc]
96	4.32[abc]
108	4.00[ab]
120	4.07[abc]
132	3.85[a]
144	3.86[a]
SEM = 0.16	

[a-d]Least squares means with different letters are significantly different (P < 0.05).

[1]Nitric oxide myoglobin formation was calculated as the ratio of R650 ÷ R570 nm. A greater number indicates more nitric oxide formation.

Table 3.11. Least squares means for muscle color[1] and surface discoloration[2] (dark storage time[3] × enhancement[4] × hour of retail display) of steaks displayed for 144 h (Normal-pH n = 6, DCN/DCE n = 8)

			Retail display hour						
Parameter	Dark storage time	Enhancement	0	12	24	72	84	132	144
Muscle Color	3 d	Normal-pH	$2.03^{ab,v}$	$1.51^{a,v}$	$1.75^{a,v}$	$2.24^{abc,v}$	$2.64^{bc,v}$	$2.90^{cd,v}$	$3.61^{d,v}$
		DCN	$6.28^{a,x}$	$6.32^{a,yz}$	$6.30^{a,xy}$	$6.31^{a,wx}$	$6.52^{a,x}$	$6.16^{a,xyz}$	$6.50^{a,y}$
		DCE	$4.47^{a,w}$	$5.43^{b,x}$	$6.04^{bc,xy}$	$6.11^{bc,wx}$	$6.14^{bc,wx}$	$6.18^{bc,xyz}$	$6.36^{c,xy}$
	6 d	Normal-pH	$2.18^{ab,v}$	$2.00^{a,vw}$	$1.92^{a,v}$	$2.64^{ab,v}$	$2.99^{bc,v}$	$3.64^{c,vw}$	$3.57^{c,v}$
		DCN	$6.75^{a,x}$	$6.45^{a,z}$	$6.45^{a,xy}$	$6.47^{a,wx}$	$6.60^{a,x}$	$6.66^{a,y}$	$6.68^{a,y}$
		DCE	$5.19^{a,w}$	$5.58^{a,xy}$	$5.94^{a,x}$	$5.92^{a,wx}$	$5.50^{a,w}$	$5.86^{a,xy}$	$5.66^{a,x}$
SEM = 0.20	9 d	Normal-pH	$2.72^{a,v}$	$2.65^{a,w}$	$3.11^{a,w}$	$2.86^{a,v}$	$3.07^{a,v}$	$4.19^{b,w}$	$4.69^{b,xy}$
		DCN	$6.82^{a,x}$	$6.71^{a,z}$	$6.80^{a,y}$	$6.66^{a,x}$	$6.75^{a,x}$	$6.82^{a,z}$	$6.88^{a,y}$
		DCE	$4.55^{a,w}$	$5.55^{b,xy}$	$5.88^{bc,x}$	$5.71^{bc,w}$	$5.58^{b,w}$	$5.49^{b,x}$	$6.45^{c,w}$
Surface Discoloration	3 d	Normal-pH	$1.31^{a,v}$	$1.17^{a,v}$	$1.14^{a,v}$	$1.25^{a,v}$	$1.39^{a,v}$	$1.75^{a,vw}$	$2.03^{a,v}$
		DCN	$1.00^{a,v}$	$1.02^{a,v}$	$1.04^{a,v}$	$1.06^{a,v}$	$1.08^{a,v}$	$1.21^{a,v}$	$1.48^{a,v}$
		DCE	$1.46^{a,v}$	$2.88^{b,wx}$	$3.01^{b,w}$	$3.40^{b,w}$	$3.19^{b,w}$	$3.31^{b,xy}$	$3.40^{b,wx}$
	6 d	Normal-pH	$1.22^{a,v}$	$1.22^{a,v}$	$1.19^{a,v}$	$1.39^{a,v}$	$1.47^{a,v}$	$2.06^{a,vw}$	$2.11^{a,v}$
		DCN	$1.02^{a,v}$	$1.04^{a,v}$	$1.04^{a,v}$	$1.10^{ab,v}$	$1.23^{ab,v}$	$2.42^{c,wx}$	$2.23^{bc,vw}$
		DCE	$1.60^{a,v}$	$3.27^{b,x}$	$3.74^{b,w}$	$3.63^{b,w}$	$3.58^{b,w}$	$4.25^{b,yz}$	$3.69^{b,x}$
SEM = 0.29	9 d	Normal-pH	$1.81^{a,v}$	$1.81^{a,vw}$	$1.81^{a,v}$	$1.39^{a,v}$	$1.61^{a,v}$	$4.14^{b,yz}$	$4.36^{b,x}$
		DCN	$1.00^{a,v}$	$1.02^{a,v}$	$1.06^{a,v}$	$1.31^{ab,v}$	$1.48^{ab,v}$	$2.06^{ab,vw}$	$2.27^{b,vw}$
		DCE	$1.44^{a,v}$	$2.88^{b,wx}$	$3.15^{b,w}$	$3.15^{b,w}$	$3.19^{bc,w}$	$4.52^{d,z}$	$4.33^{cd,x}$

[a-d]Least squares means within a row with different letters are significantly different ($P < 0.05$).

[v-z]Least squares means within a column with different letters are significantly different ($P < 0.05$).

[1]1= extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slight bright cherry-red, 5 = slightly dark cherry-red, 6 = moderately dark red, 7 = dark red

[2]1= no discoloration, 2 = minimal discoloration, 3 = slight discoloration, 4 = small discoloration, 5 = modest discoloration, 6 = moderate discoloration, 7 = extensive discoloration

[3]Total time the steaks spent in dark storage prior to retail display.

[4]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Table 3.12. Effects of dark storage time[1] and enhancement[2] on microbial growth (Normal-pH n = 6, DCN/DCE n = 8)

Dark storage time	Enhancement	Log(CFU/cm^2)
3 d	Normal-pH	4.99^{a}
	DCN	6.95^{bc}
	DCE	6.20^{b}
6 d	Normal-pH	4.89^{a}
	DCN	7.59^{c}
	DCE	6.41^{b}
9 d	Normal-pH	4.99^{a}
	DCN	6.39^{b}
	DCE	6.23^{b}
SEM = 0.21		

[a-c]Least squares means with different letters are significantly different ($P < 0.05$).
[1]Total time the steaks spent in dark storage prior to retail display.
[2]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Figure 3.1. Schematic representation of enhancement and packaging of dark-cutting loins during dark storage and repackaging of steaks for retail display (Normal-pH n = 6, DCN/DCE n = 8).

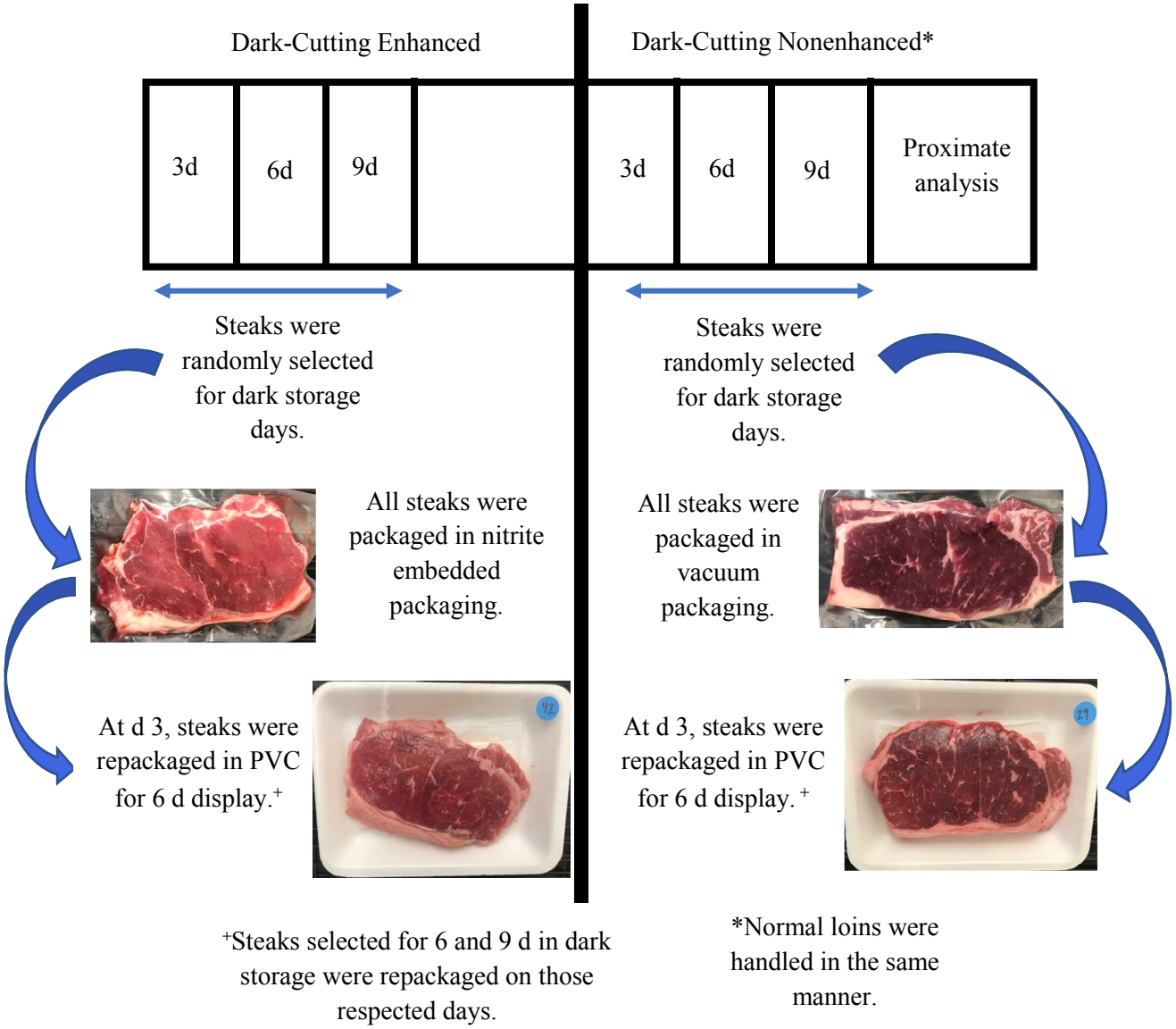
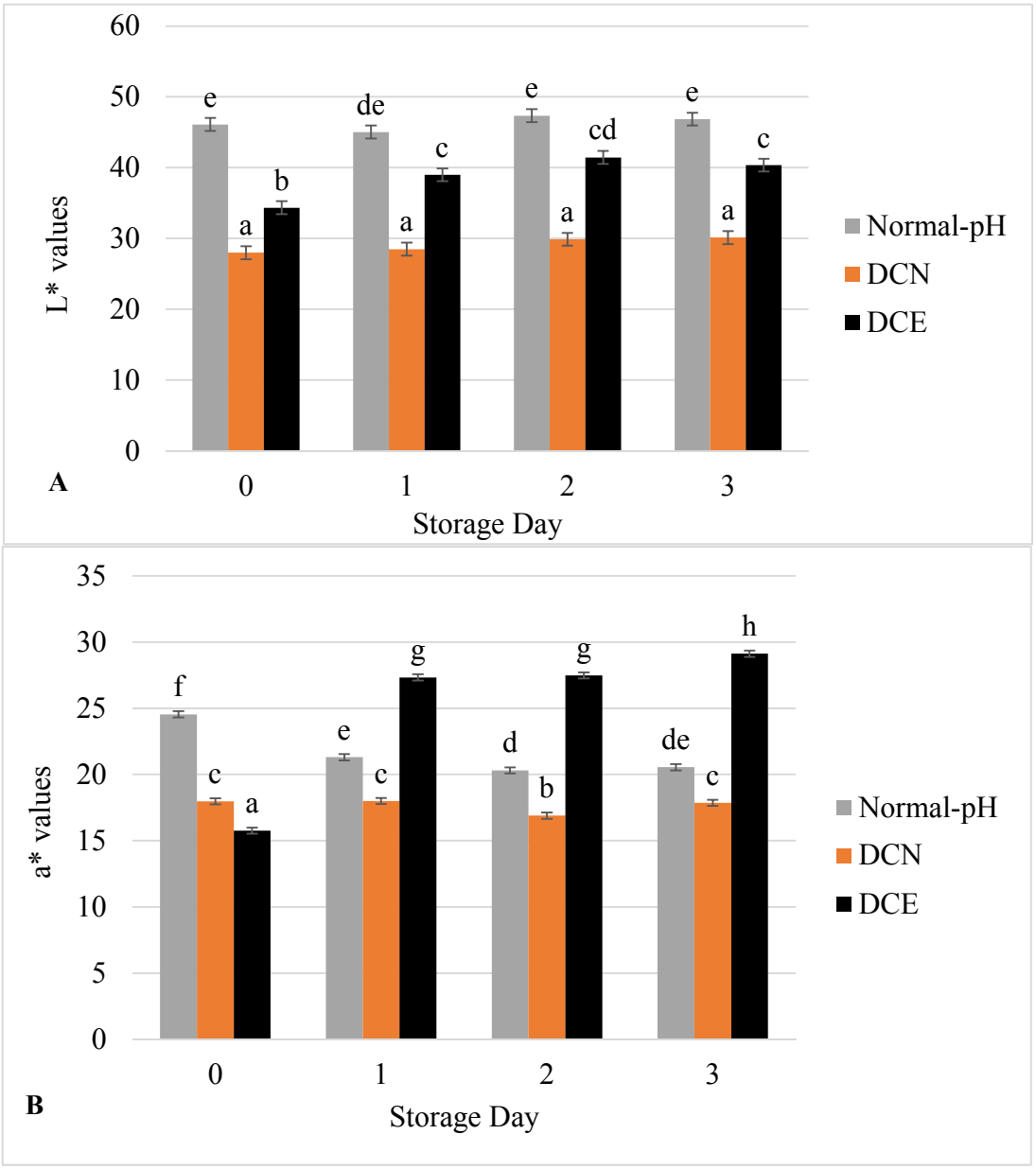
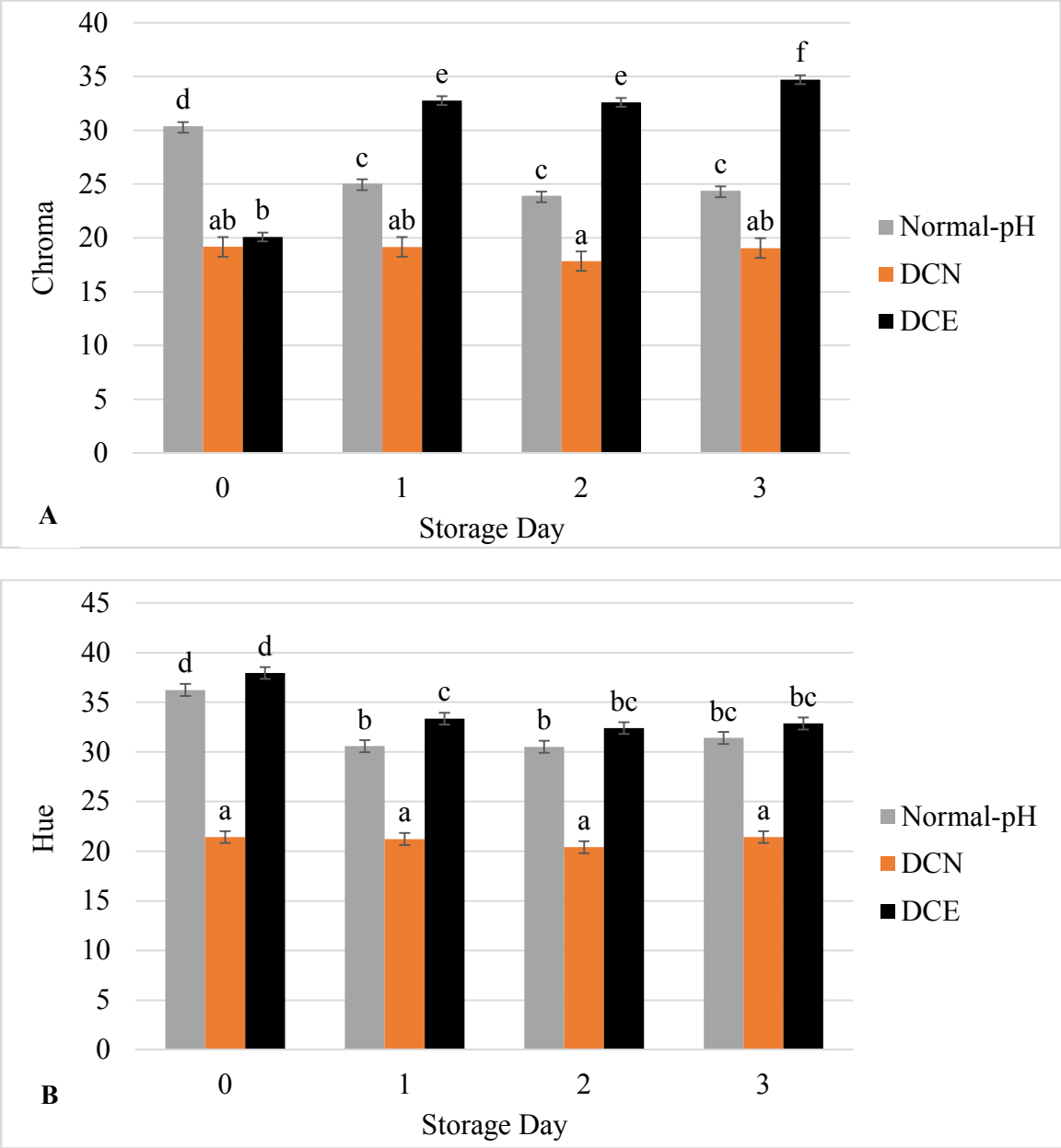


Figure 3.2. Effects of dark storage day and enhancement[1] on dark storage L* values (A) and a* values (B) (Normal-pH n = 6, DCN/DCE n = 8).



Least squares means with different letters (a-h) within attribute are significantly different ($P < 0.05$). Standard error of the mean indicated by error bars (SEM(A) = 0.91; SEM(B) = 0.24). [1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Figure 3.3. Effects of dark storage day and enhancement[1] on dark storage Chroma (A) and Hue (B) (Normal-pH n = 6, DCN/DCE n = 8).



Least squares means with different letters (a-f) within attribute are significantly different ($P < 0.05$). Standard error of the mean indicated by error bars (SEM(A) = 0.41; SEM(B) = 0.60). [1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

CHAPTER IV

EFFECTS OF GLUCONO DELTA-LACTONE ENHANCEMENT ON COOKED COLOR AND SENSORY ATTRIBUTES OF DARK-CUTTING BEEF IN NITRITE-EMBEDDED PACKAGING

Abstract

The objective of this study was to determine the effects of novel nitrite-embedded packaging (NEP) and enhancement on retail and cooked color and palatability of dark-cutting beef. Selection of dark-cutting beef strip loins (n = 10; pH > 6.0) and USDA Choice beef strip loins (normal-pH, n = 10) occurred at a commercial packing plant. Dark-cutting loins were divided into 2 sections and randomly selected as non-enhanced dark-cutting (DCN) and enhanced dark-cutting (DCE) treatments with 10% injection of the green weight. A final concentration of 0.5% glucono delta-lactone and 0.1% rosemary in the loin. Steaks (1.91 cm) were removed from nonenhanced normal-pH, DCN, and DCE loins. One steak from the normal-pH and DCN loins was packaged in poly-vinyl chloride trays, and one steak from the DCE loins was packaged in NEP. Steaks were kept in simulated retail display for 6 d with instrumental color measurements and trained color panel (n = 6) evaluation every 24 h. Remaining steaks were randomly assigned to cooked color, sensory, and Warner-Bratzler shear force. Steaks for cooked color were placed

in dark storage for 72 h after packaging the DCE steaks in NEP and the DCN and normal-pH steaks in vacuum packaging. A trained sensory panel (n = 6) evaluated sensory steaks for beef palatability. By d 6 of retail display, the DCE steaks had greater ($P < 0.05$) a* values than DCN and normal-pH steaks. There was significantly more surface discoloration on normal-pH steaks than DCE and DCN steaks by d 6 of retail display. The cooked DCE steaks had similar internal a* values to cooked normal-pH steaks ($P > 0.05$). A similar decrease in redness was observed by panelists evaluating the internal cooked color of DCE steaks. However, external cooked color of DCE was determined to have significantly more pink appearance than normal-pH and DCN steaks. The trained sensory panel determined there was no difference in tenderness and juiciness between the normal-pH and DCE steaks, paralleling with the Warner-Bratzler shear force results. In conclusion, NEP improved surface redness of DCE during retail display, and the enhancement decreased internal redness upon cooking.

Introduction

Meat color is primarily determined by the protein myoglobin. More specifically, the ligand bond to the heme ring within myoglobin and the redox state of the iron in the ring (Aberle et al., 2012). Oxymyoglobin is formed by the binding of oxygen to the heme, and by the oxygen binding, a bright-cherry red color is formed (Aberle et al., 2012). Consumers prefer the bright-cherry red color and deviations from the red color results in purchase decline (Carpenter et al., 2001). Dark-cutting (DC) beef is a deviation from the bright cherry-red color due to chronic stress prior to harvest (Lawrie, 1958; Scanga et al., 1998). Stress has been shown to decrease glycogen content in the muscle prior to harvest reducing the formation of lactic acid and leading to a greater postmortem

muscle pH (Lawrie, 1958; Scanga et al., 1998). An increase in pH leads to more repulsion between meat proteins causing muscle swelling and increased water holding. This increases the reflectance and gives a darker appearance to the human eye (Hughes et al., 2017). Previous research indicates that mitochondrial oxygen consumption is greater in DC beef (Ashmore et al., 1972). Mitochondrial oxygen consumption and muscle swelling decreases the available oxygen for bloom, and deoxymyoglobin is formed in increased concentrations. The formation of deoxymyoglobin decreases the lightness of DC beef. Additionally, a high pH increases the thermal stability of myoglobin (Hunt et al., 1999). Upon cooking, DC beef remains pink at greater internal temperatures, which gives an undercooked appearance to consumers (Cornforth et al., 1991).

Improvement of fresh and cooked color of DC beef has been shown through acidification and packaging techniques. Lactic acid enhanced DC beef has lower internal red cooked color than nonenhanced DC beef (Sawyer et al., 2009; Apple et al., 2011); however, lactic acid enhancement led to an increase in surface discoloration during retail display (Sawyer et al., 2009; Apple et al., 2011). High oxygen and carbon monoxide modified atmospheric packaging has improved retail color of DC beef (Wills et al., 2017), but increased lipid oxidation and consumer concerns are detrimental to use (Cornforth and Hunt, 2008; English et al., 2016). Alternatively, nitrite-embedded packaging (NEP) has been shown to increase red appearance of DC beef anaerobically (Ramanathan et al., 2018). Low oxygen partial pressure (6-7 mmHg) leads to the formation of metmyoglobin (Ledward, 1970; Brantley et al., 1993), and nitric oxide formed by reduction of nitrite binds to metmyoglobin. The nitric oxide metmyoglobin can be reduced by the activity of meat to form the bright-red nitric oxide myoglobin

(Siegel, 2011; Fox Jr. and Ackerman, 1968). Claus and Du (2013) and Yang et al. (2016) determined NEP increased the red appearance of the surface of normal-pH. Rosemary in combination with NEP has been shown to be effective in further improving red color of DC beef (Ramanathan et al., 2018). The implications of NEP in combination with enhancement on cooked color has not been evaluated.

The greater pH of DC beef contributes to the microbial growth DC beef. Dark-cutting beef has been shown to have more microbial growth compared to normal-pH beef (Patterson and Gibbs, 1977; Gill and Newton, 1979). Additionally, the lower glycogen content postmortem has been shown to increase spoilage rate of dark-cutting beef (Gill and Newton, 1978). The anaerobic conditions of NEP have had limited impact on the microbial growth of DC beef (Ramanathan et al., 2018). Acidification of normal-pH beef has resulted in a decline in microbial growth (Stivarius et al., 2002; Kassem et al., 2017), but the research on the implications of organic acids on the bacterial growth in DC beef has not been as extensive. A patent has indicated the potential for glucono delta-lactone (GDL) to improve DC beef color (Dolezal et al., 2013) but not the implications of enhancement on microbial growth of DC beef. The aim of this study was to evaluate the enhancement of DC beef with GDL and rosemary packaged in NEP to decrease microbial growth and improve surface color. Beyond fresh meat characteristics, the study will evaluate the effect of the enhancement and NEP on cooked color and palatability of enhanced DC beef.

Materials and Methods

Raw Materials and Processing

Three d post-harvest, ten USDA Low-Choice strip loins (normal-pH: pH = 5.33-5.47) and ten DC strip loins (pH = 6.12-6.67) were collected from a local purveyor (Tyson Foods; Amarillo, TX) and transported on ice to the Robert M. Kerr Food and Agricultural Products Center at Oklahoma State University campus in Stillwater. Loins were bisected, vacuum packaged (Walton's Vacuum Pouch; 12 × 22 pouches; 3 mil thickness; 1.3 – 1.6 oxygen transmission rate $cm^3/100\ in^2$) using Multivac C5000 vacuum packager and stored in the dark at 2°C upon arrival until use. Halves from the DC loins were randomly assigned to be nonenhanced (DCN) or enhanced (DCE). Enhanced DC loins were pumped to 110% of the green weight with a final concentration of 0.5% GDL (Glucono Delta-Lactone; PMP Fermentation Products, Inc) and 0.1% rosemary (Herbalox oleoresin rosemary; Kalsec) in the loin. Post-enhancement, loins were equilibrated for two hours prior to pH measurements of the DCE loins. Steaks were sliced (1.91 cm) from the anterior end of each strip loins with an anterior steak used for retail display using a meat slicer (Bizerba USA INC., Piscataway, NJ). Steaks were randomly selected for cooked color, trained sensory panel, and Warner-Bratzler shear force. Two posterior steaks were used for proximate composition analysis.

pH and Proximate Composition Analysis

Upon arrival to OSU, initial pH of the normal and DC strip loins was measured in three random locations using Hach HQd Portable Meter (Probe PHC10801; Hach.; Loveland, CO). The two posterior steaks were ground using a table top grinder (Big Bite Grinder, 4.5 mm, fine grind, LEM) and pressed into 140-mm sample cup. Percent

protein, fat, and moisture were determined using AOAC-approved near-infrared spectrophotometer (FoodScan Lab Analyzer, Serial No. 91753206; Foss, NIRsystem Inc.; Slangerupgrade, Denmark).

Packaging and simulated retail display

Steaks of the normal-pH and DCN loins were packaged in Styrofoam trays with polyvinyl chloride (PVC) overwrap (15,500-16,275 cm^3 O_2/m^2/24 h at 23°C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film, Koch Supplies, Kansas City, MO) using a film wrap machine (Winholt WHSS-1, 115V; Woodbury, NY). Nitrite-embedded film packaging (FreshCase; Curlon Grade A5106 Protective Packaging Film; approximately 115 mg/m^2 nitrite, 6 × 12 pouches; 7 mil thickness; <0.15 oxygen transmission rate $cm^3/100$ in^2/24 h @ 73°F, 0% FH, 1 atm; <0.5 water vapor transmission rate g/100 in^2/24 h @ 100°F, 90% RH, 1 atm; Bemis Innovation Center in Neenah, WI) was used for the DCE steaks and packaged using a Multivac C500 vacuum packager. Steaks were stored in a white-coffin-style display under continuous LED lighting (Philips LED lamps; 12 watts, 48 inches, color temperature = 3,500°K; Phillips, China) at 2 ± 1°C for 6 d.

Fresh meat color

Instrumental color

Instrumental color was evaluated every day for 6 d using a HunterLab 4500L MiniScan EZ Spectrophotometer (2.5-cm aperture, illuminant A, and 10° standard observer angle; HunterLab Associates; Reston, VA). Readings were taken in duplicate on the steak surface. The instrumental color provided CIE L*, a*, b*, and spectral data from 400-700 nm which determined surface color. The nitric oxide myoglobin formation was

determined using the reflectance ratio of 650 and 570 nm with a greater number indicating more nitric oxide formation. The CIE a* and b* were utilized to determine chroma (red intensity) $\left[\sqrt{(a^{*2} + b^{*2})}\right]$ where a larger number indicates a greater intensity. The hue angle $(\tan^{-1}(\frac{b*}{a*}))$ represents the red color present and was determined by the CIE a* and b* values (AMSA, 2012).

Visual fresh meat color

A trained panel (n = 6) evaluated surface color of the steaks each day for 6 d. Each panelist passed the Farnsworth Munsell 100-hue test. Muscle darkening (MD) was determined by panelists on d 0, 1, 2 using a 7-point scale (1 = No darkening, 7 = Very dark). Panelists evaluated steaks for muscle color (MC) on a 7-point scale (1 = Extremely bright cherry-red, 7 = Dark red), surface discoloration (SD) using a 7-point scale (1 = No discoloration (0%), 7 = Extensive discoloration (81-100%)), and worst point (WP) on a 7-point scale (1 = Bright cherry-red, 7 = Grey) for 6 d. Worst point was a randomly selected 2.54 × 2.54 cm^2 square on the surface of the steak.

Cooked meat color

After 3 d of dark storage at 2 ± 1°C, steaks were cooked using a George Foreman Grill (Lean Mean Fat Grilling Machine George Foreman Lake Forest, IL GRP99A) to 71°C then placed on ice for 5 minutes to prevent further cooking. Steaks were bisected parallel to the cooked surface to measure instrumental internal cooked color. Using a HunterLab 4500L MiniScan EZ Spectrophotometer, internal cooked color was read in duplicate across the cooked interior. Six trained panelists evaluated the internal cooked

color using a 7-point scale (1 = Very red, 7 = Tan/brown). External cooked color was evaluated by panelists on a 7-point scale (1 = Brown, 7 =Pinkish-red).

Microbiology

On d 6 of display, steaks were swabbed to determine total plate count in a 2.54 $cm^2 \times 2.54$ cm^2 square using an environmental swab (Puritan® Environmental Sampling Kit HP007-BPW; Puritan Medical Products Co LLC; Guildford, Maine). One mL from the swab container was serially diluted into 0.1% buffered peptone water (Bacto™ Peptone Ref 211677 Becton; Dickinson and Company; Sparks, MD). Aseptically one mL was plated on 3M Petrifilm Rapid Aerobic Plate Count (3M Health Care; St. Paul, MN), and the plates were incubated for 48 h at 37°C in a VWR Forced Air General Incubator (5.4 ft^3; VWR, Radnor, PA). Plates were counted to determine the total plate count per cm^2 using an Interscience Scan 100 pressure sensitive pad (Interscience, Woburn, MA).

Thiobarbituric acid reactive substances (TBARS)

All enhancements were measured for lipid oxidation on d 6 of retail display. Twenty-seven mL of trichloroacetic acid (TCA) was blended for ten s with three g of sample a Waring commercial blender (Model 33BL7; New Hartford, CT). The sample was filtered with a 42 Whatman filter paper. One mL of thiobarbituric acid (TBA) was combined with one mL of the filtrate in a test tube. The test tube was heated in a 100°C water bath for 10 min then cooled for 5 min. Using the photometric setting of the spectrophotometer (UV-2600, UV-VIS Spectrophotometer; Shimadzu; Columbia, MD), absorbance was measured at 532 nm. One mL of TCA was combined with one mL of TBA to serve as a standard.

Trained sensory panel

Utilizing the Beef Flavor Lexicon (Adhikari et al., 2011), sensory panelists were trained to evaluate the organoleptic attributes normal-pH, DCN, and DCE strip loins. The trained sensory panel was approved by the Institutional Review Board (Protocol number: AG -18-34). Nine samples were evaluated during each session by a six-member trained panel (n = 6).

Steaks were thawed for 24 h at 4°C prior to cooking on an XLT Impingement Oven (model 3240-TS; BOFI Inc., Wichita, KS) at 177°C to an internal temperature of 71°C. One-cm^3 pieces were cut from the steaks, and two of those pieces were placed in each sample cup coded with a random number. Cups maintained temperature by remaining in a warmer during sensory evaluation. Panelists cleansed their palettes between samples with deionized water and salt-free crackers. Under red lighting, samples were evaluated for juiciness (1 = extremely dry, 8 = extremely juicy), overall tenderness (1 = extremely tough, 8 = extremely tender), beef flavor (1 = not detectable, 3 = strongly detectable), metallic flavor (1 = not detectable, 3 = strongly detectable), and sour flavor (1 = not detectable, 3 = strongly detectable) by panelists.

Warner-Bratzler shear force

After steaks thawed for 24 h at 4°C, steaks were cooked on an XLT Impingement Oven (model 3240-TS; BOFI Inc., Wichita, KS) at 177°C to an internal temperature of 71°C. For 18 h, steaks were cooled at 4°C, and prior to coring, steaks reached room temperature (approximately 30 min). Parallel to the muscle fibers, six cores (1.27 cm in diameter) were removed by hand. Using the Instron Universal Testing Machine (Model

5943; Instron Corporation; Norwood, MA) with Bluehill 3 software, the maximum load (kg) was recorded for each core. The crosshead speed was 200 mm/min.

Statistical analysis

The experiment was a completely randomized block design with loin serving as the block. The ten loins served as ten replicates (N = 10). The MIXED procedure of SAS (SAS 9.4; SAS Inst.; Cary, NC) to determine least squares means and standard error. The fixed effects included in the model were enhancement, day and enhancement × day. Day was a repeated measure for instrumental and visual color analysis. Loin was a random effect. Panelists were considered a random effect for the sensory data. Significance was considered at $P < 0.05$, and significant least squares means were separated using the PDIFF option.

Results and Discussion

Proximate composition and pH

Normal-pH beef had lower ($P < 0.05$) pH and moisture content than the DCN beef (Table 4.1). Dark-cutting beef has limited glycogen content postmortem resulting in a smaller decline in postmortem pH (Lawrie, 1958; Scanga et al., 1998). Past research comparing normal-pH beef and DC beef has shown a greater pH for DC beef (Sawyer et al., 2009; Wills et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018). Hughes et al. (2017) determined high pH beef results in an increase in muscle swelling and water holding capacity. An increase in the water holding capacity aligns with the increased moisture content reported in the DCN beef in this study. Fat content between normal-pH and DCN beef was not significantly different; however, the normal-pH beef had greater

protein content than DCN beef. Preliminary data from our lab has indicated there is a decrease in pH through the enhancement of GDL and rosemary.

Retail color

Instrumental color

There was an enhancement × day effect on the L* values, a* values, chroma, and hue ($P < 0.05$, Figures 4.1-4.4). Normal-pH steaks remained lighter ($P < 0.05$) than DCE and DCN steaks throughout display (Figure 4.1). There was no significant difference between the L* values of DCE and DCN steaks throughout display indicating the enhancement had limited impacts on the lightness of the steaks. Several studies have indicated normal-pH steaks are lighter (greater L*) compared to DC steaks (English et al., 2016; McKeith et al., 2016; Stackhouse et al., 2016; Hughes et al., 2017; Wills et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018). Results by Ramanathan et al. (2018) determined DC steaks in NEP saw no difference in L* values throughout display. In combination of NEP with rosemary dip, research has shown there was no impact on lightness of DC steaks compared to DC steaks in PVC (Ramanathan et al., 2018). At low levels of lactic acid enhancement, L* values between enhanced and nonenhanced DC steaks were not significantly different (Apple et al., 2011). However, increased lactic acidification of DC steaks has shown improvement in lightness of DC steaks similar to L* values of normal-pH steaks (Sawyer et al., 2009). Although, acidification did not impact the lightness of DC steaks of this study, the final pH of DCE steaks was an average of 5.88; however, the pH remained greater than normal-pH steaks. The greater pH was speculated to keep water binding high, increasing reflectance and decreasing L*

values based on research by Hughes et al. (2017). Therefore, the change in lightness would be limited due to the pH remaining greater than normal-pH steaks.

Normal-pH steaks had the highest ($P < 0.05$) a* values on d 0 compared to the other enhancements (Figure 4.2). As display time increased, the redness of the normal-pH steaks decreased ($P < 0.05$). On d 6, normal-pH and DCN steaks had similar a* values ($P > 0.05$). Overall, DCN steaks had little variation in the a* values throughout display. Enhanced DC steaks had an increase in a* values from d 0 to d 1 with more redness ($P < 0.05$) than the DCN steaks. By d 3, there was no difference ($P > 0.05$) in a* values between the normal-pH steaks and DCE steaks. From d 1 to d 6, there was no significant increase in a* values for the DCE steaks. By d 5, the DCE steaks were redder ($P < 0.05$) than the normal-pH steaks and DCN steaks. Chroma represents the red intensity of the lean color. The normal-pH steaks had a significant decrease in red intensity throughout the display period as seen with the decrease in redness (Figure 4.3). By d 4, normal-pH and DCE steaks had similar ($P > 0.05$) chroma values indicating similar red intensities. The red intensity of DCN steaks did not change ($P > 0.05$) from d 1 to d 4; however, the red intensity decreased on d 5 and 6 but was not significantly different from the d 0 red intensity. On d 6, normal-pH steaks had similar ($P > 0.05$) chroma to the DCN steaks. However, on d 6, the DCE steaks had a greater ($P < 0.05$) red intensity compared to the normal-pH and DCN steaks. Enhanced DC steaks had a significant increase in red intensity from d 0 to d 1. After d 1 of display, there was no significant change in the red intensity of DCE steaks. Therefore, the DCE steaks had an increase in color stability compared to the normal-pH steaks.

Hue is an indication of color deviations from the true red axis with a greater number meaning further from the true red color and more discoloration. By d 5, the hue of normal-pH steaks was significantly greater than on d 0 (Figure 4.4). The normal-pH steaks shifted away from the red axis and increased discoloration during display. On d 6, the normal-pH steaks had greater ($P < 0.05$) hue and discoloration compared to the DCN and DCE steaks. Normal-pH steaks remained numerically greater than DCN and DCE steaks throughout the display. The DCN steaks had the lowest ($P < 0.05$) hue on d 0 compared to the other enhancements, and initial and final hue of DCN steaks were not different ($P > 0.05$). Enhanced DC steaks had no change ($P > 0.05$) in hue throughout the retail display. From d 1 to d 6, DCE steaks had significantly lower hue values compared to the normal-pH steaks.

There was a significant day interaction for the nitric oxide myoglobin content of the DCE steaks (Table 4.2). The ratio of R650 ÷ R570 nm indicates nitric oxide myoglobin content with a greater number representing more formation. Nitric oxide myoglobin significantly increased from d 0 to d 1 and indicates a bright red appearance as seen in the a* results. The use of NEP has been shown to form nitric oxide myoglobin in DC steaks within 24 hours and appeared bright red in color (Ramanathan et al., 2018). The NEP has increased color stability of beef upon color formation (Claus and Du, 2013; Yang et al., 2016; Ramanathan et al., 2018). Ramanathan et al. (2018) determined chroma and a* values were increased for DC steaks with the formation of nitric oxide. In the current study, the formation of nitric oxide aligned with the increase in a* value and chroma of DCE steaks in 24 h. Nitric oxide myoglobin is formed from the reduction of nitric oxide metmyoglobin (Fox Jr. and Ackerman, 1968). Dark-cutting beef has greater

metmyoglobin reducing activity than normal-pH beef (English et al., 2016; McKeith et al., 2016), and the increased reducing activity resulted in a quicker formation of nitric oxide myoglobin in comparison to normal-pH beef (Ramanathan et al., 2018). The nitric oxide myoglobin content increased with display day until d 3 then the content stabilized for the second half of the display. Ramanathan et al. (2018) determined the nitric oxide myoglobin content increased significantly from d 0 to d 3 in a retail display with addition of rosemary. The use of rosemary in NEP has been speculated to increase the rate of red color improvement by reducing the oxidation of nitric oxide myoglobin (Ramanathan et al., 2018). As an antioxidant, rosemary in beef has been established in research by Sánchez-Escalante et al. (2003) and Wills et al. (2017) to extend color stability. Enhanced DC steaks did not reach equivalent red appearance to normal-pH steaks in the expected time of 1 d. This is speculated to be because of dipping versus injection application. Dipping application increases surface water of steaks, which could increase the reflectance. Weak organic acids have been reported to improve red color of DC steaks (Sawyer et al., 2009; Stackhouse et al., 2016; Tapp et al., 2017). Dolezal et al. (2013) determined the use of GDL in combination with phosphate in DC beef resulted in increased redness compared to DC beef without the enhancement. Therefore, in combination with NEP, the redness of steaks was improved with enhancement.

Normal-pH steaks have been shown to have more redness (a*) and red intensity (chroma) than DC steaks (Apple et al., 2011; Stackhouse et al., 2016; Wills et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018). Similarly, the decline in red appearance of normal-pH steaks typically occurs in retail display (Stackhouse et al., 2016; Wills et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018) while DC steaks have shown more

color stability (Stackhouse et al., 2016; Ramanathan et al., 2018). Dark-cutting steaks typically have lower hue values than normal-pH steaks (Sawyer et al., 2009; Apple et al., 2011; Stackhouse et al., 2016). Normal-pH steaks increased in hue throughout retail storage (Stackhouse et al., 2016) as shown in the current study.

Visual color

Normal-pH steaks had no visual muscle darkening with significantly lower scores than the DCN and DCE steaks (Figure 4.5). Previous research conducted by Mitacek et al. (2018) determined DC steaks had greater darkness than normal-pH steaks in PVC. On d 0, DCE steaks had more muscle darkening compared to the DCN steaks due to the initial formation of metmyoglobin prior to the shift to nitric oxide myoglobin (Fox Jr. and Ackerman, 1968; Siegel, 2011). Darkening decreased ($P < 0.05$) within 24 h for the DCE steaks. This shift in muscle darkening for the DCE steaks parallels with the increase in a* values and the increase in nitric oxide myoglobin content seen in the present study and Ramanathan et al. (2018). Nonenhanced DC steaks did not change ($P > 0.05$) in muscle darkness from d 0-3.

As retail day increased, the muscle color score of normal-pH steaks significantly increased (Figure 4.6). The visual color of normal-pH steaks shifted to a slightly dark cherry-red color on d 6. This change in visual color aligns with the decline in a* values and chroma during display for normal-pH steaks. From d 0 to d 4, the normal-pH steaks had the lower ($P < 0.05$) muscle color than DCE and DCN steaks. The muscle color of DCN steaks did not significantly increase with display time. On d 0, DCE steaks had significantly darker red muscle color compared to normal-pH and DCN steaks. By d 1, the DCE steaks had muscle color improved ($P < 0.05$). The color continued to improve

till d 4, and the shift in muscle color from slightly dark cherry red to slightly bright cherry red color for DCE steaks. By d 5, the muscle color of the normal-pH and DCE steaks were not significantly different.

Nonenhanced DC steaks had an increase ($P < 0.05$) in discoloration from d 0 to d 6 (Figure 4.7). Enhanced DC steaks had no change in discoloration reporting minimal to slight discoloration throughout the retail display ($P > 0.05$). As retail day increased, the surface discoloration of normal-pH steaks increased ($P < 0.05$). By d 6 of display, normal-pH steaks had significantly more surface discoloration than DCE and DCN steaks. The increase in visual discoloration of normal-pH steaks were supported by the hue results.

The worst-point color supported the muscle color results. At d 0, normal-pH steaks had a bright-cherry red color at the worst point. As display time increased, the worst-point color score remained lower ($P < 0.05$) than other enhancements till d 4 (Figure 4.8). At the end of display, normal-pH steaks were closer to dark red in appearance. After d 0, the worst-point color of DCE steaks significantly decreased bringing the color closer to a dark red versus a dark red with grey appearance. On d 6, DCE steaks had a lower ($P < 0.05$) worst-point color score than normal-pH and DCN steaks. Nonenhanced DC steaks had no change in worst-point color from d 0 to d 6 ($P > 0.05$). As seen with the visual color and instrumental color results, the DCN steaks had more color stability than normal-pH steaks.

Roberts et al. (2017) reported similar trends in muscle color and surface discoloration for bison steaks in NEP. The NEP bison steaks increased in bright red appearance and remained less discolored than control packaged steaks (Roberts et al.,

2017). Nitrite spray increased red color of ribeye and round steaks (Song et al., 2015). However, limited studies have evaluated the visual color implications of beef packaged in NEP. Dark-cutting steaks have been observed to have a darker red muscle color and less discoloration than normal-pH throughout display periods (Sawyer et al., 2009; Apple et al., 2011; Stackhouse et al., 2016; Wills et al., 2017; Mitacek et al., 2018). Use of weak organic acids to impact visual color of DC beef has been heavily researched. Sawyer et al. (2009) and Apple et al. (2011) determined low concentrations of lactic acids of 0.25% and 0.35% respectively improved muscle color of DC steaks without increasing discoloration. Additionally, Stackhouse et al. (2016) reported citric acid enhancement of DC steaks improved muscle color and limited discoloration. However, greater concentrations of lactic acid increased discoloration by increased myoglobin denaturation (Sawyer et al., 2009). Previous research has not evaluated the effects of GDL on the visual color on fresh meat products. The present study had less pH decline than previously seen with the use of lactic acid (Sawyer et al., 2009; Apple et al., 2011). The smaller pH decline supports the lack of increased discoloration by acidification. Rosemary has also been seen to increase visual color stability of DC steaks (Wills et al., 2017). Therefore, rosemary could help to increase the color stability and decrease discoloration of the DCE steaks. Muscle color results indicate the enhancement in combination with nitrite-embedded packaging improved meat color.

Thiobarbituric acid reactive substances (TBARS)

The normal-pH beef had greater ($P < 0.05$) TBARS and lipid oxidation than both the DCE and DCN steaks (Table 4.3). While, the lipid oxidation of DCN and DCE steaks were not ($P > 0.05$) different. Dark-cutting beef has been noted to have lower lipid

oxidation in comparison to normal-pH in multiple studies (English et al., 2016; Wills et al., 2017). However, the TBARS for the normal-pH was still below the taste threshold of 2 mg per kg (Campo et al., 2006). Wills et al. (2017) determined DC steaks packaged in PVC had similar oxidation levels to DC steaks enhanced with 0.1% rosemary as seen in this study. Roberts et al. (2017) reported NEP bison burgers had less lipid oxidation compared to PVC control steaks; however, bison has lower oxidative stability than beef due to greater number of polyunsaturated fatty acids.

Microbial growth

The enhancement effect on microbial growth indicated normal-pH steaks had significantly lower growth compared to the DCE and DCN steaks (Table 4.4). Dark-cutting beef has been shown to have more microbial growth than normal-pH beef because of a greater pH (Gill and Newton, 1979). There was no difference ($P > 0.05$) in growth between the DCN and DCE steaks unlike previous research where the acidification of meat products by GDL reduced microbial growth (Farkas and Andrássy, 1993; Maijala et al., 1993). However, there was a numerical 1-log reduction due to the anaerobic conditions of the DCE steaks and has been previously reported in research by Yang et al. (2016), Narváez-Bravo et al. (2017), and Ramanathan et al. (2018).

Cooked color

Instrumental cooked color

There was a significant enhancement effect for the L* values, a* values, b* values, hue and chroma of the cooked steaks (Table 4.5). The DCE steaks were similar lightness compared to the normal-pH steaks ($P > 0.05$); although, the DCE steaks did not have a darker ($P > 0.05$) appearance compared to the DCN steaks. The internal a* values

of the DCN steaks was the highest. The cooked DCE steaks were not redder ($P > 0.05$) in appearance compared to the normal-pH steaks. The DCN steaks had the highest chroma and the most intense red color; however, DCE steaks were not significantly different in intensity from DCN steaks and normal-pH steaks. Hue describes a deviation from the red axis with a larger number indicating a shift from red to yellow. Therefore, in cooked steaks, the steaks having a greater hue number indicate a less internal red cooked color. The hue of DCN and DCE steaks were not significantly different, and the normal-pH steaks had the highest hue ($P < 0.05$). The hue results correspond to the a* values and chroma with normal-pH steaks having a more done appearance than DCN steaks. The b* values indicated the DCE steaks were similar to the normal-pH steaks ($P > 0.05$). In support, Sawyer et al. (2008), Sawyer et al. (2009), and Apple et al. (2011) determined b* values of DC steaks enhanced with lactic acid were similar to normal-pH steaks.

Hunt et al. (1999) evaluated the impact of pH on the denaturation of myoglobin *in vitro,* and deoxymyoglobin was determined to be more heat stable compared to metmyoglobin at greater pH levels when heated to 70°C. Dark-cutting beef has greater amounts of deoxymyoglobin than normal-pH beef (McKeith et al., 2016; Hughes et al., 2017), and the deoxymyoglobin in combination with the thermal stability provided by the greater pH allows for persistent pinking to occur in DC beef. In agreement, Sawyer et al. (2008) determined the cooked red intensity and a* values of DC steaks are more than normal-pH steaks. Persistent pinking was noted in this study as well. Therefore, the reduction of pH by acidification of DC steaks could improve cooked color through decreased stability of deoxymyoglobin. Sawyer et al. (2009) observed the enhancement of DC steaks with lactic acid at 0.5% resulted in similar L* values to normal-pH steaks as

shown in the current study. Injection of lactic acid at 0.5% or lower in DC steaks resulted in the a* values of enhanced DC steaks were not significantly different from normal-pH steaks and decreasing the persistent pinking of DC beef (Sawyer et al. 2008; Sawyer et al. 2009; Apple et al. 2011). Stackhouse et al. (2016) reported enhancement of DC steaks with citric acid at pH 3.5 resulted in a* values most similar to normal-pH steaks. Research has demonstrated cooked chroma (red intensity) decreased with acidification of DC beef with lactic acid enhancement, and the red intensity of cooked enhanced DC steaks was similar to the red intensity of cooked normal-pH steaks (Sawyer et al., 2008; Sawyer et al., 2009; Apple et al., 2011). The enhancement provided some relief to the persistent pinking of the DC steaks based on the decrease in a* values and chroma of the enhanced steaks. Dark-cutting steaks have been noted to have lower hue values and more red cooked color than normal-pH steaks (Sawyer et al., 2008). In support of acidification improving hue of DC steaks, Sawyer et al. (2009) and Apple et al. (2011) determined low levels of lactic acid can improve the hue of enhanced DC steaks to be similar to normal-pH steaks resulting in a less red cooked color. Lactic acid enhancement has been observed to increase the percent of denatured myoglobin similar to the percent of denaturation of normal-pH steaks and greater than the percent denatured in DC steaks (Sawyer et al., 2008; Sawyer et al., 2009). Sawyer et al. (2008) and Sawyer et al. (2009) attributed the increase in denaturation to the decrease in pH by the acidification of the DC beef. Therefore, an increase in denaturation could explain the cooked color changes seen in the DC steaks after acidification by GDL and concurrent pH decline in this study. Therefore, the enhancement provided some improvement to the persistent pinking of DC steaks, but the cooked color of DCE steaks was not equivalent to the normal-pH steaks.

Visual cooked color

Six panelists evaluated the internal and external cooked color on a 7-point scale [(1 = Very red, 7 = Tan/brown; 1 = Brown, 7 =Pinkish-red, respectively)]. Normal-pH had a slightly pink internal cooked color and was a similar ($P > 0.05$) internal cooked color to the DCE steaks (Table 4.6). Acidification of DC steaks using weak organic acids has improved the visual internal cooked color compared to nonenhanced DC steaks (Sawyer et al., 2008; Sawyer et al., 2009; Apple et al., 2011). High concentrations of lactic acid resulted in more brown internal color for enhanced DC steaks than normal-pH steaks (Sawyer et al., 2008; Sawyer et al., 2009); however, 0.25-0.5% lactic acid enhancement of DC steaks resulted in similar visual cooked color scores as normal-pH steaks (Sawyer et al., 2009; Apple et al., 2011). A similar reduction in a* values of DCE steaks at 0.5% GDL was reported in this study. The external cooked color of the DCE steaks was significantly pinker than the DCN and normal-pH steaks with the external color indicated as pinkish-brown. Claus and Du (2013) and Song et al. (2015) determined steaks packaged in nitrite systems had greater a* values for external cooked color compared to steaks without nitrite. Visual color scores noted steaks sprayed with nitrite solutions increased surface redness compared to steaks sprayed with water (Song et al., 2015). This corresponded with their findings of an increase in nitrosylhemochrome on the surface of cooked (Song et al., 2015). Nitrosylhemochrome is the pigment responsible for the pink color of cured meat (Macdougall et al., 1975). The formation of this pigment can explain the increase in cooked surface pink color based on visual color scores of NEP steaks.

Sensory

The Warner-Bratzler shear force (WBSF) data indicates no difference ($P > 0.05$) between enhancements for peak compression (Table 4.7). Six trained panelists evaluated the steaks for tenderness, juiciness and flavor attributes (Table 4.8). Panelists detected no difference ($P > 0.05$) in juiciness or tenderness between enhancements with tenderness agreeing with the WBSF results. Overall, all enhancements were considered between slightly tough/dry and slightly tender/juicy by the panel. Song et al. (2015) determined nitrite spray had no impact on tenderness and juiciness of normal-pH steaks based on focus group results. Research has indicated a curvilinear relationship between pH and tenderness (Wulf et al., 2002; Holdstock et al., 2014; Grayson et al., 2016). Grayson et al. (2016) reported DC steaks with a pH between 6.1-6.4 to be less tender than normal-pH steaks and DC steaks with a pH above 6.4 to be more tender than normal-pH steaks based on a trained sensory panel. Slice shear force indicated steaks with a pH above 6.4 were not different in tenderness from normal-pH steaks, and steak with a pH of 6.1-6.4 had greater slice shear force than normal-pH steaks (Grayson et al., 2016). In support of the research presented, Holdstock et al. (2014) determined the shear force and panel tenderness of normal-pH and DC steaks ($pH > 6.0$) were not significantly different from each other. However, atypical DC steaks ($pH < 6.0$) had a greater shear force and decreased tenderness compared to normal-pH and DC steaks ($pH > 6.0$; Holdstock et al., 2014). Wulf et al. (2002), in agreement, observed the pH range of 5.8-6.0 to be less tender than normal-pH steaks; they also determined the DFD *longissimus* steaks ($pH = 6.0$) had more shear force and decreased tenderness than normal-pH *longissimus* steaks by a trained panel. Past research by Apple et al. (2011) supports the results of this study.

They noted no difference in shear force values of DC steaks (pH = 6.38) and normal-pH steaks as well as DC steaks enhanced with lactic acid. Therefore, pH plays an important role in the tenderness of DC steaks. While the mean pH of DC loins in this study was 6.38, the range of pH in the DC loins selected (6.1-6.7) could have increased the variability of tenderness of the DCN steaks. In agreement, Wulf et al. (2002) noted to be more variability in tenderness of the DC steaks compared to the than normal-pH steaks. This could have resulted in the lack of difference in shear force and tenderness between normal-pH and DCN steaks seen in the current study. While the enhancement present in this study has resulted in a decline in pH over time for DCE steaks, the amount of decline has been shown to be variable resulting in final pH values near normal-pH steaks (5.6), in the high toughness range (5.8-6.4), and in a more tender range for DC steaks (above 6.4). The impact on final pH could influence the variability in shear force and tenderness seen. The variability in pH could explain the lack of difference among DCE, DCN, and normal-pH steaks. Dransfield (1981) and Wulf et al. (2002) determined there was no difference in juiciness for DC beef and normal-pH beef by a trained panel. Holdstock et al. (2014) reported juiciness among DC steaks (pH > 6.0), atypical DC steaks (pH < 6.0), and normal-pH steaks was similar as seen in this study. It is important to note that Viljoen et al. (2002) observed consumers could taste no difference in texture or juiciness of DC beef versus normal-pH beef.

Previous research on nitrite spray impact on steak flavor determined the nitrite had limited impact on the overall flavor of the samples (Song et al., 2015), and preliminary trials in our lab reported no flavor differences between DC steaks packaged in NEP and DC packaged in vacuum packaging. Therefore, the effect of NEP on sensory

was not evaluated. Nonenhanced DC steaks had significantly greater beefy flavor compared to the DCE steaks. Enhanced DC steaks had the highest sour flavor ($P < 0.05$). The decline in beef flavor and increase in sour flavor for the DCE steaks could be attributed to the flavor created by the addition of the enhancement. Glucono delta-lactone has been used as an acidulent in a variety of processed meats. In pickled goat, GDL has been shown to provide more of an acceptable sour flavor compared to the control without GDL (Gogna and Kumar, 2017). However, in bologna, which is not known for a natural sour flavor, GDL resulted in lower taste acceptability of bologna compared to the control samples (Qvist et al., 1994). In water, GDL hydrolyzes into gluconic acid (Combes and Birch, 1988; Parke et al., 1997), and the formation of gluconic acid gives a sour flavor (Parke et al., 1997). Trained panel data unpublished from our lab has indicated DC steaks enhanced with rosemary has greater sour flavor, more off flavor, and less beefy flavor than DCN steaks. Rosemary addition to ground beef patties also has been considered less acceptable in taste compared to control patties (Hashemi Gahruie et al.; Gibis and Weiss, 2012). Therefore, the increase in sour flavor of DCE steaks could be correlated to the sour flavor of GDL and partially to rosemary. The metallic flavor in the normal-pH beef was greater ($P < 0.05$) than in the DCN steaks. However, Grayson et al. (2016) and Yancey et al. (2005) determined no difference in metallic flavor between DC beef and normal-pH beef. Past research indicates that normal-pH steaks have significantly greater beef flavor than DC steaks (Yancey et al., 2005; Apple et al., 2011; Holdstock et al., 2014). Although, the beefy flavor of normal-pH steaks was similar to DCN steaks in this study. The metallic flavor could be impacting the beefy flavor of the normal-pH steaks

resulting in no difference ($P > 0.05$) between normal-pH steaks and DCN steaks for beefy flavor.

Conclusion

Nitrite-embedded packaging has the ability to improve retail display color of DC beef. Inclusion of GDL improved cooked color and did not detrimentally affect the retail color of DC beef. However, GDL resulted in increased sour flavor, and NEP resulted in a pinker external cooked color of steaks. In conclusion, the combination of NEP and acidification can be utilized to improve retail color and cooked color of DC steaks. Further research should look to reduce cooked color effects of NEP and flavor impacts of GDL.

Table 4.1. Proximate composition (%) and pH of normal-pH and dark-cutting strip loins (n = 10)

Component	Normal-pH	Dark-cutting beef	SEM
pH	5.42[a]	6.38[b]	0.03
Moisture	69.46[a]	72.61[b]	0.43
Fat	4.88[a]	3.35[a]	0.46
Protein	23.38[b]	22.38[a]	0.18

[ab]Least squares means with different letters are significantly different ($P < 0.05$).

Table 4.2. Effects of display day on the nitric oxide myoglobin formation[1] in the enhanced dark-cutting steaks packaged in nitrite-embedded packaging (n = 10)

Days of retail display	Nitric oxide myoglobin formation
0	3.32[a]
1	6.71[b]
2	6.55[b]
3	7.53[c]
4	6.67[b]
5	6.60[b]
6	6.88[bc]
SEM = 0.20	

[a-c]Least squares means with different letters are significantly different ($P < 0.05$).
[1]Nitric oxide myoglobin formation was calculated as the ratio of R650 ÷ R570 nm. A greater number indicates more nitric oxide formation.

Table 4.3. Effects of enhancement[1] on thiobarbituric acid reactive substances (n =10)

Enhancement	mg MDA/kg
Normal-pH	0.74[b]
DCN	0.40[a]
DCE	0.35[a]
SEM = 0.03	

[ab]Least squares means with different letters are significantly different ($P < 0.05$).
[1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Table 4.4. Effects of enhancement[1] on microbial growth (n =10)

Enhancement	Log(CFU/cm^2)
Normal-pH	0.61[a]
DCN	4.30[b]
DCE	3.55[b]
SEM = 0.23	

[ab]Least squares means with different letters are significantly different (P < 0.05).
[1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Table 4.5. Least squares means for instrumental cooked color attributes (enhancement[1]) of steaks stored in dark storage for 3 d (n = 10)

Enhancement	L* values	a* values	b* values	Hue	Chroma
Normal-pH	57.84[b]	24.40[a]	23.57[a]	44.15[b]	33.95[a]
DCN	53.30[a]	31.72[c]	26.30[b]	39.62[a]	41.23[b]
DCE	56.26[ab]	28.14[b]	24.93[ab]	41.60[a]	37.61[ab]
SEM	0.87	0.84	0.64	0.50	1.00

[a-c]Least squares means with different letters are significantly different (P < 0.05).
[1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Table 4.6. Least squares means for visual cooked internal[1] and external cooked color[2] (enhancement[3]) of steaks stored in dark storage for 3 d (n = 10)

Enhancement	ICC	ECC
Normal-pH	4.33[b]	1.54[a]
DCN	2.09[a]	1.68[a]
DCE	4.03[b]	4.92[b]
SEM	0.18	0.10

[ab]Least squares means with different letters are significantly different (P < 0.05).
[1]Internal cooked color (ICC), 1 = very red, 2 = slightly red, 3 = pink, 4 = slightly pink, 5 = pinkish-grey, 6 = greyish tan/brown, 7 = tan/brown
[2] External cooked color (ECC), 1 = brown, 2 = light brown, 3 = slightly brown-ish red, 4 = reddish-brown, 5 = pinkish brown, 6 = slightly pinkish-red, 7 = pinkish-red
[3]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Table 4.7. Least square means for Warner-Bratzler shear force by enhancement[1] main effect (n = 10)

Enhancement	WBS (kg)	SEM
Normal-pH	4.50	0.39
DCN	4.11	0.39
DCE	5.09	0.39

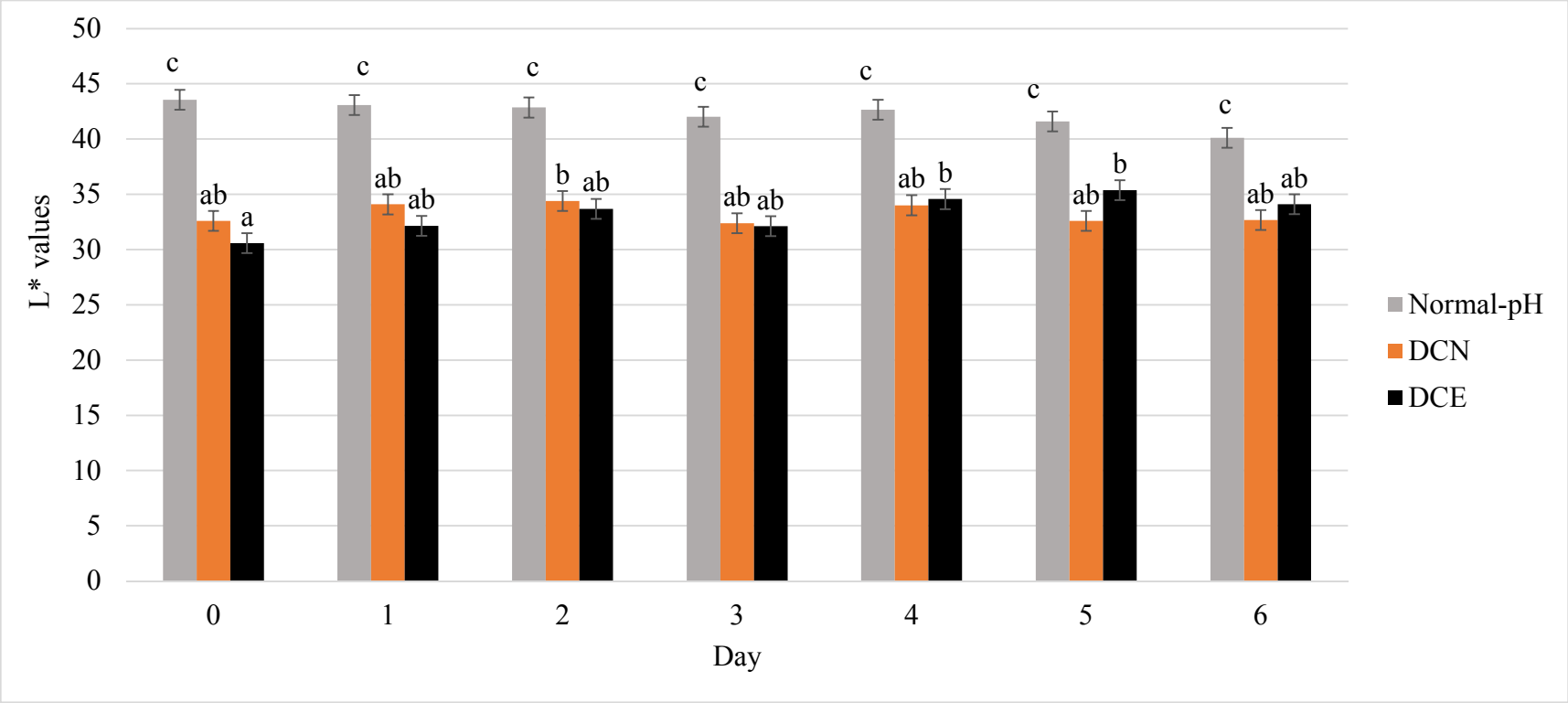
[1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution (DCE).

Table 4.8. Least squares means of trained panelists' scores[1] of beef palatability attributes by enhancement[2] main effect (n = 9)

Enhancement	Tenderness	Juiciness	Beefy flavor	Sour	Metallic
Normal-pH	4.78	4.81	1.83^{ab}	1.35^{a}	1.68^{b}
DCN	5.94	5.18	2.15^{b}	1.22^{a}	1.24^{a}
DCE	4.54	4.57	1.50^{a}	1.83^{b}	1.37^{ab}
SEM	0.35	0.32	0.14	0.12	0.11

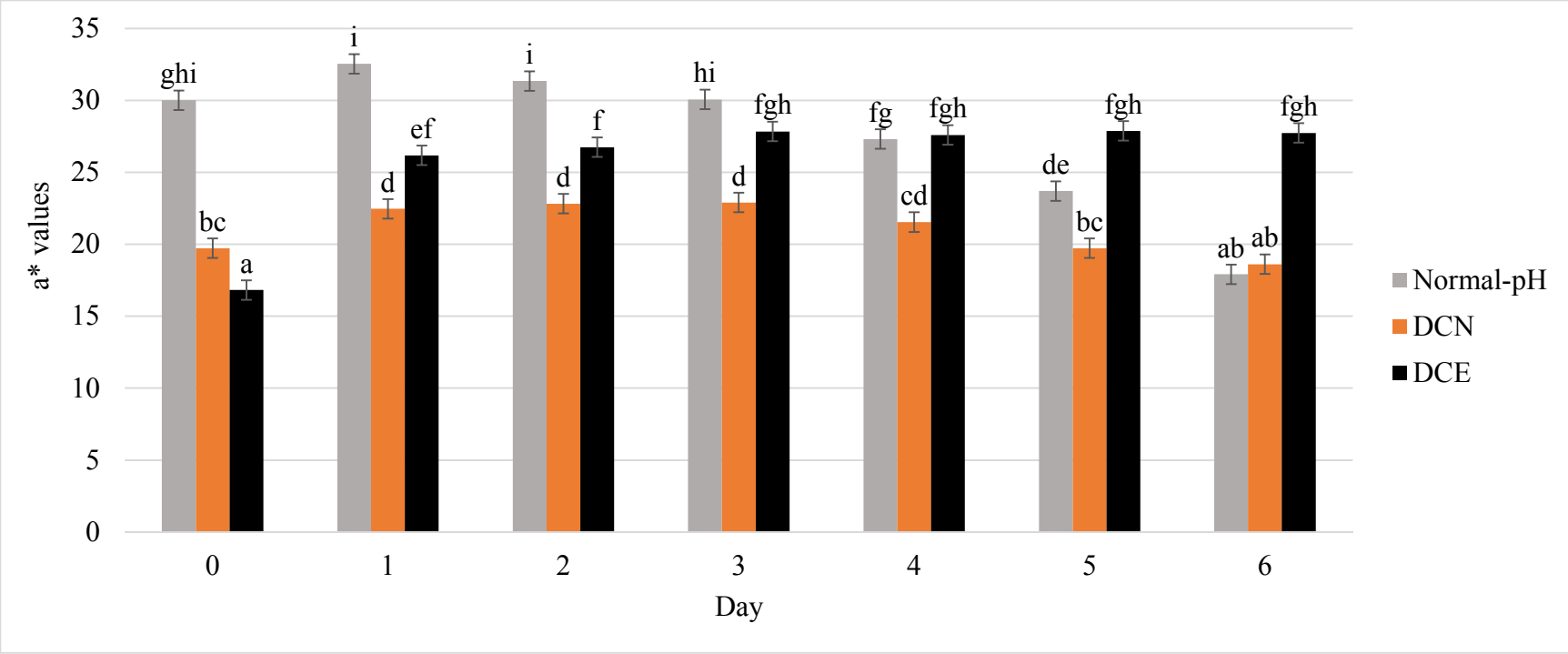
[ab]Least squares means with different letters are significantly different ($P < 0.05$).
[1]Trained panelists used a 3-point scale (1 = not detectable, 3 = strongly detectable) for beefy, sour and metallic flavor. For tenderness and juiciness, trained panelists used a 8-point scale (1 = extremely tough, 8 = extremely tender; 1 = extremely dry, 8 = extremely juicy).
[2]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution (DCE).

Figure 4.1. Least squares means of L* (enhancement × day) of steaks during a 6-d retail display (n = 10).



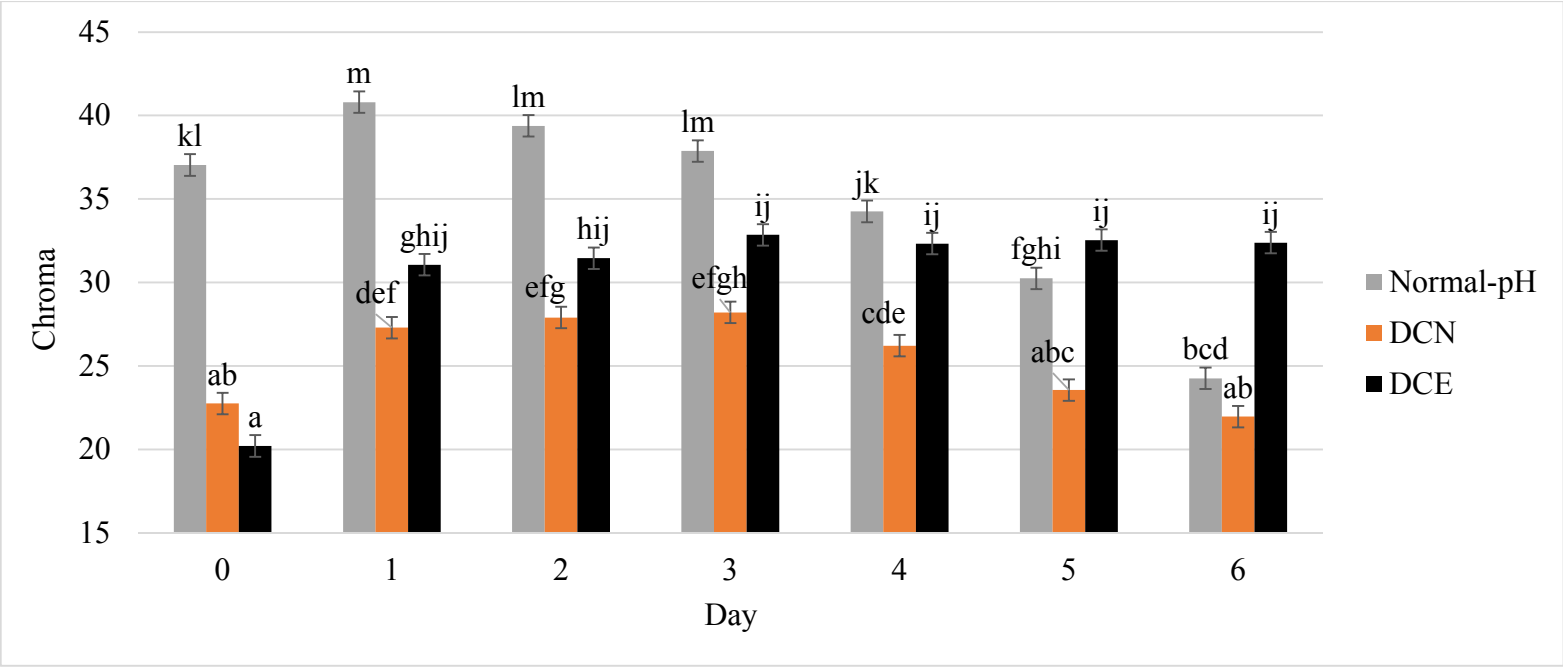
Least squares means with different letters (a-c) are significantly different (P < 0.05). Standard error of the mean indicated by error bars (SEM = 0.90). [1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Figure 4.2. Least squares means of a* (enhancement[1] × day) of steaks during a 6-d retail display (n = 10).



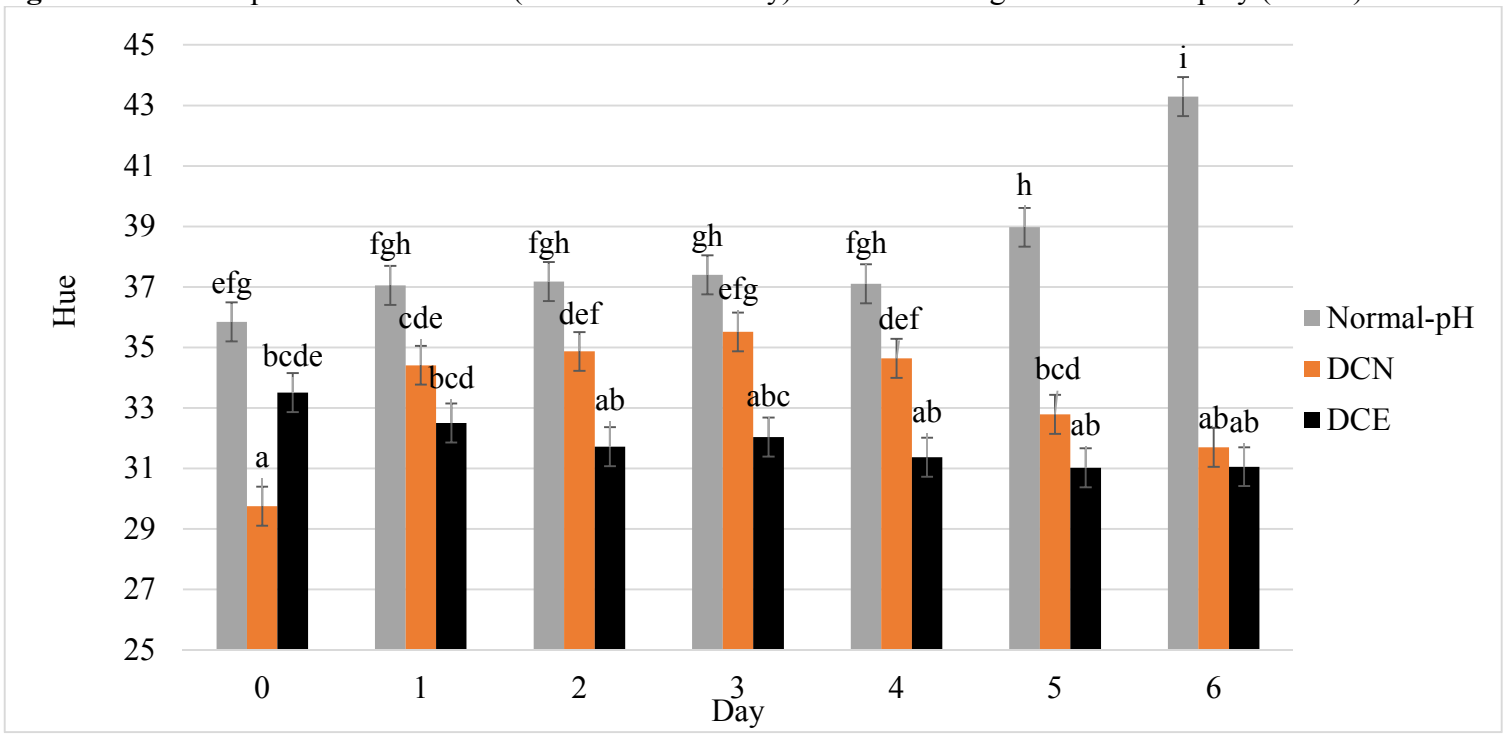
Least squares means with different letters (a-i) are significantly different ($P < 0.05$). Standard error of the mean indicated by error bars (SEM = 0.68). [1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Figure 4.3. Least squares means of chroma (enhancement[1] × day) of steaks during a 6-d retail display (n = 10).



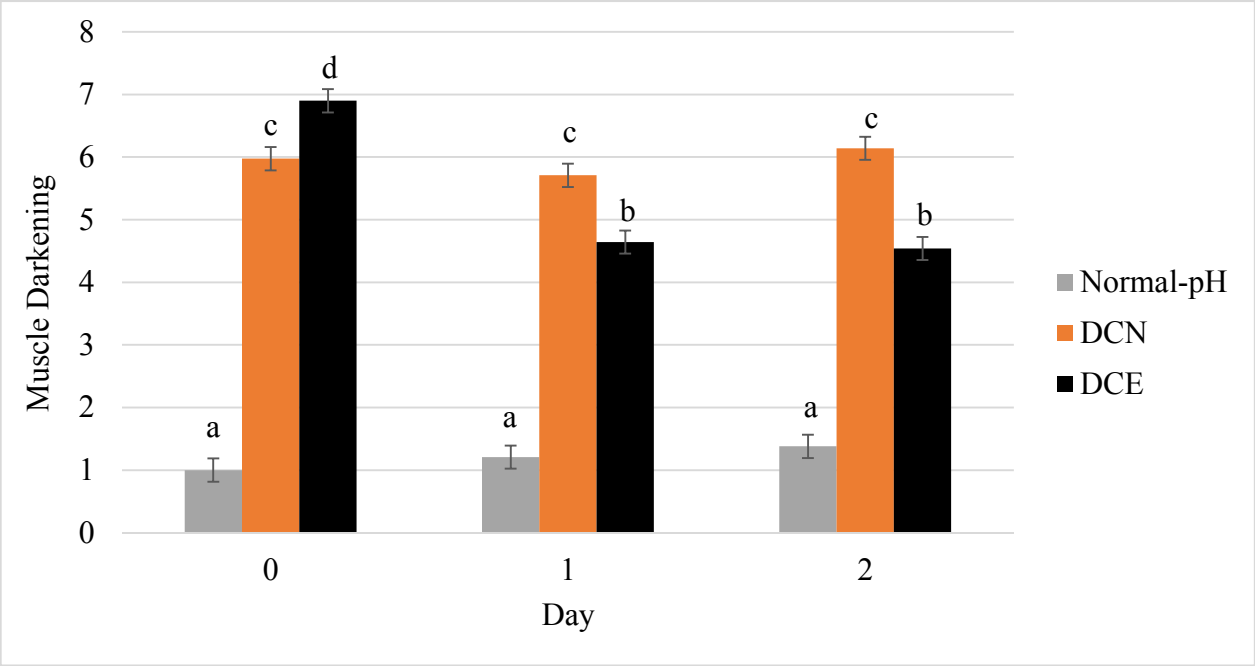
Least squares means with different letters (a-m) are significantly different (P < 0.05). Standard error of the mean indicated by error bars (SEM = 0.85). [1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Figure 4.4. Least squares means of hue (enhancement[1] × day) of steaks during a 6-d retail display (n = 10).



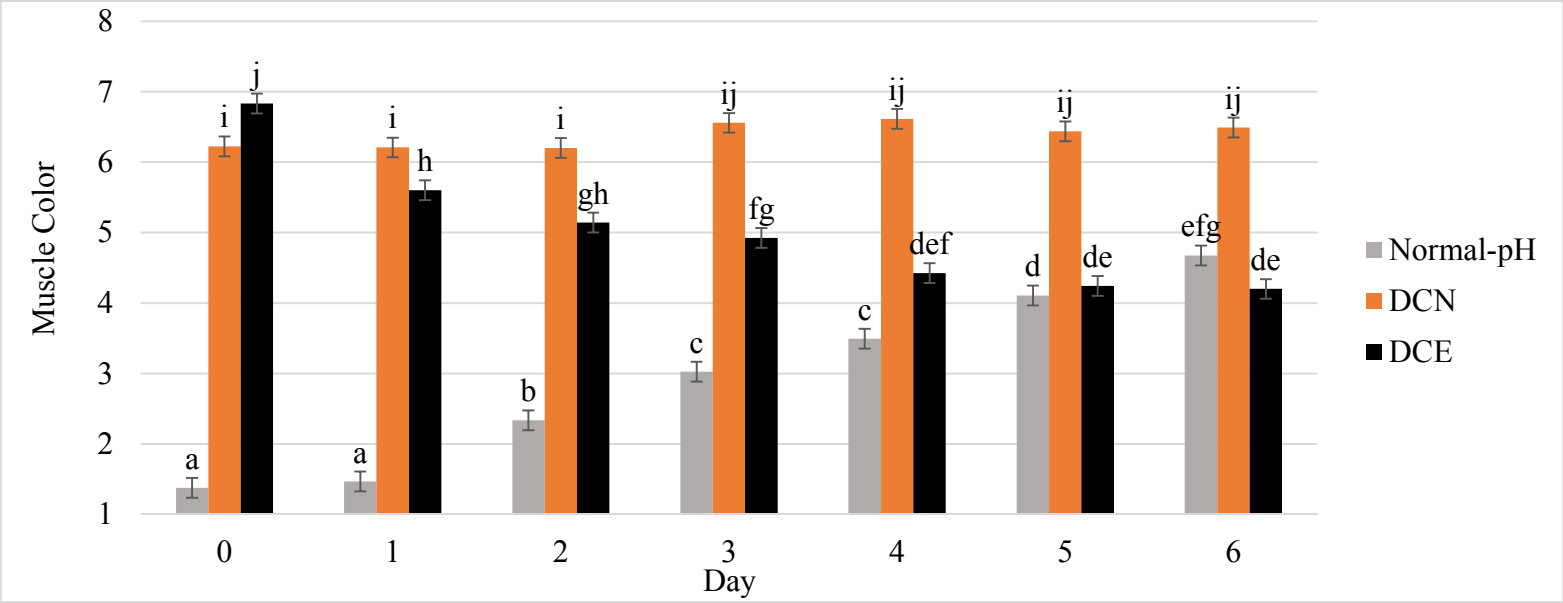
Least squares means with different letters (a-i) are significantly different ($P < 0.05$). Standard error of the mean indicated by error bars (SEM = 0.64). [1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE).

Figure 4.5. Effect of enhancement[1] and retail day on muscle darkening[2] of steaks during a 6-d retail display (n = 10).



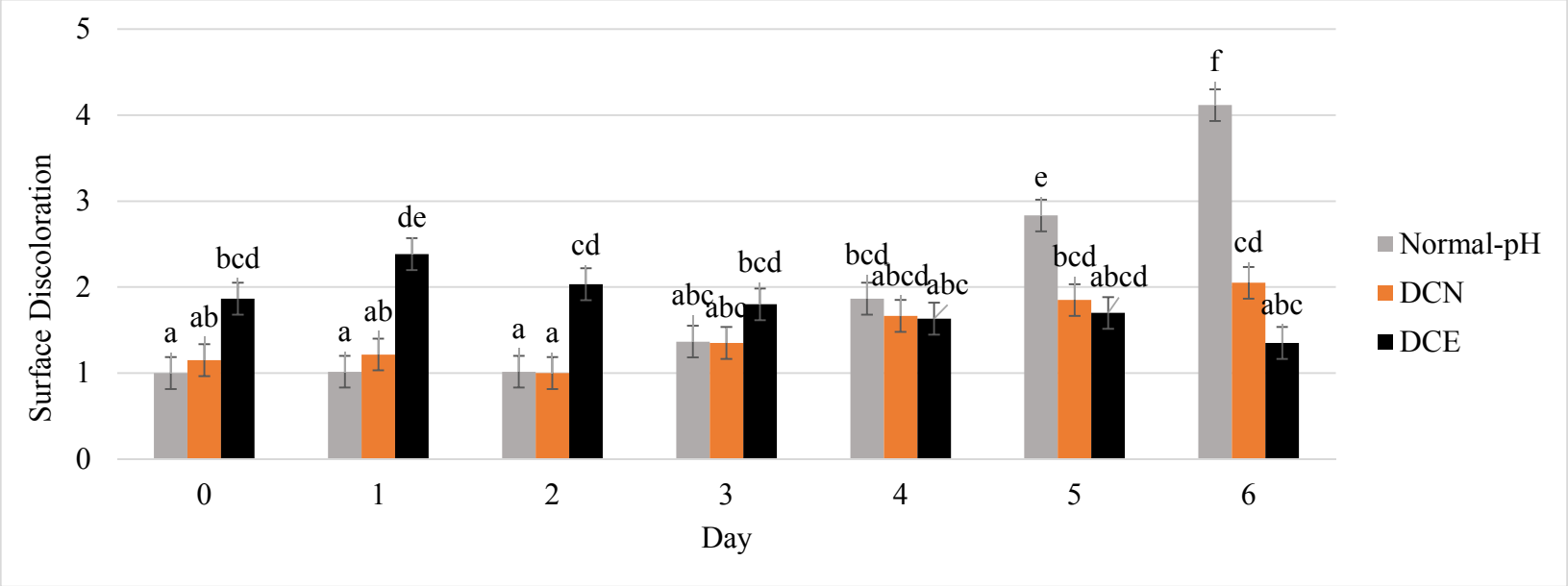
Least squares means with different letters (a-d) are significantly different (P < 0.05). Standard error of the mean indicated by error bars (SEM = 0.16). [1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE). [2]1 = no darkening, 7 = very dark

Figure 4.6. Effect of enhancement[1] and retail day on muscle color[2] of steaks during a 6-d retail display (n = 10).



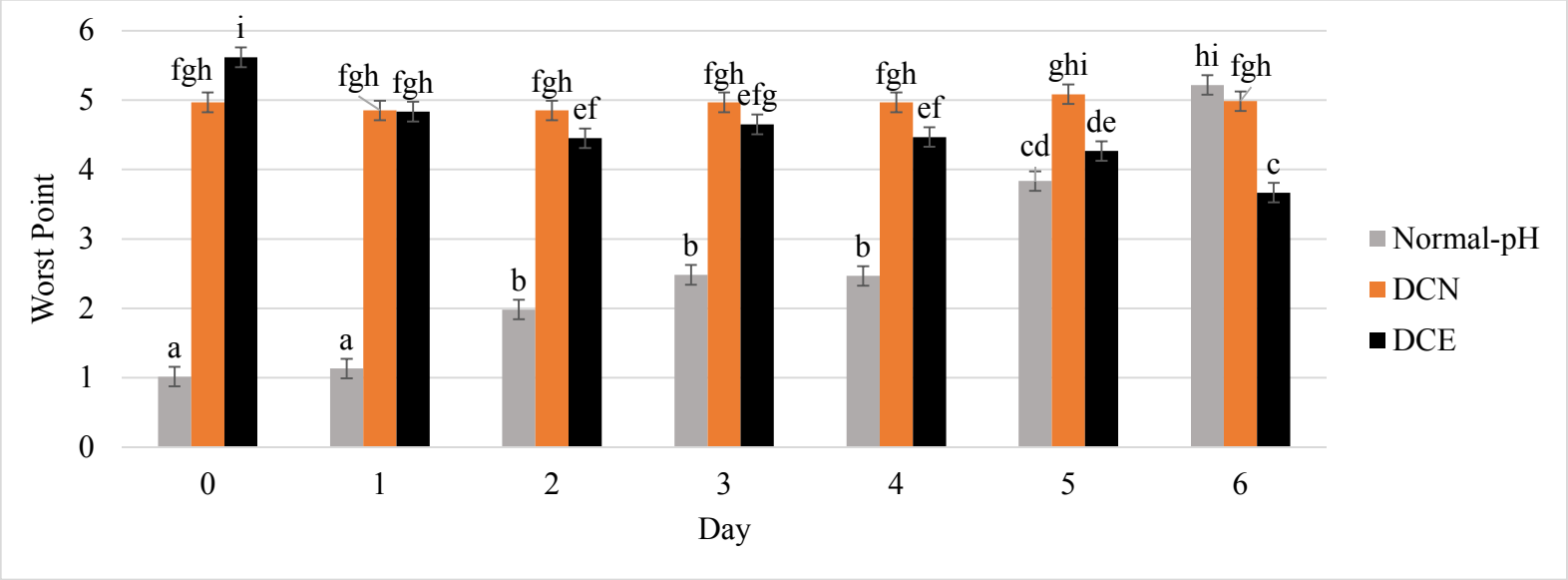
Least squares means with different letters (a-j) are significantly different (P < 0.05). Standard error of the mean indicated by error bars (SEM = 0.14). [1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE). [2]1= extremely bright cherry-red, 7 = dark red

Figure 4.7. Effect of enhancement[1] and retail day on surface discoloration[2] of steaks during a 6-d retail display (n = 10).



Least squares means with different letters (a-f) are significantly different (P < 0.05). Standard error of the mean indicated by error bars (SEM = 0.19). [1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE). [2]1= no discoloration, 7 = extensive discoloration

Figure 4.8. Effect of enhancement[1] and retail day on worst-point color[2] of steaks during a 6-d retail display (n = 10).



Least squares means with different letters (a-i) are significantly different (P < 0.05). Standard error of the mean indicated by error bars (SEM = 0.14). [1]Enhancements include normal-pH USDA Low Choice strip loin, non-enhanced dark-cutting strip loin (DCN), and dark-cutting strip loin enhanced with 0.5% glucono delta-lactone and 0.1% rosemary solution and in nitrite-embedded packaging (DCE). [2] Worst point was a randomly selected 2.54 × 2.54 cm^2 square on the surface of the steak using the scale: 1 = bright cherry-red, 7 = grey

CHAPTER V

CONCLUSION

The appearance of fresh dark-cutting beef leads to carcass discounts and negative perceptions by consumers. Therefore, improvement of the visual color of dark-cutting beef in the retail case can result in less economic loss for the beef industry and more consumer acceptance. Dark-cutting beef also has negative implications on the cooked color and palatability of beef. Enhancement of dark-cutting loins by glucono delta-lactone and rosemary increased surface redness and lightness while in nitrite-embedded packaging. However, repackaging enhanced dark-cutting steaks in traditional PVC for retail display resulted in lower color stability and decline in red appearance. The acidification of dark-cutting steaks resulted in a decline in pH and improvement of red internal cooked color compared to non-enhanced dark-cutting steaks. Sensory evaluation of enhanced dark-cutting steaks determined the enhancement had some negative implications on flavor. Future studies should look to extend the color stability of enhanced dark-cutting steaks outside of nitrite-embedded packaging and increase the palatability of the enhanced steaks by possible further enhancement.

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