# EFFECTS OF HIGH-PRESSURE PROCESSING ON DARK-CUTTING BEEF COLOR

By

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EFFECTS OF HIGH-PRESSURE PROCESSING ON

DARK-CUTTING BEEF COLOR

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Abstract: The objective was to evaluate the effects of high-pressure processing (HPP) levels on retail color, cooked color, and sensory attributes of dark-cutting beef. Eight USDA Choice (mean pH = 5.5; normal pH beef) and twelve dark-cutting (mean pH = 6.3) strip loins were obtained from a commercial packing plant within 2 d of harvest. Loins were cut into equal sections, vacuum packaged, and randomly assigned to HPP treatment of 0 (no HPP), 300, 450, and 600 megapascals (MPa). Following 48 hours of dark storage at 2°C, loin sections were cut into 1.9 cm thick steaks, placed in Styrofoam® trays overwrapped in polyvinyl chloride (PVC) film, and placed in a simulated retail display for 8 d. Remaining steaks were vacuum packaged and frozen to evaluate cooked color and sensory attributes. The surface color readings were measured every 24 hours using a HunterLab MiniScan XE Plus spectrophotometer, while a trained color panel (n = 6) evaluated discoloration, paleness, and lean color on steaks. Oxygen consumption (OC), metmyoglobin reducing activity (MRA), and lipid oxidation were evaluated on d 0, 4, and 8 of retail display. Frozen steaks were thawed and cooked to an internal temperature of 68°C and tempered to 71°C. After cooking, steaks were randomly assigned for external color measurements using a HunterLab MiniScan spectrophotometer and Warner-Bratzler shear force measurements. A trained sensory panel (n = 6) evaluated initial juiciness, sustained juiciness, tenderness, beef flavor intensity, and overall acceptability. The data were analyzed using the Glimmix Procedure of SAS. There was a significant HPP level × day of retail display interaction for all instrumental color measurements. Throughout the retail display, $L^*$ values 450 and 600 MPa treated steaks were greater ($P < 0.05$) than 300 MPa and controls. When panelists evaluated lean color and discoloration, there was a significant pressure level × day of retail display interaction. Steaks treated at 300 MPa exhibited brighter red color and lower thiobarbituric acid reactive substance values than other pressure levels and normal pH control steaks ($P = 0.0023$). HPP did not affect ($P > 0.05$) initial juiciness, sustained juiciness, beef flavor intensity, or overall acceptability. High pressure had an impact ($P < 0.05$) on external cooked color. There was no difference in redness ($a^*$) and red intensity (chroma) between HPP treated steaks and DC control steaks. In conclusion, low (300 MPa) and moderate (450 MPa) pressure levels did not affect sensory attributes nor impart a paler color to cooked steaks. The results indicate that 300 MPa can improve the redness of dark-cutting beef without affecting other quality parameters.

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# CHAPTER I

# INTRODUCTION

Meat color is the single most important quality factor influencing consumer purchasing (Mancini and Hunt, 2005). Therefore, meat color plays a vital role in consumer purchasing decisions (Carpenter et al., 2001). When purchasing beef, consumers often believe a bright-cherry red color represents a more wholesome and fresher product (AMSA, 2012). Any deviations from a bright-cherry red product can lead to fewer purchases and more food waste. Meat color is influenced by myoglobin and its interactions with small biomolecules (Suman and Joseph, 2013). Myoglobin contains a heme ring with six binding sites. The ligand-bound at the sixth binding site and redox state of heme determines the color of meat (AMSA, 2012). The consumer preferred bright-cherry red color occurs when oxymyoglobin is formed from the binding of oxygen to the sixth coordination site (Aberle et al., 2012). In anaerobic conditions, a purple color occurs when deoxymyoglobin is formed.

Dark-cutting (DC) beef is a color deviation from the characteristic bright-red appearance of beef. DC beef is known for its decreased redness and darker appearance (Wulf et al., 2002; Galloway et al., 2005; English et al., 2016; Hughes et al., 2017; Denzer et al., 2020). Due to its darker appearance, DC beef is often discriminated by consumers and leads to discounted prices and economic losses to the beef industry

(Viljoen et al., 2002). The current knowledge of DC beef indicates that pre-harvest stress leads to glycogen reserves being depleted prior to harvest, resulting in less lactic acid formation postmortem and an ultimate pH greater than normal (Lawrie, 1958; Newton and Gill, 1981; Scanga et al., 1998). The darker appearance of DC beef is a result of its higher postmortem muscle pH (Hughes et al., 2017). The higher pH allows more mitochondrial oxygen consumption, leaving less oxygen available for the myoglobin (McKeith et al., 2016). As there is less available oxygen, the formation of deoxymyoglobin increases (English et al., 2016). More deoxymyoglobin formation results in a darker surface color appearance and less desirability from consumers (Hughes et al., 2017).

High-pressure processing (HPP) is a non-thermal food processing technology that has been utilized in the food industry to sterilize and pasteurize products. HPP utilizes water as a liquid-pressure transmitting medium allowing pressure to be transmitted uniformly throughout the product. This pressure imparted by HPP results in protein denaturation and enzyme inactivation (San Martin et al., 1998). The effect of HPP on a product depends on factors such as protein susceptibility, applied pressure, and temperature, and time treated (Sun & Holley, 2010). In ready-to-eat (RTE) meat products, HPP is typically utilized at 600 MPa for a holding time of 3 minutes at refrigerated temperatures. The unfolding of muscle proteins occurs at pressures up to 300 MPa. Pressures higher than 300 MPa can result in increased denaturation and gel formation (Bajovic et al., 2012). As a result, the application of HPP to meat and meat products can influence parameters like color, texture, and water-holding capacity (Bajovic et al., 2012). Due to the negative effects on color, there has been limited

research conducted on post-rigor meat; however, research does indicate HPP increases *L** values and brightness of normal-pH beef. Dark-cutting beef has greater muscle pH, and the stability of proteins, including myoglobin, will be more than normal muscle pH. However, no research has determined the effects of HPP on dark-cutting beef color. We hypothesize that HPP will induce structural changes, increase oxygen diffusion, and increase redness in DC beef. The objective of this study was to evaluate different HPP levels on DC beef to examine changes in retail color, cooked color, and sensory attributes.

# CHAPTER II

# REVIEW OF LITERATURE

## Meat Color

Color influences consumer purchasing more than any other quality factor when purchasing meat (Mancini and Hunt, 2005). Consumers associate a bright cherry red color in beef as an indicator of wholesomeness and freshness (Carpenter et al., 2001; Suman and Hunt, 2014). The color of meat is influenced by myoglobin and its interactions with small biomolecules (Aberle et al., 2012). Myoglobin is a water-soluble protein with an iron-containing heme ring. The iron atom on the heme ring can form six bonds. Four of the bonds are with pyrrole nitrogens and the fifth bonds with the histidine. The sixth site remains available to reversibly bind ligands (Mancini and Hunt, 2005). Meat color is determined by the valence of iron and the presence or absence of ligand at the sixth site. Myoglobin can exist in four redox states: deoxymyoglobin, oxymyoglobin, carboxymyoglobin, and metmyoglobin (AMSA, 2012). Deoxymyoglobin results in a purplish-red color that occurs in low oxygen conditions when there is no ligand at the sixth coordination site of the heme iron of myoglobin (Mancini and Hunt, 2005). This purplish-red color is closely associated with muscle immediately after cutting and vacuum packaged products. Oxygenation occurs when myoglobin is exposed to oxygen resulting in diatomic oxygen bound to the sixth coordinate site of heme iron (Suman and Joseph, 2013). After oxygenation, the consumer preferred bright cherry-red

color is formed due to the formation of oxymyoglobin (Carpenter et al., 2001). Oxidation of the ferrous forms of myoglobin to ferric iron results in the formation of metmyoglobin. (Mancini and Hunt, 2005). Metmyoglobin contains a water molecule bound to the sixth coordinate of ferric heme and is unable to bind with oxygen. The presence of metmyoglobin results in a brown color that is strongly associated with discoloration (Suman and Joseph, 2013).

**Dark-Cutting Beef**

Dark-cutting (DC) beef is a color deviation from the characteristic bright-red appearance of beef. Dark-cutting beef has increased darkness and decreased redness in appearance (Wulf et al., 2002; Galloway et al., 2005; English et al., 2016; Hughes et al., 2017). Consumers often discriminate against DC beef because of its darker appearance (Viljoen et al., 2002). Due to consumer perception of DC beef, packers discount DC beef leading to more economic losses for the beef industry. In the Weekly National Carlot Report for August 23, 2021, the USDA Agricultural Marketing Services (AMS) reported the discount for DC beef to be $35.83 per CWT (100 lbs). According to the 2016 National Beef Quality Audit, approximately 1.9% of carcasses are considered DC carcasses (Boykin et al., 2017). Considering the discounts to dark-cutting carcasses, this condition accounts for a loss of $204.3 million to the US beef industry. The current knowledge implicates 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 is caused by many factors such as weather, management practices, and seasonality (Scanga et al., 1998). 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 that mitochondrial content is greater in DC beef than normal-pH beef (McKeith et al., 2016; Ramanathan et al., 2020). Greater mitochondrial activity leads to limited oxygen for myoglobin. Predominate deoxymyoglobin can lead to darker meat color (Ashmore et al., 1972; McKeith et al., 2016; English et al., 2016; Hughes et al., 2017).

The high pH of DC beef influences the muscle fibers and water holding capacity, resulting in changes to 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 a larger muscle fiber diameter and increases space for water binding (Hughes et al., 2017). In DC beef, Hughes et al. (2017) reported that a larger muscle fiber diameter increased the space between the scattering elements in the muscle. This leads to less light scattering and reflectance, resulting in less lightness of the muscle and a darker appearance (Hughes et al., 2017). In support, Lawrie (1958) determined the muscle swelling of DC beef results in a 'closed' structure. This closed structure results in less oxygen penetration and more deoxymyoglobin present (English et al., 2016; Hughes et al., 2017).

Several post-harvest techniques, such as acidification, increased oxygen content within a package, and carbon monoxide packaging, have been used to improve the redness of DC beef (Ramanathan et al., 2020). 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. Altering gas compositions within packaging can improve the appearance of high-pH meat

(Mitacek et al., 2018; Zhang et al., 2018). High oxygen and carbon monoxide-modified atmosphere packaging (HiOx-MAP and CO-MAP) has improved the redness of DC beef (Wills et al., 2017; Zhang et al., 2018). English et al. (2016) determined oxygen utilization is higher in DC steaks than in normal-pH steaks. Hence, the purpose of using MAP packaging on DC beef is to meet oxygen demand or form carboxymyoglobin to improve color.

**High pH and cooked meat qualities**

The high ultimate pH of DC beef negatively impacts the cooked color of beef products. The heating of normal-pH beef results in the denaturation of deoxymyoglobin, oxymyoglobin, or metmyoglobin, leaving a grey-cooked pigment called denatured globin ferrihemochrome (Fox, 1966). The higher ultimate pH of DC beef stabilizes myoglobin resulting in less myoglobin denaturation from cooking (Trout, 1989; Hunt et al., 1999). Less myoglobin denaturation will result in a persistent pink internal color at temperatures that would normally result in a grey-cooked appearance (Mendenhall, 1989; Trout, 1989). Persistent pinking found in higher-pH beef will negatively impact consumer perception as research has found consumers expect a grey cooked color in beef products (Cornforth et al., 1991). Research demonstrated that there is greater myoglobin content in high-pH beef than normal-pH beef (Moiseev and Cornforth, 1999; Sawyer et al., 2009; English et al., 2016; Hughes et al., 2017). Mendenhall (1998) concluded that the internal pink intensity of cooked high-pH ground beef patties increased with greater myoglobin concentration. Therefore, the higher myoglobin content as well as high pH of DC beef negatively influenced cooked color.

Research on tenderness in DC beef compared to normal-pH beef has been conflicting. The tenderness of fresh beef can be evaluated by different methods. Warner-Bratzler shear force (WBSF) is one method utilized to evaluate tenderness. In WBSF, cores are taken parallel to the muscle fiber orientation of the cooked meat. Shearing cores refer to the force per kilogram needed to cut through the core. A greater force means a tougher or less tender product. When tenderness was evaluated for different muscles of DC carcasses utilizing WBSF, muscles with pH between 5.7-6.0 were tougher than normal-pH (5.6) muscles evaluated, excluding the semitendinosus (Wulf et al., 2002). When tenderness was evaluated utilizing a trained sensory panel, DC beef was tougher than normal-pH beef (Wulf et al., 2002). When comparing different ultimate pH of dark cutters (pH of 6.1 – 6.9) utilizing the slice shear method, the pH between 6.1 - 6.4 beef was tougher (Grayson et al., 2016). In support, a trained sensory panel that evaluated DC steaks reported more tenderness in pH range of 6.7 - 6.9 than pH between 6.1 - 6.4. (Grayson et al., 2016). Based on the results from a trained sensory panel and WBSF, Grayson et al. (2016) reported a curvilinear relationship between pH and tenderness. A similar curvilinear relationship was reported by Watanabe et al. (1996). With respect to different muscles, Yu and Lee (1986) reported muscle from an intermediate pH (5.8-63) was tougher than muscle from low-pH (< 5.8) and high-pH (> 6.3) meat. This change in tenderness was reported to be a result of the pH-dependent proteolytic activity. Increased tenderness in low-pH muscle was reported to be linked to acidic proteases from the lysosome. In contrast, the improved tenderness of high-pH muscle was a result of containing more active neutral-pH calcium-dependent proteases (Yu and Lee, 1986). As a result, the tenderness of DC beef is pH-dependent. Hughes et al. (2017) reported a higher

pH will have increased water holding capacity as the greater pH will limit muscle shrinkage. Apple et al. (2011) utilized WBSF and reported no difference in tenderness between normal pH steaks and DC steaks.

Consumer acceptability research of DC beef is limited. In relation to flavor, a consumer panel found no significant difference when comparing fried DC beef steaks and fried normal-pH steaks (Viljoen et al., 2002). Although, trained panels that evaluated normal-pH and DC steaks observed more off-flavors associated with DC steaks (Wulf et al., 2002; Grayson et al., 2016). DC beef is more susceptible to microbial growth due to the greater ultimate pH. Bacterial growth favors a pH between 6.6 - 7.5 (Jay et al., 1998). Research has reported more spoilage bacteria growth in DC beef compared to normal-pH beef (Sutherland et al., 1977; Gill and Newton, 1979). Spoilage occurs in meat when bacteria attack amino acids. In aerobic conditions, bacteria are unable to attack amino acids until all glucose at the surface of meat is exhausted (Gill and Newton, 1979). Depleted glycogen levels in DC beef allow bacteria to utilize amino acids immediately, leading to a higher rate of spoilage in DC beef compared to normal-pH beef. Therefore, the deficiency in glycogen content as well as the high pH in DC beef results in more microbial growth.

**High-Pressure Processing**

High-pressure processing (HPP) is a non-thermal food processing technique. The food industry has utilized HPP to pasteurize foods such as beverages, seafood, and meat. The utilization of HPP ensures the inactivation and control of pathogenic bacteria while having minimal impacts on sensory quality and nutritional value (Bolumar et al., 2020). HPP is unique from non-thermal technologies as pressure is transmitted uniformly

through the medium and is independent of geometry (Plancken et al., 2008). The amount of HPP units in the industry is growing (Jung and Tonello-Samson, 2018). As the application of HPP increases, there have been improvements in productivity, processing costs, processing times, and energy consumption (Bolumar et al., 2020). HPP on meat products has become more common as meat products make up 25 to 30% of total high-pressure processed foods (Jung and Tonello-Samson, 2018). In the meat industry, HPP is mainly used to improve the food safety of ready-to-eat (RTE) meat products. The application of HPP to fresh meat will result in moderate to severe effects on meat appearance and other quality traits (Bolumar et al., 2020). Meat products such as hamburgers, poultry strips, oven-roasted chicken, and other fermented meats have been subjected to HPP (Bajovic et al., 2012). The efficacy of HPP on vegetative pathogenic and spoilage microorganisms in meat products depends on pressure, temperature, and holding time. Efficacy in meat products will also be affected by the product's ultimate pH, water activity, salt content, and the presence of antimicrobial compounds (Rendueles et al., 2011). Meat products are typically processed between 400 to 600MPa with a short processing time of 3 min at chilled temperatures to achieve greater than 4 log reductions for many vegetative pathogenic and spoilage microorganisms (Bolumar et al., 2020). However, these high-pressure levels will result in a change of visual color due to the denaturation of protein and oxidation of myoglobin in fresh meat. As a result, this undesirable color change has led to HPP being limited to processed meats (Warner et al., 2017). HPP induces protein denaturation leads to enzyme inactivation and dissolution of membrane-bound enzymes (San Martin et al., 2002). The induced enzyme inactivation leads to a breakdown of metabolic actions in biological systems (Kato and Hayashi,

1999). The processing conditions of pressure and temperature will impact the extent of microbial inactivation (Cheftel, 1995). The serotypes, physiological states, and characteristics of resistance to pressure will also affect the impact of HPP (Jofré et al., 2010). For pressure-resistant species, such as *Staphylococcus aureus* and *Escherichia coli*, research reports elevating temperature (>50 °C), adding antimicrobials, lowering pH, and creating anaerobic conditions by vacuum packaging can counteract these species (Garriga and Aymerich, 2009).

The application of HPP will result in pressure-induced changes to muscle proteins, oxidation reactions, and color. Protein denaturation is one of the important mechanisms for microbial inactivation, but the specific pressure levels used to reach inactivation will result in irreversible changes to the muscle protein. Research has observed different effects of HPP on different muscle structures. For instance, Cheftel and Culioli (1997) observed covalent bonds to be less sensitive to pressure changes and low compressibility, while hydrogen bonds were observed to be slightly strengthened under pressure. When meat was subjected to HPP, Rastogi et al. (2007) observed secondary structures to be sensitive to high-pressure levels (700 MPa), tertiary structures to be sensitive to pressure levels above 200 MPa, and quaternary structures to be sensitive to low pressures. Tenderization of meat by HPP is dependent on the processing conditions as well as the time postmortem when pressure is applied (Bolumar et al., 2020). The application of HPP at lower processing temperatures will increase the toughness of post rigor meat (Ma and Leward, 2004). However, HPP processing temperatures above 25°C can tenderize post rigor meat (Ma and Leward, 2004). Morton et al. (2017) observed improved tenderness when HPP was applied to prerigor beef at low

pressures. As previously mentioned, color is one of the most important factors that impact consumers' purchase intent (Mancini and Hunt, 2005). Research has indicated that HPP will cause drastic changes to fresh meat color (Ferrini et al., 2012). However, cured meat color is more stable to HPP (Bak et al., 2013). The pigment found in cured meat products, nitroslymyochromogen, is stabilized by HPP (Bak et al., 2013). As a result, more cured meat products utilize HPP than fresh meat products. The application of HPP on meat can increase lipid oxidation and lead to the presence of off-flavors. The research observed higher rates of lipid oxidation in fresh beef after HPP between 300 to 600 MPa (Ma et al., 2007; McArdle et al., 2010). The principle of the mechanisms in inducing lipid oxidation is not fully understood. Bajovic et al. (2012) speculated that membrane disruption could lead to HPP-induced oxidation. This disruption can facilitate the reaction between polyunsaturated lipids from the membrane with enzymes to catalyze lipid oxidation. HPP can also affect the myofibrillar proteins' structure leading to a modified texture due to altered properties of gel formation. Chan et al. (2011) observed functional and rheological properties of turkey meat at low pH could be improved with low-pressure treatment.

**Hypothesis and Objective**

We hypothesize that HPP will induce structural changes, increase oxygen diffusion, and increase redness in DC beef. The objective of this study was to evaluate different HPP levels on DC beef to examine changes in retail color, cooked color, and sensory attributes.

# CHAPTER III

# EFFECTS OF HIGH-PRESSURE PROCESSING ON COLOR STABILITY OF DARK-CUTTING BEEF


## Abstract

The objective of the study was to evaluate different HPP levels on the surface color of dark-cutting beef. Eight USDA Choice (mean pH = 5.5; normal pH beef) and twelve dark-cutter (mean pH = 6.3) strip loins were obtained from a commercial packing plant within 2 d of harvest. Loins were cut into equal sections, vacuum packaged, and randomly assigned to HPP treatment of 0 (no HPP), 300, 450, and 600 MPa. Normal-pH beef was not HPP treated and served as a control. After 48 hours of dark storage at 2°C, loin sections were cut into 1.9 cm thick steaks, placed in trays overwrapped in polyvinyl chloride (PVC), and placed in a stimulated retail display for 8 d. Remaining steaks were frozen and used to evaluate cooked color and sensory attributes. Surface color readings were measured every 24 hours using a HunterLab XE Plus spectrophotometer while a trained color panel (n = 6) evaluated discoloration, paleness, and lean color on steaks. Oxygen consumption (OC), metmyoglobin reducing activity (MRA), and lipid oxidation were evaluated on d 0, 4, and 8. Frozen steaks were thawed and cooked using a rationale oven to an internal temperature of 68°C and tempered to 71°C. After cooking, external color measurements using the HunterLab spectrophotometer and Warner-Bratzler shear

force was evaluated. A trained sensory panel (n = 6) evaluated initial juiciness, sustained juiciness, tenderness, beef flavor intensity, and overall acceptability. Data analysis was conducted using the Glimmix Procedure of SAS. Throughout the retail display, $L^*$ values of the higher-pressure levels (450 and 600 MPa) were greater ($P < 0.05$) than 300 MPa and controls. Steaks treated at 300 MPa exhibited lower thiobarbituric acid reactive substance values than other pressure levels and normal pH control steaks ($P < 0.05$). When examining the results of the sensory panel, HPP did not have an effect ($P > 0.05$) on initial juiciness, sustained juiciness, beef flavor intensity, or overall acceptability. There was no difference in redness, and red intensity between HPP treated steaks and DC control steaks. In conclusion, 300 MPa and 450 MPa pressure levels did not negatively affect the sensory attributes of cooked steak. The results indicate that 300 MPa can improve the redness of dark-cutting beef without affecting other quality parameters.
Keywords: DC beef, HPP, Meat color, MRA, OC, TBARS, Sensory panel, WBSF

## Introduction

Meat color is the single most important quality factor influencing consumer purchasing (Mancini and Hunt, 2005). Therefore, meat color plays a vital role in consumer purchasing decisions. When purchasing beef, consumers often believe a bright-cherry red color represents a more wholesome and fresher product (Suman and Hunt, 2014). Therefore, any deviations from a bright-cherry red product can lead to fewer purchases and food waste. Meat color is influenced by myoglobin and the interactions that occur with small biomolecules (Suman and Joseph, 2013). The consumer preferred bright-cherry red color occurs when oxymyoglobin is formed from the binding of oxygen

to the sixth coordination site (AMSA, 2012). Any deviation from the consumer-preferred color results in discounted carcass value and consumer acceptance.

Dark-cutting (DC) beef is a color deviation from the characteristic bright-red appearance of beef. DC beef is known for its decreased redness and darker appearance (Wulf et al., 2002; Galloway et al., 2005; Hughes et al., 2017). Due to its darker appearance, DC beef is often discriminated against by consumers and leads to discounted prices and economic losses to the beef industry (Viljoen et al., 2002). On April 4, 2022, the USDA AMS reported a discount on DC beef at $35.83 per CWT. The 2016 National Beef Quality Audit reported a 1.9% occurrence of dark cutters in the US. Therefore, the dark-cutting condition results in a $202.4 million loss due to discounted price (the number of heads slaughtered in 2020 was 32.8 million). The current knowledge of DC beef implicates that pre-harvest stress can cause DC beef. The pre-harvest stress leads to glycogen reserves being depleted prior to harvest, resulting in less lactic acid formation postmortem and an ultimate pH greater than normal (Lawrie, 1958; Newton and Gill, 1981; Scanga et al., 1998). The darker appearance of DC beef is a result of its greater than normal postmortem muscle pH (Hughes et al., 2017). More specifically, a greater muscle pH favors mitochondrial respiration and increased water holding capacity. Greater mitochondrial oxygen consumption leads to less available oxygen for myoglobin. Further, water greater holding capacity leads to muscle swelling; hence, less oxygen is diffused into the sub-surface (Hughes et al., 2017; Ramanathan et al., 2020). Both oxygen consumption and muscle swelling promote deoxymyoglobin and a darker color (McKeith et al., 2016; English et al., 2016). Various post-harvest techniques such as acid acidification, high-oxygen modified atmospheric packaging, carbon monoxide packaging,

and nitrite embedded packaging have been utilized to improve the color of dark-cutting beef (English et al., 2016; Denzer et al., 2020).

High-pressure processing (HPP) is a non-thermal food processing technology that has been utilized in the food industry to sterilize and pasteurize products. This pressure imparted by HPP results in protein denaturation and enzyme inactivation (San Martin et al., 1998). The effect of HPP on a product depends on factors such as protein susceptibility, applied pressure and temperature, and time treated (Sun & Holley, 2010). Implementation of HPP units in the food industry is constantly increasing as meat products currently represent about a quarter of HPP foods (Bolumar et al., 2020). The unfolding of muscle proteins occurs at pressures up to 300 MPa. Pressures higher than 300 MPa can result in increased denaturation and gel formation (Bajovic et al., 2012). As a result, the application of HPP to meat and meat products can lead to effects on quality parameters like color, texture, and water-holding capacity (Bajovic et al., 2012). Although HPP has been used in fresh beef and cooked products, limited studies have evaluated the effects of high-pH beef. We hypothesize that HPP will induce structural changes and increase oxygen diffusion into meat sub-surface and redness in DC beef. The objective of this study was to evaluate different HPP levels on DC beef color.

## Materials and Methods

### *Raw materials and processing*

Twelve DC strip loins (mean pH = 6.3, SEM = 0.08) and eight USDA Choice strip loins (mean pH =5.5, SEM = 0.09) were obtained from Greater Omaha Beef Packing (Omaha, NE) within 2 d of harvest. Strip loins were transported on ice to the meat laboratory at the University of Nebraska-Lincoln (Lincoln, NE). Upon arrival at the

University of Nebraska-Lincoln, strip loins were wet-aged for 5 d at 2°C. Each loin served as a block. After aging, using an incomplete block design, strip loins were cut into three equal sections. The anterior and posterior loin sections were equally distributed among treatments. Only enough strip loin sections to create eight replicates were used. Strip loin sections were then vacuum packaged (Flair Flexible Packaging Corporation; 12 x 14 pouches; 5 mil thickness), and randomly assigned to treatment. Dark cutting control samples were not treated with HPP. Normal pH strip loin sections were only used as a control. Using an incomplete block, DC strip loin sections were randomly assigned to one of the following treatments: control, 300, 450, or 600 MPa. All strip loin sections were vacuum packaged and transported on ice to the Food Processing Center at the University of Nebraska-Lincoln.

***High Pressure Processing (HPP)***

A commercial HPP unit was utilized to apply pressure on DC strip loin sections (Hiperbaric 55, Hiperbaric USA, Miami, FL; 55 L vessel; 200 mm diameter inside the vessel; throughput of 270 kg/h) with water as the pressurizing medium. Sections were placed in bins packed with ice to regulate processing temperature. The processing fluid temperature was approximately 4 - 8°C. All sections were processed and held for 90 s at designated pressure level. The pressurization rate of the HPP unit was between 1 – 1.5 min. After HPP treatment, all strip loin sections were transported on ice to the Robert M. Kerr Food and Agricultural Products Center at Oklahoma State University (Stillwater, OK).

***Packaging and stimulated retail display***

Following 48 hours of dark storage at 2°C, strip loin sections were cut into 1.9 cm thick steaks. Steaks were placed in Styrofoam® trays and overwrapped with PVC (15,500-16,275 $cm^3$ O2/m2 /24 h at 23°C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film; Koch Supplies; Kansas City, MO). Steaks were then placed into a coffin-style display case under a continuous light-emitting diode (LED; Philips LED lamps; 12 watts, 48 inches, color temperature = 3,500°K; Phillips, China) at 2°C for 8 d.

***Raw Color analysis***

During retail display, the 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 three times, providing the CIE $L^*$, $a^*$, and $b^*$ values. The CIE $L^*$, $a^*$, and $b^*$ values and spectral readings from 400 to 700 nm determined the surface color. Chroma $\left[\sqrt{\left(a^{*2}+b^{2}\right)}\right]$ was determined using CIE $a^*$ and $b^*$ values, representing the red intensity of the color (AMSA, 2012). The CIE $a^*$ and $b^*$ were used to determine the hue angle $\left(\tan^{-1}\left(\frac{b^*}{a^*}\right)\right)$ representing the color present (AMSA, 2012). Visual Color was examined using a panel of 6 trained panelists (n=6). All panelists passed the Farnsworth Munsell 100-hue test. On d 0, 1, and 2, panelists used a 6–point scale (1 = very dark red, 2 = dark red, 3 = red, 4 = slightly pale, 5 = moderately pale, 6 = very pale) to evaluate paleness and an 8-point scale (1 = very bright red, 2 = bright red, 3 = dull red, 4 = slightly dark red, 5 = moderately dark red, 6 = dark red to dark reddish tan, 7 = tannish red, 8 = tan

to brown) to evaluate lean color. On d 0, 1, 2, 4, 6, and 8, panelist used an 8-point scale (1 = 0%, no discoloration, 2 = 1 to 10%, 3 = 10 to 20%, 4 = 20 to 30%, 5 = 30 to 40%, 6 = 40 to 50%, 7 = 50 to 60%, 8 = 60% to 100%) to evaluate discoloration.

***pH analysis***

The initial pH of DC strip loins and USDA choice strip loins was measured at the University of Nebraska-Lincoln using a pH instrument probe (Handheld HI 99163; probe FC232; Hanna Instruments, Smithfield, RI) at three different locations of each strip loin. At Oklahoma State University, after steaks were placed on retail display, an initial pH was taken on d 0 and d 8. Sample pH was measured by blending 5 g of sample with 50 mL of distilled water. Once blended samples were placed in an incubator (VWR Forced Air General Incubator, 5.4 ft3 ; VWR, Radnor, PA) until sample temperature reached a temperature of 25 ± 0.5°C. Three pH measurements were taken using a tabletop pH probe (OrionStar A111 pH meter; Thermo Scientific, Waltham, MA).

***Thiobarbituric acid reactive substances (TBARS)***

Lipid oxidation was evaluated on d 0, 4, and 8 of retail display. A three g sample from the exterior surface was blended with 27 mL of trichloroacetic acid (TCA) in a Waring commercial blender (Model 33BL7; New Hartford, CT) for 10 s. After blending, each sample was filtered through a 42 Whatman filter paper. After filtration, 1 mL of filtrate was added with 1 mL of thiobarbituric acid (TBA) in a glass test tube. The test tube was then placed in a water bath at 100°C for 10 min and then cooled at room temperature for 5 min. Absorbance was measured at 532 nm using a spectrophotometer (UV-2600, UV-VIS Spectrophotometer; Shimadzu; Columbia, MD). 1 mL of TCA was

mixed with 1 mL of TBA to represent the standard. Lipid oxidation values were reported as mg malonaldehyde/kg meat using a validated equation (AMSA, 2012).

### *Metmyoglobin Reducing Activity (MRA)*

MRA was evaluated on d 0, 4, and 8 of the retail display using the internal steak surface. Steaks were butterfly cut to expose the interior surface and placed in sodium nitrite solution (0.3%) for 20 min. Three initial color measurements were taken using a HunterLab 4500L MiniScan EZ Spectrophotometer (2.5-cm aperture, illuminant A, and 10° standard observer angle; HunterLab Associates, Reston, VA). A modified approach used in high-pH meat was utilized to indicate MRA. The resistance to form metmyoglobin was reported as MRA (McKeith et al., 2016; Ramanathan et al., 2020). A greater K/S572 ÷ K/S525 indicates more MRA.

### *Oxygen Consumption (OC)*

OC was evaluated on d 0, 4, and 8 of retail display. The internal surface of steaks was used to evaluate OC. Steaks were butterfly cut to expose the interior surface and allowed to bloom for 1 h at 4°C. Three color measurements were taken using a HunterLab 4500L MiniScan EZ Spectrophotometer (2.5-cm aperture, illuminant A, and 10° standard observer angle; HunterLab Associates, Reston, VA). The level of oxymyoglobin on the bloom surface was used as an indicator of oxygen consumption. OC was reported using reflectance at K/S610 ÷ K/S525.

***Microbiology***

The total plate count of steaks was determined on d 0 and 8 of the retail display. 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 (BactoTM 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).

***Cooked Color analysis***

Steaks were thawed for 24 h at 4°C; steaks were then cooked to 68°C and tempered to 71°C using a Rational oven (Model SCC WE 102G, Rational AG, Landsberg am Lech, Germany) set to 204.4°C with 0% humidity. The external color was measured by taking three surface color measurements using a HunterLab 4500L MiniScan EZ Spectrophotometer (2.5-cm aperture, illuminant A, and 10° standard observer angle; HunterLab Associates, Reston, VA). The CIE $L^*$, $a^*$, and $b^*$ values and spectral readings from 400 to 700 nm determined the surface cooked color.

### *Trained Sensory Panel*

Sensory panelists were trained using the Beef Flavor Lexicon (Adhikari et al., 2011). The descriptive sensory panel was approved by the Institutional Review Board. A total of six trained panelists (n = 6) evaluated ten samples in each session. After steaks were thawed for 24 h at 4°C, steaks were cooked to 68°C and tempered to 71°C using a Rational oven (Model SCC WE 102G, Rational AG, Landsberg am Lech, Germany) set to 204.4°C with 0% humidity. Samples were cut into 1 cm × 1 cm × 1.9 cm pieces and placed into sample cups. Each sample cup contained two 1 $cm^3$ pieces. Sample cups were placed into a warming cabinet during sensory evaluation to maintain temperature. Samples were evaluated under red lighting. Panelist used 8-point hedonic scale to evaluate initial juiciness (1 = extremely dry, 8 = extremely juicy), sustained juiciness (1 = extremely dry, 8 = extremely juicy), tenderness (1 = extremely tough, 8 = extremely tender), beef flavor intensity (1 = extremely dull, 8 = extremely beefy), and overall acceptability (1 = extremely dislike, 8 = extremely like). Panelists were provided with unsalted crackers, apple juice, and deionized water to cleanse pallets between samples.

### *Warner-Bratzler shear force*

Steaks were thawed for 24 h at 4°C, steaks were then cooked to 68°C and tempered to 71°C using a Rational oven (Model SCC WE 102G, Rational AG, Landsberg am Lech, Germany) set to 204.4°C with 0% humidity. Steaks were cooled for 18 h at 4°C prior to shearing. Six cores were taken from each steak (1.27 cm in diameter) parallel to the muscle fiber orientation. The Instron Universal Testing Machine (Model 66 5943;

Instron Corporation; Norwood, MA) with Bluehill 3 software was used to evaluate the maximum load (kg) of each core. The crosshead speed was 200 mm/min.

### *Statistical analysis*

A split-plot design was utilized to determine the effects of high-pressure processing and retail storage on DC beef color. In the whole plot, an incomplete block design was used to evaluate the effects of HPP pressure levels (0, 300, 450, and 600 MPa). The whole plot experimental unit was loin sections. In the sub-plot, each loin section after HPP was allocated to either 0, 4, or 8 days of retail storage. Twelve DC strip loins and eight normal-pH strip loins served as 8 replicates. The fixed effects include pressure levels, retail days, and their interactions. The least squares means were determined using the PROC GLIMMIX procedure of SAS (SAS 9.4; SAS Inst.; Cary, NC) and were considered significant at $P < 0.05$. For the split-plot, random effects included loin, loin × whole plot treatments (Error A), and residual error (Error B). Using the PDIFF options, least squares means were separated and significant at $P < 0.05$.

## Results

### *pH analysis*

There was an HPP level × day of retail display interaction for pH. Normal control steaks had a lower pH ($P < 0.05$) than DC control steaks on d 0 and 8. By d 8 of display, there was no change ($P > 0.05$) in pH of normal control (Table 3.1). pH of DC control increased by d 8 ($P < 0.05$). On d 0, a pressure level of 450 MPa had greater pH than DC control, while other pressures showed no difference from DC control. When comparing d 0 and 8, steaks treated with HPP did not exhibit a pH change over time.

***Retail display color***

***Instrumental Color***

There was an HPP level × day of retail display interaction for $L^*$ values ($P < 0.05$). $L^*$ values indicate the brightness or darkness of the steaks. Higher values translate to a brighter steak, while lower values translate to a darker steak. DC control steaks were darker ($P < 0.05$) than normal pH steaks. HPP treated steaks had greater $L^*$ values ($P < 0.05$) than DC control. Initial color measurements on d 0 showed greater ($P < 0.05$) $L^*$ values as pressure levels were increased (Figure 3.1). Steaks treated at 600 MPa had the lightest ($P < 0.05$) color compared to all other steaks on d 0 and 6. When comparing HPP treated steaks to normal pH control, 300 MPa was the only pressure level that exhibited lower ($P < 0.05$) $L^*$ values throughout retail display. The pressure level of 450 MPa had no significant change in $L^*$ value during 6 d retail display.

There was an HPP level × day of retail display interaction resulted for $a^*$ values ($P < 0.05$). The $a^*$ values of normal control were greater ($P < 0.05$) than DC control throughout retail display (Figure 3.2). By d 3 of retail display, the pressure level of 450 MPa showed greater $a^*$ values ($P < 0.05$) than normal control, while 300 MPa treated steaks were no different ($P > 0.05$) from the normal control. From d 0 to d 3 of retail display, steaks treated at 300 MPa improved in redness. On d 6 of retail display, redness of 300 MPa and 450 MPa remained statistically similar to normal control ($P > 0.05$). After d 6 of display, DC control and 600 MPa steaks were not different ($P > 0.05$) in $a^*$ values.

There was an HPP level × day of retail display interaction for chroma values ($P < 0.05$). Chroma, also known as the "saturation index," can indicate the intensity of color.

Chroma of normal pH control was significantly greater than DC control throughout retail display ($P < 0.05$). All HPP pressure levels had higher ($P < 0.05$) chroma values than DC control (Figure 3.3). By d 3 of retail display, the pressure level of 450 MPa exhibited the greatest chroma values of all treatments evaluated ($P < 0.05$). From d 0 to d 3, there was no change ($P > 0.05$) in chroma values of the 300 MPa treatment. By d 6 of display, all HPP treatments still exhibited greater ($P < 0.05$) chroma values than DC control.

There was an HPP level × day of retail display interaction for hue angles ($P < 0.05$). Hue angle is an indicator of change from true red color to other colors in a color wheel. Normal control had significantly higher hue values than DC control throughout the retail display. All HPP levels were greater than DC control ($P < 0.05$). By d 6 of display, 300 MPa was the only pressure level with a lower ($P < 0.05$) hue than normal control (Figure 3.4). There was no statistical change in the hue of 300 MPa treatment on each day of retail display.

***Visual color***

There was an HPP level × day of retail display interaction for discoloration scores ($P < 0.05$). There was no significant increase in discoloration scores until d 2 of the retail display, when panelists evaluated higher discoloration scores for steaks treated at 600 MPa than other treatments (Figure 3.5). Normal control steaks had greater ($P < 0.05$) discoloration scores than DC control by d 4. On d 4, normal control and 600 MPa treatment discoloration scores increased significantly ($P < 0.05$). Steaks treated at 300 and 450 MPa did not significantly increase discoloration scores until d 6 ($P < 0.05$). On d 6 and 8, normal control and 600 MPa treatment continued to have increased discoloration

scores. By d 8, the treatment level of 300 MPa had the lowest discoloration scores of all steaks, while 600 MPa finished with the highest discoloration scores.

There was an HPP level × day of retail display interaction for lean color scores ($P < 0.05$). On each day evaluated, all HPP treatment levels had higher lean scores than DC control (Figure 3.6). After 1 d, 600 MPa was the only treatment to have an increase in lean score ($P < 0.05$). By d 2, lean scores were higher as pressure level increased. Higher lean scores indicate a more reddish-tan color.

Pressure level had a significant effect on paleness scores ($P < 0.05$). Panelists gave DC control the lowest scores as it indicates more of a very dark red. Normal control was significantly higher than DC control ($P < 0.05$). The lower HPP treatment of 300 MPa exhibited higher ($P < 0.05$) paleness than DC control steaks. As pressure level increased, paleness scores significantly increased (Figure 3.7).

***Thiobarbituric acid reactive substances (TBARS)***

An HPP level × day of retail display interaction represented lipid oxidation ($P < 0.05$). There was no difference in TBARS across all treatments on d 0 ($P > 0.05$). By d 4, higher pressure treatments of 450 MPa and 600 MPa showed more ($P < 0.05$) lipid oxidation than DC control (Figure 3.8). Normal control had greater ($P < 0.05$) lipid oxidation than DC control by d 8. A pressure level of 300 MPa had less ($P < 0.05$) lipid oxidation than normal control on d 8. Normal control and treatment of 600 MPa had statistically similar ($P > 0.05$) TBARS on the last day of display.

### *Metmyoglobin Reducing Activity (MRA)*

An HPP level × day of retail display interaction resulted for MRA ($P < 0.05$). MRA was represented by the resistance to form metmyoglobin (MMb). On d 0, there was no difference ($P > 0.05$) in MRA levels among all treatments (Table 3.5). On d 4 and 8, DC control steaks had higher ($P < 0.05$) MRA levels than normal control steaks. Steaks treated at 300 MPa were not different ($P > 0.05$) from DC control steaks on each pull day throughout retail display. Higher pressure levels of 450 and 600 MPa had lower ($P < 0.05$) MRA than 300 MPa and DC control steaks.

### *Oxygen Consumption (OC)*

There was an HPP level × day of retail display interaction for oxygen consumption (OC; $P < 0.05$). On each pull day in retail display, DC control steaks had greater ($P < 0.05$) OC levels than normal control steaks. On d 0, there was no difference ($P > 0.05$) in OC levels in normal control and in the 600 MPa pressure level (Table 3.3). By d 4, pressure levels of 300 and 450 MPa were no different ($P > 0.05$) from DC control steaks. On d 8, 600 MPa treated steaks had the lowest ($P < 0.05$) OC levels. DC control steaks and steaks treated at 300 MPa were similar ($P > 0.05$) on each day evaluated. OC of steaks treated at 300 MPa did not change ($P > 0.05$) throughout retail display.

### *Microbiology*

The retail day had a significant effect on microbial growth ($P < 0.05$). Microbial growth increased ($P < 0.05$) with time. Although not statistically significant, DC control steaks had greater microbial growth on d 0 and 8 than normal control steaks. On d 0,

steaks treated at 300 MPa had greater microbial growth than other HPP treatments (Table 3.7).

### *Cooked Color*

#### *Instrumental Cooked Color*

There was a significant HPP level effect for $L^*$ values, $a^*$ values, chroma, and hue of external surface of cooked steaks ($P < 0.05$). Cooked steaks treated at 600 MPa were lighter ($P < 0.05$) in appearance than all other steaks. $L^*$ values of cooked steaks treated at 300 and 450 MPa were not different ($P > 0.05$) than both control steaks (Table 3.6). Normal control steaks exhibited lower ($P < 0.05$) $a^*$ values than HPP treated steaks after cooking. There were no differences ($P > 0.05$) in redness among cooked DC control steaks and steaks treated with HPP. There was not a significant HPP level effect for $b^*$ values of cooked steaks ($P > 0.05$). Normal control steaks had the lowest ($P < 0.05$) chroma and the least red intensity of all steaks after cooking (Table 3.6). There was no difference ($P > 0.05$) in red intensity among HPP treated steaks and DC control cooked steaks. The hue of cooked steaks was highest ($P < 0.05$) among normal control steaks. Higher hue values in cooked steaks indicate a more well-done cooked color. Steaks treated at 300 and 600 MPa were statistically similar ($P > 0.05$) to DC control, while a pressure level of 450 MPa had less ($P < 0.05$) hue than DC control steaks.

#### *Warner-Bratzler shear force (WBSF)*

There was a significant HPP level effect on Warner-Bratzler shear force (WBSF) results ($P < 0.05$). WBSF measures the tenderness of a sample by using physical force. High WBSF measurements indicate more force is required to cut through the sample.

Therefore, lower values represent a more tender sample. Steaks treated at 600 MPa had the highest ($P < 0.05$) WBSF values. DC control and normal control steaks had the lowest ($P < 0.05$) WBSF values; however, 450 MPa treated steaks were not different ($P > 0.05$) from controls (Table 3.4).

### *Sensory*

Six trained panelists (n = 6) evaluated steaks for initial juiciness, sustained juiciness, tenderness, beef flavor, and overall acceptability. Attributes were evaluated using a 8-point hedonic scale. Of all attributes evaluated by panelists, only tenderness was significantly affected by HPP level ($P < 0.05$). Similar to WBSF results, 600 MPa treatment had the lowest ($P < 0.05$) tenderness scores (Table 3.5). Steaks treated at 300 and 450 MPa were statistically similar ($P > 0.05$) to normal control steaks. There were no differences ($P > 0.05$) in beefy flavor, overall acceptability, initial juiciness, and sustained juiciness of steaks.

## DISCUSSION

### *pH analysis*

The results of DC and normal pH beef agree with Sawyer et al. (2009) and Mitacek et al. (2018). Previous research has shown greater pH in DC beef than normal-pH beef (Sawyer et al., 2009; Wills et al., 2017; Mitacek et al., 2018; Ramanathan and Mancini, 2018). This is because DC beef has depleted glycogen levels postmortem resulting in less pH decline postmortem (Lawrie, 1958; Scanga et al., 1998). When evaluating the initial pH on normal-pH beef steaks after HPP, Sun et al. (2017) found no significant differences in pressure levels of 450 and 600 MPa. The current study showed

a pressure level of 450 MPa had a higher pH than DC control on d 0. Studies have indicated slight increases in pH of steaks because of HPP (McArdle et al., 2010; McArdle et al., 2011). HPP shifts the pH of non-muscle foods towards acidity; however, there has been a subsequent shift towards alkaline in muscle foods that are not fully understood (Paz et al., 2011). In the current research, greater pH in HPP products can be speculated due to protein breakdown and release of amine-containing amino acids as well as a conformational shift exposing other charged side chains.

***Retail display color***

Previous research has indicated normal-pH steaks have greater $L^*$ values than DC steaks (English et al., 2016; McKeith et al., 2016). As shown in the current study, normal-pH steaks illustrate more redness ($a^*$ values) and red intensity (chroma) compared to DC steaks (Apple et al., 2011; Stackhouse et al., 2016; Wills et al., 2017; Mitacek et al., 2018). Past research shows DC steaks have a lower hue than normal-pH steaks (Apple et al., 2011; Stackhouse et al., 2016). Hue angle represents the relative spread from true redness to yellow, green, and blue in a color wheel. An increase in hue as a result of HPP was supported by Lowder and Dewitt (2014). During retail display, normal-pH steaks see a decrease in redness and an increase in hue (Stackhouse et al., 2016). Therefore, DC steaks have illustrated more color stability than normal-pH steaks (Stackhouse et al., 2016; Ramanathan et al., 2018). HPP-induced increase in lightness has been well documented in the literature (Carlez et at., 1995; Lowder and Dewitt, 2014). Cheftel and Culioli (1997) concluded that an increase in brightness from high pressure could be a result of globin denaturation, heme displacement or release, and ferrous ion oxidation. Other research has suggested changes in water content to be responsible for

increased lightness (Ferrini et al., 2012). The current study saw HPP treated steaks have more redness than DC control steaks on initial retail display. Jung et al. (2003) noted an increase in *a** values of pressure levels up to 350 MPa and a decrease in values as pressure increases to 600 MPa. Past research indicated high pressure to decrease *a** values (Carlez et al., 1995; Kim et al., 2007) when examining normal-pH beef. This decrease in redness was attributed to reduced myoglobin content and metmyoglobin being formed at the expense of oxymyoglobin (Carlez et al., 1995).

Metmyoglobin creates a brown color that is strongly associated with discoloration in meat (Suman and Joseph, 2013). In the current study, by d 4 of retail display, MRA of steaks treated at 300 MPa was not different ($P > 0.05$) from DC control steaks. Past studies have reported a decrease in MMb with a pressure level up to 300 MPa and increases in MMb with higher pressures (Jung et al., 2003). More specifically, moderate pressures could activate the enzymatic system implicated in the reduction of MMb, while higher pressures could disturb this enzymatic system or its surrounding environment (Jung et al., 2003).

In the current research, DC controls have higher ($P < 0.05$) OC levels when compared to normal control steaks. Due to a greater than normal pH of DC beef, the decline in mitochondrial activity that occurs in normal-pH muscle is minimal. The higher mitochondrial activity promotes more oxygen consumption and a darker color. As a result, myoglobin is in a deoxygenated state (Ashmore et al., 1972; Cornforth and Egbert, 1985; Egbert and Cornforth, 1986; Renerre and Labas, 1987). Bak et al. (2012) determined that if pressure is lower than 300 MPa, deoxymyoglobin will be relatively stable to HPP. Previous studies have shown increased oxygenation as a result of low to

moderate pressures (300 to 350 MPa; Bak et al., 2012; Jung et al., 2003; Schenkova et al., 2007). At higher pressure (above 300 MPa), oxidation to metmyoglobin is reported (Carlez et al., 1995).

Instrumental color does not provide a true representation of visual color, especially in HPP meat products. The visual color analysis noted paleness in HPP products. Although the mechanistic basis for paleness is not clear, we speculate that structural changes in myofibrillar protein and sarcoplasmic proteins may contribute paleness. Application of HPP at 300 MPa improved redness of DC steaks and had similar color as normal-pH steaks. The structural changes due to HPP might have increased oxygen diffusion into meat and favored bloom.

***Thiobarbituric acid reactive substances (TBARS)***

Past research shows more lipid oxidation and greater TBARS in normal-pH steaks than DC steaks (English et al., 2016; Wills et al., 2017; Denzer et al., 2020). Normal pH control steaks had higher ($P < 0.05$) lipid oxidation on each pull day in retail display, while DC control steaks were not different ($P > 0.05$) over time in retail display. HPP has been proven to increase and accelerate lipid oxidation in beef. By d 8, all HPP treated steaks had higher ($P > 0.05$) lipid oxidation than DC control steaks. Steaks treated at 300 and 450 MPa had less ($P < 0.05$) lipid oxidation than normal control steaks, while the pressure level of 600 MPa was not different ($P > 0.05$) from normal control steaks by d 8 of retail display. Frenzel et al. (2015) reported that normal-pH steaks treated with HPP showed increased TBARS compared to steaks not processed. In support of the current study, Ma et al. (2007) concluded that a pressure level at or above 300 MPa accelerates lipid oxidation.

### *Microbiology*

A previous study noted microbial growth is greater in DC steaks than normal-pH steaks due to the higher pH (Gill and Newton, 1979). pH close to physiological conditions favors microbial growth. Limited microbial growth was expected based on HPP level. Pressure levels of 400 to 600 MPa lead to inactivation of more than four log units for vegetative pathogenic and spoilage microorganisms (Bajovic et al., 2012). This inactivation can lead to an improved shelf-life. Mcardle et al. (2010) examined HPP levels of 200, 300, and 400 MPa at different temperatures on steaks. They noted that all total plate counts were under the detection limit after one week of refrigeration. In the current study, low values on d 0 could be attributed to evaluating a freshly cut surface which would only have had bacterial exposure from the knife transfer to the fresh cut surface. To better represent microbial growth in the study, a larger swab area could be utilized in future studies.

### *Cooked Color*

Both myoglobin form and pH influence myoglobin denaturation. Hunt et al. (1999) reported deoxymyoglobin to be more heat stable than metmyoglobin at higher pH levels when cooked at 70°C. It is known that DC beef has a greater amount of deoxymyoglobin than normal pH beef (McKeith et al., 2016; Hughes et al., 2017). Based on the results (Table 4.1), normal control had lower ($P < 0.05$) $a^*$ values than DC control and HPP treated steaks. Sawyer et al. (2008) support the difference in $a^*$ values as they concluded that cooked $a^*$ values of DC steaks are higher than those of normal-pH steaks. Normal control cooked steaks illustrating lower ($P < 0.05$) chroma and higher ($P < 0.05$)

hue than DC control steaks is supported by literature (Sawyer et al., 2008). There is limited research examining the surface color of strip steaks after HPP treatment; however, more research is examining HPP treated meat using sous vide cooking. Research examining HPP treated meat often uses sous vide cooking to improve color and enhance color (Segovia et al., 2007). Sous vide method can also improve the tenderness of tough meat (Park et al., 2020) and lower cook loss and lipid oxidation (Segovia et al., 2007). Utilizing sous vide cooking, Janardhanan et al. (2022) examined different pressure levels and concluded no difference in $a^*$ values between pressure levels of cooked ground veal patties. Frenzel et al. (2015) found no difference in $b^*$ values of cooked normal-pH steaks and HPP treated steaks.

***Sensory***

As previously mentioned, tenderness was the only attribute found to be affected by pressure level ($P < 0.05$). In support, Holdstock et al. (2014) found no differences in tenderness between DC steaks and normal-pH steaks when evaluated by a taste panel. No difference between DC control and normal control could be due to variability in pH of DC strip loins. After evaluation by a trained taste panel, Wulf et al. (2002) determined no difference in juiciness between DC steaks and normal-pH steaks. No differences in initial and sustained juiciness were once again supported by Holdstock et al. (2014). The study concluded that the juiciness of DC steaks, atypical DC steaks, and normal-pH steaks was not different. When comparing HPP treated steaks to normal-pH steaks, Frenzel et al. (2015) reported no significant beef flavor and juiciness differences.

### *Warner-Bratzler Shear Force*

Past research by Apple et al. (2011) supports the current study as it found no difference in shear values between DC steaks and normal-pH steaks. It is possible the variation in pH of DC strip loins could play a factor in the tenderness of DC steaks. Studies such as Wulf et al. (2002) found more tenderness variability in DC steaks compared to normal-pH steaks. Evidence of the tenderization of meat pressurized post-rigor has not been reported. In terms of using high pressure to tenderize meat, Sun and Holley (2010) found that lower pressures (< 200 MPa) can tenderize pre-rigor meat; however, HPP on post-rigor meat must be used with a combination of higher temperatures to affect tenderness. This study found steaks treated at 600 MPa had higher ($P < 0.05$) WBSF values. In support, Sun et al. (2017) found that increasing processing pressure to 600 MPa led to slightly tougher values than treatment at 450 MPa. When focusing on a lower pressure level of 300 MPa, tenderness was not different ($P > 0.05$) to normal control. In support, Jung et al. (2000) concluded that a pressure level of 300 MPa did not improve tenderness.

## Conclusion

The application of 300 MPa improved the redness of DC steaks without affecting lipid oxidation. However, the application of 600 MPa increased paleness and discoloration of DC during retail display. Visual panelists also noted improved redness of 300 MPa DC compared to DC without any HPP application. Lower OC supported improved redness in DC at 300 MPa. When examining the sensory panel results, HPP did not affect initial juiciness, sustained juiciness, beef flavor intensity, or overall

acceptability. The application of 600 MPa was less tender than other pressure levels. In support of the sensory panel results, 600 MPa exhibited the highest WBSF values and least tenderness. After cooking, there was no difference in redness between HPP treated steaks and DC control steaks. In conclusion, 300 MPa and 450 MPa pressure levels did not negatively impact sensory attributes of cooked steaks. The current study suggests that lower levels of HPP can be used to improve redness of DC steaks.

**Table 3.1.** Effect of HPP[1] and retail day on pH of steaks during 8-d retail display

| | Pressure levels | | | | |
|---|---|---|---|---|---|
| Day | Normal pH | Dark-cutter | 300 MPa | 450 MPa | 600 MPa |
| 0 | $5.46^{f}$ | $6.52^{de}$ | $6.51^{e}$ | $6.77^{ab}$ | $6.64^{bcde}$ |
| 8 | $5.51^{f}$ | $6.81^{a}$ | $6.61^{cde}$ | $6.75^{abc}$ | $6.65^{bcd}$ |
| | | | SEM[2] = 0.05 | | |

[a-f]Least squares means with different letters are significantly different ($P < 0.05$; $n = 8$).
[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section HPP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.
[2]SEM = standard error of the mean

**Table 3.2.** Effect of HPP[1] and retail day on oxygen consumption[2] of steaks during 8-d retail display

| | Pressure levels | | | | |
|---|---|---|---|---|---|
| Day | Normal pH | Dark-cutter | 300 MPa | 450 MPa | 600 MPa |
| 0 | $0.325^{cd}$ | $0.434^{ab}$ | $0.413^{ab}$ | $0.418^{ab}$ | $0.341^{c}$ |
| 4 | $0.245^{f}$ | $0.439^{a}$ | $0.407^{ab}$ | $0.390^{b}$ | $0.273^{ef}$ |
| 8 | $0.293^{de}$ | $0.435^{ab}$ | $0.397^{ab}$ | $0.316^{cde}$ | $0.245^{f}$ |
| | SEM[3] = 0.02 | | | | |

[a-f]Least squares means with different letters are significantly different ($P < 0.05$; $n = 8$).
[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.
[2]Oxygen consumption was determined by using reflectance K/S610 ÷ K/S525 nm. A lower ratio indicates lower oxygen consumption.
[3]SEM = standard error of the mean

**Table 3.3.** Effect of HPP[1] and retail day on metmyoglobin reducing activity[2] of steaks during 8-d retail display

| | Pressure levels | | | | |
|---|---|---|---|---|---|
| Day | Normal pH | Dark-cutter | 300 MPa | 450 MPa | 600 MPa |
| 0 | $0.988^{bcd}$ | $0.985^{cd}$ | $0.973^{cd}$ | $0.995^{abc}$ | $1.005^{abc}$ |
| 4 | $0.924^{e}$ | $1.034^{a}$ | $0.994^{ab}$ | $0.988^{cd}$ | $0.930^{d}$ |
| 8 | $0.918^{e}$ | $1.030^{a}$ | $1.021^{ab}$ | $0.973^{cd}$ | $0.954^{d}$ |
| | SEM[3] = 0.02 | | | | |

[a-e]Least squares means with different letters are significantly different ($P < 0.05$; $n = 8$).
[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.
[2]Metmyoglobin reducing activity was determined using reflectance K/S ratios (572/525 nm).
[3]SEM = standard error of the mean

**Table 3.4.** Effect of HPP[1] on Warner-Bratzler shear force of steaks

| Pressure levels | WBS (kg) | SEM[2] |
|---|---|---|
| Normal pH | 2.71[c] | 0.20 |
| Dark-cutter | 2.52[c] | 0.20 |
| 300 MPa | 3.37[b] | 0.20 |
| 450 MPa | 2.98[bc] | 0.20 |
| 600 MPa | 4.92[a] | 0.20 |

[a-c]Least squares means with different letters are significantly different ($P < 0.05$; $n = 8$).
[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.
[2]SEM = standard error of the mean

**Table 3.5.** Effect of HPP[1] on trained taste panelists' scores[2] of steaks

| Pressure levels | Initial Juiciness | Sustainable Juiciness | Tenderness | Beef Flavor | Overall |
|---|---|---|---|---|---|
| Normal pH | 6.3 | 6.1 | 5.7[ab] | 6.5 | 5.7 |
| Dark-cutter | 5.5 | 5.2 | 6.5[a] | 6.5 | 6.1 |
| 300 MPa | 5.2 | 5.0 | 5.8[a] | 6.1 | 5.7 |
| 450 MPa | 5.9 | 5.6 | 6.2[a] | 6.8 | 6.1 |
| 600 MPa | 5.7 | 5.5 | 4.9[b] | 6.8 | 5.3 |
| SEM[3] | 0.28 | 0.30 | 0.30 | 0.21 | 0.27 |

[ab]Least squares means with different letters are significantly different ($P < 0.05$; $n = 8$).
[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.
[2]Trained panelist used a 8-point scale (1= extremely dry, 8 = extremely juicy) for initial and sustainable juiciness. For tenderness, beef flavor, and overall, trained panelists used a 8-point scale (1 = extremely tough, 8 = extremely tender; 1 = extremely dull, 8 = extremely beefy, 1 = extremely dislike, 8 = extremely like).
[3]SEM = standard error of the mean

**Table 3.6.** Effect of HPP[1] on cooked color of external surface of steaks

| Pressure levels | *L** values | *a** values | *b** values | Chroma | Hue |
|---|---|---|---|---|---|
| Normal pH | 35.07[b] | 15.45[b] | 18.24 | 23.92[b] | 49.69[a] |
| Dark-cutter | 36.67[b] | 18.86[a] | 20.05 | 27.62[a] | 46.68[b] |
| 300 MPa | 35.02[b] | 20.23[a] | 20.41 | 28.79[a] | 45.04[bc] |
| 450 MPa | 36.54[b] | 20.27[a] | 19.78 | 28.36[a] | 44.12[c] |
| 600 MPa | 39.97[a] | 19.03[a] | 20.18 | 27.76[a] | 46.50[b] |
| SEM[2] | 0.86 | 0.55 | 0.70 | 0.83 | 0.71 |

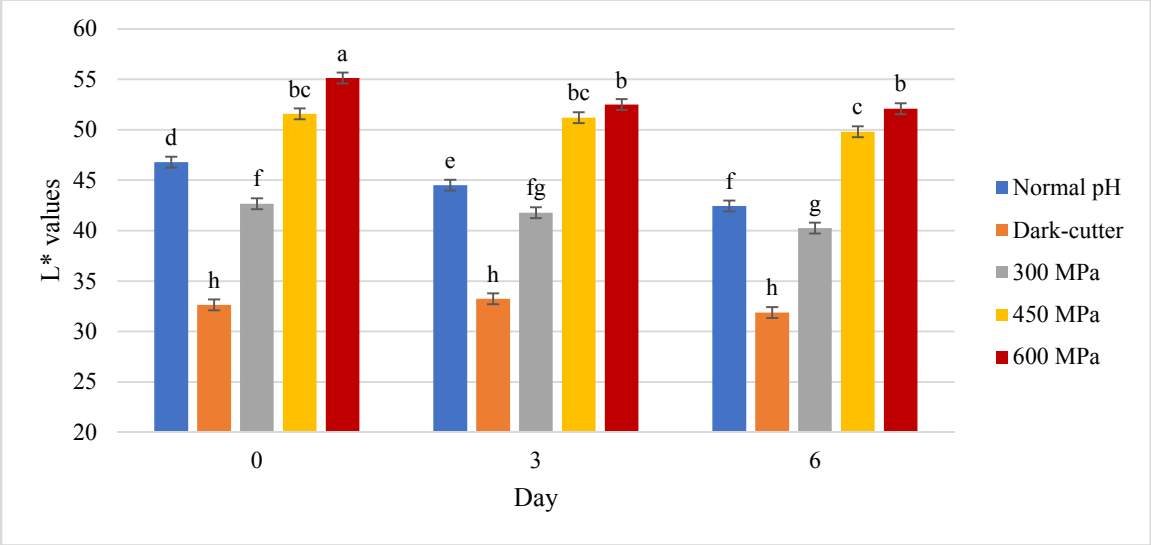
[a-c]Least squares means with different letters are significantly different ($P < 0.05$; $n = 8$).
[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.
[2]SEM = standard error of the mean

**Table 3.7.** Effect of HPP[1] and retail day on microbial growth[2] of steaks

| Pressure levels | | | | | |
|---|---|---|---|---|---|
| Day | Normal pH | Dark-cutter | 300 MPa | 450 MPa | 600 MPa |
| 0 | ND | 0.90 | 0.24 | ND | ND |
| 8 | 0.56 | 1.23 | 2.18 | 0.60 | 1.31 |
| SEM[3] = 0.59 | | | | | |

Least squares means not significantly different ($P > 0.05$; $n = 8$).
[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.
[2]Microbial growth = Log (CFU/cm$^2$)
[3]SEM = standard error of the mean
ND = not detected

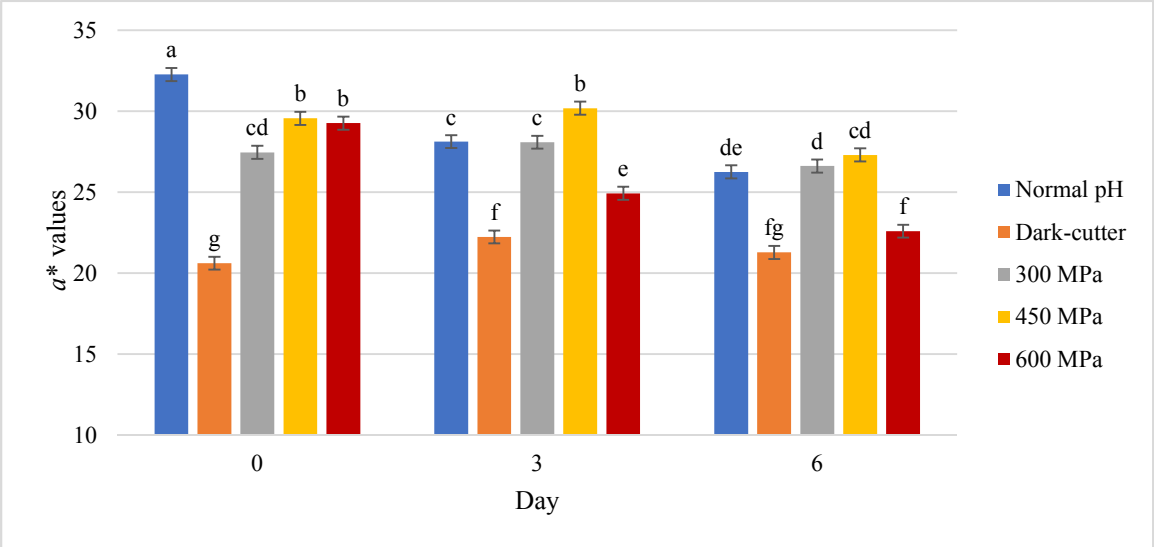
**Figure 3.1.** Effect of HPP[1] and retail day on *L** of steaks during 8-d retail display




Least squares means with different letters (a-h) are significantly different ($P < 0.05$; n = 8). Standard error of the mean indicated by error bars (SEM = 0.54).

[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.

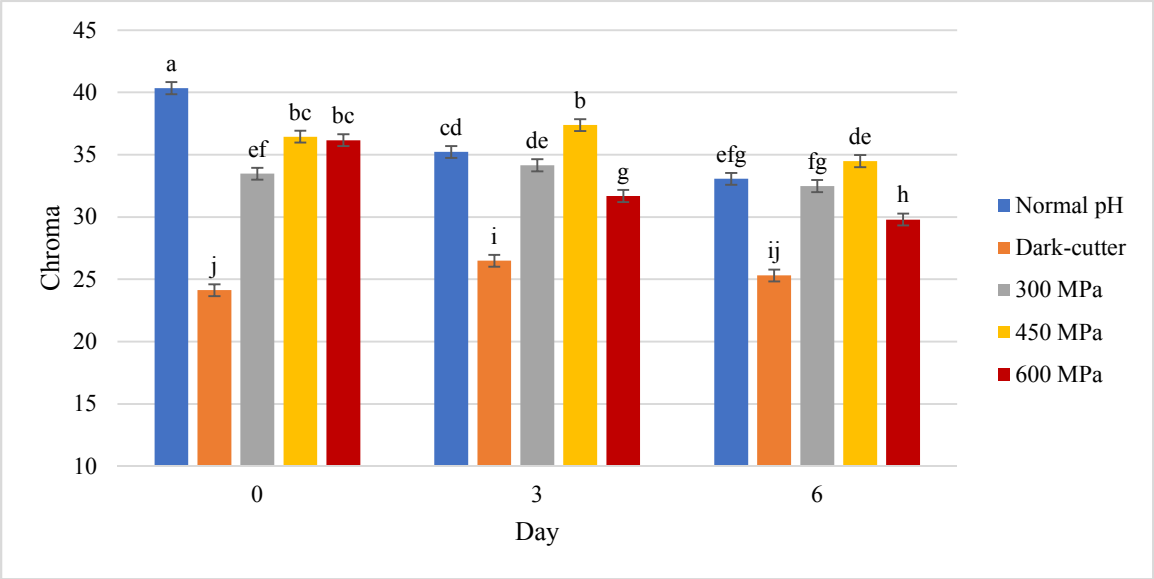
**Figure 3.2.** Effect of HPP[1] and retail day on *a** of steaks during 8-d retail display




Least squares means with different letters (a-g) are significantly different ($P < 0.05$; n = 8). Standard error of the mean indicated by error bars (SEM = 0.39).

[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.

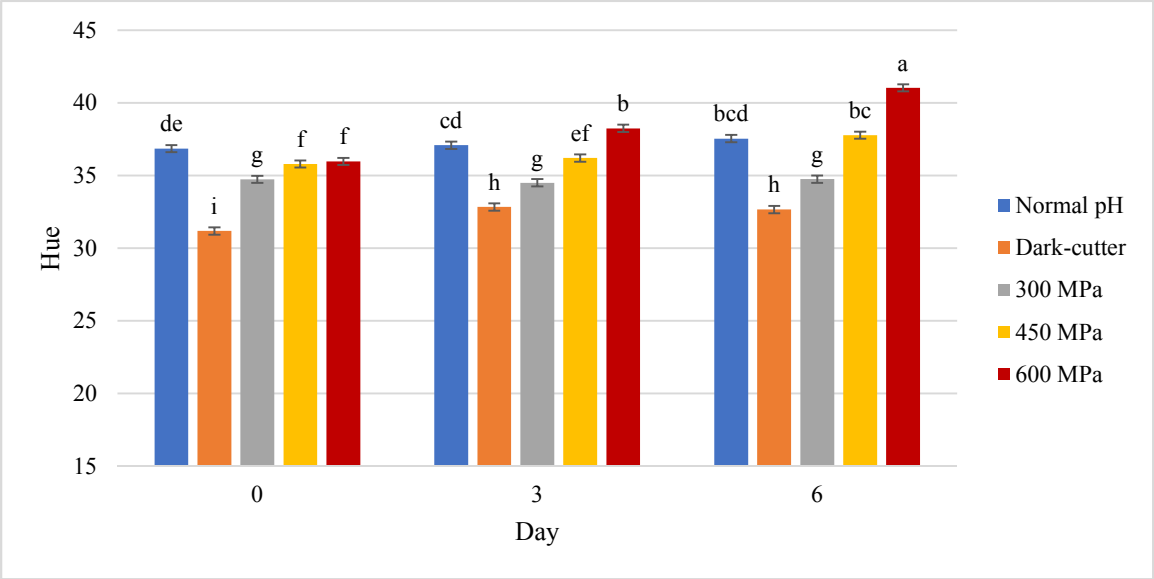
**Figure 3.3.** Effect of HPP[1] and retail day on chroma of steaks during 8-d retail display




Least squares means with different letters (a-j) are significantly different ($P < 0.05$; n = 8). Standard error of the mean indicated by error bars (SEM = 0.48).

[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.

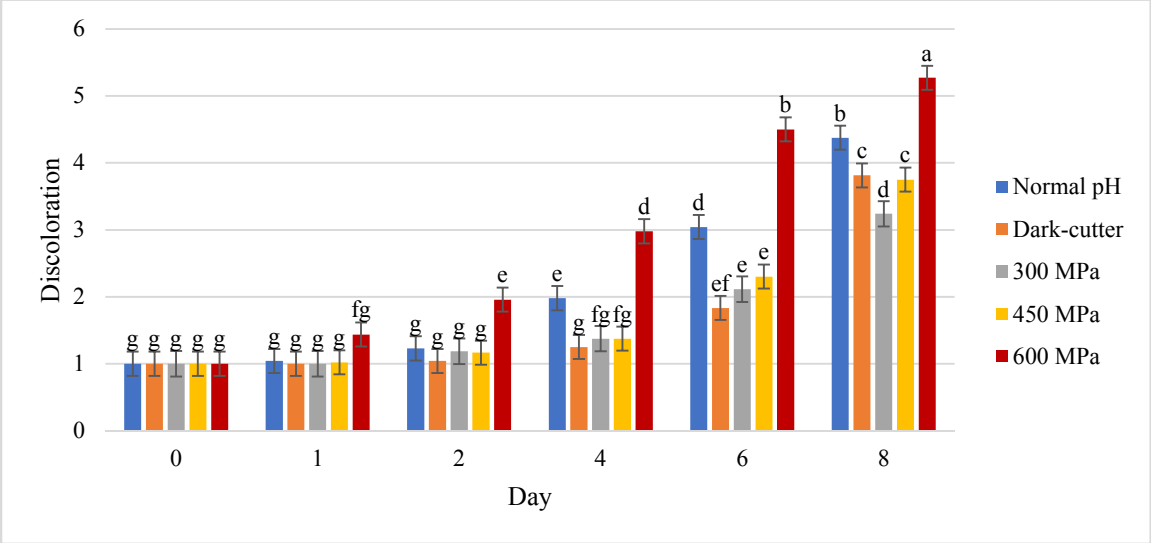
**Figure 3.4.** Effect of HPP[1] and retail day on hue of steaks during 8-d retail display




Least squares means with different letters (a-i) are significantly different ($P < 0.05$; n = 8). Standard error of the mean indicated by error bars (SEM = 0.25).

[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.

**Figure 3.5.** Effect of HPP[1] and retail day on surface discoloration[2] of steaks during 8-d retail display

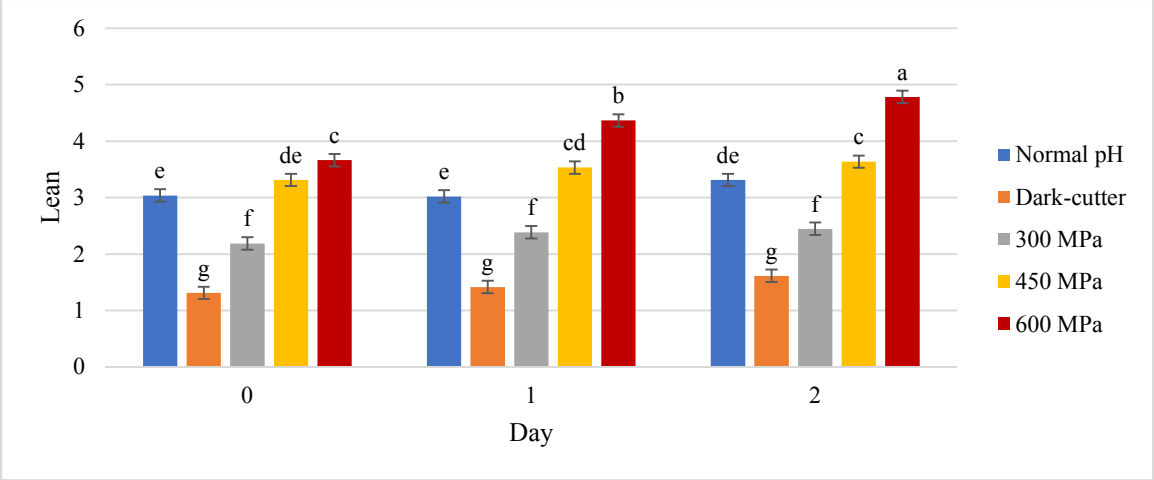



Least squares means with different letters (a-g) are significantly different ($P < 0.05$; n = 8). Standard error of the mean indicated by error bars (SEM = 0.18).

[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.

[2]1 = 0%, no discoloration, 2 = 1 to 10%, 3 = 10 to 20%, 4 = 20 to 30%, 5 = 30 to 40%, 6 = 40 to 50%, 7 = 50 to 60% 8 = 60 to 100% discoloration

**Figure 3.6.** Effect of HPP[1] and retail day on lean color[2] of steaks during 8-d retail display

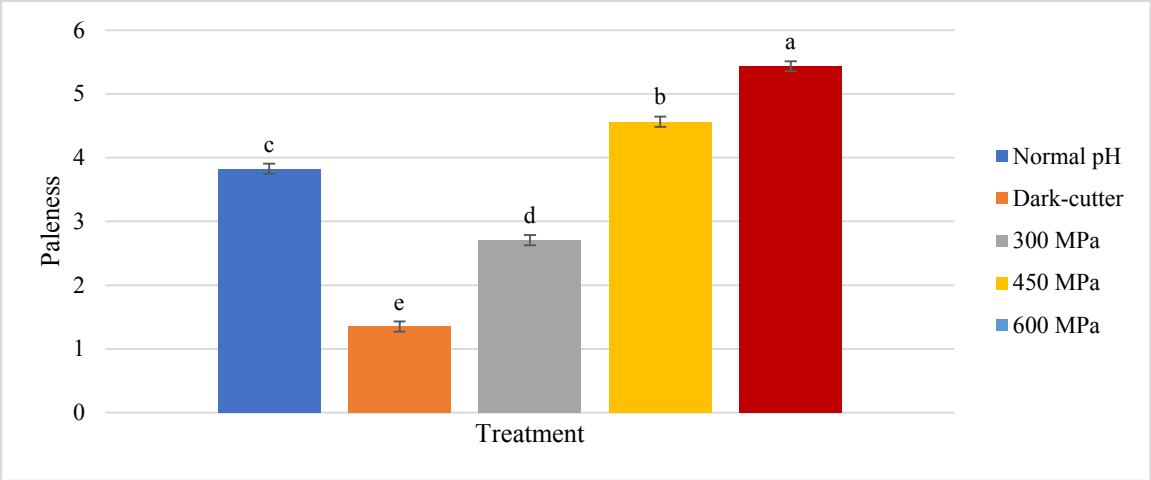



Least squares means with different letters (a-g) are significantly different ($P < 0.05$; n = 8). Standard error of the mean indicated by error bars (SEM = 0.11).

[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.

[2]1 = very bright red, 2 = Bright red, 3 = Dull red, 4 = Slightly dark red, 5 = Moderately dark red, 6 = Dark red to dark reddish tan, 7 = Tannish red, 8 = tan to brown

**Figure 3.7.** Effect of HPP[1] on paleness[2] of steaks during 8-d retail display

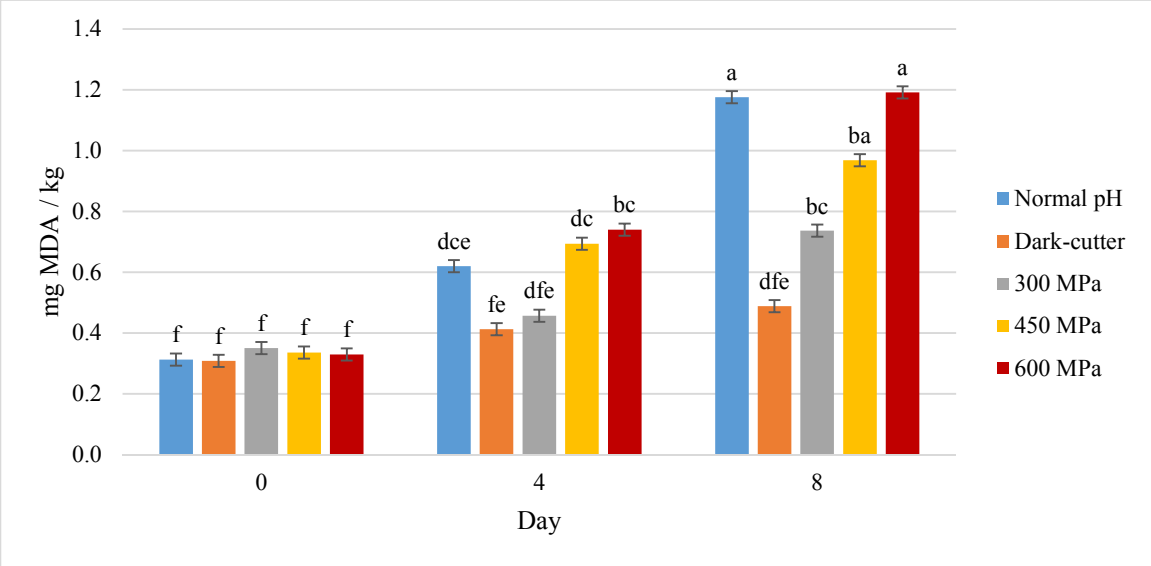



Least squares means with different letters (a-e) are significantly different ($P < 0.05$; n = 8). Standard error of the mean indicated by error bars (SEM = 0.08).

[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.

[2]1 = very dark red, 2 = dark red, 3 = red, 4 = slightly pale, 5 = moderately pale, 6 = very pale

**Figure 3.8.** Effect of HPP[1] and retail day on thiobarbituric acid reactive substances of steaks during 8-d retail display




Least squares means with different letters (a-f) are significantly different ($P < 0.05$; $n = 8$). Standard error of the mean indicated by error bars (SEM = 0.02).

[1]HPP treatments include normal-pH USDA Choice loin section (no HPP, used as a control), dark-cutting loin section (no HPP), dark-cutting loin section PP at 300 MPa, dark-cutting loin section HPP at 450 MPa, dark-cutting loin section HPP at 600 MPa.

# CHAPTER IV

# CONCLUSION

The dark appearance of DC beef leads to discrimination among consumers and discounted prices. Therefore, it is important to improve consumer acceptability of DC beef and negate economic losses to the beef industry. Improvements to visual fresh and cooked color as well as sensory attributes can be made by utilizing HPP, a non-thermal pasteurization technology. Instrumental color measurements and a trained visual color panel noted a pressure level of 300 MPa to exhibit lower L* values and paleness than other pressure levels (450 and 600 MPa). Steaks treated at 300 MPa exhibited lower thiobarbituric acid reactive substance values than other pressure levels and normal pH control steaks ($P < 0.05$). When examining the sensory panel results, HPP did not have an effect on initial juiciness, sustained juiciness, beef flavor intensity, or overall acceptability ($P > 0.05$). There was no difference in redness and red intensity between HPP treated steaks and DC control steaks. In conclusion, low (300 MPa) and moderate (450 MPa) pressure levels did not have a negative impact on cooked steaks. The results indicate that 300 MPa can improve redness of dark-cutting beef without affecting other quality parameters.

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