

Use of High-Pressure Processing to Improve the Redness of Dark-Cutting Beef

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Abstract: The objective was to evaluate the effects of high-pressure processing (HPP) levels on retail color of dark-cutting beef. Eight USDA Choice (mean pH = 5.5; normal-pH beef) and 12 dark-cutting (mean pH = 6.3) strip loins were obtained from a commercial packing plant within 2 d of harvest. Dark-cutting loins were cut into equal sections, vacuum packaged, and randomly assigned to 0 (no HPP), 300, 450, and 600 MPa of pressure for 90 s using chilled water. Following 48 h of dark storage at 2°C, dark-cutting 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. The surface color readings were measured every 24 h using a HunterLab MiniScan XE Plus spectrophotometer, whereas a trained color panel ($n = 6$) evaluated discoloration, paleness, and lean color on steaks. Lipid oxidation was evaluated on day 0, 4, and 8 of retail display, and structural changes were determined using light microscopy on day 0 of display. There was a significant HPP level \times day of retail display interaction for all instrumental color measurements. Throughout the retail display, L^* values of 450 and 600 MPa applied steaks were greater ($P < 0.05$) than 300 MPa and controls. There was a significant pressure level \times day of retail display interaction when panelists evaluated lean color and discoloration. Steaks treated at 300 MPa exhibited brighter red color and lower ($P = 0.0023$) thiobarbituric acid reactive substance values than other pressure levels and normal-pH control steaks. Light microscopy analysis indicated that HPP increased space between muscle structures. In conclusion, low (300 MPa) and moderate (450 MPa) pressure levels can improve redness of dark-cutting steaks.

Key words: dark-cutting beef, high-pH beef, beef color, high pressure processing, myoglobin

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Introduction

Meat color is the single most important sensory quality that influences consumer purchasing decisions and the value of carcasses during grading in the United States. Dark-cutting beef is a color deviation from the characteristic bright-red appearance. Dark-cutting beef is known for its high postmortem muscle pH, decreased redness, and darker appearance (Wulf et al., 2002; Hughes et al., 2017; Kiyimba et al., 2022). In the US, dark-cutting beef results in a \$202.4 million loss annually because of its discounted price (based on the

United States Department of Agriculture-Agricultural Marketing Service discount price of \$35.83 per carcass weight and 2016 National Beef Quality Audit; Boykin et al., 2017). Therefore, developing postharvest techniques will help to improve the surface color appearance and value of dark-cutting beef.

The preharvest stress depletes glycogen reserves, resulting in less lactic acid formation postmortem (Lawrie, 1958; Scanga et al., 1998; Mahmood et al., 2017). A greater postmortem muscle pH favors mitochondrial respiration and increased water-holding capacity (English et al., 2016; Hughes et al., 2017). Greater mitochondrial oxygen consumption leads to

less available oxygen for myoglobin (Tang et al., 2005; Ramanathan et al., 2013). Furthermore, increased water-holding capacity leads to a lack of muscle shrinkage; hence, less oxygen is diffused into the subsurface (Hughes et al., 2017; Ramanathan et al., 2020). Both oxygen consumption and muscle swelling promote deoxymyoglobin and a darker meat color (English et al., 2016; McKeith et al., 2016). Various postharvest techniques such as acid acidification, high-oxygen modified atmospheric packaging, carbon monoxide modified atmospheric packaging, and nitrite-embedded packaging have been utilized to improve the redness of dark-cutting beef (Sawyer et al., 2008; Wills et al., 2017; Mitacek et al., 2018; Zhang et al., 2018; Denzer et al., 2022a, 2022b; Yang et al., 2022).

High-pressure processing (HPP) is a nonthermal food processing technology that has been utilized in the food industry to sterilize and pasteurize products (Bak et al., 2019; Bolumar et al., 2021). HPP results in protein denaturation and enzyme inactivation (Hygreeva and Pandey, 2016). The effect of HPP depends on factors such as protein susceptibility, applied pressure and temperature, and time treated (Sun and Holley, 2010). The use of HPP units in the food industry is constantly increasing as meat products currently represent about a quarter of HPP foods (Bak et al., 2019). The unfolding of muscle proteins occurs at pressures up to 300 MPa. Pressures higher than 300 MPa can increase denaturation and gel formation (Bajovic et al., 2012). As a result, the application of HPP to fresh meat and meat products can affect 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 on high-pH beef. We hypothesize that HPP will induce structural changes and increase oxygen diffusion into meat subsurface and improve redness in dark-cutting beef. The objective of this study was to evaluate different HPP levels on dark-cutting beef color.

Materials and Methods

Raw materials and processing

Twelve dark-cutting strip loins (*longissimus lumborum*; mean pH = 6.3, standard error of the mean [SEM] = 0.08) and 8 USDA Choice strip loins (mean pH = 5.5, SEM = 0.09; IMPS #180; NAMP, 2002) were obtained from Greater Omaha 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 Loeffel Meat Laboratory, strip loins were wet-aged for 5 d at 2°C. Each loin served as a block. After aging, using an incomplete block design, dark-cutting strip loins were cut into 3 equal sections. The anterior and posterior dark-cutting loin sections were equally distributed among treatments. Only enough strip loin sections to create 8 replicates were used. Strip loin sections were then vacuum packaged (Flair Flexible Packaging Corporation, Calgary, Canada; 12 × 14 cm² pouches; 5 mil thickness), and randomly assigned to treatments. Dark-cutting control samples were not treated with HPP. Normal-pH strip loin sections were used as a control, and there was no HPP application. Using an incomplete block, dark-cutting strip loin sections were randomly assigned to one of the following pressure treatments: control (0), 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 for HPP application.

High pressure processing

A commercial HPP unit was utilized to apply pressure on dark-cutting strip loin sections (Hiperbaric 55, Hiperbaric USA, Miami, FL; 55 L vessel; 200-mm diameter inside the vessel; throughput of 270 kg/h) with chilled water as the pressurizing medium. Vacuum-packaged loin sections were placed in bins packed with ice so that the fluid temperature was lowered 4°C to 8°C. All sections were processed and held for 90 s at the designated pressure level. The pressurization rate of the HPP unit was between 1 and 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) for color studies.

Packaging and stimulated retail display

Following 48 h of dark storage at 2°C following HPP application, strip loin sections were cut into 1.9-cm-thick steaks. The previous study used 1.91-cm-thick steaks in beef color research (Mancini et al., 2009). Steaks were placed in Styrofoam trays and overwrapped with PVC (15,500 to 16,275 cm³ O₂/m²/24 h at 23°C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film; Koch Supplies, Riverside, MO). Steaks were then placed into a coffin-style display case under a continuous light-emitting diode (LED; Philips LED lamps; 12 W, 48 in, color temperature = 3,500°K; Philips, Amsterdam, the Netherlands) at 2°C for 8 d.

Raw color analysis

During retail display, the instrumental color of steaks was measured every 24 h for 8 d using a HunterLab 4500L MiniScan EZ Spectrophotometer (2.5-cm aperture, illuminant A, and 10° standard observer angle; HunterLab, Reston, VA). The surface of each steak was read 3 times, and the surface color was characterized by the Commission Internationale de l'Éclairage (CIE) L^* , a^* , and b^* values and reflectance from 400 to 700 nm. Chroma [$\sqrt{(a^{*2} + b^{*2})}$] was determined using CIE a^* and b^* values, representing the red intensity of the color (King et al., 2023). The CIE a^* and b^* were used to determine the hue angle ($\tan^{-1}(\frac{b^*}{a^*})$) representing the color present (King et al., 2023). However, 6 d of data were presented to avoid the complexity of including superscripts in means.

Visual color was examined using a panel of 6 trained panelists ($n=6$). All panelists passed the Farnsworth Munsell 100-hue test. On day 0, 1, and 2 of display, 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 day 0, 1, 2, 4, 6, and 8, panelists 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%, and 8 = 60% to 100%) to evaluate discoloration.

pH analysis

A handheld pH probe (Handheld HI 99163; probe FC232; Hanna Instruments, Smithfield, RI) was used to measure the initial pH (2 d postmortem) of dark-cutting strip loins and USDA Choice strip loins at the University of Nebraska-Lincoln. The pH probe was inserted at 3 different locations of each strip loin.

pH was measured by blending 5 g of sample with 50 mL of distilled water on day 0 and 8 of display. 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°C ± 0.5°C. Three pH measurements were taken using a tabletop pH probe (Orion Star A111 pH meter; Thermo Scientific, Waltham, MA).

Thiobarbituric acid reactive substances

Lipid oxidation was evaluated on day 0, 4, and 8 of retail display. A 3-g sample from the exterior surface was blended with 27 mL of trichloroacetic acid (TCA) in a Waring commercial blender (model 33BL79; Waring, New Hartford, CT) for 10 s. After blending, each sample was filtered through 42 Whatman filter paper (Cytiva, Marlborough, MA). 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). One milliliter 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 (King et al., 2023).

Light microscopy

The methodology used in the previous study was used to assess muscle structural changes on day 0 of display (Ramanathan et al., 2022). Thin sections of control dark-cutting and different HPP-level steaks were fixed in 10% neutral-buffered formalin, processed to paraffin wax blocks, sectioned at 4 µm, and stained with hematoxylin and eosin. The sections of skeletal muscle were examined by light microscopy (10× magnification). The digital images of the treatments were saved in a JPEG format.

Statistical analysis

A split-plot design was utilized to determine the effects of HPP and retail storage on dark-cutting 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 subplot, each loin section after HPP was allocated to either 0, 4, or 8 d of retail storage. Twelve dark-cutting strip loins and 8 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 Institute, 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 PDIF options, least-squares means were separated and significant at $P < 0.05$.

Results

pH analysis

There was an HPP level \times day of retail display interaction for pH. Normal-pH control steaks had lower pH ($P < 0.05$) than dark-cutting control steaks on day 0 and 8. There was no change ($P > 0.05$) in pH of normal-pH control between day 0 and 8 (Table 1). pH of dark-cutting control increased by day 8 ($P < 0.05$). On day 0, a pressure level of 450 MPa had greater pH than dark-cutting control, whereas other pressures showed no difference from dark-cutting control. When comparing day 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 \times day of retail display interaction ($P < 0.05$) for L^* values. L^* values indicate the brightness or darkness of the steaks. Higher L^* values translate to a brighter steak, whereas lower values translate to a darker steak. Dark-cutting control steaks were darker ($P < 0.05$) than normal-pH steaks. HPP-treated steaks had greater ($P < 0.05$) L^* values than dark-cutting control. Initial color measurements on day 0 showed greater ($P < 0.05$) L^* values as pressure levels were increased (Figures 1–2). Steaks treated at 600 MPa were paler ($P < 0.05$) compared with all other steaks on day 0 and 6. When comparing HPP-treated steaks with normal-pH control, 300 MPa was the only pressure level that exhibited

Table 1. Effect of high-pressure processing (HPP)¹ and retail day on pH of steaks during retail display

Day	Pressure levels				
	Normal-pH	Control dark-cutter	300 MPa	450 MPa	600 MPa
0	5.46 ^f	6.52 ^{dc}	6.51 ^e	6.77 ^{ab}	6.64 ^{bcd}
8	5.51 ^f	6.81 ^a	6.61 ^{cde}	6.75 ^{abc}	6.65 ^{bcd}

SEM² = 0.05

^{a-f}Least squares means with different letters are significantly different ($P < 0.05$; $n = 8$).

¹HPP 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, and dark-cutting loin section HPP at 600 MPa.

²SEM = standard error of the mean.

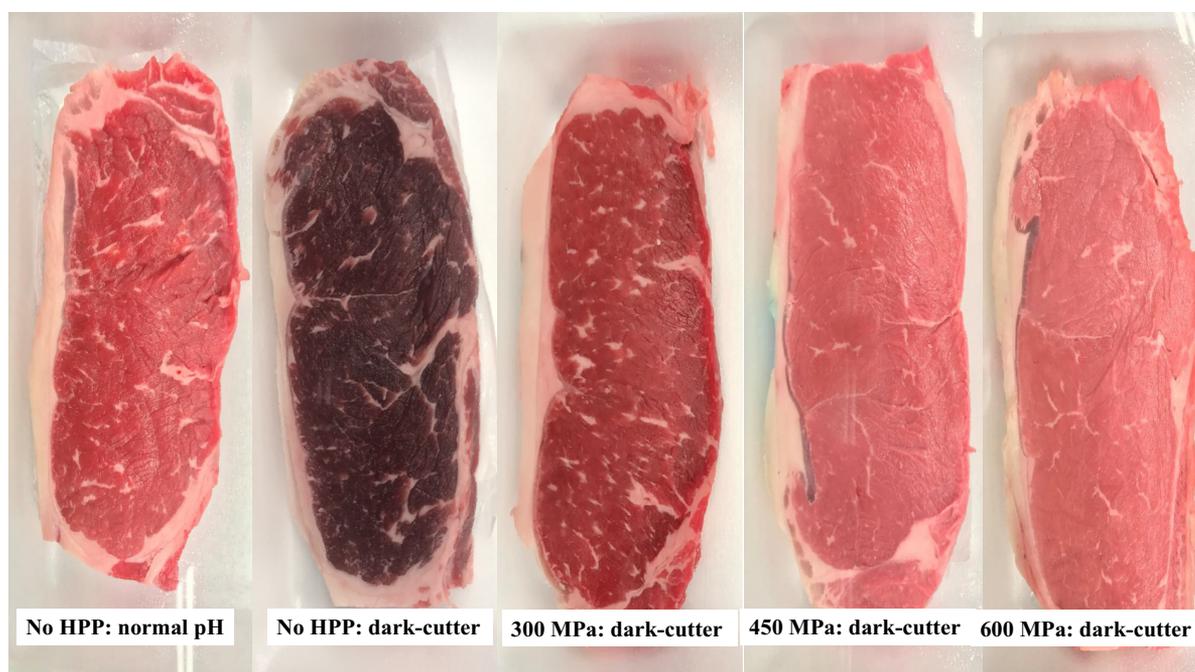


Figure 1. Effects of high-pressure processing (HPP) on surface color of dark-cutting beef on day 1 of display.

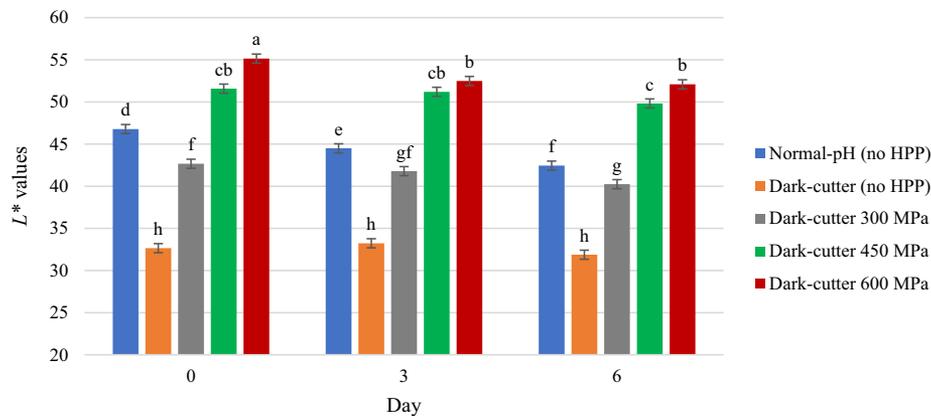


Figure 2. Effect of high-pressure processing (HPP) and retail day on L^* values of steaks during retail display. Least-squares means with different letters (a–h) are significantly different ($P < 0.05$; $n = 8$). Standard error of the mean (SEM) indicated by error bars (SEM = 0.54). HPP treatments include 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, and dark-cutting loin section HPP at 600 MPa.

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 \times day of retail display interaction that resulted for a^* values ($P < 0.05$). The a^* values of normal-pH control were greater ($P < 0.05$) than dark-cutting control throughout retail display (Figure 3). By day 3 of retail display, the pressure level of 450 MPa showed greater a^* values ($P < 0.05$) than normal control, whereas 300 MPa treated steaks were no different ($P > 0.05$) from the normal-pH control. From day 0 to 3 of retail display, steaks treated at 300 MPa demonstrated improved redness compared with dark-cutting controls. On day 6 of retail display, redness of 300 and 450 MPa remained statistically similar to normal control ($P > 0.05$). After day 6 of display, dark-cutting control and 600 MPa steaks were not different ($P > 0.05$) in a^* values.

There was an HPP level \times 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 dark-cutting control throughout retail display ($P < 0.05$). All HPP pressure levels had higher ($P < 0.05$) chroma values than dark-cutting control (Figure 4). By day 3 of retail display, the pressure level of 450 MPa exhibited the greatest chroma values of all treatments evaluated ($P < 0.05$). From day 0 to 3, there was no change ($P > 0.05$) in chroma values of the 300 MPa treatment. By day 6 of display, all HPP treatments still exhibited greater ($P < 0.05$) chroma values than dark-cutting control.

There was an HPP level \times day of retail display interaction ($P < 0.05$) for hue angle. Hue angle is an indicator of change from true red color to other colors in a color wheel. Normal-pH control had significantly

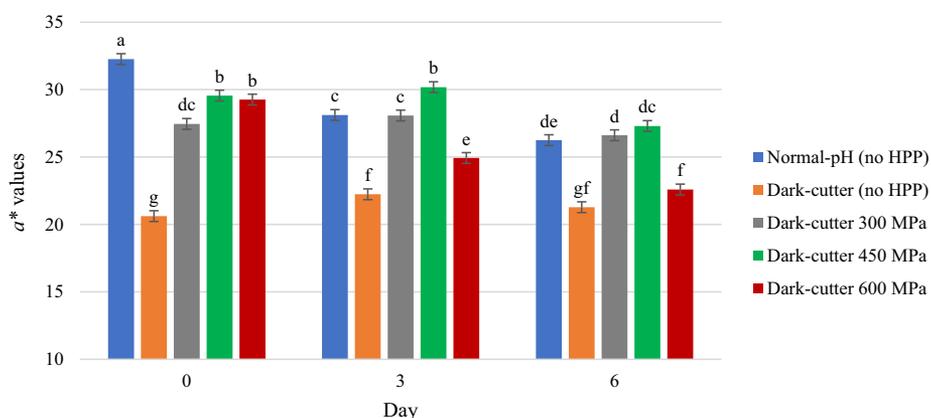


Figure 3. Effect of high-pressure processing (HPP) and retail day on a^* values of steaks during retail display. Least-squares means with different letters (a–f) are significantly different ($P < 0.05$; $n = 8$). Standard error of the mean (SEM) indicated by error bars (SEM = 0.39). HPP treatments include 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, and dark-cutting loin section HPP at 600 MPa.

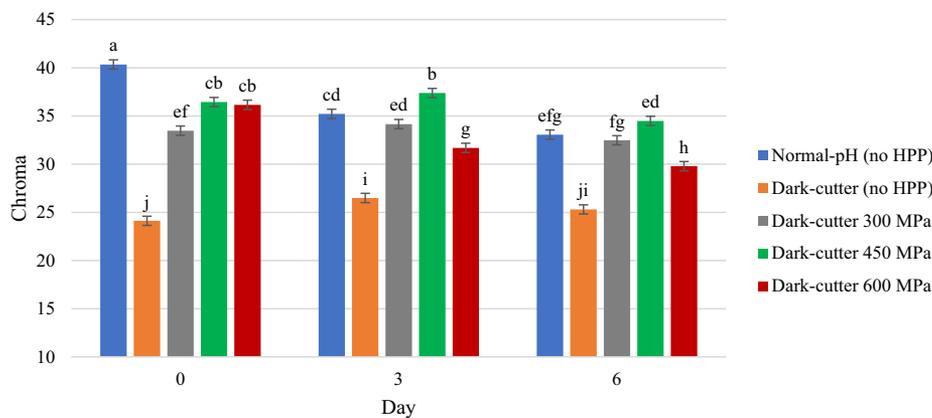


Figure 4. Effect of high-pressure processing (HPP) and retail day on chroma of steaks during retail display. Least-squares means with different letters (a–j) are significantly different ($P < 0.05$; $n = 8$). Standard error of the mean (SEM) indicated by error bars (SEM = 0.48). HPP treatments include 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, and dark-cutting loin section HPP at 600 MPa.

higher hue values than dark-cutting control throughout the retail display. All HPP levels had greater ($P < 0.05$) hue angle values than dark-cutting control. By day 6 of display, 300 MPa was the only pressure level with a lower ($P < 0.05$) hue than the normal-pH control (Figure 5). There was no statistical change in the hue of 300 MPa treatment on each day of retail display.

Visual color. Instrumental color does not provide a true representation of visual color, especially in HPP meat products. There was an HPP level \times day of retail display interaction for discoloration scores ($P < 0.05$). There was no significant increase in discoloration scores until day 2 of the retail display. On day 2 of retail display, panelists evaluated higher discoloration scores for steaks treated at 600 MPa than other treatments (Figure 6). Normal-pH control steaks had greater ($P < 0.05$) discoloration scores than dark-cutting control by

day 4. On day 4, normal-pH 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 day 6 ($P < 0.05$). On day 6 and 8, normal control and 600 MPa treatment continued to have increased discoloration scores. By day 8, the treatment level of 300 MPa had the lowest discoloration scores of all steaks, whereas 600 MPa steaks had the highest discoloration scores.

There was an HPP level \times day of retail display interaction ($P < 0.05$) for lean color scores. All HPP treatment levels had higher lean scores than dark-cutting control (Figure 7). After 1 d, 600 MPa was the only treatment to have an increase ($P < 0.05$) in lean score. By day 2, lean scores were higher as pressure levels increased. Higher lean scores indicate a reddish-tan color.

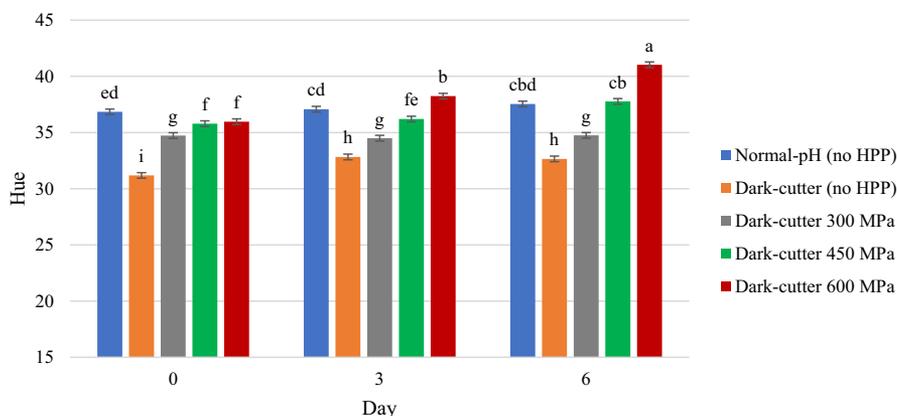


Figure 5. Effect of high-pressure processing (HPP) and retail day on hue of steaks during retail display. Least-squares means with different letters (a–i) are significantly different ($P < 0.05$; $n = 8$). Standard error of the mean (SEM) indicated by error bars (SEM = 0.25). HPP treatments include normal-pH 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, and dark-cutting loin section HPP at 600 MPa.

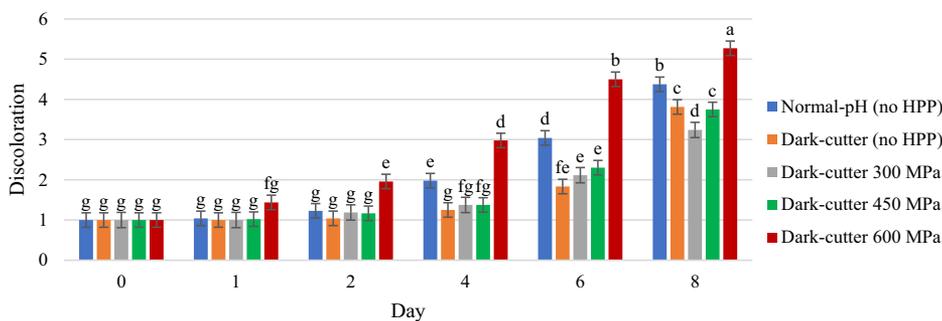


Figure 6. Effect of high-pressure processing (HPP) and retail day on surface discoloration of steaks during retail display. Least-squares means with different letters (a–g) are significantly different ($P < 0.05$; $n = 8$). Standard error of the mean (SEM) indicated by error bars (SEM = 0.18). HPP treatments 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, and dark-cutting loin section HPP at 600 MPa. Surface discoloration: 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.

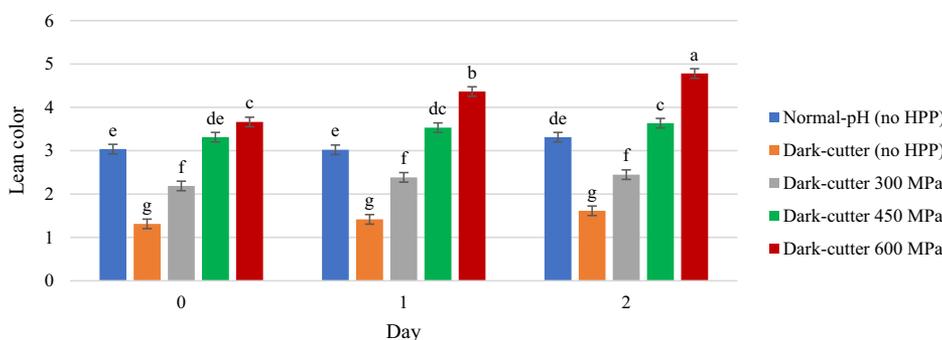


Figure 7. Effect of high-pressure processing (HPP) and retail day on lean color of steaks during retail display. Least-squares means with different letters (a–g) are significantly different ($P < 0.05$; $n = 8$). Standard error of the mean (SEM) indicated by error bars (SEM = 0.11). HPP treatments include 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, and dark-cutting loin section HPP at 600 MPa. Lean color: 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.

Only the main effects of HPP application were significant for paleness score. Panelists gave dark-cutting control the lowest scores, which indicates a very dark red. Normal control was significantly higher than dark-

cutting control ($P < 0.05$). The lower HPP treatment of 300 MPa exhibited higher ($P < 0.05$) paleness than dark-cutting control steaks. As pressure level increased, paleness scores significantly increased (Figure 8).

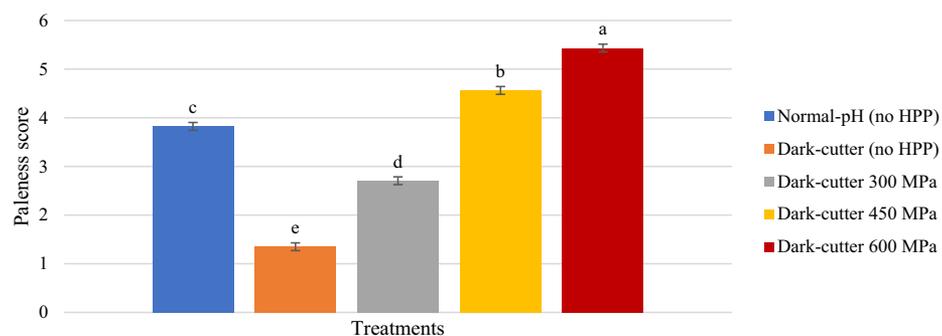


Figure 8. Effect of high-pressure processing (HPP) on paleness of steaks during retail display. Least-squares means with different letters (a–e) are significantly different ($P < 0.05$; $n = 8$). Standard error of the mean (SEM) indicated by error bars (SEM = 0.08). HPP treatments include normal-pH USDA 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, and dark-cutting loin section HPP at 600 MPa. Paleness: 1 = very dark red, 2 = dark red, 3 = red, 4 = slightly pale, 5 = moderately pale, 6 = very pale.

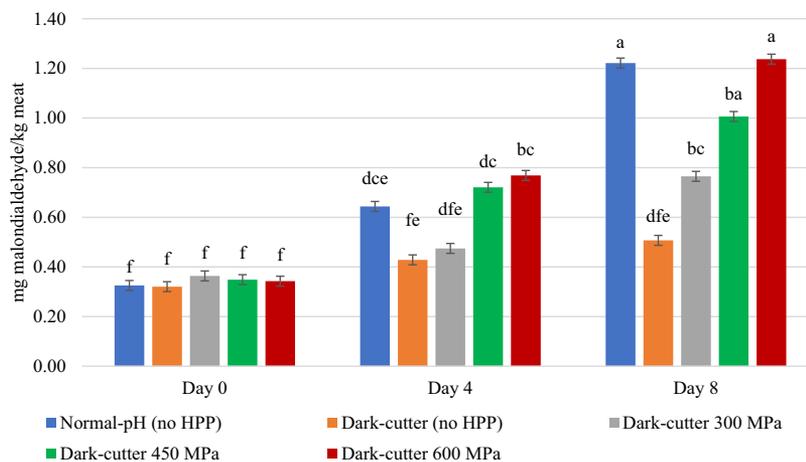


Figure 9. Effect of high-pressure processing (HPP) and retail day on thiobarbituric acid reactive substances of steaks during retail display. Least-squares means with different letters (a–f) are significantly different ($P < 0.05$; $n = 8$). Standard error of the mean (SEM) indicated by error bars (SEM = 0.02). 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, and dark-cutting loin section HPP at 600 MPa.

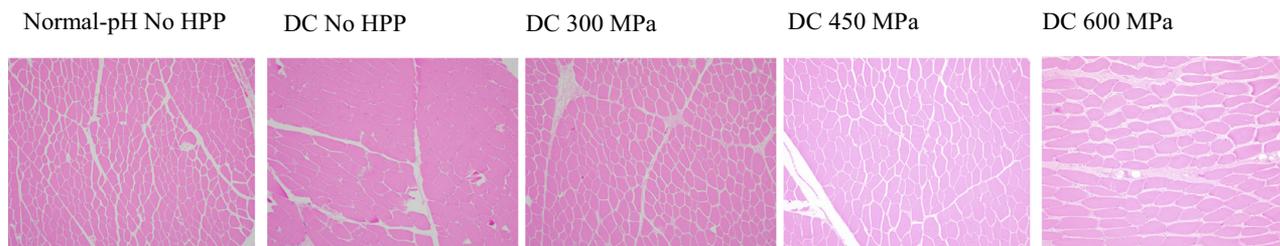


Figure 10. Effects of high-pressure processing (HPP) on muscle structural changes. Normal-pH control, without HPP, dark-cutting (DC) loin section (no HPP), DC loin section HPP at 300 MPa, DC loin section HPP at 450 MPa, and DC loin section HPP at 600 MPa. Muscles were stained using hematoxylin and eosin. Magnification = 10 \times .

Thiobarbituric acid reactive substances

An HPP level \times day of retail display interaction ($P < 0.05$) resulted for lipid oxidation. There was no difference in thiobarbituric acid reactive substances (TBARS) across all treatments on day 0 ($P > 0.05$). By day 4, higher pressure treatments of 450 and 600 MPa showed more ($P < 0.05$) lipid oxidation than dark-cutting control (Figure 9). Normal-pH control had greater ($P < 0.05$) lipid oxidation than dark-cutting control by day 8. A pressure level of 300 MPa had less ($P < 0.05$) lipid oxidation than normal-pH control on day 8. Normal-pH control and treatment of 600 MPa had statistically similar ($P > 0.05$) TBARS on the last day of display.

Light microscopy

The light microscopy analysis revealed that dark-cutting steaks had packed muscle structures (Figure 10). However, HPP application increases space between muscle structures.

Discussion

pH analysis

The results of dark-cutting and normal-pH beef agree with Sawyer et al. (2009) and Mitacek et al. (2018). Previous research has shown greater pH in dark-cutting beef than normal-pH beef (Sawyer et al., 2009; Wills et al., 2017; Mitacek et al., 2018). Dark-cutting 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) noted no significant differences in pressure levels of 450 and 600 MPa. The current study showed a pressure level of 450 MPa had a greater pH than dark-cutting control on day 0. Studies have indicated slight increases in pH of steaks because of HPP (McArdle et al., 2010, 2011). HPP shifts the pH of nonmuscle foods toward acidity; however, there has been a subsequent shift toward alkaline pH in muscle foods that are not fully

understood (Mújica-Paz et al., 2011). In the current research, greater pH in HPP products can be speculated because of 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 dark-cutting 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 with dark-cutting steaks (Apple et al., 2011; Stackhouse et al., 2016; Wills et al., 2017; Mitacek et al., 2018). Past research shows dark-cutting steaks have a lower hue than normal-pH steaks (Apple et al., 2011; Stackhouse et al., 2016). The 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 Mireles Dewitt (2014). During retail display, normal-pH steaks demonstrated a decrease in redness and an increase in hue (Stackhouse et al., 2016). Therefore, dark-cutting steaks have 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 al., 1995; Lowder and Mireles Dewitt, 2014). Cheftel and Culioli (1997) concluded that an increase in paleness from high-pressure application results from 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). In the current study, HPP-treated steaks at 300 and 450 MPa have more redness than dark-cutting control steaks on initial retail display. To the best of our knowledge, the current study is the first to report the effect of improved redness of dark-cutting steaks. However, recently, two studies reported improved redness of dark-cutting beef with HPP (Mao et al., 2023; Reesman et al., 2023). 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) when examining normal-pH beef. This decrease in redness was attributed to reduced myoglobin content and metmyoglobin being formed at the expense of oxy-myoglobin (Carlez et al., 1995). In the present research, beef at high pH might have provided more protection against pressure-induced denaturation. Furthermore,

HPP at 300 MPa may have altered myoglobin structure and promoted myoglobin oxygenation. Mitochondrial function is closely related to bloom and beef color (Tang et al., 2005; Ramanathan and Mancini, 2018). In the current study, we speculate that the role of mitochondria in improved redness at 300 and 450 MPa might be negligible. Greater pressure can denature all proteins, including mitochondrial complexes. Nevertheless, previous studies noted that metmyoglobin reductase enzymes are more active following lower-pressure applications (Jung et al., 2003). Hence, improved color stability of dark-cutting steaks treated with 300 and 450 MPa compared with 600 MPa, in part, can be due to improved metmyoglobin-reducing activity.

Light microscopy analysis indicated fewer compact structures after HPP in dark-cutting beef. A less compact structure promotes oxygen diffusion into the interior of meat. Hence, increased redness in 300 and 450 MPa might be due to increased oxygen diffusion. In support, previous studies also noted that greater oxygen levels within the package improved redness of dark-cutting steaks (Wills et al., 2017). Further, proteins are less denatured at lower-pressure levels. Hence, myoglobin might be able to bind with oxygen to form bright red color. However, greater HPP can denature myoglobin and promote metmyoglobin. In support, 600 MPa dark-cutting steaks had lower redness and chroma than 300 and 450 MPa steaks.

Thiobarbituric acid reactive substances

Past research shows more lipid oxidation and greater TBARS in normal-pH steaks than dark-cutting steaks (English et al., 2016; Wills et al., 2017; Denzer et al., 2022a). Normal-pH control steaks had higher ($P < 0.05$) lipid oxidation on each pull day in retail display, whereas dark-cutting 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 day 8, all HPP-treated steaks had higher ($P < 0.05$) lipid oxidation than dark-cutting control steaks. Dark-cutting steaks treated at 300 and 450 MPa had less ($P < 0.05$) lipid oxidation than normal-pH control steaks, whereas the pressure level of 600 MPa was not different ($P > 0.05$) from normal control steaks by day 8 of retail display. Frenzel (2015) reported that normal-pH steaks treated with HPP showed increased TBARS values compared with steaks not treated with HPP. In support of the current study, Ma et al. (2007) concluded that a pressure level at or above 300 MPa accelerated lipid oxidation. Previous research noted

that HPP increases heme content in sarcoplasm (Bak et al., 2019), which can act as a prooxidant.

Conclusions

The dark appearance of dark-cutting beef leads to discrimination among consumers and discounted prices. Therefore, it is important to improve consumer acceptability of dark-cutting beef and negate economic losses to the beef industry. 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). Dark-cutting steaks treated at 300 MPa exhibited lower lipid oxidation than those at other pressure levels and normal-pH control steaks. In conclusion, the results indicate that the use of HPP at 300 and 450 MPa can improve redness of dark-cutting beef.

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