



# Effects of Sodium Nitrite and Tocopherol Incorporated Poly(Lactic Acid) Biodegradable Films on Dark-Cutting Beef Color

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Abstract: This study aimed to develop poly(lactic acid) (PLA) biodegradable film containing sodium nitrite and  $\alpha$ -tocopherol, and to examine its effect on dark-cutting beef color attributes. Using a twin-screw extruder, PLA pellets were manufactured as a low-concentration (LC) PLA pellet containing 0.12% sodium nitrite and 0.5%  $\alpha$ -tocopherol or a high-concentration (HC) PLA pellet containing 0.6% sodium nitrite and 2.5% α-tocopherol. Extruded PLA pellets were oven-dried before being compressed and molded into film sheets using a hot press. In this study, 7 normal-pH and 7 darkcutting strip loins were selected from a commercial processor. Loins were sliced into 2.54-cm thick steaks and randomly assigned to the respective loin-type treatments. The packaging treatments include normal-pH polyvinyl chloride (PVC), dark-cutting PVC, dark-cutting vacuum, LC-PLA dark-cutting in vacuum, and HC-PLA dark-cutting in vacuum. NormalpH steaks were packaged with PVC overwrap, while dark-cutting control steaks were packaged in PVC overwrap and vacuum-sealed. The respective PLA films (LC or HC) were placed directly on the surface of the dark-cutting steak surface before vacuum packaging, resulting in LC-PLA and HC-PLA treatments. All steaks were placed in a simulated retail display maintained at  $2 \pm 1^{\circ}$ C for 6 d, during which their surface color was evaluated every 24 h using a HunterLab spectrophotometer and a trained panel (n = 6). Half of the steaks were removed from display on d 5 and cooked to 71°C on a George Foreman grill to evaluate the cooked color. The remaining steaks were evaluated on d 6 for microbial growth and lipid oxidation. A significant increase (P < 0.05) in surface redness value was observed for steaks packaged with LC-PLA and HC-PLA within the first 24 h of display. Moreover, HC-PLA steaks exhibited higher redness values (P < 0.05) than LC-PLA, dark-cutting PVC, and dark-cutting vacuum-packaged steak treatments over the entire display period. Steaks packaged with HC-PLA demonstrated greater external cooked color redness (P < 0.05) and greater internal cooked color redness (P < 0.05) than normal-pH steaks in PVC overwrap and dark-cutting steaks in PVC overwrap and vacuum packaging. Notably, LC-PLA steaks presented similar external and internal cooked color redness (P > 0.05) compared to dark-cutting steaks in control vacuum packages (without nitrite). There were no differences (P > 0.05) in aerobic plate count and lipid oxidation between nitrite-embedded and control vacuum dark-cutting treatments. Results indicate that using lower concentrations of sodium nitrite (0.12%) and  $\alpha$ -tocopherol (0.5%) in PLA films can help improve the surface color of raw dark-cutting steaks while minimizing unpleasant cooked color associated with nitrite-embedded film.

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# Introduction

Beef color plays a vital role in consumer purchasing decisions throughout the various stages of the beef supply chain (Altmann et al., 2023; Mancini et al., 2022). Specifically, with retail beef sales, consumers are willing to pay more for products that better satisfy their color expectations and exhibit a bright cherry-red color (Killinger et al., 2004). Dark-cutting beef deviates from the traditional bright cherry-red of fresh beef. Pre-slaughter stress depletes glycogen storage, resulting in less pH decline postmortem (Lawrie, 1958; Scanga et al., 1998) compared to normal postmortem muscle pH (English et al., 2016; Hughes et al., 2017). In addition to the raw color effects, the greater pH of dark-cutting beef and packaging type cause an undercooked appearance (persistent pinking) even after being cooked to 71°C (Denzer et al., 2022). This is primarily because myoglobin is more stable at higher pH values (Hunt et al., 1999), and myoglobin forms such as deoxymyoglobin, nitric oxide myoglobin, and carboxymyoglobin are stable to heat. Thus, developing technologies to improve dark-cutting beef color without affecting cooked meat color is critical to improve consumer acceptability with respect to surface color and, thus, its application in the retail market.

Various post-harvest techniques, such as antioxidant enhancement and modified atmosphere packaging, have been used to improve the color of dark-cutting beef (Mitacek et al., 2018; Wills et al., 2017). In addition, a patented nitrite-embedded packaging, which induces the formation of bright red nitric oxide myoglobin, has also been successfully used to improve the redness of darkcutting beef (Denzer et al., 2022; Ramanathan et al., 2018). However, nitric oxide myoglobin and high pH formation favor persistent pinking in cooked meat. Therefore, the generation of an undercooked appearance limits the use of nitrite-embedded packaging as an innovative technology to improve the color of dark-cutting beef. In the current research, we tested the effects of reduced nitrite levels on the raw and cooked color attributes of dark-cutting beef steaks.

Several countries, including the US, have pledged to limit the use of single-use plastics due to environmental concerns (Moshood et al., 2021). Poly(lactic acid) (PLA) is a commonly used biodegradable polymer (Bher et al., 2023; Han et al., 2018). Limited work has investigated using a PLA biodegradable film incorporating active ingredients such as nitrite within a meat system. Therefore, this work aimed to determine the impacts of PLA films incorporating sodium nitrite and  $\alpha$ -tocopherol on the color and shelf-life attributes of dark-cutting beef.

# **Materials and Methods**

# Film formulation

A twin-screw extruder (screw L/D ratio: 42; screw speed: 120 rpm; Century ZSK- 30; Traverse City, MI) was used to compound PLA resin (Ingeo<sup>™</sup> biopolymer 2003D poly [96% L-lactic acid]), sodium nitrite, and  $\alpha$ -tocopherol. Two recipes were formulated with lower and higher concentrations of the active ingredients. Lowconcentration films included 0.12% sodium nitrite and 0.5% α-tocopherol, and high-concentration films were created with 0.6% sodium nitrite and 2.5%  $\alpha$ -tocopherol. The masterbatch packaging film formulation's temperature range was between 170 and 190 °C. A BT 25 pelletizer (Scheer Bay Co.; Bay City, MI) was used to form the extruded mass into pellets after being cooled in a water bath. The pellets were oven-dried for 24 h at 50°C before being used for later processing to remove moisture. A hot press (PHI QL438-C, City of Industry, CA) operated at 182°C with an applied force of 10 tons for 3 min was used to form film sheets from 5 g samples of each respective pellet concentration recipe. After compression, the film sheets were removed from the hot press, cooled at room temperature, and stored in a refrigerator until later application.

## Oxygen permeability

A MOCON OX-TRAN<sup>®</sup> (2/22 L model; MOCON Inc., Minneapolis, MN) was used to measure the oxygen permeability of the films. The instrument was set to  $10^{\circ}$ C at  $34.3\% \pm 1\%$  relative humidity based on the intended film application conditions and the instrument limitations. The analysis was completed with three different film sets separately.

### Film transmittance analysis

A spectrophotometer (Thermo Scientific, USA) was used to collect the light transmittance of films within ultraviolet and visible light regions. Optical properties were measured in triplicate for each film recipe. Additionally, a digital micrometer (Testing Machines Inc.; Ronkonkoma, NY) with a sensitivity of 0.001 mm was used to determine film thicknesses. Film thickness was read in triplicate. The transparency value (TV) of the film samples was determined using

 $TV = log (T_{600})/x$ , where  $T_{600}$  is the transmittance at 600 nm and x is the film thickness in mm (Han and Floros, 1997).

### Thermogravimetric analysis

A Q50 TGA (TA Instruments; New Castle, DE) was used to evaluate the thermal stability of PLA films. A 10 mg sample of each film recipe was taken and tested from room temperature to 600°C with an increase of 10°C/min. Nitrogen gas was used to purge the system with a balance purge flow of 40 mL/min and a sample purge flow of 60 mL/min. Characteristic temperatures were noted during analysis in addition to residual ash content after heating. Initial decomposition temperature  $(T_{onset})$  was determined when 5% of the sample weight was lost through testing. Decomposition peak temperature  $(T_{peak})$  was noted by the peak of the derivative weight (%/°C) output curve during testing. Finally, residual ash was measured by the remaining weight percentage of the sample following testing.

# Raw materials, processing, and packaging

Beef strip loins (*longissimus lumborum*) were collected from a commercial beef processing facility, 5 d postmortem. Seven USDA Low Choice (pH = 5.40 to 5.66) and seven dark-cutting (pH = 6.16 to 6.67) strip loins were selected, and vacuum-packaged loins were transported on ice to the Robert M. Kerr Food and Agricultural Products Center at Oklahoma State University (Stillwater, OK). Loins were repackaged (CRYOVAC;  $16 \times 24$  pouches; 3 mil thickness) using a vacuum sealer (Sipromac 420A Double Chamber Vacuum Sealer; Sipromac; Drummondville, Canada) upon arrival and stored at 2°C in the dark for 2 d before being further processed.

#### pH and proximate composition analysis

A Hanna Instruments pH probe (Handheld HI 99163; probe FC232; Hanna Instruments, Woonsocket, RI) was used to measure the initial pH of normal-pH and dark-cutting loins during processing. Measurements were taken at 3 random locations of the *longissimus lumborum* muscle along the length of the loin.

The first and fifth steak from the anterior end of each loin was taken to determine the percentage protein, fat, and moisture for each strip loin. *Longissimus lumborum* muscle was ground with a tabletop grinder after trimming all visible external fat (Big Bite Grinder, coarse grind, LEM), pressed into a 140 mm sample cup, and analyzed with an AOAC-approved near-infrared spectrophotometer (FoodScan Lab Analyzer, Serial No. 91753206; Foss, NIRsystem Inc., Slangerupgrade, Denmark). Samples were read in triplicate and averaged.

### Packaging and simulated retail display

Loins were sliced into 2.54-cm-thick steaks from the anterior end with a meat slicer (Bizerba USA INC., Piscataway, NJ). Normal-pH steaks were randomly selected for polyvinyl chloride (PVC) overwrap control. Dark-cutting steaks were randomly assigned to either a PVC overwrap control, a vacuum package control, a low-concentration PLA film in a vacuum package, or a high-concentration PLA film in a vacuum package. Steaks serving as PVC control steaks for normal-pH and dark-cutting loins were placed in Styrofoam trays and overwrapped with PVC (15,500 to 16,275 cm<sup>3</sup>  $O_2/m^2$  /24 h at 23°C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film; Koch Supplies; Kansas) using a film wrap machine (Winholt WHSS-1, 115V; Woodbury, NY). Vacuum package (Walton's Vacuum Pouch;  $10 \times 10$  pouches; 3 mil thickness) treatments were vacuum sealed using a vacuum sealer (Sipromac 420A Double Chamber Vacuum Sealer; Sipromac, Drummondville, Canada). Low- and high-concentration PLA treatments had the respective film placed directly on the steak cut surface and were vacuum packaged (Walton's Vacuum Pouch;  $10 \times 10$  pouches; 3 mil thickness) using a vacuum sealer (Sipromac 420A Double Chamber Vacuum Sealer; Sipromac, Drummondville, Canada). Packaged steaks were then placed in a simulated retail display using a white coffin-style display case at  $2 \pm 1^{\circ}$ C under continuous LED lighting (Phillips LED lamps; 12 watts, 48 inches, color temperature =  $3,500^{\circ}$ K; Phillips, China) for either 5 or 6 d. Two steaks from each loin were taken for the respective packaging treatments; one steak was utilized for cooking analysis, and the other steak was used for microbial analysis and lipid oxidation. Cooking analysis took place on d 5 of the display to maximize nitric oxide myoglobin formation, whereas microbial growth and lipid oxidation were assessed on d 6.

## Retail color analysis

A HunterLab 4500L MiniScan EZ Spectrophotometer (2.5-cm aperture, illuminant A, and 10° standard observer angle; HunterLab Associates, Reston, VA) was used to measure the instrumental color of steaks every 24 h where each packaged steak surface was read in triplicate. Both CIE  $L^*$ ,  $a^*$ , and  $b^*$  values and spectral reflectance from 400 to 700 were recorded. Chroma, hue angle, and nitric oxide myoglobin formation were calculated to determine surface color. Chroma  $\left[\sqrt{(a^*)^2 + (b^*)^2}\right]$  represents the red intensity of the steak color, with a larger value indicating a brighter red color (King et al., 2023). Nitric oxide myoglobin was calculated as the ratio of reflectance of 650 and 570 nm, where a larger number indicates more nitric oxide myoglobin formation (King et al., 2023).

Both raw and cooked visual colors were evaluated for all steaks by a trained panel (n = 6) every 24 h. The Oklahoma State University Institutional Review Board (approval number: AG-18-34) approved the visual color analysis. Before participating, panelists (n = 6)passed the Farnsworth Munsell 100-hue test (King et al., 2023). Panelists determined muscle color (1 =extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark red, 6 = moderately dark red, 7 = dark red, surface discoloration (1 = no discoloration [0%], 2 = minimal discoloration [1-10%], 3 = slight discoloration [11–20%], 4 = small discoloration [21-40%], 5 = modest discoloration [41-60%], 6 =moderate discoloration [61–80%], 7 =extensive discoloration [81-100%]), and muscle darkening (1 = no darkening, 3 = slightly dark, 5 = moderatelydark, 7 = very dark) of steaks using 7-point scales throughout display.

# Microbiology

The total plate count was determined on d 0 and d 6 of storage. A 10 g muscle sample was aseptically collected from the surface of each loin (d 0) or steak (d 6) and placed in a sterile stomacher bag (VWR; Radnor, PA) in addition to 90 mL of 0.1% sterile peptone water (BactoTM Peptone Ref 211677 Becton; Dickinson and Company; Sparks, MD). Stomacher bags were placed in a stomacher (Seward; STOMACHER<sup>®</sup> 400 Circulator; 3500 model) and mixed for 30 s. One mL of the stomacher bag mixture was removed and serially diluted into 9 mL of 0.1% sterile peptone water. Each dilution was aseptically plated in duplicate by 1 mL of the solution added to 3M Petrifilm Aerobic Count plates (3M Health Care; St. Paul, MN). Plates were incubated for 48 h at 37°C in a VWR Forced Air General Incubator (0.15 m<sup>3</sup>; VWR, Radnor, PA). After 48 h, plates were removed from the incubator and enumerated to determine the total plate count based on colony forming unit per g.

#### Thiobarbituric acid reactive substances assay

Lipid oxidation was evaluated on d 0 and 6 of storage. A 3 g muscle sample was taken from the surface and blended with 27 mL of trichloroacetic acid for 10 s using a Waring commercial blender (Model 33BL7; New Hartford, CT) before being filtered with a Whatman 42 filter paper. In a test tube, 1 mL of filtrate was combined with 1 mL of thiobarbituric acid and briefly vortexed before being placed in a 100°C water bath for 10 min. Samples were cooled for 5 min before obtaining the absorbance at 532 nm using a spectrophotometer (UV-2600, UV-VIS Spectrophotometer; Shimadzu, Columbia, MD) on the photometric setting. A blank was created by mixing 1 mL of trichloroacetic with 1 mL of thiobarbituric acid. The raw absorbance value of samples was taken and multiplied by a factor of 4.16 (for 1:9 dilution of the protocol) to convert the value to mg malondialdehyde per kg meat (King et al., 2023).

# Cooked color analysis

After 5 d of retail display, steaks were cooked to an internal temperature of 71°C on a George Foreman Grill (Lean Mean Fat Grilling Machine George Foreman; Lake Forest, IL GRP99A). Steaks were placed on ice for 5 min after cooking to prevent post-temperature rise. Instrumental cooked color measurements were taken with a HunterLab 4500L MiniScan Spectrophotometer. The external cooked color was taken in triplicate across the cooked surface, while the internal color was taken in triplicate on bisected steak pieces cut perpendicular to the cooked surface near the center of the steak.

Six trained panelists determined visual cooked color using 7-point scales for both external and internal color. Visual external cooked color (1 = brown, 2 = lightbrown, 3 = slightly brownish-red, 4 = reddish-brown, 5 = pinkish-brown, 6 = slightly pinkish-red, 7 = pinkish-red) was observed on the cooked surface of each steak, while visual internal cooked color (1 = very red,2 = slightly red, 3 = pink, 4 = slightly pink, 5 = pinkish gray, 6 = grayish-tan/brown, 7 = tan/brown) was noted based on the appearance of the bisected steak pieces used to determine instrumental internal cooked color.

## Quantification of nitrite and nitrate in beef

Both nitrite and nitrate samples were quantified using a NOx Analyzer (ENO-30; Fushimi-ku Kyoto, Japan) after 5 d of storage in low- and high-concentration nitrite PLA films. The meat samples were taken from the entire thickness of the steak. The samples Smith et al.

were frozen in liquid nitrogen and pulverized using a Waring blender. Frozen samples were shipped in dry ice. Five grams of powdered sample was weighed and homogenized with 30 mL of Dulbecco's Phosphate Buffer Saline (2.5 mM EDTA and 10 mM N-ethylmaleimide) for 1 min at 10,000 rpm. Following homogenization, the slurry was centrifuged at  $10,000 \times g$  at 4°C for 8 min. The supernatant  $(400 \ \mu L)$  was transferred into a 2 mL centrifuge tube, and 400 µL of methanol was added. The samples were vortexed at high speed for 3 to 5 s and allowed to sit at 4°C for 10 min. The tubes were centrifuged for 10 min at  $13,000 \times g$  at 4°C. A 100 µL of the supernatant was placed into a 96-well plate and analyzed using an Eno-30 analyzer (high-performance liquid chromatography specific for nitrite and nitrate). The ENO-30's high sensitivity is attained by the combination of a diazo coupling technique with the extract to be measured and the separation of nitrite and then nitrate using a reverse-phase column. To separate nitrite and nitrate, the nitrate is first reduced to nitrite through a reaction with cadmium and reduced copper inside a reduction column. The two resolved peaks are then mixed with Griess reagent (dinitrogen trioxide,  $N_2O_3$ , generated from acidified nitrite that reacts with sulfanilamide) in-line to form the classical diazo compound, which can be detected spectrophotometrically. This system allows for easy sample preparation, little if any cross-reactivity, and high throughput when coupled with an auto-sampler. The system is adaptable to a wide range of nitrite and nitrate concentrations regardless of their respective ratios and operates at a sensitivity level of  $1 \text{ nM} \times 100 \text{-}\mu\text{L}$  injections for each anion with no interference from protein or other colored species. The results from raw steak samples were reported in parts per million (ppm).

## Statistical analysis

The effects of active PLA films incorporating sodium nitrite and  $\alpha$ -tocopherol on dark-cutting beef color were determined based on a randomized complete block design. The treatments applied to dark-cutting loins consisted of either a control treatment in PVC overwrap, a control treatment in vacuum packaging, or the low- or high-concentration active PLA films within vacuum packages. Each loin represented a block, and the 7 replicates were served by the 7 strip loins. Packaging treatment, retail display time, and their interactions were the fixed effects. The random effect was each loin, and day was a repeated measure observed in the study. The repeated measures

covariance-variance structure was determined by evaluating the AICC output. The GLIMMIX procedure of SAS (SAS 9.4; SAS Inst., Cary, NC) was used to determine the least-squares means, and significance was considered at P < 0.05. The least-squares means were separated using the PDIFF option (least-squares difference) and were significant at P < 0.05. Normal-pH steak values were reported as averages, and the significant differences were established based on standard error.

# **Results and Discussion**

The overall goal of the study was to determine the transfer of nitrite from biodegradable film to meat and to study the effect on color. An anaerobic condition is required to form a bright red color with nitrite film. In the current research, an oxygen-impermeable layer was not included in the biodegradable layer. Therefore, nitrite-containing PLA film was placed on meat, followed by packaging in a vacuum bag to create anaerobic conditions. Hence, the film characterizing analysis tested both PLA films and vacuum packaging materials.

# Oxygen transmission rate

There was no effect (P > 0.05) of PLA film type on the oxygen transmission rate (Table 1). Testing was completed by layering PLA films with the commercially available vacuum package. Thus, the PLA film layer was expected to have a limited effect on the oxygen transmission rate since the commercial vacuum package film created the main barrier with the adjusted testing approach. Therefore, low- and high-concentration PLA films exhibited similar (P > 0.05) oxygen transmission rate values.

**Table 1.** Oxygen transmission rate1of PLA filmslayered with control vacuum film

Film	OTR
Low-concentration <sup>2</sup> w/ vacuum	5.89
High-concentration <sup>3</sup> w/ vacuum	6.57
$SEM^4 = 0.29$	

<sup>1</sup>Oxygen transmission rate (OTR) units given as  $cm^3 \times mm/m^2 \times day$ .

 $^{2}$ Low-concentration poly(lactic acid) (PLA) film compounded with PLA, 0.12% sodium nitrite, and 0.50%  $\alpha$ -tocopherol.

 $^3\text{High-concentration PLA}$  film compounded with PLA, 0.6% sodium nitrite, and 2.5%  $\alpha\text{-tocopherol}.$ 

 $^{4}$ SEM = standard error of the mean.

### Film fransparency

There was no difference (P > 0.05) between lowand high-concentration PLA film transparency values when sampled alone and when layered with the vacuum package. However, there were differences (P < 0.05) between vacuum and PLA films (Table 2). While transparency values between the PLA film recipes were not different (P > 0.05), there was a visual

**Table 2.** Transparency value<sup>1</sup> of films<sup>2</sup>

Film	TV
Control vacuum	25.57ª
Low-concentration PLA	10.86 <sup>b</sup>
High-concentration PLA	10.49 <sup>b</sup>
Low-concentration w/ vacuum	7.07 <sup>c</sup>
High-concentration w/ vacuum	6.70 <sup>c</sup>
$SEM^3 = 0.63$	

<sup>a-c</sup>Least-squares means with different letters are significantly different (P < 0.05).

<sup>1</sup>A greater transparency value (TV) indicates a clearer sample and is calculated by  $TV = \log (T_{600})/x$ , where  $T_{600}$  represents transmittance at 600 nm and x represents the film thickness.

<sup>2</sup>Films consisted of a commercial vacuum package film; a lowconcentration poly(lactic acid) (PLA) film compounded with PLA, 0.12% sodium nitrite, and 0.5%  $\alpha$ -tocopherol; a high-concentration PLA film compounded with PLA, 0.6% sodium nitrite, and 2.5%  $\alpha$ tocopherol; the low-concentration PLA film with a layer of commercial vacuum film; and the high-concentration PLA film with a layer of commercial vacuum film.

 $^{3}$ SEM = standard error of the mean.

color difference (Figure 1). Previous work indicated increased yellowness in films incorporating  $\alpha$ -tocopherol (Byun et al., 2010; Hwang et al., 2012; Manzanarez-López et al., 2011). The current work showed that the high-concentration PLA film recipe exhibited a more yellow appearance than the low-concentration PLA films because of the greater  $\alpha$ -tocopherol content (Figure 1). In addition, increased transparency was previously noted with the addition of sodium nitrite in films (Chatkitanan and Harnkarnsujarit, 2020). However, in this study, sodium nitrite content within the PLA films did not (P > 0.05) affect transparency (Table 2).

### Film thermal stability

There was no effect (P > 0.05) of PLA film type on onset temperature, decomposition temperature, or residual ash percentage (Table 3), suggesting similar thermal stability properties were observed between low- and high-concentration PLA films. Both film recipes also noted a single-phase degradation (Figure 2). Previous research has shown that the addition of  $\alpha$ -tocopherol (at 2.2% and 4.4% concentrations) did not impact the thermal stability of PLA films (Gonçalves et al., 2011). Alternatively, Chatkitanan and Harnkarnsujarit (2020) reported sodium nitrite within thermoplastic starch films significantly decreased the thermal stability of films. Further testing of film recipes containing PLA and PLA in combination with each active ingredient individually will help to determine the



**Figure 1.** Images of color differences between poly(lactic acid) (PLA) film recipes<sup>1</sup>. <sup>1</sup>Low-concentration PLA films were compounded with PLA, 0.12% sodium nitrite, and 0.5%  $\alpha$ -tocopherol, and high-concentration PLA films were compounded with PLA, 0.6% sodium nitrite, and 2.5%  $\alpha$ -tocopherol. PLA films were placed directly on display surface of steaks before being vacuum packaged (Walton's Vacuum Pouch; 10 × 10 pouches; 3 mil thickness).

American Meat Science Association.

Table 3.	Thermal	stability	values	of PLA	films <sup>1</sup>
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Film	$T_{onset}^2$	$T_{peak}^{3}$	Residual Ash <sup>4</sup> (%)
Low-concentration	296.69	350.52	4.75
High-concentration	265.70	329.42	4.99
SEM <sup>5</sup>	8.13	6.61	1.52

<sup>1</sup>Low-concentration poly(lactic acid) (PLA) films were compounded with PLA, 0.12% sodium nitrite, and 0.5%  $\alpha$ -tocopherol, and high-concentration PLA films were compounded with PLA, 0.6% sodium nitrite, and 2.5%  $\alpha$ -tocopherol.

 $^{2}T_{onset}$  is the initial decomposition temperature and is noted when 5% of sample weight is lost during heating.

 ${}^{3}T_{peak}$  is determined by the peak of the derivative weight (%/°C) curve.  ${}^{4}$ Residual ash is the remaining weight percentage at the completion of heating.

 $^{5}$ SEM = standard error of the mean.



**Figure 2.** Thermogravimetric response<sup>1</sup> of low<sup>2</sup>- and high<sup>3</sup>concentration poly(lactic acid) (PLA) films. <sup>1</sup>Weight (%) results are given as a continuous line and derivative weight (%/°C) is shown as a dashed line. <sup>2</sup>Low-concentration PLA films were compounded with PLA, 0.12% sodium nitrite, and 0.5%  $\alpha$ -tocopherol. <sup>3</sup>High-concentration PLA films were compounded with PLA, 0.6% sodium nitrite, and 2.5%  $\alpha$ -tocopherol.

impact sodium nitrite and  $\alpha$ -tocopherol have on thermal stability.

#### pH and proximate composition

In the current work, dark-cutting loins had greater pH (P < 0.05) than normal-pH loins (Table 4). Previous research also noted that dark-cutting beef has greater pH values than normal-pH beef (Mitacek et al., 2018; Ramanathan et al., 2018; Sawyer et al., 2009; Wills et al., 2017). Limited glycogen content postmortem leads to less pH decline in dark-cutting beef, resulting in greater pH values than normal-pH beef (Lawrie, 1958; Scanga et al., 1998). Both loin types showed similar (P > 0.05) protein content percentages (Table 4). However, there was a significant loin-type effect on the moisture and fat content of loins.

**Table 4.** Proximate composition (%) and pH of normal-pH and dark-cutter strip loins

Component	Normal-pH	Dark-cutter	SEM <sup>1</sup>
pН	5.55 <sup>b</sup>	6.40 <sup>a</sup>	0.06
Moisture (%)	74.11 <sup>b</sup>	76.28 <sup>a</sup>	0.27
Fat (%)	4.69 <sup>a</sup>	2.58 <sup>b</sup>	0.31
Protein (%)	22.30 <sup>a</sup>	22.15ª	0.17

<sup>a,b</sup>Least-squares means with different letters within each row are significantly different (P < 0.05).

The experiment was replicated seven times (n = 7).

 $^{1}$ SEM = standard error of the mean.

Dark-cutting loins exhibited greater (P < 0.05) moisture and lower (P < 0.05) fat percentages than normal-pH loins (Table 4). The high-pH of dark-cutting beef results in a lack of muscle fiber shrinkage and increases water holding capacity (Hughes et al., 2017), which parallels the results shown within this study. Furthermore, the fat percentage difference noted between normal-pH and dark-cutting loins can be attributed to the dark-cutting loins being selected primarily for their pH value rather than their marbling content.

# Residual nitrite and nitrate content due to contact with nitrite-containing PLA film

High-concentration PLA transferred more nitrite and nitrate to meat than low-concentration PLA film (Table 5). Previous study also noted the migration of active ingredients, such as sorbic acid, from PLA film to the food matrix. However, migration of sorbic acid was greater when 95% ethanol was used than 10% ethanol (Rodríguez-Martínez et al., 2016).

### Retail display color

A packaging type × day interaction (P < 0.05) was observed for  $L^*$  values during the display (Figure 3). Normal-pH steaks in PVC showed greater  $L^*$  values (P < 0.05) compared with all other treatments throughout the display duration. Previous research showed greater deoxymyoglobin formation occurred in darkcutting beef than in normal-pH beef, resulting in decreased  $L^*$  values (English et al., 2016; Hughes et al., 2017; McKeith et al., 2016).

A packaging type × day interaction (P < 0.05) was observed for  $a^*$  and chroma values during the display (Figures 4 and 5). Normal-pH steaks in PVC overwrap exhibited a redder (P < 0.05) appearance than all other treatments from d 0 to d 5 of the simulated retail display. Previous work also observed greater  $a^*$  and

Table 5. Residual nitrite and nitrate (in ppm) in the dark-cutting steak surface after packaging in PLA film for 5  $d^1$ 

	Nitrite	Nitrate
Low-concentration (0.12%) film	1.07 <sup>b</sup>	19.04 <sup>b</sup>
High-concentration (0.6%) film	1.26 <sup>a</sup>	33.00 <sup>a</sup>
Standard error of the mean	0.06	1.81
<i>P</i> value	0.03	0.0002

<sup>a,b</sup>Least-squares means with different letters within each column are significantly different (P < 0.05).

<sup>1</sup>Low-concentration poly(lactic acid) (PLA) films were compounded with PLA, 0.12% sodium nitrite (1,200 parts per million [ppm]), and 0.5% α-tocopherol, and high-concentration PLA films were compounded with PLA, 0.6% sodium nitrite (6,000 ppm), and 2.5% α-tocopherol. NOx analyzer is a high-performance liquid chromatography-based technique specifically designed for nitrite and nitrate.

chroma values from normal-pH steaks than darkcutting steaks in the aerobic display (Apple et al., 2011; Mitacek et al., 2018; Ramanathan et al., 2018; Stackhouse et al., 2016; Wills et al., 2017). Moreover, greater pH in dark-cutting steaks decreases light reflectance and increases oxygen consumption compared to normal-pH steaks (Hughes et al., 2017; McKeith et al., 2016); hence, lower  $a^*$  and chroma values are reported in dark-cutting steaks (Hunt et al., 1999; Reesman et al., 2023). Redness increased ( $a^*$  and chroma values) in both low- and high-concentration PLA film treatments within the first 24 h of the display. In support, a previous study using patented nitrite-film also reported increased redness in dark-cutting beef (Denzer et al., 2020, 2022). In addition, Ramanathan et al. (2018) noted that the addition of an antioxidant (rosemary) to nitrite film

exhibited greater color stability through limiting oxidation of nitric oxide myoglobin in dark-cutting beef. Low- and high-concentration PLA film steaks possessed significantly greater  $a^*$  values than dark-cutting steaks in vacuum packaging alone after d 2 of the display.

There was a packaging type × day interaction (P < 0.05) for nitric oxide myoglobin formation (Table 6). Low- and high-concentration PLA film treatments showed significant increases in nitric oxide myoglobin formation throughout the display. Moreover, following d 1, high-concentration PLA film steaks consistently presented significantly greater (P < 0.05) levels of nitric oxide myoglobin formation than steaks packaged in low-concentration PLA films throughout the entire display period.

There was a packaging type  $\times$  day interaction (P <0.05) for muscle color (Figure 6), surface discoloration (Figure 7), and muscle darkening (Figures 8 and 9). A significantly brighter cherry-red appearance (P <0.05) was noted for normal-pH steaks in PVC than all other treatments up to d 4 of retail display. Additionally, high-concentration film steaks exhibited brighter cherry red (P < 0.05) muscle color than darkcutting steaks in PVC and vacuum packaging without nitrite. No discoloration was noted for all steaks in vacuum packaging during retail display, including lowand high-concentration PLA film steaks. However, both normal-pH and dark-cutting steaks in PVC exhibited discoloration during display. Normal-pH steaks in PVC demonstrated a more significant discoloration than dark-cutting steaks in PVC from d 4 to d 6. Moreover, high-concentration PLA film steaks showed decreased (P < 0.05) muscle darkness throughout the



**Figure 3.** Least-squares means of external *L*\* values (packaging type<sup>1</sup> × day) of steaks during a 6 d retail display. Least-squares means with different letters (a–m) are significantly different (P < 0.05). Standard error of the mean indicated by error bars (SEM = 1.10). The experiment was replicated seven times (n = 7). <sup>1</sup>Packaging treatments included normal-pH steaks in polyvinyl chloride (PVC) overwrap, dark-cutter steaks in PVC overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration poly(lactic acid) (PLA) film (compounded with PLA, 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package.

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Figure 4. Least-squares means of external  $a^*$  values (packaging type<sup>1</sup> × day) of steaks during a 6-d retail display. Least-squares means with different letters (a–r) are significantly different (P < 0.05). Standard error of the mean indicated by error bars (SEM = 0.81). The experiment was replicated seven times (n = 7). <sup>1</sup>Packaging treatments included normal-pH steaks in polyvinyl chloride (PVC) overwrap, dark-cutter steaks in PVC overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration poly(lactic acid) (PLA) film (compounded with PLA, 0.12% sodium nitrite, and 0.5%  $\alpha$ -tocopherol) in a vacuum package.



**Figure 5.** Least-squares means of external chroma (packaging type<sup>1</sup> × day) of steaks during a 6-d retail display. Least-squares means with different letters (a–n) are significantly different (P < 0.05). Standard error of the mean indicated by error bars (SEM = 1.04). The experiment was replicated seven times (n = 7). <sup>1</sup>Packaging treatments included normal-pH steaks in polyvinyl chloride (PVC) overwrap, dark-cutter steaks in PVC overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration poly(lactic acid) (PLA) film (compounded with PLA, 0.12% sodium nitrite, and 0.5%  $\alpha$ -tocopherol) in a vacuum package.

display than dark-cutting steaks packaged in PVC and vacuum package.

Roberts et al. (2017) observed that bison steaks in nitrite-embedded packaging increased redness and experienced less discoloration than control packaged steaks. Furthermore, ribeye and round steaks sprayed with a nitrite solution showed improved red color (Song et al., 2015). Previous research also noted darker muscle color and less discoloration in dark-cutting steaks than normal-pH steaks during retail display (Apple et al., 2011; Mitacek et al., 2018; Sawyer et al., 2009; Stackhouse et al., 2016; Wills et al., 2017). Initial darkness of dark-cutting steaks in low-and high-concentration film packages can be caused by metmyoglobin formation during the interconversion to nitric oxide myoglobin (Fox Jr. and Ackerman, 1968; Siegel, 2011). The decreased muscle darkening observed by high-concentration PLA film steaks in the current work is due to the formation of bright red nitric oxide myoglobin (Ramanathan et al., 2018; Denzer et al., 2022).

### Total plate counts and lipid oxidation

There were no differences (P > 0.05) in total plate count between normal-pH and dark-cutting loins on d 0

**Table 6.** Effects of display day on nitric oxide myoglobin formation<sup>1</sup>

Day	Low-concentration PLA <sup>2</sup>	High-concentration PLA <sup>3</sup>
0	3.50 <sup>j</sup>	2.23 <sup>k</sup>
1	4.09 <sup>i</sup>	4.55 <sup>de</sup>
2	4.14 <sup>hi</sup>	4.69 <sup>cd</sup>
3	4.18 <sup>ghi</sup>	4.88 <sup>bc</sup>
4	4.34 <sup>efg</sup>	5.09 <sup>b</sup>
5	4.55 <sup>def</sup>	5.61 <sup>a</sup>
6	4.77 <sup>bcd</sup>	5.77 <sup>a</sup>
$SEM^4 = 0.15$		

<sup>a-k</sup>Least-squares means with different letters are significantly different (P < 0.05).

The experiment was replicated seven times (n = 7).

 $^1 \rm Nitric oxide myoglobin formation was calculated as the ratio of R650 <math display="inline">\div$  R570 nm. A greater number indicates more nitric oxide myoglobin formation.

 $^2Low-concentration poly(lactic acid) (PLA) film compounded with PLA, 0.12% sodium nitrite, and 0.5% <math display="inline">\alpha$ -tocopherol.

 $^3\text{High-concentration PLA}$  film compounded with PLA, 0.6% sodium nitrite, and 2.5%  $\alpha\text{-tocopherol}.$ 

 $^{4}SEM =$  standard error of the mean.

(Table 7). However, there was a significant packaging type effect for d 6 microbial growth analysis as darkcutting steaks in PVC exhibited greater (P < 0.05) microbial growth than all other treatments (Table 7). Previous research reported a 1-log reduction for aerobic plate counts with nitrite-embedded packaging (Narváez-Bravo et al., 2017; Ramanathan et al., 2018). However, there was no difference (P > 0.05) in aerobic plate count between nitrite-embedded and control vacuum treatments. Thus, the differences in microbial growth in the current work may be attributed to the anaerobic environment rather than the active ingredients. Therefore, evaluating anaerobic plate counts for future studies may be beneficial to better understand the impact of nitrite and alpha-tocopherol addition within PLA films on total plate counts.

In addition, there was no difference (P > 0.05) between normal-pH and dark-cutting loin steaks for lipid oxidation on d 0 (Table 7). Nonetheless, a packaging type effect (P < 0.05) was noted on d 6, with normal-pH steaks in PVC overwrap possessing greater (P < 0.05) lipid oxidation values than all other treatments. Dark-cutting beef has lower lipid oxidation values than normal-pH beef (English et al., 2016; Wills et al., 2017). The addition of sodium nitrite in previous research reduced lipid oxidation compared to their respective control treatment (Roberts et al., 2017; Chatkitanan and Harnkarnsujarit, 2020). However, Denzer et al. (2022) observed that greater pH of darkcutting beef minimizes packaging impact (PVC vs. vacuum) on lipid oxidation.

## Cooked color

Cooked color is primarily due to myoglobin denaturation and Maillard reaction (Hunt et al., 1999; Trevisan et al., 2016). Denaturation of myoglobin leads to either red ferro- or brown ferrihemochorme. The pH of meat influences the denaturation of myoglobin. Deoxymyoglobin, carboxy myoglobin, and nitric oxide myoglobin are resistant to denaturation,



**Figure 6.** Least-squares means of muscle color<sup>1</sup> evaluated by a trained panel (packaging type<sup>2</sup> × day) of steaks during a 6-d retail display. Least-squares means with different letters (a–t) are significantly different (P < 0.05). Standard error of the mean indicated by error bars (SEM = 0.22). The experiment was replicated seven times (n = 7). <sup>1</sup>Muscle color evaluated using a 7-point scale (1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark red, 6 = moderately dark red, 7 = dark red). <sup>2</sup>Packaging treatments included normal-pH steaks in polyvinyl chloride (PVC) overwrap, dark-cutter steaks in PVC overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration poly(lactic acid) (PLA) film (compounded with PLA, 0.12% sodium nitrite, and 0.5%  $\alpha$ -tocopherol) in a vacuum package.

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■ Normal-pH PVC ■ Dark-cutter PVC ■ Dark-cutter vacuum ■ Low-concentration PLA ■ High-concentration PLA

Figure 7. Least-squares means of surface discoloration<sup>1</sup> evaluated by a trained panel (packaging type<sup>2</sup> × day) of steaks during a 6-d retail display. Least-squares means with different letters (a–h) are significantly different (P < 0.05). Standard error of the mean indicated by error bars (SEM = 0.56). The experiment was replicated seven times (n = 7). <sup>1</sup>Surface discoloration evaluated using a 7-point scale (1 = no discoloration [0%], 2 = minimal discoloration [1–10%], 3 = slight discoloration [11–20%], 4 = small discoloration [21–40%], 5 = modest discoloration [41–60%], 6 = moderate discoloration [61–80%], 7 = extensive discoloration [81–100%]). <sup>2</sup>Packaging treatments included normal-pH steaks in polyvinyl chloride (PVC) overwrap, dark-cutter steaks in PVC overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with high-concentration PLA film (compounded with PLA, 0.12% sodium nitrite, and 0.5%  $\alpha$ -tocopherol) in a vacuum package.



**Figure 8.** Least-squares means of muscle darkening<sup>1</sup> evaluated by a trained panel (packaging type<sup>2</sup> × day) of steaks during a 6-d retail display. Least-squares means with different letters (a–r) are significantly different (P < 0.05). Standard error of the mean indicated by error bars (SEM = 0.30). The experiment was replicated seven times (n = 7). <sup>1</sup>Muscle darkening evaluated using a 7-point scale (1 = no darkening, 3 = slightly dark, 5 = moderately dark, 7 = very dark). <sup>2</sup>Packaging treatments included normal-pH steaks in polyvinyl chloride (PVC) overwrap, dark-cutter steaks in PVC overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration poly(lactic acid) (PLA) film (compounded with PLA, 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package.

while metmyoglobin and oxymyoglobin are very susceptible to heat (Denzer et al., 2020; Djimsa et al., 2017). Greater heat stability was noted with deoxymyoglobin at greater pH levels than metmyoglobin when heated to 70°C (Hunt et al., 1999). There was a packaging type effect (P < 0.05) on both external (Table 8) and internal (Table 9) cooked color  $a^*$  values. Greater (P < 0.05) external cooked color redness ( $a^*$  values) was exhibited by high-concentration PLA film steaks compared to all other treatments. In

addition, high-concentration PLA film steaks possessed significantly greater (P < 0.05) internal cooked color a\* values than normal-pH and dark-cutter steaks in PVC and dark-cutter steaks in vacuum packaging. Previous research also observed increases in  $a^*$  values and redness for steaks packaged within nitrite packaging (Claus and Du, 2013; Song et al., 2015). Song et al. (2015) observed an increase in nitrosylhemochrome on the surface of cooked steaks that had previously been packaged in nitrite packaging. Nitrosylhemochrome is the distinctive pink pigment given by cured meats (MacDougall et al., 1975). Therefore, the higher cooked color redness of the high-concentration PLA film packaged steaks can be attributed to the greater formation of nitrosylhemochrome on the steak surface. There was no difference (P > 0.05) in external and internal cooked color redness between low-concentration PLA film steaks and dark-cutting steaks in vacuum packaging. Greater cooked color redness and cooked color  $a^*$  values are noted with dark-cutting steaks compared to normal-pH steaks (Sawyer et al., 2009). Furthermore, there was a packaging type effect (P < 0.05) on both external (Table 8) and internal (Table 9) cooked color chroma values. High-concentration PLA film steaks exhibited greater (P < 0.05) external cooked color chroma values than all other treatments. Additionally, low-concentration PLA film and dark-cutting steaks in vacuum packages showed similar (P > 0.05) external and internal cooked color chroma values.

A packaging type effect (P < 0.05) was observed for external (Table 8) and internal (Table 9) cooked color hue angle values. Smaller (P < 0.05) external and internal cooked color hue angle values were



**Figure 9.** Visual effect of packaging treatments<sup>1</sup> on steaks at the beginning and end of retail display. <sup>1</sup>Packaging treatments included normal-pH steaks in polyvinyl chloride (PVC) overwrap, dark-cutter steaks in PVC overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration poly(lactic acid) (PLA) film (compounded with PLA, 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with PLA, 0.6% sodium nitrite, and 2.5% α-tocopherol) in a vacuum package.

**Table 7.** Effects of packaging type<sup>1</sup> on total plate count and lipid oxidation on d 6

Treatment	Total plate count (Log CFU/mL)	Lipid oxidation (mg MDA/kg)
Normal-pH PVC	0.40 <sup>b</sup>	0.56 <sup>a</sup>
Dark-cutter PVC	1.86 <sup>a</sup>	0.42 <sup>b</sup>
Dark-cutter vacuum	0.26 <sup>b</sup>	0.43 <sup>b</sup>
Low-concentration PLA	0.38 <sup>b</sup>	0.38 <sup>b</sup>
High-concentration PLA	0.24 <sup>b</sup>	0.32 <sup>bc</sup>
$SEM^2 = 0.28$		0.04

<sup>a,b</sup>Least-squares means with different letters are significantly different (P < 0.05).

The experiment was replicated seven times (n = 7). Day 0 microbial growth for normal-pH and dark cutter loins were 0.09 and 0.60 CFU/mL, respectively (SEM = 0.18). Day 0 thiobarbituric acid reactive substances for normal-pH and dark-cutter loins were 0.39 and 0.41 mg MDA/kg, respectively (SEM = 0.04).

<sup>1</sup>Packaging treatments included normal-pH steaks in polyvinyl chloride (PVC) overwrap, dark-cutter steaks in PVC overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration poly(lactic acid) (PLA) film (compounded with PLA, 0.12% sodium nitrite, and 0.5%  $\alpha$ -tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with PLA, 0.6% sodium nitrite, and 2.5%  $\alpha$ -tocopherol) in a vacuum package.

 $^{2}$ SEM = standard error of the mean.

expressed by high-concentration PLA film steaks compared to all other treatments. Furthermore, low-concentration PLA film steaks and dark-cutting steaks in vacuum packaging demonstrated similar (P > 0.05) external and internal cooked color hue angles.

There was a packaging type effect (P < 0.05) on the external cooked color panel evaluation (Table 8 and Figure 10). The trained panel determined the high-concentration PLA film steaks exhibited a redder pink (P < 0.05) external cooked color compared to all other treatments. Alternatively, panelists observed a similar (P > 0.05) external cooked color appearance between low-concentration PLA film steaks and darkcutting steaks in vacuum packaging. Additionally, there was no significant packaging type effect on internal cooked color panel evaluation scores (Table 9). In the current research, dark-cutting cooked steaks had numerically greater redness than normal-pH cooked steaks. However, visual panelists were not able to observe visual differences in redness. A packaging type effect (P < 0.05) was shown for external cooked color  $L^*$  values (Table 8). However, there was no packaging type effect (P > 0.05) on internal cooked color  $L^*$  values (Table 9).

Although raw color was improved with lowconcentration nitrite film, cooked color was not comparable to normal-pH steaks packaged in PVC. Hence, future studies will aim to decrease nitrite levels within packaging film and to decrease heat stability by lowering muscle pH with organic acid enhancement. In addition, including an oxygen-impermeable layer to PLA films will eliminate the need for vacuum packaging to create anaerobic conditions.

# Conclusions

The marketability of meat is heavily influenced by its color. Using commercially available nitriteembedded packaging has shown significant improvements in retail color redness. However, this intervention has shown the formation of a red pigment when the meat is cooked. In this study, the utilization of PLA films containing sodium nitrite and  $\alpha$ -tocopherol is a viable option for improving the retail color of dark-cutting beef. Furthermore, the lower concentrations of sodium nitrite

Packaging Type	L* Value	a* Value	Chroma	Hue Angle	Panel Evaluation <sup>2</sup>
Normal-pH PVC	55.57ª	11.32 <sup>c</sup>	21.69 <sup>c</sup>	58.55ª	1.54°
Dark-cutter PVC	49.30 <sup>b</sup>	12.68 <sup>c</sup>	23.05 <sup>c</sup>	56.13 <sup>ab</sup>	1.77 <sup>cb</sup>
Dark-cutter vacuum	50.10 <sup>b</sup>	15.03 <sup>b</sup>	25.94 <sup>b</sup>	54.48 <sup>bc</sup>	1.61°
Low-concentration PLA	51.62 <sup>ab</sup>	14.87 <sup>b</sup>	25.79 <sup>b</sup>	54.37°	2.21 <sup>b</sup>
High-concentration PLA	51.87 <sup>ab</sup>	23.18 <sup>a</sup>	31.60 <sup>a</sup>	42.45 <sup>d</sup>	4.99 <sup>a</sup>
SEM <sup>3</sup>	1.82	0.64	1.15	1.03	0.16

**Table 8.** Effects of packaging type<sup>1</sup> on external cooked color attributes

<sup>a-d</sup>Least-squares means with different letters within each column are significantly different (P < 0.05). Steaks were cooked after 5 d of retail display.

The experiment was replicated seven times (n = 7).

 $^{1}$ Packaging treatments included normal-pH steaks in polyvinyl chloride (PVC) overwrap, dark-cutter steaks in PVC overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration poly(lactic acid) (PLA) film (compounded with PLA, 0.12% sodium nitrite, and 0.5%  $\alpha$ -tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with PLA, 0.6% sodium nitrite, and 2.5%  $\alpha$ -tocopherol) in a vacuum package.

<sup>2</sup>Panel evaluation for exterior color was given on a 7-point scale (1 = brown, 2 = light brown, 3 = slightly brownish-red, 4 = reddish-brown, 5 = pinkish-brown, 6 = slightly pinkish-red, 7 = pinkish-red).

 $^{3}$ SEM = standard error of the mean.

<b>Table 9.</b> Effects of packaging type	e <sup>1</sup> on internal cooked color attributes
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Packaging Type	L* Value	a* Value	Chroma	Hue Angle	Panel Evaluation <sup>2</sup>
Normal-pH PVC	53.94	16.79 <sup>b</sup>	25.78 <sup>b</sup>	49.81 <sup>a</sup>	4.11
Dark-cutter PVC	56.50	17.36 <sup>b</sup>	26.14 <sup>b</sup>	48.83 <sup>a</sup>	4.40
Dark-cutter vacuum	55.52	19.80 <sup>b</sup>	28.97 <sup>ab</sup>	47.33 <sup>a</sup>	4.02
Low-concentration PLA	55.21	20.84 <sup>ab</sup>	29.50 <sup>ab</sup>	45.23 <sup>a</sup>	3.17
High-concentration PLA	57.25	23.09 <sup>a</sup>	31.62 <sup>a</sup>	43.35 <sup>b</sup>	3.02
SEM <sup>3</sup>	1.61	1.4	1.48	1.51	0.51

a,bLeast-squares means with different letters within each column are significantly different (P < 0.05). Steaks were cooked after 5 d of retail display.

The experiment was replicated seven times (n = 7).

<sup>1</sup>Packaging treatments included normal-pH steaks in polyvinyl chloride (PVC) overwrap, dark-cutter steaks in PVC overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration poly(lactic acid) (PLA) film (compounded with PLA, 0.12% sodium nitrite, and 0.5%  $\alpha$ -tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with PLA, 0.6% sodium nitrite, and 2.5%  $\alpha$ -tocopherol) in a vacuum package.

<sup>2</sup>Panel evaluation for interior color was given on a 7-point scale (1 = very red, 2 = slightly red, 3 = pink, 4 = slightly pink, 5 = pinkish-gray, 6 = grayish-tan/brown, 7 = tan/brown).

 $^{3}$ SEM = standard error of the mean.



**Figure 10.** Visual effect of packaging treatments<sup>1</sup> on steaks cooked to 71°C on a George Foreman grill after 5 d retail display. <sup>1</sup>Packaging treatments included normal-pH steaks in polyvinyl chloride (PVC) overwrap, dark-cutter steaks in PVC overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration poly(lactic acid) (PLA) film (compounded with PLA, 0.12% sodium nitrite, and 0.5%  $\alpha$ -tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with PLA, 0.6% sodium nitrite, and 2.5%  $\alpha$ -tocopherol) in a vacuum package.

(0.12%) and  $\alpha$ -tocopherol (0.5%) in PLA films significantly improved retail color redness while possessing minimal changes to cooked color appearance compared to dark-cutting steaks in vacuum packaging. There was no impact of sodium nitrite and  $\alpha$ -tocopherol on microbial growth and lipid oxidation. Therefore, this work demonstrated that developing a packaging system utilizing both fully industrial compostable film containing nitrite can improve redness of dark-cutting beef.

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