EFFECTS OF SODIUM NITRITE AND TOCOPHEROL INCORPORATED POLY(LACTIC ACID) FILMS ON DARK-CUTTING BEEF

By

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Abstract: This study aimed to determine the effects of poly(lactic acid)-(PLA) films incorporating sodium nitrite and α-tocopherol on dark-cutting beef. A low-concentration (LC) PLA pellet was compounded with 0.12% sodium nitrite and 0.5% α-tocopherol. In addition, a high-concentration (HC) PLA pellet incorporated 0.6% sodium nitrite and 2.5% α-tocopherol. Extruded PLA pellets were oven dried before use. Pellets were compressed and molded using a hot press to form film sheets for packaging application. Steaks from seven dark-cutting loins (n = 7) were randomly assigned to either a polyvinyl chloride (PVC) overwrap, or a control, LC-PLA, or HC-PLA vacuum package. Normal-pH loins (n = 7) were sliced, and steaks were randomly chosen for PVC overwrap. Steaks were placed in simulated retail display for 6 d. Surface color was evaluated every 24 h with instrumental analysis and a trained color panel (n = 6). On d 5 of the display, half of the steaks were removed from the display and cooked to 71°C on a George Foreman grill to evaluate the cooked color. The remaining steaks on d 6 were used to evaluate microbial growth and lipid oxidation. The data were analyzed using the GLIMMIX procedure of SAS and considered significant at $P < 0.05$. There was a significant increase in redness for steaks in both LC-PLA and HC-PLA within the first 24 h of display. Additionally, HC-PLA steaks showed increased ($P < 0.05$) redness throughout the entire duration of display than all other dark-cutting steak treatments. Moreover, LC-PLA steaks showed greater redness ($P < 0.05$) than dark-cutting steaks in vacuum packaging during display. Once cooked, steaks packaged with HC-PLA showed greater ($P < 0.05$) external cooked color redness than all other treatments. In addition, HC-PLA steaks presented greater ($P < 0.05$) internal cooked color redness than dark-cutting steaks in vacuum and PVC overwrap and normal-pH in PVC. However, LC-PLA steaks demonstrated similar ($P > 0.05$) external and internal cooked color redness to dark-cutting steaks in vacuum packaging. In conclusion, the application of LC-PLA improved the retail display color without significantly impacting the cooked color of dark-cutting beef.

Keywords: dark-cutting beef, biodegradable film, nitrite-embedded packaging

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CHAPTER I

INTRODUCTION

Consumer purchasing decisions are influenced by meat color (Altmann et al., 2023). Thus, beef color is a critical variable throughout various stages of the beef chain (Mancini et al., 2022). Especially in retail, consumers who prefer bright cherry red beef are willing to pay more for products that satisfy their color expectations (Killinger et al., 2004). Myoglobin is the protein responsible for meat color and is composed of a heme ring with six binding sites (King et al., 2023). Meat color depends on the redox state of the heme iron and the ligand bond on the sixth binding site. A bright cherry-red color is formed with a predominant oxymyoglobin form and is attributed to the binding of oxygen and a reduced heme molecule. Alternatively, under anaerobic conditions, a purple pigment is formed called deoxymyoglobin.

Dark-cutting beef is a color defect that alters the visual and muscle characteristics of meat. The darker appearance of dark-cutting beef is attributed to a greater muscle pH (Hughes et al., 2017). Stress prior to slaughter depletes glycogen stores and results in less pH decline postmortem resulting in a higher ultimate muscle pH than normal-pH beef (Lawrie, 1958; Scanga et al., 1998). Greater pH directly correlates to greater mitochondrial oxygen consumption and water holding capacity (Ashmore et al., 1972). Therefore, less oxygen is available to bind to myoglobin leading to greater deoxymyoglobin formation (English et al., 2016; Hughes et al., 2017). Moreover, microbial growth is favored with the higher pH found in dark-cutting beef

than in normal-pH meat (Patterson and Gibbs, 1977; Gill and Newton, 1979). In addition to the effects on raw color, the greater pH of dark-cutting beef results in persistent pinking during cooking as myoglobin is more stable at a greater pH (Hunt et al., 1999). Accordingly, developing technologies to improve dark-cutting beef color and alter biochemical attributes is essential for future applications of dark-cutting beef within the retail space.

Modified atmospheric packaging and antioxidant/acid-enhancement have been used to improve dark-cutting beef appearance. For example, research completed by Sawyer et al. (2009) and Apple et al. (2011) have shown an improvement in L^* values (decrease in muscle darkening) of dark-cutting beef enhanced with lactic acid. Moreover, studies utilizing glucono delta lactone have also demonstrated improvements in dark-cutting beef color (Dolezal et al., 2013; Denzer et al., 2022). In lieu of enhancement, nitrite-embedded packaging (NEP) has been associated with increased redness by forming bright red nitric oxide myoglobin. Under the vacuum environment of the NEP, the low oxygen partial pressure induces the formation of metmyoglobin (Ledward, 1970; Brantley et al., 1993). Furthermore, nitrate is a potent oxidant that can convert myoglobin to metmyoglobin. Consequently, metmyoglobin binds with nitric oxide to form nitric oxide metmyoglobin, which is then reduced to nitric oxide myoglobin and presents a bright red pigment (Fox Jr. and Ackerman, 1968; Siegel, 2011). Greater muscle pH and metmyoglobin reducing ability of dark-cutting meat also favor faster nitric oxide myoglobin formation. The commercially available NEP bags utilize synthetic polymers to achieve this active packaging matrix.

Packaging has become a significant focus of attention regarding waste reduction in terms of private household consumption (Thøgerson, 1996). Therefore, as environmental concerns continue to rise, expanding the use of biodegradable packaging has become an area of focus (Moshood et al., 2021). Hence, lengthy work has been completed to investigate future applications of biodegradable packages within the food industry (Han et al., 2018). One emerging biodegradable polymer is poly(lactic acid) - (PLA); however, due to its brittleness and low barrier

properties (Mensitieri et al., 2011), applications thus far have been limited. In addition, there has been limited application of biodegradable plastics on meat in the industry due to the high moisture, variable lipid and protein content, as well as sensitivity to oxygen exhibited by meat. The application of biodegradable film, specifically incorporating PLA, with active ingredients, has yet to be investigated within a meat system. Therefore, this work aimed to determine the effects of PLA films with varying levels of sodium nitrite and α-tocopherol on the color and shelf-life attributes of dark-cutting beef.

CHAPTER II

REVIEW OF LITERATURE

Meat Color

Meat color is primarily attributed to myoglobin and comprises a globin portion and a heme ring that can form six bonds. Four of the six bonds are formed with nitrogen pyrrole rings, the fifth with a histidine, and the sixth with a ligand. Meat color depends on the ligand bound on the sixth binding site and the redox state of the heme iron (King et al., 2023). Deoxymyoglobin is formed when heme iron is ferrous and no ligand is attached. Deoxymyoglobin can be found when the oxygen level within a package is very low or, in the case of the interior of a steak. Through oxygen exposure, also known as oxygenation, oxymyoglobin is formed as oxygen binds to the ferrous iron. Metmyoglobin is formed by oxidation as water binds to the sixth binding site of the ferric iron to form a brown pigment. In addition, consumer acceptability changes with myoglobin form. Consumers prefer the bright cherry-red color of oxymyoglobin and discriminate against any color deviation (Altmann et al., 2023). Thus, meat color plays a vital role in the different stages of the beef supply chain (Mancini et al., 2022), as consumers are willing to pay more for bright cherry red beef (Killinger et al., 2004). Beef products not meeting these consumer standards are likely discounted or removed from the case. Consequently, the US beef industry loses approximately 3.73 billion dollars annually due to discoloration of ground beef and steaks (Ramanathan et al., 2022). Thus, understanding beef color is essential to mitigate economic losses

by improving color and color stability of fresh product.

Dark-cutting Beef

One deviation from the customary bright cherry-red beef is dark, firm, and dry (DFD) or dark-cutting beef. The dark color attribute of dark-cutting beef relates to increased darkness and decreased redness when compared to normal-pH beef (Wulf et al., 2002; Galloway et al., 2005; English et al., 2016; Hughes et al., 2017). Since dark-cutting beef deviates from a traditional bright cherry-red appearance, consumer acceptability diminishes due to the darker appearance (Viljoen et al., 2002). Consequently, packers discount dark-cutting beef, leading to major economic losses within the beef industry. As reported in the 2016 National Beef Quality Audit (NBQA), approximately 1.9% of carcasses graded were considered dark-cutting in the United States (Boykin et al., 2017). Based on calculations from the 2016 NBQA, the beef industry loses roughly $202 million annually due to dark-cutting carcasses (Kiyimba et al., 2023). Dark-cutting meat is due to pre-harvest stress due to seasonality, weather, and management practices (Scanga et al., 1998). With stress, glycogen reserves are depleted prior to harvest and, in turn, decrease the lactic acid formation postmortem (Lawrie, 1958; Newton and Gill, 1981; Scanga et al., 1998). Limited lactic acid formation postmortem results in greater pH than normal-pH muscle (pH = 5.4 - 5.7). Increased meat pH strongly influences fresh- and cooked-color and microbial growth. Previous research has determined mitochondrial abundance increases with the degree of darkness of the dark-cutting condition (McKeith et al., 2016; Kiyimba et al., 2020; Ramanathan et al., 2020). Thus, more actively respiring mitochondria limit the oxygen available to bind with myoglobin resulting in more deoxymyoglobin formation and, in turn, darker meat color (Ashmore et al. 1972; McKeith et al., 2016; English et al., 2016). Furthermore, greater pH limits muscle shrinkage, leading to larger muscle fiber diameter through the repulsion of the myofilaments, causing greater space for water binding (Hughes et al., 2017). Swollen muscle fibers decrease the

space between muscle bundles; thus, decreasing overall reflectance and making beef appear darker (Hughes et al., 2017).

Cooked meat color is due to myoglobin denaturation and Maillard reaction. The presence of ferrohemochrome gives the brown color to meat. Various factors, such as pH, myoglobin form, and added ingredients, can influence myoglobin denaturation. In addition to the effects on fresh meat color impact, the cooked color of dark-cutting beef is also negatively impacted due to its undercooked appearance. The heating of normal-pH meat products results in myoglobin denaturation, resulting in a cooked grey pigment (Fox, 1966). However, myoglobin is more stable to a temperature at greater pH (pH $\geq$ 6.0) (Trout, 1989; Hunt et al., 1999). Thus, high-pH meat results in a persistent pink internal color even at temperatures that should cause the cooked grey appearance (Mendenhall, 1989; Trout, 1989). The deviation to this internal pink or red pigment is interpreted as undercooked as it differs from the traditional cooked appearance (Cornforth et al., 1991). Hence, the persistent pink-cooked interior of dark-cutting beef negatively impacts consumer perception.

With greater ultimate pH, there is an increased concern for microbial growth. Bacterial growth of spoilage bacteria has been shown to occur more readily in dark-cutting beef than normal-pH beef (Patterson and Gibbs, 1977; Gill and Newton, 1979). A more neutral pH from 6.6 to 7.5 provides a more suitable environment for bacterial survival and replication (Jay et al., 2005). Additionally, glycogen content in muscle impacts microbial activity. Bacteria can use the remaining glycogen in muscle as glucose (Newton and Gill, 1981). When the remaining glucose is depleted, bacteria use amino acids and cause spoilage. Thus, since dark-cutting meat contains lesser amounts of glycogen (Lawrie, 1958; Scanga et al., 1998), the spoilage rate is increased since amino acids are more readily utilized by bacteria (Gill and Newton, 1979). Therefore, combining high-pH and low glycogen content in dark-cutting meat results in more microbial growth and spoilage.

Antioxidants

Antioxidants are compounds that prevent lipid and protein oxidation and help extend the shelf life of oxygen-sensitive foods. Specifically, antioxidants help extend the shelf life of meat by limiting rancidity and color changes (Karre et al., 2013). Within a meat system, lipid oxidation products can stimulate the oxidation of oxymyoglobin, which increases the formation of metmyoglobin (Chan et al., 1997). Thus, limiting lipid oxidation can reduce metmyoglobin formation and increase the color stability of meat.

Natural antioxidants in the form of extracts and powders are an emerging intervention used by meat processors (Ali et al., 2018). Grape seed extract used on beef patties improved color stability by limiting myoglobin oxidation to metmyoglobin while also improving the overall redness of raw color (Yang et al., 2022). Moreover, a spray treatment applied to steaks containing vitamin C alone or paired with either α-tocopherol, taurine, or rosemary successfully decreased metmyoglobin formation while improving redness (*a** values) (Djenane et al., 2002). Furthermore, the addition of green tea extract and rosemary in ground beef patties packaged in modified atmospheric packaging improved retail color (*a** values/redness) compared to untreated patties (Yoder et al., 2021). Therefore, natural antioxidants have been shown to be effective in controlling meat products' oxidation and color stability.

Among natural antioxidants, α-tocopherol (vitamin E) is commonly used within the food industry to limit oxidation. Alpha-tocopherol can be found in many foods, including vegetable oils as a lipid-soluble antioxidant (Min and Boff, 2002), and it is known as an effective radical chain breaker in lipid oxidation (Manzanarez-López et al., 2011). Alpha-tocopherol added to oxymyoglobin lowered metmyoglobin accumulation and lipid oxidation *in vitro* (Yin et al., 1993). Additionally, Yin et al. (1993) concluded α-tocopherol indirectly protects water-soluble components like oxymyoglobin from prooxidant free radicals by directly affecting the lipid

phase. Thus, as α-tocopherol has shown promise in improving color stability. However, limited work has examined the effects of utilizing α-tocopherol in combination with sodium nitrite on meat color.

Packaging

Packaging is very important within the food industry as it helps maintain product quality and safety throughout storage, transportation, retail, and end-use (Han et al., 2018). Various materials, such as glass, metal, paper, and plastics, can be used to achieve these quality and safety standards. However, plastics possess many advantages compared to other materials, including low cost, lightweight, ease of moldability, and variable barrier and physical properties (Coles et al., 2003). In recent decades, food technologists have evolved packaging from focusing on simple barriers and preservation to packaging systems that enhance safety, convenience, and environmental impact (Stilwell, 1991). One specific advancement is incorporating active ingredients such as antioxidants, antimicrobials, redox-sensitive compounds, or nitrite into packaging material. Active packaging creates a system designed to lengthen the shelf life by interacting with the product directly and/or indirectly.

Active packaging

Various active packaging technologies have been investigated and formulated to enhance the safety and security of food products (Rooney, 1995). Some of these technologies include oxygen scavengers and antioxidant-releasing systems. Regarding oxygen-sensitive foods, shelf life relies heavily on reducing oxygen exposure within the packaging system. Therefore, to reduce available oxygen within the food system, oxygen scavengers are commonly used in the form of sachets within the package of these products (Gómez-Estaca et al., 2013). Oxygen scavengers help to eliminate oxygen by harnessing the dissolved oxygen with a chemical reaction; thus, oxygen is unavailable to react and damage products within the packaging system

(Vermeien et al., 2003). Moreover, polymers, excluding glass, can interact with various molecules within the packaging system. These molecules can be sorb, diffused, and desorbed across their structure. With this principle, antioxidant-releasing systems actively release antioxidant compounds from the polymers to protect oxygen-sensitive foods (Gómez-Estaca et al., 2013). Antioxidants within meat packaging films have been shown to improve color and color stability as well as reduce lipid oxidation during retail display. Specifically, Lorenzo et al. (2014) noted that films containing 2% oregano extract inhibited metmyoglobin formation and decreased lipid oxidation values in foal meat over 15 days of storage compared with a control a green tea extract film counterpart. Additionally, films with varying concentrations of olive leaf extract improved fresh color redness and reduced lipid oxidation in pork (Moudache et al., 2017). Furthermore, films containing BHA, BHT, rosemary, or α-tocopherol showed less changes in *a** values of beef steaks throughout 8 d of storage, indicating more color stability (Moore et al., 2003).

Nitrite Embedded Packaging

Freshcase® nitrite film or nitrite-embedded packaging (NEP) is a patented active technology implemented in the industry (Siegel, 2011). Nitrite-embedded packaging has been utilized to meet consumer demands for bright cherry-red beef. The structure of NEP bags consists of three layers. An outer layer on the bag's exterior is followed by a barrier layer used to establish an anaerobic environment when vacuum sealed. Finally, an internal sealant layer contains nitrite crystals that interact with the meat surface (Siegel, 2011). Nitrite-embedded packages create an anaerobic environment where nitrite is available to interact with the meat surface (Siegel, 2011). Due to the limited oxygen available and the low partial pressure of the anaerobic environment, there is an increase in metmyoglobin formation (Mancini and Hunt, 2005). Nitrous acid from the added nitrite is converted to nitric oxide by the natural reducing ability of meat (Fox Jr. And Ackerman, 1968). Nitric oxide binds to metmyoglobin and induces the formation of nitric oxide

metmyoglobin. Nitric oxide metmyoglobin is then reduced to nitric oxide myoglobin (Fox Jr. and Ackerman, 1968) and imposes a bright red pigment on meat (Siegel, 2011; Claus and Du, 2013). Contrary to the improved raw color redness, NEP has shown increased cooked color redness of steaks compared to control treatments (Claus and Du, 2013). When heat is applied, the nitric oxide myoglobin formed in NEP results in nitrosylhemochrome, causing this red-cooked pigment (Fox, 1966; Claus and Du, 2013). However, limited research has been applied to identify approaches to mitigating red-cooked color appearance. Additionally, there is a concern for residual concentration on the surface of the meat with the addition of nitrite. Considering the total steak weight and the percentage of nitrite bound within the steak surface, residual nitrite remains below FDA recommendation. Therefore, residual nitrite is not a significant concern for lower-concentration applications (Claus and Du, 2013).

Nitrite-embedded packaging has been used in various studies to improve fresh meat cuts' retail color. With bison steaks, applying NEP improved color stability (Roberts et al., 2017; Narváez-Bravo et al., 2017). Nitrite-embedded packaging improved steak redness of *longissimus lumborum* and *psoas major* muscles (Claus and Du, 2013). Specifically, with dark-cutting beef, NEP improved dark-cutting steak color throughout retail display (Denzer et al., 2022). Furthermore, steaks packaged in NEP possessed greater surface color redness when compared with steaks packaged with polyvinyl chloride (PVC) overwrap (Ramanathan et al., 2018). However, due to variances of reducing ability between steaks caused by factors such as postmortem age and pH, the color development within a NEP system can differ significantly (Claus and Du, 2013; Ramanathan et al., 2018). For example, with normal-pH beef, Yang et al. (2016) noted the NEP increased redness in 5 d, while Claus and Du (2013) observed a redder appearance after 4 d. Additionally, previous research on dark-cutting steaks indicated nitric oxide myoglobin formed within 24 h due to the greater reducing ability of the higher pH meat (Ramanathan et al., 2018).

Additionally, studies have examined the effect of pairing NEP with other active ingredients. Ramanathan et al. (2018) determined that adding rosemary to dark-cutting beef within a NEP package possessed a redder appearance (greater *a** values) when compared with dark-cutting steaks only packaged with NEP by d 3 of storage. Moreover, it was noted that dark-cutting steaks treated with glucono delta lactone (GDL) in a NEP displayed more significant retail color redness than untreated dark-cutting steaks in NEP and control USDA Choice steaks (Denzer et al., 2022). In addition, Denzer et al. (2022) noted that applying the GDL enhancement resulted in enhanced dark-cutting steaks presenting a similar cooked color to USDA Choice steaks.

Significance of Biodegradable packaging

As a result of President Biden's Executive Order 14057 outlining future federal sustainability efforts, Secretary of the Interior Deb Haaland issued Secretary's Order 3407. This order aims to phase out single-use plastics on public lands by 2032. Therefore, the transition from traditional commercial plastics is imminent. Commercial plastics are comprised of petroleum-based synthetic polymers. Examples of these synthetic polymers include low-density polyethylene, high-density polyethylene, polypropylene, polytetrafluoroethylene, and nylon (polyamide) (Han, 2005). Petroleum-based polymers are inherently resistant to microbial attack and cannot be degraded naturally when discarded in the environment (Madhavan Nampoothiri et al., 2010). As concerns regarding environmental sustainability issues gain relevance, polymers derived from bio-based sources, which are more easily recyclable and biodegradable, have been developed to reduce plastic pollution (Thøgerson, 1996). Such bio-based polymers include poly(lactic acid)-(PLA), poly(hydroxyl-alkanoates), poly(3-hydroxybutyrate) and thermoplastic starches. However, the major drawbacks associated with these newly developed biodegradable polymers include their limited rheological characteristics. For example, PLA is known to be brittle and possess low barrier properties, which limits its application (Mensitieri et al., 2011). On

top of physical limitations, biodegradable materials are known to be more expensive than their petroleum-based counterparts.

Biodegradable active packaging

While there has been limited application of biodegradable films incorporating sodium nitrite on beef, there has been work done to determine the effects on pork. Chatkitanan and Harnkarnsujarit (2020) used thermoplastic starch (TPS) compounded with sodium nitrite and linear low-density polyethylene (LLDPE). Nitrite levels at 1 and 5% showed to enhance and stabilized redness with vacuum-packaged pork in addition to limiting metmyoglobin formation (Chatkitanan and Harnkarnsujaritl, 2020). Moreover, further research investigating different sodium nitrite concentrations (0.25, 0.5, 1, and 2%) in TPS/ LLDPE films showed improved redness (*a** values) up to 4 times (with 2% nitrite concentration) the LLDPE control film (Chatkitanan and Harnkarnsujarit, 2021). However, there has not been a film developed which utilized PLA, sodium nitrite, and an antioxidant for the application to improve beef color.

Final Remarks

Management and processing practices continue to focus on improving meat quality and display color in retail cases. However, a sizeable economic burden is still caused by the discoloration of ground beef and steak (Ramanathan et al., 2022). Therefore, pioneering advancements which improve color and maintain color stability are essential to reduce losses within the industry. For example, interventions to mitigate the effects of color defects like dark-cutting beef are still being investigated. As concerns for the environment continue to rise and restrictions on single-use plastics become more imminent, the development of biodegradable packaging has increased in popularity. Thus, incorporating biodegradable films with active ingredients to achieve a redder retail color appearance and improve the color stability of dark-

cutting meat is a novel step forward to bridge the gap between economic and environmental sustainability.

Hypothesis and Objective

We hypothesized that using biodegradable PLA films with sodium nitrite and α- tocopherol will improve the retail color of dark-cutting beef.

This study aimed to evaluate the impact of sodium nitrite and α-tocopherol incorporated PLA films on the changes in retail color, cooked color, microbial growth, and lipid oxidation of dark-cutting beef steaks.

CHAPTER III

METHODOLOGY

Film Formulation

Poly(lactic acid) (Ingeo™ biopolymer 2003D poly (96% L-lactic acid)), α- tocopherol, and sodium nitrite were compounded by a twin-screw extruder (screw L/D ratio: 42; screw speed: 120 rpm; Century ZSK- 30; Traverse City, MI). Two batches were compounded with lower and higher concentrations of the active ingredients. The low-concentration film recipe incorporated 0.12% sodium nitrite and 0.5% α- tocopherol, while the high-concentration included 0.6% sodium nitrite and 2.5% α- tocopherol. The temperature ranged between 170 and 190°C in the different zones for the masterbatch formulation. The extruded mass was cooled in a water bath before being pelletized with a BT 25 pelletizer (Scheer Bay Co.; Bay City, MI). Pellets were oven dried at 50°C for 24 h before being compression molded. Film sheets were formed with 5 g of the respective pellet recipe using a hot press (PHI QL438-C, City of Industry, CA, USA). The hot press was operated at 182°C with an applied force of 10 tons for 3 min before sheets were removed and cooled at room temperature.

Oxygen Permeability

Oxygen permeability of the films was measured using a MOCON OX-TRAN® (2/22 L model; MOCON Inc.; Minneapolis, MN) at 10°C at 34.3 ± 1% relative humidity. Temperature

and humidity settings were determined by the intended conditions of film application and the instrument limitations. Three different sets of each film treatment were analyzed separately

Film Transmittance Analysis

Light transmittance was collected in ultraviolet and visible light wavelength regions on a spectrophotometer (Thermo Scientific, USA.). The transparency value (TV) of the film 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). The optical properties were measured in triplicate for each film treatment.

A digital micrometer (Testing Machines Inc.; Ronkonkoma, NY) with a sensitivity of 0.001 mm was used to determine film thickness. Film thicknesses were read in triplicate and averaged.

Thermogravimetric Analysis (TGA)

The thermal stability of active PLA films was evaluated using a Q50 TGA (TA Instruments; New Castle, DE, USA). Testing was performed on 10 mg samples of each film recipe from room temperature to 600°C, increasing at a rate of 10°C/min. Nitrogen purge gas was utilized with a balance purge flow of 40 mL/min and a sample purge flow of 60 mL/min. Characteristic temperatures like initial decomposition temperature (T_{onset}) and decomposition peak temperature (T_{peak}) as well as the residual ash percentage were determined. Initial decomposition temperature was noted when 5% of the sample weight was lost during testing. The decomposition peak temperature was indicated at the peak of the derivative weight (%/°C) output curve during testing. Residual ash was characterized by the remaining weight percentage of the sample at the completion of heating.

Raw Materials, Processing, and Packaging

Seven dark-cutting strip loins (pH = 6.16 - 6.67) and seven USDA Low Choice strip loins (pH = 5.40 - 5.66) were collected at no more than 5 d postmortem from a Tyson Fresh Meats facility in Amarillo, TX. Strip loins were transported on ice to the Robert M. Kerr Food and Agricultural Products Center at Oklahoma State University in Stillwater, Oklahoma. Upon arrival, loins were repackaged (CRYOVAC; 16 × 24 pouches; 3 mil thickness) using a vacuum sealer (Sipromac 420A Double Chamber Vacuum Sealer; Sipromac; Drummondville, Canada). Loins were stored in the dark at 2°C until use 2 d following collection.

pH and Proximate Composition Analysis

Initial pH on dark-cutting and normal-pH loins was measured at the time of processing in three random locations of the *longissimus lumborum* muscle along the length of the loin using a Hanna Instruments pH probe (Handheld HI 99163; probe FC232; Hanna Instruments). Percent protein, fat, and moisture were determined for each strip loin using the first and fifth steak from the anterior end of the loin. Steaks were trimmed of all external fat, and the *longissimus lumborum* muscle was ground using a tabletop grinder (Big Bite Grinder, course grind, LEM). The samples were pressed into a 140 mm sample cup and analyzed using an AOAC-approved near-infrared spectrophotometer (FoodScan Lab Analyzer, Serial No. 91753206; Foss, NIRsystem Inc.; Slangerupgrade, Denmark).

Packaging and Simulated Retail Display

Dark-cutting and normal-pH loins were sliced into 2.54-cm thick steaks from the anterior end using a meat slicer (Bizerba USA INC., Piscataway, NJ). Dark-cutting steaks were randomly assigned to one of the following treatments: polyvinyl chloride overwrap (PVC) control, vacuum package control, low-concentration nitrite film in a vacuum package, or high-concentration nitrite film in a vacuum package. Normal-pH steaks were randomly chosen for a PVC overwrap control

treatment. Each treatment contained two steaks from the respective loin type, as one steak was used for cooking analysis on d 5 of display and the other for laboratory analysis on d 6 of display. Cooking analysis occurred on d 5 as nitric oxide myoglobin formation was expected to have already formed. PVC control steaks for both dark-cutting and normal-pH loins were placed in Styrofoam trays and overwrapped with PVC (15,500-16,275 cm^3 O_2/m^2 /24 h at 23°C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film; Koch Supplies; Kansas) using a film wrap machine (Winholt WHSS-1, 115V; Woodbury, NY). Vacuum control steaks were vacuum packaged (Walton's Vacuum Pouch; 10 × 10 pouches; 3 mil thickness) using a vacuum packager (Sipromac 420A Double Chamber Vacuum Sealer; Sipromac; Drummondville, Canada). Low- and high-concentration film treatments had the respective biodegradable film placed directly on the cut steak surface and were vacuum packaged (Walton's Vacuum Pouch; 10 × 10 pouches; 3 mil thickness) using a vacuum packager (Sipromac 420A Double Chamber Vacuum Sealer; Sipromac; Drummondville, Canada). Packaged steaks were placed in a white coffin-style display case and stored under continuous LED lighting (Phillips LED lamps; 12 watts, 48 inches, color temperature = 3,500°K; Phillips, China) at 2 ± 1°C for either 5 d or 6 d.

Retail Color Analysis

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). Steak display surface was read in triplicate, and CIE L^*, a^*, and b^* values and spectral reflectance from 400 to 700 nm were obtained to determine surface color. The red intensity of the color was represented with Chroma $\left[\sqrt{(a^*)^2 + (b^*)^2}\right]$ (King et al., 2023). For dark-cutting steaks with biodegradable films, nitric oxide myoglobin formation was determined by the ratio of reflectance at 650 and 570 nm where a greater number indicates more nitric oxide myoglobin formation (King et al., 2012).

Visual color was evaluated every 24 h by a trained panel (n = 6) for all steaks during display. The visual color analysis was approved by the Oklahoma State University Institutional Review Board (approval number: AG-18-34). All panelists passed the Farnsworth Munsell 100-hue test. Panelists determined muscle color appearance 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), surface discoloration 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%)), and muscle darkening using a 7-point scale (1 = no darkening, 3 = slightly dark, 5 = moderately dark, 7 = very dark) throughout the duration of retail display.

Microbiology

The total plate count of each loin was determined on d 0, and all steaks were evaluated on d 6. A 10g sample of the steak surface was aseptically collected and placed in a sterile stomacher bag (VWR; Radnor, PA) with 90 mL of 0.1% sterile peptone water (BactoTM Peptone Ref 211677 Becton; Dickinson and Company; Sparks, MD). Stomacher bags were closed and placed in a stomacher (Seward; STOMACHER® 400 Circulator; 3500 model) for 30 s. One mL from the mixed stomacher bag was serially diluted into 9 mL of 0.1% sterile peptone water. One mL from each dilution was aseptically plated in duplicate on 3M Petrifilm Aerobic Count plates (3M Health Care; St. Paul, MN). Plates were incubated at 37°C for 48 h in a VWR Forced Air General Incubator (5.4ft^3; VWR, Radnor, PA). Plates were removed and counted after 48 h to determine the total plate count (colony forming units per mL).

Thiobarbituric Acid Reactive Substances (TBARS)

Lipid oxidation values were measured on d 0 and 6 of storage. Twenty-seven mL of trichloroacetic acid (TCA) was blended with 3 g of sample (taken from the steak surface) for 10 s using a Waring commercial blender (Model 33BL7; New Hartford, CT). The blended sample was filtered with 42 Whatman filter paper. One mL of the filtrate was combined with 1 mL of thiobarbituric acid (TBA) in a test tube. The test tube was heated for 10 min in a 100°C water bath. The samples were cooled for 5 min before reading absorbance at 532 nm using a spectrophotometer (UV-2600, UV-VIS Spectrophotometer; Shimadzu; Columbia, MD) on the photometric setting. One mL of TCA combined with 1 mL of TBA was used as the standard. Raw absorbance values were taken and converted to mg malondialdehyde/kg meat by multiplying by a factor of 4.16 (for 1:9 dilution) based on calculations for lipid oxidation in King et al. (2023).

Cooked Color Analysis

After 5 d of retail display, steaks were cooked using a George Foreman Grill (Lean Mean Fat Grilling Machine George Foreman; Lake Forest, IL GRP99A) to 71°C. Immediately after cooking, steaks were placed on ice for 5 min to prevent post-temperature rise. External color readings were taken in triplicate across the cooked surface using a HunterLab 4500L MiniScan Spectrophotometer. Additionally, steaks were bisected perpendicular to the cooked surface near the center of the steak. Internal color readings were obtained in triplicate across the bisected steak portions using a HunterLab 4500L MiniScan Spectrophotometer. Hue angle $\left[\tan^{-1}\left(\frac{b^*}{a^*}\right)\right]$ was computed with CIE *a** and *b** values where a larger hue angle indicates a greater cooked color appearance as it has a greater distance from the true red axis (King et al., 2023).

The visual cooked color was evaluated by six trained panelists using 7-point scales. Visual external color (1 = brown, 2 = light brown, 3 = slightly brownish-red, 4 = reddish-brown, 5 = pinkish-brown, 6 = slightly pinkish-red, 7 = pinkish-red) was assessed using the cooked

surface of the display side of each steak. This assessment aided in determining the impact of active PLA films on visual cooked color appearance since the active PLA films were only placed on the display surface of each steak. Visual internal color (1 =very red, 2 = slightly red, 3 = pink, 4 = slightly pink, 5 = pinkish gray, 6 = grayish-tan/ brown, 7 = Tan/brown) was determined based on the appearance of the bisected steak pieces previously used to determine internal instrumental cooked color. All panelists passed the Farnsworth Munsell 100-hue test. The visual color panel was approved by the Institutional Review Board.

Statistical Analysis

A randomized complete block design was utilized to determine the effects of active PLA films incorporating sodium nitrite on dark-cutting beef color during retail display. Dark-cutting loins were applied to either a control treatment in PVC, a control treatment in vacuum, or the low- or high-concentration active PLA films. Seven replicates were served by seven strip loins and each loin represented a block. The fixed effects included packaging treatment, retail display time, and their interactions. Each loin served as a random effect and the day was a repeated measure. The repeated measures covariance-variance structure was determined by evaluating the AICC output. The least square means were determined using the GLIMMIX procedure of SAS (SAS 9.4; SAS Inst.; Cary, NC) and considered significant at $P < 0.05$. With the PDIFF option, the least square means were separated and significant at $P < 0.05$. Normal-pH steaks are reported as averages, and significant differences were determined based on standard error.

CHAPTER IV

RESULTS

Oxygen Transmission Rate

There was no significant film effect on the oxygen transmission rate (Table 1). Thus, both low- and high-concentration PLA films demonstrated similar ($P > 0.05$) impacts on oxygen transmission rates.

Film Transparency

There was a significant film effect on film transparency (Table 2). The vacuum package alone possessed a greater ($P < 0.05$) transparency value than all other films. Furthermore, there was no difference ($P > 0.05$) between low- and high-concentration PLA film transparency values for the PLA films alone as well as when layered with the vacuum package.

Thermal Stability

There was no effect ($P < 0.05$) of film recipe on onset temperature, decomposition peak temperature, or the residual ash percentage when evaluated for thermal stability (Table 3). Therefore, low- and high-concentration PLA films demonstrated similar ($P < 0.05$) thermal stability properties. Moreover, a single-phase degradation model was observed by both film recipes (Figure 2).

pH and Proximate Composition

Dark-cutting loins demonstrated greater ($P < 0.05$) pH than the normal-pH loins (Table 4). Both normal-pH and dark-cutting loins possessed similar ($P > 0.05$) protein content percentages for proximate analysis. However, there was a significant muscle color effect on both moisture and fat content of the loins. The dark-cutting loins exhibited greater ($P < 0.05$) moisture and less ($P < 0.05$) fat content than normal-pH loins.

Retail Display Color

There was a packaging type × day interaction ($P < 0.05$) for L^* values for the duration of retail display (Figure 3). Normal-pH steaks packaged in PVC displayed greater L^* values ($P < 0.05$) than all other packaging types during display. In the first 24 h of display, there was an increase ($P < 0.05$) in lightness (L^* values) for all treatments. Nonetheless, all treatments maintained L^* values ($P > 0.05$) from d 2 until the conclusion of display.

There was a packaging type × day interaction ($P < 0.05$) for a^* values during simulated retail display (Figure 4). Throughout retail display, normal-pH steaks packaged in PVC presented a redder ($P < 0.05$) appearance (a^* values) compared with all other treatments up to d 5. Moreover, both low- and high-concentration PLA film steaks showed greater redness ($P < 0.05$) than dark-cutting steaks in vacuum packaging following d 2. Both low- and high-concentration PLA film steaks exhibited an increase in a^* values within the first 24 h of display ($P < 0.05$). However, low-concentration PLA film steaks possessed stable ($P > 0.05$) a* values for the remainder of display. Alternatively, high-concentration PLA film steaks revealed increased redness ($P < 0.05$) throughout display duration and were comparable ($P > 0.05$) to normal-pH steaks packaged in PVC by d 6. Finally, high-concentration PLA film steaks revealed greater ($P < 0.05$) a^* values following d 1 of display compared with low-concentration PLA film steaks.

There was a packaging type × day ($P < 0.05$) interaction for chroma values throughout retail display (Figure 5). Normal-pH steaks in PVC showed greater chroma ($P < 0.05$) values up to d 5 of retail display than all other treatments. Both low- and high-concentration PLA film steaks presented greater ($P < 0.05$) red intensity after d 0 of display. Furthermore, low- and high-concentration PLA film steaks showed significant increases in red intensity within the first 24 h of display and presented a continual increase in red intensity for the remainder of display. Finally, high-concentration PLA film steaks presented greater ($P < 0.05$) chroma values following d 1 of retail display compared with low-concentration PLA film steaks.

There was a significant packaging type × day interaction for nitric oxide myoglobin formation during the study (Table 5). Both low-concentration and high-concentration PLA film steaks demonstrated increases ($P < 0.05$) in nitric oxide myoglobin values for the duration of retail display. Steaks packaged with active PLA films exhibited an increase ($P < 0.05$) of nitric oxide myoglobin within the first 24 hours and showed a continual rise of nitric oxide myoglobin formation throughout display. Finally, by d 1, steaks packaged with high-concentration PLA films presented greater ($P < 0.05$) nitric oxide myoglobin formation compared with low-concentration PLA film steaks.

There was a significant packaging type × day interaction for muscle color (Figure 6), surface discoloration (Figure 7), and muscle darkening (Figure 8) evaluated by the trained color panel. Normal-pH steaks in PVC were evaluated to have a brighter cherry red appearance ($P < 0.05$) up to d 4 of retail display when compared with all other treatments. Additionally, after d 0, high-concentration PLA film steaks presented brighter ($P < 0.05$) cherry red muscle color than dark-cutting steaks in PVC and in vacuum packaging. Moreover, both low- and high-concentration PLA film steaks exhibited an increase in brightness ($P < 0.05$) during the first 24 hours of retail display.

There was no discoloration noted for all steaks packaged in vacuum packaging throughout retail display. However, normal-pH steaks in PVC presented greater ($P < 0.05$) discoloration from d 4 to d 6 of display than dark-cutting steaks in PVC. Moreover, there was noted discoloration for dark-cutting steaks packaged in PVC.

Normal-pH steaks in PVC presented less muscle darkening ($P < 0.05$) than all other treatments up to d 4 of display. Additionally, high-concentration PLA film steaks showed a decrease ($P < 0.05$) in muscle darkness throughout display with comparable ($P > 0.05$) muscle darkness to that of normal-pH steaks packaged in PVC on d 5 and d 6.

Microbial Growth and Lipid Oxidation

There was no significant difference between normal-pH and dark-cutting loins on d 0 microbial growth analysis (Table 6). However, for d 6 microbial growth analysis, there was a packaging type effect ($P < 0.05$) where dark-cutting steaks in PVC showed greater ($P < 0.05$) microbial growth compared with all other packaging treatments (Table 6).

There was no difference ($P > 0.05$) between normal-pH and dark-cutting loins on lipid oxidation for d 0 analysis (Table 7). Normal-pH steaks in PVC revealed greater lipid oxidation for d 6 analysis (Table 7).

Cooked Color

There was a significant treatment effect on external cooked color L^* values (Table 8). Normal-pH steaks in PVC presented greater ($P < 0.05$) L^* values compared to dark-cutting steaks in both PVC and vacuum package. Moreover, there was no treatment effect ($P > 0.05$) on internal cooked color L^* values (Table 8).

There was a significant packaging type effect on both external (Table 8) and internal (Table 8) cooked color a^* values. High-concentration PLA film steaks exhibited greater ($P <$

0.05) external cooked color redness (*a** values) than all other treatments. Moreover, high-concentration PLA film steaks presented greater ($P < 0.05$) internal cooked color *a** values than normal-pH steaks in PVC and dark-cutter steaks in PVC and vacuum packaging. However, there was no significant difference ($P > 0.05$) in external and internal *a** values between low-concentration PLA film steaks and dark-cutting steaks in vacuum packages.

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 red intensity than all other treatments. There was no significant difference in internal red intensity between low- and high-concentration PLA film steaks and dark-cutting vacuum packaged steaks.

There was a significant packaging type effect on external (Table 8) and internal (Table 9) hue angle values. High-concentration PLA film steaks expressed smaller ($P < 0.05$) external and internal cooked color hue angles than all other treatments. Moreover, steaks with low-concentration PLA demonstrated similar ($P > 0.05$) external and internal cooked color hue angles to dark-cutting steaks in vacuum packaging.

There was a significant packaging type effect on external cooked color panel evaluation (Table 8). Panelists determined high-concentration PLA film steaks exhibited a redder pink ($P < 0.05$) external cooked color appearance than all other treatments. However, panelists noted the low-concentration PLA film steaks presented a similar ($P > 0.05$) external cooked color appearance to dark-cutting steaks packaged in vacuum packages. There was no packaging type effect ($P > 0.05$) on the internal cooked color panel evaluation (Table 9).

Table 1. Oxygen transmission rate[1] (OTR) of PLA films layered with control vacuum film

Film	Oxygen Transmission Rate
Low-concentration[2] w/ vacuum	5.89
High-concentration[3] w/ vacuum	6.57
SEM[4] = 0.29	

[1]Oxygen transmission rate units given as $cm^3 \times mm / m^2 \times day$.
[2]Low-concentration PLA film compounded with poly(lactic acid) and 0.12% sodium nitrite and 0.50% α-tocopherol.
[3]High-concentration PLA film compounded with poly(lactic acid) and 0.6% sodium nitrite and 2.5% α-tocopherol.
[4]SEM = standard error of the mean.

Table 2. Transparency value[1] (TV) of films[2]

Film	Transparency Value
Control vacuum	25.57[a]
Low-concentration PLA	10.86[b]
High-concentration PLA	10.49[b]
Low-concentration w/ vacuum	7.07[c]
High-concentration w/ vacuum	6.70[c]
SEM[3] = 0.63	

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

[1]A greater transparency value 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

[2]Films consisted of a commercial vacuum package film, a low-concentration PLA film compounded with poly(lactic acid), 0.12% sodium nitrite and 0.5% α-tocopherol, a high-concentration PLA film compounded with poly(lactic acid), 0.6% sodium nitrite and 2.5% α-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.

Table 3. Thermal stability values of PLA films[1]

Film	T_{onset}[2]	T_{peak}[3]	Residual Ash[4] (%)
Low-concentration	296.69	350.52	4.75
High-concentration	265.70	329.42	4.99
SEM[5]	8.13	6.61	1.52

[1]Low-concentration PLA films were compounded with poly(lactic acid), 0.12% sodium nitrite and 0.5% α-tocopherol and high-concentration PLA films were compounded with poly(lactic acid), 0.6% sodium nitrite and 2.5% α-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.

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

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

[a-b]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.

Table 5. Effects of display day on nitric oxide myoglobin formation[1]

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

[a-k]Least squares means with different letters are significantly different ($P < 0.05$). The experiment was replicated seven times (n = 7).

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

[2]Low-concentration PLA film compounded with poly(lactic acid), 0.12% sodium nitrite and 0.5% α-tocopherol.

[3]High-concentration PLA film compounded with poly(lactic acid), 0.6% sodium nitrite and 2.5% α-tocopherol.

[4]SEM = standard error of the mean.

Table 6. Effects of packaging type[1] on microbial growth on d 6

Treatment	Log (CFU/mL)
Normal-pH PVC	0.40[b]
Dark-cutter PVC	1.86[a]
Dark-cutter vacuum	0.26[b]
Low-concentration PLA	0.38[b]
High-concentration PLA	0.24[b]
SEM[2] = 0.28	

[a-b]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, respectfully (SEM = 0.18).
[1]Packaging treatments included normal-pH steaks in polyvinyl chloride overwrap, dark-cutter steaks in polyvinyl chloride overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration PLA film (compounded with poly(lactic acid), 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with poly(lactic acid), 0.6% sodium nitrite, and 2.5% α-tocopherol) in a vacuum package.
[2]SEM = standard error of the mean.

Table 7. Effects of packaging type[1] on thiobarbituric acid reactive substances (mg malondialdehyde/ kg meat) on d 6

Packaging Type	mg MDA/kg
Normal-pH PVC	0.56[a]
Dark cutter PVC	0.42[b]
Dark cutter vacuum	0.43[b]
Low-concentration PLA	0.38[b]
High-concentration PLA	0.32[b]
SEM[2] = 0.04	

[a-b]Least squares means with different letters are significantly different ($P < 0.05$).
The experiment was replicated seven times (n = 7).
Day 0 thiobarbituric acid reactive substances for normal-pH and dark-cutter loins were 0.39 and 0.41 mg MDA/kg, respectfully (SEM = 0.04).
[1]Packaging treatments included normal-pH steaks in polyvinyl chloride overwrap, dark-cutter steaks in polyvinyl chloride overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration PLA film (compounded with poly(lactic acid), 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with poly(lactic acid), 0.6% sodium nitrite, and 2.5% α-tocopherol) in a vacuum package.
[2]SEM = standard error of the mean.

Table 8. Effects of packaging type[1] on external cooked color attributes

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

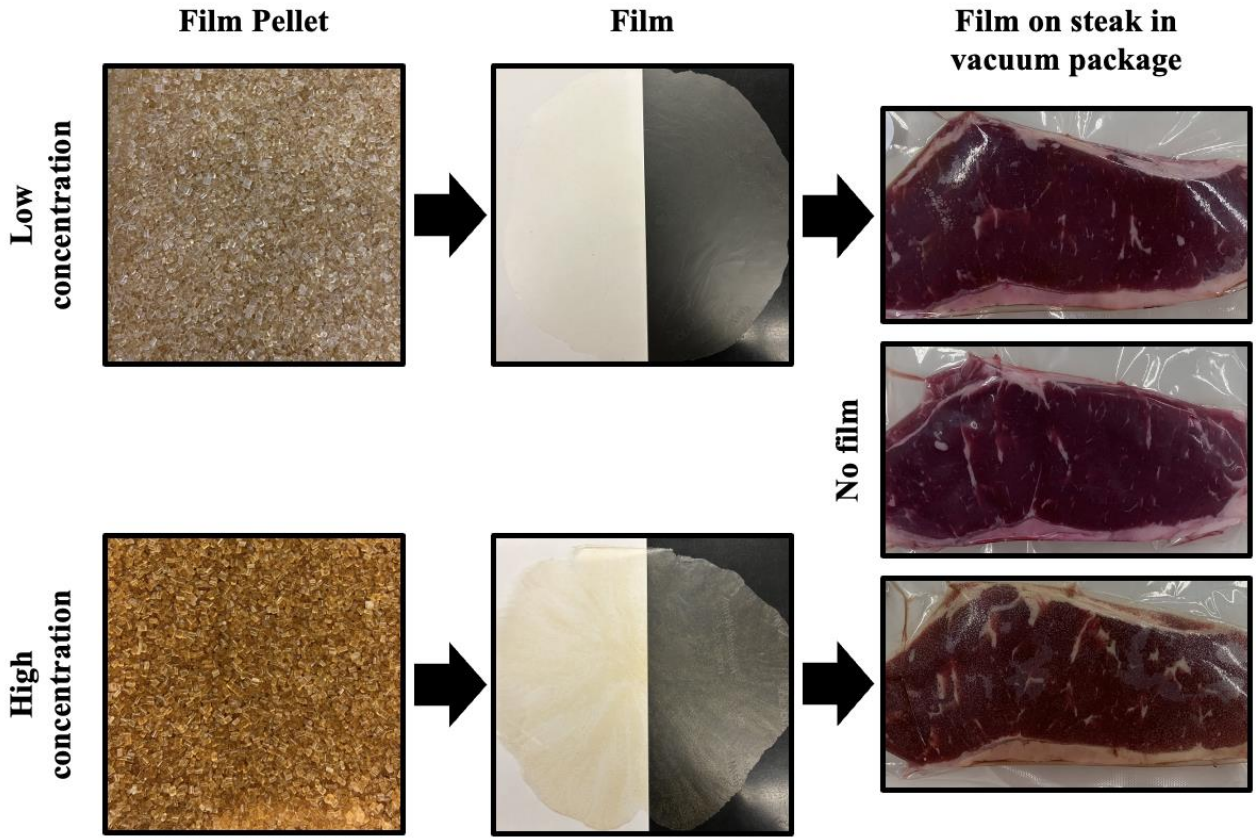
Least squares means with different letters within each column are significantly different ($P < 0.05$).
The experiment was replicated seven times (n = 7).
[1]Packaging treatments included normal-pH steaks in polyvinyl chloride overwrap, dark-cutter steaks in polyvinyl chloride overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration PLA film (compounded with poly(lactic acid), 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with poly(lactic acid), 0.6% sodium nitrite, and 2.5% α-tocopherol) in a vacuum package.
[2]Panel evaluation for exterior color was given on a seven-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.

Table 9. Effects of packaging type[1] on internal cooked color attributes

Packaging Type	*L** value	*a** value	Chroma	Hue angle	Panel Evaluation[2]
Normal-pH PVC	53.94	16.79[b]	25.78[b]	49.81[a]	4.11
Dark-cutter PVC	56.50	17.36[b]	26.14[b]	48.83[a]	4.40
Dark-cutter vacuum	55.52	19.80[b]	28.97[ab]	47.33[a]	4.02
Low-concentration PLA	55.21	20.84[ab]	29.50[ab]	45.23[a]	3.17
High-concentration PLA	57.25	23.09[a]	31.62[a]	43.35[b]	3.02
SEM[3]	1.61	1.4	1.48	1.51	0.51

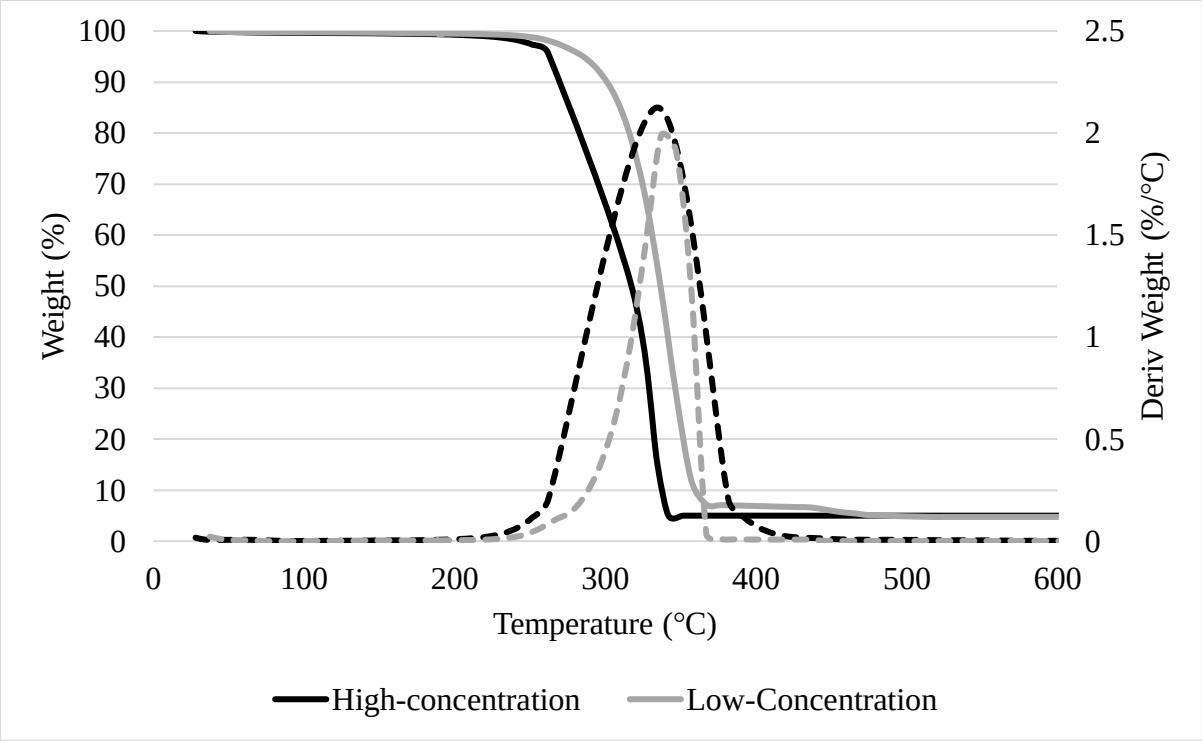
[a-b]Least squares means with different letters within each column are significantly different ($P < 0.05$).
The experiment was replicated seven times (n = 7).
[1]Packaging treatments included normal-pH steaks in polyvinyl chloride overwrap, dark-cutter steaks in polyvinyl chloride overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration PLA film (compounded with poly(lactic acid), 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with poly(lactic acid), 0.6% sodium nitrite, and 2.5% α-tocopherol) in a vacuum package.
[2]Panel evaluation for interior color was given on a seven-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 1. Images of color differences between PLA film recipes[1].



[1]Low-concentration PLA films were compounded with poly(lactic acid), 0.12% sodium nitrite, and 0.5% α-tocopherol and high-concentration PLA films were compounded with poly(lactic acid), 0.6% sodium nitrite, and 2.5% α-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).

Figure 2. Thermogravimetric response[1] of low[2]- and high[3]-concentration PLA films.

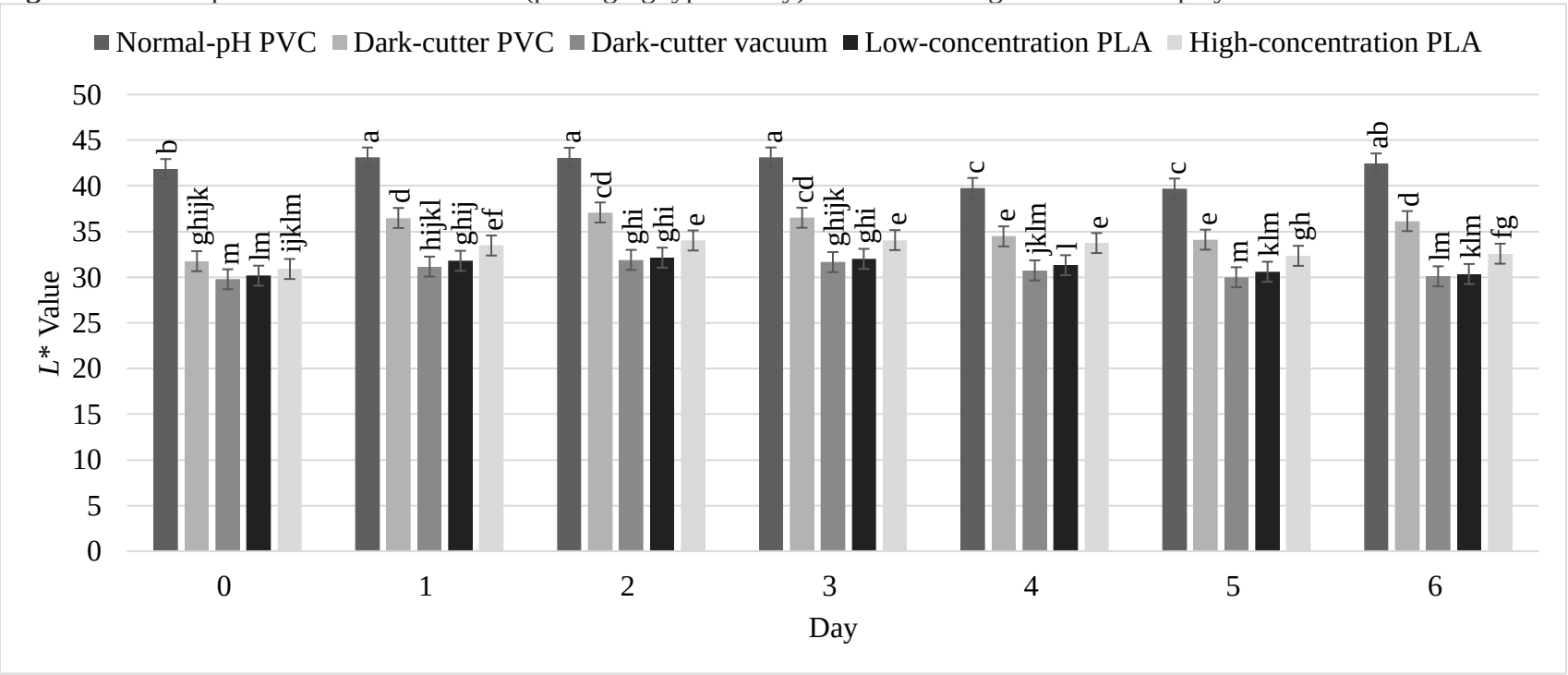


[1]Weight (%) results are given as a continuous line and derivative weight (%/°C) is shown as a dashed line.

[2]Low-concentration PLA films were compounded with poly(lactic acid), 0.12% sodium nitrite, and 0.5% α-tocopherol.

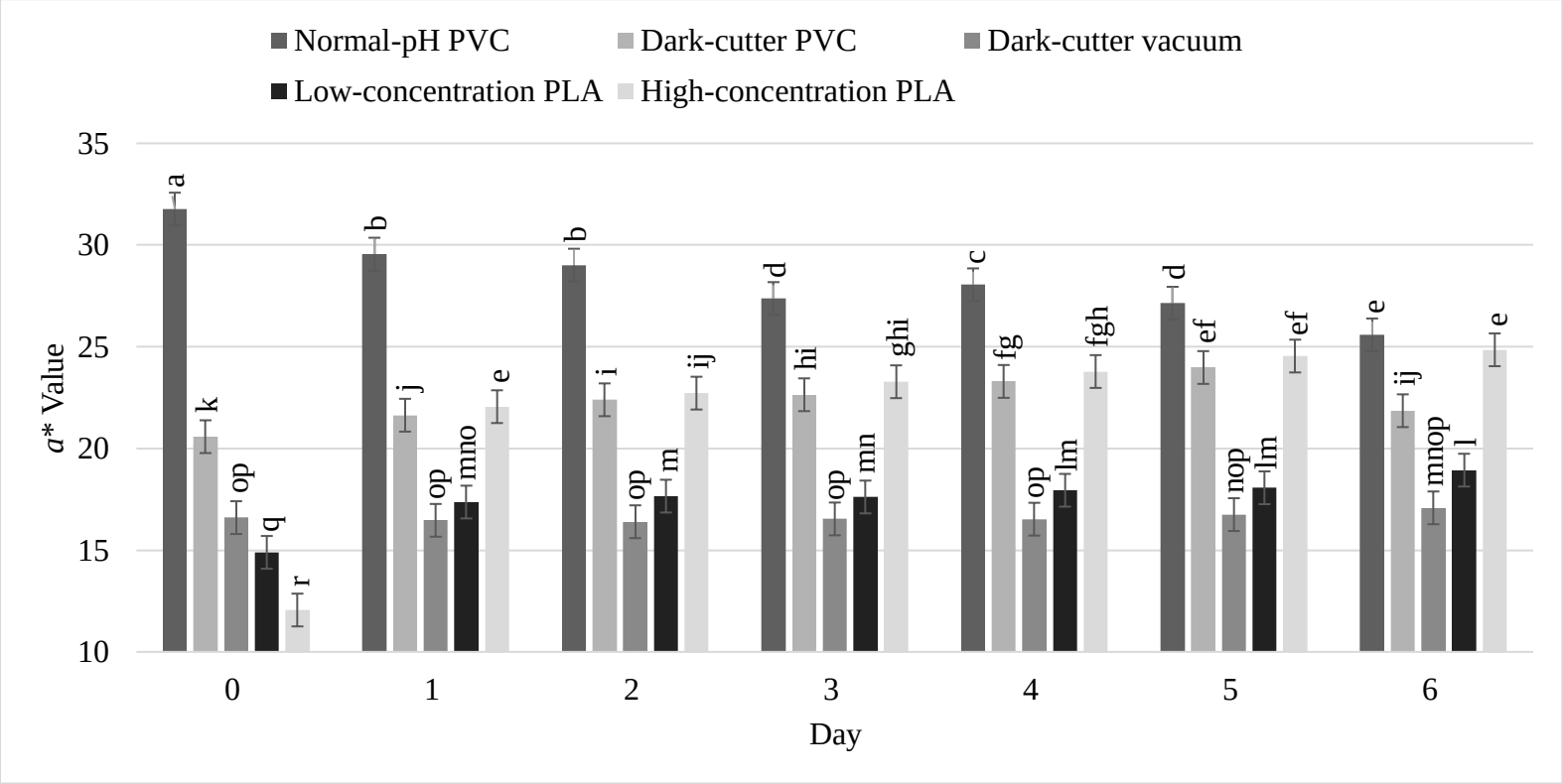
[3]High-concentration PLA films were compounded with poly(lactic acid), 0.6% sodium nitrite, and 2.5% α-tocopherol.

Figure 3. Least squares means of *L** values (packaging type[1] × day) of steaks during a 6 d retail display.



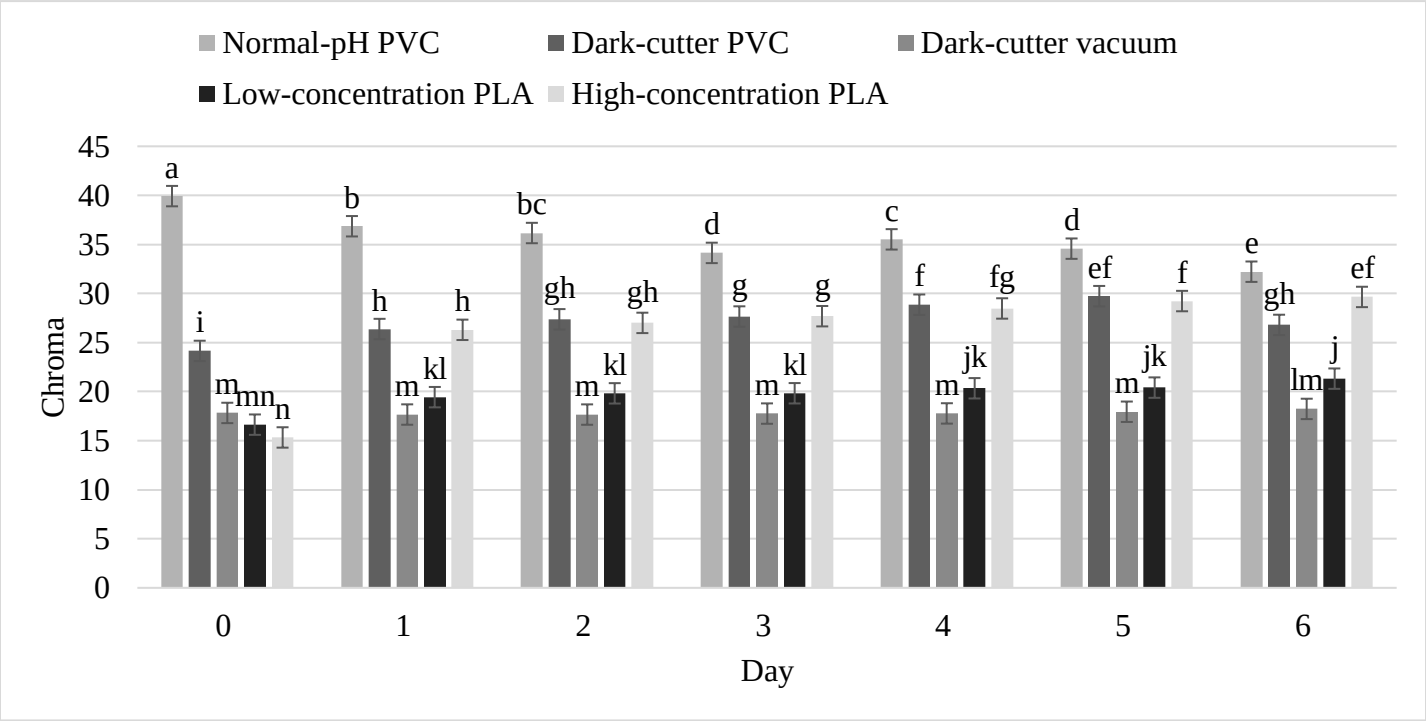
Least squares means with different letters (a - m) are significantly different ($P < 0.05$).
Standard error of the mean indicated by error bars (SEM = 1.10).
The experiment was replicated seven times (n = 7).
[1]Packaging treatments included normal-pH steaks in polyvinyl chloride overwrap, dark-cutter steaks in polyvinyl chloride overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration PLA film (compounded with poly(lactic acid), 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with poly(lactic acid), 0.6% sodium nitrite, and 2.5% α-tocopherol) in a vacuum package.

Figure 4. Least squares means of *a** values (packaging type[1] × day) of steaks during a 6-d retail display.



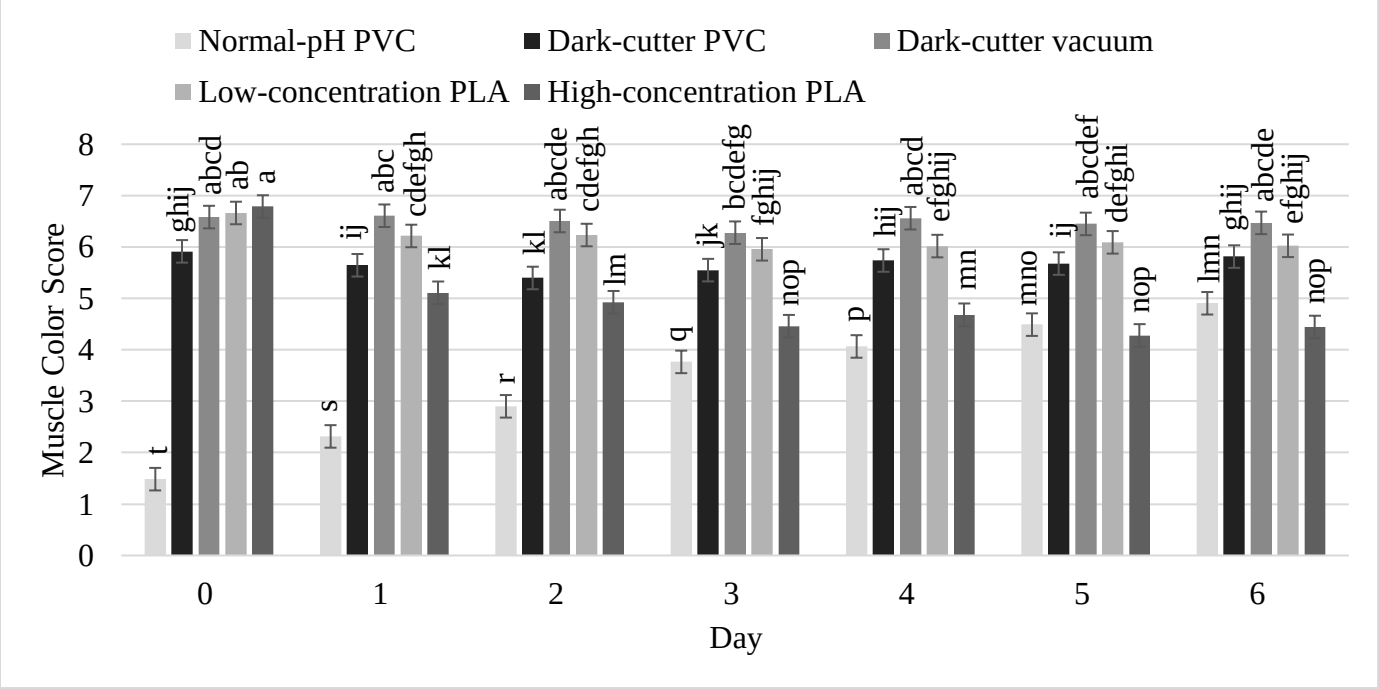
Least squares means with different letters (a – r) are significantly different ($P < 0.05$).
Standard error of the mean indicated by error bars (SEM = 0.81).
The experiment was replicated seven times (n = 7).
[1]Packaging treatments included normal-pH steaks in polyvinyl chloride overwrap, dark-cutter steaks in polyvinyl chloride overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration PLA film (compounded with poly(lactic acid), 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with poly(lactic acid), 0.6% sodium nitrite, and 2.5% α-tocopherol) in a vacuum package.

Figure 5. Least squares means of chroma (packaging type[1] × day) of steaks during a 6-d retail display.



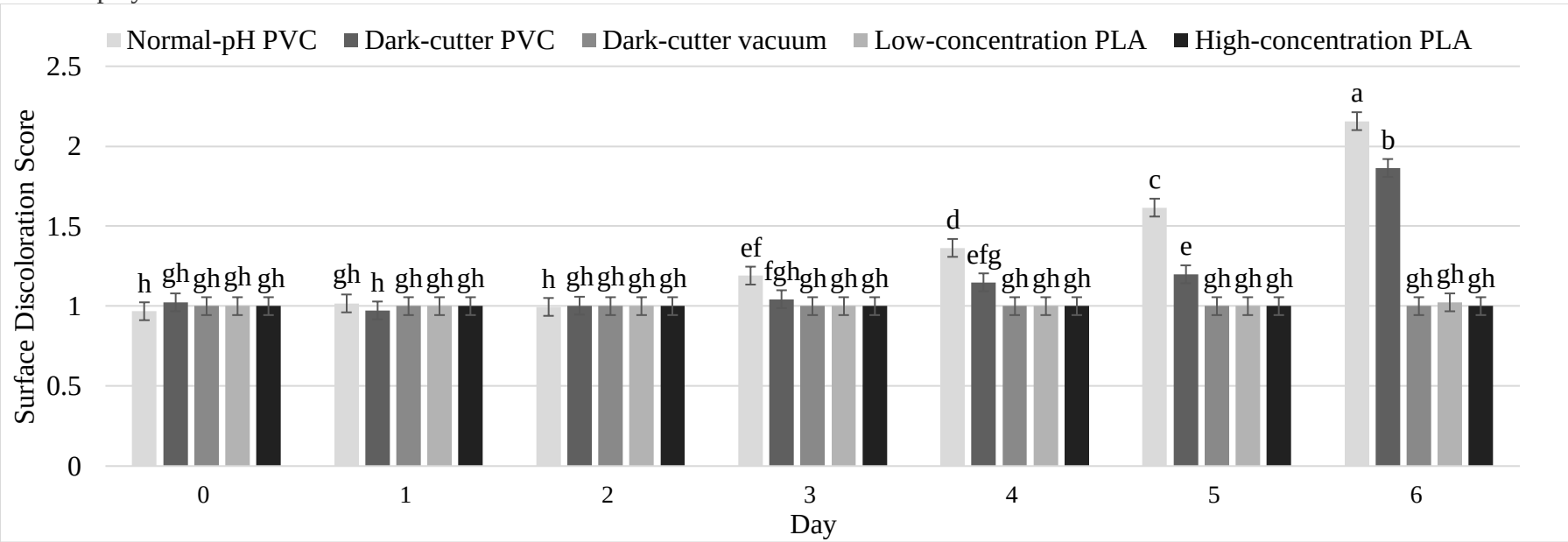
Least squares means with different letters (a – n) are significantly different ($P < 0.05$).
Standard error of the mean indicated by error bars (SEM = 1.04).
The experiment was replicated seven times (n = 7).
[1]Packaging treatments included normal-pH steaks in polyvinyl chloride overwrap, dark-cutter steaks in polyvinyl chloride overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration PLA film (compounded with poly(lactic acid), 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with poly(lactic acid), 0.6% sodium nitrite, and 2.5% α-tocopherol) in a vacuum package.

Figure 6. Least squares means of muscle color[1] evaluated by a trained panel (packaging type[2] × day) of steaks during a 6-d retail display.



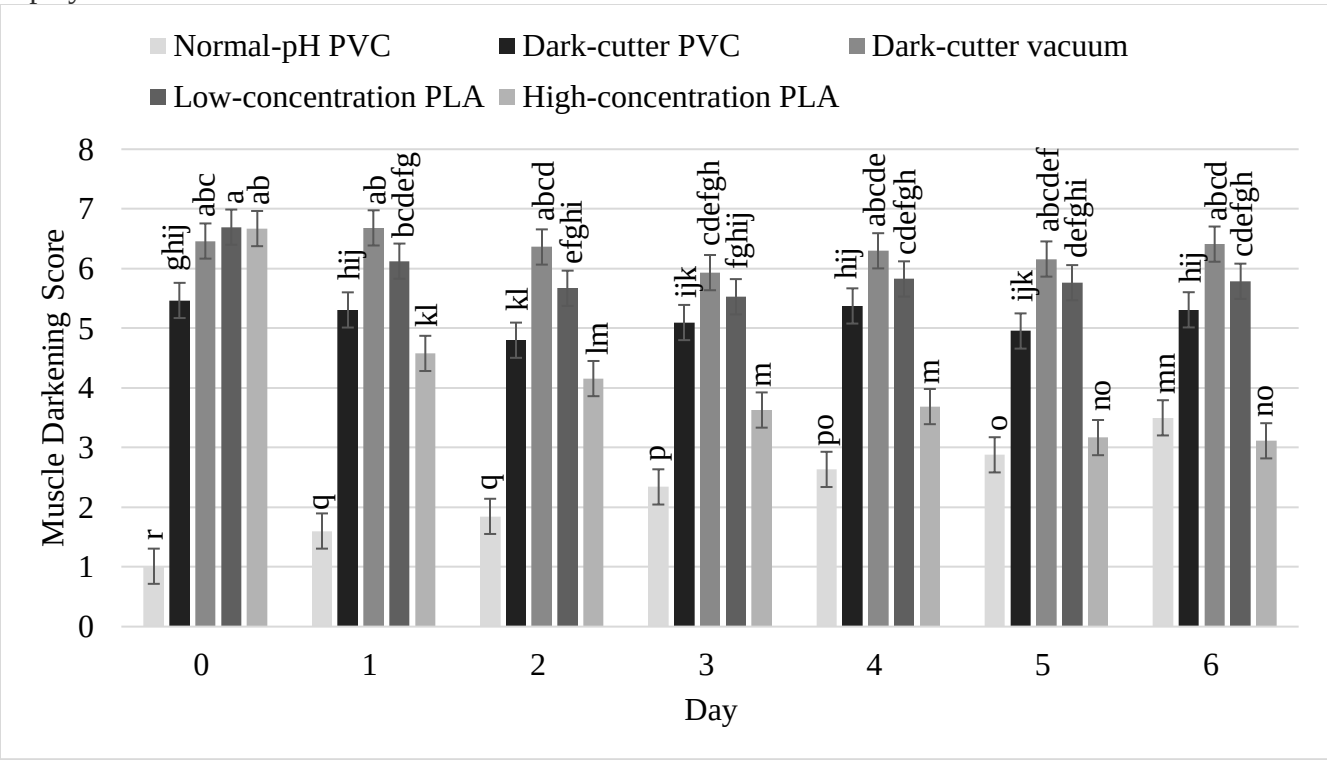
Least squares means with different letters (a – t) are significantly different ($P < 0.05$).
Standard error of the mean indicated by error bars (SEM = 0.22).
The experiment was replicated seven times (n = 7).
[1]Muscle color evaluated using a 7-point scale (1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark red, 6 = moderately dark red, 7 = dark red).
[2]Packaging treatments included normal-pH steaks in polyvinyl chloride overwrap, dark-cutter steaks in polyvinyl chloride overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration PLA film (compounded with poly(lactic acid), 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with poly(lactic acid), 0.6% sodium nitrite, and 2.5% α-tocopherol) in a vacuum package.

Figure 7. Least squares means of surface discoloration[1] evaluated by a trained panel (packaging type[2] × 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).
[1]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%)).
[2]Packaging treatments included normal-pH steaks in polyvinyl chloride overwrap, dark-cutter steaks in polyvinyl chloride overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration PLA film (compounded with poly(lactic acid), 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with poly(lactic acid), 0.6% sodium nitrite, and 2.5% α-tocopherol) in a vacuum package.

Figure 8. Least squares means of muscle darkening[1] evaluated by a trained panel (packaging type[2] × day) of steaks during a 6-d retail display.



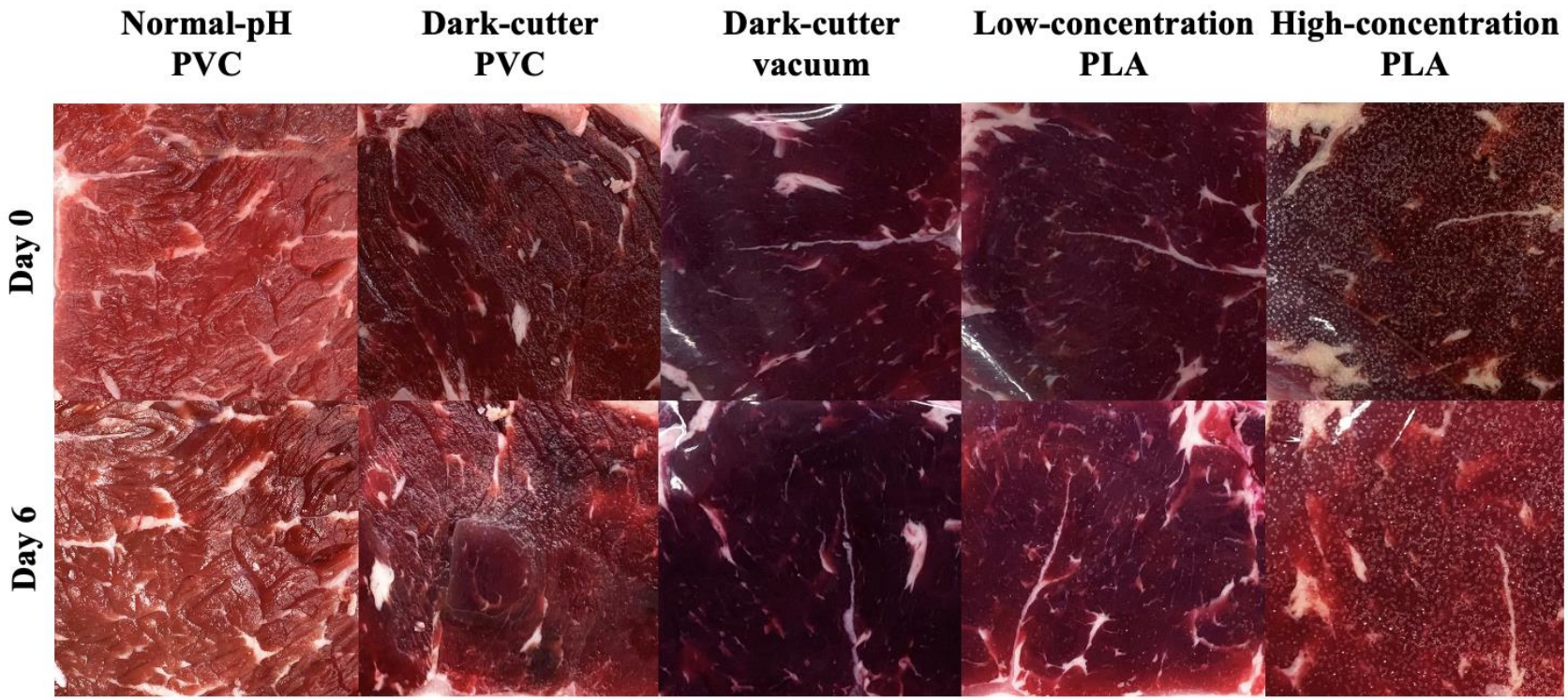
Least squares means with different letters (a – r) are significantly different ($P < 0.05$). Standard error of the mean indicated by error bars (SEM = 0.30).

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

[1]Muscle darkening evaluated using a 7-point scale (1= no darkening, 3= slightly dark, 5= moderately dark, 7= very dark).

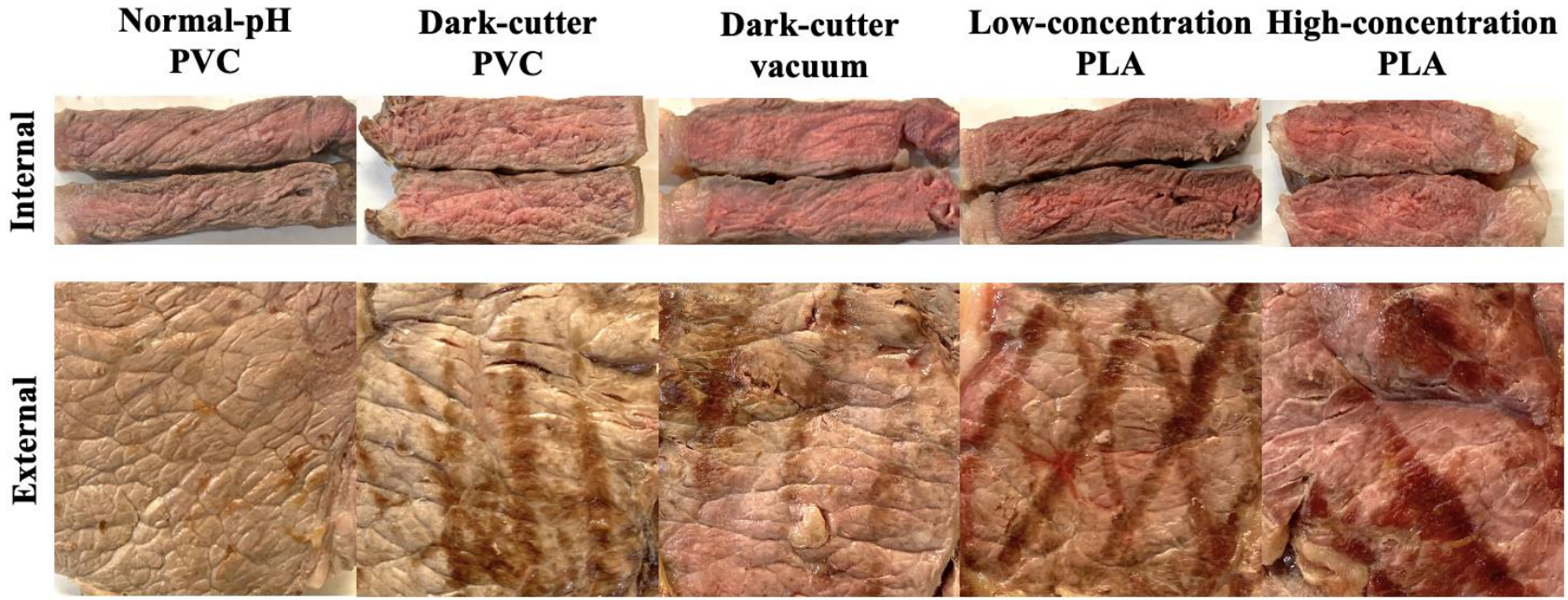
[2]Packaging treatments included normal-pH steaks in polyvinyl chloride overwrap, dark-cutter steaks in polyvinyl chloride overwrap, dark-cutter steaks in vacuum package, dark-cutter steaks with low-concentration PLA film (compounded with poly(lactic acid), 0.12% sodium nitrite, and 0.5% α-tocopherol) in a vacuum package, and dark-cutter steaks with high-concentration PLA film (compounded with poly(lactic acid), 0.6% sodium nitrite, and 2.5% α-tocopherol) in a vacuum package.

Figure 9. Visual effect of packaging treatments[1] on steaks at the beginning and end of retail display.



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

Figure 10. Visual effect of packaging treatments[1] on steaks cooked to 71°C on a George Foreman grill after 5 d retail display.



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

CHAPTER V

DISCUSSION

Film Properties

In the current study, there was no change ($P > 0.05$) in oxygen permeability between PLA films with low- and high-concentration sodium nitrite and α- tocopherol (Table 1). Initial testing of films caused instrumental failure due to insufficient oxygen barrier properties. Final testing was completed by layering films with a commercial vacuum package as would be used for the application of the current research. Based on the adjusted testing, the PLA film layer is expected to have a limited effect on the OTR since the main barrier layer of the commercial vacuum package film is associated with high barrier properties.

While there was no significant difference in transparency between low- and high-concentration PLA film recipes (Table 2), there was a visual color difference noted (Figure 1). Previous studies noted increased yellowness with α-tocopherol incorporation in film (Byun et al. (2010), Manzanarez- López et al. (2010), and Hwang et al. (2012)). Thus, the high-concentration PLA film recipe presented a more yellow appearance compared to the low-concentration PLA films as it possessed greater α-tocopherol content (Figure 1). Other research has shown improved transparency with films incorporating sodium nitrite (Chatkitanan et al., 2020). However, that was not observed within this study.

Similar to the current research, previous research showed the addition of α-tocopherol (at 2.2 and 4.4% concentrations) had no effect on the thermal stability (Table 3) of poly(lactic acid) films (Gonçalves et al., 2011). In contrast to the results of this study, Chatkitanan et al. (2020) reported the addition of sodium nitrite significantly decreased the thermal stability of thermoplastic starch films. Examining further film recipes containing poly(lactic acid) and poly(lactic acid) paired with either sodium nitrite or α-tocopherol alone will aid in determining each active ingredient's impact on thermal stability.

pH and Proximate Composition

Past research determined dark-cutting beef showed greater pH values when compared with normal-pH beef (Sawyer et al., 2009; Wills et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018). This difference in pH between normal and dark-cutting beef is associated with dark-cutting beef having limited glycogen content postmortem resulting in less postmortem pH decline (Lawrie, 1958; Scanga et al., 1998). Consequently, high muscle pH increases muscle swelling and water holding capacity (Hughes et al., 2017), which aligns with the increased moisture content of dark-cutting loins compared with normal-pH loins. In the current research, dark-cutting loins were selected based on pH rather than by marbling score. Hence, differences seen in fat content between normal-pH and dark-cutting loins can be attributed to the dark-cutting loins likely possessing less marbling than the USDA Low Choice loins selected as the normal-pH control.

Retail Display Color

Upon blooming, dark-cutting beef in the current study demonstrated smaller *L** values than normal-pH beef (Figure 3). In previous research, dark-cutting beef demonstrated greater deoxymyoglobin formation than normal-pH beef (English et al., 2016; McKeith et al., 2016; Hughes et al., 2017). Thus, the greater presence of deoxymyoglobin in dark-cutting beef results in a darker (decreased *L** values) than normal-pH beef (English et al., McKeith et al., 2016; Hughes

et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018). Moreover, the limited change in *L** values for all treatments throughout display noted in the current study was similar to the results reported by Zhang et al. (2018). Finally, nitrite-embedded packaging had a limited impact on *L** values of dark-cutting steaks (Denzer et al., 2022; Ramanathan et al., 2018).

Greater *a** values and chroma were exhibited by normal-pH steaks compared with dark-cutting steaks in display settings with aerobic packaging (Apple et al., 2011; Stackhouse et al., 2016; Wills et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018). Similar results were noted in this study with both aerobic and anaerobic packaging utilized in a retail setting. In support, Hunt et al. (1999) observed lower *a** values from ground beef patties containing deoxymyoglobin than those containing oxymyoglobin. Previous research indicated dark-cutting steaks possess more deoxymyoglobin compared with normal-pH steaks (McKeith et al., 2016; Hughes et al., 2017); thus, the difference in *a** values between normal-pH and dark-cutting beef seen within the current work are in conjunction with previous observations. Moreover, John et al. (2005) and Lagerstedt et al. (2011) noted normal-pH steaks in high oxygen-modified atmospheric packaging displayed greater a* values than those packaged in vacuum packaging. Steaks packaged in low- and high-concentration PLA films showed the formation of nitric oxide myoglobin in the first day of retail display as indicated by an increase in *a** values, chroma, and the derived wavelength ratio representing nitric oxide myoglobin formation. These results parallel Ramanathan et al. (2018) as dark-cutting steaks in nitrite packaging showed improved redness (*a** values) and chroma during display with significant increases by d 1 of display. Moreover, Ramanathan et al. (2018) similarly noted the addition of an antioxidant (rosemary) showed to increase color stability by reducing nitric oxide myoglobin oxidation. Furthermore, in the transformation to nitric oxide myoglobin, metmyoglobin is formed by the application of nitrite films (Fox Jr. and Ackerman, 1968) leading to decreased redness in low- and high-concentration PLA treatments on d 0.

Bison steaks in NEP studied by Roberts et al. (2017), showed an increase in bright red appearance and remained less discolored than control packaged steaks. Moreover, nitrite spray applied to ribeye and round steaks was shown to improve red color (Song et al., 2015). Additionally, dark-cutting steaks have exhibited a darker red muscle color and less discoloration than normal-pH steaks during retail display periods (Sawyer et al., 2009; Apple et al., 2011; Stackhouse et al., 2016; Wills et al., 2017; Mitacek et al., 2017). However, limited studies have investigated the effects of visual color on steaks packaged in nitrite film containing various nitrite concentrations.

Research completed by Mitacek et al. (2018) observed dark-cutting steaks to have greater darkness than normal-pH steaks in PVC. Day 0 darkness of dark-cutting steaks in low- and high-concentration films can be attributed to the initial formation of metmyoglobin in the conversion to nitric oxide myoglobin (Fox Jr. And Ackerman, 1968; Siegel, 2011). The shift in muscle darkening observed by steaks packaged in high-concentration PLA film parallels the increase in *a** values and nitric oxide myoglobin content and aligns with results noted by Ramanathan et al. (2018).

Microbial Growth and Lipid Oxidation

In the current work, dark-cutting steaks in PVC possessed significantly greater microbial counts compared with all other treatments (Table 6). Previous studies reported a 1-log reduction in aerobic plate counts with nitrite packaging. (Narváez-Bravo et al., 2017; Ramanathan et al., 2018). However, the current worked showed no impact between steaks packaged with active PLA film containing sodium nitrite and the steaks in vacuum packages alone. Thus, the limited microbial growth may be attributed to the vacuum environment rather than sodium nitrite addition. Analyzing lactic acid bacteria counts will be beneficial for future studies investigating the films used in this study as they are utilized in anaerobic conditions.

Dark-cutting beef has lower lipid oxidation compared with normal-pH beef (English et al., 2016; Wills et al., 2017). Moreover, Roberts et al. (2017) observed less lipid oxidation with bison burgers in nitrite-embedded packaging than control steaks with PVC overwrap. Similar results were reflected within the current study where normal-pH steaks in PVC showed greater ($P < 0.05$) lipid oxidation compared with dark-cutting steaks in PVC, control vacuum packaging, as well as low- and high-concentration PLA films (Table 7). Contrary to the current work, the addition of sodium nitrite in previous research reduced lipid oxidation compared with a control treatment within their respective meat products (Roberts et al., 2017; Chatkitanan and Harnkarnsujarit, 2020). However, in agreement with Denzer et al. (2022), the greater pH of dark-cutting beef may have limited the packaging type effect on lipid oxidation.

Cooked Color

Denaturation of myoglobin is impacted by pH (Hunt et al., 1999). At greater pH levels, deoxymyoglobin was determined to have greater heat stability compared with metmyoglobin when heated to 70°C (Hunt et al., 1999). Thus, dark-cutting beef allows for persistent pinking with its combination of greater pH and greater deoxymyoglobin content than normal-pH beef. In support, previous research noted greater cooked red intensity and a^* values observed with dark-cutting steaks compared to normal-pH steaks (Sawyer et al., 2008). Claus and Du (2013) and Song et al. (2015) noted increases in a^* values and redness for steaks in nitrite systems. In support, Song et al. (2015) determined an increase in nitrosylhemochrome on the surface of cooked steaks. Nitrosylhemochrome is the pigment that gives cured meat its distinctive pink color (Macdougall et al., 1975). Thus, nitrosylhemochrome formation can explain the increase in pink color after cooking on the surface of steaks packaged in nitrite packaging systems. Consequently, the combination of dark-cutting steaks with PLA films containing high-levels of nitrite resulted in a redder cooked color appearance.

CHAPTER VI

CONCLUSION

Color is a vital factor in the marketability of meat. The appearance of dark-cutting beef results in product discounts and low consumer appeal. In addition, a redder internal cooked color appearance has been observed with dark-cutting beef. Thus, improvements to retail and cooked color of dark-cutting beef are needed. While commercially available nitrite-embedded packaging has been shown to significantly improve retail color redness of dark-cutting beef, the formation of an external cooked red pigment occurs when heated. Thus, identifying the optimized level of nitrite within the packaging, which can significantly improve the retail color redness of dark-cutting beef while mitigating the cooked color impact, is essential to further developing this intervention. Furthermore, as environmental concerns continue to rise, the development of biodegradable packaging is an important to step towards meeting the world's sustainability goals. The use of poly(lactic acid) films incorporating sodium nitrite and α-tocopherol is a viable option to improve the retail color of dark-cutting beef. Low-concentrations of sodium nitrite (0.12%) and α-tocopherol (0.5%) in poly(lactic acid) films significantly improved retail color redness while having minimal changes to cooked color of dark-cutting steaks compared to dark-cutting steaks in vacuum packaging. The current study was unable to determine the direct impact of sodium nitrite and α-tocopherol on shelf life attributes such as microbial growth and lipid oxidation. However, previous work has shown the ability of sodium nitrite and α-tocopherol to limit the degradative

reactions seen with microbial growth and lipid oxidation in meat systems. Therefore, this study demonstrated that developing a packaging system with biodegradable aspects and active ingredients can help improve the retail color redness and color stability of dark-cutting beef. In turn, these improvements can lead to limited retail waste as steaks maintain an ideal red color throughout the display duration. Future work should investigate the impacts of PLA films incorporating sodium nitrite and α-tocopherol on normal-pH beef.

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