Quality Parameters on Beef Steaks and Ground Beef Using Thermoforming Vacuum Packaging

by

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ABSTRACT

Meat surface color is a critical attribute influencing consumer purchasing decisions, as it serves as a visual indicator of product freshness and wholesomeness. Changes in surface color during retail display periods can significantly affect consumer intent to purchase. Vacuum packaging, traditionally utilized for extended storage of meat products, is increasingly employed alongside freezing to mitigate meat quality deterioration. This document is comprised of three studies focused on the effect of thermoforming vacuum packaging on the shelf life of beef steaks *Longissimus dorsi* (L.D.) and ground beef on the surface color and overall quality during storage display.

The effect of three different thermoforming vacuum packaging films on bloom development in beef steaks transitioning from frozen to fresh conditions was evaluated through this surface color measurements taken every 4 hours during simulated retail display. Revealed that steak color became lighter, redder, and more yellow as bloom time increased ($p < 0.05$). Spectral values, including chroma, red-to-brown ratio, and deoxymyoglobin values, also increased significantly with bloom time ($p < 0.05$), suggesting that this type of packaging positively impacts surface color during the initial 8 hours post-thawing.

The second study focused on beef steaks packaged in VPA (250 µ nylon/EVOH/enhanced polyethylene coextrusion) film remained lighter and redder over time ($p < 0.05$), while those in VPB (250 µ nylon/EVOH/enhanced polyethylene coextrusion) and VPC (125 µ nylon/EVOH/enhanced polyethylene coextrusion) films darkened. Yellowness, hue angle, and chroma values were highest in steaks stored in VPC film ($p < 0.05$). Additionally, steaks in VPC films showed greater levels of metmyoglobin and oxymyoglobin and lower deoxymyoglobin ($p < 0.05$). Lipid oxidation, although influenced by packaging treatment, was more significantly affected by storage time,

which also impacted purge loss, cook loss, and Warner-Bratzler shear force (WBSF) ($p < 0.05$). The study findings suggest that vacuum packaging is viable for extended storage of beef steaks beyond 60 days, with varying effects based on packaging type.

Lastly, ground beef stored at frozen temperatures prior to refrigerated display period using three different thermoforming vacuum packaging was not associate with significant differences in lipid oxidation among the packaging treatments ($p = 0.0744$). However, oxidation levels increased throughout the storage period ($p < 0.0001$). Additional surface color including lightness, redness, yellowness, hue angle, and myoglobin redox forms. Packaging treatment and storage day significantly influenced by both packaging treatment and storage duration ($p < 0.05$). The interaction of treatment and storage day also impacted on chroma, Delta E, and the ratio a*:b* ($p < 0.05$). These findings indicate that thermoforming vacuum packaging can effectively reduce the rate of color and oxidative deterioration in ground beef during extended storage and display, thereby enhancing product quality and shelf-life.

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CHAPTER I

Literature Review

1. Introduction

Packaging plays a crucial role of meat products in retail stores and is directly associated with meat discoloration. The color of meat is a significant attribute that represents freshness and quality, playing a major role in consumer purchasing decisions. Bright cherry-red color is often associated with freshness, largely due to the formation of oxymyoglobin, which occurs when meat is exposed to oxygen. In contrast, a purplish color indicates the presence of deoxymyoglobin, which forms when meat is not exposed to oxygen. Consumers tend to associate a higher concentration of deoxymyoglobin with poor quality or spoilage, even though the meat may still be safe to eat. Proper packaging techniques are vital for preserving the fresh appearance of meat by controlling exposure to oxygen, moisture, and light which are considered as primary factors responsible for color changes. Consequently, effective packaging not only prolongs shelf life but also maintains the visual appeal of meat, allowing retailers to reduce waste and meet consumer expectations. When meat appears less vibrant, consumers may reject it, leading to significant food waste in retail, which in turn contributes to unnecessary losses throughout the food supply chain.

2. Meat Color

Meat color is considered one of the most influential factors used by consumers at the time of purchase. Therefore, this attribute may be influenced by several variables that interact during storage and display conditions. (MacDougall, 1981; Faustman 1990). Myoglobin is the principal protein responsible for meat color.

Mancini (2013) defined myoglobin as a sarcoplasmic protein located in muscle tissue, playing a crucial role influencing the color of meat due to its heme iron, which is

situated at its core. The iron atom at the center of the heme ring can form multiple bonds, four of which are linked to the nitrogen atoms of the pyrrole groups. Moreover, this globular protein is composed of 153 amino acid units, connected by brief non-helical segments. According to Mancini and Hunt (2005) it has been reported that the interaction between two specific histidine residues and the heme group in myoglobin plays a crucial role in shaping the protein's structure, contributing to the stability of meat surface color. The determinants of meat color are the chemical state of the iron and the type of molecules bound to the ferric part of the meat. The redox state of iron determines meat color by producing three possible chemical forms of myoglobin: deoxymyoglobin, oxymyoglobin, and metmyoglobin (Mancini and Hunt, 2005).

Initial stages of metmyoglobin formation begin with the deoxygenation of oxymyoglobin. Protons enter the heme cavity and cause the protonation of dioxygen, leading to removal of a single electron from the heme ring, resulting in a superoxide dissociating from the heme to ultimately result in the creation of metmyoglobin (Shikima, 1998).

Deoxymyoglobin is another myoglobin redox form which visually appears as a purplish-red hue, arising from the presence of ferrous heme iron and a sixth coordination position. As the term suggests, this myoglobin variant is linked to muscle-base food items shielded from oxygen exposure and can be attributed to meat stored in low oxygen atmospheric conditions or associated with muscle tissue (Mancini 2013). However, Yusa and Shikama (1987) reported that reducing the partial pressure of oxygen increased the concentration of deoxymyoglobin. Furthermore, partial pressure changes may explain the higher formation of metmyoglobin. The greater redox instability of myoglobin at lower oxygen pressure is demonstrated by the fact that deoxymyoglobin oxidizes more rapidly

than oxymyoglobin. Oxymyoglobin forms when deoxymyoglobin is exposed to oxygen, a crucial step in the development of the vibrant cherry-red color.

The fading of color in beef steaks stored in refrigerated display cases appears to result from the combined influence of oxygen levels, temperature and specific lighting (Renerre, 1990). Display lighting can influence pigment photo-oxidation as the reaction caused by lighting serve as a catalyst for the creation of metmyoglobin (Renerre and Labadie, 1993). Setser et al. (1973) reported a correlation between oxygen levels and illuminating wavelength, observing that the least oxymyoglobin loss occurred at 0% oxygen and 577 nm. In contrast, oxygen levels between 20% and 100%, combined with a wavelength of 254 nm, resulted in the highest oxymyoglobin loss. Numerous investigations have explored the impact of light on the discoloration of fresh meat though these findings have been contradictory. Consistent with display lighting impact on surface color, Zachariah and Satterlee (1973), along with the work of Solberg and Frank (1971), illustrated that visible light falling within the range of 500 to 600 nm led to a slight yet noteworthy rise in the buildup of metmyoglobin on the surface color of meat.

Additionally, Lentz (1971) noted that the surface color of frozen beef remained appealing for up to 3 months when stored in darkness but deteriorated within just 3 days when exposed to light under lighting conditions of 1600 to 2100 lux. The speed at which surface color deteriorates is a combination of light intensity, wavelength distribution and the light permeability of the packaging material (Andersen et al.1989). Consistent with lighting conditions impacting the surface color, Lentz (1979) reported that frozen retail beef cuts subjected to varying levels of light (ranging from 0 to 200 lux), could be stored from 1 to over 90 days. Contingent on both light intensity and storage temperature. Satterlee and Hansmeyer (1974) concluded that low-wavelength visible light intensified

the oxidation process, transforming the red color which is associated with oxymyoglobin to brown indicating a greater presence of metmyoglolobin.

Additional investigation into the chemical state of myoglobin has concluded that more components are influential in contributing to the meat surface color. Ramanathan et al. (2020) assessed the oxidation rates of oxymyoglobin in muscle tissue from seven distinct species, quantifying histidine levels, and determined that the formation of metmyoglobin followed this order: equine > turkey > chicken > porcine > venison > ovine > bovine. Krzywicki (1982) created a series of equations to gauge the relative ratios of myoglobin redox states in liquid meat extracts, and these equations have found extensive applications that are still in use today.

3. Influences of meat color

Meat color can be influenced by different factors before and after harvesting, providing a wide range of colors in the superficial part of the muscle. Consumers often use this parameter as an indicator of freshness and quality, which influences their purchasing decisions.

4. Temperature

Extending storage life in food products is closely linked to temperature. Wolfe (1980) has documented that extending shelf-life of meat products. Hinge upon prudent management of temperature regulation. Although the importance of storage temperature in relation to shelf life is well established, there remains some degree of uncertainty about how the interaction between these factors affects the color stability, retail characteristics, and overall shelf life of beef products (Jeremiah and Gibson, 2001). Frozen meat products experience greater quality deterioration during storage that at any other point in the production process (Fu and Labuza 1997).

5. Transportation temperature

Regulations are enforcing stricter limits on energy consumption, recommending storage and transportation temperatures below -18°C. However, it is common for frozen meat to undergo temperature fluctuations during these processes. Shi *et al.* (2018) have reported that conventional freezing storage (-18°C) is widely used for long-term beef preservation. However, it is quite common for frozen meat to encounter temperature variations during these phases. Chrystall (1972) studies indicate that temperature fluctuations are the primary cause of the physicochemical alterations responsible for food quality deterioration and reduced shelf-life period. The pace of deterioration will be contingent on the storage temperature, the magnitude of thermal fluctuations, and the specific characteristics of the product itself (Fennema, 1966; Chrystall, 1972).

6. Storage temperature

Frozen storage has long been employed as a method to preserve the safety and quality of meat products. Among the various factors impact in safety and shelf life on the meat products, the freezing temperatures stands out as one of the most critical elements for prolonging the shelf-life of highly perishable foods like fresh meat (Rahman *et al.* 2011). Superchilling storage, where products are kept at temperatures approximately 1° to 2°C below their initial freezing point, has also been employed for the preservation of meat products (Lu *et al.* 2018). However, implementing the superchilling storage method necessitates rigorous temperature control, which can be challenging to sustain consistently within the meat processing industry. The superchilling approach tends to consume a significant amount of energy during storage, and instances of temperature deviations are frequent. Therefore, it is crucial to select an appropriate storage temperature for beef that aligns with the prevailing regulatory requirements of the meat processing industry.

7. Crystal formation during storage

Freezing is a method that allows for the extended storage of foods, and upon thawing, these foods can be used as if they were fresh products (Hui *et al.* 2004). While freezing temperatures effectively halt microbial spoilage and slow down certain physico-chemical alterations, it's important to note that food quality can still be influenced by the freezing process (Utrera *et al.* 2014). The harm caused by freezing is connected to the formation of ice crystals, which can result in structural disruptions caused by physical fractures and the osmotic pressure created by the concentration of solutes outside the cells (Zaritzky 2012).

Crystallization is likely the most significant purely physical transformation in frozen meat during storage time. Consequently, smaller crystals tend to dissipate more rapidly compared to those of larger size (Burke and Turnbull, 1952). For instance, Zaritzky (2012) noted that the growth in the size of these ice crystals, referred to as recrystallization, is a phenomenon that occurs during frozen storage and can lead to further physical damage in the cellular structure. Deterioration of muscular fibers can be effectively minimized by keeping low and uniform temperatures, even though commercially this is difficult to achieve. For this reason, recrystallization of ice has been studied in foods. The degree of quality deterioration typically correlates with the size and placement of crystals formed during the freezing and subsequent frozen storage processes, primarily being influenced by temperature (Scott and Heldman, 1990).

These physical alterations result in the release of mitochondrial and lysosomal enzymes into the sarcoplasm, facilitating contact between pro-oxidants like metals, heme pigments and vulnerable macromolecules. This interaction ultimately triggers specific chemical reactions, primarily involving protein denaturation, proteolysis, lipolysis and

lipid oxidation (Zaritzky 2012). The phenomenon of lipid oxidation induced by frozen storage has been a subject of extensive research in the field of muscle food.

8. Bloom Time

Blooming refers to the alteration in color resulting from the oxygenation of myoglobin when a meat surface comes into contact with oxygen. Blooming is a characteristic that is highly appreciated by consumers and, being a visual effect, influences the purchase intention of red meat, including lamb and veal (Hopkins *et al.*, 1996). The magnitude of these color alterations is influenced by time and, as a result, time is frequently incorporated into measurement protocols to assess meat color and allow comparisons between samples (Pearce, 2009). A recent review on bloom in red meat concluded that bloom is mainly influenced by the oxygen transmission rate (OTR) of the meat, as the partial pressure of oxygen tends to remain relatively constant, depending on the packaging system (Jacob, 2020). Modified atmosphere packaging is a recent technology that effectively decreases the variability caused by blooming and, in turn, improves both color and stability.

Blooming is a process influenced by the amount of oxygen in contact with the meat surface. Higher oxygen concentration can accelerate the dispersion of oxygen into muscle tissue, promoting increased formation of oxymyoglobin (Suput *et al.* 2013). This, in turn, brings about a surface color transition from purple to a vibrant red hue. This process proves to be more efficient in conditions that enhance oxygen solubility while reducing the enzymatic activity of muscle tissue. Furthermore, meat undergoing extended aging periods may exhibit a more rapid and pronounced surface color blooming (Irurueta *et al.*, 2008). Consumers typically assess meat when it displays its peak surface color bloom, often upon removal from packaging or during storage. Changes in surface color

can significantly impact consumer perceptions of quality driving both consumer choice and satisfaction (Wyrwisz *et al.* 2016).

Measurement time alone may not cover all meat color fluctuations attributed to bloom. Although measurement equipment is likely to continue relying on light reflectance, the accuracy of these devices has recently been enhanced to better capture bloom development in meat (Khari *et al*., 2012) and the relationship between instrumental measurements and consumer perception of color (Girolami *et al*., 2013).

9. Packaging Platforms

Meat packaging is a constantly evolving field, and its innovative developments have been the subject of recent scrutiny and review. The packaging methods employed for fresh meat at the point of sale are undergoing transformation, primarily driven by the shift towards centrally packaged meats and the growing consumer demand for enhanced quality, safety, and convenience (Thoden van Velzen and Linnemann, 2008). The way a product looks when it's displayed for sale significantly shapes consumers' perceptions of its quality and, consequently, impacts their purchasing choices. For beef, Issanchou (1996) identified packaging and color as the two most crucial visual indicators that consumers rely on when selecting meat products.

Packaging fulfills two distinct yet equally essential roles with regard to the product (Sara, 1990). First and foremost, must safeguard and enclose the contents from the manufacturing facility to the end user. Whether it encompasses liquid detergent, frozen meat, ripe peaches, or talcum powder, the packaging's primary objective is to maintain the contents in optimal condition until they are used, regardless of the duration between the initial packing and consumption, and regardless of the handling processes, shipping methods, or environmental conditions it may encounter during transportation.

Secondly, packaging tends to make the product stand out on the competition on the supermarket shelf, capturing the consumer's attention, creating a positive impression, and ultimately convincing the consumer to add that product to their cart (Sara 1990). Frequently, the actual product inside the packaging is hidden from view, either because it doesn't have an appealing appearance in its raw state or because preservation requirements dictate that it be shielded from light. Consequently, the packaging becomes the primary interface connecting the product and the consumer.

Vacuum packaging is a commonly employed method to preserve beef by maintaining anaerobic conditions during storage. A study by Avilés et al. (2013) evaluated the effect of vacuum packaging on color development and stability in beef steaks, noting that in commercial settings, beef primal cuts generally remain vacuum sealed in optimal conditions throughout the aging process until retail processing. In numerous research projects, primal cuts of meat are vacuum-sealed and transported from the commercial beef packing plant to the research facility for subsequent processing, such as deboning and fat trimming.

Occasionally, this meat may undergo additional vacuum-sealing processes due to scheduling constraints, manpower limitations, a limited quantity of primal cuts, the specific demands of experimental design, or even occasional packaging errors. In this regard, Holdstock *et al.* (2014) reported that packaging process may reduce the meat's ability to undergo the blooming process and maintain consistent color stability, especially when it has been vacuum-packaged multiple times.

10. Vacuum Packaging

Currently, the most commonly adopted method for extended the shelf-life of fresh meat by creating an oxygen-deficient environment is through the use of vacuum packaging. In the USA, it's estimated that approximately 97% of all beef is processed and

transported as a vacuum-packaged product (Humphreys, 1996). Effective evacuation of the air within the packaging is crucial to reduce oxygen levels to below 500 parts per million (ppm) and prevent irreversible browning caused by residual oxygen. The rapid exclusion of oxygen from the meat surface, immediately after the carcass is divided into primal, preserves the meat's ability to reoxygenate once it is displayed in retail packaging (Walsh *et al.* 2022).

Vacuum packaging consists of placing primal or subprimal cuts of meat into plastic bags or pouches and extracting the air by means of a nozzle-type vacuumizing machine or by use of a vacuumizing chamber (Seideman and Durland, 1983).

Seideman (1975) outlined several benefits associated with vacuum packaging including: (a) minimizing weight loss by preventing dehydration of meat surfaces. Typically experienced in open refrigeration systems; (b) preserving the natural color of the muscle in its freshest state by excluding oxygen; (c) ensuring enhanced hygiene by eliminating external contamination; (d) extending the edibility period compared to non-vacuum packaged beef; and (e) creating an optimal environment for the aging process of beef.

The meat industry encounters a major challenge in communicating the benefits of traditional vacuum packaging methods to consumers, despite the clear advantages these platforms offer. To address this issue, numerous studies have been conducted to test non-traditional packaging options with consumers. Rikert *et al.* (1957), found that meat stored in a vacuum of 20 in. or higher showed quicker initial loss and return of redness compared to samples stored under less than 20 inches of vacuum. However, Fredholm (1963) contested this, stating that meat stored in a vacuum for 14 days did not always regain a bright red color upon exposure to oxygen. In that study, some surfaces maintained a greyish-brown discoloration despite the vacuum storage.

Although vacuum packaging seems to eliminate residual air spaces, there might still be some presence of oxygen inside the package, particularly if the surface has been exposed to oxygen. The remaining oxygen can lead to the formation of brown metmyoglobin within a few hours, as the oxidation rate exceeds enzymatic reduction at low oxygen levels (Seideman and Durland, 1983). However, packaging films with low oxygen permeabilities result in a rapid rise in metmyoglobin formation right after packaging. However, after 2-4 hours, there won't be any additional increase in metmyoglobin production (Pirko, 1957). The innovative concept of package structures has been termed as smart, interactive, and active packaging. These refer to packaging types that modify the packaging conditions, enhancing shelf life, safety, or sensory properties of the food while preserving its quality (Skandamis and Nychas, 2002).

11. Food waste

The challenge of food wastage, particularly concerning perishable animal proteins, is intensifying. Food waste, particularly the waste of perishable animal proteins, is on the rise. In high-income countries, the availability of animal protein surpasses the population's needs (Ederer *et al*., 2023), leading to significant food loss. For instance, about 26% of fresh meat produced in the U.S. is discarded each year at the retail and consumer levels (Gunders, 2012). In 2022, the U.S. produced approximately 8.94 billion kilograms of beef for retail consumption (USDA ERS, 2023), with an estimated 194.7 million kilograms lost annually (Ramanathan *et al*., 2022). A key factor in this wastage is surface discoloration, which accounts for around 2.55% of the total beef discarded. This loss leads to an economic impact of $3.7 billion per year for the beef industry (Ramanathan *et al.,* 2022). A significant portion of this wastage is linked to consumers' strong preference for fresh beef with a bright cherry-red color, as they are often reluctant to buy meat that doesn't conform to these visual expectations (Viana *et al*. 2005).

Conclusion

Effective packaging plays a critical role in preserving the visual appeal of fresh meat, especially its bright cherry-red color, which consumers associate with freshness and quality. The challenge lies in maintaining this color, as factors like oxygen exposure, light, and temperature fluctuations can lead to discoloration, contributing to significant food waste. By addressing these issues through improved packaging technologies and storage methods, the meat industry can extend shelf life, reduce waste, and meet consumer expectations, ultimately benefiting both retailers and the environment.

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CHAPTER II

Bloom Development from Frozen to Fresh of Vacuum Packaged Steaks

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Abstract:

Meat surface color is a key attribute that influences the purchase of meat products in stores because consumers consider color to be an indicator of wholesomeness. Changes in surface color of meat during display can be detrimental to consumer purchase intent in the retail setting. Vacuum packaging is a technology often reserved for extended storage of meat and food products. To extend storage conditions of fresh meats, freezing is commonly used to reduce meat quality deterioration while retail use of vacuum packaging for meat products is increasing. Therefore, the current study evaluated the influence of thermoforming vacuum packaging on bloom development in beef *Longissimus dorsi* (L.D.) steaks undergoing the transition from frozen to fresh storage conditions. Surface color of the steak was measured objectively every 4 hours after removing steaks from frozen storage and placing on display cases to recreate a retail storage exposure that similar to what is common in supermarkets. Steak surface color became lighter, redder, and more yellow ($p < 0.05$) as bloom time increased. Calculated spectral values chroma, red-to-brown, and calculated relative values of deoxymyoglobin increased ($p < 0.05$) with increasing bloom time. Current results suggest that thermoforming vacuum packaging positively influences surface color of beef steaks during the first 8 hours after increasing storage temperatures.

Keywords: Beef, Blooming, Instrumental color, Vacuum packaging

1. Introduction

Fresh meats are a group of global commodities stored using a variety of packaging systems for retail display in supermarkets [1]. Consumers select meat products based on characteristics such as surface color, price, and consumer convenience. Selection criteria suggests that the color of meat is a primary indicator of quality and freshness. Consumers perceive red meat to be fresher and higher quality in contrast to discolored cuts that are commonly considered to be of poor quality [2].

Blooming in meat surface color is caused by oxygen exposure to the cut surface and can be altered by the diffusion of oxygen into the muscle tissue through factors such as temperature and pressure [3]. Elevated oxygen concentrations can increase the rate of oxygen dispersion into the muscle tissue, leading to greater oxymyoglobin development, and causing a surface color change from purple to bright red [4]. However, this process is also more effective under conditions that increase oxygen solubility and decrease the enzymatic activity of muscle tissue recovers the original bright-red color. Additionally, it has been demonstrated that meat undergoing prolonged aging can experience surface color blooming more quickly and intensely [5]. Consumers often view meat at its peak surface color bloom, typically after being removed from its packaging or during storage, surface color can alter the consumer perception of quality [3].

Discoloration is one of the major quality changes that should limit the shelf-life on meat products, the selection of suitable packaging system would be retard or prevent this unfavorable quality change during storage and distribution [6]. Recently the meat industry makes use of a wide range of packaging, differing in the properties that constitute each of them.

Vacuum packaging is a technique used throughout the meat and food industry to ex-tend meat storage life and to maintain meat quality during the transition from frozen

storage to fresh display at refrigerated temperatures ranging from 4 °C to 6 °C [7,8]. Variability in packaging methods for fresh meat has been well documented to alter the surface blooming process of beef steaks [9]. However, various packaging forms have been adopted by the meat industry, one such method, modified atmosphere packaging (MAP) with a gas mixture (80% O_2 and 20% CO_2) has been shown to be an effective packaging method for beef that supports a stable bright-red color during storage [10]. Vacuum skin packaging (VSP) uses a top cover film shrink wrapped around the meat surface resulting in a pack-aging method that can sustain a longer storge period but limits bloom development [11].

Therefore, the objectives of this study were to evaluate the influence of thermoforming vacuum packaging on blooming time when steaks transition from frozen to fresh storage conditions.

2. Materials and Methods

2.1 Raw materials

Beef ribeye rolls (Institutional Meat Purchasing Specifications No. 112A) were purchased from a commercial meat processor, transported to the Auburn University Lambert-Powell Meat Laboratory, and stored in refrigerated conditions (2°C ± 1.25°C) for 21 days (Model LEH0630, Larkin, Stone Mountain, GA, USA). After aging, ribeye rolls (N = 20) were fabricated into steaks. Steaks were cut 2.54-cm-thick using a BIRO bandsaw (Model 334, Biro Manufacturing Company, Marblehead, Ohio, USA). To mimic industry applications of steak fabrication, the cut surface of each steak was allowed to bloom for 30 min in atmospheric conditions at 2°C ± 1.25°C before packaging.

2.2 Packaging Treatments and Simulated Display Conditions

Steaks (n = 4/ribeye roll) were assigned randomly to a packaging treatment. Each steak was packaged individually using a Variovac Optimus (OL0924, Variovac, Zarrentin am Schaalsee, Germany). Steaks were placed into one of three different thermoformaing packaging films (TA, TB and TC) and sealed with a non-forming layer (NF) using commercial packaging guidelines (WINPAK, Winnipeg, MB, Canada). Packaging film com-ponents, oxygen transmission rate (OTR) and vapor transmission rates (VPR) are presented in Table 1.

Packaged steaks were placed into cardboard boxes and stored in a blast freezer (Model LHE6950, Larkin, Stone Mountain, GA, USA) for seven days at -20°C ± 1.50°C to simulate frozen distribution from manufacturer to retailer at the Auburn University Lambert-Powell Meat Laboratory. Frozen steaks were placed into a three-tiered, multi-deck, lighted display case Avantco (Model 178GDC49HCB, Turbo Air Inc., Long Beach, CA, USA) operating at 3.0 °C ± 1.5 °C. Lighting within the retail case consisted of cool LED strips (TOM-600-12-v4-3, Philips Xitanium 40W-75W, Korea) with a lighting intensity of 2297 lux (ILT10C, International Light Technologies, Peabody, MA, USA).

2.3 Instrumental Color

Instrumental surface color was measured every 4 hours with a HunterLab MiniScan EZ colorimeter, Model 45/0 LAV (Hunter Associates Laboratory Inc., Reston, WV, USA) through the packaging film. Prior to surface color readings, the colorimeter was standardized using a black and white tile covered with the packaging films to confirm instrument accuracy.

Instrumental color values were determined from the average of three readings using illuminant A, a 10° observer, and a 31.8mm aperture to measure the lightness (L*), redness (a*), and yellowness (b*) of each steak. In addition, the hue angle was calculated as follows: tan−1 (b*/a*), with a greater value indicative of the surface color shifting from red to yellow. Chroma (C*) was calculated as √ a*2 + b*2 where a larger value indicates a more vivid color. Lastly, reflectance values within the spectral range 400 to 700 nm were used to capture the surface color changes from red to brown by calculating the reflectance ratio of 630 nm:580 nm and the relative calculated percentages of deoxymyoglobin (%DMb = {2.375 × [1 − ({A473 − A700}/{A525 − A700})]} × 100), metmyoglobin (%MMb = {[1.395 − ({A572 − A700}/{A525 − A700})]} × 100) and oxymyoglobin (%OMb = 100 – (%MMb + %DMb). Surface color measurement was conducted according to American Meat Science Association (AMSA) Meat Color Measurement Guidelines [12].

2.4 Statistical Analysis

Data was analyzed as a completely randomized design using the GLIMMIX model procedures of SAS (version 9.2; SAS Inst., Cary, NC, USA). Least square means were generated and significant ($\alpha = 0.05$) F-values were separated using a pair-wise t-test (PDIFF option).

3. Results and Discussion

3.1 Instrumental Color

Subprimals were aged for 21 days, cut into steaks, and subsequently stored frozen for 7 days before collecting objective color measurements. Surface color measured over the period of 32 hours indicated there was no interaction ($p > 0.05$) for packaging treatment × bloom time on beef steaks. Nonetheless, steak surface color became lighter

($p < 0.05$) with-in 4 hours of removing steaks from frozen storage temperatures (Table 2). Additionally, steak redness was darker initially (0 hour) but with increasing time surface redness increased ($p < 0.05$). Similar results were reported in a previous study evaluating blooming using longissimus lumborum indicating lightness (L*) values increase after 5 hours in retail display exposure [13]. Current results agree with previous studies noting similar objective color development observations on fresh beef semimembranosus displaying surface color changes occurring within increasing time [3]. Furthermore, additional results agree with the current results noting increases in lightness, redness, and yellowness of aged beef steaks, suggesting the blooming ability of vacuum packaged beef steaks can occur [14,15]. Surface color changes in red meat such as beef have been well documented to influence consumer purchase decisions. Such decisions based on surface color are often related to retail cuts frequently packaged in oxygen permeable films. It is plausible that the changes in partial pressure within a vacuum package after freezing and thawing can alter the surface color of the steaks whereby causing color values to increase.

Steaks packaged in treatment films TA and TB were lighter ($p < 0.05$), less red and appeared less yellow after removal from frozen storage temperatures (Table 3). However, steaks packaged in TC appeared darker, redder ($p < 0.05$) more yellow than steaks pack-aged in TA or TB packaging films. Previous objective color results agree with the current results, where surface color differences did not occur across time of exposure in these parameters [16]. Additionally, previous studies have reported that vacuum packaging film thickness did not alter fresh meat surface color during the first 24 hours [17]. Nevertheless, limited documentation throughout the literature on blooming duration from frozen to refrigerated temperatures exist. Current results suggest that

barrier properties within packaging films are instrumental in the changes of surface color that occur in vacuum packaged beef steaks.

There was no interactive impact ($p > 0.05$) of time and packaging film on the calculated relative spectral values for hue angle, red-to-brown (RTB), and chroma (C*). Mean values of calculated spectral values are presented in table 4. Steak surface color became more ($p < 0.05$) vivid (C*) with increase bloom time during refrigerated storage. Chroma variations observed are consistent with results reported in previous studies where filets presented the same trend for more vivid surface color was observed at the beginning of the evaluation [17]. However, in another study that evaluated the influence of vacuum pack-aging on blooming in *Longissimus thoracis* steaks hue angle values differed throughout the display, whereas red-to-brown color values did not differ [18]. Increases in spectral values of surface color suggest that steak surface color was dark during frozen storage, but surface color bloomed and became redder when storage temperature increased. Interestingly, thermoforming packaging can influence in surface color on steaks from frozen to fresh temperatures [19], surfaces redness can be maintained in the thicker packaging during retail period.

Furthermore, calculated relative spectral values of myoglobin forms differed ($p < 0.05$) as bloom time increased (Table 4). Declining metmyoglobin (MMb) and oxymyoglobin (OMb) values with increasing time is similar to previous studies that evaluated longissimus muscle of lamb where the transition between frozen to thawed (i.e. fresh) resulted in diminished MMb and OMb values [20]. However, as expected, deoxymyoglobin values (DMb) increased with storage time, and this is consistent with previous studies reporting an increase in DMb for lamb steaks stored in vacuum [21]. Furthermore, another study using longissimus thoracis steaks from Nellore and Aberdeen Angus reported similar changes in myoglobin redox forms that are consistent with our

current results [22]. Information regarding the bloom of steaks moving from frozen temperatures to refrigerated temperatures is limited throughout the literature. However, the limited previous literature that does exist supports the current findings of bloom development in vacuum packaged beef. Unlike alternative retail packaging methods such as modified atmosphere or breathable polyvinyl chloride overwrap, vacuum packaging in combination with colder storage temperatures can create a redder surface color with less surface discoloration than red meats stored in warmer temperatures [18].

Relative spectral values for packaging treatments did not differ ($p > 0.05$) apart from vividness (C*) and OMb presented in table 5. Current results agree with earlier studies that evaluated veal cuts using film-wrapped and vacuum packages which demonstrated that the thinner the packaging films, the better the chance of presenting a more striking color in a shorter time compared to thicker packaging [23]. However, additional findings indicate that using oxygen-impermeable films for storing frozen beef might offer benefits to instead meat quality [24].

Interestingly, it should be noted that throughout the 32 hours of bloom from frozen to fresh display, packaging treatments did not affect hue angle, red-to-brown, MMb, or DMb values ($p > 0.05$). These results differ from previous literature evaluating vacuum packaging films that can cause meat color parameters to change over time [25]. Mitochondrial oxygen consumption has previously been linked to redox change leading to the conversion of MMb and OMb to DMb [26]. However, current results agree with previous research utilizing different cuts of fresh chevon, suggesting that the calculated spectral values do not tend to undergo major changes in the first hours of exposure to atmospheric gases such as oxygen though they are inconsistent with, another study which reported myoglobin redox forms did not differ throughout the first 48 hours of display

[27, 28]. Such reported differences highlight the need for continued research focusing upon the impact of vacuum packaging on blooming during the transition of frozen to fresh storage of meat and points to the likelihood that differences exist in the utility of vacuum packaging related to species and breed from which the meat product is derived.

4. Conclusions

Vacuum packaging and storage temperatures can influence the blooming process of beef steaks regardless of aging (>22 days). Surface color, specifically redness increased as storage temperature and duration increased. Herein, instrumental color measurements were monitored for a retail display period of 32 hours to evaluate blooming evolution over a typical commercial setting. However, no decline in bloom on the surface color of the steaks was observed during this period. Therefore, additional studies should be directed at extending the duration of surface color bloom time measurements that may alter red meat color using thermoforming vacuum packaging for retail cuts.

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TABLES

Table 1. Influence of bloom time on instrumental surface color blooming of beef steaks.

Trt. [3]	Components	OTR [1]	VPR [2]
TA	250 µ nylon/EVOH/enhanced polyethylene coextrusion	0.1 cc/sq. m/24 h	2.5 g/sq. m/24 h
TB	250 µ nylon/EVOH/enhanced polyethylene coextrusion	0.1 cc/sq. m/24 h	2.0 g/sq. m/24 h
TC	125 µ nylon/EVOH/enhanced/ polyethylene coextrusion	0.6 cc/sq. m/24 h	4.9 g/sq. m/24 h
NF[4]	110 µ nylon/EVOH/enhanced/ polyethylene coextrusion	0.7 cc/sq. m/24 h	6.0 g/sq. m/24 h

Table 2. Influence of bloom time on instrumental surface color blooming of beef steaks.

	Time (Hour)									
Objective Color [1]	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**28**	**32**	**SEM**
Lightness (L*)	37.08^{b}	37.20^{b}	41.62^{a}	42.18^{a}	42.64^{a}	42.46^{a}	42.46^{a}	42.07^{a}	41.80^{a}	0.472
Redness (a*)	12.34^{e}	14.58^{d}	14.96^{d}	15.97^{c}	16.75^{b}	18.17^{a}	18.17^{a}	18.48^{a}	18.73^{a}	0.281
Yellowness (b*)	9.32^{c}	11.47^{ab}	11.28^{b}	12.00^{a}	11.94^{a}	11.41^{ab}	11.41^{ab}	11.41^{ab}	11.51^{ab}	0.224

[1] Objective Color: L* values are a measure of darkness to lightness (larger value indicates a lighter color); a* values are a measure of redness (larger value indicates a redder color); and b* values are a measure of yellowness (larger value indicates a more yellow color). $^{a-e}$ Means within a row lacking a common letter differ ($p < 0.05$). SEM: Standard error of the mean.

Table 3. Influence of packaging film treatments on instrumental surface color blooming of beef steaks.

	Packaging Treatments[1]			
Objective Color[2]	**TA**	**TB**	**TC**	**SEM***
Lightness (L*)	41.45[a]	41.28[a]	40.44[b]	0.385
Redness (a*)	16.12[b]	16.20[b]	17.06[a]	0.230
Yellowness (b*)	10.99[b]	11.10[b]	11.83[a]	0.183

[1] Packaging treatments: TA (250µ nylon/EVOH/enhanced polyethylene coextrusion), TB (250 µ nylon/EVOH/enhanced polyethylene coextrusion), and TC (125µ nylon/EVOH/enhanced/polyethylene coextrusion). [2] Objective Color: L* values are a measure of darkness to lightness (larger value indicates a lighter color); a* values are a measure of redness (larger value indicates a redder color); and b* values are a measure of yellowness (larger value indicates a more yellow color). [a–b] Mean values within a row lacking common superscripts differ ($p < 0.05$). * SEM, Standard error of the mean.

Table 4. Influence of bloom time for calculated relative spectral values on beef steaks.

	Time									
Spectral Values[1]	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**28**	**32**	**SEM**
C*	15.54^{d}	18.66^{c}	18.86^{c}	20.14^{b}	20.72^{b}	21.55^{a}	21.55^{a}	21.85^{a}	22.11^{a}	0.218
Hue Angle (°)	37.0^{ab}	38.37^{a}	37.17^{ab}	37.25^{ab}	35.6^{b}	32.30^{c}	32.30^{c}	31.87^{c}	31.68^{c}	0.813
RTB	1.71^{e}	2.01^{d}	2.01^{d}	2.12^{d}	2.73^{ab}	2.84^{a}	2.48^{c}	2.70^{abc}	2.57^{bc}	0.083
MMb (%)	42.25^{a}	40.75^{a}	37.11^{b}	34.35^{b}	30.42^{c}	28.07^{cd}	24.59^{de}	24.00^{e}	22.91^{e}	1.259
DMb (%)	26.90^{f}	36.08^{e}	46.68^{d}	48.81^{d}	54.25^{c}	57.34^{bc}	61.30^{ab}	63.96^{a}	66.04^{a}	1.839
OMb (%)	30.85^{a}	23.18^{b}	16.21^{dc}	16.84^{c}	15.34^{cde}	14.59^{de}	14.11^{e}	12.04^{f}	11.05^{f}	0.684

[1]Spectral Values: Chroma (C*), is a measure of total color where a larger number indicates a more vivid color; Hue angle (°), represents the change from the true red axis where a larger number indicated a greater shift from red to yellow; Red-to-brown (RTB), calculated as 630 nm reflectance / 580 nm reflectance which represents a change in the color of red to brown (larger value indicates a redder color); Calculated percentages of metmyoglobin (MMb), deoxymyoglobin (DMb), oxymyoglobin (OMb) using relative spectral values. $^{a–f}$ Mean values within a row lacking common superscripts differ ($p < 0.05$). * SEM, Standard error of the mean.

Table 5. The influence of packaging treatment of calculated spectral values on beef steaks.

	Packaging Treatments[1]			
Spectral Values[2]	**TA**	**TB**	**TC**	**SEM**
C*	19.64[b]	19.74[b]	20.95[a]	0.178
Hue (°)	34.62	34.72	35.19	0.664
RTB	2.30	2.38	2.38	0.068
MMb (%)	31.78	31.62	31.42	1.028
DMb (%)	51.64	50.39	51.75	1.501
OMb (%)	16.58[b]	17.99[a]	16.83[b]	0.559

[1] Packaging treatments: TA (250 µ nylon/EVOH/enhanced polyethylene coextrusion), TB (250 µ nylon/EVOH/enhanced polyethylene coextrusion), and TC (125 µ nylon/EVOH/enhanced/polyethylene coextrusion). [2] Spectral values: Chroma (C*), is a measure of total color where a larger number indicates a more vivid color; Hue angle (°), represents the change from the true red axis where a larger number indicated a greater shift from red to yellow; Red-to-brown (RTB), calculated as 630 nm reflectance / 580 nm reflectance which represents a change in the color of red to brown (larger value indicates a redder color); Calculated percentages of metmyoglobin (MMb), deoxymyoglobin (DMb), oxymyoglobin (OMb) using relative spectral values. [a–b] Mean values within a row lacking common superscripts differ ($p < 0.05$). * SEM, Standard error of the mean.

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CHAPTER III

Extended Storage of Beef Steaks Using Thermoforming Vacuum Packaging

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Abstract:

Extended storage duration often results in negative quality attributes of fresh or frozen beef steaks. This study focused on evaluating the fresh and cooked meat quality of beef steaks stored using vacuum packaging for 63 days. Steaks 2.54 cm thick were packaged into one of three thermoforming films VPA (250 µ nylon/EVOH/enhanced polyethylene coextrusion), VPB (250 µ nylon/EVOH/enhanced polyethylene coextrusion), or VPC (125 µ nylon/EVOH/enhanced/ polyethylene coextrusion). Steaks placed in VPA were lighter (L*) and redder (a*) in surface color ($p < 0.05$) as the display period increased, whereas steaks packaged in VPB and VPC became darker. Yellowness, hue angle (Hue◦), and chroma (C*) values were greater ($p < 0.05$) in steaks using VPC film as the storage period increased. Calculated spectral values of red to brown were greater ($p < 0.05$) for steaks in VPA and VPB than in VPC. However, steaks placed in VPC films contained greater ($p < 0.05$) forms of metmyoglobin and oxymyoglobin and lower calculated relative values of deoxymyoglobin. In addition, packaging treatment altered ($p > 0.05$) lipid oxidation, but storage time had a greater ($p < 0.05$) influence on purge loss, cook loss, and Warner-Bratzler shear force (WBSF). Current results suggest that the use of vacuum packaging for extended storage of beef steaks (>60) days is plausible.

Keywords: Color; cook loss, lipid oxidation; storage life; vacuum packaging; warner-bratzler shear force.

1. Introduction

Packaging is a fundamental part of the food industry that is used to create a product that is not only functional but also convenient for the consumer. Vacuum packaging for fresh meat throughout the various segments of the meat and food industry is increasingly popular in the United States and is under continual innovation [1]. There is a need for centralized packaging methods to increase demand for greater quality and safety of meat cuts for the consumer [2]. Using vacuum packaging requires the placement of beef cuts into plastic bags or pouches and evacuating the atmosphere from within the package. Vacuum packaging can increase the storage life of meat and reduce retail losses, enhance distribution, and maintain meat quality [3].

Thermoforming packaging utilizes heat and pressure to mold a pouch inline using plastic film. After filling the pouch with fresh or cooked meat, a second layer is applied by voiding the atmosphere of the package and sealing with heat. Conventional packaging methods for retail use consisting of polyvinyl chloride (PVC) film and an expanded polystyrene tray have declined in use by almost 46% [1]. Using permeable films for fresh meat, such as PVC, results in greater exposure of the meat surface to detrimental gases, such as oxygen. Plastic films used in thermoforming applications can limit the transmission rate of atmospheric gases to the meat surface and lengthen the stability of surface color on fresh meat products [4].

Meat quality greatly influences the marketability of beef, and research continues to highlight surface color as a factor that consumers continue to use in determining freshness and safety at the time of purchase [3]. Consumers associate and prefer the bright cherry-red color of fresh beef, in contrast to a purplish-red color linked to vacuum-packaged meat as an indicator of wholesomeness [5]. Preferences for a desired red surface color have led to discarding meat that does not meet this parameter and does not guarantee its

marketability [6]. It is well known throughout the literature that altering the color of the surface of meat causes profound consumer rejections of the meat at the retail counter [7]. Industry methods have been adopted to minimize this effect, such as controlling the age of fresh beef through packaging and maintaining refrigeration standards that may alter meat characteristics during storage [8]. With advances in technologies, vacuum packaging has caused improvements in the surface color of the meat by maintaining a brighter red surface color for longer periods [4].

Water, as one of the primary components of meat, can be greatly altered by refrigerated storage temperatures, packaging methods, and storage duration [9]. Moisture losses occurring throughout the many phases of meat logistics from farm to consumer have been linked to negative changes in cooking yields, organoleptic properties, and even objective tenderness measurements [10]. Cooking and storing meat can cause tremendous losses of moisture in the meat, ultimately reducing the fragmentation of muscle proteins [10].

Guidelines for cookery and color evaluation highlight a myriad of methodologies for measuring meat quality attributes [11,12]. However, there are no specific guidelines or best practices in storing meat apart from refrigeration for protecting consumer food and meat products [13]. Storing meat products for extended periods has influenced objective tenderness values through moisture loss or degradation of myofibrillar proteins [14–16]. Improvements in packaging technologies can enhance the rate at which fresh meat characteristics change during periods of storage prior to consumption.

Therefore, the objective of this study was to evaluate the influence of thermoforming vacuum packaging on the fresh and cooked characteristics of beef steaks after wet aging for 21 days.

2. Materials and Methods

2.1 Muscle Fabrication

Beef boneless ribeye rolls (Institutional Meat Purchasing Specifications No. 112A) were purchased from a commercial meat processor and transported to the Auburn University Lambert-Powell Meat Laboratory and placed in refrigerated (2 ◦C ± 1.25 ◦C) storage (Model LEH0630, Larkin, Stone Mountain, GA, USA). Following 21 days of wet aging, ribeye rolls were removed from their individual vacuum packaging and fabricated. Ribeye rolls (N = 20) were cut to obtain 12 beef steaks 2.54 cm thick using a BIRO bandsaw (Model 334, Biro Manufacturing Company, Marblehead, OH, USA). Steaks from each ribeye roll were allocated randomly to one of three packaging treatments. On days 0, 7, 14, 21, 28, 35, and 42, steaks were removed from the refrigerated display case and measured for instrumental color, lipid oxidation, purge loss, cook loss, and Warner-Bratzler shear force.

2.2 Packaging Treatments

After cutting, beef steaks (n = 80/treatment) were allowed to bloom to simulate an industry steak cutting application for 30 min at 2 ◦C (±1.25 ◦C). After bloom time, each steak was packaged individually into an assigned packaging film using a Variovac Optimus system (OL0924, Variovac, Zarrentin am Schaalsee, Germany). Beef steaks were placed in one of three different thermoforming packaging films—VPA, VPB, or VPC—and sealed with a non-forming layer (NFL) using commercial packaging guidelines (WINPAK, Winnipeg, MB, Canada). Packaging film components, oxygen transmission (OTR), and vapor transmission rates (VPR) of the packaging treatments are presented in Table 1.

2.3 Simulated Storage Periods

Initially, packaged steaks were stored frozen in the absence of light at −20 °C (±1.50 °C) for seven days to simulate frozen distribution from manufacturer to retailer at the Auburn University Lambert-Powell Meat Laboratory. Steaks were placed into cardboard boxes, sealed shut, and stored in a blast freezer (Model LHE6950, Larkin, Stone Mountain, GA, USA). After frozen dark storage, steaks were placed into a refrigerated, multi-deck, lighted display case Avantco (Model 178GDC49HCB, Turbo Air Inc., Long Beach, CA, USA) operating at 3.0 °C ± 1.5 °C. Thawed steaks were displayed under constant light for 42 days. The lighting within the retail case consisted of cool LED strips (TOM-600-12-v4-3, Philips Xitanium 40 W–75 W, Korea) with a lighting intensity of 2297 lux (ILT10C, International Light Technologies, Peabody, MA, USA).

2.4 Instrumental Color

Instrumental color readings were measured with a HunterLab MiniScan EZ colorimeter, Model 45/0 LAV (Hunter Associates Laboratory Inc., Reston, WV, USA) according to American Meat Science Association (AMSA) Meat Color Measurement Guidelines [17] as described previously by this laboratory [8].

2.5 Lipid Oxidation

Steaks were sampled for 2-thiobarbituric acid reactive substances (TBARS) as previously described [4,18], and mg of malonaldehyde/kg of fresh meat was calculated by using the value of 12.21 obtained from a standard curve using a known malonaldehyde solution measured across multiple absorbencies [18].

2.6 Purge Loss

Purge loss reduces the weight of a product, is unappealing for many consumers in retail packaging, and ultimately decreases purchase stimulation. Purge loss was collected on fresh (thawed) steaks throughout the refrigerated storage period of 42 days. Steaks were removed from their packaging treatment, blotted dry with a paper towel, and weighed on a balance (Model PB3002-S, Mettler Toledo, Columbus, OH, USA). Purge loss calculations were performed using [(packaged weight − steak weight) ÷ packaged weight × 100].

2.7 Cook Loss and Warner-Bratzler Shear Force

Steaks were removed from packages, excess moisture was blotted dry with a paper towel, and then steaks were weighed (initial weight). Steaks were cooked in a convection oven (Vulcan, Baltimore, MD, USA) preheated to 177 °C until the internal temperature of each steak reached 70 °C. Internal steak temperature was monitored with a data logging thermometer (Therma K-Plus, American Fork, UT, USA). Cooked steaks were cooled to room temperature, and final weights were recorded. Cook loss percentages were calculated as follows: [(weight of raw meat samples − post-cook weight of samples) ÷ weight of raw meat samples × 100].

Objective tenderness was measured using Warner-Bratzler shear force (WBSF) with a texture analyzer (Model TA-XT Icon, Texture Technologies Corp., New York, NY, USA). A load cell of 294 N and a crosshead speed of 50 mm/min sheared each core once. Seven cores of 1.27 cm were removed parallel to the muscle fiber from each steak and each core was sheared perpendicular to the fiber direction using previously described methods [11]. The maximum peak force recorded during analysis was reported as Newton (N) of shear force.

2.8 Statistical Analysis

The current study was conducted and analyzed as a completely randomized design. Data were analyzed using the GLIMMIX model procedures of SAS (version 9.2; SAS Inst., Cary, NC, USA). Treatment served as the fixed effect, and replication as the lone random effect for meat characteristics instrumental color, lipid oxidation, purge loss, cook loss, and WBSF. Least square means were generated, and significant ($\alpha = 0.05$) F-values were separated using a pair-wise t-test (PDIFF option).

3. Results and Discussion

3.1 Instrumental Color

Sub-primals in this experiment were wet aged for 21 days before being fabricated into steaks and displayed in multi-deck cases for 42 days. Limited research on extended storage (>60 days) of fresh beef is available in the literature; therefore, this was a novel opportunity to evaluate changes in fresh meat color over long storage periods. Anticipating large changes in myoglobin state in these long-stored meat products, instrumental color readings were used to measure the surface color changes between different pigment forms [19]. There was an interactive impact ($p < 0.05$) of the packaging method and storage period on the fresh surface color (Table 2).

From day 0 to 42, steaks packaged in VPC packaging film were darker ($p < 0.05$) than beef steaks packaged using VPA or VPB (Table 2). Regardless of packaging treatment, L* values initially increased ($p < 0.05$) through day 21. However, as the duration of storage increased, steaks in VPC became darker. Lightness is a characteristic of fresh meat as it blooms, and during lighted display and limited oxygen conditions, oxymyoglobin formation can be altered [20]. An increase in L* using VPA and VPB films is likely the result of film thickness limiting oxygenation of myoglobin and

mitochondria resulting in more light scattering on the surface of the steak. Similar changes in lightness were reported in previous studies using vacuum-packaged ground beef over a 14-day simulated display period [20,21]. However, previous literature on the storage of vacuum-packaged whole-muscle cuts after extended wet aging and subsequent fresh storage is limited.

An interaction between packaging film and storage day for objective redness values occurred (Table 2). Steaks were redder ($p < 0.05$) after day 35 of storage when using VPA and VPB consisting of greater barrier properties and a concentration of OMB on the surface of the steaks. However, there were some similarities ($p > 0.05$) among packaging films for redness values from day 0 to 28 of the storage period. Steaks packaged in VPC were less red ($p < 0.05$) and became more yellow ($p < 0.05$) as storage time increased. Similar findings were reported when using vacuum packaging to store foal meat for 14 days in retail display cases [22]. Additionally, the current results tend to agree with others that have evaluated retail color characteristics of vacuum-packaged longissimus lumborum and noted an increase in redness (a*) and yellowness (b*) values over retail storage [23].

As expected, hue angle values lacked considerable differences ($p > 0.05$) among all packaging films through the first 21 days of storage (Table 3). However, by day 28 until 42 of the study, steaks packaged in VPC were further ($p > 0.05$) from the true red axis suggesting surface color deterioration was occurring. Surface vividness (C*) was greater ($p < 0.05$) for steaks packaged in VPC than either in VPA or VPB. The changes in vividness suggest that the film thickness in VPA and VPB reduced the rate at which atmospheric gases, such as oxygen, could pass through the film to the surface of the steak and alter the percentage of OMB. It should be noted that current results are similar to

previous work on vacuum-packaged beef loins, suggesting that hue angle and vividness stability values deteriorated after peaking during storage [24].

Red-brown ratios (RTB) were calculated from objective measurements of spectral reflectance from 400 to 700 nm. An interaction ($p < 0.05$) for packaging method × day of display is presented in Table 3. Initially (day 0), regardless of the packaging film, RTB values did not differ ($p > 0.05$). However, by day 35, steaks packaged in VPC had a browner surface color ($p < 0.05$). Red-to-brown ratios for beef steaks using VPB packaging film showed a greater color shift ($p < 0.05$) in contrast with the steaks packaged in VPA and VPC films. Furthermore, during the display period, red-to-brown values declined after day 28 (peak) as steaks shifted from a redder to browner surface color. Previous studies have reported similar color shifting of calculated values regardless of packaging method, and it is reasonable that the shift from red to brown is a function of greater metmyoglobin formation throughout the retail display period [4]. It is not surprising that calculated spectral values for instrumental surface color in fresh beef meat are expected to shift from red to brown as the exposure time to atmospheric gases increases. Changes in RTB appear to be related to packaging thickness and the volume of oxygen exposure over time on the surface of the meat.

Calculated relative values of metmyoglobin (MMb) were lower ($p < 0.05$) from day 21 to 42 when using VPA and VPB films (Table 4), whereas steaks packaged in VPC appeared to have a greater ($p < 0.05$) percentage of MMb measured objectively on the surface from day 7 to 42. Calculated relative values suggest the increase in metmyoglobin formation is associated with the oxygen transmission rate that occurred but was not measured throughout the storage period. Previous studies have mentioned that a cause of MMb formation can be accelerated by water loss and heme concentration, but fresh meat in a properly packaged condition should not discolor because of the purge [5].

As the term suggests, deoxymyoglobin can be associated with muscle foods that are not exposed to oxygen, and this myoglobin form can be identified either in vacuum packaged meat or within the interior of freshly cut meat [24]. Similar to relative MMb values, DMb values in beef steaks packaged using VPA and VPB vacuum-packaged film were greater ($p < 0.05$) than values calculated for steaks in VPC (Table 4). As expected, when using relative values to calculate myoglobin forms of muscle foods, as one form increases (i.e., MMb or OMb) the other forms should decline. Current results tend to agree with results reported on beef steaks using polyvinyl chloride (PVC) overwrap exposed to 35 days in retail display, where the DMb formation was less overall but also increased over time [25].

It is well known that gases, particularly oxygen, from within the atmosphere can react with meat pigments to form a bright red color in contrast to darker purple or brown colors that lack vividness. Calculated OMb values were greater ($p < 0.05$) and declined throughout the entire storage period (Table 4). However, from day 0 to 42, the greatest ($p < 0.05$) decline in relative values of OMb occurred in steaks packaged in VPC. It is quite possible that the changes in relative myoglobin values, especially OMb, are associated with the oxygen transmission rate (OTR) of each packaging film. Oxygenation is a process that occurs when myoglobin is exposed to oxygen and the development of oxymyoglobin causes a cherry-red surface color—this process is commonly referred to as bloom [25].

3.2 Lipid Oxidation

Lipid oxidation was measured through the quantification of malonaldehyde (MDA) per kilogram of fresh muscle. There was an interactive effect ($p < 0.05$) of the packaging method × day of display on the lipid oxidation of fresh beef steaks (Table 5). 2-

Thiobarbituric acid reactive substance (TBARS) values were greatest for steaks packaged in VPC on day 0 and the least ($p < 0.05$) for steaks packaged using VPB on day 14. It is well known that lipid oxidation values will increase over refrigerated storage periods in fresh and cooked meat products. Current results agree with previous findings, which show the same change in TBARS values in beef cuts aging time during display time using vacuum packaging [26]. Finally, lipid oxidation of fresh steaks using PVC packaging film was reported on days 0 and 14. Surprisingly, for the last retail display day, VPA-packaged film had a higher value in MDA than VPB and VPC films. This contradicts previous studies, which show that greater amounts of oxygen across the packaging material can result in increased catalysis of lipid oxidation [27].

3.3 Purge Loss

Measurement of purge loss is commonly reported as a percentage of the meat weight that is lost due to the fluid that is released from the tissue during retail display and is time dependent. An interaction between packaging treatments and storage duration did not occur ($p > 0.05$). Moisture loss was the greatest ($p < 0.05$) on day 42 of the storage period (Table 6). Beef steaks displayed an increasing loss of moisture during storage that may be attributed to the variation in storage temperatures that can occur as a result of display cabinet defrost cycles or operating temperatures. Similar findings were obtained using vacuum packaging methods on the shelf life of chicken where the film thickness had not influenced purge loss when samples were exposed to a stable temperature [27]. Other studies supported that using beef loin cuts to evaluate three packaging methods caused less purge loss when sub-primals were placed in vacuum packages at the end of the storage period [28].

3.4 Cook Loss and Warner-Bratzler Shear Force

During cooking, meat can lose a large proportion of its mass, which can be attributed to moisture losses prior to and during the cooking process. There was no interaction between the packaging and storage period ($p < 0.05$) for purge loss, cook loss, or WBSF. As expected, purge loss in packaged steaks increased ($p < 0.05$) with increasing storage time (Table 6), whereas cook loss was greater ($p < 0.05$) in steaks after 28 days of storage (Table 6). These shifts in moisture losses can be caused by the combination of storage time and temperature or cooking conditions, which ultimately can influence the objective tender-ness values. Results in the current study agree with previous studies reporting that moisture loss in different retail beef cuts can be altered as storage time increases [29]. Nonetheless, additional aging of meat has shown that lower cook loss can occur in beef cuts aged over 50 days [30].

Changes in moisture during storage and cooking have been well documented to alter meat tenderness. Objective tenderness can be measured via Warner-Bratzler shear force (WBSF) and is often reported in newtons (N) of force. WBSF values were the greatest in steaks on day 21 ($p < 0.05$), but steaks became more tender as storage duration increased (Table 6). Countless studies have concluded that the tenderness and juiciness of meat are affected by heat exposure, and these sensory factors can influence customer satisfaction. Past studies using aging in beef loins around 42 days reported similar trends, where WBSF decreased linearly as the aging period increased [27]. In addition, another study evaluated the tenderness properties of aging beef and concluded that the shear force would decrease when the storage time prior to cooking increased [28].

Previous literature suggests that sous vide-processed beef has more space between the muscle fibers in comparison to raw beef or boiled beef [2]. As the internal muscle temperature increases the internal space within meat becomes thinner causing connective

tissue to dissolve allowing for more space between the muscle fibers. In summary of this previous study, sous vide samples had an increase of shrinkage which coincides with the greater loss of water previously reported [2,30].

4. Conclusions

Vacuum packaging film thickness does alter the oxygen transmission rate and subsequent influences the fresh characteristics of beef steaks stored for extended periods (> 60 days). However, with improvements in vacuum packaging technologies fresh meat can appear redder through objective measurements. As expected, storage duration was a con-tributing factor that caused differences in purge loss, cook loss, and WBSF, but additional research is needed to further identify the mechanism of these changes. Future studies should be directed towards assessing the organoleptic traits of steaks stored for extended periods by eliciting consumer and trained panelist input on vacuum-packaged fresh beef steaks.

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TABLES

Table 1. Vacuum packaging specifications for thermoforming films.

Trt. [3]	Components	OTR [1]	VPR [2]
VPA	250 µ nylon/EVOH/enhanced polyethylene coextrusion	0.1 cc/sq. m/24 h	2.5 g/sq. m/24 h
VPB	250 µ nylon/EVOH/enhanced polyethylene coextrusion	0.1 cc/sq. m/24 h	2.0 g/sq. m/24 h
VPC	125 µ nylon/EVOH/enhanced/ polyethylene coextrusion	0.6 cc/sq. m/24 h	4.9 g/sq. m/24 h
NFL [4]	110 µ nylon/EVOH/enhanced/ polyethylene coextrusion	0.7 cc/sq. m/24 h	6.0 g/sq. m/24 h

[1] OTR: Oxygen transmission rates. [2] VPR: Vapor transmission rates. [3] Packaging treatments defined as (VPA, VPB, VPC). [4] NFL (Non-forming film).

Table 2. Interactive impact of packaging method × day on surface color (L*, a*, b*) values during 42 days of refrigerated storage.

Packaging Treatment[1]									
Day	**Lightness (L*)**			**Redness (a*)**			**Yellowness (b*)**		
	VPA	**VPB**	**VPC**	**VPA**	**VPB**	**VPC**	**VPA**	**VPB**	**VPC**
0	37.76^{f}	37.94^{f}	35.72^{g}	12.23^{jk}	11.72^{k}	13.09^{j}	9.04^{i}	8.92^{i}	9.99^{h}
7	42.25^{de}	45.53^{cde}	42.35^{cde}	20.72^{i}	21.49^{hi}	22.38^{g}	10.82^{g}	10.95^{g}	11.90^{f}
14	44.75^{ab}	46.29^{a}	45.10^{ab}	22.91^{fg}	22.30^{gh}	24.16^{e}	11.67^{f}	11.39^{fg}	12.98^{e}
21	46.23^{a}	45.78^{a}	44.79^{ab}	23.37^{ef}	23.58^{ef}	24.20^{e}	11.64^{f}	11.83^{f}	13.80^{d}
28	41.38^{bc}	41.03^{e}	38.44^{f}	28.15^{a}	27.91^{a}	27.52^{ab}	14.92^{bc}	15.09^{b}	17.79^{a}
35	42.79^{cd}	42.52^{cde}	38.34^{f}	27.33^{ab}	26.68^{bc}	25.24^{d}	14.25^{d}	14.25^{cd}	17.77^{a}
42	43.77^{bc}	42.97^{cd}	37.15^{fg}	26.40^{c}	25.83^{cd}	23.52^{ef}	13.76^{d}	13.70^{d}	17.64^{a}
SEM		0.577			0.315			0.243	

[1]Packaging treatments: VPA (250 µ nylon/EVOH/enhanced polyethylene coextrusion), VPB (250 µ nylon/EVOH/enhanced polyethylene coextrusion), and VPC (125 µ nylon/EVOH/enhanced/polyethylene coextrusion). L* values are a measure of darkness to lightness (larger value indicates a lighter color); a* values are a measure of redness (larger value indicates a redder color); and b* values are a measure of yellowness (larger value indicates a more yellow color). $^{a–k}$ Mean values within a color measurement lacking common superscripts differ ($p < 0.05$). SEM, Standard error of the mean.

Table 3. Interactive impact of packaging method × day on calculated spectral values during 42 days of refrigerated storage.

	Packaging Treatment[1]								
Day	**Hue Angle (°)**			**Chroma (C*)**			**Red-to-Brown (RTB)**		
	VPA	**VPB**	**VPC**	**VPA**	**VPB**	**VPC**	**VPA**	**VPB**	**VPC**
0	36.29^{a}	37.24^{a}	37.61^{a}	15.28^{n}	14.78^{n}	16.55^{m}	1.69^{k}	1.61^{k}	1.84^{k}
7	27.56^{efgh}	26.94^{efgh}	27.98^{efg}	23.40^{l}	24.14^{kl}	25.36^{ij}	3.14^{fghi}	3.34^{fg}	3.44^{ef}
14	26.94^{fgh}	27.05^{efgh}	28.23^{def}	25.72^{hij}	25.05^{jk}	27.43^{g}	3.15^{ghij}	2.97^{j}	3.22^{fghi}
21	26.45^{gh}	26.61^{gh}	26.67^{c}	26.12^{hi}	26.39^{h}	27.87^{g}	3.00^{ij}	3.10^{ghij}	3.07^{hij}
28	27.94^{efg}	28.44^{de}	32.92^{c}	31.87^{ab}	31.74^{bc}	32.79^{a}	4.16^{ab}	4.30^{a}	3.97^{bc}
35	27.52^{efgh}	28.10^{ef}	35.17^{b}	30.82^{cd}	30.25^{de}	30.88^{cd}	3.87^{cd}	3.82^{cd}	3.26^{fgh}
42	27.51^{efgh}	27.91^{efgh}	36.93^{a}	29.78^{ef}	29.24^{f}	29.43^{ef}	3.65^{de}	3.67^{de}	2.97^{j}
SEM		0.531			0.344			0.087	

[1]Packaging treatments: VPA (250 µ nylon/EVOH/enhanced polyethylene coextrusion), VPB (250 µ nylon/EVOH/enhanced polyethylene coextrusion), and VPC (125 µ nylon/EVOH/enhanced/polyethylene coextrusion). The hue angle (°) represents the change in color from the true red axis (a larger number indicates a greater shift from red to yellow). C* (Chroma) is a measure of total color (a larger number indicates a more vivid color). RTB is the reflectance ratio of 630 nm ÷ 580 nm and represents a change in the color of red to brown (a larger value indicates a redder color). $^{a–n}$ Mean values within a color measurement lacking common superscripts differ ($p < 0.05$). SEM, Standard error of the mean.

Table 4. Calculated spectral values for the interactive impact of packaging method × storage day.

Day	Packaging Treatment[1]								
	Metmyoglobin (MMb)			Deoxymyoglobin (DMb)			Oxymyoglobin (OMb)		
	VPA	VPB	VPC	VPA	VPB	VPC	VPA	VPB	VPC
0	41.5^{b}	43.60^{a}	41.32^{b}	8.97^{jk}	9.03^{k}	9.20^{j}	49.52^{b}	47.37^{c}	49.48^{b}
7	15.95^{j}	15.29^{j}	17.30^{hij}	32.12^{i}	33.45^{hi}	33.44^{fg}	51.93^{a}	51.27^{a}	49.27^{b}
14	18.08^{hi}	19.33^{fgh}	21.08^{fg}	35.67^{fg}	34.25^{gh}	34.74^{def}	46.25^{c}	46.41^{c}	44.18^{d}
21	21.21^{f}	20.92^{fg}	25.46^{e}	36.27^{ef}	36.06^{ef}	32.53^{g}	42.52^{def}	43.02^{de}	42.01^{ef}
28	16.58^{ij}	15.96^{j}	26.29^{e}	43.09^{a}	43.10^{a}	35.92^{d}	39.33^{ghi}	40.94^{fg}	37.79^{i}
35	18.56^{hi}	19.10^{gh}	32.38^{d}	43.03^{ab}	41.28^{bc}	29.73^{h}	37.96^{hi}	39.61^{hg}	37.90^{i}
42	19.30^{fgh}	20.81^{fg}	36.78^{c}	41.40^{c}	39.10^{cd}	25.26^{i}	39.30^{ghi}	40.08^{g}	37.96^{hi}
SEM	1.062			0.577			0.613		

[1]Packaging treatments: VPA (250 µ nylon/EVOH/enhanced polyethylene coextrusion), VPB (250 µ nylon/EVOH/enhanced polyethylene coextrusion), and VPC (125 µ nylon/EVOH/enhanced/polyethylene coextrusion). Relative values of metmyoglobin (MMb), deoxymyoglobin (DMb), and oxymyoglobin (OMb) using spectral values. a–k Mean values within a color measurement lacking common superscripts differ ($p < 0.05$). SEM, Standard error of the mean.

Table 5. Interactive impact of packaging method × day of display of TBARS on beef steaks during 42 days of refrigerated storage.

	Storage Day							
	0	**7**	**14**	**21**	**28**	**35**	**42**	**SEM ***
VPA [2]	0.84^{de}	0.85^{de}	0.91^{bcde}	0.86^{de}	0.92^{bcde}	0.85^{de}	0.93^{bcde}	0.103
VPB	0.91^{bcde}	0.90^{cde}	0.80^{e}	1.03^{b}	0.95^{bcd}	0.89^{cde}	0.88^{cde}	0.106
VPC	1.16^{a}	0.88^{cde}	1.01^{bc}	0.96^{bcd}	0.98^{bc}	0.84^{de}	0.88^{cde}	0.102

[1] TBARS: 2-thiobarbituric acid reactive substances are reported as mg/kg of malonaldehyde in fresh tissue. A larger value is indicative of greater oxidation. [2] Packaging treatments: VPA (250 µ nylon/EVOH/enhanced polyethylene coextrusion), VPB (250 µ nylon/EVOH/enhanced polyethylene coextrusion), and VPC (125 µ nylon/EVOH/enhanced/polyethylene coextrusion). [a–e] Mean values lacking a common superscript differ ($p < 0.05$). * SEM, Standard error of the mean.

Table 6. Effect of storage day on purge loss, cook loss, and Warner-Bratzler shear force (WBSF) of beef steaks during 42 days of refrigerated storage.

	Storage Day							
	0	**7**	**14**	**21**	**28**	**35**	**42**	**SEM ***
PL (%)	7.05^{bc}	6.41^{c}	7.03^{bc}	8.08^{a}	7.50^{ab}	8.24^{a}	8.28^{a}	0.285
CL (%)	25.79^{bcd}	22.84^{d}	23.24^{cd}	24.50^{bcd}	35.09^{a}	30.03^{abc}	30.05^{ab}	2.315
WBSF (N)	17.17^{abc}	18.25^{ab}	18.03^{abc}	19.12^{a}	13.77^{d}	15.79^{bcd}	15.33^{cd}	0.939

Purge loss is expressed in percentage (PL), cook loss is expressed in percentage (CL%), and Warner-Bratzler shear force is reported in Newton (WBSF (N)). $^{a–d}$ Mean values within a row lacking common superscripts differ ($p < 0.05$). * SEM, Standard error of the mean.

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CHAPTER IV

Vacuum Packaging can Protect Ground Beef Color and Oxidation during Cold Storage

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Abstract:

Storing ground beef at frozen temperatures prior to refrigerated display when using thermoforming vacuum packaging is not a common manufacturing practice. However, limited data on thermoforming packaging film and its interaction with meat quality suggests that more information is needed. The current study aimed to identify the influences of thermoforming packaging on the surface color and lipid oxidation of ground beef. Ground beef was portioned into 454 g bricks and packaged into one of three thermoforming films: T1 (150 µ polyethylene/EVOH/polyethylene coextrusion), T2 (175 µ polyethylene /EVOH/polyethylene coextrusion), and T3 (200 µ polyethylene/EVOH/polyethylene coextrusion), stored for 21 days at −20.83 °C (±1.50 °C), and displayed for 42 days at 3.0 °C ± 1.5 ◦C. There were no statistical differences for the packaging treatment of lipid oxidation (p = 0.0744), but oxidation increased throughout storage day (p < 0.0001). The main effects of treatment and day resulted in altered (p < 0.05) surface lightness (L*), redness (a*), yellowness, hue angle (°), red-to-brown (RTB), and relative myoglobin for met-myoglobin (MET), deoxymyoglobin (DMB), and oxymyoglobin (OMB). Surprisingly, there was an interaction between treatment and day for the calculated relative values of chroma (p = 0.0321), Delta E (p = 0.0155), and the ratio of a*:b* (p < 0.0001). These results indicate that thermoforming vacuum packaging can reduce the rate of deterioration that occurs to ground beef color and the rate of oxidation.

Keywords: Ground beef, instrumental color, lipid oxidation, storage period, vacuum packaging.

1. Introduction

Storing meat products is a vital stage in delivering protein options to the consumer within the retail or food service sector of the U.S. Seldom do manufacturers recommend freezing ground beef during logistical patterns and retail display. Frozen portioned consumer meat products will often deteriorate rapidly if stored frozen prior to refrigerated store display, altering the surface color and compromising consumer purchasing. Despite efforts to stimulate consumer demand for beef, ground beef sales are increasing at a rate of 39.2% of total dollars and representing under half the consumer purchases of retail sales in the U.S. [1-2]. It has been estimated that 53.7% of total ground beef in the retail arena is sold in poly-vinyl chloride packaging which is well-documented for causing rapid color decline and greater discarding [3]. Recently, ground beef preference has increased consumer purchase rates, and despite a 1.8% decline in pounds sold during December of 2023, ground beef is purchased more often than whole-muscle cuts [3]. Regardless of variability in fresh meat quantities in the U.S., logistical management of perishable meats for consumer products like ground beef can be difficult. No research has evaluated the com-bination of freezing, thawing, and refrigerated storage for fresh beef during retail consumer presentation.

Meat products sold within the U.S. meat industry may vary in storage duration, sur-face color, or even flavor profile. Storing meat in frozen temperatures prior to retail offering may curtail the volume of fresh ground beef that is discarded at the retail level when using creative packaging methods [4]. Unfortunately, little is known about the surface color development following frozen storage of ground beef. Every year, it is estimated that over 1.3 billion pounds of ground beef is manufactured for retail purposes, occupying more shelf space per linear foot in retail stores than any other fresh meat products [2]. Considering the versatility and widespread consumption of ground beef in

the U.S., it is often cataloged as the undisputed leader [4]. Therefore, identifying methods for extending storage duration without altering surface color both in-store and at-home for consumers of ground beef is needed.

Ground beef leads the progression of packaging technologies within the domain of red meats due to greater food flexibility, creating an easy way to justify such developments comparing meat cuts and other food products [4]. Traditionally, consumers have relied on color as the predominant signal of freshness and quality, often seeking retail packaging that highlights a vibrant cherry red shade of the surface color [5]. Unfortunately, the use of vacuum packaging methods has been limited because barrier properties of the film limits interactions of the meat surface with oxygen and conversion of surface colors that visually appear redder. However, it has been noticeable that a bright-cherry-red can be achieved as a favorable surface color of ground beef even when placed in vacuum packaging [6].

Changes to the surface color of fresh and frozen meat is dependent on the concentration of meat pigments, oxidation of these pigments and physical characteristics such as light scattering [7]. Fresh meat color can be determined by the relative behavior of the three myoglobin derivatives [8]. Certain reduced forms of myoglobin such as metmyoglobin can result in a brown color often associated with deterioration of fresh beef quality by consumers [7]. Identifying solutions to reduce meat color deterioration are of significant consideration throughout the global meat industry, as metmyoglobin concentrations exceeding 40% can exert a negative influence on consumer purchasing behavior [9]. Historically, the perceptions of fresh beef in vacuum pouches have often been perceived as purplish red color, causing consumers to seek alternative protein choices due to surface color appearing brighter red. However, improvements in plastic film construction have created new methods for packaging fresh meat using vacuum

packaging, though the foundational information regarding new packaging films on beef color is limited.

The deterioration of food quality can be attributed to lipid oxidation, which is facilitated by heme compounds [10]. A major cause of flavor deterioration in meat is the oxidation of unsaturated fatty acids [11]. Fatty acids in meat are composed mostly of triglycerides and phospholipids, which can be affected by the packaging method leading to storage stability of frozen meat [12]. A method often used to quantify lipid oxidation is the use of 2-thiobarbituric acid reactive substances (TBARS), resulting in malondialdehyde (MDA) equivalents, derived from tetraethoxypropane and identified as a by-product that occurs during the lipid oxidation process throughout storage of fresh and cooked meats [13-14].

Packaging methods for fresh meat at the point of sale are undergoing changes, primarily influenced by a shift toward centrally packaged meats and a growing consumer demand for enhanced quality, safety and convenience [15]. Vacuum-packaged meat has been conventionally employed to extend the freshness of beef over long-distance transportation and storage periods [16]. Vacuum packaging using thermoforming films involves enclosing a product in a package with or without barrier properties, then evacuating residual air to inhibit the growth of aerobic spoilage organisms, minimize shrinkage, prevent oxidation, and preserve color quality [17].

The objectives of the current study were to evaluate the lipid oxidation and surface color changes to vacuum-packaged ground beef bricks through 42 days at refrigerated storage under constant light exposure in retail cases following 21 days of frozen storage.

2. Materials and Methods

2.1 Raw Materials

Beef chuck-eye rolls (USDA Institutional Meat Purchasing Specification #116A) were purchased from a commercial meat processing facility, transported to the Auburn University Lambert-Powell Meat Laboratory under refrigerated conditions 1.5 °C ± 0.5 °C, then stored for 24 h prior to grinding and packaging. At the time of grinding, coarse meat (227.27 kg) was allocated randomly to 1 of 3 treatments (N = 64.09 kg/treatment) and coarse ground once through a 9.525 mm plate (SPECO 400, Shiller Park, IL, USA) using a commercial meat grinder (Model AFMG-48, The Biro Manufacturing Company, Marblehead, OH, USA). Three batches (n = 21.36 kg/batch) of coarse ground beef per treatment were then ground once through a 3.18 mm plate (SPECO 400, Schiller Park, IL, USA). After grinding, ground beef was portioned into 454 g bricks using a vacuum stuffer (Model-VF608plus, Handtmann, Biberach, Germany). Ground beef bricks were stored in the absence of light at frozen temperatures −20.83 °C (±1.50 °C) for 21 days to simulate the logistical transportation of U.S. ground beef. Bricks were then transferred to refrigerated temperatures 3.0 °C ± 1.5 °C for instrumental color and lipid oxidation measurements to occur on days 0, 7, 14, 21, 28, 35, and 42 during refrigerated conditions.

2.2 Packaging Treatments

The packages of ground beef (n = 47 bricks/batch) were sealed using a Variovac Optimus (OL0924, Variovac, Zarrentin am Schaalsee, Germany). Ground beef bricks were placed in one of three different thermoforming packaging films (T1, T2, and T3) and sealed with a standard non-forming layer constructed with the following parameters: 75 µ nylon/EVOH/enhanced/poloefin plastomer coextrusion with an oxygen transmission rate of 0.10 cc/sq. m/24 h and a vapor transmission rate of 4.0 g/sq. m/24 h, using

commercial vacuum packaging procedures (WINKPAK, Winnipeg, MB, Canada). Packaging film specifications for vapor (VPR) and oxygen transmission rates (OTR) are presented in Table 1. The forming parameters of packaging film were conducted using 110 ◦C ± 1.5 °C of heat and 0.650 bar of pressure, and the sealing of packages was completed using 5 bar of vacuum and 135 °C ± 1.10 °C. After packaging, ground beef brick packages were individually labelled to identify their respective treatment and batch, placed into a cardboard box, and stored in the absence of light.

2.3 Simulated Storage Periods

Ground beef bricks were stored in the absence of light at −20.83 °C (±1.50 °C) for 21 days to simulate a frozen period of distribution. Bricks were placed into cardboard boxes and stored in a blast freezer (Model LHE6950, Larkin, Stone Mountain, GA, USA). At the conclusion of frozen storage, bricks were placed into a refrigerated (Day 0), multi-deck, lighted display case (Avantco, Model 178GDC49HCB, Turbo Air Inc., Long Beach, CA, USA), operating at 3.0 °C ± 1.5 °C. After 21 days of frozen storage, ground beef bricks were exposed under constant lighting for 42 days. Lighting within the case consisted of cool LED strips (TOM-600-12-v4-3, Philips Xitanium 40 W–75 W, Seoul, Korea) with a lighting intensity of 2297 lux (ILT10C, International Light Technologies, Peabody, MA, USA).

2.4 Lipid Oxidation

Throughout the 42-days of refrigerated storage ground beef bricks were sampled (n = 5 bricks/batch/day) for 2-thiobarbituric acid reactive substances (TBARS) as previously described [18]. Briefly, duplicate 2.0 g (± 0.5) ground beef was homogenized into a uniform sample in duplicate and mixed with 8 mL of cold (1 °C) 50 mM phosphate buffer

(pH 7.0) containing 0.1% ethylenediamine-tetraacetic acid (EDTA), 0.1% n-propyl gallate, and 2 mL of trichloroacetic acid (Sig-ma-Aldrich, Saint Louis, MO, USA). Homogenized samples were filtered through a Whatman No. 1 filter paper into borosilicate glass tubes and du-plicate 2 mL aliquots of clear filtrate was transferred into 10 mL test tubes. Filtrate was mixed with 2 mL of 0.02 M 2-thiobarbituric acid reagent (BeanTown Chemical, Hudson, NH, USA) and placed into a hot water bath (100 °C) for 20 min. After the hot water bath, tubes were transferred to an ice bath for 15 min. Absorbance of each sample was measured at 533 nm with a spectrophotometer (VWR UV-1600 VWR International, Radnor, Pennsylvania, USA) and multiplied using a factor of 12.21 to derive the TBARS value (mg of malonaldehyde/kg of fresh meat). The value of 12.21 was obtained previously from a standard curve using a known malonaldehyde solution measured across multiple absorbances [18].

2.5 Instrumental Color

Instrumental color was measured with a HunterLab MiniScan EZ colorimeter, Model 45/0 LAV (Hunter Associates Laboratory Inc., Reston, WV, USA) conforming to American Meat Science Association (AMSA) Meat Color Measurement Guidelines [19]. Surface color values were collected on 36 bricks/treatment (n = 12 bricks/batch/treatment) on days 0, 7, 14, 21, 28, 35, and 42 through the packaging film. Prior to surface color readings, the colorimeter was standardized using a black and white tile covered with the packaging films to confirm instrument accuracy.

Objective color values were determined from the average of three readings per package using illuminant A, a 10° observer and a 31.88 aperture for lightness (L*), redness (a*) and yellowness (b*) of each brick. Furthermore, calculated values of hue angle (°) were determined by: tan−1 (b*/a*), and chroma (C*) was calculated using the √ a*2 +

b*2. Reflectance values from 400 to 700 nm were used to record surface color changes from red to brown using the reflectance ratio of 630nm:580nm. In addition, relative values of myoglobin redox forms such as deoxymyoglobin (%DMb = {2.375 × [1 − ({A473 − A700}/{A525 − A700})]} × 100), metmyoglobin (%MMb = {[1.395 − ({A572 − A700}/{A525 − A700})]} × 100) and oxymyoglobin (%OMb = 100 – (%MMb + %DMb) were calculated after measuring objective surface color readings using the handheld colorimeter. Delta E values indicated the total color change over a period and calculated as ΔE = [(ΔL*)2 + (Δa*)2 + (Δb*)2]1/2, in addition, ratios of a*:b* were calculated (a* ÷ b*) and indicate greater redness and less discoloration. Surface color measurements and relative calculations of color data was conducted according to American Meat Science Association (AMSA) Meat Color Measurement Guidelines [19]. Visual surface color variation for packaging treatment and day of storage are provided for reference (Figure 1).

2.6 Proximate Analysis and pH Value

Using a near-infrared (NIR) approved spectrophotometer (Food Scan™, FOSS Analytical A/S, Hilleroed, Denmark) and data processing ISIscan™ Software (version 4.8, Höganäs, Sweden), one brick per batch was measured for proximate analysis (protein, moisture, and fat). Lastly, pH values were obtained by weighing 2 g of ground beef into a plastic centrifuge tube, adding 20 mL of deionized water, and homogenizing (Kinematica CH-6010, Brinkmann Instruments, Inc., Westbury, NY, USA) for 45 s. Afterwards, pH was measured using a pH meter (ModelHI99163, Hanna Instruments, Woonsocket, RI, USA) equipped with a glass electrode. The calibration of the pH meter was completed (pH 4.0 and pH 7.0) using 2-point standard buffers (Thermo Fisher Scientific, Chelmsford, MA, USA) prior to sampling. Mean values for proximate analysis and pH of ground beef within each treatment are presented for reference (Table 2). Results

for proximate analysis are merely presented for reference support of objective surface color measurements.

2.7 Statistical Analysis

Data were analyzed as a completely randomized block design using the GLIMMIX model procedure of SAS (version 9.2; SAS Inst., Cary, NC, USA). Batch was included in the model as the random effect, and packaging treatment and day were the fixed effects. Least square means were computed for the variables, and significant ($p \leq 0.05$) F-values were separated using a pair-wise t-test (PDIFF option).

3. Results and Discussion

3.1 Instrumental Color

After frozen storage for 21 days, ground beef bricks were stored in retail display cases, and color was analyzed objectively through the surface of the packaging film every 7 days for 42 days of refrigerated storage. There was no interaction of packaging treatment × storage day for lightness ($p = 0.5925$), redness ($p = 0.0919$), or yellowness ($p = 0.8965$) on the surface of the packaged ground beef (Table 3). A lack of interaction for the objective surface color suggests that the surface color of ground beef may have been protected from deterioration through the combination of colder storage temperatures and packaging technologies such as greater barriers reducing the OTR. Main effects for the lightness of fresh ground beef may have been influenced by the exposure to illuminated display affecting oxymyoglobin formation and the properties of the packaging film.

Regardless of sampling day, ground beef stored in packaging treatments T1 and T3 was lighter ($p = 0.0116$) than ground beef packaged using T2 (Table 3), whereas redness values (a*) were greater ($p < 0.0001$) for T2 and T3. Current findings agree with previous

research, where the objective redness values were greater in ground beef using vacuum packaging, in contrast to those obtained using either modified atmosphere packaging (MAP) or overwrap [20]. Similar to lightness, ground beef surface yellowness values were greater for T1 ground beef bricks ($p < 0.0001$). Changes in the surface color of the packages are likely attributed to the relationship of oxygen with the meat product, thereby accelerating the oxidation process. Previous research on ground beef tends to differ with the current findings. When identifying packaging with high and low oxygen transmission rates, few to no statistical differences for surface yellowness have been reported [20]. Variation in objective color for packaging treatments may be accredited to the elevated oxygen transmission rates associated with the packaging films of T1 and T2, allowing for greater concentrations of oxygen to pass through the barrier levels of the film to the surface of the meat or, conversely, due to a protective effect on the surface deterioration, as seen when using T3 by limiting oxygen exposure.

Storage day greatly influenced ground beef lightness ($p < 0.0001$), with bricks appearing darkest on day 0 and lightest on day 35 (Table 3). Previous research has reported that surface lightness is greatest after only 14 days of evaluation [21]. Ground beef bricks were redder ($p < 0.0001$) on days 14 and 21, but darkest on day 0. The current results of redness agree with those previously reported using a traditional PVC package on ground beef, where the highest point in this parameter occurred at 14 days [21]. Yellowness values (b*) increased from day 14 ($p < 0.0001$), which contrasts with another study that evaluated the stability of ground beef in traditional packaging with regard to storage duration, which reported a decline on day 28 [21].

The surface redness of beef products during retail storage has been instrumental in altering consumer purchasing. The relative spectral values of redness calculated as hue angle and red-to-brown offer another resource to evaluate surface color changes that are

specific to redness. Calculated spectral redness for ground beef bricks did not result in a packaging treatment × day of storage interaction for hue angle ($p = 0.3306$) or red-to-brown ($p = 0.4393$). However, significant impacts on main effects for the packaging treatment and storage day of the ground beef did occur (Table 4).

Hue angle represents the objective progression of surface color from red to yellow, with greater angles as a measure of declining redness. Ground beef placed in T1 packaging treatment had greater hue angle values ($p < 0.0001$) than ground beef in treatments T2 or T3. It is likely that the greater hue angle for T1 can be attributed to the greater oxygen permeability of the packaging film, allowing for a greater association of oxygen with the surface of ground beef during storage. Comparable hue angles were reported in a study focused on the color of beef from mature cows during display using a high-oxygen-atmosphere package, which reported that hue angle values were greater as oxygen levels increased [22]. Additionally, throughout the storage time, hue angle was greatest ($p < 0.0001$) on day 7 and again at day 42, which agrees with previous research, in which values increased after 5 days of storage [23]. It has been documented that prolonged frozen storage leads to increased discoloration and a decrease in redness, consistent with the penetration of oxygen [22]. Current results suggest that frozen storage prior to refrigerated display contributes significantly to meat surface color changes [22]; without a doubt, more research is needed to identify these changes that occur during different storage temperature when using thermoforming vacuum packaging.

Red-to-brown measurement was obtained by calculating the ratio reflectance at 630:580 nm from the spectral values; this is also frequently used to determine the surface redness of meat. In contrast to hue angle measurements, ground beef packages in T2 and T3 were redder ($p < 0.0001$) than ground beef in T1. It appears, based on the surface color differences, that a greater percentage of oxygen was able to pass through T1 layers within

the packaging film, and it is likely that the protective barriers of T2 and T3 caused less dissociation of oxygen and preserved the red surface color. Current results suggest that ground beef packages in thermoforming appear to have a greater impact on red-to-brown than those previously reported in a study that evaluated the color stability of ground beef packaged in a low-carbon-monoxide atmosphere [23]. Storage day influences on red-to-brown values ($p < 0.0001$) increased through 14 days of storage (Table 4) and then declined through the remaining 28. This increasing action and subsequent decline contradict what has been reported previously, which is that the red-to-brown decline occurs immediately on the first day of storage [24].

Surface color vividness is a result of calculated relative spectral values and is reported as Chroma. An interaction ($p = 0.0321$) of packaging treatment × storage day occurred during the current study (Figure 2). During the evaluation period, treatment T1 demonstrated the highest saturation point by day 21, contrasted with treatment T2, which exhibited a gradual decrease in values throughout the time of evaluation. However, ground beef stored in the T3 packaging film showcased a comparatively stable development as opposed to the other treatments, particularly notable at the conclusion of the 42-day assessment period. Similar saturation index performance was observed in a study where the shelf-life and stability of ground beef packaged in a traditional overwrap for 28 days were evaluated [21].

The relative values of myoglobin forms were calculated using objective measurements obtained throughout the storage period. There was no significant interaction between packaging treatment × storage duration for metmyoglobin ($p = 0.2810$), deoxymyoglobin ($p = 0.2284$), or oxymyoglobin ($p = 0.10339$). However, main effects for each calculated relative value of myoglobin recorded from objective spectral values are presented for treatment and the days of storage in Table 5.

Packaging treatment caused an effect on the calculated relative values of myoglobin (Table 5). The packaging of ground beef in T2 and T3 resulted in less ($p < 0.0001$) calculated metmyoglobin, in contrast with treatment T1. However, packaging treatment (T3) resulted in the greatest relative value of oxymyoglobin (OMb) during storage ($p = 0.0460$). Vacuum packaging of fresh meat has been criticized for limiting surface color changes during storage. The current results of objective surface color for relative myoglobin forms indicate that OTR and VPR are influential in the transition of surface color. Packaging film T1 was constructed with fewer barrier properties associated with the altered forms of myoglobin. The altered surface color of myoglobin forms could ultimately change consumer perceptions of surface color. Specifically, previous research has revealed a markedly diminished level of metmyoglobin and deoxymyoglobin content when using vacuum packaging, but an inverse relationship with the amount of relative oxymyoglobin [25].

The day of storage also contributed to changes in the calculated relative forms of myoglobin within the packaged ground beef (Table 5). Main effects differed for metmyoglobin values and were greater upon removal from frozen storage on day 0 ($p < 0.0001$) and then declined by more than 45% through the first 7 days of refrigerated storage. Surprisingly, oxymyoglobin values were greatest ($p < 0.0001$) on day 7 of storage and remained relatively greater than expected during a prolonged storage period of 42 days. Packaging technology is improving, as noted by the estimated values of relative myoglobin, specifically oxymyoglobin. Oxymyoglobin has been historically referenced for influencing the consumer purchasing of fresh meat because it is associated with a redder surface color.

Current results suggest that packaging films such as thermoforming can protect the surface color variables and that the film OTR is influential in changes that occur to fresh

meat color. Differences in the relative values of met- and oxymyoglobin agree with previous research when using vacuum packaging methods for storing retail beef loin cuts, but current results different for deoxymyoglobin levels, which reported increasing calculated values of deoxymyoglobin during storage [26].

3.2 Calculate Relative Pigments

Delta E was calculated from objective color readings to assess the total color change that occurred on the meat surface during the storage period [19]. Calculations for relative pigments aid in supporting the traditional values of L*, a*, and b*. Because the surface color was exposed to frozen temperatures, it was possible that color deterioration could have occurred more rapidly in the current study. Delta E was used to confirm that, even if color change was minimal, as recorded by the colorimeter, the change could be visualized. There was an interactive effect for the packaging method × day of storage on calculated delta E ($p = 0.0155$; Figure 3). Ground beef packaged in T1 had greater surface color changes throughout storage than ground beef packaged in T2 and T3, an effect that may be due to the high permeability (lower OTR and VPR) that characterizes these treatments, as a high permeability tends to promote a slower color change compared to packaging with very low permeability [22]. Current findings are consistent with previous research, which has reported that the extended frozen storage of beef steaks in packaging with varying permeability levels leads to increased surface color changes, attributed to reduced metmyoglobin activity and resulting in greater discoloration over time [27]. More research needs to be conducted that evaluates the total color changes that occur during storage and the mechanisms aside from packaging film oxygen transmission rate that cause these changes.

Larger a*:b* ratios indicate more redness and less discoloration [19]. Focusing only on surface redness (a*) may impede our understanding of the overall surface color changes that are linked to the hues of red. Surface redness in fresh meat such as beef, pork, and lamb has been well supported in the literature as to their influence on consumer purchasing intent. The current use of calculated ratios for redness supports the findings in this submission that packaging barriers are instrumental in stabilizing the red hues of beef, even when altered during frozen storage. An interaction between packaging film and storage day for the ratios of a*:b* values occurred ($p < 0.0001$). Ground beef packaged in T1 had more discoloration than ground beef packaged using T2 or T3 films ($p < 0.0001$), indicating that the barrier properties of the packaging films can accelerate or stabilize surface color changes (Figure 4). Greater a*:b* ratio values can be adjudicating to the packaging permeability of atmospheric gases such as oxygen [19]. Protecting surface color during storage is paramount to ensuring consumer acceptance at the time of purchase. These results agree with previous research where the surface discoloration of beef steaks utilizing low permeability packaging increased when extended storage duration occurred [21]. A rise in the use of vacuum packaging in the U.S. at the retail counter suggests agreement with the current results that ground beef can withstand temperature and excessive storage duration [3].

3.3 Lipid Oxidation

Lipid oxidation was measured through the quantification of malonaldehyde (MDA) within the fresh ground beef throughout the storage periods. There was no interaction of treatment × day on lipid oxidation in ground beef during the current study ($p = 0.4104$, results not presented). The main effect of packaging treatment did not alter lipid oxidation ($p = 0.0744$) of the ground beef bricks (Figure 5). These results suggest

that the OTR of the vacuum packaging film plays a significant role in the inhibition of the lipid oxidation in ground beef regardless of the combined storage duration that occurred in frozen and fresh conditions. Unfortunately, lipid oxidation was not measured during the 21 days of frozen storage, but it is possible that the storage of meat products at low temperatures can reduce oxidative effects when they are protected by a package [28]. During fresh storage, packaging treatment T3 had numerically lower TBAR values during storage. This trend is likely attributed to the greater barrier properties of this film, which would be expected to reduce OTR and VPR. Current results agree with prior research on beef cuts with different packaging methods, where authors have reported that using vacuum packaging generates lesser TBAR values compared to beef cuts stored using either wrapped or CO_2 packaging methods [29]. Nevertheless, additional research is needed to identify the growth of specific microorganisms that occur during the storage of beef products stored in thermoforming packaging and the subsequent association to lipid oxidation. However, the objectives of the current research were aimed at only surface color and lipid oxidation characteristics that occurred during frozen storage prior to refrigerated storage.

The storage duration of vacuum-packaged ground beef significantly altered lipid oxidation values ($p < 0.0001$). Lipid oxidation in the bricks of ground beef were greatest on day 21 ($p < 0.0001$) and least on day 0 of the storage period (Figure 6). Interestingly, regardless of the 21-day frozen storage and the 42-day fresh combined storage duration, lipid oxidation did not exceed 1.0 mg of malonaldehyde, which has been previously linked to detectable oxidation flavors by consumers [30]. Changes in lipid oxidation likely occurred due to the combination of case lighting causing the rapid deterioration of fat in the ground beef bricks and the increased storage temperature when bricks were moved from −20 °C to 3.0 °C. In contrast, whole muscle using packaging films offering

OTR protection can likely reduce TBAR values even more than in the current study [29]. Previous research on the lipid oxidation potential of beef, chicken, and pork has indicated that raw red meats are more prone to lipid oxidation due to a greater presence of heme pigments [31]. It has also been reported that lipid oxidation values in frozen raw muscles were greater for beef compared to pork or chicken, suggesting that myoglobin acts as a major catalyst for lipid oxidation during storage [31].

Current findings for TBARS align with some studies that suggest that the oxidative behavior of beef increases from the first day of exposure to constant lighting within the retail case and grows larger as storage duration increases [9]. Likewise, extending storage periods to greater than 21 days has been associated with increased lipid oxidation in ground beef patties [32]. Nevertheless, present findings suggest that, after a combined 21 days of dark frozen storage and 42 days of fresh storage with exposure to retail case lighting, malonaldehyde (MDA) levels remain below the range detectable by consumers, as rancid taste in beef has been documented to be noticeable above 1.0 mg malonaldehyde/kg tissue [33].

4. Conclusions

Storing ground beef at frozen temperatures prior to refrigeration and fresh display when using thermoforming vacuum packaging did not cause disruption to lipid oxidation. However, storage duration demonstrated that lipid oxidation will increase, yet vacuum packaging will allow for lipid oxidation to remain within acceptable thresholds, even after 63 days of total storage. The barrier components of the packaging films can stabilize surface color attributes from rapid deterioration normally observed in aerobic packaging such as poly-vinyl chloride film or even modified atmosphere. Thermoforming is a promising new packaging platform used for consumer retail meats. However, changes to packaging film properties that alter the interaction of meat proteins with atmospheric gases, leading to changes in surface color, suggests that more results are needed. Nevertheless, more research should prioritize the investigation of microbial populations and sensory taste attributes when extended storage conditions are considered, regardless of frozen or fresh temperatures.

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TABLES

Table 1. Vacuum packaging components and treatment allocation for thermoforming and non-forming films.

Treatment	Components[1]	OTR [2]	VPR [3]
T1	150µ polyethylene/EVOH/polyethylene coextrusion	0.6 cc/sq. m/24 h	3.2 g/sq. m/24 h
T2	175µ polyethylene /EVOH/ polyethylene coextrusion	0.5 cc/sq. m/24 h	2.8 g/sq. m/24 h
T3	200µ polyethylene /EVOH/ /polyethylene coextrusion	0.4 cc/sq. m/24 h	2.4 g/sq. m/24 h

[1]Packaging treatment composition. [2]OTR: Oxygen transmission rates. [3]VPR: Vapor transmission rates.

Table 2. Relative mean values for proximate analysis and ultimate pH of ground beef.

	Packaging Treatments[1]		
	T1	**T2**	**T3**
pH	5.84	5.80	5.79
Protein (%)	22.86	22.62	22.47
Fat (%)	15.20	15.22	15.62
Moisture (%)	68.62	68.49	68.16

[1]Packaging treatments are defined as follows: T1 (150µ polyethylene/EVOH/polyethylene coextrusion), T2 (175µ polyethylene /EVOH/ polyethylene coextrusion), and T3 (200µ polyeth-ylene /EVOH/ /polyethylene coextrusion).

Table 3. Influence of packaging film treatments on instrumental surface color of ground beef.

	Surface Color Parameters[1]		
	Lightness (L*)	**Redness (a*)**	**Yellowness (b*)**
Packaging Treatment[2]			
T1	47.87^{a}	20.46^{b}	13.51^{a}
T2	47.24^{b}	21.44^{a}	12.90^{b}
T3	47.70^{a}	21.76^{a}	12.65^{b}
Storage Day[3]			
0	40.92^{Z}	20.29^{Y}	13.49^{V}
7	46.88^{Y}	21.62^{VW}	12.17^{Y}
14	48.22^{X}	22.29^{V}	12.66^{X}
21	48.91^{W}	22.02^{V}	13.06^{WX}
28	49.70^{V}	21.19^{WX}	13.10^{VW}
35	49.74^{V}	20.69^{XY}	13.20^{VW}
42	48.84^{WX}	20.44^{XY}	13.45^{VW}
p-value (Day)*	<0.0001	<0.0001	<0.0001
p-value (Treatment)**	0.0116	<0.0001	<0.0001
p-value (Day × Treatment)***	0.5925	0.0919	0.8965
SEM (Day)*	0.223	0.284	0.150
SEM (Treatment)**	0.146	0.186	0.098

[1]Surface Color Parameters: L* values are a measure of darkness to lightness (larger value indicates a lighter color); a* values are a measure of redness (larger value indicates a redder color); and b* values are a measure of yellowness (larger value indicates a more yellow color). [2]Packaging treatments are defined as follows: T1 (150µ polyethylene/EVOH/polyethylene coextrusion), T2 (175µ polyethylene /EVOH/ polyethylene coextrusion), and T3 (200µ polyethylene /EVOH/ /polyethylene coextrusion). [3]Storage Day: refers to 42-days of refrigerated storage with constant light exposure in the retail cases following 21 days of frozen storage. $^{a–b}$ Mean values within the main effect of treatment for color measurements lacking common superscripts differ ($p < 0.05$). $^{V–Z}$ Mean values within the main effect of day for color measurement lacking common superscripts differ ($p < 0.05$). p-value*: packaging treatment main effect, p-value**: effect of storage day main effect, and p-value***: packaging treatment × storage day interaction. SEM*: standard error of the mean for packaging treatment, SEM**: standard error of the mean for storage day.

Table 4. Impact of packaging film on calculated relative spectral values of ground beef.

	Calculated Relative Spectral Parameters[1]	
	Hue Angle (°)	**Red-to-Brown (RTB)**
Packaging Treatment[2]		
T1	33.61^{a}	2.59^{b}
T2	31.07^{b}	2.83^{a}
T3	30.19^{b}	2.91^{a}
Storage Day[3]		
0	33.90^{W}	2.80^{Y}
7	29.38^{Z}	3.13^{W}
14	29.61^{Z}	3.01^{WX}
21	30.67^{YZ}	2.88^{XY}
28	31.75^{XY}	2.59^{Z}
35	32.6^{WX}	2.52^{Z}
42	33.90^{W}	2.49^{Z}
p-value (Day)*	<0.0001	<0.0001
p-value (Treatment)**	<0.0001	<0.0001
p-value (Day × Treatment)***	0.3306	0.4393
SEM (Day)*	0.658	0.069
SEM (Treatment)**	0.431	0.045

[1]Calculated Relative Spectral Parameter: refers to Hue angle (°) represents the change in color from the true red axis (a larger number indicates a greater shift from red to yellow), RTB is the reflectance ratio of 630 nm ÷ 580 nm and represents a change in the color of red to brown (a larger value in-dicates a redder color). [2]TRT: packaging treatments are defined as follows: T1 (150µ polyeth-ylene/EVOH/polyethylene coextrusion), T2 (175µ polyethylene /EVOH/ polyethylene coextru-sion), and T3 (200µ polyethylene /EVOH/ /polyethylene coextrusion). [3] Storage Day: refers to 42-days of refrigerated storage with constant light exposure in the retail cases following 21 days of frozen storage. [a–b] Mean values within the main effect of treatment for color measurements lacking common superscripts differ ($p < 0.05$). [W-Z] Mean values within the main effect of day for color measurement lacking common superscripts differ ($p < 0.05$). p-value*: packaging treatment main effect, p-value**: effect of storage day main effect, and p-value***: packaging treatment × storage day interaction. SEM*: standard error of the mean for packaging treatment, SEM**: standard error of the mean for storage day.

Table 5. Calculate spectral values for the packaging treatment effect on myoglobin forms of ground beef.

	Calculated Spectral Values for Myoglobin Forms[1]		
	Metmyoglobin (MMb)	**Deoxymyoglobin (DMb)**	**Oxymyoglobin (OMb)**
Packaging Treatment[2]			
T1	24.49^{a}	29.06^{c}	46.45^{b}
T2	21.22^{b}	31.25^{b}	47.52^{a}
T3	20.00^{b}	32.72^{a}	47.28^{ab}
Storage Day[3]			
0	29.65^{U}	22.51^{Z}	47.84^{V}
7	16.30^{W}	33.26^{VW}	50.44^{U}
14	17.85^{W}	35.14^{U}	47.02^{V}
21	18.68^{W}	34.15^{UV}	47.18^{V}
28	23.00^{V}	31.81^{WX}	45.20^{W}
35	23.97^{V}	30.63^{XY}	45.40^{W}
42	23.90^{V}	29.58^{Y}	46.53^{VW}
p-value (Day)*	<0.0001	<0.0001	<0.0001
p-value (Treatment)**	<0.0001	<0.0001	0.0460
p-value (Day × Treatment)***	0.2810	0.2284	0.1033
SEM (Day)*	1.036	0.617	0.470
SEM (Treatment)**	0.678	0.404	0.308

[1]Calculated spectral values for myoglobin forms: metmyoglobin (MMb), deoxymyoglobin (DMb), and oxymyoglobin (OMb) using spectral values. [2]TRT: packaging treatments are defined as follows: T1 (150µ polyethylene/EVOH/polyethylene coextrusion), T2 (175µ polyethylene /EVOH/ polyethylene coextrusion), and T3 (200µ polyethylene /EVOH/ /polyethylene coextrusion). [3] Storage Day: refers to 42-days of refrigerated storage with constant light exposure in the retail cases following 21 days of frozen storage. [a–b] Mean values within the main effect of treatment for color measurements lacking common superscripts differ ($p < 0.05$). [U-Z] Mean values within the main effect of day for color measurement lacking common superscripts differ ($p < 0.05$). p-value*: packaging treatment effect, p-value**: effect of storage day, and p-value***: packaging treatment × storage day interaction. SEM*: standard error of the mean for packaging treatment, SEM**: standard error of the mean for storage day.

FIGURES

Figure 1. Surface color of ground beef during refrigerated retail display. Packaging treatments are defined as follows: T1 (150µ polyethylene/EVOH/polyethylene coextrusion), T2 (175µ polyethylene /EVOH/ polyethylene coextrusion), and T3 (200µ polyethylene /EVOH/ /polyethylene co-extrusion).

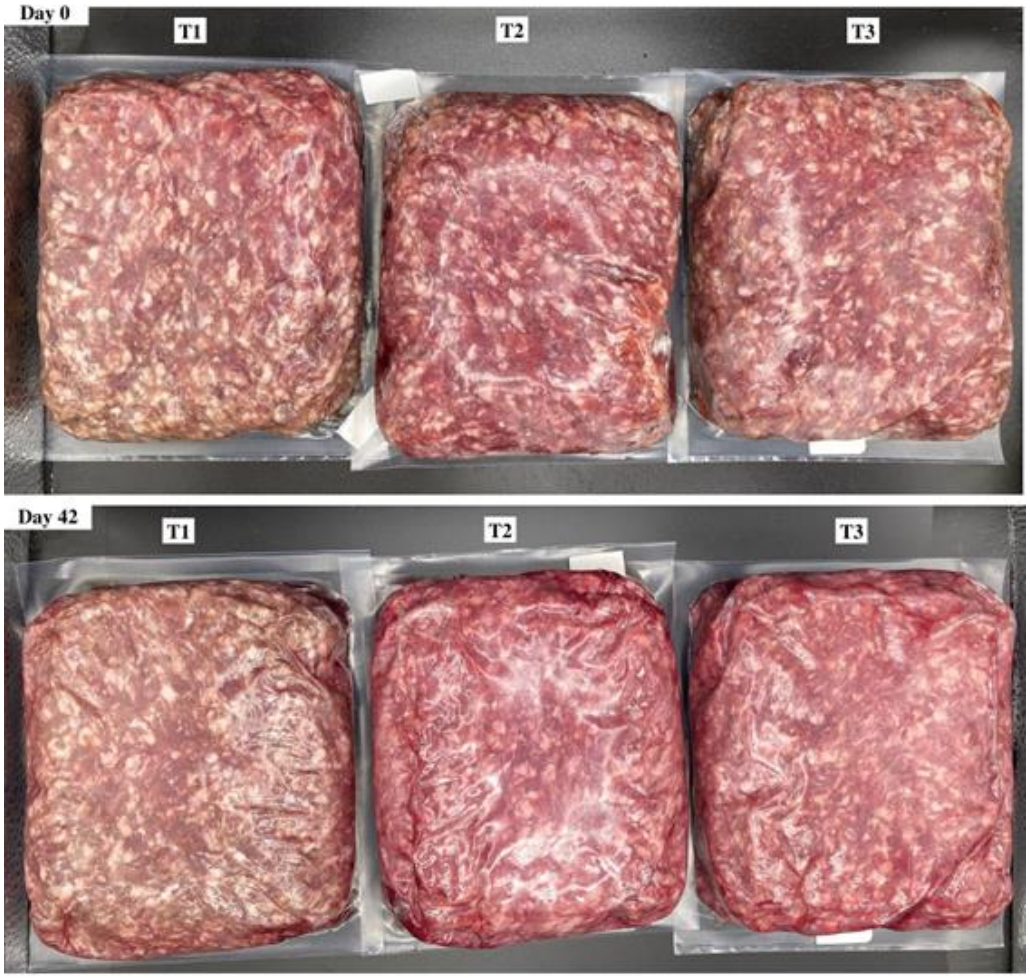


Figure 2. Calculated chroma values for the interactive impact of packaging method × storage day. C* (Chroma) is a measure of total color (a larger number indicates a more vivid color) a–f Mean values within a color measurement lacking common superscripts differ ($p < 0.05$). SEM, Standard error of the mean.

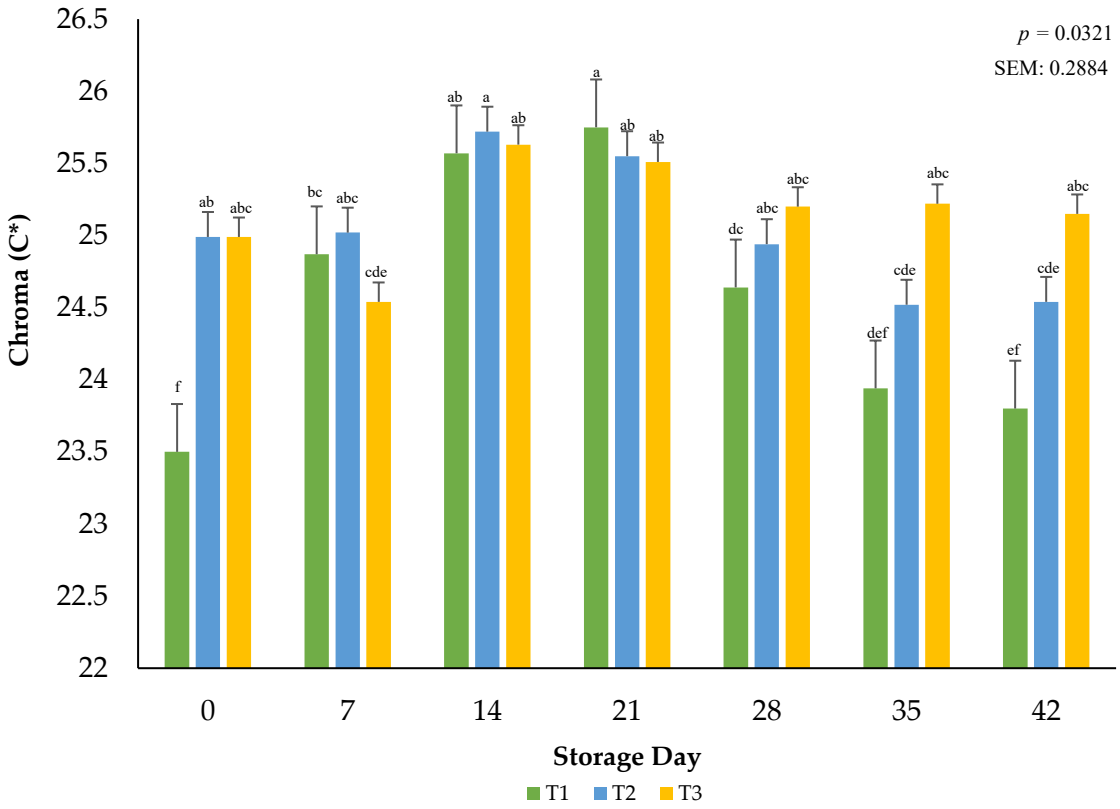


Figure 3. Calculated Delta E values for the interactive impact of packaging method × storage day. Delta E: Total color change over a selected period of time. a–k Mean values within a color measurement lacking common superscripts differ ($p < 0.05$). SEM, Standard error of the mean.

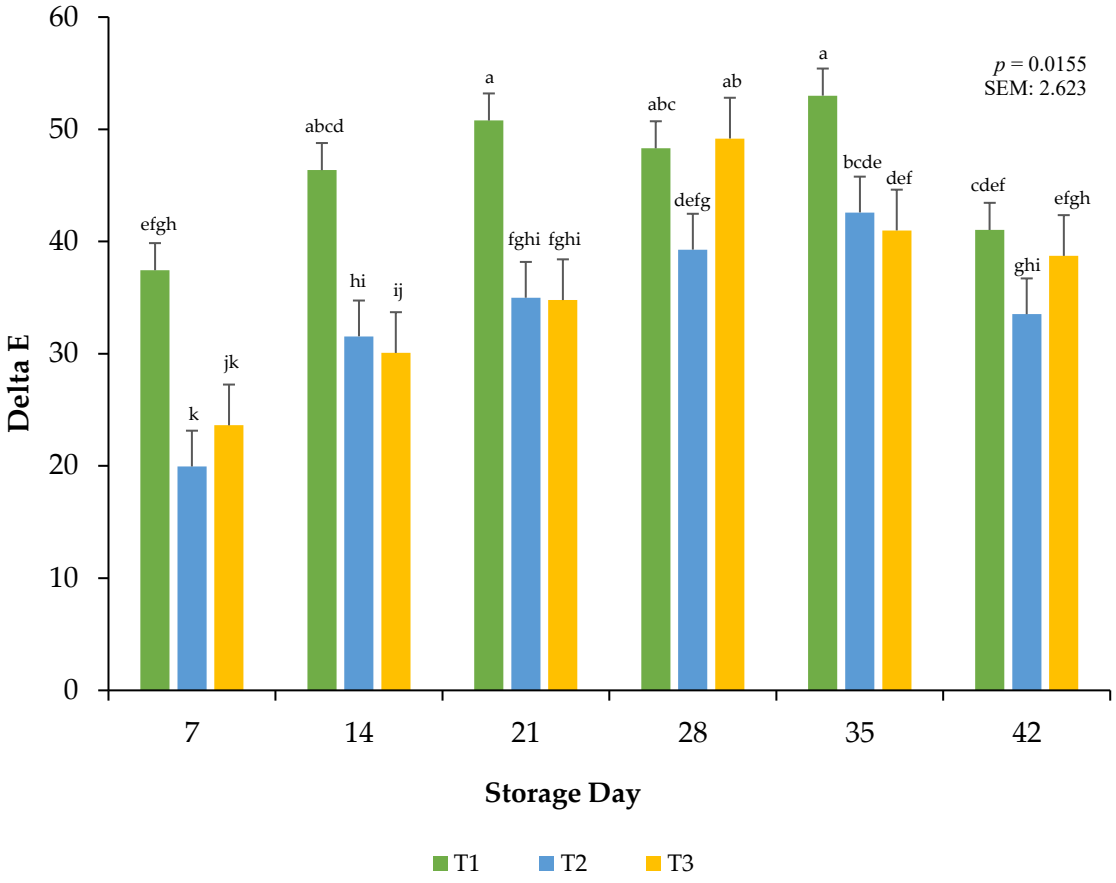


Figure 4. Calculated ratios of a*:b* for the interactive impact of packaging method × storage day. Larger ratios indicate more redness and less discoloration. [a–m] Bars lacking a common superscript differ ($p < 0.05$). SEM, Standard error of the mean.

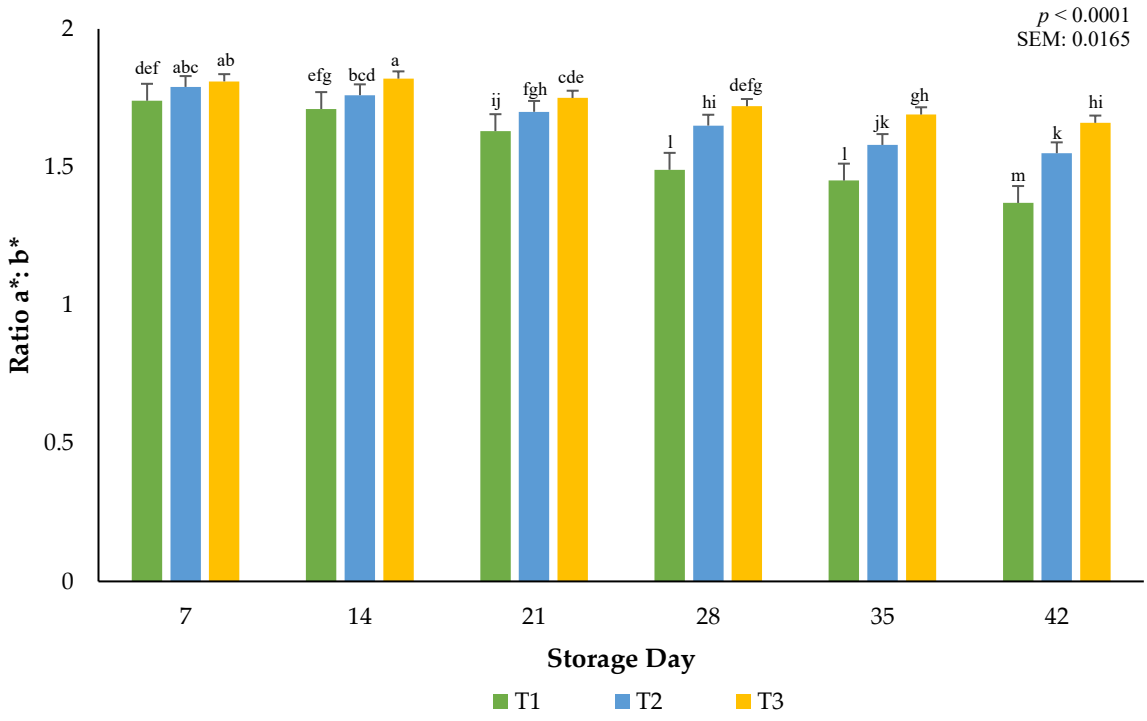


Figure 5. Influence of packaging film treatments on lipid oxidation values of ground beef bricks. Packaging treatments are defined as follows: T1 (150µ polyethylene/EVOH/polyethylene coextrusion), T2 (175µ polyethylene /EVOH/ polyethylene coextrusion), and T3 (200µ polyethylene /EVOH/ /polyethylene coextrusion).

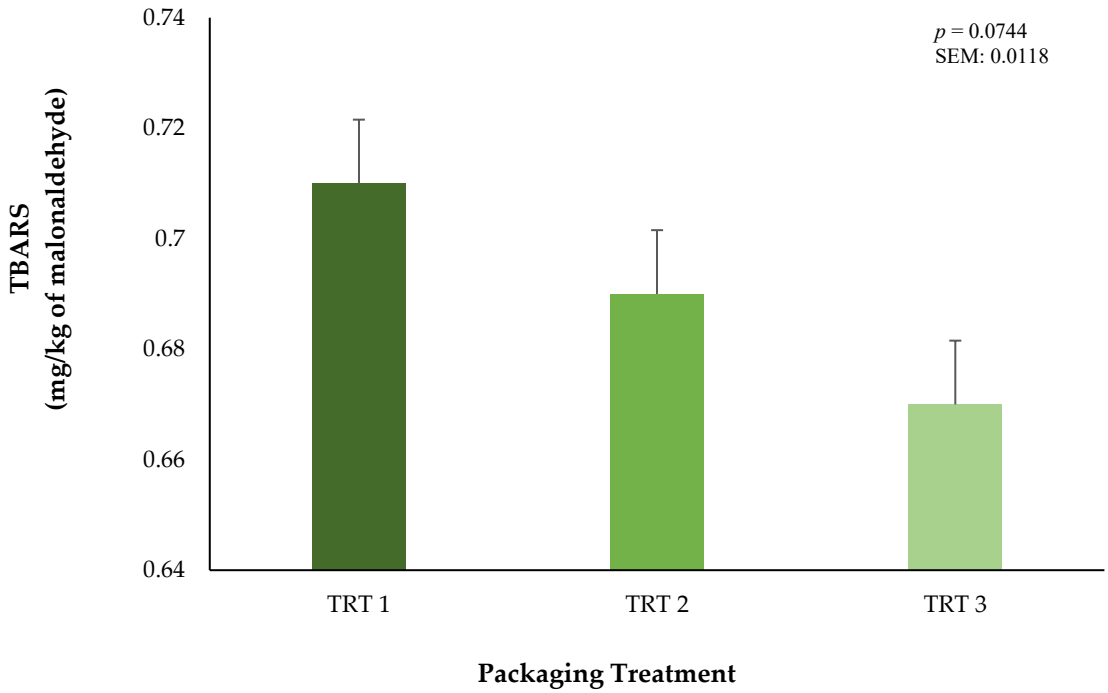
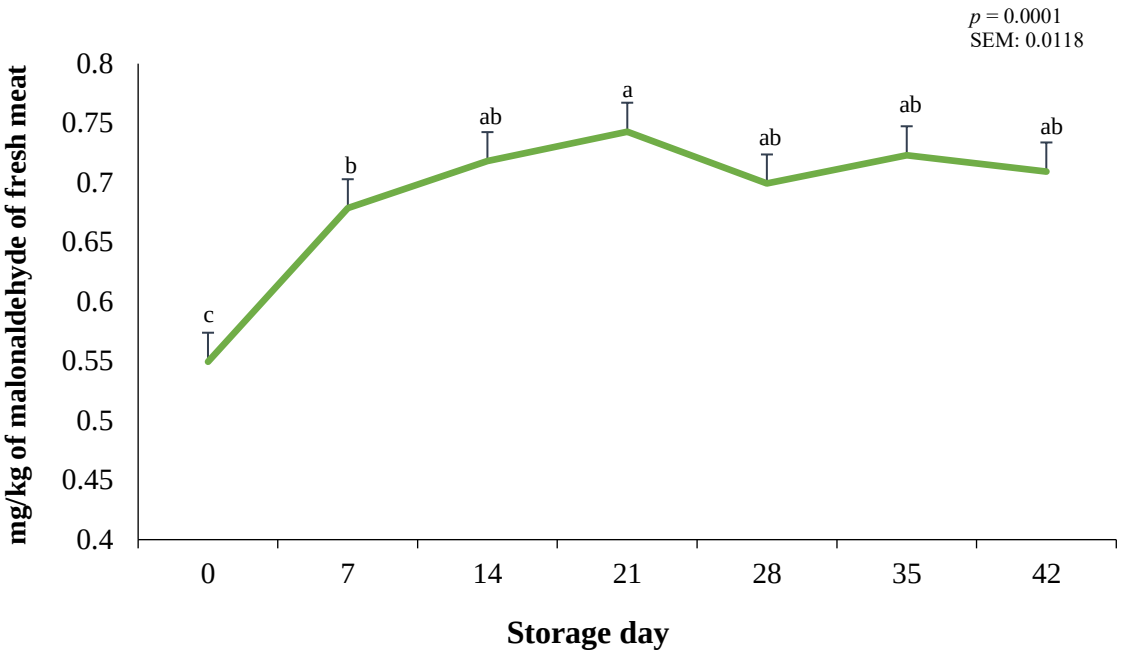


Figure 6. Impact of storage day on 2-thiobarbituric acid reactive substances on ground beef bricks during 42 days of refrigerated storage.



APPENDICES

APPENDIX A

Thiobarbituric Acid Reactive Substances (TBARS)

Chemicals:

Water – HPLC grade or distilled deionized water Potassium phosphate (monobasic) KH2PO4 potassium phosphate (dibasic) K2HPO4 Ethylenediaminetetraacetic acid (EDTA) n-Propyl gallate (PG) Trichloroacetic acid (TCA) 2-Thiobarbuturic acid (TBA) 1, 1, 3, 3, Tetraethoxypropane (TEP)

Reagents:

- 50mM phosphate buffer – pH 7.0, shelf-life = 2 weeks

Prepare 50mM monobasic potassium phosphate solution – weight out 3.40g KH2PO4, place in a 500 ml volumetric flask, dissolve and bring to volume with distilled-deionized water (pH will be approximately 4.5).

Prepare 50mM dibasic potassium phosphate solution – weight out 8.71g K2HPO4, place in a 1 L volumetric flask, dissolve and bring to volume with distilleddeionized water (pH will be approximately 8.5). Prepare at least 4 L of the dibasic solution each time.

Using a 2 L beaker, combine approximately 500 ml of dibasic and 100 ml of monobasic solutions. Mix and monitor the pH of the combined solution as you continue to add more of each solution until the volume is in excess of 1 L. The pH of this solution will be slightly greater than 7.0.

Add 1.0g of EDTA and 1.0g of PG. Allow the solution to mix for one hour, as PG is extremely slow to dissolve.

- **30% TCA**

Use extreme care when making, as TCA is corrosive (clean up any spills immediately). Weigh 300g of TCA into a 2 L beaker, add 1000 ml of distilled deionized water. If less is needed, weigh out 30g and add 100 ml of distilled deionized water.

- **0.02M TBA**

Make fresh daily (250 ml is enough for 125 samples). Weigh out 0.7208g TBA, and place into a 250 ml volumetric flask. Add 250 ml of distilled deionized water. The use of low heat while mixing will accelerate the dissolving process, but use extreme caution as too much heat will destroy the solution.

Analysis:

General notes: Prepare and turn on water bath-set temperature at 100 ºC. It takes approximately 1 h for the water bath to reach the desired temperature. If a sipper unit is being used, it is necessary to prepare at least 3 blanks and then run at least one working standard with each run.

For raw meat samples:

1. Weigh out 2.0g (1.95 to 2.05g) of minced meat into a labeled 50 ml disposable centrifuge tube. Record the exact weight of the sample.

2. Add 8 ml of prepared phosphate buffer to the tube.

3. Add 2 ml of TCA to the tube and homogenize for 20 to 30 secs.

4. Filter homogenate through a Whatman (No. 4) filter paper, collecting the clear filtrate into labeled tubes. (It is OK to stop at this point, but the tubes containing the filtrate must be sealed and stored in a refrigerator).

5. Remove 2 ml of the sample filtrate and place it into a labeled glass test tube. Prepare duplicate tubes for each sample at this point (i.e., tube “A” and tube “B”).

6. Add 2 ml of TBA to each tube including the blanks and standard.

7. Cover tubes with aluminum foil and place them into the hot water bath for 20 min.

8. Remove tubes from hot water bath and place into the ice water bath for 15 min.

9. Read absorbance at 533 nm

10. Multiply absorbance by 12.21

11. Report TBARS as mg/kg of malonaldehyde.

Standards for standard curve

Dilute each of the following amounts of TEP working solution in 50 ml volumetric flasks with distilled water.

TEP	Concentration of "Standard"	Absorbance
1 ml (4.4 μg)	0.088 μg/ml	0.03
2 ml (8.8 μg)	0.176 μg/ml	0.06
4 ml (17.6 μg)	0.352 μg/ml	0.123
5 ml (22.0 μg)*	0.44 μg/ml	0.150
10 ml (44.0 μg)	0.88 μg/ml	0.30
20 ml (88.0 μg)	1.76 μg/ml	0.60
40 ml (176.0 μg)	3.52 μg/ml	1.20

*This standard should have an Absorbance in the proximity of 0.150. Range may be 0.130 to 0.170, depending upon the accuracy of solutions and dilutions.

References:

Kuntapanit, C. 1978. Beef muscle and adipose lipid deterioration as affected by nutritional regime, vacuum aging, display, and carcass conditioning. Ph.D. dissertation. Kansas State University. pg. 117.

Witte, V. C., Krause, G. F., & Bailey, M. E. 1970. A new extraction method for determining 2- thiobarbituric acid values for pork and beef during storage. *Journal of Food Science, 35*, 582-585.

APPENDIX B

Chapter II: Packaged beef steaks pictures from hour 0 through hour 42.

APPENDIX C

CHAPTER III: Packaged beef steaks pictures from hour 0 through hour 42.