

The Influence of Packaging Methodology on Fresh Beef Surface Color

by

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Abstract

Packaging techniques and materials used throughout the meat industry are evolving as technology is developed. Most notably, the rise in use of thermoforming vacuum packaging within the fresh meat industry has prompted research to focus on alternative packaging methods for fresh meat. The comparison of packaging materials and method of packaging were investigated to determine the effects on fresh beef surface color and storage period during two simulated retail display periods of fresh beef. The first study evaluated vacuum packaging films recycle-ready (RRF), standard barrier (STB), and enhanced barrier (ENB) on ground beef surface color. Packaging type resulted in significant differences ($P < 0.05$) where ground beef packaged in ENB had greater values for lightness (L^*), redness (a^*), chroma (C^*), red to brown (630/580), and calculated relative values of deoxymyoglobin. In the second study, whole muscle beef strip loin steaks were assigned to either vacuum-ready packaging (VRF) or polyvinyl chloride (PVC) overwrap packaging. Steaks packaged in PVC had greater ($P < 0.05$) redness (a^*) values until day 5, whereas VRF steaks had greater ($P < 0.05$) a^* values from days 10 to 35. PVC steaks had greater ($P < 0.05$) values for hue angle from days 5 through 35. Furthermore, lipid oxidation for VRF packaged steaks was less ($P < 0.05$) from day 10 to the conclusion of the study. The results from these studies suggest that packaging materials, can influence fresh beef surface color.

Table of Contents

Abstract.....ii

Table of Contents.....iii

List of Tables..... v

List of Figures..... vi

CHAPTER I..... 1

 Literature Review..... 1

 1) Introduction.....1

 2) Meat Color.....2

 3) Shelf Life..... 8

 4) Packaging Platforms..... 10

 5) Food Loss and Food Waste.....20

 6) Conclusion..... 22

Literature Cited..... 24

FIGURES.....32

CHAPTER II.....35

 Surface Color Variations of Ground Beef Packaged Using Enhanced, Recycle Ready,
 or Standard Barrier Vacuum Films..... 35

 1) Introduction.....37

 2) Materials and Methods.....39

 3) Results and Discussion..... 42

 4) Conclusions.....45

References.....47

TABLES.....	52
CHAPTER III.....	56
Vacuum Packaging Can Extend Fresh Color Characteristics of Beef Steaks during Simulated Display Conditions.....	56
1) Introduction.....	58
2) Materials and Methods.....	60
3) Results and Discussion.....	65
4) Conclusions.....	72
References.....	74
TABLES & FIGURES.....	80
APPENDICES.....	86
APPENDIX A.....	87
Thiobarbituric Acid Reactive Substances (TBARS).....	88
APPENDIX B.....	91
PVC Overwrap Beef Loin Steaks Sensory Ballot.....	92
APPENDIX C.....	93
VAC-Packed Beef Loin Steaks Sensory Ballot.....	94
APPENDIX D.....	95
CHAPTER II Packaged ground beef fresh surface color pictures.....	96
APPENDIX E.....	97
CHAPTER III Packaged steak fresh surface color pictures.....	98

List of Tables

CHAPTER II TABLES

Table 2.1 Influence of packaging film on color values of vacuum-packaged ground beef during a simulated retail display.....	53
Table 2.2 Influence of retail display (d) on color values of vacuum-packaged ground beef.....	54

CHAPTER III TABLES

Table 3.1 The interactive impact of packaging method × day of display for instrumental surface color values on fresh beef strip loin steaks during a simulated retail display.....	81
Table 3.2 Interactive influence of packaging method × day of display for expert surface color evaluation on fresh beef strip loin steaks during a simulated retail display.....	82

List of Figures

CHAPTER I FIGURES

Figure 1.1 Different States of Myoglobin.....33

CHAPTER III FIGURES

Figure 3.1 Interactive influence of packaging method × day of display for 2-Thiobarbituric acid reactive substances (TBARS) on beef strip loin steaks during a simulated retail display. Bars lacking common letters differ ($p \leq 0.05$)..... 83

Figure 3.2 Interactive influence of packaging method × day of display for purge loss (%) on beef strip loin steaks during a simulated retail display. Bars lacking common letters differ ($p \leq 0.05$).....84

Figure 3.3 The interactive influence of packaging method × day of display for fresh muscle pH on beef strip loin steaks. Bars lacking common letters differ ($p \leq 0.05$)..... 85

**This Chapter is formatted to fit the style and guidelines for the peer-reviewed Journal of
Meat Science**

CHAPTER I

Literature Review

1) Introduction

Vacuum packaging was used initially as a method to package primal and sub primal cuts for distribution from packing plants to retail outlets; this process was identified as boxed beef (Seideman and Durland, 1983). However, beef is still vacuum packaged for distribution and, the use of vacuum packaging is a likely alternative for marketing fresh beef in retail stores. Kelly (2015) reported an increase in vacuum packaged meat products of 14% from 2002 to 2015. There has also been an increase in the use of modified atmosphere packaging (MAP) in retail stores. Kelly (2015) also reported that MAP packaging had a 3% increase from 2002 to 2015. The most typical or traditional platform of packaging used in retail stores is polyvinyl chloride (PVC) foam tray overwrapping. The transition from traditional packaging is due to the advancements in non-traditional packaging types. Kelly (2015) indicates that PVC overwrap packaging use decreased in retail settings from 51% in 2002 to 33% in 2015. It is likely that the decline in PVC has been caused by factors such as shelf-life extension, availability of packaging materials, or demands for a more sustainable packaging method. Nonetheless, it appears the conversion from packaging methods to a non-traditional packaging platform can offer greater shelf-life stability for fresh meat products.

Despite the advantages of non-traditional packaging platforms, one of the greatest challenges the meat industry faces can be informing consumers of the advantages in non-traditional packaging. In this regard, there have been several studies testing non-traditional packaging with consumers. A study conducted by Wezemaal et al. (2011) evaluated the

response of European consumers to vacuum and MAP packaging platforms. Results suggested vacuum packaging was accepted by 73% of the participants and MAP was accepted by over 54.7% of the participants. An additional study by Lynch et al. (1986) explored consumer acceptance of vacuum packaged ground beef with consumers at the grocery store point-of-sale. Consumers were divided across two groups and were either provided supplemental information regarding vacuum packaging or received no additional information. The results concluded that informed consumers were more likely to purchase vacuum packaged ground beef. Furthermore, the results on informing consumers suggests that consumers will accept and purchase a product that is not bright-cherry-red at the time of purchasing.

2) Meat Color

Meat color has been deemed by many to be the most important attribute that consumers take into consideration at the time of purchasing fresh beef. Therefore, this attribute is still regarded as an instrumental factor for consumers purchasing fresh meat to this day (Hood, 1980; Carpenter et al., 2001; Mancini and Hunt 2005; Henriott et al., 2020). Myoglobin is the protein identified within meat proteins that is responsible for meat surface color. More specifically, gases bind to the heme iron portion of the myoglobin protein which determines the color of meat. Generally, in fresh beef, there are four chemical states of myoglobin. When myoglobin is bound to oxygen it has been identified as oxymyoglobin which is present as a bright-cherry-red color. Oxymyoglobin is indicative of fresh meat presented in PVC packaging which is often found in retail stores. Carboxymyoglobin is often the result of MAP packaging, and most often used in case ready applications for retail stores. Fresh meat presented in MAP packaging is typically gas

flushed with carbon monoxide gas that when bound to myoglobin presents a stable, bright-cherry-red color.

Vacuum packaged beef reduced levels of oxygen exposure whereby impeding the heme iron portion of myoglobin to bind with oxygen or other gases and resulting in a meat that appears dark purple in color. This chemical state of myoglobin is called deoxymyoglobin. Moreover, through all these packaging platforms, the color ultimately deteriorates and metmyoglobin can eventually form resulting from extended periods of time or even temperature abuse (Claus et al., 2007). When meat products reach the point of discoloration by the accumulation of metmyoglobin, it is typically discounted in retail settings or even disposed of due to lack of consumers interest in purchasing a fresh meat product that is unappealing. Oxidation of the heme iron molecule within the myoglobin structure of fresh meat results in the formation of metmyoglobin (Figure 1.1).

Influencers of meat color

There are several factors that can affect meat color that occur in either pre- or post-harvest conditions. In addition, influencing factors of meat color can attribute to an enormous amount of variation in surface color for either whole muscle or ground products. Fresh ground meat products tend to develop metmyoglobin during shorter storage periods. It is likely that the rapid surface color changes in ground meat products is attributed to greater exposed surface area when compared to whole muscle products (Uboldi et al., 2015). The greater amount of surface area of fresh ground meat product allows for greater amounts of oxidation to take place due to the binding of more oxygen.

Species

Protein species is a major influencer of color because species-to-species meat color can vary depending on the total amount of myoglobin within the muscle tissue (Seideman et al., 1984). For instance, Ginger et al., (1954) evaluated the amount of myoglobin in beef and pork and found on average beef muscle contained 4.7 mg/g of tissue, whereas pork muscle contained 1.0 mg/g of tissue of myoglobin. Poultry, lamb, goat, and seafood are all considered meat proteins and the surface color of the meat can be altered.

Age

In addition to the species variations in meat color, animal age at the time of slaughter can be a major factor that can alter meat surface color. As animals age the content of myoglobin increases resulting in older animals containing greater amounts of myoglobin which results in a darker meat color surface (Schweihofer, 2014). Cho et al., (2015) conducted a study evaluating Korean Hanwoo cattle at varying ages and the effect on surface color. Results report that there is a positive correlation of slaughter age with myoglobin concentration. Furthermore, a study by Humada et al., (2014) reported increased myoglobin content with increasing age of Pirenacia bulls. In another study using 4- and 11-month-old lambs the myoglobin content was measured to be 3.3 and 4.9 (mg/ml) respectively (Kim et al. 2012). Yu et al., (2017) reported results in a study using pigs slaughtered at different growth stages having varying concentrations of myoglobin within the *longissimus dorsi.*, and Jaborek et al., (2018) evaluated the effect of animal age on meat characteristics of sheep. Results suggest that mature ewes had greater redness values compared to yearling ewes and ewe lambs.

Diet

When feeding cattle, it is known that grass-fed beef typically has darker, red-colored meat when compared to grain-fed cattle (Schroeder et al., 1980; Crouse et al., 1984). The diet of beef cattle is important because it affects the color of beef and ultimately the consumer's decision to purchase that product. There have been several studies evaluating the diet of beef cattle and its subsequent effect on meat surface color (Schroeder et al., 1980; Crouse et al., 1984; Sapp et al., 1999; Priolo et al., 2000). In a study by Realini et al., (2004), the researchers examined an effect of pasture vs. concentrate feeding beef on meat color. Results of diet constituents indicate that beef cattle finished on pasture displayed darker *longissimus dorsi* color than cattle finished using a diet comprised of concentrates. An additional study of beef cattle diets examined different varieties of barley inclusion within finishing diets on meat surface color (Boles et al., 2005). Results indicate that beef surface color can be altered by the variety of barley that is incorporated into the finishing diet phase.

Temperature

As evident by previous research, many factors can influence fresh meat color, specifically, the surface color of fresh beef proteins. In addition, temperature can affect color of meat during storage, with warmer storage temperatures associated with greater oxidation of myoglobin (Seideman et al., 1984). When myoglobin is oxidized, it will begin the conversion to metmyoglobin resulting in surface discoloration in temperature-abused meat products. Rosenvold and Wiklund (2011) concluded that storage temperature is the second most important factor to affect meat color in addition to storage duration. Gill and Jones (1992) reported that the optimal storage temperature to obtain the greatest duration of

shelf life is -1.5°C , just before meat begins to freeze. Jeremiah and Gibson (2001) used retail ready beef steaks stored at varying temperatures. Steaks stored at 5°C had greater amounts of surface discoloration and metmyoglobin accumulation than steaks stored at -1.5°C . In another study using varying packaging methods of ground beef patties stored in temperature abusive conditions (10°C), warmer storage temperatures resulted in greater amounts of surface discoloration for beef patties packed using high oxygen MAP and traditional PVC packaging (Rogers et al., 2014). Lastly, a study investigated the effects of temperature abuse on the surface color of lamb loin showing that ideal temperatures ($-1.5^{\circ}\text{C} - 2^{\circ}\text{C}$) had significantly greater color than lamb loins exposed to short and long-term temperature abuse (Rosenvold and Wiklund, 2011).

Lighting

Another influential factor affecting meat color is lighting during the storage period in retail store. Mancini and Hunt (2005) concluded that display case lighting is not considered often though it can alter the color presentation of meat products. Factors like photo-oxidation, color of light, and the heat produced by lighting sources should be considered when evaluating and measuring meat color. Three typical lighting types are currently used during the display of fresh meat currently. Lighting sources include florescent, incandescent, and light emitting diode (LED) lighting. Though many studies have yet to elude the optimal lighting for displaying fresh meat in retail settings. A study by Steele et al., (2016) evaluated the effects of two lighting types, LED and florescent, on several types of fresh meats. Researchers concluded that under LED lighting conditions, beef products had less visual discoloration and pork loin chops had greater lightness (L^*) values than products displayed under florescent lighting. Barbut et al., (2001) evaluated the

influence of incandescent, fluorescent, and metal halide lighting on beef, pork, and chicken and observed that beef, pork, and chicken displayed a more desirable color under incandescent lighting. In another study by Djenane et al., (2001) utilizing fresh beef steaks product packaged in MAP packaging, and displayed under standard supermarket low-UV fluorescent, and the standard supermarket fluorescent with a UV filter. Fresh beef color on the 28th day of retail display with the standard supermarket low-UV fluorescent lighting had redness (a^*) values near 4. However, when using the supermarket fluorescent light with a UV filter, a^* values were approaching 10. Larger redness values are an indicator of a redder color which is often more desirable to consumers at the time of purchase.

Additionally, metmyoglobin formulation for meat products under the standard supermarket fluorescent lighting were 70% at day 17 and 90% at the conclusion of the study. Whereas the beef products under the supermarket fluorescent light with a UV filter accumulated only 40% metmyoglobin after day 28 of the study. Therefore, lighting should be equally considered along with temperature and species when identifying storage methods in the retail setting.

Muscle pH

The conversion of muscle to meat begins at the time of harvest when an animal is exsanguinated. The flow of blood provides the muscle with oxygen, though once the abundance of oxygen is depleted, the muscle shifts from aerobic to anaerobic conditions and the accumulation of lactic acid within the muscle begins. With the production of lactic acid, the pH drops from 7.0 to 5.5. An example of the effects of pH on meat color is in pork following the slaughter process. If postmortem muscle pH declines rapidly, it may denature the myoglobin proteins which are responsible for pigment development and may result in a

lighter, less intense, surface color, often referred to as Pale, Soft, and Exudative (PSE; Lonergan, 2008). Additionally, when the muscle pH rate of decline is delayed the surface color of meat can (especially) pork can result in a condition referred to as dark, firm, and dry (DFD). DFD occurs due to a lack of glycogen in the muscle at the time of slaughter which hinders the production of lactic acid during postmortem muscle activities caused by a limited decline in muscle pH. The lack of pH decline in muscle after slaughter can result in pH greater than 6.0 instead of within the normal pH range of 5.5 to 5.8 and a subsequent result of pork appearing as a darker red color (Buege, 2003). In a study by Zhang et al., (2018), investigating the rate of pH decline and the correlation to beef color. Results concluded that the muscles with an ultimate pH of 5.40 to 5.79 had the greatest rate of postmortem pH decline, and beef muscles were lighter and redder which is more desirable to consumers. Moreover, the muscle groups that declined the least resulted in pH values ranging from 6.87 to 6.54, and muscle surface color-maintained redness, yellowness, and chroma values similar to the initial postmortem values that were measured, ultimately resulting in a dark red color that is often classified as a dark cutter within the beef industry.

3) Shelf Life

The term shelf life has been defined in many ways over the years, however, when it comes to meat shelf life, Delmore (2020) defines it as the duration of time before spoilage organisms cause meat to become unpalatable and unsafe for human consumption. Fresh meat is a highly perishable item therefore, it is pertinent to understand the factors that affect meat shelf life. There are several factors that can influence the shelf life of fresh meat, which include duration of storage, temperature of storage, accumulation of spoilage

organisms, and packaging type (Laleye et al., 1984; Bağdatlı & Kayaardi, 2014; Rogers et al., 2014; Luzardo et al., 2016).

Storage temperature and packaging type can aid in the hindrance of spoilage organisms which ultimately can help extend the duration of storage for fresh meat products. Additionally, to help extend shelf-life, meat products can be sold as fully cooked which can help extend the time of shelf stability, though, even fully cooked products must have an expiration date for consumers at the point of retail purchase. It is imperative for the meat industry to continue controlling and identify alterations to these factors when it comes to extending the shelf life of fresh meat.

The extension of meat shelf life can be paramount to feeding the nation and the expected population growth in the year 2050. Kuck and Schnitkey (2021) reported that the 2020 consumption of beef per capita was 83 pounds, whereas, in 2019 67 pounds of pork and 112 pounds of chicken were consumed. With the growing population the consumer requirement for available fresh meat will continue to rise. To help satisfy the demand for fresh meat proteins, the extension of meat shelf life is a necessity.

There have been many studies that have investigated the duration of fresh meat shelf life and its ability to remain suitable for human consumption. These studies have evaluated countless simulated retail display conditions such as lighting, packaging, temperature, and ingredient technologies (Hunt et al., 2004; Martin et al., 2013; Limbo et al., 2013; Uboldi et al., 2013; Steele et al., 2016). In addition, the process of freezing beef has been considered to extend the shelf life of meat products (Iskandar et al., 2019).

Temperature can affect the duration of storage by storing meat in super chilled or partially frozen (1 to 2°C below initial freezing point) or frozen conditions (-18°C) which

can drastically extend the shelf life of meat products. Temperature is a method that can slow or reduce the growth of microorganism bacteria. When meat products are stored at super-chilled temperatures, microbial growth can be reduced (Magnussen et al., 2008). The same effects on microorganism survival are seen when meat products are stored at freezing temperatures which can extend the shelf life of meat. Magnussen et al., (2008) reported that storing meat at super-chilled temperatures can extend the shelf life 1.4 to 4 times longer than traditional chilling temperatures (1.1 - 4.4°C). Ding et al., (2020) investigated varying temperatures (-1°C, -2°C, and -3°C) for super-chilling pork and reported that pork stored at -3°C resulted in lower microbial plate counts. Furthermore, pork stored at -3°C had lower TBARS values than pork stored at the other super-chilled temperatures.

4) Packaging Platforms

Packaging is an inevitable step in the merchandizing of fresh meat and has two ultimate functions. The first function of meat packaging is to protect the product from contaminants and to contain the product (Sara, 1990). Secondly, packaging plays an important step in the marketing of meat products product sold to consumers in the retail setting. If the packaging is unappealing, then research has concluded that consumers will not purchase the product (Sara, 1990). In addition to consumer purchase intent, the application or presentation within a retail setting will determine what packaging method is used. Each packaging platform has a designated target consumer group and end-user purpose within the meat industry. Packaging platforms may alter the purchase decision of consumers by altering the meat product presentation, surface color, storage duration, or safety.

Polyvinyl Chloride (PVC) Overwrap

PVC tray overwrap packaging has been the packaging of choice by consumers due to the appearance of meat products within this packaging platform. The PVC film used is oxygen permeable allowing oxygen to permeate through the film and bind to the heme iron portion of myoglobin protein in meat. This chemical state of myoglobin is identified as oxymyoglobin and can appear as a bright cherry red color which has often been correlated to freshness by the consumer (Gupta et al., 2018). The packaging method of PVC is often regarded as the most popular packaging platform in the retail setting because of the bright cherry red color presented. Montgomery et al., (2003) stated the primary attribute that influences consumers at the time of purchasing is meat color. Consumers correlate product color with freshness and wholesomeness when purchasing fresh meat products in the retail setting (Mancini and Hunt, 2005). Though color is not sole factor influencing meat freshness or wholesomeness, it is still the influential factor a consumer uses when making a purchasing decision.

PVC overwrap packaging does have some downfalls regarding shelf life. The FDA (2021) recommends that fresh meat (beef, veal, lamb, and pork) steaks, chops, and roasts have a shelf life of 3 to 5 days once purchased and stored in the consumers home refrigerator. Whereas ground products may have a shelf life of 1 to 2 days when stored at 4°C. When freezing these meat products, it is recommended that fresh meat (beef, veal, lamb, and pork) steaks and roasts may have a shelf life of 6 to 12 months, and chops are 4 to 6 months. When electing to freeze ground products, the shelf life may increase to 3 or 4 months according to FDA (2021). Current PVC packaging platform offers a relatively short shelf life when compared to other types of packaging platforms which include MAP and

vacuum packaging. However, when using PVC and MAP packaging methods, freezer burn can occur resulting in negative visual and taste attributes.

Consumer freezing of PVC overwrapped meat products may lead to a greater increase in the disposal of these product due to color alterations and eating experiences. Freezer burn occurs when moisture on the surface of frozen foods is loss due to sublimation or when ice turns into gas skipping the transition to water, leaving the surface of food dehydrated (Schmidt and Lee, 2009). Moreover, Schmidt and Lee (2009) describe the accumulation of freezer burn as a combination of several factors, however, packaging materials is one of the greatest factors contributing to the detrimental influence of meat products during extended frozen storage periods. A couple of the more prominent factors affecting the accumulation of freezer burn may include excess head space atmosphere within the package and packaging materials that have a greater water-vapor transmission rate. These common factors attributed to freezer burning in meat produces are both confounding attributes associated with PVC overwrap packaging methods.

Modified Atmosphere Packaging (MAP)

MAP packaging is a packaging platform whereby a package is flushed with an atmospheric gas or gas mixture. The goal of MAP packaging is to help extend the packaged product shelf life with the overall objective to maintain a desirable surface color that will appeal to consumers. Fresh beef that is packaged in MAP packaging is typically gas flushed with a single, Bi, or Tri-gas. These gases typically consist of carbon monoxide, carbon dioxide, nitrogen, and in some limited occurrences the package may contain oxygen.

Carbon monoxide can bind to the heme iron portion of the myoglobin molecule causing the fresh meat to appear as a bright, cherry-red surface color resulting in a chemical state referred to as carboxymyoglobin (McMillin, 2008). The use of carbon dioxide in MAP packaging is typically used to inhibit the growth of microorganisms (McMillin, 2008). Whereas nitrogen gas is typically used as an inert filler within the packaging atmosphere to prevent the collapse of the packaging film onto the surface of the meat or food product. When nitrogen is used within a packaging atmosphere it has been reported that the meat product color, safety, or texture are not impacted due to nitrogen's inert properties (McMillin, 2008).

Storage duration of meat packaged in MAP systems can have varied storage periods. Cornforth and Hunt (2008) reported that meat packaged in high oxygen MAP packaging may have a shelf life of 10 to 14 days. Increasing the concentration of oxygen within the package (HiOX) MAP package for whole muscle beef cuts may have a shelf-life period of 12 to 16 days according to (Belcher, 2006). In addition, modified atmosphere packaging can also be created with a low oxygen atmosphere using reduced levels of oxygen to reduce the rate of oxidation and extend the shelf life of meat products. One example of a low oxygen MAP packaging gas mixture is a Bi gas package atmosphere comprised of nitrogen and carbon dioxide. It has been reported that beef packaged in a bi-gas mixture of 70% nitrogen and 30% carbon dioxide may result in a shelf life of 25 to 35-days (Delmore, 2020). Lastly, another form of MAP packaging using a low oxygen tri gas atmosphere mixture may include nitrogen, carbon dioxide, and carbon monoxide. The gas atmosphere ratio within the package can be 69.6% nitrogen, 30% carbon dioxide, and 0.4%

carbon monoxide and has been reported to provide ground beef a shelf life of 28 to 35 days (Delmore, 2020).

Master (Mother) Bag Packaging

Mother bag or masterpack use is like MAP packaging, using a barrier pouch flushed with a gas atmosphere combination of oxygen, nitrogen, carbon monoxide, or carbon dioxide. In most instances, fresh meat products are packaged first using PVC overwrapping methods and then placed into the mother bag whereby allowing the gases to permeate through the PVC film and support fresh meat color stability. More specifically, master packing uses packaging technologies that are breathable in a low oxygen pouch. In addition to PVC packaging films, there are also vacuum packaging materials that can be used to package meat products that contain breathable components that are successful in mother bag packaging application.

The process of mother bag packaging allows gas flushing to influence product color by reducing spoilage organisms, limiting oxygen deterioration, and improving color stability. The mother bag essentially allows for a customizable environment depending on what gas blend is used to flush the pack. The use of mother bag packaging is influenced by the demand of case ready meat products throughout the retail setting. Belcher (2006) identified several conclusions on the increase usage of pre-packaged meat products arriving at the retail store. The growth in case ready usage trends include 1) a reduction of labor in retail stores with growing consumer demand of fresh meat products, 2) a greater convince for consumers, and 3) the improvement in food safety due to the reduction in product handling and repackaging. Mother bag packaging gas flush can vary depending on its use, however, Delmore (2020) reported that a typical gas atmosphere mixture for mother bag

packaging is often a bi-gas flush with varying amounts of carbon dioxide (100% to 80%) along with nitrogen. Meat products within a mother bag packaging system may have a shelf life of 10 to 14 days during dark storage and 2 to 7 days once the mother bag is opened and the packaged meat placed into in a retail setting for consumer purchase (Delmore, 2020).

Traditional Vacuum Packaging and Shrink Bags

During the process of vacuum packaging, products are placed into barrier film materials and all the atmosphere surrounding the product within the package is evacuated before the packaging material is heat sealed. Vacuum packaging creates an anerobic atmospheric environment within the package which can impede microbial growth and lipid oxidation thus extending the shelf life of meat products.

In traditional vacuum packaging, fresh meat is placed into a bag/pouch. Typically, these bags are composed of multi-layered films and classified as barrier films. Barrier films limit the volume of atmospheric gases from entering or escaping the package. Each packaging film, bag, or pouch is designed with a specific level of atmospheric gas transmission that may occur within a specific amount of time. Initially, the residual amounts of oxygen can be minimal, however, current packaging films within the industry aren't completely impenetrable by oxygen.

Over the duration of display, the transmission of oxygen in and out of the packaging is defined as oxygen transmission rate (OTR). Additionally, in traditional vacuum packaging films, there are components within the film that surround the packaged product to allow for excess purge accumulate. It has been concluded in previous research that during retail display shelf life can be reduced because the accumulated purge provides a permissive environment for microbial growth (Stella et al., 2018).

Vacuum packaging can impact the color of fresh meat as a result of the anerobic state within the package that can cause fresh meat cuts to appear as a purplish-red color. Moreover, vacuum packaged fresh meat surface color can influence consumer purchasing decisions as consumers rate the surface color as a great influencer in their purchasing decision (Suman et al., 2014). Vacuum packaging has been reported to significantly extend the shelf life of fresh meat. Delmore (2020) concluded that vacuum packaged beef primals and subprimals may have a shelf life of 35 to 45 days under normal refrigerated storage conditions (4°C), and meat products refrigerated at -2 to 1°C and can last from 3 to 83 days. Furthermore, whole muscle vacuum packaged beef that is frozen at -18°C may have a shelf life of up to 12 months (Delmore, 2020).

Vacuum Skin Packaging

Vacuum skin packaging is one of the newest types of vacuum packaging that is gaining popularity in the case ready sector of the meat industry (Stella et al., 2018). In the process of vacuum skin packaging, fresh meat is placed into a plastic tray where it is covered by thermoformed film to form perfectly over the product. This process creates an anerobic environment and no excess space for the accumulation of purge during retail display. Moreover, a study by Kamenik et al., (2014) demonstrated that beef and pork meat packaged in vacuum skin packaging has the least amount of purge loss among the other packaging types. In a study by Vazquez et al., (2004), researchers compared the shelf life of beef retail cuts packaged in traditional and skin vacuum packaging platforms. The results showed that for all the retail cuts packaged in the vacuum skin packaging there were lower microbial counts than in traditional vacuum packaging.

Thermoforming

Thermoforming vacuum packaging is another method that utilizes vacuum packaging technology. Constructing the thermoformed package utilizes two rolls of film loaded on a rollstock machine. The first film is defined as a forming layer and the film enters a forming die where heat and pressure are applied to the film causing it to form to the forming die to create a pouch. Once the pouch is created, it can be loaded with fresh meat products before it enters the sealing box. The meat product within the formed pouch enters the sealing box of the rollstock machine where the second layer of film is introduced. The second film is often referred to as the non-forming layer throughout the meat packaging industry. The non-forming layer is pressed against the forming layer when the atmosphere within the chamber of the rollstock machine voided and then subsequently the pouch is heat sealed. The shelf life of thermoforming vacuum packages and traditional bag/pouch vacuum packages are similar. However, the major difference in using thermoforming instead of bags or pouches for vacuum packaging is the visual appearance of the package.

Active Packaging Technologies

Active packaging is one of the newest forms of packaging technologies which can be applied to current packaging platforms. Sand (2020) defines active packaging as a system that actively releases or absorbs compounds within the packaging from either the product or the headspace atmosphere. Active packaging exists in many forms and the objective of active packaging is to extend the shelf life of food and meat products.

Some of the current techniques used in modern active packaging technologies have been used for years, though they have been modified recently to be more efficient and

effective. Active packaging can be divided into two groups characterized as 1) absorbers including oxygen scavengers and moisture scavengers or 2) antioxidant releasers and carbon dioxide emitting packaging systems (Kostova et al., 2019). Oxygen scavengers are often used to remove any residual oxygen within packaging system atmospheres that may be present in MAP trays or mother bag packaging applications. Previously, scavengers were placed as a sachet within the tray or mother bag pouch, however, with developments in active packaging, this technology can be imbedded into the package film or structures (Sand, 2020). Absorption of residual oxygen within fresh meat packaging aims to reduce the amount of oxidation occurring thereby preserving the meat color stability and extending the shelf life (Chounou et al., 2012; Roberta, 2020). Substances used for active packaging are constructed containing iron, ascorbic acid, photosensitive dyes, unsaturated hydrocarbon dienes, and palladium (Yildirim et al., 2017).

Moisture scavengers are used to reduce the residual moisture within packaging platforms. Moisture scavengers are typically used in MAP trays with shelf-stable products such as jerky or in addition to oxygen scavengers within overwrap packaging platforms. Moisture scavengers can be constructed using materials that appear as packets or pads. The packet scavengers are used to control the moisture within the headspace of packages while pads are used to absorb moisture from meat products. In recent years the inclusion of moisture absorbers into films and packaging structures has been improved (Gaikwad et al., 2018; Sand, 2020).

Moisture control in the packaging of fresh meat is essential to extending the shelf-life storage period. Greater amounts of moisture within a package can possibly create an improved environment for microorganism's growth leading to a reduction storage periods

of meat products (Yildirim et al., 2017). Secondly, moisture control impacts product presentation in fresh meat as it is often viewed as the accumulation of purge liquid within the package during the storage period of a retail setting. In a study by Droval et al., (2012), researchers concluded that consumers preferred products with less moisture loss or purge the within package.

Antioxidants

The use of antioxidants impregnated within the meat packaging film has increased in recent years in an effort to help extend shelf life by preventing lipid oxidation. There are two classes of approved food contact antioxidants in use throughout the industry currently and are defined as synthetic or natural. Synthetic antioxidants include butylated hydroxy-toluene (BHT) and butylated hydroxy anisole (BHA) while natural antioxidants include polyphenols, tocopherols, plant extracts, and essential oils. Natural antioxidants have been increased in popularity compared to synthetics due to growing concerns of health issues associated with their use and because consumers demand natural products (Barbosa-Pereira et al., 2014).

Research has concluded that antioxidant impregnated films can improve the surface color of fresh meat and extend shelf-life storage period (Moore et al., 2003; Camo et al., 2008; Junior et al., 2014). Additionally, the increased use of essential oils has been noted in active packaging due to their ability to not only provide antioxidant properties but have also been linked to antimicrobial reductions (Mahmoudzadeh et al., 2016; Pateiro et al., 2018). Packaging atmospheres containing carbon dioxide has been reported to help inhibit the number of microorganisms in the packaging of fresh meat which is the purpose of using carbon dioxide in MAP and mother bag packaging (Djenane and Roncalés 2018).

Moreover, the development of carbon dioxide emitters in active packaging has allowed the shelf life of fresh meat to be extended by inhibiting the growth of microorganisms (Holck et al., 2014; Fang et al., 2017).

5) Food Loss and Food Waste

Food loss can be defined as the decrease in edible food mass and food loss can take place at the production, postharvest, and processing stages (Gustavsson et al., 2011).

However, food waste is defined as food loss at the end of the food chain and is linked to retailers and consumers (Gustavsson et al., 2011). These reductions in meat or food waste/loss can be associated with different sectors throughout the meat industry as waste/loss has occurred at the slaughterhouse, meat processor, and even rendering plants where by-products of food production are produced, resulting in the loss of edible protein (Jayathilakan et al., 2012).

During slaughter and processing, the creation of food loss is inevitable. Animal processing creates a large volume of raw material that are of low economic value, such as bones, tendons, skin, contents of the gastro-intestinal tract, blood, and internal organs (Jayathilakan et al., 2012). Though not all of these by-products are a complete loss in value to the agriculture industry. Rendering often generates by-products that are either sold to foreign countries or sold to create a myriad of products from pet food to automobile tires. The by-products that are produced by the harvesting of animals allows processors to recoup some monetary loss that would otherwise be a complete loss if there weren't secondary markets for these products to be sold in. The amount of food waste seen at the retail and consumer level is excessive. Buzby et al., (2014) reported that 133 billion pounds of the 430 billion pounds of available food in the United States was wasted. Additionally, the 133

billion pounds of food loss was comprised of 43 billion pounds lost at the retail level and 90 billion pounds at the consumer level. Moreover, Buzby et al., (2014) also concluded that meat, poultry, and fish was the number one food group in terms of total value loss approaching \$48 billion in the United States. The meat industry has been working vigorously to identify methods for reducing the volume of waste and loss occurring annually within the United States. It is apparent that a method for quickly reducing food waste or loss may be linked to enhancing packaging platforms that extend the shelf life of meat products and ultimately reduce the amount of waste at the retail and consumer levels.

Sustainability in Packaging

Many advancements have been made over the years with packaging of meat products. The use of various packaging platforms has provided researchers within the meat industry that there is a great opportunity to reduce the amount of food waste in America. However, given the amount of meat produced in the United States alone, it requires an even greater amount of plastic and packaging materials.

The packaging material that remains instrumental in creating meat and food packaging is not a sustainable option for the meat industry because it lacks recyclable or biodegradable materials. Dilkes-Hoffman et al., (2018) suggested that it could be beneficial to find a packaging material that is recyclable or biodegradable to replace current packaging materials. Nonetheless, in recent years, efforts to develop recyclable films are changing the meat packaging industry as it aspires to improve sustainable production practices. The development of recyclable packaging films would be a partial fix to reduce the number of polymers and packaging materials that end up in our landfills. Though, Barlow and Morgan (2013) and Dilkes-Hoffman et al., (2018) indicate that recycling multi-

layer films at the present time remains difficult because the packaging film layers cannot be separated.

Given the difficulty in using films that are easily recyclable, many researchers and packaging companies have evaluated the use of biodegradable materials. The use of biodegradable materials solves many of the sustainability problems for the meat and food industry. Biodegradability allows for food products to be packaged in packaging materials that will not contaminate the packaged food while effectively containing the food product within a package. Additionally, the packaging material that is contaminated by food products can then be disposed of normally or even composted. Barlow and Morgan (2013) concluded that biodegradable materials can be disposed of with food material and degrade in the same manner. Interestingly, the United Kingdom promotes composting and recycling to reduce the amount of biodegradable material that enters landfills (DEFRA, 2006).

6) Conclusion

The shelf life of fresh meat can become a complex topic when considering the various components that can alter shelf life such as pH, lighting, temperature, microorganisms, and packaging. Shelf life can be a solution to many of the challenges that the meat industry faces. These challenges include feeding a population that is expected to reach 9 billion by the year 2050. In this growth of population, food shortages, food waste, and sustainability are arenas of focus for the meat industry to address delivery increase the volume of packaged food for consumers. With the improvements in meat color because of technology improvements in packaging platforms and the increasing knowledge for parameters that control meat color, the possibility of extending the shelf life of fresh meat can become a reality. Even with the developments in recyclable and biodegradable

materials, the meat industry continues to address sustainable meat and food activities with an expectation for improving the shelf life of meat products. It is important that the meat industry continues to inform consumers about technologies for improving the safety and wholesomeness of their food options in order to help consumers make conscience decisions when purchasing meat products.

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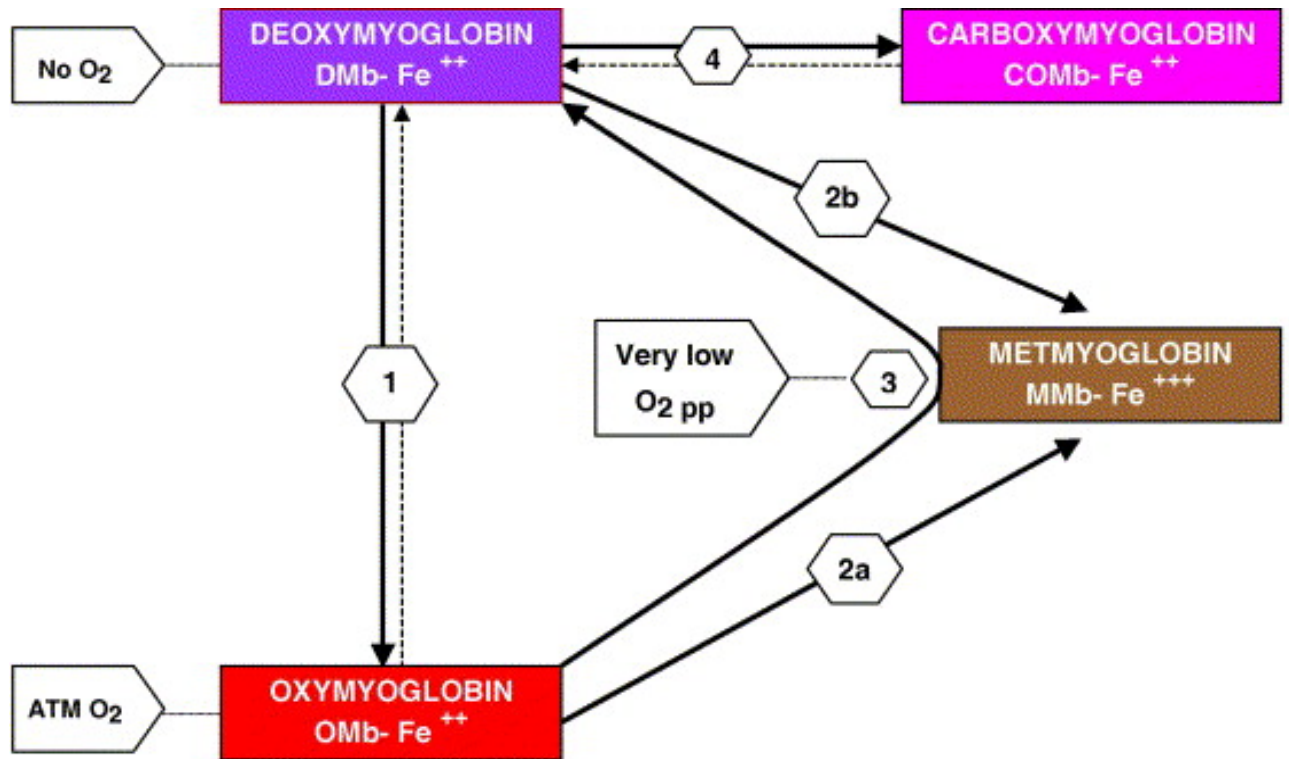
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FIGURES

Figure 1.1 Different States of Myoglobin



(From Mancini and Hunt, 2005)

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**Surface Color Variations of Ground Beef Packaged Using Enhanced, Recycle Ready,
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Chapter II

Surface Color Variations of Ground Beef Packaged Using Enhanced, Recycle Ready, or Standard Barrier Vacuum Films

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Abstract:

With current meat industry efforts focused on improving environmental influencers, adopting sustainable packaging materials may be an easier transition to addressing the sustainability demands of the meat consumer. With the growing popularity of vacuum-packaged meat products, the current study evaluated instrumental surface color on fresh ground beef using vacuum packaging films, recycle-ready film (RRF), standard barrier (STB) and enhanced barrier (ENB). Ground beef packaged using ENB barrier film was lighter (L^*), redder (a^*) and more vivid (chroma) than all other packaging treatments during the simulated display period ($p < 0.05$). By day 12 of the simulated retail display, the ground beef surface color became lighter (L^*), more yellow (b^*), less red (a^*), less vivid (chroma) and contained greater forms of calculated metmyoglobin, oxymyoglobin ($p < 0.05$). The current results suggest that barrier properties of vacuum packaging film for ground beef are pivotal for extending the surface color during fresh shelf-life conditions.

Keywords: ground beef; instrumental color; shelf life; vacuum packaging

1. Introduction

When consumers purchase fresh meat, a primary attribute influencing the consumer's purchasing decision is meat color [1]. As meat is stored in refrigerated conditions following harvest, fabrication, or portioning, surface color variations are dependent on the chemical state of myoglobin. With the presence of oxygen, the chemical state of myoglobin can appear as a bright, cherry-red color, often correlated to freshness by the retail consumer [2] at the time of selection and purchase. Vacuum packaging is often not the preferred packaging platform of choice within the retail consumer market setting by meat industry retailers due to surface color variations that are presented under vacuum. It is widely known that beef packaged in a vacuum platform displays a purple-red color identified as deoxy-myoglobin. Before vacuum packaging, fresh beef appears as a bright, cherry-red (oxy-myoglobin) color due to surface exposure to oxygen. When packaging fresh meat with limited exposure to oxygen (one to two days post packaging), the oxygen-bound myoglobin is converted to deoxymyoglobin [3] as a result of a reduction in partial pressure of the package. The reduction in partial pressure within a vacuum package of fresh meat can result in a shift in color form of myoglobin from a bright red oxygenated color to a purplish red deoxygenated appearance. It has been reported that 74% of consumers utilized color as an important attribute influencing their purchasing intent of meat [4]. Moreover, when half of the consumers are informed on vacuum-packaged beef and its purple-red color, consumers are more likely to purchase vacuum-packaged beef over non-barrier polyvinyl chloride (PVC) packaged beef [4]. Because of the unique fresh characteristics, ground beef is a product that often has a reduced shelf life because of the manufacturing process creating a greater surface area for oxidative and degradative reactions [5].

Vacuum packaging has been a resource often used for decades when packaging meat to achieve extended storage periods and meet the fresh and frozen product shelf-life expectations throughout the meat industry. To achieve vacuum packaging platforms, the use of plastic film is inevitable, and it is required to protect consumer foods from contaminants while maintaining product shelf life and potentially reducing food waste [6]. Efforts in previous years have mainly focused on shelf life and surface color of fresh beef. Countless studies have evaluated fresh meat under retail conditions using packaging platforms such as the following: polyvinyl chloride (PVC) with an overwrapped foam tray [7–9] appearance; modified atmosphere packaging (MAP) with various gasses [10–12]; newer efforts using PVC overwrapping with mother bag packaging [13–15] that combines MAP gases and atmospheric transmission of PVC. In recent years, efforts have suggested the use of high-barrier, multi-layer, biodegradable food packaging could be beneficial as a replacement to current multi-layered film packaging, which lacks the ability to be recycled [16].

It is evident with the growing trends in use of vacuum packaging, the investigation of recyclable materials and various films used within the vacuum packaging platform is necessary. Thermoforming vacuum packaging is constructed by the forming of multi-layered films with heat, pressure, and forming duration [17]. Moreover, many combinations of materials exist in the formation of these multi-layer films; some of these materials include amorphous polyethylene terephthalate (A-PET), polyolefins (PO), ethylene vinyl alcohol (EVOH), polyvinylidene di-chloride (PVdC), and Nylon [17–20]. Although films typically comprised of PO and EVOH have been constructed with the intent to be recycled downstream of the consumer [17], with the development of recyclable films, a challenge

remains present due to limitations in the recycling process of flexible multi-layered films. A difficulty with recycling multi-layered films is the delamination of the individual layers within the constructed film which is currently not economically viable [17,18]. Nonetheless, efforts have been focused on identifying a viable option to recycle multi-layered films which include the technique of compatibilization, which can be done to produce a blended material [18]. In addition to compatibilization, the process of chemical recycling has been investigated as an option to recycle multi-layer packaging [17]. As technology within the packaging landscape of fresh meat evolves, the investigation of viable options in recycling multi-layer films is still essential to identifying the shelf-life performance of packaging films that could serve as a recycle ready option for the meat industry. Therefore, the objectives of this study were to evaluate the instrumental changes in surface color of fresh ground beef packaged in enhanced (ENB), standard (STB), and recycle-ready (RRF) vacuum packaging films and stored under simulated retail conditions.

2. Materials and Methods

2.1. Raw Materials

Coarse ground beef (80:20; lean:fat) packaged in 4.5 kg chubs (DuraChub, WINPAK, Winnipeg, MB, Canada) with an oxygen transmission rate of 0.9 cc/sg. m/24 h) was purchased from a commercial meat processing facility. Fresh, never-frozen chubs were transported under refrigerated conditions $1.5\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$ in the absence of light to the Auburn University Lambert Powell meat laboratory. Coarse ground beef was stored in the absence of light at $2.0\text{ }^{\circ}\text{C} \pm 1.0\text{ }^{\circ}\text{C}$ for 48 h prior to grinding and packaging. At the time of grinding, coarse ground beef (36 kg) was allocated randomly to 1 of 3 treatments ($n = 12$ kg/treatment) and ground once using a commercial meat grinder (Model 4346, Hobart

Corporation, Troy, OH, USA) 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).

2.2. Packaging

Ground beef bricks ($n = 5/\text{treatment}/\text{rep}$) were placed into a commercial packaging film (WINPAK, Winnipeg, MB, Canada) consisting of an enhanced barrier (175 μm nylon, enhanced EVOH, and polyethylene: ENB), standard barrier (175 μm nylon, EVOH, and Polyethylene: STB), and recycle ready film (175 μm polyolefins and EVOH: RRF). The non-forming film used for all packages was comprised of (75 μm polyester, EVOH, and polyethylene). The oxygen transmission rates (OTR) for each packaging treatment were as follows: ENB (0.2 cc/sq. m/24 h); STB (0.4 cc/sq. m/24 h); RRF (0.5 cc/sq. m/24 h).

However, the moisture vapor transmission rates for each treatment were as follows: ENB (3.3 g/sq. m/24 h); STB (3.3 g/sq. m/24 h); RRF (2.8 g/sq. m/24 h). Packages of ground beef bricks were sealed using a Variovac Optimus (OL0924, Variovac, Zarrentin am Schaalsee, Germany). After packaging, ground beef brick packages were individually identified and placed into dark storage at $2.2\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$ for 120 h.

2.3. Retail Display

Packaged ground beef was stored in the absence of light at $2.2\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$ for 120 h to simulate logistic conditions from manufacturer to retailer. Following dark storage, ground beef packages were placed into a three-tiered, lighted display case Turbo Air (Model 60DXB-N, Turbo Air Inc., Long Beach, CA, USA) operating at $3.0\text{ }^{\circ}\text{C} \pm 1.5\text{ }^{\circ}\text{C}$ with three 25 min defrost cycles occurring each day. Shelf-life timeline for measuring surface color began (Day 0) at the time of displaying ground beef packages under constant lighting for

simulated retail conditions. The 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) on each shelf. Ground beef packages were randomly dispersed throughout the display case shelves and rotated daily to simulated consumer packaging shifting that occurs at the retail counter.

2.4. Instrumental Color

Throughout the 15-day simulated retail display period, instrumental surface color was measured on packages of ground beef ($n = 75$) with a HunterLab MiniScan EZ colorimeter, Model 45/0 LAV (Hunter Associates Laboratory Inc., Reston, WV, USA). Prior to surface color readings, the colorimeter was standardized using a black and white tile covered with the packaging films to confirm instrument accuracy. Surface color readings were captured each day of the simulated display period at 17:00. Instrumental color values were determined from the mean of three readings on the surface of each ground beef through the intact package using illuminant A, with an aperture of 31.8 mm, and a 10° observer to measure lightness (L^*), redness (a^*) and yellowness (b^*) of each ground beef package [21]. In addition, 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: $\sqrt{a^{*2} + b^{*2}}$ 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 percentages of deoxymyoglobin ($DMB = \{[1.395 - (\{A_{572} - A_{700}\}/\{A_{525} - A_{700}\})]\} \times 100$), metmyoglobin ($MMB = \{2.375 \times [1 - (\{A_{473} - A_{700}\}/\{A_{525} - A_{700}\})]\} \times 100$),

and oxymyoglobin ($OMB = DMB - MMB$) according to [22] Meat Color Measurement Guidelines.

2.5. Statistical Analysis

All data were analyzed as a completely randomized design using the ground beef package as the experimental unit with 25 replications of each treatment. The ANOVA was generated using the GLIMMIX model procedure of SAS (version 9.2; SAS Inst. Inc. Cary, NC, USA) using day of simulated retail display as a repeated measure, with packaging, day, and packaging \times day interaction as the fixed effects. Least squares means were generated, and, when significant ($p < 0.05$) F-values were observed, least squares means were separated using pair-wise t-test (PDIFF option).

3. Results and Discussion

There were no ($p > 0.05$) interactive effects for packaging film \times day throughout the simulated retail display period on surface color values of vacuum-packaged ground beef. Ground beef displayed using ENB barrier packaging film was lighter ($p < 0.05$) L^* than ground beef packaged using STB or RRF (Table 2.1). Moreover, ground beef packages became lighter ($p < 0.05$) as the duration of storage time increased (Table 2.2). Similar results for surface lightness (L^*) have been noted when using various packaging methods such as vacuum-packaged, overwrapping, or the addition of gasses within the package of fresh ground meat [23]. Additionally, fresh meat under lighted display in a limited oxygen package has been reported to impact the formation of oxymyoglobin formation [24]. Changes that occurred to the surface color lightness (L^*) are likely a function of deoxymyoglobin formation that occurred during the simulated display period. Furthermore,

declining changes in lightness are similar with previous studies reporting vacuum-packaged ground beef became darker over a 20-day simulated display period following temperature abuse [23]. Ground beef packages were redder and more vivid ($p < 0.05$) when displayed using ENB packaging film, whereas RRF packages were more yellow (b^*) and had a greater ($p < 0.05$) hue angle (Table 2.1). Nonetheless, redness and vividness declined ($p < 0.05$) as storage duration in refrigerated display increased (Table 2.2). A decrease in redness (a^*) values suggest fresh meat products may be less accepted by consumers, due to the meat product presenting a darker red surface color. Previous studies have evaluated the influence of storage period and packaging method on beef steaks (*M. longissimus dorsi*) [25]. Similar results in vacuum-packaged steaks indicate a^* values may decline throughout the storage period [25]. Moreover, similar results for vacuum-packaged beef loins have been reported to the current study, indicating chroma values (surface color vividness) will decline as the duration of display increases [26].

Instrumental spectral reflectance data from 400 to 700 nm was used to calculate relative values for the red to brown ratio (630/580 nm), metmyoglobin (MMB), deoxymyoglobin (DMB), and oxymyoglobin (OMB) of ground beef surface color changes. The red to brown ratio for ground beef packaged using ENB barrier packaging film was greater ($p < 0.05$) than ground beef packaged in STB or RRF films (Table 2.1). Additionally, throughout the duration of the retail display period, the red to brown (630/580 nm) values declined ($p < 0.05$), resulting in a shift from a redder to browner surface color (Table 2.2). As noted in previous research, red to brown values tend to decline regardless of packaging method [27]. It is plausible the shift in calculated red to brown values is a function of greater metmyoglobin formation over the course of the extended display period. Moreover, relative

calculated values of oxymyoglobin captured through instrumental measurements indicated vacuum packages of ground beef in RRF film were greater ($p < 0.05$) than packages of ground beef in STB or ENB packaging films, respectively (Table 2.1). Surprisingly, calculated values of OMB increased ($p < 0.05$) in vacuum-packaged ground beef as the day of simulated display increased (Table 2.2). Interestingly, ground beef packaged using RRF films resulted in greater ($p < 0.05$) calculated relative values for MMB and OMB than ground beef packaged in STB or ENB packaging films (Table 2.1). However, as the time of display in days increased, calculated MMB increased ($p < 0.05$) and DMB values declined (Table 2.2). Changes recorded in calculated relative values of DMB, MMB, and OMB may be attributed to the oxygen transmission rates of the packaging films. In addition, it has been reported that the ratio of myoglobin forms in fresh meat can be influenced by the available oxygen, oxygen consumption rate, autoxidation of myoglobin or the reducing ability of metmyoglobin [28,29]. Current results for calculated myoglobin forms are similar to previous reports when evaluating vacuum-packaged fresh meats [30]. Furthermore, vacuum packaging of fresh meat can result in residual quantities of oxygen that may influence the autoxidation of DMB and MMB during extended storage periods [31]. However, it is plausible vacuum packaging resulted in a greater regeneration of NADH which has been reported to delay discoloration of fresh meats [32]. Nonetheless, it is widely known that a greater percentage of surface MMB greatly influences consumer purchasing intent at the retail counter. Regardless of the shelf-life duration for meat products, surface color and spoilage organism may contribute to the changes associated with a shift in surface color from DMB to MMB [3]. Therefore, the addition of continued research evaluating visual surface color of vacuum-packaged fresh meats is necessary.

4. Conclusions

Evaluation of vacuum packaging films for ground beef platforms indicated that ENB film provided a significant packaging solution for sustaining the fresh surface color of ground beef during a simulated retail display. When using ENB packaging films a reduction in surface color variation across the 15-day simulated retail display was noted when compared to STP and RRF packaging films. It is plausible the lack of color stability in ground beef surface color with RRF packaging film may have been impacted by the EVOH layer that exists within the layers of the packaging film. Furthermore, research evaluating RRF film is needed to identify the feasibility of vacuum packaging fresh meat, extension of shelf-life, reduction in lipid oxidation and visual surface color changes that may occur with a packaging film intended to be recycled after consumer use.

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TABLES

Table 2.1. Influence of packaging film on color values of vacuum-packaged ground beef during a simulated retail display.

	TRT ¹			
	ENB	STB	RRF	SEM *
Lightness (L*) ²	48.94 ^a	48.38 ^b	48.11 ^c	0.044
Redness (a*) ²	21.02 ^a	18.39 ^b	16.92 ^c	0.096
Yellowness (b*) ²	13.47 ^c	14.04 ^b	14.64 ^a	0.028
C* ³	25.02 ^a	23.26 ^b	22.55 ^c	0.067
Hue (°) ⁴	32.90 ^c	37.93 ^b	41.58 ^a	0.188
RTB ⁵	2.61 ^a	2.17 ^b	1.91 ^c	0.016
MMB (%) ⁶	25.22 ^c	33.66 ^b	39.83 ^a	0.321
DMB (%) ⁶	66.73 ^a	50.29 ^b	40.68 ^c	0.525
OMB (%) ⁶	8.05 ^c	16.05 ^b	19.49 ^a	0.218

¹ Packaging treatments are defined as follows: enhanced EVOH + polyethylene (ENB); nylon + EVOH + polyethylene (STB); and polyolefins + EVOH (RRF). ² 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). ³ C* (Chroma) is a measure of total color (larger number indicates a more vivid color). ⁴ Hue (°) angle represents the change in color from the true red axis (larger number indicates a greater shift from red to yellow). ⁵ RTB is the reflectance ratio of 630 nm / 580 nm and represents a change in the color of red to brown (larger value indicates a redder color). ⁶ Calculated percentages of oxymyoglobin (OMB), deoxymyoglobin (DMB), and metmyoglobin (MMB) using relative spectral values. ^{a-c} Mean values within a row lacking common superscripts differ ($p < 0.05$). * SEM, Standard error of the mean.

Table 2.2. Influence of retail display (d) on color values of vacuum-packaged ground beef.

	Instrumental Value								
	L* ¹	a* ¹	b* ¹	C* ²	Hue (°) ³	RTB ⁴	MMB (%) ⁵	DMB (%) ⁵	OMB (%) ⁵
Day 0	47.36 ^g	22.05 ^a	13.35 ^{h,i}	25.86 ^a	31.39 ^g	3.05 ^a	20.81 ^h	69.85 ^a	9.34 ^{i,j}
Day 1	48.03 ^f	21.66 ^{a,b}	13.20 ⁱ	25.42 ^b	31.53 ^g	2.88 ^b	22.06 ^h	69.18 ^a	8.76 ^j
Day 2	48.27 ^{e,f}	21.37 ^{b,c}	13.39 ^h	25.28 ^{b,c}	32.26 ^g	2.78 ^b	22.75 ^h	67.05 ^a	10.21 ^{g,h,i}
Day 3	48.57 ^d	20.80 ^{c,d}	13.71 ^{f,g}	24.99 ^{c,d}	33.63 ^f	2.59 ^c	25.78 ^g	63.31 ^b	10.91 ^{g,h}
Day 4	48.60 ^{c,d}	20.61 ^d	13.68 ^g	24.82 ^d	33.82 ^f	2.53 ^{c,d}	26.96 ^{f,g}	62.72 ^b	10.32 ^{g,h,i}
Day 5	48.98 ^a	20.45 ^d	13.88 ^f	24.80 ^d	34.47 ^f	2.44 ^d	28.33 ^f	61.77 ^b	9.90 ^{h,i,j}
Day 6	48.96 ^{a,b}	19.78 ^e	14.07 ^e	24.37 ^e	35.84 ^e	2.31 ^e	30.65 ^e	57.79 ^c	11.56 ^g
Day 7	48.98 ^{a,b}	18.62 ^f	14.26 ^d	23.58 ^f	37.97 ^d	2.10 ^f	34.78 ^d	51.36 ^d	13.85 ^f
Day 8	48.95 ^{a,b}	18.04 ^f	14.35 ^{c,d}	23.18 ^{f,g}	39.07 ^d	2.02 ^f	36.24 ^d	48.82 ^d	14.93 ^f
Day 9	48.84 ^{a,b,c}	17.42 ^g	14.50 ^{a,b,c}	22.80 ^g	40.35 ^c	1.91 ^g	38.36 ^c	44.55 ^e	17.09 ^e
Day 10	48.71 ^{b,c,d}	16.81 ^h	14.55 ^{a,b}	22.37 ^h	41.47 ^{b,c}	1.85 ^{g,h}	39.69 ^{b,c}	41.90 ^{e,f}	18.41 ^{d,e}
Day 11	48.46 ^{d,e}	16.42 ^{h,i}	14.63 ^a	22.13 ^h	42.29 ^{a,b}	1.80 ^{h,i}	40.87 ^b	39.84 ^{f,g}	19.29 ^{c,d}
Day 12	48.09 ^f	15.54 ^j	14.34 ^{c,d}	21.26 ^j	43.13 ^a	1.67 ^j	43.50 ^a	34.43 ^h	22.07 ^a
Day 13	48.11 ^f	16.04 ^{i,j}	14.45 ^{b,c}	21.69 ⁱ	42.48 ^{a,b}	1.74 ^{i,j}	41.59 ^{a,b}	37.88 ^g	20.53 ^{b,c}
Day 14	48.27 ^{e,f}	16.03 ^{i,j}	14.37 ^{c,d}	21.64 ^{i,j}	42.39 ^{a,b}	1.75 ^{i,j}	41.14 ^b	38.03 ^g	20.83 ^{a,b}
SEM *	0.097	0.214	0.063	0.149	0.419	0.035	0.718	1.174	0.488

¹ 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 C* (Chroma) is a measure of total color where a larger number indicates a more vivid color. ³ Hue (°) angle represents the change from the true red axis where a larger number indicates a greater shift from red to yellow. ⁴ 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). ⁵ Calculated percentages of oxymyoglobin (OMB), deoxymyoglobin (DMB), and metmyoglobin (MMB) using relative spectral values. ^{a-j} Mean values within a column lacking common superscripts differ ($p < 0.05$). * SEM, Standard error of the mean.

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Chapter III

Vacuum Packaging Can Extend Fresh Color Characteristics of Beef Steaks during Simulated Display Conditions

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Abstract:

Packaging technology is evolving, and the objectives of this study were to evaluate instrumental surface color, expert color evaluation, and lipid oxidation (TBARS) on beef *longissimus lumborum* steaks packaged in vacuum-ready packaging (VRF) or polyvinyl chloride (PVC) overwrap packaging. Paired strip loins (Institutional Meat Purchasing Specifications # 180) were cut into 2.54 cm-thick steaks and assigned randomly to one of two packaging treatments, VRF or PVC. Steaks packaged in VRF were lighter in color ($p < 0.05$) as the display period increased, whereas steaks packaged in PVC became darker ($p < 0.05$). Redness (a^*) values were greater ($p < 0.05$) for PVC steaks until day 5, whereas VRF steaks had a greater ($p < 0.05$) surface redness from day 10 to 35 of the display period. Calculated spectral values of red to brown were greater ($p < 0.05$) for steaks in VRF than PVC. In addition, expert color evaluators confirmed VRF steaks were less brown and less discolored ($p < 0.05$) from day 5 to 35 of the display. Nonetheless, lipid oxidation was greater ($p < 0.05$) for PVC steaks from day 10 through day 35 of the display. Results from this study suggest that the use of vacuum packaging for beef steaks is plausible for maintaining surface color characteristics during extended display periods.

Keywords: instrumental color; overwrapped packaging; simulated retail display; TBARS; vacuum packaging

1. Introduction

Vacuum packaging using form-and-fill technology is a packaging method that is becoming one of the most prominent packaging systems in use within the retail meat industry [1]. Unfortunately, previous research focused on form-and-fill vacuum packaging for use with fresh meat storage in a retail setting is limited. Previous efforts in vacuum packaging uses for fresh meat have focused on using bag or skin technologies [2]. Form-and-fill packaging systems use one film to construct a pouch with time, pressure, and heat. After forming the pouch, meat products are placed into the pouch and a second film is overlaid and sealed within the vacuum chamber. Furthermore, vacuum packaging has accounted for 40% of packaging types within meat cases, with most products packaged using a roll-stock machine [1]. It has been noted that PVC overwrapped packaged beef has decreased in use by 46% from 2018 to 2021 [1].

While the meat surface color is still regarded as one of the greatest determining factors consumers utilize when purchasing fresh beef in the retail setting [3,4], packaging technologies are pivotal in maintaining the surface color of fresh meat. PVC is a packaging method used with fresh meat that allows oxygen and other gasses to permeate through the film in large quantities allowing oxygen to bind with myoglobin. The oxymyoglobin state of beef is often correlated with a fresher and more wholesome product by consumers due to a bright cherry red color [5]. Creating a shift from the current industry's primary packaging methods of PVC to vacuum packaging is unclear; however, many advantages such as the extension of shelf life and color stability may exist with the use of vacuum packaging in fresh meat applications. Vacuum packaging allows meat products to remain more color stable over extended periods of time within retail coolers [6]. Reportedly, vacuum

packaging has been known to extend the storage period of fresh meat products by reducing the amount of residual oxygen within the package [7,8].

With the ability to extend fresh meat storage through the use of vacuum packaging, it is a packaging system that is quickly becoming an essential part of the solution for meeting sustainability programs and reducing food waste for the meat industry. Food waste has been characterized as edible food that is not consumed and often discarded by consumers or retailers [9]. It has also been reported that meat, poultry, and fish were the top food groups contributing to an estimated food loss approaching \$48 billion in 2010 [9]. In addition, approximately 43 billion pounds of food at the retail level and 90 billion pounds at the consumer level have not been consumed [9]. Aside from food loss and food waste issues that still reside within the meat and food industry, there exist excessive food packaging materials entering the waste management system destined for landfills. In 2017, there were approximately 26.3 billion pounds of beef, 25.6 billion pounds of pork, and 42.2 billion pounds of chicken that American meat companies processed [10].

The packaging of fresh meat products is a necessity for the purpose of maintaining a fresh and wholesome product during retail display for consumer purchases. With the volume of packaging necessary to address the meat industry's demand of packaged meat products, it is essential that a packaging option be investigated for extending storage times of meat products. New packaging technologies could assist in reducing the volume of markdowns and throwaways that occur at the retail counter. A large percentage of fresh meat has been packaged with a form-and-fill roll stock machine, which utilizes multi-layered packaging films [11]. Multi-layered vacuum packaging is constructed with a wide variety of materials that can include amorphous polyethylene terephthalate, polyolefines,

ethylene vinyl alcohol, polyvinylidene di-chloride, and nylon [10–13]. Currently, the ability to recycle multi-layered films lacks economic viability due to the nature of the film layering [14].

Nonetheless, multi-layered vacuum packaging films are growing in popularity for vacuum packaging platforms; unfortunately, these packaging films are often constructed without sustainable or recycle-ready materials. Limitations in recycle-ready packaging materials can create difficulties downstream from the consumer with sustainable meat packaging due to challenges in the delamination process of multi-layered films [10,15]. Nevertheless, an investigation into using multi-layered films is a necessity to extend the fresh-meat shelf life. With a need for greater storage periods of fresh meat by retailers, customers, and consumers, the agriculture industry could focus its efforts on becoming more sustainable through innovative developments of packaging materials for meat and meat products. Therefore, the objectives of the current study were to investigate the feasibility of using VRF vacuum packaging film in place of PVC overwrapping on beef strip loin steaks and the subsequent impacts on surface color characteristics during a simulated retail display period.

2. Materials and Methods

2.1. Raw Materials

Cattle ($n = 7$) were harvested under simulated commercial conditions according to USDA humane slaughter standards at the Auburn University Lambert Powell Meat Laboratory after a 12 h rest period. After harvest, carcasses were chilled for 48 h at 2 °C. Following carcass chilling, beef carcasses were subsequently fabricated into left- and right-side paired (IMPS # 180) boneless beef strip loins, vacuum packaged (3 mil, Clarity

Vacuum Pouches, Kansas City, MO, USA), and stored in the absence of light for 10 days to simulate boxed beef fabrication and logistics. After aging, beef strip loins were cut into 2.54-cm-thick steaks (N = 112 steaks/packaging treatment) using a BIRO bandsaw (Model 3334, BIRO Manufacturing Company, Marblehead, Ohio, USA). At the time of steak cutting, steaks from each loin were allocated randomly to one of two packaging treatments, VRF or PVC. The allocated steaks were placed onto a plastic tray and allowed to bloom for 30 min prior to packaging.

2.2. Packaging and Simulated Display Conditions

After steak portioning, steaks allocated to vacuum packaging (VRF) were packaged using a Reiser form-and-fill vacuum packaging machine (Optimus OL0924, Variovac, Zarrentin, Germany) and sealed. Steaks were packaged in VRF packaging films (O₂ transmission rate = 0.8 cc/sq. m²/24 h/atm). Steaks allocated to traditional overwrapping (PVC) were placed onto a foam tray (2s, Genpak, Charlotte, NC, USA) with an absorbent moisture pad (DRI-LOC AC-50, Novipax, Oak Brook, IL, USA) and wrapped by hand with a polyvinyl chloride film (O₂ transmission rate = 14,000 cc O₂/m²/24 h/atm). Packaged steaks were placed onto lighted shelves within a refrigerated retail display case (Model TOM- labels 60DXB-N, Turbo Air Inc., Long Beach, CA, USA). Packages of steaks were displayed for 35 days at 3 °C ± 1.2 °C, and the case temperature throughout the display period was monitored with temperature data recorders (Model-TD2F, ThermoWorks, American Fork, UT, USA) placed on the center of each display shelf. Packages of steaks were displayed on shelves under continuous LED lighting with an intensity of 2297 lux for each shelf. Lighting intensity was measured (ILT10C,

International Light Technologies, Peabody, MA, USA) throughout the duration of the simulated display period. Additionally, packages of steaks were distributed evenly across all shelves and rotated daily from side to side and front to back to simulate consumer movement. Fresh meat characteristics of instrumental color, surface color, lipid oxidation, purge loss, and pH were measured on days 0, 5, 10, 15, 20, 25, 30, and 35 throughout the simulated display period.

2.3. Instrumental Color

Throughout the 35-day simulated retail display period, the instrumental surface color was measured on packaged steaks ($n = 28$) with a HunterLab MiniScan EZ colorimeter, Model 45/0 LAV (Hunter Associates Laboratory Inc., Reston, WV, USA). Prior to surface color readings, the colorimeter was standardized using a black and white tile. Instrumental color values were determined from the mean of three readings through the surface of each unopened package using illuminant A, an aperture of 31.8 mm, and a 10° observer. Packages of steaks were evaluated for lightness (L^*), redness (a^*), and yellowness (b^*) using the Commission Internationale de l' Eclairage guidelines for surface color [16]. In addition, the hue angle was calculated as $\tan^{-1}(b^*/a^*)$, with a greater value indicative of the surface color shifting from red to yellow. Chroma (C^*) was calculated as $\sqrt{a^{*2} + b^{*2}}$, where a larger value indicates a more vivid color. Lastly, reflectance values within the spectral range of 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 values of deoxymyoglobin ($DMb = \{[1.395 - (\{A_{572} - A_{700}\}/\{A_{525} - A_{700}\})]\} \times 100$), metmyoglobin ($MMb = \{2.375 \times [1 - (\{A_{473} - A_{700}\}/\{A_{525} - A_{700}\})]\} \times 100$), and

oxymyoglobin (OMb = DMb – MMb) according to color guidelines previously described [17].

2.4. Expert Color Evaluation

A five-member, expert color panel was used to evaluate the surface color of packaged beef boneless strip steaks during the simulated retail display period. Color measuring experts used anchors for scoring surface color discoloration previously described and modified from meat color guidelines [12]. At 16:00 h on the day of simulated display, experts rated surface color changes for steaks (n = 28) every 5 days for 35 days of refrigerated storage. Surface color ratings were created for steaks packaged under vacuum (VRF) for the initial beef color (1 = extremely bright purple-red, 2 = bright purple-red, 3 = moderately bright purple-red, 4 = slightly purple-red, 5 = slightly dark purple, 6 = moderately dark purple, 7 = dark purple, 8 = extremely dark purple), whereas packages of PVC overwrapped steaks were rated for the initial beef color (1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright, 4 = slightly bright cherry-red, 5 = slightly dark cherry-red, 6 = moderately dark red, 7 = dark red, 8 = extremely dark red). Both VRF- and PVC-packaged steaks were rated for the amount of browning (1 = no evidence of browning, 2 = dull, 3 = grayish, 4 = brownish gray, 5 = brown, and 6 = dark brown) and percent (%) discoloration (1 = no discoloration [0%], 2 = slight discoloration [1–20%], 3 = small discoloration [21–40%], 4 = modest discoloration [41–60%], 5 = moderate discoloration [61–80%], 6 = extensive discoloration [81–100%]).

2.5. Purge Loss and Fresh Muscle pH

Prior to conducting lipid oxidation analysis, steaks were removed from their respective packaging materials, blotted dry, and weighed on an analytic balance (PB3002-S, Mettler Toledo, Columbus, OH, USA). Purge loss was calculated as $[(\text{packaged steak weight} - \text{steak weight}) \div \text{packaged steak weight} \times 100]$. After capturing the purge loss for each steak, fresh muscle pH was measured in duplicate with a glass electrode inserted into two random locations within the steak and attached to a pH meter (Model-HI99163, Hanna Instruments, Woonsocket, RI, USA). Prior to measuring, the pH probe was calibrated (pH 4.0 and 7.0) using 2-point standard buffers (Thermo Fisher Scientific, Chelmsford, MA, USA) and again after 10 readings.

2.6. Lipid Oxidation

Packaged steaks ($n = 56$) were removed from their packaging material and sampled for 2-thiobarbituric acid reactive substances (TBARS) using a previously described method [18]. Steaks were trimmed of all external fat and connective tissue then minced together to form a uniform sample of the entire steak. Approximately 2 g of minced muscle was homogenized with 8 mL of cold (1 °C) 50 mM phosphate buffer (pH of 7.0 at 4 °C) containing 0.1% EDTA, 0.1% n-propyl gallate, and 2 mL trichloroacetic acid (Sigma-Aldrich, Saint Louis, MO, USA). Homogenized samples were subsequently filtered through Whatmann No. 4 filter paper, and duplicate 2-mL aliquots of the clear filtrate were transferred into 10-mL borosilicate tubes, mixed with 2 mL of 0.02 M 2-thiobarbituric acid reagent (Sigma-Aldrich, Saint Louis, MO, USA) then boiled for 20 min. After boiling, tubes were placed into an ice bath for 15 min. Absorbance was measured at 533nm with a

spectrophotometer (Turner Model–SM110245, Barnstead International, Dubuque, IA, USA) and multiplied using a factor of 12.21 to obtain the TBARS value (mg malonaldehyde/kg of meat).

2.7. Statistical Analysis

Data were analyzed with the GLIMMIX procedures of SAS (ver. 9.4; SAS Institute Inc. Cary, NC, USA) with treatment serving as the lone fixed effect and replication serving as the random effect for instrumental color, expert color, lipid oxidation, purge loss, and pH. All data were analyzed in a modified randomized design with steak serving as the experimental unit. For expert surface color rating data, the expert color panelist was included as a random factor, and panelist \times day of display was included as a random, repeated factor (with a first-order autoregressive covariance structure). Least-squares means were generated, and when significant ($p \leq 0.05$) F-values were observed, least squares means were separated using a pair-wise t-test (PDIFF option).

3. Results and Discussion

3.1. Instrumental Beef Color

The instrumental surface color of packaged steaks was measured throughout a 35-day simulated retail period. An interaction of the packaging method \times day of display on steak surface lightness (L^*) occurred (Table 3.1). Steaks packaged in PVC were lightest ($p < 0.05$) on day 0 and became darker as the length of display period increased (Table 3.1). However, from day 20 through day 35 of the display, steaks packaged using VRF were lighter ($p < 0.05$) than steaks packaged using PVC methods (Table 3.1). Additionally,

surface redness (a^*) for beef steaks packaged in PVC were redder ($p < 0.05$) from day 0 through day 15 of the display period (Table 3.1), whereas steaks packaged in VRF became significantly redder ($p < 0.05$) until the conclusion of the study on day 35 (Table 3.1). Greater a^* values are indicative of a redder fresher color and have a greater consumer appeal at the time of the consumers' purchasing decision. PVC-packaged steaks maintained greater ($p < 0.05$) values for yellowness (b^*) throughout the duration of simulated retail display than steaks packaged in VRF (Table 3.1). The changes in surface color for steaks packaged using VRF indicated surface lightness and redness were more stable throughout the entire simulated retail period than steaks packaged in PVC. As expected during a simulated retail period, fresh steaks packaged in an oxygen-rich permeable method such as PVC will have a brighter surface color initially. Similar findings have reported that ground beef packaged using PVC methods resulted in greater L^* values on day 0, along with greater a^* and b^* through only 50% of the display period [5] when displayed up to 35 days. Moreover, ground beef patties when packaged with PVC materials have recorded similar results, indicating a^* values will decline within the first 5 days of the display period [19]. However, a^* values for ground beef patties packaged using a vacuum packaging platform have been reported to increase throughout a display period [19]. Furthermore, a study evaluating the surface color of beef steaks indicated a^* values were greater for vacuum packaging rather than other packaging types at the conclusion of a 35-day study [20]. It appears the results for b^* values of ground beef and steaks are consistent with the current study, resulting in a decline during a 5-day retail storage period when packaged in PVC. Regardless of the fluctuation of yellowness, the current and previous results suggest b^* values are less stable regardless of the packaging method [5,19–21].

There was a packaging method \times day of display interaction for surface color chroma (C^*) and hue angles (Table 3.1). The instrumental surface color of steaks packaged in PVC was more vivid ($p < 0.05$) on day 0 but C^* values declined as the duration of display increased. However, steaks packaged with VRF became more vivid ($p < 0.05$) from day 25 through 35 of the simulated retail display period (Table 3.1). In addition, steaks packaged with PVC had greater ($p < 0.05$) hue angles indicative of a surface color shift from red to yellow from day 5 through 35. It appears that the reduction in oxygen exposure for steaks in VRF packages protected the surface color of steaks by sustaining the vividness and reducing the shift from red to yellow. Similar results for fresh packaged beef C^* and hue angle values have been reported to decline during the initial 10 days of a simulated display period when using an oxygen-rich packaging method such as PVC [22]. Changes in surface color values for the hue angle and C^* can be used as a great indicator for observing meat discoloration in retail display settings [19–24]. Interestingly, C^* (vividness) for steaks packaged in VFR in the current study differ from previous C^* results that did not differ throughout a 35-day display period [23]. It should be noted that as the percentage of oxygen exposure to the steak surface increases a reduction in the hue angle and C^* will likely occur during retail display periods as the surface color shifts from red to brown with the formation of metmyoglobin [22–24].

The interactive influence for packaging method \times day of display remained for calculated spectral values of red to brown (630:580 nm) and relative forms of myoglobin (Table 3.1). Red to brown values were greater ($p < 0.05$) for steaks packaged in PVC until day 5 of the simulated display period. However, from day 10 through 35, PVC-packaged steaks' surface color showed a greater shift from red to brown. Steaks packaged in VRF

had less ($p < 0.05$) discoloration from red to brown after day 5 through day 35 (Table 3.1). Previous studies have [25] reported similar findings indicating a decline in calculated red to brown values throughout 7 days of simulated display for beef packaged in PVC [25]. It is expected that calculated spectral values for the surface color of fresh beef will shift from a brighter red to brown as the duration of a simulated retail display increases. Steaks packaged in VRF had the greatest ($p < 0.05$) amount of calculated metmyoglobin (MMb) on day 0 (Table 3.1). However, as expected from days 5 to 35, steaks packaged using PVC had greater ($p < 0.05$) calculated relative values for MMb. As expected, steaks packaged in VRF had greater ($p < 0.05$) calculated deoxymyoglobin (DMb) values throughout the entire simulated retail display period (Table 3.1) because of limited oxygen exposure. Interestingly, calculated relative values of oxymyoglobin (OMb) were greater ($p < 0.05$) for steaks packaged using PVC packaging materials throughout the entire simulated retail display period (Table 3.1). The results for calculated spectral values reported are likely due to the oxygen permeability of the PVC package resulting in greater exposure of the steak surface to an oxygen-rich atmosphere. Greater formations of MMb in PVC have been associated with greater amounts of lipid oxidation [26,27] and the relationship of oxidation during the transition of myoglobin pigment from OMb to MMb [26–28].

3.2. Expert Color Evaluation

Fresh steaks were evaluated by experts for visual surface color variations during a simulated retail display for up to 35 days. However, the evaluation of steaks packaged in aerobic PVC packaging materials was discontinued after day 20 due to total surface color deterioration. An interaction of the packaging method \times day of display occurred for the

surface color evaluation (Table 3.2). Trained expert evaluators noted that values for the initial beef color, amount of browning, and surface discoloration deteriorated ($p < 0.05$) for steaks packaged in PVC from day 5 through day 20 (Table 3.2). The surface color of steaks packaged in PVC materials became darker, with a greater amount of browning, and a greater percentage of discoloration as the duration of display increased. As a result of significant surface discoloration, PVC-packaged steaks used for expert color evaluation were discarded on day 20 of the display period. The changes in visual surface color are influential in driving consumer purchasing intent and the lack of storage for PVC steaks may contribute to greater throwaway by the retailer. Steaks packaged in VRF had initial beef colors that decreased ($p < 0.05$), and the amount of browning and surface discoloration were less ($p < 0.05$) than steaks packaged in PVC throughout the duration of the study (Table 3.2). Interestingly, steaks packaged in VRF were darker at day 0, but the visual steak color turned brighter purple red with less browning and surface discoloration throughout a 35-day simulated retail period. Results from the current study agree with previous findings when using vacuum packaging. Beef's surface color tends to remain visually stable throughout the duration of the study, whereas high-oxygen packaging of fresh beef can show rapid color deterioration [29]. The color stability of fresh beef is dependent on controlling countless factors such as pH, temperature, light, lipid oxidation, residual oxygen, MMb-reducing systems, reducing equivalents, and the oxygen consumption rate [30,31]. It is plausible that the transformation from OMb to MMb in PVC-packaged steaks was due to greater amounts of lipid oxidation. Furthermore, limited surface color variation of steaks packaged in VRF may be attributed to a lack of residual

oxygen within the packaging, influencing and reducing the amount of oxidation occurring in vacuum-packaged fresh beef products.

3.3. Lipid Oxidation

There was an interactive effect of the packaging method \times day of display for lipid oxidation on fresh beef steaks (Figure 3.1). The packaging method did not alter ($p > 0.05$) lipid oxidation through day 5 of the simulated retail display period. However, from days 10 through 35 of the storage period, lipid oxidation was greater ($p < 0.05$) for steaks packaged using PVC methods. Lipid oxidation of fresh steaks using PVC packaging from the current study agrees with previous simulated retail storage studies measuring an expected storage period in a retail setting of 3 to 7 days [32]. The exposure to greater amounts of oxygen across the packaging material can result in increased catalysis of lipid oxidation [33,34]. Moreover, greater lipid oxidation can be correlated to reduced consumer palatability due to the deterioration of the surface color and accumulation of off flavors [35]. Unfortunately, the evaluation of sensory taste characteristics was not completed during the current study, but future studies on the extended storage of fresh beef influencing lipid oxidation and sensory characteristics would be warranted.

3.4. Purge Loss

A packaging method \times day of display interaction occurred for the purge loss of fresh beef steaks (Figure 3.2). The purge loss was greatest ($p < 0.05$) for steaks packaged in PVC materials on day 25 of the simulated display period and the lowest on day 0. The packaging method influenced the purge loss on day 0, with steaks packaged in VRF having a greater ($p < 0.05$) percentage of moisture loss. It is plausible that the method of vacuum

packaging using the form-and-fill machine caused more moisture to be pressed out of the steak at the time of package sealing. However, the purge loss in vacuum-packaged meat products can result in an unappealing visual appearance for consumers due to the accumulation of purge in the packaging [36,37]. The results from the current study differ from previous results where values for purge loss using vacuum-packaging platforms were greater than PVC or alternative packaging such as modified atmosphere packaging platforms [38].

3.5. pH

The interactive influence of the packaging method \times day of display for fresh muscle pH values is presented in Figure 3.3. Fresh muscle pH values were recorded within muscle pH values (5.1 to 5.8) throughout the duration of the simulated display period. Values for fresh muscle pH were greatest ($p < 0.05$) on day 10 in steaks packaged using PVC methods. At the time of harvest and before chilling, carcasses were rinsed with an FDA-GRAS (U.S. Food and Drug Administration-Generally Recognized as Safe) organic acid (lactic acid). The combination of vacuum packaging and the organic carcass wash may have contributed to the decline in fresh muscle pH of VRF-packaged steaks, causing a shift in the visual and instrumental surface color variations reported within the current study. Furthermore, it is plausible that pH values for VRF declined due to an increase in lactic acid bacteria that can be present in vacuum-packaged fresh meats. With limited residual oxygen within the vacuum package, favorable conditions for anaerobic lactic acid bacteria may have caused fresh muscle pH to decline as lactic acid bacteria populations increased

[5,39]. In addition, lactic acid bacteria can be associated with low-pH (<5.8) vacuum-packaged meats due to a lower residual oxygen environment [40].

4. Conclusions

It is feasible that the storage of beef strip loin steaks using vacuum packaging, VRF, can provide a longer, fresh, refrigerated storage period than steaks packaged in traditional PVC packaging. It is evident that VRF displayed a more color-stable product throughout the duration of simulated retail display. Additionally, VRF maintained less oxidation throughout the display period, whereas steaks packaged in PVC tended to have greater oxidation leading to greater amounts of surface discoloration in beef products. The current results suggest that the vacuum-packaged film used within the current study is an acceptable replacement to traditional packaging methods of PVC for packaging whole muscle beef steaks for up to 35 days of refrigerated retail storage. However, additional research should be considered to evaluate the sensory taste profiles of vacuum packaging used for extended storage periods and the implications for flavor characteristics of beef steaks.

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TABLES & FIGURES

Table 3.1. The interactive impact of packaging method × day of display for instrumental surface color values on fresh beef strip loin steaks during a simulated retail display.

	Day								SEM*
	0	5	10	15	20	25	30	35	
PVC									
L* ¹	46.85 ^a	45.42 ^{abc}	44.26 ^{cde}	44.37 ^{bcde}	43.31 ^{de}	43.08 ^{def}	42.47 ^{efg}	40.63 ^g	0.713
a* ¹	29.57 ^a	25.40 ^b	19.33 ^d	15.93 ^e	15.69 ^e	15.79 ^e	15.93 ^e	15.99 ^e	0.704
b* ¹	21.33 ^a	19.97 ^b	17.71 ^c	15.13 ^d	14.29 ^{de}	14.03 ^{de}	13.69 ^e	13.44 ^e	0.392
C* ²	36.47 ^a	32.32 ^b	26.31 ^c	22.07 ^{efg}	21.41 ^g	21.31 ^g	21.16 ^g	21.01 ^{gh}	0.672
Hue (°) ³	35.76 ^d	38.22 ^{cd}	43.24 ^{ab}	43.81 ^a	42.87 ^{ab}	42.16 ^{ab}	41.08 ^{abc}	40.20 ^{bc}	1.185
RTB ⁴	5.28 ^a	3.94 ^b	2.57 ^{de}	1.92 ^f	1.99 ^f	2.03 ^f	2.07 ^f	2.22 ^{ef}	0.143
MMb ⁵	20.40 ^{ef}	28.08 ^d	40.18 ^{abc}	43.22 ^a	41.09 ^{ab}	38.81 ^{abc}	37.14 ^{bc}	34.59 ^c	2.081
DMb ⁵	4.89 ^f	7.68 ^{ef}	14.27 ^e	24.25 ^d	33.14 ^c	34.91 ^c	35.74 ^c	38.74 ^c	3.161
OMb ⁵	74.71 ^a	64.25 ^b	45.55 ^c	32.53 ^d	25.77 ^e	26.28 ^e	27.12 ^e	26.67 ^e	1.815
VRF									
L* ¹	41.17 ^{fg}	42.56 ^{efg}	43.32 ^{de}	44.86 ^{abcd}	45.41 ^{abc}	46.37 ^{ab}	46.58 ^a	45.84 ^{abc}	0.713
a* ¹	15.72 ^c	19.54 ^d	19.56 ^d	19.89 ^{cd}	20.26 ^{cd}	20.55 ^{cd}	20.91 ^{cd}	21.59 ^c	0.704
b* ¹	11.03 ^{fg}	9.69 ^h	9.85 ^h	10.26 ^{gh}	10.48 ^{gh}	11.07 ^{fg}	11.59 ^f	12.12 ^f	0.392
C* ²	19.26 ^h	21.82 ^{fg}	21.91 ^{fg}	22.39 ^{efg}	22.82 ^{efg}	23.36 ^{def}	23.92 ^{de}	24.77 ^{cd}	0.672
Hue (°) ³	35.13 ^d	26.41 ^e	26.71 ^e	27.28 ^e	27.31 ^e	28.26 ^e	29.00 ^e	29.32 ^e	1.185
RTB ⁴	2.12 ^f	3.18 ^c	3.10 ^c	2.90 ^{cd}	2.85 ^{cd}	2.70 ^d	2.57 ^{de}	2.69 ^d	0.143
MMb ⁵	34.97 ^c	14.54 ^g	15.20 ^{fg}	17.20 ^{fg}	18.62 ^{efg}	20.62 ^{ef}	23.56 ^{de}	24.18 ^{de}	2.081
DMb ⁵	53.28 ^b	86.97 ^a	87.40 ^a	88.35 ^a	88.95 ^a	86.65 ^a	82.89 ^a	83.98 ^a	3.161
OMb ⁵	11.75 ^f	2.69 ^h	2.90 ^h	5.67 ^{gh}	7.57 ^{fgh}	7.26 ^{fgh}	6.63 ^{gh}	8.16 ^{fg}	1.815

¹ 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 C* (Chroma) is a measure of total color (larger number indicates a more vivid color).³ Hue (°) angle represents the change from the true red axis (larger number indicates a greater shift from red to yellow).⁴ RTB calculated as 630 nm ÷ 580 nm, which represents a change in the color of red to brown (larger value indicates a redder color).⁵ Calculated percentages of deoxymyoglobin (DMb), metmyoglobin (MMb), and oxymyoglobin (OMb) using relative spectral values. ^{a–h} Mean values within a row and a packaging method lacking common superscripts differ (p ≤ 0.05). * SEM, Standard error of the mean. Bold font, the packaging methods investigated.

Table 3.2. Interactive influence of packaging method × day of display for expert surface color evaluation on fresh beef strip loin steaks during a simulated retail display.

	Day of Simulated Display							SEM*	
	5	10	15	20	25	30	35		
PVC¹									
Initial Beef Color	1.20 ^b	3.34 ^f	4.48 ^c	5.09 ^c	6.50 ^b	--	--	--	0.154
Amount of Browning	1.00 ^c	1.79 ^c	3.94 ^b	3.75 ^b	4.52 ^a	--	--	--	0.080
Surface Discoloration	1.09 ^{efg}	1.79 ^d	3.12 ^c	4.20 ^b	4.76 ^a	--	--	--	0.073
VRF²									
Initial Beef Color	7.34 ^a	5.57 ^c	4.17 ^c	4.15 ^c	3.41 ^f	3.42 ^f	3.52 ^f	2.79 ^g	0.154
Amount of Browning	1.00 ^c	1.17 ^{dc}	1.30 ^d	1.00 ^c	1.23 ^d	1.00 ^c	1.00 ^c	1.20 ^{dc}	0.080
Surface Discoloration	1.03 ^{fg}	1.21 ^{ef}	1.01 ^g	1.01 ^g	1.23 ^c	1.00 ^g	1.00 ^g	1.06 ^{efg}	0.073

¹ PVC color anchors: Initial Beef Color (1 = Extremely bright cherry-red to 8 = Extremely dark red); Amount of Browning (1 = No Evidence of Browning to 6 = Dark Brown); Surface Discoloration (1 = No discoloration (0%) to 6 = Extensive discoloration (81–100%)). ² VRF color anchors: Initial Beef Color (1 = Extremely bright purple red to 8 = extremely dark purple red); Amount of Browning (1 = No Evidence of Browning to 6 = Dark Brown); Surface Discoloration (1 = No discoloration (0%) to 6

= Extensive discoloration (81–100%). ^{a–h} Mean values within a row and packaging method lacking common superscripts differ ($p \leq 0.05$). * SEM, Standard error of the mean. Bold font, the packaging methods investigated.

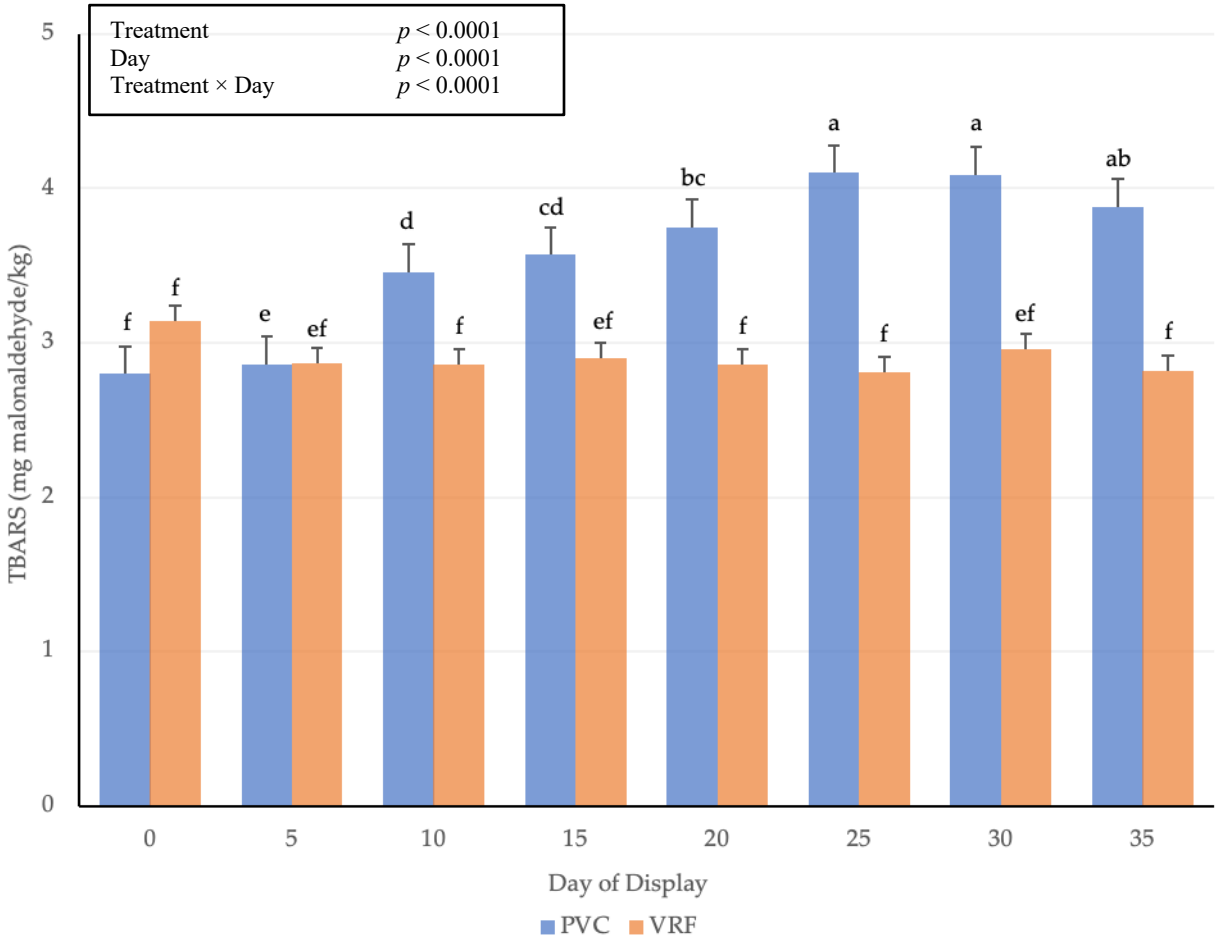


Figure 3.1. Interactive influence of packaging method \times day of display for 2-Thiobarbituric acid reactive substances (TBARS) on beef strip loin steaks during a simulated retail display. Bars lacking common letters differ ($p \leq 0.05$).

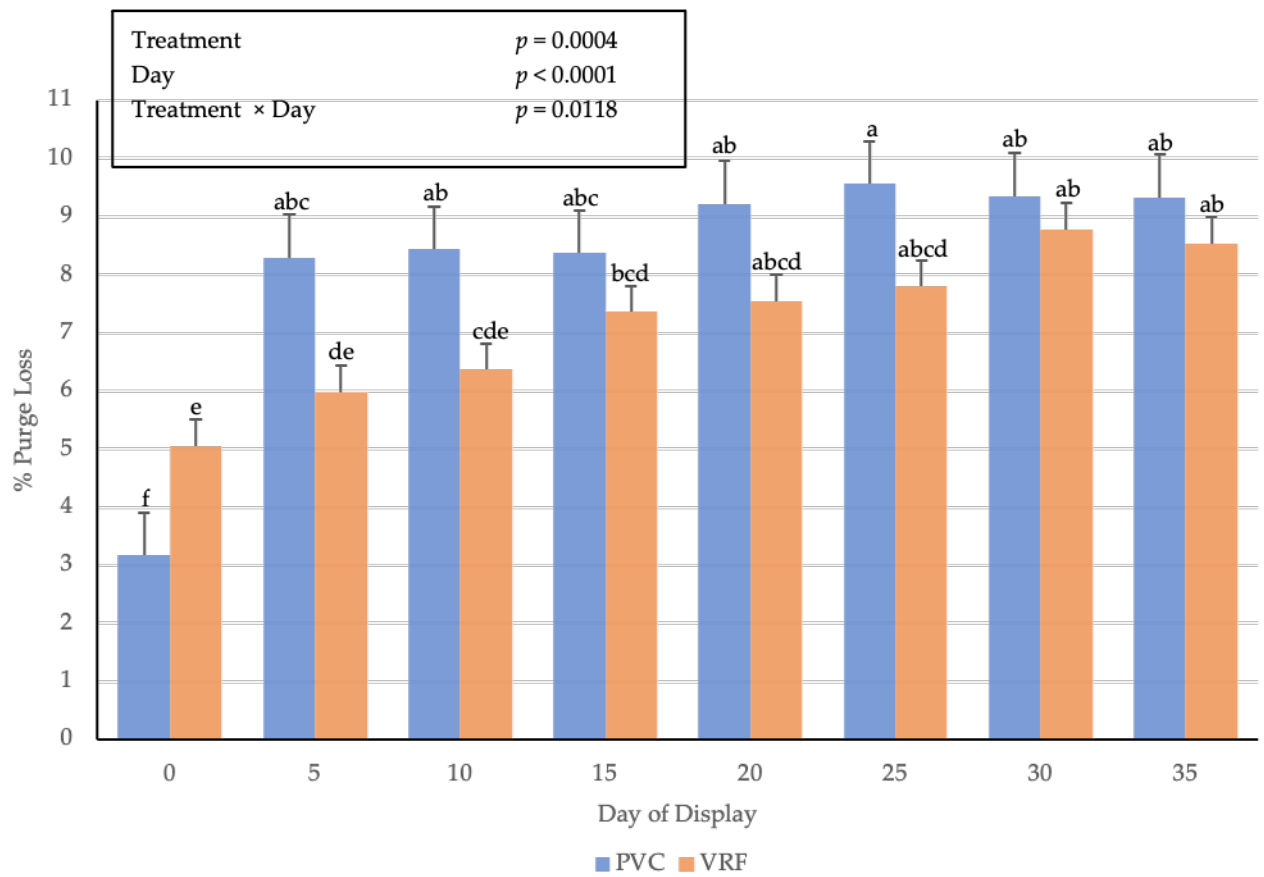


Figure 3.2. Interactive influence of packaging method \times day of display for purge loss (%) on beef striploin steaks during a simulated retail display. Bars lacking common letters differ ($p \leq 0.05$).

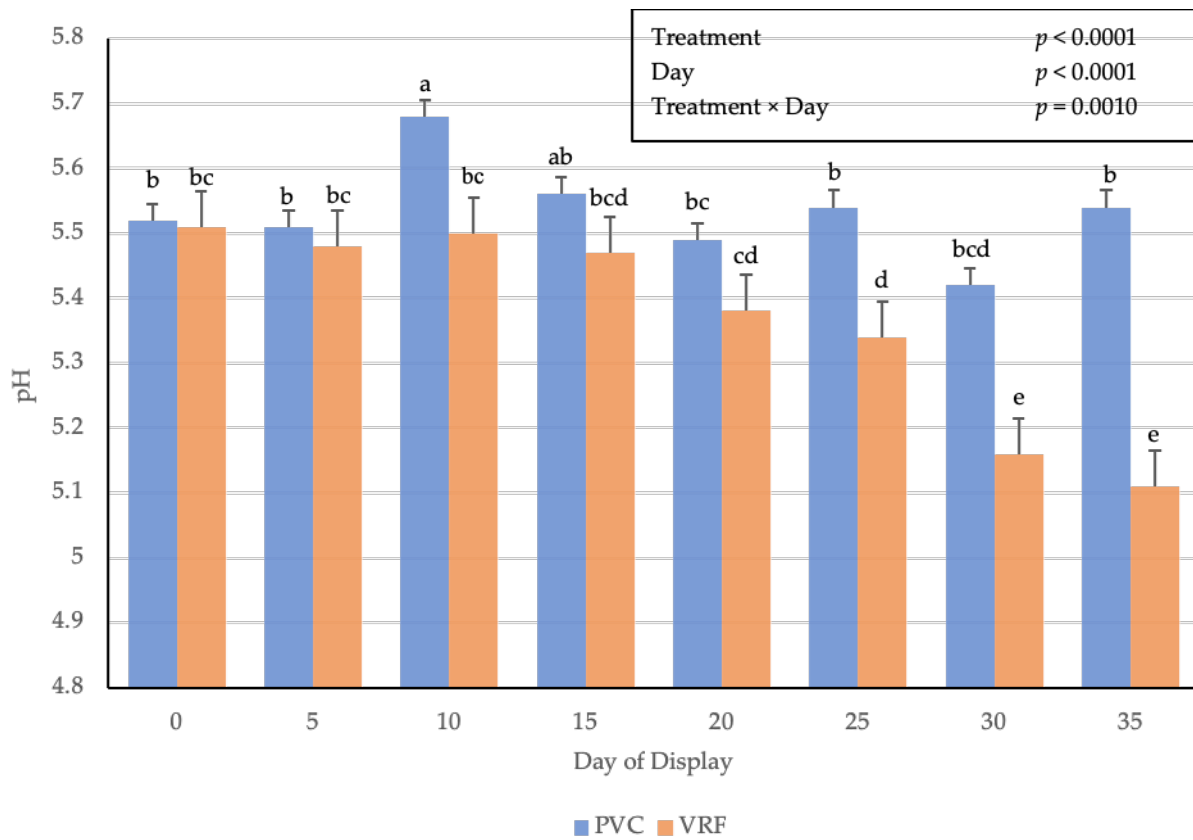


Figure 3.3. The interactive influence of packaging method × day of display for fresh muscle pH on beef strip loin steaks. Bars lacking common letters differ ($p \leq 0.05$).

APPENDICES

APPENDIX A

Thiobarbituric Acid Reactive Substances (TBARS)

Chemicals:

Water – HPLC grade or distilled deionized water
Potassium phosphate (monobasic) KH_2PO_4
Potassium phosphate (dibasic) K_2HPO_4
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 KH_2PO_4 , 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 K_2HPO_4 , place in a 1 L volumetric flask, dissolve and bring to volume with distilled-deionized 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

Store all reagents under refrigerated conditions, but do not store solutions in the coldest regions of the refrigerator as some of these solutions will freeze at low temperatures.

Analysis:

General notes: Prepare and turn on water bath-set temperature at 100 °C. It takes approximately 1h 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. Prepare three "Blank" tubes, using 2 ml of distilled-deionized water.
7. Prepare one "Standard" tube, using 2 ml of phosphate buffer. (Note: after this point, time is extremely critical. Make sure that the water bath is at the correct temperature and level prior to continuing).
8. Add 2 ml of TBA to each tube including the blanks and standard.
9. Cover tubes with aluminum foil and place them into the hot water bath for 20 min.
10. Remove tubes from hot water bath and place into the ice water bath for 15 min.
11. Read absorbance at 533 nm
12. Multiply absorbance by 12.21
13. Report TBARS as mg/kg of malonaldehyde.

Standards:

1, 1, 3, 3 tetraethoxypropane (TEP) Stock standard solution
0.02M solution-0.44g (0.5 ml) to 100 ml of distilled water (2×10^{-5} moles/ml)

Working standard solution

Dilute 0.5 ml of TEP stock standard to 500 ml (2×10^{-8} moles/ml).

Standards for standard curve

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

<u>TEP</u>	<u>Concentration of "Standard"</u>	<u>Absorbance</u>
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.

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APPENDIX B

APPENDIX C

Panelist: _____

Date: _____
(DDMMYY)

Vac-Packed Beef Loin Steaks

Sample Number	Initial Beef Color	Amount Of Browning	% Discoloration
	1 = Extremely bright purple-red 2 = Bright purple-red 3 = Moderately bright purple-red 4 = Slightly purple-red 5 = Slightly dark purple 6 = Moderately dark purple 7 = Dark purple 8 = Extremely dark purple	1 = No evidence of browning 2 = Dull 3 = Grayish 4 = Brownish-gray 5 = Brown 6 = Dark brown	1 = No discoloration (0%) 2 = Slight discoloration (1-20%) 3 = Small discoloration (21-40%) 4 = Modest discoloration (41-60%) 5 = Moderate discoloration (61-80%) 6 = Extensive discoloration (81-100%)

APPENDIX D

CHAPTER II: Packaged ground beef pictures.

Day 1



Day 14



ENB

STB

RRF



APPENDIX E

CHAPTER III: Packaged steak pictures.

DAY 0



DAY 35

