Influence of Storage Temperature on Vacuum Packaged Steak Characteristics

by

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Abstract

Meat consumers utilize beef color as one of the sole indicators of product freshness. However, if the product has been previously frozen for preservation, changes may occur during thawing that hinders the consumers perception of quality and alter the safety of the product. The effect of freezing beef steaks prior to being displayed in a retail setting on their sensory attributes has not been thoroughly investigated. Therefore, the objective of the first study was to determine the effect of three different vacuum packaging films (MB, MFS, or MDF) on instrumental color values of boneless ribeye steaks during frozen storge. Throughout the frozen display period, steaks packaged in MDF were significantly ($p < 0.05$) lighter (L*) more vivid (C*) and possessed redder values for red-to-brown (RTB), oxymyoglobin (OMb), and hue angle from day 7 to 25. In the second study, previously frozen beef steaks with their respective vacuum packaging films were evaluated for fresh characteristics focused on the quality and safety of the product. Vacuum packaging material resulted in significant differences where steaks packaged in MB had greater ($p < 0.05$) values for redness (a*) and chroma (C*). Additionally, at the end of the 25 days of simulated retail display, MB steaks had fewer ($p < 0.05$) microbial aerobic spoilage organisms $\log_{10}$ CFU/g and no significant ($p > 0.05$) increase in lipid oxidation values. Results from these studies suggest that packaging materials can influence frozen color characteristics, as well as aid in retarding the detrimental effects caused by frozen storage which occur after placing steaks in simulated fresh retail conditions.

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CHAPTER I

LITERATURE REVIEW

1.0 Introduction

It had been understood since the early 1900's that, to assess and understand shelf life and meat quality, the end goal is to meet consumers evolving and consistent demands for meat products (Miller, 2020). Beef has been identified as a popular protein choice due to its dense mineral, vitamin, and protein content. Currently, the United States is presumed to remain the world's largest beef producer and second-largest exporter (USDA, 2022). To successfully meet the expectations of global beef production, the variation in meat storage (fresh vs. frozen) should be evaluated. To combat variables that can negatively affect frozen meat, vacuum packaging has become a universal method for high value products, particularly for imported or exported meat to aid in shelf life stability (Wang et al., 2018). Disruptions in meat manufacturing, distribution, and retail services can result in the alternative option of freezing meat products for frozen retail purchase. Ambiguous times that were brought on by the Covid-19 pandemic resulted in disruption to the commerce logistics of meat items for consumers, therefore utilizing effective packaging for extended storage periods is an important consideration for the industry to evaluate.

Vacuum packaging is a useful option for freezing meat while maintaining organoleptic properties and extending shelf life. Due to the removal of atmospheric gasses from the product, shelf life will increase, microbial growth will slow, and if packaged correctly, the product will not experience "freezer burn." Moreover, Kelly (2015) described that an increased usage from 2015 was seen in vacuum packaged meats of 14% from 2002. Unfortunately, the use of vacuum packaging for beef products results in a purplish surface color which is not typically accepted by

consumers. Despite the advantages that vacuum packaging offers, the lack of proper education of consumers surrounding various packaging methods is a challenge that the industry faces today. Continuous improvements in the processing and packaging of frozen meat products are vital for the agricultural community to in turn, benefitting the producers, processors, and the consumers alike. Additionally, it had been reported that consumers are more likely to purchase products that provide a longer shelf life if they are educated on the packaging technology being used (McMillin, 2018). While freezing meat does offer a handful of advantages, undesirable hindrances can occur that can alter perceived quality during the thawing and subsequent cooking stage. Ledward and MacFarlane (1971) reported that during the freeze-thaw cycle, surface color shifts from red to brown at a rapid pace. During the slacking or thawing phase, microbial organisms have the potential to regain activity and proliferate when storage temperatures increase (Löndahl & Nilsson, 2003). Additional meat characteristics can be affected by the freezing and thawing process such as changes in surface color, pH, objective tenderness, moisture loss, and lipid oxidation (Aberle et al., 1989). Considering these issues, choosing the proper packaging material and method that facilitates the preservation of desirable quality characteristics during frozen storage and during the thaw cycle is of importance in the packaging and meat industry.

1.1 Consumer Purchasing Preferences and Influencing Factors

Consumers purchasing behavior is a thoroughly researched and investigated topic as a result of meat and meat products being a valuable source of protein in human diets. However, the combination of intrinsic and extrinsic factors that influence consumers are still not fully understood. Consumer behavior is influenced by three major factors: psychological, sensory, and marketing (Font-i-Furnols & Guerrero, 2014). Producers and retailers need to understand the

influence of these factors simply because they are the last step in the production chain - consumer satisfaction must be their main goal. The most influencing factor impacting consumer purchasing of fresh meat retail cuts in a display case is associated with the intrinsic cue of surface color (Jeremiah et al., 1972; Ngapo et al., 2007). Therefore, to maintain desirable meat color throughout cold storage, transportation, and retail-display, proper preservative systems are required (Jeremiah & Gibson, 2001). Inevitably, meat will begin to discolor due to a combination of environmental factors and chemical components of meat. As a result, the product may be discounted, further processed into another product, or disposed of or condemned by the retailer or consumer. It has been reported that 15% of retail beef is discounted in the United States due to discoloration resulting in an economic loss of $1 billion annually (Mancini & Hunt, 2005; Smith et al., 2000).

Surface meat color can be determined in a subjective manner (descriptive panelists) or by determining the ratios and relative amounts of three derivatives of myoglobin: oxymyoglobin (OMb), metmyoglobin (MMb), and deoxymyoglobin (DMb) (Abdallah et al., 1999). Discoloration occurs due to the accumulation of metmyoglobin on the surface due to increased oxidation of oxymyoglobin and deoxymyoglobin (Henriott et al., 2020). Several factors affect color including postmortem glycolysis rate, fat content, relative myoglobin percentages, pH, species, age, packaging materials, and temperature.

However, the eating experience is not exclusively related to color appearance (Carpenter et al., 2001). At the time of purchase, preference of meat goes beyond the color and appearance of the product as perceived texture, off-flavors, and odors also play a role in consumer purchasing intent (Risvik, 1994). More recently, consumers have voiced their desire to live a more pro-environmental and healthy lifestyle. Those who express a higher environmental

concern coupled with wanting to buy eco-friendly labeled foods are willing to pay more for a lower sodium product with a carbon footprint label (Rondoni & Grasso, 2021). Additionally, attitudes and beliefs an individual possess have a major influence on consumer purchasing behavior. Claret et al. (2014) found that consumer perception of a product can be influenced by their perception of the way it was produced, handled, and distributed. The type of packaging method used for display also can have an influential impact on a consumer at the time of purchase. Carpenter et al. (2001) examined various beef packaging systems (modified atmosphere packaging (MAP), vacuum skin packaging (VSP), and polyvinyl chloride overwrap (PVC) and their impact on consumer preferences. They found that consumers are more inclined to buy the PVC beef due to the bright cherry-red color of products in this packaging. As the meat industry shifts towards MAP and VSP packaging, overcoming the hurdle of consumer preference for that bright cherry-red color that PVC initially demonstrated is a challenge that packaging technology the meat industry may have to conquer. The beef industry continues to strive to deliver consistent, shelf-stable, and wholesome products to consumers while investigating packaging methodologies to meet those standards and reducing the loss in monetary profits.

1.2 Quality & Shelf Life Changes During Freezing & Storage

Freezing meat provides consumers and retailers with the opportunity to extend the shelf life; however, it has been reported that freezing can have an unfavorable influence on color and other quality characteristics (Abdallah et al., 1999; Farouk & Swan, 1998; MacDougall, 1982). Freezing has been defined as a decrease in the molecular motion of the existing molecules withing an environment (Rahman et al., 2007). Freezing of muscle foods is primarily referring to the decrease in motion of water molecules that exist in various forms in the muscle. Moreover, freezing meat is primarily used to extend product shelf life; frozen meats may have double the

storage period compared with never frozen refrigerated meats (Lentz, 1979). As early as 1909, it was reported that a temperature of -9 °C were adequate for storing beef products for up to 554 days (Richardson & Scherubel, 1909). This coincides closely with Qian et al. (2018), who reported frozen storage during shelf life of beef products can be extended from 84 to 126 days at -6°C and -9°C, respectively. Finally, Fernandes et al. (2013) reported a storage temperature of -18°C were acceptable for maintaining quality and preventing spoilage in lamb for more than a year. However, the quality and shelf life characteristic of frozen meat is influenced by freezing rate and method, storage temperature and time, method of packaging and materials, as well as the intensity of light during storage and display. The compound annual growth rate for frozen meat in the United States is 4.9% and the U.S. market segment is expected to grow to $33.9 billion by 2027 due to consumers and the industry shift towards frozen products (Coherent Market Insights, 2022). The emerging market of frozen meat products further echoes the necessity of understanding how to maintain quality and shelf-life characteristics of beef during freezing and frozen storage.

Natural Chemistry of Myoglobin

Meat color has long been the key factor in consumer acceptability for fresh meat products and is still deemed the most influential factor to this day (Herriott et al., 2020). In addition, surface meat color aids the USDA graders to determine carcass maturity and overall quality. Myoglobin (Mb) is the sarcoplasmic heme protein responsible for color (Appendix A) and consists of two parts: the protein segment (globin) and iron containing non-protein portion (heme ring). Myoglobin is a water-soluble protein that is comprised of 8 α-helices with a prosthetic heme group linked by non-helical sections that enwraps the heme. The ability for Mb to bind to oxygen is based on the presence of heme located within the heme crevice, but this coupled with

the resonant nature of the double bonds in the heme group gives Mb the ability to absorb light, thus creating a visible pigment (Sumam & Joseph, 2013). The heme group is comprised of one iron that can either be in a reduced (ferrous, Fe^{2+}) or oxidized (ferric, Fe^{3+}) form. The color of fresh meat is influenced by the fact that myoglobin can exist in four states: oxymyoglobin (OMb), deoxymyoglobin (DMb), carboxymyoglobin (COMb), and metmyoglobin (MMb). OMb, COMb, and DMb exist in the ferrous state, whereas MMb exists in the ferric form. In living muscle and pre-fabrication meat, myoglobin exists in the OMb state. Once meat has been exposed to oxygen via cutting or further processing, it will enter the ferrous oxymyoglobin state. Oxymyoglobin will present a bright cherry-red pigment on the surface of the meat due to oxygen binding to the heme iron. Inevitability, meat surface color will deteriorate and enter the metmyoglobin phase which is responsible for an undesirable brown meat hue. This phenomenon is known as autoxidation, when the ferrous forms of myoglobin are oxidized to the ferric form of metmyoglobin, water will occupy the sixth ligand, leading to a brown discoloration of the meat surface but, the rate of autoxidation is dependent on species (Foucat et al., 1994). Within the same species, variation in color stability occurs in different muscle groups due to differing biochemical (enzyme activity and mitochondrial density) and physical characteristics (Bekhit & Faustman, 2005). Typically, metmyoglobin can be reduced back into the deoxymyoglobin state and then oxygenated into oxymyoglobin to present a bright cherry-red color. However, this can be no longer possible if the reducing components of metmyoglobin are depleted and/or when available oxygen is decreased (Hood, 1980). This discoloration is associated with discounts which results in a loss of more than one billion dollars annually for the meat industry (Faustman & Cassens, 1990). Finally, the carboxymyoglobin formation has been of recent interest due to packaging systems that use low levels of carbon monoxide (Hunt et al., 2008). The chemical

structural concept of carboxymyoglobin, when in the presence of CO, will be in the ferrous state and appear as a bright cherry red color (Herriott et al., 2020). The chemical structural concept of carboxymyoglobin is not fully understood; however, it has been stated that DMb is more readily converted to COMb than to OMB or MMb (Mancini & Hunt, 2005). Discoloration occurs due to the accumulation of metmyoglobin on the surface due to increased oxidation of oxymyoglobin and deoxymyoglobin (Henriott et al., 2020). Several researchers have further investigated other factors that influence fresh meat color such as genetics, pH, dietary components, slaughter techniques, age, water holding capacity, and temperature (Apple et al., 2013; Crouse et al.,1984; Cho et al., 2015; Huff-Lonergan & Lonergan, 2005; O'Sullivan et al., 2003). With combined efforts to better understand the factors that affect fresh meat color, it is hopeful new technologies and methods will aid in this improvement.

Effect of Freezing on Myoglobin

Muscle foods can be stored under freezing conditions for extended periods of time. However, quality characteristics like color will decrease over time. The formation of ice crystals during freezing leads to denaturation of myoglobin, muscle fiber disruption, and a significant reduction in the myoglobin redox system due to the damage of the protein, which is often associated with a decrease in color stability (Henriott et al., 2020; Jeong et al., 2011). Furthermore, variation in lightness on the surface of meat is due to differences in the rate of ice crystal formation (Voyle, 1974). It has been reported by several authors that freezing alters myoglobin levels and formation in meat due to a myriad of reasons as is further described below (Ben Abdallah et al., 1999; Jeong et al., 2011; Lanari et al., 1990). The measurement of instrumental color is a method that applies an illuminant to the surface of the meat and records light wavelength reflectance and reports the three CIE tristimulus values: L* (lightness), a*

(redness), b*(yellowness). Additionally, indicators of surface color discoloration can be seen with lower a*/b* values and high values of hue angle (tan^{-1} b*/a*) (AMSA, 1991). Calculation of wavelength is also recorded at the ratio of 630/580 nm which provides an indication of a shift from red to brown (Khliji et al., 2010). The L*, a*, and b* system correlates best with sensory, visual, and objective analysis of color (Ferreira et al.,1994). The species and location of muscle should be considered when collecting instrumental color measurements (O'Neill et al., 2003; Norman et al., 2004; Sammel et al., 2006).

A handful of related studies have investigated instrumental color values on fresh and frozen muscle food. Vieira et al. (2009) reported lower L*, a*, and b* values for beef steaks that were frozen for 90 days when compared to 30-day frozen steaks. In a similar study, bovine *Longissimus* was stored at -18°C for 9 months which showed a significant ($p < 0.05$) decrease in L* (Cho et al., 2015). Additionally, Brewer and Harbers (1991) examined instrumental color in frozen ground pork and reported a decrease in oxymyoglobin and a* values as frozen storage time increased. A decrease in color stability parameters was observed in frozen beef samples that were stored for one month at 10°C (Farouk & Swan, 1997a). A handful of studies that investigated frozen beef with various packaging platforms, found that vacuum packaging resulted in better color stability when compared to oxygen permeable packages (Bhattacharya & Hanna, 1989; Brewer & Wu, 1993; Lanari, 1989) and decreased oxidative rancidity (Lanari et al., 1989). In addition, it has been stated that frozen beef had lower a* (redness) values than the never-frozen beef (da Silva Bernardo, et al., 2020; Kim et al., 2015; Kim et al., 2017; Setyabrata & Kim, 2019; Vieira et al., 2009). These findings agree that lower a* values in frozen beef is a result of increased susceptibility of myoglobin oxidation due to freezing. However, Henriott et al. (2020) suggests beef steaks that are frozen in a high oxymyoglobin state will display a

superior bright cherry-red color coupled with higher percentages of oxymyoglobin throughout extended frozen storage than those beef steaks packaged in a deoxymyoglobin state.

Additionally, the color of frozen meat varies and is highly dependent on the rate of freezing. James and James (2002) described the direct relationship between meat color and freezing rate, stating that the faster the rate of freezing, the lighter the product will be. Conversely, Farouk et al. (2003) did not find an effect of frozen storage temperature on most sensory properties in frozen beef when comparing various fat content and diet of the animal (pasture versus grain). In addition to the freezing rate, it has been reported that partial freezing, frozen and stored at -5°C, will increase oxygenation capacity, suggesting that freezing temperatures can also have an impact on color stability (Lanari & Zaritzky, 1991). Therefore, because the temperature has a direct correlation with surface discoloration, it is vital for producers and retailers to consider this factor during storage and distribution.

Another influential factor that will undoubtedly affect meat color is the type and intensity of lighting during storage and retail display. A multitude of factors have been considered when evaluating and measuring meat surface color such as the color of light, photo-oxidation, as well as potential heat being produced by emitting light sources. Currently, there are three different types of lighting that are used during the display of fresh meat. Lighting sources include incandescent, light-emitting diode, and florescent. In broad terms, Renerre and Labadie (1993) described that light could be a potential catalyst for myoglobin oxidation rate. In agreement, it has been shown that even faster oxidation and discoloration can occur if energy spikes at certain wavelengths of lighting emissions (Iverson, 1985). Furthermore, it has been reported that frozen beef packaged in oxygen-impermeable packaging aids in minimizing discoloration, lipid oxidation, and when stored in the absence of light, it remained visibly more attractive

(MacDougall, 1982). In general, a greater pigment oxidation occurs when meat is stored under light versus when stored in dark conditions. Frozen prepackaged beef showed an increase in color stability with decreasing light intensity and temperatures above -29°C (Lentz, 1979). Franke and Solberg (1971) stored meat under various wavelengths of light and reported that illuminated meat contained 5.5% more metmyoglobin than those stored in the absence of light. These findings suggest that it is equally important to consider the type of lighting in addition to other factors such as temperature and species when determining storage and transportation methods for fresh and frozen meat products.

Quality Characteristics and Freezing

Quality does not have a straightforward definition because it is determined by consumer preferences. Nonetheless, meat scientists have defined meat quality based upon factors such as nutrients, color, water holding capacity (WHC), tenderness, composition, flavors, and contamination (Joo et al., 2013). In general, consumers rank meat color as the largest indicator of quality with tenderness and juiciness rated as the most important factors of quality for cooked meat (Glitsch, 2000). Quality priorities in the industry also include food safety, lean-to-fat ratio, and how cattle are raised although, eating satisfaction and visual characteristics are still of importance (BQA, 2016). Improper or prolonged frozen storage may alter physical properties, such as texture, drip loss, and chemical mechanisms such as protein denaturation and oxidation, lipid oxidation; as well as organoleptic properties of meat for instance flavor and other sensory characteristics (Pérez-Palacios, et al., 2010). With decades of research focused on fresh meat color stability and its major influencing factors, it is of equal importance for research efforts to be focused on frozen and previously frozen meat quality and shelf life characteristics.

Water Holding Capacity

The ability of muscle foods to retain moisture is arguably one of the most important quality attributes for both the consumer and the industry. Unacceptable moisture loss can cause reductions in primal and carcass weights loss of product yield during further processing, and impaired sensory attributes of the final cooked product (Apple et al., 2013; Oeckel et al., 1999). Another issue associated with high moisture loss is a shortened shelf life due to the opportunity for microorganisms to proliferate in the exudate, subsequently resulting in increased oxidative pressure and safety concerns (Oswell et al. 2021). Additionally, the moisture loss from the muscle contains vitamins, minerals, proteins, and salts which can diminish the nutritive value. Hamm (1960) defined water-holding capacity of meat as its ability to retain its inherent water during processing and/or force application (grinding, cutting, packaging, etc.). This vital quality characteristic can be described in several ways, but in fresh products, it is often described as drip loss or purge loss. Lean muscles contain approximately 75% water with other components comprised of protein (20%), lipids or fat (5%), carbohydrates (1%), and vitamins and minerals (1%) (Huff-Lonergan & Lonergan, 2005). Many elements have an effect on the water-holding capacity of meat including but are not limited to: genetics, preslaughter animal management, nutrition, carcass/meat chilling, stunning methods, packaging, storage temperature, pH decline, cooking, and end point temperature (Apple et al., 2013; Farouk & Swan, 1998; Huff-Lonergan & Lonergan, 2005).

The majority of water is located in the muscle cells of fresh meat. Due to water being a dipolar molecule, it is attracted to charged molecules and polar molecules without charges. There are four different categories of water that is present within the muscle cells: bound water, immobilized water, free water, and extracellular water. Bound water is directly associated with

protein and is held tightly by myofibrillar proteins with reduced mobility and can only be removed by intense drying methods, not including conventional cooking (Fennema, 1996). Additionally, bound water represents about 4-5% of the total water in the muscle and tends to be resistant to freezing temperatures (-40°C or lower) (Fennema, 1996; Huff-Lonergan & Lonergan, 2005). Moreover, it has been reported that bound water represented about 0.5 g water per 1 g of protein (Bertram et al., 2002). The second type of water is immobilized water, which is held within myofibril by steric effects and is the largest portion of water found within the muscle. Immobilized water lacks high mobility like bound water; however, immediately after the completion of rigor mortis, it can be removed by drying and/or converted into ice during the freezing process (Fennema, 1996). Immobilized water is the main type of water that is found within the muscle, and it is important for the meat industry to maintain immobilized water to maximize consumer satisfaction as well as for profitability purposes. The third type of water found within the muscle cells is free water which flows unimpeded from muscle tissue and is held by weak capillary forces (Apple et al., 2013). The fourth and final type of water that has an impact on the water-holding capacity is extracellular water, which represents the water that escapes the muscle cell as purge sometime after rigor mortis is complete (Offer & Knight, 1998). Water can be lost from raw meat by evaporation or by the exudation from the cut surfaces as drip (Offer & Knight, 1998). Prior to any further processing of raw meat products, it has been reported that during rigor mortis the water holding capacity of muscle fibre is drastically altered, resulting in the relocation of water from the muscle into the extracellular space (Gill et al., 1984).

Relationship Between Freezing and Water Holding Capacity (WHC)

Due to the muscle containing roughly 75% water, the formation of ice crystals during freezing is inevitable. Huff- Lonergan and Lonergan (2006) reported that when large ice crystals

form during the freezing stage, damage can occur in the myofibrillar structures which, in turn, will decrease the WHC of the muscle. However, controlling the rate or speed of freezing has been shown to influence the WHC of muscle food. It has been recommended to introduce freezing temperatures once rigor mortis has been completed to avoid toughening and increased drip loss due to thaw rigor (Dang et al., 2021). Additionally, freezing muscle foods at an accelerated rate will decrease purge loss due to ice crystals forming intramuscularly, thus cells do not experience detrimental damage and can maintain moisture better (Paul & Child, 1937; Ramsbottom & Koonz, 1940). Postmortem aging can have an impact on the water holding capacity of the lean muscles. A few related studies stated that the water holding capacity of meat will increase postmortem aging (Davis et al., 2004; Straadt et al., 2007). Additionally, it has been suggested that postmortem proteolysis could be responsible for improving the water holding capacity of aged meats (Huff-Lonergan, 2005). These findings are consistent with Ruiz de Huidobro et al. (2003) who reported that water holding capacity increased significantly ($p < 0.05$) during the postmortem aging process. Honikel et al. (1981) stated that the WHC is highest in prerigor muscle. However, to preserve this state for months it was suggested that rapid freezing of bovine muscle paired with processing without prior thawing can aid in conserving the high WHC. Genetics have also been reported to have a major influence on the WHC and cook loss of beef. Uytterhaegen et al. (1994) examined WHC and cook loss characteristics of Belgian Blue White double-muscled (DM) bulls with bulls from the same breed that possesses normal muscle conformation. The authors reported that the normal conformation bulls had a significantly higher ($P < 0.001$) drip and cook loss at 8-day postmortem when compared to the DM bulls. Due to WHC being an important attribute, continuous research should be conducted to further understand the encompassing aspects of maintaining it.

1.3 Thawing Effects on Quality and Shelf Life Characteristics

Thawing is an important process following the freezing of meat and is directly related to quality characteristics. Fresh meat can be stored in frozen temperatures for extended periods of time and then subsequently sold either in a frozen state or as chilled meat that has been thawed. Thawing of meat from a domestic and commercial standpoint is typically completed at refrigerated temperatures of about 4°C. Additional methods include thawing at room temperatures that can range up to 49°C, microwaving, or submerging in tap water (Eastridge & Bowker, 2011; Rahman et al., 2014). Other methods used for thawing include heat convection, thawing by cooking, radio frequency, impingement, and ohmic (Anderson & Singh, 2006; Hong et al., 2007; Moody et al., 1978). The Food and Drug Administration and U.S. Department of Agriculture Food Safety and Inspection Services deem thawing at either refrigerated temperatures, microwave thawing, immersing in cold water, and cooking from a frozen state to be safe methods of thawing (FDA, 2005; USDA – FSIS 2005). The freezing and thawing of muscle foods alters the biochemical and physical quality characteristics of the meat. However, the water fraction and distribution of water within meat is mainly influenced during the freeze and thawing cycle, which can decrease various quality and shelf life characteristics of the product.

Water Holding Capacity and Cook Loss

For consumers, it is often necessary to freeze meat products to be consumed at a later date; however, the process of freeze-thaw can have a negative impact on water holding capacity and cook loss. Cook loss can be measured by a simple method that evaluates the amount of fluid released from lean muscle post cooking. During cooking, between 20% and 40% of its weight can be lost due to an assortment of reasons (Martens et al., 1982). The variation in cooked yield

loss is of economic concern to meat processors and consumers. Cooking is defined as the heating of meat to a sufficiently high temperature to denature proteins (Davey & Gilbert, 1974). The high temperatures that are reached during the cooking process have a major effect on the physical and chemical properties of meat such as textural changes and decreases in cook yield. Both temperature and time are vital factors in the process of cooking due to heat transfer, protein denaturation, and occasionally protein solubilization (Purslow et al., 2016). It is well established that increasing cooking temperatures alter salt content, pH, and sarcomere length of the muscle influence the end weight loss of cooking (Offer & Knight, 1988; Tornberg, 2005). Changes in myofibrillar proteins coupled with tissue shrinkage and moisture loss commence at 40°C and continue until coagulation occurs at around 65°C (Jones et al., 1977). It has been proposed that shrinkage of collagen fibers has a major impact on cook loss; however, it has been found that there is no significant correlation between collagen and cook loss in eleven major beef muscles with varying collagen concentrations (Rhee et al., 2004). Although water holding capacity has been reported to increase during aging due to the swelling of muscle fibers, this does not translate to cook loss yield. It has been reported that increased cook loss is higher in meat that has been aged for at least 3 to 6 days when compared to unaged meat (Purslow et al., 2016). This finding is consistent with previous literature showing that cook loss increases with aging duration (Shanks et al., 2002; Straadt et al., 2007; Warner et al., 2005). The relationship between water holding capacity and cook loss has been examined and it was reported that because cooking induces structural changes of the muscle, it will have a negative impact on WHC (Tornberg, 2005). Shrinkage of muscle fibers during the cooking process results in extensive water loss; and, it has been concluded that water is expelled in response to the pressure being exerted by the shrinking of connective tissues (Offer, 1984).

Low-field nuclear magnetic resonance (LF-NMR) has proven to be a non-invasive technique that aids in monitoring the behavior of water of meat during the cooking process (Micklander et at., 2002). Studies that have utilized LF-NMR have reported that a myriad of changes occur amongst the water component distribution at different temperatures. Micklander et al. (2002) described those major changes in the water matrix of meat at 42, 46, 57, 66, and 76°C. A previous study that used LF-NMR reported that approximately 20% of all water in lean muscle does not freeze at -10°C (Belton et al., 1972). Moreover, a study using the same method examined the effects that a freeze-thaw cycle has on lean muscle, and it was reported that cook loss significantly increased whereas WHC significantly decreased after one freeze-thaw cycle (Cheng et al., 2019). Understanding how freeze-thaw cycles and thermal protein denaturation effects water distribution and properties in meat can impact ideal cooking techniques as well as endpoint temperatures.

Effect of Thawing on Color and Lipid Oxidation

It is well known that color plays a major role in consumer preference and has been previously linked to other characteristics such as lipid oxidation. Lipid oxidation is a complex reaction that occurs between fatty acids and oxygen, which results in oxidative deterioration of the meat, the development of off flavors and rancidity (Dave & Ghaly, 2011). During the process of freezing and thawing, myoglobin proteins will denature which can lead to autoxidation and the subsequent loss of desirable color pigmentation (Leygonie et al., 2012). This hypothesis has been investigated and confirmed by many authors who compared the ability of meat to resist oxidation to metmyoglobin during refrigerated storage post a freeze-thaw cycle (Abdallah et al., 1999; Farouk & Swan, 1998; Lanari et al., 1990; Lanari & Zaritzky, 1991). Repeated freezing and thawing can occur in domestic kitchens, during transportation, or in restaurants. Surface

color significantly deteriorates over multiple freeze-thaw cycles and can influence lipid oxidation, pH, and water holding capacity as well. Ali et al. (2015) found that after 5 or 6 freeze-thaw cycles, there was a significant ($p < 0.05$) decrease in WHC, L*, a* and B* values, and a significant increase in lipid oxidation values in chicken breasts. However, there are contradictions amongst previous research that have investigated the effect of the freeze-thaw cycle on the surface color of meat. For instance, it has been reported that temperature abuse during any point of the freeze-thaw cycle can accelerate lipid oxidation as well as influence surface discoloration in meat (Hansen et al., 2004; Moore & Young, 1991). On the contrary, Carballo et al. (2002) found that varying freeze-thaw treatments on a mixture of swine M. *biceps femoria*, M. *semimembranosus*, M. *semitendinosus*, M. *gracilis*, and M. *abductor* had no significant ($p > 0.05$) effect on yellowness, redness, or brightness of meat mixtures. Furthermore, it has been stated that all forms of oxidation are associated with one another (Leygonie et al., 2012). Therefore, lipid oxidation and protein oxidation have been investigated separately and together. Similar studies concluded that the production of pro-oxidants from oxidation interact with oxymyoglobin, thus resulting in metmyoglobin development (Farouk & Swan, 1998; Leygonie et al., 2012). Lipid oxidation is a factor that can alter the nutritional and sensory quality of muscle foods in raw and cooked meat during refrigeration or frozen storage (Gomez et al., 2003). Lipid oxidation is considered an effective quality measurement because certain levels can lead to rancidity (Moody et al., 1978). Freezing and thawing can lead to depletion of NADH (nicotinamide adenine dinucleotide hydride) from the mitochondria caused by ice crystal formation, which plays an important role in the metmyoglobin reducing activity cycle, thus, accelerating surface color discoloration (Abdallah et al., 1999; Henriott et al., 2020). In a similar study investigating the freeze-thaw cycle on pork loins confirmed that freezing and thawing did

accelerate color deterioration of the pork loins; however, the authors did not find lipid oxidation to be linked to the discoloration (Jeong et al., 2006). Similar findings were reported by Ledward and MacFarlane (1971) who found lipid oxidation and metmyoglobin concentration to not be directly related during the thawing stage of beef. However, it has also been reported that the duration of thaw time and anatomical location of the muscle has a major influence on lipid oxidation (Fioramonti et al., 2017; Ledward, 1971).

1.4 Temperature

Display and storage temperature are considered to have one of the largest impacts on meat color stability (MacDougall, 1982). Moreover, color stability of beef gradually deteriorates as chilled storage is extended (Jones et al.,1991). Previous research has found that storage life is respectively obtained at 100, 70, 50, and 15% when stored at optimum temperatures of -1.5°C, 0°C,5°C and 10°C (Gill & Shand, 1993). Additionally, bloom (development of redness) will increase in storage temperature within the range of 1°C to 29°C (Rickert et al., 1957). MacDougall and Taylor (1975) found that when temperatures are lowered between 3 and 5°C, surface discoloration will decrease by 50% when compared to storage temperatures of 7°C. In a similar study that investigated ground beef patties stored at a slightly abusive temperature of 10°C with various packaging methods, warmer temperatures led to a greater amount of surface discoloration in beef patties stored in high oxygen MAP and PVC packaging rather than being stored in low oxygen MAP or vacuum packaging (Rogers et al., 2014). Lastly, a study investigated the effects of several temperatures (6.6°C, 2.2°C, 4.4°C, and 0°C) on the surface color of beef wrapped in PVC film and the product had a significant decrease in desirable visual color with increasing storage temperatures (Lanier et al., 1977). Shelf life is equally as important as surface color stability. Jeremiah and Gibson (2001) examined three different beef retail cuts

(*longissimus lumborum, psoas major,* and *semimembranosus*) stored at (-1.5°C, 2°C, or 5°C) for 24 weeks and found that shelf life can be more than doubled by storage at subzero temperatures (-1.5°C).

Kropf (1980) described that retail display cases vary in temperature by 4.5°C from the air inlet and air discharge. This variation in temperature of retail display cases means higher shelves are recorded to have warmer temperatures. The FDA (2021) states that any fresh meat retail cuts (beef, lamb, pork, and veal) have a shelf life of 3 to 5 days after purchasing when stored under refrigerated conditions (4°C). Furthermore, when freezing these meat products, the FDA (2021) states that shelf life may increase to 3 to 4 months. If a consumer elects to freeze fresh meat retail cuts (beef, lamb, pork, and veal) shelf life can be extended for up to 6 to 12 months when stored at -18°C in vacuum packaging, whereas the shelf life of chops is extended 4 to 6 months. Temperature control is of paramount importance, particularly with vacuum packaged meat because temperature abuse can result in a favorable environment for spoilage and unwanted microorganism proliferation (Rickert et al., 1957). Temperature has a profound effect on microbiological growth in meat products. Sammel et al. (2006) reported that chilling rates will decrease aerobic reducing activities as a result, affecting the color stability of the meat. James and James (2002) stated that even though some pathogens are capable of surviving at 0°C or lower, food safety risks are substantially reduced when temperature of meat is maintained below 5°C. This can be a challenge for retailers to maintain throughout the cold chain process, particularly because when meat products are stored in bulk, they are far less sensitive to small heat inputs compared to smaller batches in distribution for retailers or when meat is stored in open display cases (Aidani et al., 2014). Ramsbottom et al. (1940) investigated storage temperatures ranging between 10°C and -30°C and reported that when frozen meat is stored at -

12.2°C there was a reduction in purge loss, inhibition of lipid oxidation, and meat demonstrated minimized color changes after thawing. A similar study by Watts (1954) found that oxidation rates doubled for every 10°C rise in storage temperature. These findings coincide with Brown and Mebine (1969), who found a 40-to-50-fold slower oxidative reaction rate at -2°C than at 22°C. In anaerobically packaged meat, temperature control is of great importance because temperature abuse provides a favorable environment for microbial growth (Seideman & Durland, 1983). Furthermore, holding meat at abusive elevated temperatures (> 7°C) even when packaged in oxygen permeable films, is dangerous from a microbial growth standpoint (Jeremiah & Gibson, 2001).

1.5 Common Causes of Spoilage and Preservation Methods

Spoilage can be blamed for one of the main causes of meat waste and loss, especially in North American and Europe (FAO, 2012). However, the root cause of this occurrence is mostly due to packaging methods and pre- and post-mortem handling. Spoilage of meat and meat products represents a major portion of annual losses for retailers and meat producers (Nattress et al., 2001). Interventions are set in place to prevent any contamination pre- and post-mortem as well as during further processing. Although contamination and spoilage of muscle foods still occurs, these prevention techniques are still essential.

Pre-slaughter

When an animal is exposed to high levels of stress prior to slaughter, such as during particularly long periods of transportation, the glycogen content is reduced as part of the animal stress response (Dave & Ghaly, 2011). The breakdown of glycogen via anaerobic glycogen pathways in the animals' muscles results in the production of lactic acid, which reduces the

muscle to an ultimate pH of 5.6. In cases where limited glycogen breakdown can occur, the pH remains high, which will result in dark, firm, and dry (DFD) meat, also known as a 'dark cutter' in beef. DFD meat is not only unappealing to consumers, but also has a shorted shelf life; spoilage bacteria generally show preferential growth at near-neural pH levels, and DFD meat retains more water that can be used for bacterial processes (Dave & Ghaly, 2011). Additionally lower amounts of bacteria are required to produce off-flavors at a higher pH (Addis, 2015). Severe short-term stress results in pale, soft, and exudative (PSE) meat. PSE meat presents a lower pH than normal and results in excess water in the extracellular space available for the benefit of microorganisms. In essence, ante-mortem the focus should be to minimize stress by maximizing animal welfare, which will decrease the likelihood of producing an unsatisfactory product and extend the shelf life.

Post-slaughter

Post-mortem handling, packaging, and storage of meat products can introduce or affect spoilage by three main mechanisms: autolytic enzymatic spoilage, microbial spoilage, and lipid oxidation (Addis, 2015). Meat preservation is a necessity when considering transportation time and avoiding unfavorable changes in the texture, color, and nutritive value. The central goals of preservation methods are to minimize oxidation and microbial spoilage. Current meat preservation methods have been separated into three categories: temperature control, water activity management, and the use of chemical or bio preservatives (Bagamboula et al., 2004). The intestinal tract and hide of the animal are the main sources of pathogens, whereas spoilage microorganisms are primarily environmental. Thus, ensuring proper sanitation and hygiene practices are implemented is paramount for preventing any contamination. Storage temperature and packaging method additionally have an immense impact on the rate and type of bacterial

growth. The basic goal of chilling, cooling, and freezing techniques is to limit or reduce the spoilage rate of microorganisms. Chilling is the first and lowest temperature that is typically implemented immediately post-slaughter and during transportation and storage. Chilling of the carcass occurs when it reaches ≤ 4°C and this is critical for a myriad of reasons such as shelf life, food safety, appearance and maintaining nutritional components (Zhou et al., 2010). This method can be employed in two ways: immersion chilling and air chilling. Immersion chilling, particularly for poultry carcasses, is when the product is immersed in chilled water (0 - 4°C). Air chilling, primarily used for large animals though it is also used in poultry, occurs when the carcass is misted with water in a room that is equipped with circulating chilled air (Carroll & Alvarado, 2008). It has been reported that air-chilling minimized microbial spoilage and carcass surface temperature is reduced at a quicker rate which improves carcass drying (Ockerman et al., 2014; Sanchez et al., 2002). Seideman et al. (1984) found that exponential bacteria growth is commonly associated with beef packaged in oxygen permeable film when compared to beef stored in oxygen impermeable film such as vacuum packaging. Furthermore, these authors reported that vacuum packaging inhibited aerobic spoilage bacteria due to oxygen converting into carbon dioxide which is an inhibitor for most aerobic microorganisms.

Bacterial Discoloration

The proliferation of certain microorganisms that develop as storage time increases can have detrimental effects on meat color. The favorable pH for the growth of spoilage microorganisms on fresh meat ranges from 5.5 to 7.0 (Addis, 2015). There are two myoglobin derivatives that are responsible for a green pigmentation. Sulfmyoglobin is the first myoglobin derivative capable of altering fresh meat color, giving it a green hue. This pigment shift occurs from bacterial production of hydrogen sulfide, or the growth of hydrogen sulfide-producing

microorganisms and is associated with an alteration in the heme structure (Faustman & Cassens, 1990). The most common bacterial by-products are hydrogen sulfide (H_2S) and hydrogen peroxide (H_2O_2), which can react with an unstable part of myoglobin resulting in sulfmyoglobin and choleglobin, these processes can also accelerate oxidation. It has been reported that this cause of discoloration via bacterial growth occurs during the logarithmic growth phase (Seideman et al., 1984). Specifically with vacuum packaged meats, *Pseudomonas mephitica* is commonly associated with the green discoloration but when the packaging is opened it will become oxygenated and transform into oxysulfmyoglobin which exudes a red color (Seideman et al., 1984). However, in aerobically stored retail meat products the green discoloration is associated with the bacterial by-product, hydrogen peroxide and is associated with the second myoglobin derivative, choleglobin. The production of hydrogen peroxide will interact with myoglobin in the ferrous or ferric state (Yong et al., 2018) and this formation is favorable between a pH of 4.5 to 6.0 (Faustman & Cassens, 1990). It has been suggested that hydrogen peroxide could either be produced by the muscle itself or through an interaction between bacteria with the oxygen molecule of oxymyoglobin and ascorbic acid (Fox, 1966).

Water Activity

Water activity (a_w) refers to the ratio between the vapor pressure of the food itself and the vapor pressure of distilled water under ideal conditions (FDA, 2018). Managing and controlling water activity in food products is one of the oldest methods of preservation. The object of controlling water activity is to restrict the availability of water to inhibit microbial growth. Furthermore, it has been stated that controlling water activity can maintain physical characteristics such as odor, flavor, texture, moisture migration and shelf life; and prolong enzymatic activity (Sandulachi, 2012). The average water activity of meat and meat products

ranges from 0.99 to 0.70 (Duckworth, 2012). Water activity can be altered and controlled through various means including the addition of chemicals, refrigeration, salting, drying, and curing. Refrigeration has been shown to significantly influence water activity in meat. Clemente et al. (2009) found that when previously frozen raw pork meat was stored at higher temperatures, a higher water activity was recorded. Additionally, refrigeration temperatures during carcass cooling are vital in the surface drying and is directly correlated with water activity which in turn, reduces water activity and inhibits surface bacteria growth (Aberle et al., 1989).

Antimicrobial Preservatives

Antimicrobial preservatives are ingredients that are used to extend shelf life of meat and meat products by reducing the opportunity for microbial proliferation. Common antimicrobial compounds, or chemical preservatives, that are used today include organic acids, sulfites, chlorides, and nitrites (Davidson et al., 2005). Organic acids such as lactic, citric, and acetic acid are used to wash carcasses after processing. A wash at 1.5% can lead to a significant ($p < 0.05$) decrease in aerobic and coliform counts (Davidson et al., 2005). With the additional use of vacuum packaging, it has been reported that increasing the organic acid wash to 2.0% can significantly decrease anaerobic, aerobic, and lactic acid bacteria counts; though, some discoloration can occur (Caccuarelli et al., 1983). Nitrites and sulfites contribute to preservation as well as the color stability of meat and are effective against certain microorganisms (Davidson et al., 2005). Freezing of meat does not fully prevent oxidative spoilage and/or microbial spoilage, thus the addition of some chemical preservatives paired with stable refrigerated temperatures can maximize stability (Addis, 2015). This use of antioxidants (phenolic and phosphates) can facilitate in the delay of lipid oxidation and has been successful (Davidson et al., 2005; Smitzis et al., 2010).

1.6 Packaging

Packaging is ubiquitous and indispensable in society today. It has been defined as the container which preserves, maintains, and protects food products from adversaries while maximizing performance for the consumer (Robertson, 2012). Additionally, packaging is important for marketing meat products, thus, if it is perceived as unappealing the consumer will not purchase the product (Sara, 1990). Packaging types and films range from air-permeable packaging that is used for short-term storage and display, vacuum packaging with barrier materials, bulk-gas flushing, and MAP for longer term storage or display. The choice of the proper packaging method to maintain quality and shelf life characteristics varies amongst products, intended storage temperature, and their estimated time spent in distribution. Concerns and challenges within the meat industry have galvanized the industry to evolve and assess present and past packaging solutions and methods. For instance, the durability of packaging materials has been thoroughly researched to ensure the physical nature of meat products are resistant to tampering, wear, and convenient for consumers (Holman et al., 2018; Liebmann et al., 2012; Rodrigues et al.; 2017, Xiu et al.; 2017). Common plastics possess properties that are suitable for food packaging and common polymers that are used for food packaging include low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), polytetrafluoroethylene (PTFE), and nylon (McMillin, 2008). Additionally, polyesters like polyvinyl chloride (PVC), polyvinylidene chloride (PVDC), polystyrene, polyamide, ethylene-vinyl alcohol (EVOH), and ethylene vinyl acetate (EVA) are also commonly used in food packaging (Robertson, 2012). Common packaging platforms used today can consist of one or more polymers or polyesters which include polyvinyl chloride (PVC) overwrap, modified

atmosphere packaging (MAP), traditional vacuum packaging and shrink bags, and vacuum skin packaging.

Vacuum Packaging

The introduction of vacuum packaging for the distribution and storage of chilled meat products is arguably one of the greatest inventions and investments the meat industry has made. Vacuum packaging is the process of placing the product into a barrier film, removing all the atmosphere surrounding it for the packaging material to then be heated and sealed, creating a water and airtight package. In doing so, an anaerobic environment is created which impedes lipid oxidation and inhibits microbial proliferation thus, extending shelf life. Once beef carcasses are broken down into primal and subprimal cuts they can be further broken down into boneless and bone-in cuts making them available for vacuum packaging. The first advantage noticed when implementing this practice was that it considerably reduced refrigeration space for distribution and storage (Robertson, 2012). Additionally, it was soon discovered that vacuum packaging greatly extended shelf life. Delmore (2020) stated that vacuum packaged beef primals and subprimals could have an extended storage for up to 45 days when stored at 4°C, whereas frozen whole muscle has an estimated shelf life of 12 months when stored at -18°C. Due to the anaerobic environment that vacuum packaging provides, fresh meat color appears as a purplish-red color which can ultimately influence consumers purchasing decisions. However, manipulation of the rate in which oxygen can permeate through the packaging and react with myoglobin, i.e., altering the oxygen transmission rate (OTR), results in a cherry red color. OTR is the rate at which oxygen can permeate through packaging film and be tailored to a specific products need by utilizing films that possess high or low OTR which could optimize specific characteristics. Frozen meat is highly susceptible to dehydration; however, proper packaging

materials can be used to combat moisture loss and temperature fluctuations during storage. Furthermore, the rate of permeation of water vapor that occurs through packaging material can have a major influence on preservation and shrinkage of the product. Snyder and Ayres (1961) reported that pigments can be lost in purge and result in a darker surface color of the meat. However, this type of discoloration can be controlled by selecting a packaging film that has low moisture vapor loss. A handful of studied have concluded that frozen beef in vacuum packaging provides beef with better color stability than packaged in oxygen permeable packaging (Bhattacharya & Hanna, 1989; Lanari et al., 1990; Brewer & Wu, 1993). In agreement, Seidman et al. (1976) found that vacuum packaged meat prolonged the shelf life of beef retail cuts compared with those packaged in oxygen permeable film. Similarly, Jul (1984) reported that packaging beef in low permeability films results in decreased oxidative rancidity but also delayed surface discoloration over time. More specifically, it was suggested that frozen beef color was best maintained for 18 months in low-density polyethylene vacuum packaging film at 0.0015 to 0.0020 μm thick (Lundquist, 1972). A similar study that evaluated ground beef in frozen storage reported samples packaged in loose-fitting polyethylene bags had greater rate constants for drip and cook loss when compared to those stored in vacuum packaging (Bhattacharya & Hanna, 1989).

Pros and Cons of Other Packaging Methodologies

Meat products in a refrigerated retail self-serve setting were first packaged in Styrofoam trays that were overwrapped with polyvinyl chloride (PVC) film (McMillin, 2008). PVC is a stretch film that is air-permeable and is widely used in the packaging of fresh meat in a retail setting. This packaging allows for myoglobin and oxygen binding to form oxymyoglobin

pigments resulting in the red color consumers desire, which is why it has been the most widely adopted packaging method in retail. However, myoglobin will reach its reducing capacity quickly and brown metmyoglobin pigments will form therefore limiting shelf life. Beyond color stability, microbiological contamination levels are accelerated in PVC overwrapped meat products. Brooks et al. (2008) examined spoilage and safety characteristics of ground beef in various packaging treatments and found that traditional overwrap (PVC) had a significantly greater ($p < 0.05$) psychrophilic aerobic, *Lactobacillus*, and total coliform counts during storage periods when compared to other packaging methods. PVC film also possesses a relatively higher water vapor transmission rate (WVTR) which helps prevent excess condensation to form inside the film in fresh products (Robertson, 2012). However, if a consumer elects to freeze a PVC overwrapped product freezer burn is likely to occur due to the combination of the high WVTR and excess head space in the packaging. Freezer burn is the result of ice turning into gas and bypassing the water stage, or when moisture on the surface of frozen foods is loss due to sublimation, ultimately causing dehydration of the product (Schmidt & Lee, 2009). This type of packaging is not ideal for freezing products and further quality characteristics and shelf life diminishes during the freeze-thaw cycle.

Another common packaging technique is referred to as MAP, which consists of substituting the air surrounding the product with specific atmospheric gas or mixture and then enclosing the product in a sealed vapor-barrier film. The gases that are typically used in MAP for the purposes of extending shelf life and maintaining desirable surface color include one or more of the following: carbon monoxide (CO), carbon dioxide (CO_2), nitrogen (N), and occasionally oxygen (O_2). This packaging system commonly uses ~20% CO_2, ~80% N, and <0.4% CO (Aberle et al., 1989). Variations within these ratios can be seen when using a high-oxygen versus low-oxygen

MAP. Typical ratios found with high-oxygen MAP includes 80% O_2 and 20% CO_2, whereas a low-oxygen ratio has 30% O_2 and 70% CO_2 (Lorenzo & Gomez, 2012).

1.7 Advances in meat packaging

Packaging materials and their attributes are directly related to food quality so different packaging materials and considerations have recently emerged to maintain the desired characteristics of meat during storage and display. Recent innovations in the packaging industry include reclosable packaging, active packaging methods, the use of antioxidants and essential oils, and recyclable or biodegradable packaging materials (McMillin, 2017). Active packaging is a method that provides an interaction between the product, package, and packaging environment to deliver desirable characteristics of the product. Fang et al. (2017) reported that the United States defines active packaging as any packaging system that protects the food from degradation or contamination by providing a barrier to external conditions while interacting with the internal environment to manage the atmosphere within the package. Chemoactive or bioactive components are commonly used in active packaging (McMillin, 2017). Active packaging can exist is various forms with the common goal of extending shelf life with the most important systems for meat and meat products including, O_2 scavengers, CO_2 emitters/generators, and impregnated films with antioxidant or antimicrobial compounds.

The key objective of using antimicrobial active packaging is to not only extend shelf life but also to ensure the food safety of meat and meat products. It has been described that antimicrobial packaging has four categories: 1) incorporation of antimicrobial compounds into sachets or pads inside the packages, 2) impregnated films via co-extrusion, 3) coating of the packaging with a matrix that is a carrier for antimicrobial agents that will be released onto the surface of the food via evaporation, or 4) the addition of polymers that are fundamentally

antimicrobial (Fang et al., 2017). Inherently, the antimicrobial agents that are used include carbon dioxide, silver ions, bacteriocins, organic acid, and spices that have proven to be effective in retarding the growth of lactic acid bacteria and the proliferation of common meat microbiota (Suppakul et al., 2003). It has been reported that a 50% increase in shelf life can be attributed to the use of antimicrobial agents in active packaging (Zhang et al., 2015).

Lipid oxidation can result in the development of off flavors and odors, color changes, and can promote the growth of aerobic bacteria and molds due to high levels of oxygen in MAP packaging. Therefore, the use of oxygen scavengers can aid in reducing residual oxygen within the packaging atmosphere and function as an antioxidant. Absorption of residual oxygen in fresh meat packaging reduces the amount of oxygen to 0.1vol% and subsequently reduces the rate of oxidation (Dey & Sudarsan, 2019). Recent research has investigated the use of antioxidant impregnated films and describes an improvement in the surface color of fresh meat, as well as an, extend shelf life (Camo et al., 2008; Júnior et al., 2014). Carbon dioxide emitting and/or generating systems have been proven to have inhibitory effects of aerobic bacteria and fungi through direct antimicrobial effects and reducing relative oxygen thus, a CO_2 system has been viewed as a technique complementary to oxygen scavengers (Suppakul et al., 2003). Moreover, Lövenklev et al. (2004) reported that at high concentrations of CO_2, *Clostridium botulinum* toxin exhibits a higher proliferation rate. The commercial use of CO_2 emitters in fresh meat products are comprised of either calcium hydroxide and sodium hydroxide, or potassium hydroxide which can be used to remove excess carbon dioxide during storage to prevent busting of the packaging material (Fang et al., 2017).

1.8 Conclusion

In essence, the continuous shift of consumer preferences and expectations is a challenge the meat industry continuously faces driving the motivation to identify methods for packaging frozen and fresh meat products that maintain quality and safety characteristics. Giving attention to the various components that alter shelf life such as packaging, water holding capacity, pre and post slaughter intervention, lighting, and temperature further complicates the improvements that must be made to continue to providing consumers with a wholesome and shelf stable product. Additional research should be considered to evaluate these components and how they interact with emerging packaging platforms and technology. Choosing the proper packaging material and method that facilitates the preservation of desirable quality characteristics during frozen, fresh and the freeze-thaw cycle is important to ensure disruptions that may occur do not encumber the industry. Finally, providing consumers and retailers with the proper education surrounding recent packaging innovations is paramount for the progression of the meat industry.

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Chapter II

Influence of Vacuum Packaging on Instrumental Surface Color Characteristics of Frozen Beef Steaks

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Abstract:

Consumers often consider beef color as a sole indicator of product freshness at the time of purchase. However, disruptions in meat manufacturing may cause manufacturers to create frozen retail meat products, but changes to surface color during frozen storage may be deleterious. Therefore, the objective for the current study was to determine the effect of vacuum packaging on instrumental surface color values of boneless ribeye steaks during frozen storage. Steaks were cut 2.54-cm-thick, assigned to one of three packaging films, allowed to bloom for 30 min and immediately frozen. Throughout the 25 days of frozen storage, instrumental surface color values were collected. Steaks packaged using MDF were lighter ($p < 0.05$) but became darker as storage time increased. However, redness (a*) values were greater ($p < 0.05$) for steaks packaged using MDF from day 10 to 25, as well as more yellow (b*) from day 7 to 25 ($p < 0.05$). Furthermore, steaks packaged in MDF were more ($p < 0.05$) vivid (C*) and possessed redder values for red-to-brown (RTB), oxymyoglobin (OMb), and hue angle from day 7 to 25 of the simulated storage period. These data indicate that choice of vacuum packaging film impacted instrumental surface color of frozen retail cuts with MDF packaging more effectively maintaining optimal color throughout the duration of this study.

Keywords: Beef; Frozen storage; Instrumental color; Vacuum packaging

1. Introduction

The design of packaging materials selected for meat products may alter the chemical state of myoglobin which suggests choice of packaging material could influence product appeal and storage stability. Vacuum packaging provides beef products with an anaerobic environment which protects against spoilage but limits the heme iron in myoglobin availability to bind with oxygen resulting in a meat surface that appears dark purple in color. Given the increasing pressure to produce more food in the face of anticipated population growth, preserving meat quality may be a challenge as overcoming the inefficiencies in the current supply chain due to the deterioration of meat products is hampered by the limited packaging resources which could be deployed to prolong storage life. Presently, storage frozen meat in vacuum packaging is the most common method for high value meat products, particularly for imported or exported meat to aid in storage stability [1]. Therefore, the need for extending cold chain storage or frozen meat logistics is inevitable. To meet this need, it is imperative to develop novel packaging technologies which allow cold storage time to be lengthened without negatively impacting product surface color. Current packaging technologies used to store, display, or transport beef products include modified atmosphere packaging (MAP), vacuum skin technology, active packaging systems, and overwrap, however, meat primals are often packaged into vacuum bags for transport to retail venues and for export purposes [2]. There has been extensive research indicating vacuum packaging can extend and aid in stabilization of storage characteristics of beef when compared to other packaging methods [3-7]. Recent efforts comparing beef products in vacuum-packaging to packaging materials such as polyvinyl chloride (PVC) and polyethylene, reported a reduction in oxidation levels and a greater color stability during retail and frozen display periods in products packaged in PVC or polyethylene [5,7]. Consequently, when beef is

vacuum packaged, the color quickly changes from a bright red form of oxymyoglobin to a purple form of deoxymyoglobin (DMb) [8]. However, a deoxymyoglobin (purple) pigment is more likely to be rejected by consumers at the point of sale though this behavior can be reversed if consumers are informed that such packaging technology provides for a much longer storage period [2]. Selecting the appropriate vacuum packaging to increase storage duration of beef products requires consideration of the oxygen transmission rate (OTR) which dictates the amount of oxygen that can permeate the packaging film. A minimum OTR is necessary to allow for a sufficient oxygen infiltration necessary to maintain oxymyoglobin pigments at a level which stably promotes the consumer preferred [2]. Additionally, packaging materials used in frozen meat applications contain barrier properties for preventing moisture loss, oxidation, and aid in color stability during a storage period [9]. Freezing meat is primarily used to extend product shelf life, and when compared to the storage of fresh meat products, frozen meats may have twice the storage life of refrigerated meats [10]. Shelf life of fresh red meat is limited by microbial contamination, and extended shelf life of fresh meat products may be achieved by delaying microbial spoilage with frozen storage [11]. Previous research indicates that freezing meat products may reduce microbial growth and cause chemical fluctuations that alter the product quality with a greater impact on surface color [8]. Moreover, it has been reported that vacuum-packages can be used to avoid the negative impact that storing meat frozen has on meat quality [12]. Much research has examined the influence of storage temperature and duration of storage to determine the optimal storage conditions to maintain product quality for frozen meat products this remains a primary limitation to frozen storage [3,6-7,10,14-17]. Importantly, it has been previously reported that packaging materials with reduced oxygen and moisture vapor transmission rates can result in maintaining perceived consumer quality of meat products during

frozen storage [13]. Therefore, the objective of this study was to determine the effect of vacuum packaging film on instrumental surface color changes that occur on boneless ribeye steaks during frozen storage.

2. Materials and Methods

2.1. *Muscle Fabrication*

Beef boneless ribeye rolls (IMPS #122A) were purchased from a commercial meat processor and transported under refrigeration (2°C) to the Auburn University Lam-bert Powell Meat Laboratory for processing. Ribeye rolls (N = 18) were cut into 2.54-cm-thick steaks (n = 12 steaks/ribeye roll) using a BIRO bandsaw (Model 334, Biro Manufacturing Company, Marblehead, Ohio, USA). Four steaks from each ribeye roll were randomly selected and allocated to one of three packaging treatments.

2.2 *Packaging Treatments*

After cutting, steaks were allowed to bloom for 30 min at 2°C, crust frozen at -23°C for 45 minutes, and then packaged using a rollstock form and fill packaging machine (Model OL0924, Variovac, Zarrentin, Germany). At the time of packaging, steaks were packaged in one of three commercial packaging films (WINPAK, Winnipeg, MB, Canada) consisting of a high barrier (MB) comprised of 150μm of nylon, enhanced ethylene-vinyl alcohol (EVOH), and polyethylene. Steaks packaged in low barrier films were constructed with 150um polypropylene and polyolefin plastomer (MFS) or a combination of 150μm polyolefin, and polyethylene (MDF). Oxygen transmission rates (OTR) for each packaging treatment consisted of: MB (0.5 cc/sq. m/24h); MFS (1100 cc/sq. m/24h); and MDF (1287 cc/sq. m/24h). In addition, the moisture vapor transmission rates for each packaging treatment accounted for: MB (3.9 g/sq.

m/24h); MFS (2.9 g/sq. m/24h); and MDF (3.5 g/sq. m/24h). After packaging, steaks were placed flat onto a plastic tray and stored in a blast freezer (-23°C) for 120 min.

2.3. *Simulated Frozen Storage*

Packages of frozen steaks were stored in an upright, two door, reach-in, commercial freezer (Model AF49EX, Arctic Air, Eden Prairie, MN, USA) for 25 days at -13°C. Packaged steaks were stored in the absence of light for the duration of the simulated storage period. Storage temperatures during the frozen stimulated display period were monitored using a data recording device (Model-TD2F, Thermoworks, American Fork, UT, USA) with probes placed within the center of each shelf. Packages of steaks were distributed evenly across all shelves within the freezer and rotated throughout the storage period.

2.4. *Instrumental Color*

Instrumental color readings were measured on day 0, 5, 10, 15, 20 and 25 by scanning the surface of each steak (N= 216) through the packaging according to guidelines [18] previously described. Surface color values were collected using a HunterLab MiniScan XE Plus Colorimeter (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA, USA) calibrated against a standard black and white glass tile each day immediately before data collection. The L* (lightness), a* (redness), b* (yellowness) values of each steak were determined from the average of three readings using Illuminant A10, with a 10° observer and a 25-mm diameter aperture and the Commission Internationale de l' Eclariage (CIE L*a*b*) color scale [19]. Chroma (C*) was calculated using the following equation with a larger value indicative of a more vivid color. Hue angle was calculated as: with a greater value indicative of the surface color shifting from red to yellow. Reflectance values within the spectral range 400 to 700 nm were used to calculate the

surface color changes from red to brown by with the reflectance ratio of 630 nm:580 nm, and the relative percentages of deoxymyoglobin (DMb = {[1.395 – ({A572 – A700} / {A525 – A700})]} × 100), metmyoglobin (MMb = {2.375 × [1 – ({A473 – A700} / {A525 – A700})]} × 100), and oxymyoglobin (OMb = DMb - MMb) according to American Meat Science Association [18].

2.5. *Statistical Analysis*

The current study was conducted and analyzed as a completely randomized design with packaged steak serving as the experimental unit and 72 replications of each treatment. Data were analyzed using the GLIMMIX model procedure of SAS (version, 9.2; SAS Inst. Inc. Cary, NC, USA). Packaging treatment served as the lone fixed effect and replication serving as the random effect for instrumental color. Day of simulated frozen storage served as a repeated measure, with packaging, day, and packing × day interaction as the fixed effects. Least squares means were generated, and, when significant ($p \leq 0.05$) F-values were observed, least squares means were separated using the pair-wise t-test (PDIFF option).

3. Results and Discussion

3.1. *Instrumental color*

Currently fresh, never-frozen product represents a large portion of meat sold to consumers in the United States. However, with a growing focus on extending cold chain storage or frozen meat logistics, it is imperative to investigate color changes that may occur during cold storage in the face of the paucity of published studies addressing surface color variations that are known to occur in frozen beef steaks. To address this, we measured instrumental color values of beef ribeye steaks were measured during a simulated frozen storage period. There was an interaction ($p < 0.05$) for packaging treatment × day of simulated frozen display that occurred for

surface lightness values (Table 1). Frozen steak surface color was lighter ($p < 0.05$) for steaks packaged using MDF initially (day 0), but steaks packaged in MFS and MDF were darkest ($p < 0.05$) as the duration of frozen storage time increased beyond day 20 (Table 1). Frozen steaks were redder and more yellow ($p < 0.05$) when packaged using MDF film. It is plausible the surface color changes reported on frozen steaks may be attributed to oxygen transmission rate for each packaging film. The OTR for MDF film was greater than MDF or MB which presumably allowed for greater myoglobin binding with oxygen resulting in greater percentages of oxymyoglobin throughout frozen storage. Higher redness values are often indicative of a redder fresher surface color in fresh meat and have a pro-found positive influence on the consumer at the time of purchase. These data therefore support the hypothesis that MDF film promotes a superior surface color in frozen steaks. Given the surface color of beef during frozen storage has not been extensively investigated, these data represent an important addition to the literature and indicate that further research into the use of MDF is warranted. Limited previous research noted similar trends that the effect of freezing on beef color stability will result in a decrease of instrumental color parameters of lightness, redness, and yellowness [20]. These surface color changes have been attributed to the lack of myoglobin oxidation as the duration of frozen storage time increases, surface color changes occur due to the lack of myoglobin oxidation [20]. However, chilled then frozen storage lamb longissimus muscle was reported to have more stable redness (a*), chroma and hue angle values when compared to never frozen chilled lamb meat [21]. Additionally, it has been reported that when beef is frozen in vacuum packaging detrimental effects like protein denaturation and a declining in myoglobin activity, can result in darker redness (a*) and yellowness (b*) values as storage time increases [22]. Our observation in the current study that redness and yellowness values declined as storage time increased in frozen

ribeye steaks is consisted with the limited results focusing on color changes in other meat cuts that appear in the literature. Interestingly, it has been reported that surface color deterioration is less likely to occur when beef steaks reach rigor at higher temperatures prior to frozen storage at temperatures around 35°C [23-24]. Nevertheless, surface color values for redness and yellowness are correlated to color deterioration and as these values decrease, the formation of metmyoglobin will ultimately change the color from red to a brownish-red [13]. The lack of published studies focusing on frozen surface color of beef steaks suggest additional research efforts are necessary to identify the mechanisms of underlying color changes in frozen beef steaks and the potential for packaging films such as MDF to disrupt them to stabilize surface color. A packaging method × day of frozen display ($p < 0.05$) interaction for surface col-or chroma (C*), hue angles, red to brown (630:580nm) and calculated forms of myo-globin (Table 2). Frozen steak surface color was more vivid ($p < 0.05$) for steaks packaged in MDF throughout the entire storage period. Furthermore, steaks packaged with MB had the greatest ($p < 0.05$) shift from red to yellow (Hue°) and red to brown (RTB) as the duration of frozen storage time increased beyond day 7. Calculated values were greater for oxymyoglobin ($p < 0.05$) when packaged using MDF film from day 7 to 25 of frozen storage. Whereas the calculated value of deoxymyoglobin and metmyoglobin increased ($p < 0.05$) for steaks packaged using MB and MFS films throughout the entire display period. It is plausible that because of the oxygen transmission rate of films MDF and MFS that a greater shift calculated color values occurred. These results support the hypothesis that packaging films can influence surface color in frozen steaks and that MDF promotes a more optimal surface color over time in storage.

Studies have reported that vacuum packaging for frozen beef storage provides the product with a more stable color than alternative oxygen permeable packaging [24-27]. However, very

limited previous research has been conducted examining vacuum-packaging film materials and their impact on frozen beef color. Due to MB film limiting the exposure of packaged meat to oxygen because of the low OTR of MB, the observed increase in deoxymyoglobin observed in the present study for MB packaged steaks was unexpected. However, a decrease in myoglobin oxygenation overtime has been reported to disrupt the mitochondrial respiration in skeletal muscle which can result in myoglobin remaining in the DMb state, and a correspondingly darker pigmented surface color [23]. Differences in oxygen penetration between packaging films has been reported with higher rates allowing for more oxygen to penetrate the surface of the meat during freezing and frozen storge, resulting in greater oxymyoglobin values [17]. Our observation that oxymyoglobin values increased with increasing frozen storage time in steaks packaged using higher oxygen permeable vacuum-packaging films is consistent with the few previously published studies in the literature. However, it has been reported that vividness (C*) and a reduced shift from red to yellow (Hue°) was associated with reduced oxygen exposure packaging as storage time increased for beef steaks which is not consistent with the results of this current study [5]. Moreover, it has also been reported that an increase in hue angle can be influenced by the gradual oxidation of myoglobin resulting in a greater accumulation of metmyoglobin over time [25]. This observation is consistent with results in this study when comparing the hue angle and MMb increase shown in the steaks packaged in MB. Generally, the few published studies to date indicate that vacuum-packaging film materials can influence frozen surface color of beef steaks and the present study extends this literature. Contradictions that do exist amongst available studies point to the continued need for supplementary research aimed at identifying mechanisms by which packaging film materials alter the oxidative state of myoglobin

in order to better understand the interaction between these films and surface color changes during cold storage.

4. Conclusions

The results presented here suggest that oxygen transmission rates of thermoforming vacuum packaging film used for frozen storage of beef steaks should be considered to minimize surface color changes throughout a frozen storage period. Our data indicates that steaks packaged in MDF film possessing a greater oxygen transmission rate experienced a more stable surface color throughout storage compared to films with lower oxygen transmission rates. These findings support the use of MDF film for preserving optimal surface color when using vacuum packaging for frozen storage or transportation purposes. However, additional research should be conducted to evaluate the sensory taste profile, surface color after frozen storage, and cookery of frozen, vacuum packaged steaks.

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TABLES

Table 2.1 The interactive impact of packaging treatment × day on instrumental color values of vacuum-packaged, frozen, beef ribeye steaks during simulated storage.

	Day						
	0	7	10	15	20	25	SEM
MB[1]							
L*[2]	43.70^{bcd}	44.03^{bc}	42.57^{defgh}	42.99^{cdef}	43.00^{cdef}	42.88^{cdefg}	0.4323
a*[2]	27.81^{a}	23.77^{d}	22.38^{f}	19.06^{g}	16.86^{h}	15.38^{i}	0.3684
b*[2]	21.66^{bc}	19.56^{gh}	20.04^{efg}	17.37^{j}	16.42^{k}	15.58^{l}	0.2984
MFS[1]							
L*[2]	44.37^{b}	42.03^{fgh}	41.78^{gh}	42.15^{fgh}	41.55^{h}	41.43^{h}	0.4323
a*[2]	24.83^{c}	24.35^{cd}	24.57^{cd}	23.59^{de}	22.59^{ef}	21.84^{f}	0.3684
b*[2]	19.07^{hi}	19.27^{ghi}	20.02^{fg}	19.34^{gh}	18.79^{hi}	18.49^{i}	0.2984
MDF[1]							
L*[2]	47.06^{a}	43.52^{bcde}	42.48^{efgh}	43.22^{bcdef}	42.13^{fgh}	41.49^{h}	0.4323
a*[2]	27.79^{a}	26.68^{b}	28.26^{a}	26.67^{b}	25.30^{c}	24.51^{cd}	0.3684
b*[2]	21.11^{bcd}	20.66^{def}	22.88^{a}	21.75^{b}	20.86^{cde}	20.59^{def}	0.2984

[1] Packaging treatments are defined as: (MB) nylon + enhanced ethylene-vinyl alcohol + polyethylene; (MFS) polypropylene + polyolefin plastomer; and (MDF) polyolefin + polyethylene.
[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).
[a-l] Mean values within day of display and packaging method lacking common superscripts differ ($p < 0.05$).

Table 2.2 The interactive impact of packaging treatment × day on instrumental surface color values of vacuum packaged beef ribeye steaks during a frozen storage.

	Day						
	0	7	10	15	20	25	SEM
MB[1]							
C*[2]	35.28^{ab}	30.83^{fgh}	30.09^{hi}	25.84^{k}	23.58^{l}	21.94^{m}	0.4590
Hue (°)[3]	37.71^{ij}	39.71^{def}	42.20^{c}	42.68^{c}	44.49^{b}	45.56^{a}	0.2461
RTB[4]	5.04^{a}	3.54^{fg}	3.19^{h}	2.44^{i}	1.99^{j}	1.81^{j}	0.0971
MMb[5]	25.89^{l}	33.81^{ef}	37.71^{d}	40.10^{c}	45.14^{b}	47.22^{a}	0.5545
DMb[5]	6.51^{b}	3.74^{cd}	4.44^{c}	4.83^{c}	6.46^{b}	8.52^{a}	0.4171
OMb[5]	70.45^{ab}	61.65^{h}	56.93^{i}	53.95^{j}	47.07^{k}	42.76^{l}	0.7287
MFS[1]							
C*[2]	31.34^{fgh}	31.07^{fgh}	31.71^{efg}	30.52^{ghi}	29.39^{ij}	28.64^{j}	0.4590
Hue (°)[3]	37.34^{jk}	38.35^{hi}	39.18^{fg}	39.33^{fg}	39.74^{def}	40.25^{d}	0.2461
RTB[4]	4.26^{bc}	3.96^{d}	3.97^{d}	3.66^{ef}	3.43^{fgh}	3.27^{gh}	0.0971
MMb[5]	25.68^{l}	29.67^{ij}	30.75^{hi}	30.46^{hij}	32.98^{ef}	33.94^{e}	0.5545
DMb[5]	4.04^{cd}	2.33^{ef}	2.14^{ef}	2.06^{ef}	1.94^{ef}	2.29^{ef}	0.4171
OMb[5]	70.18^{ab}	67.52^{cde}	66.59^{def}	66.96^{cde}	64.61^{fg}	63.08^{gh}	0.7287
MDF[1]							
C*[2]	34.93^{bc}	33.76^{cd}	36.37^{a}	34.43^{bc}	32.81^{de}	32.02^{ef}	0.4590
Hue (°)[3]	37.01^{k}	37.66^{jk}	38.93^{gh}	39.16^{fg}	39.48^{efg}	40.04^{de}	0.2461
RTB[4]	4.79^{a}	4.49^{b}	4.92^{a}	4.33^{b}	4.01^{cd}	3.86^{de}	0.0971
MMb[5]	26.15^{l}	28.06^{k}	28.95^{jk}	29.40^{ijk}	31.39^{gh}	32.37^{fg}	0.5545
DMb[5]	3.02^{de}	1.99^{ef}	2.07^{ef}	1.78^{f}	1.48^{f}	1.44^{f}	0.4171
OMb[5]	70.83^{a}	69.78^{ab}	68.78^{bc}	68.58^{bcd}	66.83^{cde}	65.83^{ef}	0.7287

[1] Packaging treatments are defined as: (MB) nylon + enhanced ethylene-vinyl alcohol + polyethylene; (MFS) polypropylene + polyolefin plastomer; and (MDF) polyolefin + polyethylene.
[2] 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 reflectance/580 nm reflectance, which represents a change in 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-m] Mean values within day of display and packaging method lacking common superscripts differ ($p < 0.05$).

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Chapter III

Vacuum Packaging Maintains Fresh Characteristics of Previously Frozen Beef Steaks during Simulated Retail Display

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Abstract:

The impact of frozen storage on beef steaks prior to the retail setting may result in changes to the quality and safety of the packaged meat. Therefore, the objective of the current study was to evaluate fresh characteristics on previously frozen steaks during a simulated retail display. Steaks were allocated to one of three packaging treatments (MB, MDF, MFS) and stored frozen (−13 ◦C) for 25 days in the absence of light. After thawing, steaks were stored in a lighted retail case at 3 ◦C and evaluated for instrumental surface color, pH, purge loss, lipid oxidation, and microbial spoilage organisms throughout the 25-day fresh display period. There was an increase ($p < 0.05$) for aerobic plate counts and lipid oxidation from day 20 through 25 on steaks packaged in MFS and MDF, respectively. Steaks packaged in MB were redder ($p < 0.05$) and more vivid (C*) as storage time increased. Whereas lipid oxidation was greater ($p < 0.05$) throughout the entire display for steaks packaged in MFS and MDF. It is evident that barrier properties of MB limiting oxygen exposure of the steak preserved fresh meat characteristics after frozen storage. Results from the current study suggest that vacuum packaging films can aid in retarding detrimental effects caused by frozen storage after placing the steaks in fresh retail conditions.

Keywords: beef; instrumental color; lipid oxidation; self-life; slacked thaw; vacuum packaging

1. Introduction

Meat products are highly perishable; therefore, strategies have been explored for countless decades to extend the fresh shelf life of red meat [1]. Cold storage is one such strategy frozen meat can extend storage life and the possible reduction in quality losses that occurs to fresh meat products [2,3]. Consumers may consider freezing meat purchases to prolong the interval between purchase and consumption. However, prior to consumer purchase the meat industry may consider frozen meat storage. If freezing meat prior to retail or foodservice use, this technique has often been used to extend storage periods, manage supply chain, or facilitate distribution channels. However, despite the benefits, the process of frozen storage often requires a considerable amount of logistical planning that manufacturers or retailers may not find ideal.

During freezing, meat products undergo a physical transformation when water is converted into ice crystals, upon thawing, a transformation to a pre-frozen state occurs [4]. Freezing meat discourages food-borne pathogens by creating an unstable environment for microorganism growth, organism can regain activity as storage temperatures increase [5]. Despite the unavoidable physical changes, it is imperative the frozen-stored and thawed meat will retain the quality attributes consumers associate with fresh meat [6]. Moisture lost from the muscle during thawing can promote microbial growth as a as packaging purge increases [7]. Thus, the FDA and USDA-FSIS have indicated to consumers meat should be thawed using refrigerated temperature at 4.44 ◦C or below to discourage microbial growth [8,9]. In addition to microbial growth, other important meat characteristics that are affected by freezing and thawing procedures also include: moisture loss, protein denaturation, lipid oxidation, surface color, pH, objective tenderness, and purge loss [10,11].

At the point of sale, the visual appearance of beef products represents the most important characteristic influencing consumer purchasing decisions with a characteristic cherry-red color being highly desirable [12,13]. Vacuum packaging limits meat surface exposure to oxygen resulting a dark purple. However, when permeability of the packaging film increases, greater concentrations of oxygen can all the meat surface to possess a bright, cherry-red color.

Often, the most overlooked packaging factor to consider when selecting packaging materials for meat products is the oxygen transmission rate (OTR) of the packaging film. Packaging film OTR reflects the potential for oxygen and other atmospheric gases to bind with myoglobin and form surface color pigments, thus there may be an optimal OTR which would promote the reddish surface color that consumers prefer at the time of purchase [14]. Additionally, moisture vapor barrier properties may also influence storage of fresh and frozen meat [15]. Although freezing meat offers consumers a product that reflects the same nutritional quality as fresh products, physical and biochemical changes that occur to meat during freeze storage can negatively affect critical organoleptic properties like surface color [14].

Purge loss is inevitable in fresh meat given the inherent conversion of muscle to meat driving such processes as rigor mortis and postmortem muscle pH [15]. Retaining moisture in meat products is important to limit the loss of salable weight and protein at the time of consumer purchase [16]. Furthermore, there is a mechanistic relationship between pH and water holding capacity as the loss of hydrogen ions (H+) can accelerate pH decline and reduced water-holding capacity can lead to unacceptable purge loss [7,16]. Increased thawing time at elevated temperatures may also increase purge loss promoting an increase in microbial proliferation [17]. To date, limited research investigating the impact of packaging methods and materials on meat quality has been published [18]. Therefore, the objective for the current study was to determine

the effect of vacuum packaging on shelf-life characteristics of boneless ribeye steaks that have been previously frozen.

2. Materials and Methods

2.1. Muscle Fabrication

Beef boneless ribeye rolls (IMPS #122A) were purchased from a commercial meat processor and transported under refrigeration (2 ◦C) to the Auburn University Lambert Powell Meat Laboratory for processing. Using pack date on each box not exceeding 10 days from the time of packaging, ribeye rolls were selected for steak cutting. Ribeye rolls (N = 18) were fabricated into 2.54-cm-thick steaks (n = 12 steaks/ribeye roll) with a BIRO bandsaw (Model 334, Biro Manufacturing Company, Marblehead, OH, USA). Steaks from each ribeye roll were randomly selected and allocated to one of three packaging treatments.

2.2. Packaging Treatments

After cutting, steaks were allowed to bloom to simulate an industry application for 30 min at 2 ◦C, crust frozen at −23 ◦C for 45 min, and then packaged with a form and fill packaging machine (Model OL0924, Variovac, Zarrentin, Germany). Steaks were packaged in one of three commercially available packaging films (WINPAK, Winnipeg, MB, Canada) consisting of a high barrier and or low barrier film. The high barrier film (MB) was comprised of 150 µm of nylon, enhanced ethylene-vinyl alcohol (EVOH), and polyethylene. Steaks packaged in low barrier films were constructed with 150 µm polypropylene and polyolefin plastomer (MFS) or a combination of 150 µm polyolefin and polyethylene (MDF). Oxygen transmission (OTR) of the packaging treatments consisted of: MB (0.5 cc/sq. m/24 h); MFS (1100 cc/sq. m/24 h); and MDF (1287 cc/sq. m/24 h). In addition, moisture vapor transmission of each packaging film was measured: MB (3.9 g/sq. m/24 h); MFS (2.9 g/sq. m/24 h); and MDF (3.5 g/sq. m/24 h).

Packaged steaks were placed flat on a tray (76.2 cm × 60.96 cm) and stored in a blast freezer (−23 ◦C) for 120 min.

2.3. Simulated Storage Periods

Initially, steaks were placed in a two-door, reach-in, commercial freezer (Model AF49EX, Arctic Air, Eden Prairie, MN, USA) for 25 days at −13 ◦C. Packaged steaks were stored in the absence of light for the duration of the simulated frozen storage period. Temperature during the frozen storage period was monitored using a data recording device (Model-TD2F, Thermoworks, American Fork, UT, USA) with probes placed within the center of each shelf. Throughout the storage period frozen steaks were rotated across all shelves. Following the 25-day frozen dark storage, packaged steaks were transferred to an LED lighted, refrigerated, 3-tiered, case (Model TOM-60DX-BN, Turbo Air Inc., Long Beach, CA, USA) to simulate a fresh retail setting. Packaged steaks were displayed at 3 ◦C ± 1.2 ◦C and data loggers (Model- TD2F, Thermoworks, American Fork, UT, USA) recorded storage temperatures. Continuous lighting intensity (2297 lux) of case shelves was recorded (Model ILT10C, International Light Technologies, Peabody, MA, USA) throughout the fresh display period. During fresh display, steaks were placed across all shelves and rotated on the shelving to simulate consumer movement. On days 0, 7, 10, 15, 20, and 25 steaks were removed from the refrigerated display case and measured for instrumental color, lipid oxidation, purge loss, pH, and spoilage organisms.

2.4. Instrumental Color

Fresh instrumental color readings were measured through the packaging on day 0, 5, 10, 15, 20, and 25 by scanning the surface of each steak through the packaging according to guidelines previously described [19]. Surface color values were collected using a HunterLab MiniScan XE

Plus Colorimeter (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA, USA) calibrated against a standard black and white glass tile each day immediately before data collection. The L* (lightness), a* (redness), and b* (yellowness) values of each steak were determined from the average of three readings using Illuminant A10, with a 10◦ observer and a 25 mm diameter aperture and the Commission Internationale de l' Eclariage (CIE L*a*b*) color scale [20]. Chroma (C*) was calculated using the following equation: $\sqrt{a^{*2} + b^{*2}}$ with a more vivid color resulting from a great value. Additionally, hue angle was calculated as: $\tan^{-1}(b^*/a^*)$ where a greater value represents the surface color shifting from red to yellow.

2.5. Microbial Analysis: Aerobic Plate Count

The total number of viable non-pathogenic aerobic microorganisms was determined using standard methods [21]. Duplicate 5 g samples were removed aseptically from each package. Samples were placed in a stomacher bag containing a sterile filter (3M Corp., St. Paul, MN, USA) and 50 mL of Butterfield's Buffer (3M Corp., St. Paul, MN, USA). Stomacher bags were agitated for 1 min. After stomaching, a 10-fold dilution series was completed for microbial analysis. Serial, duplicate platings were placed onto aerobic (APC) plates, Petrifilm® (3M Corp., St. Paul, MN, USA), and incubated at 36.0 ◦C for 48 hours in an incubator chamber (Model IB-05G, Lab Companion, Yuseong-gu, Daejeon, Korea) prior to enumeration. Microbial counts were recorded as colony-forming units per gram (CFU/g) [21]. Incubation temperature was recorded using a data logger (Model-TD2F, Thermoworks, American Fork, UT, USA) placed in the geometric center of each self.

2.6. Lipid Oxidation

Packages of fresh steaks were sampled for 2-thiobarbituric acid reactive substances (TBARS) as previously described [22]. Steaks were minced into a uniform sample of the entire steak with a hand-held knife. In duplicate, 2 g ± 0.5 g of each minced steak was pulverized with 8 mL of cold (1 ◦C) of 50 mM phosphate buffer (pH of 7.0 at 4 ◦C) containing 0.1% ethylenediaminetetraacetic acid (EDTA), 0.1% n-propyl gallate, and 2 mL trichloroacetic acid (Sigma-Aldrich, Saint Louis, MO, USA). Samples were filtered through a 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 (BeanTown Chemical, Hudson, NH, USA) and boiled at 100 ◦C for 20 min. After boiling, tubes were placed into an ice bath for 15 min. Absorbance of each sample was measured at 533 nm with a spectrophotometer (Turner Model-SM110245, Barnstead International, Dubugue, IA, USA) and multiplied using a factor of 12.21 to derive the TBARS value (mg of malonaldehyde/kg of fresh meat). The value of 12.21 was obtained previously from a standard curve using a known malonaldehyde solution measured across multiple absorbencies [22].

2.7. Fresh pH

Muscle pH was measured on steaks throughout the fresh display period in duplicate using a steel electrode attached to a pH meter (Model HI199163, Hanna Instruments, Woonsocket, RI, USA) inserted into the steak at two random locations. Prior to collecting fresh muscle pH values, the pH meter was calibrated using 2-point (4.0 and 7.0) buffers (Thermo Fisher Scientific, Chelmsford, MA, USA) and after every 5 readings.

2.8. Purge Loss

Steaks were removed from their package treatment, blotted dry with a paper towel, and weighed on a balance (Model PB3002-S, Mettler Toledo, Columbus, OH, USA). Purge loss calculations is as follows: [(packaged weight − steak weight) ÷ packaged weight × 100)].

2.9. Statistical Analysis

The current study was conducted and analyzed as a completely randomized design with packaged steaks serving as the experimental unit. Data were analyzed using the GLIMMIX model procedure of SAS (version, 9.2; SAS Inst. Inc., Cary, NC, USA). Packaging treatment was used as a lone fixed effect and replication represented the random effect for instrumental surface color, APC, TBARS, pH, and purge loss. Day of simulated display served as a repeated measure, whereas packaging treatment, day, and packaging treatment × day interaction were fixed effects. Least square means were generated, and when significant ($p \leq 0.05$) F-values observed, least square means separation occurred by using the pair-wise t-test (PDIFF option).

3. Results and Discussion

3.1. Instrumental color

Following frozen dark storage, instrumental color of vacuum-packaged steaks was recorded throughout a 25-day fresh display period. There was no interaction ($p > 0.05$) for packaging method × day of display for fresh surface color lightness (L*; values not reported). However, throughout the fresh display there was an interaction ($p < 0.05$) for packaging method × day of simulated display for redness (a*), yellowness (b*), chroma (C*) and hue angle (Table 1). Steaks packaged using MB film were redder ($p < 0.05$) from day 7 through 25 of the fresh simulated retail display period. However, steaks packaged in MFS and MDF films were more

yellow ($p < 0.05$) initially, but as storage time increased past day 20, yellowness values declined for all packaging methods. This data suggest that the use of MB film may promote a better visual color during retail display post frozen storage compared to MFS and MDF films.

A decline in a* values of thawed meat has been attributed to myoglobin denaturation occurring during colder storage temperatures, but surface redness can increase after thawing when myoglobin is stored in a favorable oxygen binding environment [23]. A similar study conducted examining the relationship between frozen and fresh beef color values reported increased anaerobic refrigerated storage duration can result in a rapid decline of a* values which has also been linked to an increase in lipid oxidation [24]. Our results are consistent with a previous study that evaluated the surface color of meat following a frozen storage period and reported declining b* values throughout a refrigerated storage time after frozen storage [25]. However, another study reported b* values did not differ after thawing at different temperatures when measuring the surface color of beef Longissimus dorsi [26]. Interestingly, duration of storage time may negatively influence the percentage of oxymyoglobin or metmyoglobin causing a detrimental impact on redness values for steaks possessing greater percentages of oxymyoglobin [27]. Nevertheless, it has been reported that increasing oxygen saturation prior to freezing can result in greater oxidation after thawing, and a loss of reducing enzymes through exudate contributing to a deterioration in color stability [13,27]. Storage temperature of frozen black wildebeest resulted in yellowness (b*) values increasing during the refrigerated storage time, but these surface color changes of game meats may have occurred because of inherent darker color and greater muscle pH values related to game muscle [28]. Results from the current study support the hypothesis that surface color may be negatively altered after storage in frozen and subsequent refrigerated temperatures.

Consistent with observed redness and yellowness values for thawed beef steaks, instrumental surface color was also more ($p < 0.05$) vivid (C*) for steaks packaged in MB from day 7 through day 25 of the fresh display period (Table 1). In contrast, hue angle values for MFS and MDF increased ($p < 0.05$) throughout the entire display period indicative of color shifting from red to yellow for these packaged steaks. The current results for C* differ from previous results that have reported the combination of chilled-then-freezing beef loins can cause an increase in C* values with increased storage time [29]. However, additional studies have reported that storing frozen beef in oxygen impermeable films resulted in maintenance of a more desirable color, reduced off-flavors and less lipid oxidation [30,31]. In the current study, packaging steaks using MB film constructed with the lowest OTR rating appeared to confer protection against deterioration throughout the storage periods to thawed beef steaks resulting in greater surface color stability. It has been reported that color changes throughout frozen storage in marinated raw beef meat can be due myoglobin denaturation caused by lipid oxidation [32]. Additionally, it has been reported that a decrease in lightness values during the freeze–thaw cycle is associated with a surface light reflectance attributable to water loss [32]. Color shifting in frozen meat may be caused by physical processes such as drip loss, or when water molecules freeze resulting in a shift of fat, total protein, and water/protein ratio chemical concentrations on the surface layer after thawing [33]. It is plausible that the duration of frozen storage time, packaging materials, and refrigerated storage temperature will affect color stability. Further research is needed determine the impact of intrinsic and extrinsic factors on meat color stability when storage temperatures are altered. Regardless, these data point to a potential advantage for MB packaging film.

3.2. Aerobic Plate Count Changes

An interaction ($p < 0.05$) for packaging treatment × day of retail display occurred for spoilage organisms (Table 2). Regardless of packaging treatment, spoilage organisms increased ($p < 0.05$) throughout the simulated refrigerated display period. However, spoilage organism growth was hindered ($p < 0.05$) when using MB for packaging steaks after day 20. Interestingly, it should be noted that throughout the duration of the current study, there were no packaging treatments that crossed the 6 log CFU/g threshold thus no packing treatments associated with detrimental effects on the wholesomeness and safety of fresh meats in the current study.

Results agree with previous findings related to varying storage treatments (ex. chilled only, frozen only, or chilled then frozen) where like in the current study, the storage of beef loins did not cause an increase in spoilage microorganisms [29]. Furthermore, reduced the growth of microbial populations has been reported for meat when stored at colder temperatures (−12 ◦C to −18 ◦C) [29]. Nonetheless, an increased microbial count is to be expected after freezing and thawing because of exudate formation coupled with an increase in moisture and the amount of nutrients available to support microbial proliferation [7]. Packaging materials that are constructed to limit OTR can reduce oxygen transmission thus reducing aerobic microbial proliferation and potentially extend shelf life. Limited microbial growth in the current study agrees with a previous study that examined packaging OTR and the subsequent influence on aerobic spoilage organisms [34].

3.3. *Lipid Oxidation*

There was an interactive effect of packaging method × day of display for lipid oxidation on thawed beef steaks (Figure 1). Lipid oxidation increased ($p < 0.05$) in steaks packaged using

MDF and MFS films throughout the 25-day simulated retail display. Increases in lipid oxidation values agree with previous findings that evaluated vacuum stored meat products. The reduced lipid oxidation reported in MB-versus MDF, and MFS films is expected as a reduced OTR would reduce exposure of the steak to oxidation throughout the frozen and fresh storage periods. Consistent with this hypothesis, an accelerated rate of lipid oxidation associating with a greater amount of oxygen exposure has been reported across packaging materials [24–26,28,29,32,33,35]. In a study examining minced porcine muscles stored in vacuum packaging, lipid oxidation tended to accelerate after thawing as peroxidation giving rise to rapid secondary lipid oxidation and increased TBARS values were reported [36]. In the current study, it appears the MB film confers a greater protection against lipid oxidation than the use of either MDF or MFS films.

3.4. Fresh pH

The interactive ($p < 0.05$) of packaging method × day of display for pH values of thawed steaks is presented in Figure 2. Postmortem muscle pH can be instrumental in indicating the quality of fresh meat surface color and optimal pH values may hinder microbial growth. In the current study, a significant decrease ($p < 0.05$) in pH occurred across packaging treatments throughout the stimulated storage periods. It is worth noting that in this study, fresh muscle pH values did not decline below values that would be expected to detrimentally influence surface color values.

It has been previously stated that greater pH values have been attributed to the denaturation of buffer proteins with the increase of solute concentration occurring in frozen storage [37]. It is plausible that an increase of OTR for steaks packaged in MDF influenced the growth in lactic acid bacteria often noted in vacuum-packaged meats [38]. A similar reduction in

muscle pH was reported when evaluating frozen vacuum packaged meat after frozen storage [25]. Moreover, the loss of free moisture from the meat products during the defrosting phase can result in a greater concentration of solutes within the package, plausible causing a decline in pH of thawed meat [37]. Conversely, packaging methods of frozen beef sirloins did not appear to influence muscle pH [11]. However, lactic acid bacteria are generally associated with a decline in muscle pH.

3.5. Purge Loss

There was no interactive effect for packaging treatment × day on purge loss of packaged steaks (values not reported). Purge loss after frozen storage and throughout fresh, refrigerated storage increased ($p < 0.05$) from day 0 to 25 regardless of packaging (Figure 3). It is plausible that the rise in purge loss could be attributed to the decline in muscle pH that occurred across all packaging treatments causing greater amounts of free and bound water to be lost during storage.

Purge loss, specifically water holding capacity, is related to the available moisture properties residing within microfibrillar proteins [11]. Furthermore, moisture losses occurring in meat have been linked to storage temperatures and temperature variation can influence moisture loss during storage periods [40]. Given this, purge loss remains a crucial factor to consider when selecting packaging materials or storage temperatures due to the monetary impact throughout the meat industry even though we observed no differences in purge loss related to packaging film for previously frozen, beef ribeye steaks across MB, MSF, and MDF films.

5. Conclusions

Results presented here supports the hypothesis that when selecting vacuum-packaging film during the storage of beef products, oxygen transmission and moisture vapor transmission

rate of the film should be considered. The potential influence caused by packaging film composition can alter surface color, and wholesome characteristics throughout a freeze-thaw cycle of beef steaks. It is plausible that steaks packaged in film possessing reduced oxygen and moisture transmission rates may have a more stable surface color, reduced lipid oxidation, and hindered aerobic microorganism growth. However, to enhance the consumer acceptance of vacuum packaging, additional educational opportunities should be provided to consumers and producers on the various impacts of freezing and thawing of vacuum packaged red meats. Furthermore, evaluating the sensory profile of meat products after freeze–thaw cycles when using vacuum packaging films for red meat storage is needed.

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TABLES AND FIGURES

Table 3.1 The interactive impact of packaging method× day) on instrumental color values.

	Day						
	0	7	10	15	20	25	SEM
MB[1]							
a*[2]	13.61^{e}	22.11^{a}	20.45^{b}	20.15^{b}	17.52^{c}	15.76^{d}	0.330
b*[2]	13.94^{fg}	13.17^{hi}	13.03^{hi}	13.77^{gh}	13.59^{ghi}	12.88^{hi}	0.251
C*[3]	19.69^{e}	25.79^{a}	24.27^{b}	24.45^{b}	22.25^{d}	20.40^{e}	0.326
Hue (°)[4]	45.87^{d}	30.85^{i}	32.52^{h}	34.41^{g}	38.03^{f}	39.51^{f}	0.716
MFS[1]							
a*[2]	17.21^{c}	10.46^{gh}	10.46^{gh}	10.78^{fgh}	10.03^{hi}	10.17^{ghi}	0.345
b*[2]	16.28^{a}	16.25^{ab}	15.33^{cd}	14.66^{ef}	13.94^{fg}	13.92^{fgh}	0.251
C*[3]	23.76^{bc}	19.94^{e}	18.61^{f}	18.32^{fg}	17.25^{h}	17.29^{gh}	0.344
Hue (°)[4]	43.63^{e}	54.83^{b}	55.85^{b}	53.90^{b}	54.36^{b}	53.69^{b}	0.716
MDF[1]							
a*[2]	17.04^{c}	11.25^{fg}	9.19^{i}	10.29^{gh}	10.69^{fgh}	11.98^{f}	0.345
b*[2]	15.79^{bc}	16.05^{ab}	14.99^{de}	14.34^{fg}	12.99^{hi}	12.75^{i}	0.251
C*[3]	23.31^{c}	19.65^{e}	17.62^{fgh}	17.72^{fgh}	16.94^{h}	17.56^{fgh}	0.344
Hue (°)[4]	43.06^{e}	55.24^{b}	58.58^{a}	54.34^{b}	50.45^{c}	47.06^{d}	0.716

[1] Packaging treatments: (MB) nylon + enhanced ethylene-vinyl alcohol + polyethylene; (MFS) polypropylene + polyolefin plastomer; and (MDF) polyolefin + polyethylene. [2] a* values measure redness (larger value indicates a redder color); and b* values measure yellowness (larger value indicates a more yellow color). [3] Chroma measures total color (larger number indicates a more vivid color). [4] Hue angle is the change from the true red axis (larger number indicates a greater shift from red to yellow). [*] SEM, standard error of the mean.

[a–i] Mean values within day of display and packaging method lacking common superscripts differ ($p < 0.05$).

Table 3.2 Interactive effect of packaging method × day on aerobic (APC) spoilage organism growth.

	Day					
	0	**10**	**15**	**20**	**25**	**SEM***
MB[1]	>0.001^{e}	0.57^{d}	1.25^{ab}	0.85^{bcd}	0.57^{d}	0.142
MFS[1]	0.04^{e}	0.56^{d}	0.86^{bcd}	1.46^{a}	1.03^{bc}	0.142
MDF[1]	>0.001^{e}	0.83^{cd}	0.85^{cd}	1.47^{a}	1.44^{a}	0.142

[1] Packaging treatments: (MB) nylon + enhanced ethylene-vinyl alcohol + polyethylene; (MFS) polypropylene + polyolefin plastomer; and (MDF) polyolefin + polyethylene. * SEM, standard error of mean. $^{a–e}$ Mean values within day of display and packaging treatment lacking common superscripts differ ($p < 0.05$).

Figure 2.1 Interactive effect of treatment × day of display for 2-Thiobarbituric acid reactive substances (TBARS). Bars lacking common letters differ ($p \leq 0.05$).

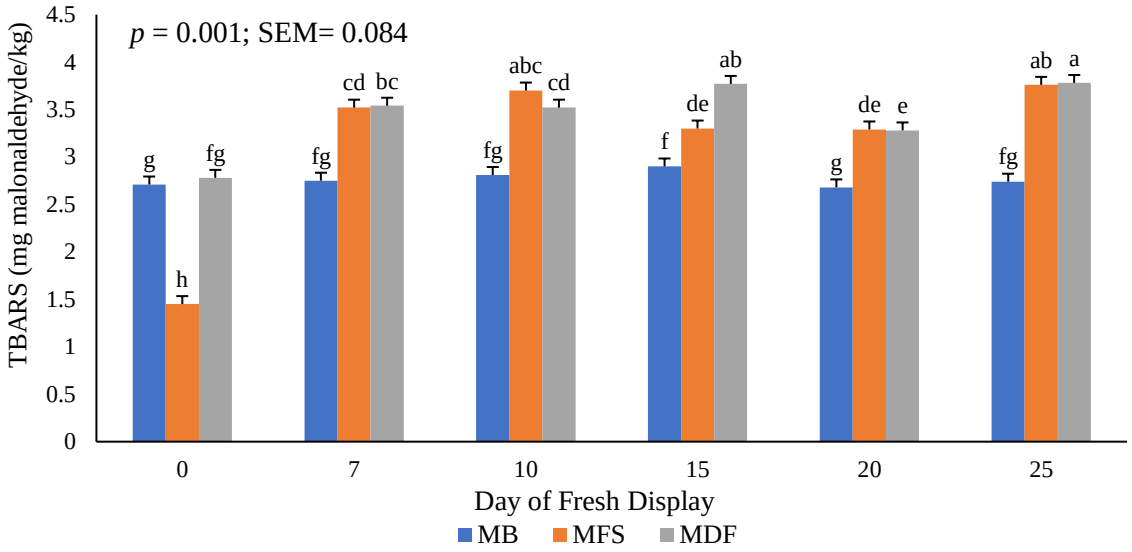


Figure 2.2 Interactive effect of packaging method × day of display fresh pH values. Bars lacking common letters differ ($p \leq 0.05$).

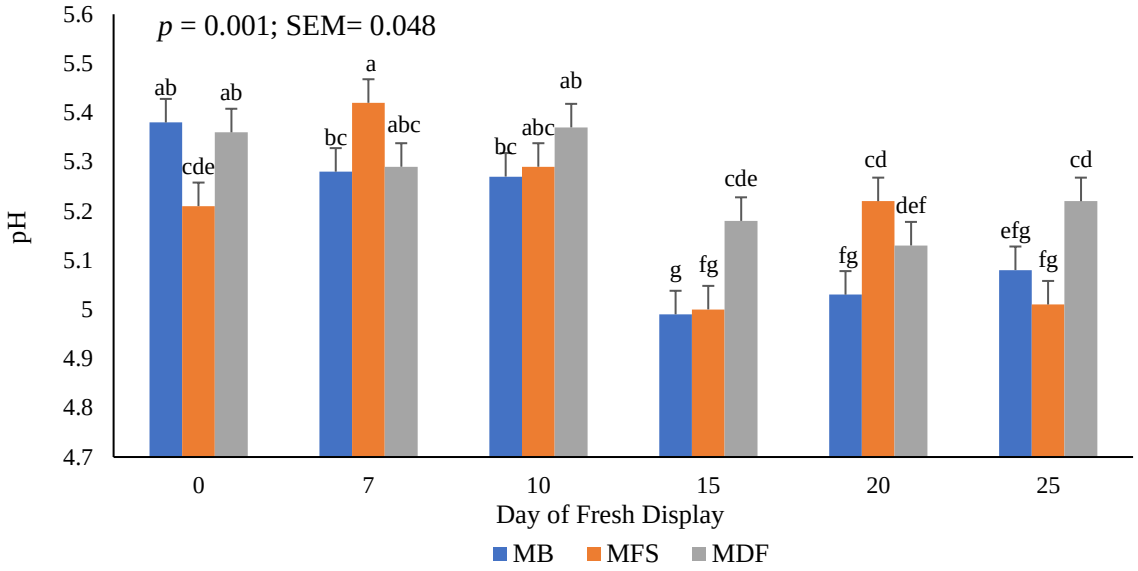
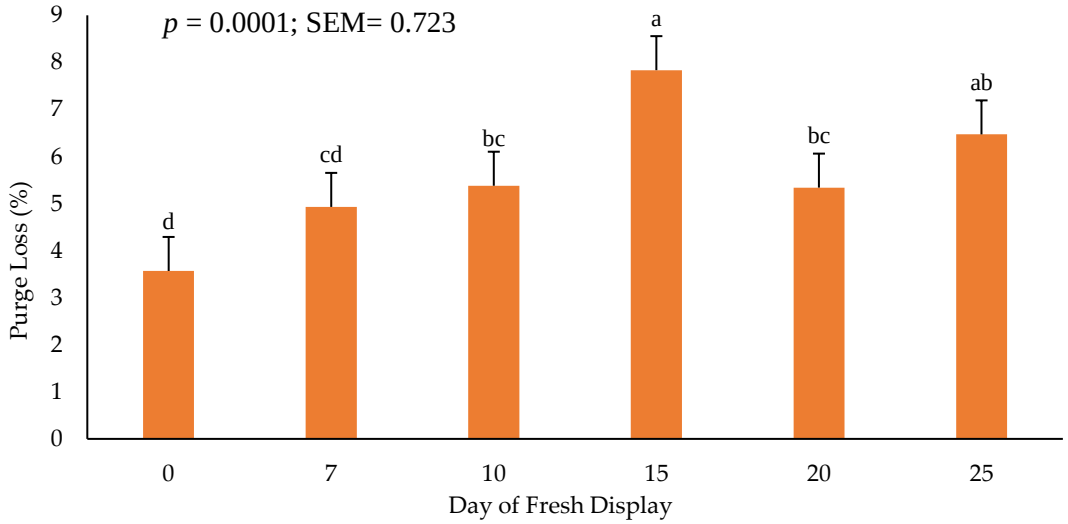


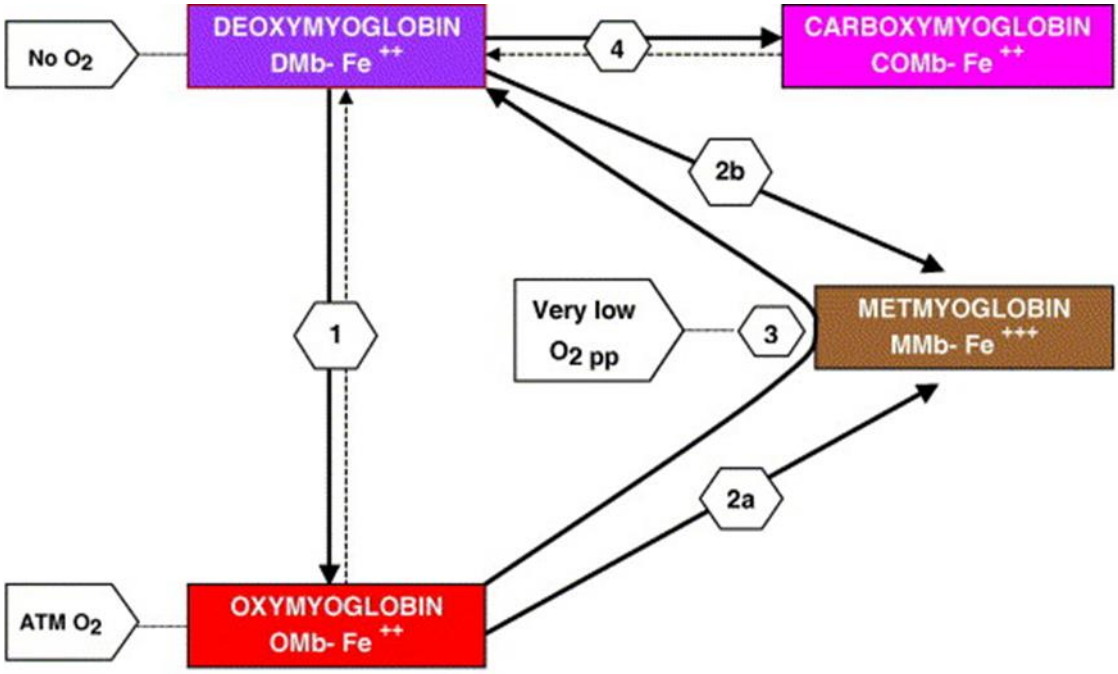
Figure 2.3. Influence of display day on purge loss (%) on refrigerated beef ribeye steaks. Bars lacking common letters differ ($p \leq 0.05$)



APPENDICES

APPENDIX A

Myoglobin Meat Color Triangle



Rx 1 (Oxygenation): DMb + O_2 → OMb

Rx 2a (Oxidation): OMb + [oxygen consumption or low O_2 partial pressure] − e^- → MMb

Rx 2b (Oxidation): [DMb – hydroxyl ion – Hydrogen ion complex]+ O_2 → MMb + O_2^-

Rx 3 (Reduction): MMb + Oxygen consumption + metmyoglobin reducing activty → DMb

Rx 4 (CarboxyMb): DMb + carbon monoxide → COMb

APPENDIX B

Thiobarbituric Acid Reactive Substances (TBARS)

Chemicals:

Water – HPLC grade or distilled deionized waterPotassium 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-deionizedwater (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 (pHwill 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 moreof 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 isextremely 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 deionizedwater. 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 placeinto 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 coldestregions 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 ºC. It takes approximately 1h for the water bath to reach the desired temperature. If a sipper unit is being used, it is necessaryto 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 centrifugetube. 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 mustbe sealed and stored in a refrigerator).
5. Remove 2 ml of the sample filtrate and place it into a labeled glass test tube. Prepareduplicate 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, timeis extremely critical. Make sure that the water bath is at the correct temperature and levelprior 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 withdistilled water.

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

*This standard should have an Absorbance in the proximity of 0.150. Range may be 0.130 to0.170, depending upon the accuracy of solutions and dilutions.

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APPENDIX C

Aerobic Plate Count Method

Materials & Equipment:

5g sample of Raw Product

3M Sample Bag W/ Filter Sterile

50mL 3M Butterfield's Buffer

Stomacher Lab Blender

2 Glass test tubes with 9mL of 3M Butterfield's Buffer (autoclaved)

1mL Pipetting tips and Pipette

Vortex Mixer

Petrifilm® aerobic plate count (APC) plates

3M Petrifilm Spreader Incubation chamber (36.0 °C)

Procedure:

1. Extract 5-gram sample from respective packaging material
2. Place sample in 3M Sample Bag W/ Filter Sterile
3. Add 50mL 3M Butterfield's Buffer to sample bag
4. Place sample bag and contents in stomacher lab blender for 60 seconds
5. With pipette extract 1mL from sample bag and plate sample on APC plate
6. Use 3M Petrifilm Spreader to spread sample evenly
7. With pipette extract 1mL from sample bag and place in dilution two tube
8. Vortex dilution two tube
9. With pipette extract 1mL and place in dilution three tube
10. With pipette extract 1mL from dilution two tube and plate sample on APC plate
11. Use 3M Petrifilm Spreader to spread sample evenly
12. Vortex dilution three tube
13. With pipette extract 1mL from dilution three tube and plate sample on APC plate
14. Use 3M Petrifilm Spreader to spread sample evenly
15. Vortex dilution four tube
16. With pipette extract 1 mL from dilution four tube and plate sample on APC plate
17. Use 3M Petrifilm Spreader to spread sample evenly

18. Vortex dilution five tube
19. With pipette extract 1 mL from dilution five tube and plate sample on APC plate
20. Use 3M Petrifilm Spreader to spread sample evenly
21. Vortex dilution six tube
22. With pipette extract 1 mL from dilution six tube and plate sample on APC plate
23. Use 3M Petrifilm Spreader to spread sample evenly
24. Vortex dilution seven tube
25. With pipette extract 1 mL from dilution seven tube and plate sample on APC plate
26. Use 3M Petrifilm Spreader to spread sample evenly
27. Vortex dilution eight tube
28. With pipette extract 1 mL from dilution eight tube and plate sample on APC plate
29. Use 3M Petrifilm Spreader to spread sample evenly
30. Vortex dilution nine tube
31. With pipette extract 1 mL from dilution nine tube and plate sample on APC plate
32. Use 3M Petrifilm Spreader to spread sample evenly
33. Vortex dilution ten tube
34. With pipette extract 1 mL from dilution ten tube and plate sample on APC plate
35. Use 3M Petrifilm Spreader to spread sample evenly
36. Incubate APC plates at 36°C ± 1°C for 48h
37. Interpret plates by counting colonies

References:

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