

Influence of Packaging Film and Beef Trimmings on Ground Beef Shelf-Life

by

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ABSTRACT

Fresh beef storage in the retail setting can be presented in a variety of packaging methods. The focus of this study was to identify the influence of packaging film and lean blends on fresh ground beef surface color during a simulated retail display period of 21 days. The influence of packaging had no impact ($P < 0.05$) on initial beef color, amount of browning, or percent of surface discoloration. Moreover, packaging materials were able to support storing ground beef for up to 21 days in simulated retail display settings. Across the simulated day of display 0, 7, 14, and 21 visual sensory panelists recorded a greater percentage of discoloration for all treatments. Ground beef packages formulated with a greater percentage of CULL beef trimmings resulted in the greatest ($P < 0.05$) increase in percent discoloration. Additionally, packaging film MB2 (0.2 cc/sq. m/24hr.) consistently resulted in fewer ($P < 0.05$) microbial aerobic spoilage organisms \log_{10} CFU/g counts across lean trimming formulations blends and day of simulated retail display. These results suggest that the ground beef utilized in this study can be considered safely consumed as packaging materials prevent microorganisms from exceeding a 6-log spoilage threshold limit. More importantly, the surface color variation was minimal and could be visually appealing to consumers after 21 days of retail display conditions.

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**This Thesis is formatted to fit the style and guidelines for the peer-reviewed
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CHAPTER I

LITERATURE REVIEW

ABSTRACT

Vacuum packaging is a common packaging method used to store meat products for extended periods of time. By extending the shelf life of meat products, this maintains consumer appeal and enables the meat industry to reduce waste and be more sustainable for consumers and retailers. Additionally, shelf-life stability advancements not only ease the constantly growing pressure to feed more people in the world, but allow for higher efficiency to do so with fewer resources. Through technology advancements, such as the application of essential oils being utilized during vacuum packaging, these applications show real promise for improving shelf-life for the food industry. A recent study describing the use of chitin nanofibril and Ajowan essential oil simultaneously in raw beef wrapping concluded that a more desirable color and shelf-life stability are obtainable during retail display. Additionally, a trending topic among packaging enthusiasts and environmentalists currently are recycling and sustainability of the earth pertaining to use of plastics and expanded polystyrene (EPS). Vacuum packaging creates a scenario for the meats industry to become more eco-friendly by utilizing recyclable materials. Several companies such as Klöckner Pentaplast and SEALPAC have recently contributed to increased innovation by creating recyclable materials that can be utilized in vacuum and form-fill-seal packaging. Through recognizing the deficiencies in the food industry such as meat waste, loss, and major causes of meat spoilage, the assumption can be made that changes in the industry are imminent. For these reasons, combined with shelf-life extension and sustainability, vacuum packaging makes a strong case to be utilized as the primary packaging for retail food applications.

INTRODUCTION

The time that meat can be stored safely for consumption is pertinent to the meat industry. Product safety does not end when the meat product leaves the plant or grocery store setting but instead when the product is actually consumed. Not only does the meat industry lose money when meat spoils, so does the customer when they get the product home and do not consume it in a timely manner. In the United States, meat, poultry, and fish account for \$48 billion in value of food loss at the retail and consumer levels annually (Buzby et. al, 2014). Fresh meats account for 30% of the food loss for the total value of \$161.6 billion in 2010 (Buzby et. al, 2014). In meat loss, roughly 30% occurs at the retail counter and 70% is lost at the consumer level (Buzby et. al, 2014). Especially in times of uncertainty, like the world experienced with the onset time of the Covid-19 pandemic, as consumers rush to grocery stores to buy large quantities of perishable items, storage is an important consideration.

Vacuum packaging is a common method used to store meat products for extended periods of time. Vacuum packaging removes air from the product, thus allowing for longer storage times due to less aerobic microbial growth. Additionally, it allows for longer aging times and less undesirable meat discoloration. Unfortunately, without any additives or coloring agents, the lack of oxygen leads to a purplish color of meat, which is not appealing to the typical consumer. In contrast, overwrap and modified atmospheric packaging (MAP) tend to present a more desirable product visually but their shelf life is just a fraction of what vacuum packaging provides. But as consumers shift towards buying large quantities, such as during a pandemic these items have roughly 3 to 5 d to be consumed or frozen once they arrive home. However, freezing in the original overwrap or MAP packaging renders the meat susceptible to “freezer burn.” To best suit the needs of both retailers and consumers, combining some of the benefits of

each packaging method can assist in solving many problems in the meat supply chain. Therefore, this exposition is aimed to:

- Highlight the need for packaging advancements in shelf-life stability.
- Review some major causes of meat spoilage and preservation.
- Investigate new upcoming technology.

MEAT LOSS VS. MEAT WASTE

A major issue in the meats industry is meat loss and waste, which are two different terms that have to do with meat products not being efficiently utilized. Meat loss is associated with “any food that is discarded, incinerated or otherwise disposed of along the food supply chain from slaughter up to, but excluding the retail level, and does not re-enter in any other productive utilization, such as feed or seed” (Rosmini et al., 2004). This can even include moisture loss, cook loss and other loss associated with mold and pests (Buzby et al., 2014). On the other side we have meat waste being described as, “deviates from what is considered optimal, for example in terms of shape, size, and color, is often removed from the supply chain during sorting operations” along with, “foods that are close to, at or beyond the ‘best-before date are often discarded by retailers and consumers” (Rosmini et al., 2004). Both loss and waste are issues in the meats industry causing an estimated 2.7 billion pounds of meat products to be discarded in the U.S. having an economic impact of around \$8.8 billion (Lipinski et al., 2013). Thus, the need for packaging advancements in shelf-life stability.

Advancements in shelf-life stability will not only ease the constantly growing pressure to feed more people in the world but allow for higher efficiency to do so with fewer resources. If food loss and waste can be reduced by half (24% to 12%) by the year 2050, the world would need 1,314 trillion kcals of less food per year (Lipinski et al., 2013). It is also projected by

cutting waste and loss in half, this could bridge the gap between the estimated need for food and the current production expected in 2050 (Lipinski et al., 2013). With the vast majority of food waste and loss in developed countries like the US happening at the handling/storage (9% of total waste/loss) and consumption (28% of total waste/loss) stages (Lipinski et al., 2013). Meat loss and waste is an estimated 19% of the total food loss and waste as of 2009 (Lipinski et al., 2013). Decreasing meat waste and loss could be a leading strategy in an attempt to find ways for more sustainable meat products. Identifying a solution in meat vacuum packaging not only affects red meat products but potentially chicken, fish, processed, and other meats as well.

CAUSES OF SPOILAGE AND COMMON PRESERVATION

There have been many studies and attempts previously made to correct this deficit in the meats industry. There are several different ways of preserving meats for various amounts of storage times, each having unique associated advantages and disadvantages. Spoilage is often the root cause behind meat loss and waste with our current packaging techniques. Post-mortem handling and packaging of meat products are affected by spoilage mechanisms such as microbial spoilage (decomposition of meat from microorganisms), lipid oxidation (also known as chemical though the degradation of lipids, proteins, and carbohydrates), and autolysis enzymatic spoilage (also known as physical when meat becomes brittle and falls apart (Addis, 2015). If meat products did not have spoilage risks associated, this packaging dilemma would be irrelevant and meat would have a much longer, more stable shelf life. However, from a practical standpoint we are challenged with having to find a sustainable solution in improving vacuum packaging shelf life and appearance to decrease meat waste and loss.

Several methods of current vacuum packaging shelf-life extension methods are outlined. The first and most obvious method of shelf-life extension in vacuum packaged meats in the

literature is temperature. Low temperature anticipates slowing or limiting spoilage by creating an environment that is favorable to inhibit microbial growth.

Temperature

There are three natures of low-temperature methods, consisting of chilling, freezing, and super chilling. The first and highest temperature of the trio is chilling, typically used directly post-slaughter, during transport and storage. Chilling is essential to meat safety, shelf life, and appearance and typically has a temperature around 4°C. Vacuum packaged beef is expected to have a shelf life of roughly 35 to 45 d at chilled temperatures (Delmore, 2009). Additionally, freezing is a method of shelf-life extension as it allows meat to sustain characteristics of fresh meat once thawed. Meat generally contains about 50 to 75% of its weight in water, depending on species, making it suitable for freezing due to water turning to ice (Heinz, 2007). The lower the temperature, the faster the rate of freezing for meat becomes, and at -20°C, around 98% of the water freezes. At -5°C, about 75% of water is frozen in meat products (Rosmini et al., 2004). With these colder temperatures, microbial growth can be slowed and at low temperatures even stopped. Unfortunately, it comes with consequences due to quality changes such as oxidative rancidity and ice crystallization as they affect spoilage (Zhou et al., 2010). Vacuum packaged beef at the frozen temperatures have an approximate shelf life of a recommended 12 months (Delmore, 2009). The final temperature method used to control meat shelf life is super chilling. Defined as the temperature below the initial freezing point of 1 to 2°C, this higher temperature prevents ice crystals from forming (Bahuaud et al, 2008). This method is desirable due to being able to sustain up to four times longer shelf life than conventional chilling (Magnussen et al., 2008). Temperature will continue to be an important factor when it comes to the shelf life of all meat products, because it is a variable that is readily controlled and is easily monitored.

Controlling Water Activity

An additional method of meat preservation is controlled water activity, as defined by water which supports the growth of microorganisms (Addis, 2015). Water activity can be controlled through different means such as drying, refrigeration, or adding chemicals. Common additives are sodium chloride and sugar as they bind up free water and inhibit cell growth (Ray, 2004). Sodium chloride has the capability to stop the growth of *Pseudomonas spp.* a Gram-negative bacteria that grows best in aerobic conditions. When the water activity is reduced with adding 4% sodium chloride (Doyle, 1999). The other agent used is sugar, as it can bind to moisture and reduce water activity. Common sugars used are dextrose, sucrose, brown sugar, corn syrup, lactose, honey, molasses, and starches. All are typically used to enhance the taste due to the addition of salts to the meat products (USDA, 2005). Using salt and sugars alter the meat product sensory experience, which throws off flavor and taste changes caused by the additives.

Chemical Approaches

The third common method of extending shelf life is through chemical approaches. These microbial spoilage methods are listed as sodium chloride, nitrites, sulphites, lactic acid, and sorbic acid (Addis, 2015). First, sodium chloride is generally used in ground beef during refrigeration to extend shelf life as it reduces microbial growth when combined with sodium lactate (Sallam and Samejima, 2004). Another additive used is nitrites. Nitrites are able to control color in beef, lipid oxidation, odor, and anaerobic bacteria (Sindelar and Houser, 2009). Next is sulphites, as which are effective against aerobic Gram-negative bacilli, molds, and yeast in meat (Ray, 2004). Lactic acid is another antimicrobial often used to defend against pathogenic organisms such as *Clostridium botulinum* due to the ability to reduce pH levels (Doores, 2009). Lastly, sorbic acid and its salts are a common meat preservative inhibiting bacteria and fungi

through depression of internal pH (Davidson et al., 2005). Since freezing of meats cannot prevent oxidative spoilage, other additives that are used such as, phenolic antioxidants and phosphates, assist in inhibiting oxidative spoilage (Jay et al., 2005). Phenolic antioxidants are referred to as synthetic with their purpose to delay, retard or prevent effects of oxidation spoilage in meat (Simitzis, 2010). The other oxidative spoilage inhibitor is phosphates, have several functions on meat. These functions include, enhancing water-binding capacity, improved emulsification of fats, stabilizing the protein-fat-water system, retard rancidity, and binding iron into the system all reducing oxidation in meat (ICLPP, 2006). The final type of chemical method controlling spoilage is an autolytic enzymatic process. These are salts and acids used to inhibit or slow the deterioration and spoilage of fats, carbohydrates, and proteins after the death of an animal. Much like the controlled water activity method of meat preservation, large amounts of chemical methods can affect the taste, flavoring, and aroma of meat. These methods work but are limited by the amount of product that is able to be applied to the meat. If it was feasible to soak or coat products in the various microbial inhibitors discussed above then a much longer shelf life could be achieved. However, doing so would render the meat nearly inedible in some applications due to the undesirable sensory effects. Thus, there is potential for future innovation and advancements to be made regarding meat packaging.

PACKAGING INNOVATION

Innovation in the food industry is not a luxury, it is an essential aspect of the ever-growing need to feed the world with a quality protein source. Two recent innovations in the meat industry include essential oils and packaging sustainability. Both are linked to each other in the movement for a more natural and sustainable world that consumers are increasingly motivated to

adopt. It is this ideology and consumer desire that drives innovation for the future as we as an industry strive to produce the safest and highest quality products possible.

Essential Oils

Innovative applications of essential oils are being utilized by vacuum packaging and show real promise for the food industry. However, essential oils as a whole are not necessarily new groundbreaking advancements in meat packaging. The identification of new essential oils that can be utilized and combinations of these substances are recent advancements. The attractiveness of essential oils for the meat and food industry follows the consumer tendencies. As consumers are leaning towards more natural foods and ingredients, essential oils are a clear solution to this desire in an effort to reduce foodborne illness organisms (Azarifar et al., 2020).

A recently published study combined “chitin nanofibril” and “Ajowan essential oil” simultaneously in raw beef wrapping for the first time (Azarifar et al., 2020). Chitin is described as a microfibrillar material that originates from crustaceans such as shrimp crab shells. Additionally, Ajowan essential oil is obtained from the ajwain seed of a plant with the botanical name of *Trachyspermum ammi*. This study investigated the combination in association with microbial, chemical, and sensory characteristics of beef over a 12-day storage period. The following bacteria were examined, *Pseudomonas spp.*, lactic acid bacteria, and *Staphylococcus aureus*. It was concluded that samples with chitin nanofibril and Ajowan essential oils significantly lowered log CFU/g counts across the board when compared to the control (Azarifar et al., 2020). Moreover, the control surpassed the recommended maximum bacteriological limit of 7 log CFU/g by day six and the treated samples having values of 4.5 and 5.1 log CFU/g over 15 days of storage (Azarifar et al., 2020). Additionally, films with antimicrobial inclusion (pH 6.12) appeared effective as they exhibited lower pH values when compared to control samples

(pH 6.7) (Azarifar et al., 2020). Moreover, color was improved with the treated films, highlighted by a^* values (redness color). The control sample exhibited an a^* value of 7.83 compared to the chitin and Ajowon with 11.20 respectively after 12 d of storage (Azarifar et al., 2020). Although this study was conducted with wrapped films instead of vacuum sealing, adding chitin and Ajowon to the vacuum seal were demonstrated to provide potential advancement in shelf-life extension.

Packaging Applications

A trending topic among packaging enthusiasts and environmentalists are recycling and sustainability of the earth pertaining to plastic and expanded polystyrene (EPS) use. Potentially the strongest argument in the eyes of the consumer for vacuum sealing or fill-form-seal applications is the clear advantage in sustainability. Several companies have developed recyclable meat packaging and ,as recently as of late 2018, an Australian supermarket chain converted to this type of packaging and pledged by 2020 to make all its meat packaging recyclable (Fortune, 2018). This decision stems from the grocery store chain, “understanding the important role that packaging plays in maintaining food safety, supporting product longevity and reducing food waste. At the same time, we are committed to reducing our impact on the environment and continue to look for opportunities to increase the content of recycled material” (Fortune, 2018). This attitude keeps both sustainability and food safety in mind as grocers strive to improve the packaging sector. It is only a matter of time until other regions of the world adopt such thinking. This directly affects the meat industry as the majority of retail meats are traditionally sold on a polystyrene tray with plastic overwrap.

Currently, Maine and Maryland as states are implementing a ban on polystyrene containers along with major cities consisting of but not limited to, New York, San Diego, Miami

Beach, Seattle and Washington, DC (Valinsky, 2019). Additionally, the California legislature has an amendment to the California Integrated Waste Management Act of 1989 that “not less than 75% of solid waste generated be source reduced, recycled or composted by 2030” (A.B. 1080). Additionally, this amendment identifies fresh meats, poultry, fish, and deli counters as local agencies it intends on regulating packaging materials (A.B. 1080).

Companies such as Klöckner Pentaplast group are helping lead the charge in total recyclable packaging as they have made the transition in their form-fill-seal products. They offer packaging for deli, fresh meat, and convenience segments (Ellipse, 2020). This packaging allows for different barrier transmission rates, extended shelf life, cost savings, and a recyclable solution (Ellipse, 2020). Another company that has innovated by creating what they call “Flatskin” is SEALPAC. This packaging application utilizes vacuum sealing combined with up to a 75% reduction in plastic to elevate their sustainability (Meating Point Magazine, 2018). Both of these recent advancements in food packaging are a step in the direction of sustainability through reducing packaging waste. Their importance will exponentially grow in the years to come as consumers demand more recycling and government leaders implement more legislation supporting this movement.

CONCLUSION

In conclusion, vacuum sealing and various film applications are possible solutions for future use within the meat industry and will continue to grow in popularity as the meat industry evolves. A move to identify alternative methods for packaging meat products could provide industry improvements in processing efficiencies and environmental sustainability. These packaging methods create an opportunity to extend shelf life of meat products while maintaining consumer appeal at the grocery store. These attributes benefit the consumer, producer, and

retailer by way of reducing waste and saving money. Extending fresh and frozen shelf life for meat consumers is vastly important as the availability of packaged meat products could be altered based on changing consumer buying patterns. By recognizing the deficiencies in the food industry such as meat waste, loss, and major causes of meat spoilage the conclusion can be made that changes in the industry are imminent to achieve sustainability. For these reasons vacuum packaging makes a strong case to be utilized as the primary packaging for retail food applications.

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Chapter II

Running Title: Ground Beef Shelf Life

INFLUENCE OF PACKAGING FILM AND BEEF TRIMMINGS ON GROUND BEEF SHELF LIFE

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ABSTRACT:

Fresh beef storage in the retail setting can be presented in a variety of packaging methods and identifying the best alternative such as vacuum packaging to current traditional methods could potentially increase shelf life and reduce meat waste. The objective of this study was to identify the influence of packaging film in conjunction with lean trimmings on fresh ground beef surface color during a simulated retail display period. There were no differences ($P > 0.05$) in surface color redness (a^*), yellowness (b^*), chroma, or hue angle regardless of packaging film or lean trimmings. However, thiobarbituric acid reactive substances (TBARS) were greater ($P < 0.05$) for packages containing CULL beef trimmings regardless of packaging film. In addition, pH values of ground beef packages did not differ ($P > 0.05$) among packaging film or lean trimming blends. Microbial spoilage organisms were greatest ($P < 0.05$) on day 21 of the simulated display period. Sensory panelist ratings for initial beef color did not differ ($P > 0.05$) throughout the simulated retail display period (Day 0, 7, 14, or 21) regardless of beef trimmings or packaging film. Furthermore, the percentage discoloration of surface color was unaffected ($P > 0.05$) by the display period or beef trimmings. These results indicate that ground beef presented in a simulated retail setting using an alternative packaging platform, such as vacuum packaging is plausible.

KEYWORDS: Ground Beef, Instrumental Color, Shelf Life, TBARS

1.0 INTRODUCTION

Purchasing intent specifically during the Covid-19 pandemic has resulted in excessive pressure on meat production from the farmer to retail establishments. Current meat packaging in the retail setting is not designed for extended storage, forcing consumers to visit retail outlets regularly. Furthermore, reductions in processing/manufacturing volumes of fresh beef with added limitations on purchases by retailers have resulted in a significant strain on the availability of meat for the consumer. Consumers of fresh meat products are highly influenced by surface color. Moving forward, it is plausible greater interest in bulk purchasing of protein sources could also occur in the retail setting. In the United States; meat, poultry, and fish account for \$48 billion of food loss occurring at the retail and consumer levels annually (Buzby et. al, 2014). Fresh meat has accounted for 30% of the food loss in the United States at a value of \$161.6 billion in 2010 (Buzby et. al, 2014). Of the meat loss occurring annually, 30% has been identified at the retail counter and 70% is lost at the consumer level (Buzby et. al, 2014). With modifications in packaging technology for fresh meat and a deeper understanding of color stability in beef surface color as influenced by packaging technologies, it is plausible to reduce these annual losses that occur in the beef industry.

Current beef industry methods used by retailers for packaging fresh beef occurs in expanded polystyrene (EPS) trays with polyvinyl chloride (PVC) film or in some instances placed inside a tray and gas-flushed with a modified atmosphere (MAP). These packaging methods are intended to influence color only, are not designed for extended storage in either a store or consumer refrigerator, or freezer. These packaging methods often result in product being discarded before it is sold by the retailer or eaten by the consumer. Investigating alternative

packaging strategies could lend itself to greater beef purchases due to extended storage in a refrigerated or frozen setting for the consumer.

The objective of this experiment was to identify alternative packaging films and their influence on beef trimmings shelf life stability for vacuum packaged fresh meats in a simulated retail display setting.

2.0 MATERIALS AND METHODS

2.1 Raw Materials

Fresh beef trimmings representative of FED (White Oak Pastures Inc., Bluffton, GA) and CULL (Golden State Foods, Opelika, AL) beef cattle were procured from commercial meat processing facilities in the Southeast. Beef trimmings were transported in insulated coolers to the Auburn University Lambert-Powell Meats Laboratory. Trimmings were stored in a refrigerated walk-in cooler (2 °C) in the absence of light for 24 h until grinding and packaging occurred. Beef trimmings were identified as either from FED or CULL cattle and ground once through a 9.525 mm plate (SPECO 400, Schiller Park, IL) using a commercial meat grinder (Model 4346, Hobart Corporation, Troy, OH). Coarse ground beef (FED = 170.1 kg, CULL = 102.06 kg) was allocated to one of eight treatments batches (34.02 kg/treatment).

Treatments:

TRT 1-MB1 75% CULL:25% FED	TRT 5-MB2 75% CULL:25% FED
TRT 2-MB1 50% CULL:50% FED	TRT 6-MB2 50% CULL:50% FED
TRT 3-MB1 25% CULL:75% FED	TRT 7-MB2 25% CULL:75% FED
TRT 4-MB1 100% FED	TRT 8-MB2: 100% FED

Treatments were then mixed for 2 min in a commercial meat grinder (Model 4346, Hobart Corporation, Troy, OH) and finely ground once through a 3.18 mm plate (SPECO 400, Schiller

Park, IL) creating three replications (11.34 kg/replication) of each treatment. Once all products were ground, beef was portioned into 454 g bricks using a vacuum stuffer (Model – VF608plus, Handtmann, Biberach, Germany). After portioning, treatments were packaged into vacuum packaging materials using a Reiser roll-stock packaging machine (Optimus OL0924, Variovac, Zarrentin, Germany). Each treatment produced a total of 150 ground beef portions resulting in 25 packages per replication and a total 600 packages of ground beef. Half (300 packages) of the formed bricks were packaged in a barrier film being MB-175M (0.4 cc/sq. m/24hr) (WINPAK Ltd, Winipeg, Manitoba, Canada) whereas the remaining portions (300 packages) were packaged in a barrier film being MB2-175 (0.2 cc/sq. m/24hr.) (WINPAK Ltd, Winipeg, Canada) and placed into refrigerated tiered display cases for simulated retail display. The barrier films differed only in the rate at which oxygen can transfer thru the barriers (MB-175M (0.4 cc/sq. m/24hr) vs. MB2-175 (0.2 cc/sq. m/24hr.)). The thickness of film (0.7 mil) is identical along with the moisture vapor transmission rates (3.3 g/sq. m). Packages were comprised of either MB-175M (0.4 cc/sq. m/24hr.) or MB2-175M (0.2 cc/sq. m/24hr.) as forming film layers and non-forming film consisted of MB75NF (1.0 cc/sq. m/24hr.)

2.2 Experimental Design

This experiment was an 8×4 complete factorial design with eight treatment combinations (TRT 1-MB1 75% CULL:25% FED; TRT 2-MB1 50% CULL:50% FED; TRT 3-MB1 25% CULL:75% FED; TRT 4-MB1 100% FED; TRT 5-MB2 75% CULL:25% FED; TRT 6-MB2 50% CULL:50% FED; TRT 7-MB2 25% CULL:75% FED; TRT 8-MB2: 100% FED) and four days (0, 7, 14, 21). The treatment combinations were assigned as completely randomized design for this study. Three replications of twenty-five packages of ground beef were vacuum packaged for each treatment (TRT) and packaging film (PKG film). Packages were

stored in simulated retail conditions at 3 to 5°C in a Turbo Air coffin style cooler (Model TOM-labels 60DXB-N, Turbo Air Inc., Long Beach, CA, USA) under continuous LED lighting. Nine packages were randomly selected (Day 0) from each of the treatments to be analyzed for instrumental color. Three packages were randomly selected from each treatment to be utilized in sensory panel color observation and were placed in a separate retail display. Two packages were randomly selected each pull day (0, 7, 14, or 21) for pH analysis, aerobic plate count, and proximate analysis. Lastly, three samples were randomly selected and placed in a freezer (Model-5706, Thermo Fisher Scientific, Marrieta, OH, USA) storage at -80 °C for lipid oxidation analysis. Storage temperatures were monitored using (Model-TD2F, ThermoWorks, American Fork, UT, USA) and packages were evenly distributed amongst the retail display and rotated from side to side and front to back within the cooler each day.

2.3. Packaging and Display

One packaging method was utilized in this experiment with two different variables of material. Vacuum packaging on a roll stock machine (MODEL-Optimus OL0924, Variovac, Zarrentin, Germany) was used with the sealing layer kept constant on all treatments MB 75NF (3.0 mils thickness; Oxygen Transmission 1.0 cc/sq. m; Moisture Vapor Transmission 4.0 g/sq. m; WinPak, Winnipeg, MB, Canada). Two different forming layers, MB-175 (7.0 mils thickness; Oxygen Transmission 0.4 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m; WinPak, Winnipeg, MB, Canada), and MB2-175 (7.0 mils thickness; Oxygen Transmission 0.2 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m; WinPak, Winnipeg, MB, Canada) were used in this study. All packages were displayed at 3 to 5 °C and monitored with temperature loggers (Model-TD2F, ThermoWorks, American Fork, UT, USA) on display shelves under continuous

LED lighting. Lighting intensity on the LED display case shelves averaged 2297 lux (ILT10C, International Light Technologies, Peabody, MA, USA).

2.4. Proximate Analysis and pH Value

Samples for proximate analysis (protein, moisture, fat, and collagen) were obtained on the day of packaging (d 0) and subsequent measuring points throughout retail display days (7, 14, and 21). Analysis was conducted using a near-infrared (NIR) with AOAC (2007.4) approved spectrophotometer (Food Scan™, FOSS Analytical A/S, Hilleroed, Denmark), and data processing was determined using ISIScan™ Software. Values were reported on a percent (%) basis. Ground beef pH was measured in duplicate after mixing, grinding, and packaging were completed with a pH electrode attached to a pH meter (Model-HI99163, Hanna Instruments, Woonsocket, RI, USA). The pH meter was calibrated using 2-point standard buffers (pH 4.0 and 7.0).

2.5. Visual Color Evaluation

A nine-member, trained color panel was used to evaluate the surface color of packaged ground beef during the simulated retail display period. Color panelists were recruited and trained using AMSA (1991) meat color measurement guidelines. At 1600 hours, surface color was evaluated on day 0, 7, 14, and 21 or initial beef color (1 = Light purple red, 2 = slight purple red, 3 = moderately light purple red, 4 = red, 5 = slightly dark purple, 6 = moderately dark purple red, 7 = dark purple red, and 8 = extremely dark purple red), amount of browning (1 = no evidence of browning, 2 = dull, 3 = grayish, 4 = brownish gray, 5 = brown, and 6 = dark brown), and percent (%) discoloration (1 = no discoloration [0%], 2 = slight discoloration [1-10%], 3 = small discoloration [11-25%], 4 = modest discoloration [26-50%], 5 = moderate discoloration [51-75%], 6 = extensive discoloration [76-99%], and 7 = total discoloration [100%]).

2.6. Instrumental color measurement

Instrumental color (L^* , a^* , and b^*) of ground beef packages were measured through the packaging film at three different locations on each package using a HunterLab MiniScan XE Plus Spectrocolorimeter, Model 45/0-L (Hunter Associates Laboratory Inc., Reston, WV, USA). Samples were read using illuminant A/10° observer and evaluated for CIE (L^* , a^* and b^*) color values. Hue angle, which describes the hue or color of ground beef was calculated ($\tan^{-1}(b^*/a^*)$), as was the chroma value ($((a^{*2} + b^{*2})^{1/2})$), which describes the brightness or dullness of color. Instrument calibration was completed prior to use on each sampling day 0, 7, 14, or 21 using black and white tiles.

2.7. Thiobarbituric acid reactive substance (TBARS)

On days 0, 7, 14, and 21 of simulated retail display, ground beef was removed from the packaging material to be sampled for 2-thiobarbituric acid reactive substances (TBARS) using the method of Beuge and Aust (1978). Approximately 4 g of ground beef was homogenized with 8 ml of cold (1 °C) of 50 mM phosphate buffer (pH of 7.0 at 4 °C) containing 0.1% EDTA, 0.1% n-propyl gallate, and 2 ml trichloroacetic acid (SUPPLIER, State, USA). Homogenized samples were filtered through Whatmann No. 4 filter paper and duplicate 2-ml aliquots of the clear filtrate were transferred into 10-ml borosilicate tubes, mixed with 2 ml of 0.02 M 2-thiobarbituric acid reagent (SUPPLIER, City, State, USA) then boiled for 20 min. After boiling, tubes were placed into an ice bath for 15 min. Absorbance was measured at 533nm with a spectrophotometer (Turner Model – SM110245, Barnstead International, Dubuque, IA, USA) and multiplied using a factor 12.21 to obtain the TBARSA value (mg malonaldehyde/kg of meat).

2.8. Aerobic Plate Counts

The total number of viable non-pathogenic aerobic microorganisms in ground beef samples was determined using standard methods. Two 5-gram samples were removed from two randomly selected packages per treatment. Ground beef was placed in a stomacher bag with filter 3M Sample Bag W/ Filter Sterile (3M Corp., St. Paul, MN, USA) with 50 mL of 3M Butterfield's Buffer (3M Corp., St. Paul, MN, USA). Stomacher bags were stomached for 60 S. Once the samples were stomached, the solution was serial diluted three times. Subsequent duplicate platings were made on Petrifilm® (3M Corp., St. Paul, MN, USA) aerobic plate count (APC) plates. Plates were then incubated at 35.5°C in a Lab Companion incubation chamber (Model IB-05G, Lab Companion, Yuseong-gu, Daejeon, Republic of Korea) and APC plates were read at 48h. Counts were recorded as colony-forming units per gram (CFU/g).

2.9. Statistical Analysis

Data were analyzed with linear models and linear mixed models using the GLIMMIX procedure of SAS (ver. 9.4; SAS Institute Inc., Cary, NC). For analysis of color ratings data, panelist was included as a random factor, and panelist × replication was included as a random, repeated factor (with a first-order autoregressive covariance structure). Fixed effects evaluated were treatment blends, temperature and packaging film. Least squares means were computed for all variables, and when significant ($P \leq 0.05$) F -values were observed, least squares means were separated using pair-wise t -tests (PDIFF option).

3.0 Results

3.1. Instrumental Analysis of Fresh Ground Beef

Instrumental analysis of ground beef packages stored in simulated retail display conditions for thiobarbituric reactive substances (TBARs), pH, moisture, protein, fat, and collagen are presented in Table 1. There were no differences for pH ($P > 0.05$) regardless of

packaging method or lean trimmings. Raw beef trimmings used for this study originated from beef carcasses that are considered normal postmortem muscle pH ranges (5.8 to 5.3). There was no interactive effect of packaging film \times storage time for lipid oxidation ($P < 0.05$). However, the main effect for TBARs values (Table 1) were greater ($P < 0.05$) for lean trimmings containing a greater percentage of fat. Moreover, ground beef formulations consisting of a greater percentage of CULL beef trimmings (Table 1) produced more ($P < 0.05$) lipid oxidation regardless of packaging materials. There were no differences ($P < 0.05$) for protein, fat, and collagen (Table 1) regardless of beef trimmings, packaging materials, or day of simulated display. Moisture (Table 1) of ground beef packages used during this simulated study were greater ($P < 0.05$) for packages containing a greater percentage of FED beef trimmings.

3.2. Fresh Beef Color

Fresh beef color of ground beef packages stored in simulated retail display were investigated for instrumental color; lightness (L^*), redness (a^*), yellowness (b^*) and trained sensory panel; initial beef color, amount of browning, percent of discoloration, found in tables 2-6. There were no significant differences for instrumental or sensory panel evaluations ($P < 0.05$) in terms of packaging materials utilized, differences ($P < 0.05$) were found amongst lean trimming blends. It was not surprising that the TRT blends with a greater percentage of FED trimmings received the lowest ($P < 0.05$) lightness (L^*) values (Table 2) and highest ($P < 0.05$) chroma values (Table 2), which may be observed in grass-fed cattle. Conversely, TRT blends with a greater percentage of CULL trimmings displayed the greatest ($P < 0.05$) L^* and hue angle values. Packaging materials had no significant ($P < 0.05$) impact on instrumental fresh color (Table 2) of the 1lb. ground beef bricks. Packaging (Table 3) had no significant differences ($P < 0.05$) between materials for initial beef color, amount of browning, or percent of discoloration.

Based on these results either packaging material would be sufficient in storing ground beef for up to 21 days in retail display. In terms of TRT blends, differences observed between CULL and FED samples. Samples higher in the percentage of CULL trimmings (Table 3) revealed a lower ($P < 0.05$) initial beef color score, indicative of a more light reddish color. However, samples with a greater percentage of FED trimmings (Table 3) displayed a significantly lower ($P < 0.05$) amount of browning and % of discoloration scores, especially in the 100% FED samples. This can be explained through these samples being more color stable throughout the 21 days of retail display. Across sample day 0, 7, 14, and 21 panelists observed an increasing percent of discoloration for all TRT (Table 6). Samples with a higher percentage of CULL trimmings revealed the greatest ($P < 0.05$) increase in percent discoloration (Table 6) This was evident with the 75 CULL / 25 FED treatments as they had significantly greater ($P < 0.05$) discoloration of samples.

3.3. Aerobic Changes

Analysis of ground beef packages stored in simulated retail display conditions for microbial spoilage organisms (APC) of ground beef were presented in Table 7. Retail display storage of ground beef was maintained at 3 to 5°C and for dilution-3, all TRTs (75% CULL, 25% FED; 50% CULL, 50% FED; 25% CULL, 75% FED; and 100% FED) and packaging films (MB1 and MB2) started off at levels between 0.96-1.94 \log_{10} CFU/g and experienced an increase during retail display time (Table 7). Maximum growth of $\sim 3.24 \log_{10}$ CFU/g was observed on day 21 of display storage (Table 7). Across beef trimming blends differences were observed but the pattern was inconsistent across different display times. Moreover, the 25 CULL:75 FED beef trimmings had the greatest ($P < 0.05$) aerobic growth at day 21 of storage. Aerobic counts among packaging films differed across display times. Packaging film MB2 (0.2 cc/sq. m/24hr.)

consistently displayed fewer \log_{10} CFU/g spoilage organisms across TRT blends and days of simulated retail display. This is evidenced by an approximately 0.1 to 0.4 difference in \log_{10} CFU/g between the packaging films (MB1 and MB2).

4.0 Discussion

In this study, packaging films with varying oxygen transmission rates (OTR) differing in lean trimming formulation of ground beef were placed into a simulated retail display setting in an effort to investigate new ground beef offerings for the retail consumer. Instrumental analysis of fresh surface color of vacuum packaged ground beef was not drastically altered in either packaging film ($P < 0.05$), but beef trimmings did have a greater influence ($P < 0.05$) on the surface color. This is consistent with previous studies which have identified several factors such as animal diet, breed type, processing, manufacturing, logistical temperatures, and retail storage temperature conditions can influence surface color of meat, particularly beef, once simulated display has commenced (Ball et al., 2015; Jakobsen et al., 2000; Bruce et al., 2004). Decrease in lightness (L^*) values across lean trimming blends ($P < 0.05$) indicates that the FED trimmings comprised of grass-fed cattle are consistent with findings in previous work (Bruce et al., 2004, Apaoblaza et al., 2020, and Vitale, M., et al. 2014) with grass finished beef cattle. Increase in redness (a^*) values across lean trimming blends from FED cattle in the present study are also similar to findings of Bruce et al. (2004) and Vitale, M., et al. (2014) in grass-finished beef cattle. These improvements in instrumental redness values can be attributed to the lack of internal fat (subcutaneous, intermuscular, and intramuscular) that is associated with lean trimmings from CULL and grass-influenced beef (Bruce et al., 2004). This is conclusive with a previous study on effects of quality grade (Premium Choice vs. Select) on display color of ground beef patties by Garner et al. (2014). The greater fat content of beef trimmings can result

in lighter surface color due to less myoglobin, whereas beef trimmings with less fat content produces redder surface color as a result of greater myoglobin content present in lean trimmings (Lee et al., 2000).

Lipid oxidation (TBARS) was altered ($P < 0.05$) through the use of packaging materials (MB1 vs. MB2) and beef trimmings. Results for TBARS during a display period in a vacuum package for extended periods are consistent with previous beef simulated shelf-life studies (Ball et al., 2015; Hughes et al., 2015; Chen et al., 2020) which resulted in greater lipid oxidation (TBARS values) as storage time duration increased. Generally, lipid oxidation increases over time are predominantly affected by temperature and oxygen concentration (Jakobsen, Marianne, & Bertelsen, 2000) which the current study attempted to control through variations in OTR of packaging materials. Vacuum packaged meats can sustain longer display periods without adverse implications to lipid oxidation (Jakobsen, Marianne, & Bertelsen, 2000, and Chen et al., 2020). These studies reported that initial bacteria load coupled with temperature has vast effects on lipid oxidation. Storage temperatures, especially super-chilled storage ($-1.5\text{ }^{\circ}\text{C}$) are noted to have the ability to inhibit the increase of bacteria load when compared to chilled storage ($2\text{ or }5\text{ }^{\circ}\text{C}$) however, if initial bacteria load is already great then temperature becomes less of a bacteria control method (Chen et al., 2020).

Packing films with varying oxygen transmission rate (OTR) properties were compared for their influence on reducing aerobic spoilage microorganisms in an effort to support an extended shelf-life for vacuum packaged ground beef. A higher OTR film is commonly used throughout the industry and has only one ethylene vinyl alcohol copolymer (EVOH) barrier resulting in an OTR of 0.4 cc/sq. m/24hr . A lower OTR film has two ethylene vinyl alcohol copolymer (EVOH) barriers allowing for an OTR of 0.2 cc/sq. m/24hr . Ground beef displayed in

high OTR film at the end of display (d 21) had greater aerobic (APC) organism growth than the low OTR film. Similar results have been noted by Rogers et al. using various packaging methods of fresh meat. Thus, it is plausible that the lower OTR film would decrease aerobic spoilage, and potentially increase shelf life. Lower OTR film (MB2; 0.2 cc/sq. m/24hr) had lower log₁₀ CFU/g growth than the higher OTR film (MB1; 0.4 cc/sq. m/24hr). These findings are similar to previous research by McSharry et al. 2020 focused on packaging OTR as an influencer of aerobic spoilage. The authors identified high barrier packaging to have the greatest potential to reduce aerobic spoilage. When consumers are selecting meat products in the retail setting, specifically beef they tend to place heavy emphasis on visual appearance (Hood and Riordan, 1973). The presence of vacuum packaged beef products offered to consumers in the retail setting is often limited to niche marketed products (Wagyu, Grass-finished, or Subprimals) at the grocer or club store outlet. The current study evaluated sensory color panelist ratings of vacuum packaged ground beef for anchors of initial beef color, amount of browning, and percent discoloration. These characteristics are important due to the extended shelf-life that vacuum packaging offers and identifying optimal storage times. The current study did not observe significant changes in surface color throughout the display period that might be considered unacceptable by consumers when using trained color panelists. Surface color of ground beef packaged in vacuum packaging throughout the current 21-day study declined as rated by trained panelists. This decline in surface color is similar to other studies that note fresh meat color will eventually degrade regardless of packaging methods, antioxidant ingredient use, or storage temperature (McSharry et al., 2020, Wang et al., 2021, Suman et al., 2010). Improvements in temperature storage environment and vacuum packaging fresh meat products in the retail setting could potentially lead to minimizing markdowns and throwaways in the retail setting or by the

consumer. However, additional investigation to support storage temperatures and perception of vacuum packaging use in fresh meats by consumers is warranted.

5.0 Implications

Vacuum packaging (rollstock) barrier films for ground beef may be an option for use in the retail setting. These results suggest that fresh color properties of ground beef for instrumental surface color can withstand extended storage (up to 21 days) in a simulated retail setting. Surface color redness (a^*) variation was minimal throughout the simulated display period regardless of beef trimmings or packaging materials. Consumers place tremendous emphasis at the time of purchase on surface color, particularly on redness of the surface color at the time of purchase. These results suggest that minimal surface color variations (emphasis on redness) in fresh ground beef are not largely impacted by a vacuum packaging platform. Under normal retail display conditions (3 to 5 °C) all ground beef blends and packaging films were acceptable in terms of initial beef color, amount of browning, and % discoloration. All blends were less than $\sim 3 \log_{10}$ CFU/g, which is below the threshold (6 \log_{10} CFU/g) for concerns related to spoilage and safety for consumers. Under these conditions packaging methods maintained safety for consumption and visual appeal to consumers at 21 days of retail display. Thus, proving to be sufficient in satisfying consumer concerns and reducing the potential for waste and loss of beef products.

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TABLES

TABLE 1. INFLUENCE OF PACKAGING FILM¹ AND LEAN TRIMMINGS² ON INSTRUMENTAL ANALYSIS OF GROUND BEEF

TRAIT	75 CULL / 25 FED (MB1)	75 CULL / 25 FED (MB2)	50 CULL / 50 FED (MB1)	50 CULL / 50 FED (MB2)	25 CULL / 75 FED (MB1)	25 CULL / 75 FED (MB2)	100 FED (MB1)	100 FED (MB2)	SEM
TBARS ³	1.46 ^a	1.40 ^{ab}	1.34 ^b	1.37 ^{ab}	1.31 ^{bc}	1.29 ^{bc}	1.22 ^c	1.30 ^{bc}	0.028
pH ⁴	5.47	5.49	5.53	5.56	5.61	5.56	5.59	5.60	0.012
MOISTURE ⁵	68.18 ^b	68.50 ^b	68.06 ^b	67.85 ^b	69.61 ^a	70.04 ^a	69.79 ^a	69.84 ^a	0.017
PROTEIN ⁶	21.42	21.87	22.15	22.28	23.12	22.65	22.62	22.73	0.015
FAT ⁷	15.97	15.81	15.82	16.32	13.49	13.51	12.98	12.76	0.109
COLLAGEN ⁸	4.77	5.27	4.94	5.08	4.62	4.55	3.95	4.04	0.091

¹ Packaging film properties for MB1 (MB-175: 7.0 mils thickness; Oxygen Transmission 0.4 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m) and MB2 (MB2-175: 7.0 mils thickness; Oxygen Transmission 0.2 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m).

² Lean trimmings sourced from commercial processor blended prior to representing FED beef and CULL beef trimmings of four treatments, where treatments represent a percentage split of contribution from these sources.

³ TBARs, Thiobarbituric acid reactive substances used to measure lipid oxidation.

⁴ pH, measure of acidity or basicity of a solution.

⁵ MOISTURE, content of moisture in ground beef samples.

⁶ PROTEIN, content of protein in ground beef samples.

⁷ FAT, content of fat in ground beef samples.

⁸ COLLAGEN, content of collagen in ground beef samples.

*SEM, Standard Error of the Mean.

^{a-c} Mean values and standard deviations in the same row with different superscripts are significantly different ($P < 0.05$).

TABLE 2. INFLUENCE OF PACKAGING FILM¹ AND LEAN TRIMMINGS² ON INSTRUMENTAL FRESH COLOR OF GROUND BEEF DURING A SIMULATED RETAIL DISPLAY SHELF LIFE

TRAIT	75 CULL / 25 FED (MB1)	75 CULL / 25 FED (MB2)	50 CULL / 50 FED (MB1)	50 CULL / 50 FED (MB2)	25 CULL / 75 FED (MB1)	25 CULL / 75 FED (MB2)	100 FED (MB1)	100 FED (MB2)	SEM
L* ³	46.44 ^{ab}	46.99 ^a	45.27 ^b	46.18 ^c	43.99 ^d	43.56 ^d	42.40 ^e	41.97 ^e	0.206
a* ³	21.65	21.12	22.63	23.37	22.69	23.01	23.55	23.11	0.661
b* ³	13.88	14.10	14.07	13.64	13.58	13.90	13.67	12.98	0.076
CHROMA ⁴	25.77	25.51	26.68	25.55	26.46	26.91	27.23	26.51	0.108
HUE ANGLE (°) ⁵	32.86	34.05	32.00	32.61	30.97	31.32	30.17	29.37	0.149

¹ Packaging film properties for MB1 (MB-175: 7.0 mils thickness; Oxygen Transmission 0.4 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m) and MB2 (MB2-175: 7.0 mils thickness; Oxygen Transmission 0.2 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m).

² Lean trimmings sourced from commercial processor blended prior to representing FED beef and CULL beef trimmings of four treatments, where treatments represent a percentage split of contribution from these sources.

³ 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).

⁴ Chroma is a measure of total color (a larger number indicates a more vivid color).

⁵ Hue angle represents the change from the true red axis (a larger number indicates a greater shift from red to yellow).

*SEM, Standard Error of the Mean.

^{a-c} Mean values and standard deviations in the same row with different superscripts are significantly different ($P < 0.05$).

TABLE 3. INFLUENCE OF PACKAGING FILM¹ AND LEAN TRIMMINGS² ON SENSORY PANELIST RATINGS FOR SURFACE COLOR OF GROUND BEEF DURING A SIMULATED RETAIL DISPLAY

TRAIT	75 CULL / 25 FED (MB1)	75 CULL / 25 FED (MB2)	50 CULL / 50 FED (MB2)	50 CULL / 50 FED (MB2)	25 CULL / 75 FED (MB1)	25 CULL / 75 FED (MB2)	100 FED (MB1)	100 FED (MB2)	SEM
INITIAL BEEF COLOR ³	4.19 ^b	4.19 ^b	4.31 ^b	4.33 ^b	4.39 ^{ab}	4.39 ^{ab}	4.64 ^a	4.64 ^a	0.293
AMOUNT OF BROWNING ⁴	1.36 ^{ab}	1.42 ^a	1.31 ^{abc}	1.32 ^{ab}	1.27 ^{abcd}	1.24 ^{bcd}	1.16 ^{cd}	1.14 ^d	0.093
% OF DISCOLORATION ⁵	1.22 ^{ab}	1.30 ^a	1.25 ^{ab}	1.23 ^{ab}	1.20 ^{ab}	1.19 ^{ab}	1.14 ^b	1.14 ^b	0.077

¹ Packaging film properties for MB1 (MB-175: 7.0 mils thickness; Oxygen Transmission 0.4 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m) and MB2 (MB2-175: 7.0 mils thickness; Oxygen Transmission 0.2 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m).

² Lean trimmings sourced from commercial processor blended prior to representing FED beef and CULL beef trimmings of four treatments, where treatments represent a percentage split of contribution from these sources.

³ Initial Beef Color (1 = Light purple red, 2 = slight purple red, 3 = moderately light purple red, 4 = red, 5 = slightly dark purple, 6 = moderately dark purple red, 7 = dark purple red, and 8 = extremely dark purple red).

⁴ Amount of browning (1 = No Evidence of Browning, 2 = Dull, 3 = Grayish, 4 = Brownish Gray, 5 = Brown, and 6 = Dark Brown).

⁵ Percent (%) discoloration (1 = No Discoloration (0%), 2 = Slight Discoloration (1 to 10%), 3 = Small Discoloration (11 to 25%), 4 = Modest Discoloration (26 to 50%), 5 = Moderate Discoloration (75%), 6 = Extensive Discoloration (76 to 99%), and 7 = Total Discoloration (100%).

*SEM, Standard Error of the Mean.

^{a,b,c,d} Mean values and standard deviations in the same row with different superscripts are significantly different ($P < 0.05$).

TABLE 4. INFLUENCE OF PACKAGING FILM¹ AND LEAN TRIMMINGS² ON TRAINED SENSORY PANEL INITIAL BEEF COLOR³ OF GROUND BEEF DURING A SIMULATED RETAIL DISPLAY DAY

STORAGE PERIOD (DAYS)	75 CULL / 25 FED (MB1)	75 CULL / 25 FED (MB2)	50 CULL / 50 FED (MB1)	50 CULL / 50 FED (MB2)	25 CULL / 75 FED (MB1)	25 CULL / 75 FED (MB2)	100 FED (MB1)	100 FED (MB2)	SEM
DAY 0	4.11	4.18	4.45	4.06	4.44	4.36	4.71	4.70	0.319
DAY 7	4.15	4.03	4.15	4.24	4.28	4.39	4.50	4.68	0.328
DAY 14	4.36	4.30	4.36	4.59	4.35	4.42	4.67	4.77	0.319
DAY 21	4.12	4.24	4.24	4.44	4.49	4.40	4.70	4.38	0.328

¹ Packaging film properties for MB-175 (7.0 mils thickness; Oxygen Transmission 0.4 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m) and MB2-175 (7.0 mils thickness; Oxygen Transmission 0.2 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m).

² Lean trimmings sourced from commercial processor blended prior to representing FED beef and CULL beef trimmings of four treatments, where treatments represent a percentage split of contribution from these sources.

³ Initial Beef Color (1 = Light purple red, 2 = slight purple red, 3 = moderately light purple red, 4 = Red, 5 = slightly dark purple, 6 = moderately dark purple red, 7 = dark purple red, and 8 = extremely dark purple red).

*SEM, Standard Error of the Mean.

TABLE 5. INFLUENCE OF PACKAGING FILM¹ AND LEAN TRIMMINGS² ON TRAINED SENSORY PANELISTS RATINGS FOR THE AMOUNT OF BROWNING³ OF GROUND BEEF DURING A SIMULATED RETAIL DISPLAY DAY

STORAGE PERIOD (DAYS)	75 CULL / 25 FED (MB1)	75 CULL / 25 FED (MB2)	50 CULL / 50 FED (MB1)	50 CULL / 50 FED (MB2)	25 CULL / 75 FED (MB1)	25 CULL / 75 FED (MB2)	100 FED (MB1)	100 FED (MB2)	SEM
DAY 0	1.27	1.30	1.18	1.36	1.06	1.15	1.00	1.03	0.114
DAY 7	1.27	1.32	1.41	1.16	1.23	1.07	1.05	1.02	0.121
DAY 14	1.27	1.30	1.15	1.27	1.21	1.15	1.15	1.15	0.115
DAY 21	1.64	1.75	1.52	1.47	1.62	1.60	1.45	1.35	0.121

¹ Packaging film properties for MB-175 (7.0 mils thickness; Oxygen Transmission 0.4 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m) and MB2-175 (7.0 mils thickness; Oxygen Transmission 0.2 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m).

² Lean trimmings sourced from commercial processor blended prior to representing FED beef and CULL beef trimmings of four treatments, where treatments represent a percentage split of contribution from these sources.

³ Amount of browning (1 = No Evidence of Browning, 2 = Dull, 3 = Grayish, 4 = Brownish Gray, 5 = Brown, and 6 = Dark Brown).

*SEM, Standard Error of the Mean.

TABLE 6. INFLUENCE OF PACKAGING FILM¹ AND LEAN TRIMMINGS² ON TRAINED SENSORY PANELIST RATINGS ON THE PERCENT DISCOLORATION³ OF GROUND BEEF DURING A SIMULATED RETAIL DISPLAY DAY

STORAGE PERIOD (DAYS)	75 CULL / 25 FED (MB1)	75 CULL / 25 FED (MB2)	50 CULL / 50 FED (MB1)	50 CULL / 50 FED (MB2)	25 CULL / 75 FED (MB1)	25 CULL / 75 FED (MB2)	100 FED (MB1)	100 FED (MB2)	SEM
DAY 0	1.00	1.03	1.00	1.03	1.00	1.00	0.99	1.00	0.096
DAY 7	1.06	1.23	1.29	1.10	1.10	1.08	1.00	1.07	0.102
DAY 14	1.27	1.24	1.21	1.30	1.18	1.12	1.15	1.12	0.096
DAY 21	1.54	1.75	1.53	1.47	1.51	1.58	1.41	1.36	0.101

¹ Packaging film properties for MB-175 (7.0 mils thickness; Oxygen Transmission 0.4 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m) and MB2-175 (7.0 mils thickness; Oxygen Transmission 0.2 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m).

² Lean trimmings sourced from commercial processor blended prior to representing FED beef and CULL beef trimmings of four treatments, where treatments represent a percentage split of contribution from these sources.

³ Percent (%) discoloration (1 = No Discoloration (0%), 2 = Slight Discoloration (1-10%), 3 = Small Discoloration (11-25%), 4 = Modest Discoloration (26-50%), 5 = Moderate Discoloration (51-75%), 6 = Extensive Discoloration (76-99%), and 7 = Total Discoloration (100%).

*SEM, Standard Error of the Mean.

TABLE 7. INFLUENCE OF PACKAGING FILM¹ AND LEAN TRIMMINGS² ON MICROBIAL SPOILAGE ORGANISMS (APC)³ OF GROUND BEEF DURING A SIMULATED RETAIL DISPLAY

STORAGE PERIOD (DAYS)	75 CULL / 25 FED (MB1)	75 CULL / 25 FED (MB2)	50 CULL / 50 FED (MB1)	50 CULL / 50 FED (MB2)	25 CULL / 75 FED (MB1)	25 CULL / 75 FED (MB2)	100 FED (MB1)	100 FED (MB2)	SEM
DAY 0	1.20 ^{cd}	0.96 ^d	1.69 ^{ab}	1.26 ^{bcd}	1.32 ^{bcd}	1.37 ^{bcd}	1.94 ^a	1.42 ^{bc}	0.054
DAY 7	2.55 ^{ab}	2.66 ^{ab}	2.98 ^a	2.31 ^b	2.37 ^b	2.65 ^{ab}	2.60 ^{ab}	2.32 ^b	0.054
DAY 14	2.92 ^{ab}	3.11 ^a	2.62 ^{bc}	2.52 ^{bc}	2.42 ^c	2.38 ^c	2.32 ^c	2.30 ^c	0.054
DAY 21	2.64 ^{bcd}	2.59 ^{bcd}	2.75 ^{bc}	2.42 ^{cde}	3.24 ^a	3.02 ^{ab}	2.20 ^e	2.31 ^{de}	0.054

¹ Packaging film properties for MB1 (MB-175: 7.0 mils thickness; Oxygen Transmission 0.4 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m) and MB2 (MB2-175: 7.0 mils thickness; Oxygen Transmission 0.2 cc/sq. m/24hr; Moisture Vapor Transmission 3.3 g/sq. m).

² Lean trimmings sourced from commercial processor blended prior to representing FED beef and CULL beef trimmings of four treatments, where treatments represent a percentage split of contribution from these sources.

³ Log₁₀ colony-forming units/cm.

*SEM, Standard Error of the Mean.

^{a-c} Mean values and standard deviations in the same row with different superscripts are significantly different ($P < 0.05$).

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APPENDICES

APPENDIX A

Thiobarbituric Acid Reactive Substances (TBARS)

Chemicals:

Water – HPLC grade or distilled deionized water

Potassium phosphate (monobasic) KH_2PO_4

Potassium phosphate (dibasic) K_2HPO_4

Ethylenediaminetetraacetic acid (EDTA)

n-Propyl gallate (PG)

Trichloroacetic acid (TCA)

2-Thiobarbuturic acid (TBA)

1, 1, 3, 3, Tetraethoxypropane (TEP)

Reagents:

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

Prepare 50mM monobasic potassium phosphate solution – weight out 3.40g KH_2PO_4 , place in a 500 ml volumetric flask, dissolve and bring to volume with distilled-deionized water (pH will be approximately 4.5).

Prepare 50mM dibasic potassium phosphate solution – weight out 8.71g K_2HPO_4 , place in a 1 L volumetric flask, dissolve and bring to volume with distilled-deionized water (pH will be approximately 8.5). Prepare at least 4 L of the dibasic solution each time.

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

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

30% TCA

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

0.02M TBA

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

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

Analysis:

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

For raw meat samples:

1. Weigh out 2.0g (1.95 to 2.05g) of minced meat into a labeled 50 ml disposable centrifuge tube. Record the exact weight of the sample.
2. Add 8 ml of prepared phosphate buffer to the tube.
3. Add 2 ml of TCA to the tube and homogenize for 20 to 30 secs.
4. Filter homogenate through a Whatman (No. 4) filter paper, collecting the clear filtrate into labeled tubes. (It is OK to stop at this point, but the tubes containing the filtrate must be sealed and stored in a refrigerator).
5. Remove 2 ml of the sample filtrate and place it into a labeled glass test tube. Prepare duplicate tubes for each sample at this point (i.e., tube "A" and tube "B").
6. Prepare three "Blank" tubes, using 2 ml of distilled-deionized water.
7. Prepare one "Standard" tube, using 2 ml of phosphate buffer. (Note: after this point, time is extremely critical. Make sure that the water bath is at the correct temperature and level prior to continuing).
8. Add 2 ml of TBA to each tube including the blanks and standard.
9. Cover tubes with aluminum foil and place them into the hot water bath for 20 min.
10. Remove tubes from hot water bath and place into the ice water bath for 15 min.
11. Read absorbance at 533 nm
12. Multiply absorbance by 12.21
13. Report TBARS as mg/kg of malonaldehyde.

Standards:

1, 1, 3, 3 tetra ethoxy propane (TEP)

Stock standard solution

0.02M solution-0.44g (0.5 ml) to 100 ml of distilled water (2×10^{-5} moles/ml)

Working standard solution

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

Standards for standard curve

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

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

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

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APPENDIX B

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 10mL of 3M Butterfield's Buffer (autoclaved)
1mL Pipetting tips and Pipette
Vortex Mixer
Petrifilm® aerobic plate count (APC) plates
3M Petrifilm Spreader
Incubation chamber (35.5°C)

Procedure:

1. Extract 5-gram sample from ground beef packaging
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. Incubate APC plates at 35°C ± 1°C for 48h
16. Interpret plates by counting colonies

References:

3M, P. (n.d.). *3M Petrifilm Aerobic Count Plates and*.
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APPENDIX C

Panelist: _____

Date: _____
(DDMMYY)

Ground Beef Bricks

Sample Number	Initial Beef Color	Amount of Browning	% Discoloration
	8= Extremely dark purple red 7= Dark purple red 6= Moderately dark purple red 5= Slightly dark purple red 4= Red 3= Moderately light purple red 2= Slight purple red 1= Light purple red	6 = Dark Brown 5 = Brown 4 = Brownish Gray 3 = Grayish 2 = Dull 1= No Evidence of Browning	7= Total Discoloration (100%) 6= Extensive Discoloration (76-99%) 5= Moderate Discoloration (51-75%) 4= Modest Discoloration (26-50%) 3= Small Discoloration (11-25%) 2= Slight Discoloration (1-10%) 1= No Discoloration (0%)