EVALUATING THE OCCURRENCE OF PERSISTENT PINKING IN GROUND BEEF PATTIES OF DIFFERING FAT PERCENTAGES PACKAGED IN CARBON MONOXIDE MODIFIED ATMOSPHERE PACKAGES

By

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EVALUATING THE OCCURRENCE OF

PERSISTENT PINKING IN GROUND BEEF PATTIES

OF DIFFERING FAT PERCENTAGES

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ATMOSPHERE PACKAGES

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Title of Study: EVALUATING THE OCCURRENCE OF PERSISTENT PINKING IN GROUND BEEF PATTIES OF DIFFERING FAT PERCENTAGES PACKAGED IN CARBON MONOXIDE MODIFIED ATMOSPHERE PACKAGES

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Abstract: The objective of this study was to gain a better understanding of the occurrence of persistent pinking by evaluating ground beef with different fat percentages packaged with carbon monoxide modified atmosphere packaging (CO-MAP). Three cases of fine ground beef (IMPS 136) with targeted lean/fat percentages of 73/27, 81/19, and 93/7 were collected from a commercial packing facility. Ground beef was stored for 10 d. After storage, each ground beef chub was opened and reground with a 3 mm stainless steel grinder plate together to form one composite batch of each fat percentage. After grinding, 225 g patties were weighed, formed utilizing a patty maker, and placed onto foam trays (n = 81) with an absorbent pad and overwrapped with polyvinylchloride film (PVC). The trays were randomized within each fat percentage and were then placed in master bags (n = 27) in groups of three with one oxygen scavenger per bag. Twenty-seven master bags were flushed with a tri-gas blend of 0.4% CO, 30% CO_2, and balance N_2. Master bags were then held in dark storage until pull day: 1 d, 8 d, or 15 d. On each pull day, 9 master bags were removed from dark storage, and the trays were placed in a display case for retail display evaluation for 3 d. Headspace analysis was conducted before each master bag was opened. Instrumental and objective color measurements were collected on d 0 – 2 of retail display. Visual color and surface discoloration were analyzed daily by a trained panel (n = 6). The formation of carboxymyoglobin (COMb) was confirmed by measuring ratio of absorbance at 543 nm/581 nm. Patties were cooked to an internal temperature of 71°C. Internal cooked color was evaluated by a trained panel (n = 6) along with instrumental color reading post cooking. The results of the study showed the 93/7 patties had higher a* values throughout retail display over all pull days. All patties had formed COMb by retail display, regardless of storage day or fat percentage. The 93/7 patties had consistently browner internal subjective cooked color scores compared to the 73/27 patties. The 73/27 patties from all pull days were significantly ($P < 0.05$) redder than the 93/7 patties, and on pull d 1 and 15, the 81/19 and 73/27 patties had significantly ($P < 0.05$) redder cooked a* values than the 93/7 patties. COMb was formed in CO-MAP regardless of ground beef fat percentages or pull day. Additionally, ground beef with more fat (73/27) had more pinking after cooking to recommended internal temperature compared to lower fat blends. With consumer confusion on pinking within meat products post cooking, further research should be conducted to better understand the relationship of fat content of ground beef and dark storage times resulting in persistent pinking.

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CHAPTER I

INTRODUCTION

Consumers are fueled by many things when purchasing meat, with the most influential being meat color. A bright cherry red color in beef is associated with wholesomeness and freshness (Suman and Joseph, 2013). With increased time in the retail case and dark storage, potential for surface discoloration increases. Discoloration can deter consumers from purchasing products. The formation of metmyoglobin can be combatted in numerous ways, one being packaging techniques.

Modified atmosphere packaging (MAP) is a type of case ready packaging that modifies the gas composition surrounding a product (McMillin, 2008). One example of MAP utilizes a tri-gas blend of CO_2, N_2 and CO (CO-MAP) being flushed into a master bag containing retail ready packaged trays. CO-MAP can increase the shelf life of a product by stabilizing color and reducing lipid oxidation and microbial spoilage (Brooks et al., 2008). Meat color is stabilized within the master bag by CO binding with myoglobin and forming carboxymyoglobin (COMb; Sørheim et al., 1999).

COMb has greater stability than oxymyoglobin due to the CO remaining tightly bound to myoglobin even after cooking, leading to one major issue known as persistent pinking (Cornforth and Hunt, 2008). Persistent pinking is a term used when a product is cooked to a safe endpoint temperature, but the internal color remains pink (Ryan et al., 2006). For consumers who rely on meat color as an indicator of doneness, this can become an issue of food quality or satisfaction because of overcooking.

The objective of this study was to gain a better understanding of the occurrence of persistent pinking by evaluating ground beef of fat percentages packaged with CO-MAP.

CHAPTER II

REVIEW OF LITERATURE

Meat color

Consumers use meat color to make purchasing decisions more than any other quality factor (Mancini and Hunt, 2005). Therefore, research to improve, enhance or maintain desirable meat color is crucial to the industry. Scientists are continually finding ways to extend the bright cherry red color that consumers desire when purchasing meat for their household. Understanding the chemistry behind meat color is important when moving forward with research. Myoglobin is the major pigment responsible for meat color (Suman and Joseph, 2013). The state of myoglobin's central heme iron and the ligand bound at the free binding site will determine the visible color (Mancini and Hunt, 2005). There are four major chemical forms of myoglobin: deoxymyoglobin, oxymyoglobin, metmyoglobin, and carboxymyoglobin.

Oxymyoglobin and carboxymyoglobin have no distinguishable difference in appearance, both producing a bright cherry red color. However, carboxymyoglobin is more stable than oxymyoglobin due to myoglobin having a greater affinity to bind carbon monoxide than oxygen (Suman and Joseph, 2013). When oxygen is bound at the sixth binding site of the central heme iron the result is oxymyoglobin and when it is bound to carbon monoxide the result is carboxymyoglobin, while there is no ligand bound for deoxymyoglobin (Mancini and Hunt, 2005). Deoxymyoglobin, oxymyoglobin, and carboxymyoglobin are all in a ferrous state (Mancini and Hunt, 2005). Deoxymyoglobin is commonly associated with vacuum packaged meat color and forms a purplish red color with iron in the reduced state (Mancini and Hunt, 2005). Consumers in the US distrust vacuum packaged beef which displays the purple color (Meischen, Huffman, and Davis, 1987). This is likely due to the lack of understanding of the purplish color because consumers are accustomed to seeing fresh meat packaged in an air permeable package that allows the formation of oxymyoglobin resulting in a bright cherry red color (Djenane and Roncales, 2018). Metmyoglobin in a ferric state results from oxidation of the three other forms of myoglobin (Mancini and Hunt, 2005). Metmyoglobin is associated with meat discoloration due to the brown color formed (Suman and Joseph, 2013). Meat that has been exposed to oxygen for extended periods of time will discolor and often influence the consumer's impression of safety and quality of the product (Mancini and Hunt, 2005). Metmyoglobin has a water molecule bound at the sixth coordinate of the ferric heme and is incapable of binding oxygen (Suman and Joseph, 2013). Meat with 20% surface metmyoglobin is discriminated by consumers (MacDougall, 1982). Bright red beef outsells discolored beef with 20% metmyoglobin by a ratio of 2:1 (Hood and Riordan, 1973). Oxygen presence, temperature, pH, and microbial growth, among others, are all factors which can lead to possible metmyoglobin formation (Mancini and Hunt, 2005). For example, residual oxygen within a mother bag and temperature abuse during storage causes metmyoglobin to form. Consequently, significant efforts have been developed by the beef industry which prolong the shelf life and color stability of beef (Rogers et al., 2015).

Ground beef

According to the Code of Federal Regulations 319.15, ground beef can be defined as chopped fresh and/or frozen beef with or without seasoning, without more than 30% fat, and no added water, phosphates, binders, or extenders. Ground beef is the most consumed beef product in the US and accounts for nearly half of the total beef consumption (National Cattlemen's Beef Association, 2012). Ground beef takes up more space in the retail case than any other product with 1.3 billion pounds of ground beef produced each year for retail (Schulz, 2021). Ground beef is easy to prepare, versatile, and a relatively inexpensive protein source. Ground beef is utilized in many capacities: fast food restaurants, school lunch programs, military programs, and households (Troutt et al., 1992). According to some analysts, ground beef has increased up to possibly 50% in consumption over the past several years (Green, 2012). This could be due to high unemployment rate between 2008 and 2012, and it being a cheaper protein source than other options (Green, 2012). In 2020, ground beef accounted for over 46% of the total US retail beef consumed (Schulz, 2021).

Case Ready Packaging

Meat packaging is used to increase product safety and quality while extending shelf life. Thus, one way to extend shelf life and keep products the bright cherry red color consumers desire for longer periods of time is by utilizing different packaging techniques (Hotchkiss, 1988). The main concerns when storing fresh beef are color, lipid oxidation, and microbial growth (Esmer et al., 2011). As technology advances so do packaging technologies, often making it possible to extend shelf life. The variables that influence the shelf-life properties of packaged meat are product type, gas mixture, package, headspace, packaging equipment, and storage temperature (Hotchkiss, 1988). One common packaging type used is vacuum packaging. This packaging type forms deoxymyoglobin within the package due to low or no oxygen presence (Mancini and Hunt, 2005). Vacuum packaging is used mainly for primals, which are the larger sections of a carcass that are later broken down into retail cuts. The primals are shipped to retailers or further processors where they are prepared for retail by opening, fabricating into retail cuts, and placing into trays with overwrap. Meat was first seen on display in self-service meat cases with a bright red bloomed color in an air permeable package. The bright cherry red color is now associated with meat freshness making it important to maintain to ensure consumer satisfaction (McMillin, 2008). Not all retailers have the capability of receiving primals and cutting their own retail cuts, therefore they must receive the product in a case ready package. This technique of in house cutting and packaging in air permeable overwrap has been gradually replaced with case ready packaging or centralized operations (McMillin, 2008). In 2007, 64% of the packages in the US fresh meat self-service cases were from case ready packaging (Crews, 2007). There are many advantages of case ready packaging such as: uniformity of cuts in plants, quality improvements, less discoloration, fewer off flavors, and increased space and time utilization in retailers (Cole, 1986). Case ready packaging is the fabrication of retail items at a processing plant or somewhere outside of the retailer, where they are then transported and subsequently displayed in retail cases with little or no manipulation of each individual package after removal from shipping case (McMillin, 1994). One example of this used today is modified atmosphere packaging with case ready trays placed within a master bag where bloom is prolonged until removal from bag and displayed at the retail store.

Master Bag Modified Atmosphere Packaging

The most common case ready packaging type is modified atmosphere packaging (MAP), a package that modifies the gas composition surrounding the product and within the package (McMillin, 2008). In the packaging process, headspace air is removed, gas is flushed in, and then the package is sealed rapidly with a heat bar (Cornforth and Hunt, 2008). The typical gases most used within MAP packaging are oxygen, nitrogen, and carbon dioxide (Djenane and Roncales, 2018). Nitrogen helps prevent the master bag from collapsing and aids in preventing bacterial growth and preventing aerobic spoilage, but anaerobic bacteria growth can still occur (Nassu et al., 2010). Carbon dioxide also reduces growth of aerobic spoilage bacteria while allowing myoglobin to stay in its reduced form (Isdell et al., 1999). There are several types of gas mixtures used within MAP packaging. High oxygen MAP (80% O_2 and 20% CO_2) was the most widely used form of MAP in the early 2000's before master bag technology (Eilert, 2005). Although high oxygen MAP can extend meat's bright cherry red color, there are some issues linked with high oxygen environments. One major concern being increased lipid oxidation which creates rancid flavors within the product (Esmer, 2011). Another problem is premature browning: where cooked product appears to brown at below normal internal cooking temperatures, and consumers eat product prior to complete cooking (Hague et al., 1994). This poses a food safety concern due to pathogens not being killed with proper cooking temperatures (Lyon et al., 2000).

In 2002 to reduce the incidence of premature browning and prolong shelf-stable bright cherry-red color, the FDA approved the use of 0.4% carbon monoxide (CO) in MAP systems (John et al., 2005). Although, the ability for CO to increase the red color stability of meats was recognized over 100 years ago (Church, 1994). It is important to note, CO poses a threat during the packaging process as it is highly flammable and toxic (Arvanitoyannis, 2012). Due to the potential toxic effect of CO, it is a controversial topic and still banned in the EU to be used in their food processing. With CO, shelf-life of meat can be increased to 21 d rather than the 14 d of high oxygen MAP (Suman and Joseph, 2013). Without the use of CO in MAP, issues with meat re-blooming would arise (Hunt et al., 2004). Also, low CO compared to high oxygen packaging has improved flavor due to less oxidation (Cornforth and Hunt, 2008). CO-MAP has been found to reduce lipid oxidation and microbial spoilage and is a viable option for extending the shelf life for ground beef (Brooks et al., 2008). Since metmyoglobin formation is an oxidative process, CO can be considered an antioxidant due to its ability to inhibit metmyoglobin formation (Lanier et al., 1978). CO-MAP has advantages over traditional overwrapped packages as it results in decreased growth of pathogens and less spoilage. Although, CO-MAP does have disadvantages, one being persistent pinking, or masking spoilage due to enhanced color stability (Cornforth and Hunt, 2008).

Oxygen scavengers within MAP

With case ready packaging, oxygen still can be present. To reduce oxygen levels, oxygen scavengers, a type of active packaging, are added to master bags to reduce oxygen levels. Oxygen scavengers are in a sachet form and work by oxidizing iron when exposed to oxygen (Cruz et al., 2012). By using an effective oxygen scavenger, myoglobin is maintained in the deoxymyoglobin state in storage, and then converted to oxymyoglobin when removed from the bag at the retail store (Arteaga Custode et al., 2017). Residual oxygen must be less than 0.15% to prevent metmyoglobin formation (Mancini and Hunt, 2005). Metmyoglobin is not accepted by consumers due to its brown color, so limiting its formation is valuable (Mancini and Hunt, 2005). Meat discoloration can be slowed with oxygen levels being reduced to below 0.01% by the scavengers (Cruz et al., 2012). With the use of scavengers, oxygen should be removed by 1-1.5% per hour (McMillen, 2008). With this oxygen reduction, scavengers continue to keep oxygen reduced for up to 21 d in storage (Arteaga Custode et al., 2017). By keeping exposure to oxygen down in storage, overall red color in ground beef is increased and shelf life is extended compared to ground beef stored without an oxygen scavenger. (Arteaga Custode et al., 2017). Since grinding exposes more of the meat to air and increases surface area, ground beef is prone to discolor faster than other cuts of beef (Ellis et al., 2002).

Persistent pinking use of CO

CO-MAP can enhance the red color stability of meat due to the formation of carboxymyoglobin (COMb) which has a higher stability compared to oxymyoglobin (Lanier, 1978). However, the increased stability leads to concerns surrounding CO used in fresh meat packaging due to the CO remaining tightly bound to myoglobin even after cooking, persistent pinking can occur (Cornforth and Hunt, 2008). Persistent pinking is a term used when a product is cooked to a safe endpoint temperature, but the internal color remains pink (Ryan et al., 2006). This is a concern for consumers since they could be purchasing product under the impression it is fresh, but bacterial numbers could have already reached spoilage levels (Cornforth and Hunt, 2008). Due to these concerns early on, there was little to no commercial use of CO with fresh red meats. Gradually, research investigating time, temperatures, and MAP packaging slowly grew (Cornforth and Hunt, 2008). In 1985 Norway's processors began using CO-MAP first in commercial packaging of fresh meats (Cornforth and Hunt, 2008). They learned with high levels of CO_2 and anaerobic conditions, lower levels of CO were needed to maintain an acceptable red color (Sørheim et al., 1999). Research showed that consumers did not accept product packaged with CO levels at 2% due to the color looking artificial (Sørheim et al., 1999). Although, CO-MAP prolongs the bright cherry red color, it will still eventually reach spoilage. In CO-MAP ground beef can maintain bacterial levels lower than spoilage for 4-5 weeks, and acceptable red appearance for at least 8 weeks (Hunt et al., 2004, Jayasingh et al. 2001). Additionally, it is common for consumers to believe a brown internal cook color ensures a cooked safe product for consumption, and a pink center for a ground patty needs additional cooking (Ryan et al., 2006). Therefore, it is important for consumers not to base doneness of cooked beef solely on interior color and visual appearance due to inaccuracy (Suman, 2016). The USDA-Food Safety and Inspection Service recommends using a food thermometer to ensure safety and to determine desired doneness (Food Safety and Inspection Service). Some consumers stated that when in doubt about doneness overcooking is better than undercooking (Koeppl, 1998). Communicating the importance of using a meat thermometer to consumers is essential, especially with the problem of persistent pinking present in the industry (Cornforth and Hunt, 2008). By using a meat thermometer consumers will receive a higher quality product without overcooking.

Ground beef fat content

In retail, there are primal specific ground beef blends offered as well as common lean/fat ratios: 73% lean/27% fat, 80% lean/20% fat, and 90% lean/10% fat (Savell, 2020). As lipid content increases, lipid oxidation also increases in ground beef. Lipid oxidation is deterioration caused by aerobic storage negatively affecting the flavor with the development of oxidative rancidity (Wang, et al., 2021). Lipid content can be used to predict lipid oxidation more reliably than visual discoloration (Wang, et al., 2021). Ground beef with higher fat contents will have greater rates of discoloration and lipid oxidation (Wang, et al., 2021). Texture, fat content, and palatability all can impact ground beef based on primal sourcing. Research has found ground beef sourced from dairy-type cow inside rounds had greater display color life in high-oxygen modified atmosphere packaging compared to ground beef sourced from beef-type cow inside rounds (Savell, 2020). Across the different fat percentages, cook loss percentage is fairly similar, but in leaner ground beef the loss consists of more moisture compared to fat while in fatter ground beef, more fat is lost (Savell, 2020). Studies show higher fat contents in ground beef will also have higher tenderness, juiciness, and flavor ratings than ground beef with lower fat contents (Savell, 2020). All these factors influence consumer's eating experience and in turn purchasing habits making it important to balance what is desired by the customer while provided a high quality, safe product.

Conclusion

Meat color influences consumers' purchasing decisions, making it important to maintain the bright cherry red color they desire. One way to do this is to use different packaging techniques. MAP is one form of case ready packaging that can be utilized to extend shelf life in fresh meat products. High oxygen packaging has proven to increase red color in meat, but with it comes issues like premature browning. CO-MAP also has both advantages and disadvantages with the main disadvantage being persistent pinking. Its many advantages include extended shelf life, decreased microbial growth and lipid oxidation, and increased bright cherry red color stability. Ground beef has remained a popular choice for beef consumers over the years; thus, it is important to improve packaging types and further develop them to benefit the meat industry.

CHAPTER III

EVALUATING THE OCCURRENCE OF PERSISTENT PINKING IN GROUND BEEF PATTIES OF DIFFERING FAT PERCENTAGES PACKAGED IN CARBON MONOXIDE MODIFIED ATMOSPHERE PACKAGES

ABSTRACT

The objective of this study was to gain a better understanding of the occurrence of persistent pinking by evaluating ground beef with different fat percentages packaged with carbon monoxide modified atmosphere packaging (CO-MAP). Three cases of fine ground beef (IMPS 136) with targeted lean/fat percentages of 73/27, 81/19, and 93/7 were collected from a commercial packing facility. Ground beef was stored for 10 d. After storage, each ground beef chub was opened and reground with a 3 mm stainless steel grinder plate together to form one composite batch of each fat percentage. After grinding, 225 g patties were weighed, formed utilizing a patty maker, and placed onto foam trays (n = 81) with an absorbent pad and overwrapped with polyvinylchloride film (PVC). The trays were randomized within each fat percentage and were then placed in master bags (n = 27) in groups of three with one oxygen scavenger per bag. Twenty-seven master bags were flushed with a tri-gas blend of 0.4% CO, 30% CO2, and balance N2. Master bags were then held in dark storage until pull day: 1 d, 8 d, or 15 d. On each pull day, 9 master bags were removed from dark storage, and the trays were placed in a display case for retail display evaluation for 3 d. Headspace analysis was conducted

before each master bag was opened. Instrumental and objective color measurements were collected on d 0 – 2 of retail display. Visual color and surface discoloration were analyzed daily by a trained panel (n = 6). The formation of carboxymyoglobin (COMb) was confirmed by measuring ratio of absorbance at 543 nm/581 nm. Patties were cooked to an internal temperature of 71°C. Internal cooked color was evaluated by a trained panel (n = 6) along with instrumental color reading post cooking. The results of the study showed the 93/7 patties had higher a* values throughout retail display over all pull days. All patties had formed COMb by retail display, regardless of storage day or fat percentage. The 93/7 patties had consistently browner internal subjective cooked color scores compared to the 73/27 patties. The 73/27 patties from all pull days were significantly ($P < 0.05$) redder than the 93/7 patties, and on pull d 1 and 15, the 81/19 and 73/27 patties had significantly ($P < 0.05$) redder cooked a* values than the 93/7 patties. COMb was formed in CO-MAP regardless of ground beef fat percentages or pull day. Additionally, ground beef with more fat (73/27) had more pinking after cooking to recommended internal temperature compared to lower fat blends. With consumer confusion on pinking within meat products post cooking, further research should be conducted to better understand the relationship of fat content of ground beef and dark storage times resulting in persistent pinking.

INTRODUCTION

Consumers are fueled by many things when purchasing meat, with the most influential being meat color. A bright cherry red color in beef is associated with wholesomeness and freshness (Suman and Joseph, 2013). With increased time in the case, potential for surface discoloration increases. Discoloration can deter consumers from purchasing products. The formation of metmyoglobin can be combatted in numerous ways, one being through packaging techniques.

Modified atmosphere packaging (MAP) is a type of case ready packaging that modifies gas composition surrounding a product (McMillin, 2008). One example of MAP utilizes a tri-gas blend of CO_2, N_2 and CO (CO-MAP) being flushed into a master bag containing retail ready packaged trays. CO-MAP can increase the shelf life of a product by stabilizing color and reducing lipid oxidation and microbial spoilage (Brooks et al., 2008). Meat color is stabilized within the master bag by CO binding with myoglobin and forming carboxymyoglobin (COMb) (Sørheim et al., 1999).

COMb has greater stability than oxymyoglobin due to the CO remaining tightly bound to myoglobin even after cooking leading to one major issue known as persistent pinking (Cornforth and Hunt, 2008). Persistent pinking is a term used when a product is cooked to a safe endpoint temperature, but the internal color remains pink (Ryan et al., 2006). For consumers who rely on meat color as an indicator of doneness, this can become an issue of food quality or satisfaction because of overcooking.

The objective of this study was to gain a better understanding of the occurrence of persistent pinking by evaluating ground beef with different fat percentages packaged with CO-MAP.

MATERIALS AND METHODS

Product Collection

Three cases of fine ground beef (IMPS 136) with targeted lean/fat percentages of 73/27, 81/19, and 93/7 were collected from Creekstone Farms in Arkansas City, KS. The case of 81/19 was specifically ground chuck (IMPS 137). Product was then transported to Oklahoma State University (Stillwater, OK) with the average ambient temperature of 0.05°C ± 1.0°C during transport measured with EasyLog EL-USB-2-LCD data logger (Lascar Electronics, Erie, PA, USA).

Packaging and Storage

Upon arrival to Oklahoma State University, ground beef was stored for 10 d at an average of 3.0°C ± 0.5°C. After storage, each ground beef chub was opened and reground with a 3 mm stainless steel grinder plate (BIRO Model meat grinder, Biro Manufacturing Co., Marblehead, OH) together to form one composite batch of each fat percentage. Proximate analysis measurements for protein, fat, and moisture were conducted with a FOSS FoodScan™ (FOSS Analytics North America, in Eden Prairie, MN) and averaged as shown in Table 1. The blend of 93/7 was close to target with a fat level of 8.7%. The 81/19 and 73/17 blends were similar; 73/27 blend was below targeted fat with 22.4% fat and the 81/19 ground chuck was 21.6% fat.

After grinding, 225 g patties were weighed formed by hand with a ½ pound patty maker (KitchenArt Adjust-A-Burger Press, Delhi, India). Patty dimensions were 10.80 cm x 2.75 cm. Patties (n = 81) were placed into Styrofoam trays (NoviPro; 16.5 cm x 21.75 cm x 3.22 cm) obtained from NoviPax (Oak Brook, IL), with an absorbent pad and overwrapped with polyvinyl chloride (PVC) and heat sealed (Intertek Heat Seal, model 600A, Intertek USA Inc., Houston, TX). After the patties were packaged in trays, they were randomly assigned a number (n = 81) to be stored 1 d, 8 d, or 15 d in dark storage. The trays were placed in master bags in groups of three with one oxygen scavenger (Multisorb FreshPax® CR) per bag. Master bags (Bemis, Master beef z-0300, 22.75 in x 29 in) were flushed with M-Tek CORR-VAC® MARK-III (M-Tek Corporation, Elgin, IL, USA) with a tri-gas blend of CO, CO_2, and N_2 (Airgas NI FGCD30CM415A; 0.42% CO, 30.01% CO_2, balance N_2). Master bags were then held in dark storage in Meat 65126 totes (Tosca®, Atlanta, GA, USA) at 0.05°C ± 1.0°C until their respective pull days (1 d, 8 d, or 15 d) for 3 d retail display.

pH

The pH of the ground beef was measured at grinding, and at the end of each retail display using a Hanna pH meter (model HI 99163, Hanna Instruments Inc., Smithfield, RI). Three measurements were taken each time and averaged for statistical analysis.

Retail Display and Headspace Analysis

On each pull day (1 d, 8 d, or 15 d) 9 master bags were removed from dark storage. Headspace analysis was conducted using a Mocon PAC CHECK® Model 333 Triple Gas Analyzer (Minneapolis, MN, USA) measuring O_2, CO_2 and CO. The packages were removed and placed in retail display. Each package was placed in a Hussmann IM1SL retail case set at 2.0°C ± 2.0°C and was displayed in retail cases lit with Philips LED T8 Lamps (model number 9290011240B-453597) manufactured in Niles, OH. Retail lights and ceiling lights in the retail room remained on consistently throughout the retail display time. During retail display, visual and instrumental color measurements were taken every 24 h. Packages were rotated randomly once a day to reduce variation from the case. At the end of display, one raw patty was selected per master bag for raw lipid oxidation, and the other two patties were assigned for cooking.

Visual Color Analysis

Visual color and surface discoloration were evaluated by a six-member trained panel once daily while in retail display with scales based on AMSA (2012) guidelines.

Panelists were trained using base pictures for reference on color and percent discoloration. Patty color was evaluated on a scale of 1 to 8: 1 being very light red or grayish-pink, 8 being dark red or grayish pink, and the ideal color score 5 being bright cherry red. Surface discoloration was evaluated on a percentage basis on a scale from 1 to 6, with 1 being no discoloration (0%) and 6 being extensive discoloration (81-100%).

Instrumental Color Analysis

Instrumental color was measured quantitatively with a portable, reflected-color measurement spectrophotometer. Measurements were taken on d 0 and every 24 h throughout retail display. Three readings were recorded at random locations on each patty's surface with the HunterLab MiniScan® EZ 4500L (2.5-cm aperture, illuminant A, and 10° standard observer angle; Reston, VA, USA), and readings were averaged. L* values measure lightness, white to black; higher L* values indicate lighter products. Redness is measured by a* values with a higher a* value indicating a redder product, and a positive b* value indicating a more yellow color with negative values representing blue. The Hunterlab instrument was standardized prior to each use with white and black tiles.

Cooking

Patties were cooked on an XLT Impingement Oven (model 3240-TS, BOFI Inc., Wichita, KS) to the USDA recommended endpoint internal temperature of 71°C (Food Safety and Inspection Service). Patties were weighed raw prior to cooking and post cooking to measure cook loss percentage. Patties were placed in ice for 5 min post cooking to prevent further cooking. The patties were then removed from the ice for analysis.

Internal Cooked Color

Patties were cut horizontally; L*, a*, absorbance, and reflectance of patty interior was measured using HunterLab MiniScan® EZ 4500L (2.5-cm aperture, illuminant A, and 10°standard observer angle; Reston, VA, USA) from 400 to 700 mm. Three readings were taken on each patty and averaged for L* and a* measurements. Averages were also taken from 3 readings of reflectance and absorbance wavelengths from 400 to 700 nm at 10 nm increments and were used for calculations. Internal subjective cooked color was also evaluated visually on a scale of 1 to 7, with 1 being very red and 7 being tan/brown.

Lipid Oxidation

Evaluation of lipid oxidation was conducted at the end of retail display according to a modified procedure from Witte et al. (1970). Samples (three g) of ground beef patties were collected and blended with 27 mL trichloroacetic acid (TCA) solution. Samples were blended using a Waring commercial blender (Model 33BL7; New Hartford, CT) for 1 min and then filtered through Whatman (#1) filter paper. One mL of trichloroacetic acid (TCA) solution and 1 mL filtered solution were combined and placed in a boiling water bath for 10 min. After incubation samples were cooled at room temperature for 5 min and absorbance was measured with a Shimadzu UV-2600 PC spectrophotometer at 532 nm.

Statistical Analysis

A split plot design was used to evaluate the color of ground beef patties with differing fat percentages packaged in a master bag packaging system flushed with CO. The whole plot being fat percentages and the sub plot being pull day. The experimental unit was the master bag (n = 27) including 3 patties of the same fat percentage; master bags were randomly assigned to various pull days. Simple means were calculated for proximate analysis. All other data was analyzed using PROC GLIMMIX of SAS 9.4 (SAS Institute Inc., Cary, North Carolina), where main effects were pull day, fat percentage, and their interactions. Non-significant interactions were removed from the model. Least squares means were calculated; where ANOVA testing indicated significance, means were separated using the PDIFF option and deemed significant when $P < 0.05$.

RESULTS AND DISCUSSION

pH

There was a significant ($P < 0.05$) interaction of pull day × fat percentage for pH values. The pH values in table 2 were significantly ($P < 0.05$) higher on d 1 for the 93/7 and 81/9 patties. The pH values for the 73/27 patties were similar to all patties from pull d 8 and the 81/19 and 73/27 patties on pull 15. The 93/7 patties on pull d 15 were significantly ($P < 0.05$) lower in pH than all other patties. These could be due to high amount of lean in this blend under anaerobic conditions within master bag inherently causing lactic acid formation and lowering pH (Nissan et al. 1996).

Trained Display Color

There was a significant ($P < 0.05$) interaction of fat percentage × retail day on display color. As shown in table 3, the 93/7 patties had a bright cherry red appearance indicated at level 5 on d 1 and increased in darkness on d 2 and 3. As retail display increases at each storage day, display color scores of beef patties decrease with the bright cherry red color diminishing with it (Harlan, 2011). The 81/19 and 73/27 patties were similar throughout all of retail display, having significantly ($P < 0.05$) lighter red color than the 93/7 patties. The lighter red color could be due to the 81/19 patties being made from ground chuck compared to the 93/7 and 73/27 patties made from ground beef trim. The similarities of the 81/19 and 73/27 in redness values were expected based on results of our analytical evaluation to determine fat percentage.

Trained Surface Discoloration

There was a significant ($P < 0.05$) interaction of fat percentage × retail day on surface discoloration. On d 1 of retail display, there was no discoloration for patties from all 3 fat percentages shown in Table 4. This is due to MAP limiting oxygen exposure and therefore limiting discoloration for products in MAP (Arteaga Custode et al., 2017). Discoloration increased on d 2 slightly, with the only significant increase being with the 73/27 patties which were still within acceptable levels of discoloration being below 20%. A score above 2.0 for surface discoloration would most likely be discounted in retail store, due to consumers discriminating against meat with 20% surface metmyoglobin, and rejecting the product when discoloration reaches 40% (MacDougall, 1982; McMillin, 2008). This is because color serves as the major indicator of freshness and quality of product (Mancini and Hunt, 2005). As myoglobin pigments oxidize, discoloration occurs shifting meat away from the bright cherry red color that is most accepted by consumers (Jeong and Claus, 2011). By d 3 in retail, the 81/19 and the 73/27 patties were significantly more discolored than 93/7 patties and well above levels of acceptability. The 93/7 patties were the least discolored by retail d 3, this is to be expected since ground beef with higher fat contents has greater rates of discoloration (Wang, et al., 2021).

Retail L* values

There was a significant main effect (*P* < 0.05) of retail day (Table 5) when evaluating L* values. Patties on retail d 1 were significantly the lightest (*P* < 0.05), increasing in darkness on d 2. This is to be expected with a decrease in L* values over time in retail display with packages (Jayasingh et al. 2001).

There was a significant main effect (*P* < 0.05) of fat content on L* values shown in Table 5. The 73/27 ground beef patties had the lightest appearance (*P* < 0.05), and the 93/7 patties had the darkest (*P* < 0.05). The more fat within in ground beef patties will affect meat color and lightness values (Kerth, 2015); increased fat results in lighter color evaluation and higher L* values. Researchers have shown the highest L* values in the higher fat percentages and the lowest L* values in leaner ground beef patties (Troutt et al., 1992).

Retail a* values

There was a significant (*P* < 0.05) interaction of fat percentage × retail day for a* values shown in table 6. There was no significant interaction on pull day for a* values. Thus, patties packaged within CO-MAP will have a prolonged bright cherry red color (Hunt et al., 2004). On d 1 patties from all fat percentages were significantly redder than all other retail days. As display continued, the a* values decreased which is to be expected. According to Hunt et al. (2004), a decrease in a* values will occur during display of product packaged in modified atmosphere packaging with CO. After extended exposure to oxygen, product's a* values will decrease (Isdell, 1999). On all three retail days, the 93/7 patties were significantly redder (*P* < 0.05) than the 73/27 and 81/19 patties. The 81/19 and 73/27 patties were similar (*P* > 0.05) in a* values throughout retail display, as expected since ground beef chubs purchased for these two fat blends were similar when tested analytically, 21 and 22% fat, respectively. Troutt et al. 1992 showed leaner ground beef results in higher a* values throughout retail display.

Effects of Storage on Carboxymyoglobin Formation

The spectra from the HunterLab MiniScan was taken and analyzed to further clarify myoglobin type formed by patties within CO MAP. Figure 1 shows 2 peaks. Carboxymyoglobin can be confirmed with a peak being at 540 and oxymyoglobin with a peak at 580. By taking a ratio of absorbances at 543 and 581, carboxymyoglobin can also be confirmed when the ratio has a value greater than one. This was confirmed in all patties packaged with CO-MAP, by using spectra data from HunterLab MiniScan upon putting into retail display. There was no significance in main effects: pull day and fat percentage, or their interaction.

Thiobarbituric Acid Analysis

Absorbance levels for all raw and cooked ground beef were well below detectable levels of lipid oxidation, data not shown in tabular form. A score over 2 g/kg malondialdehyde (MDA) can be associated with off-flavors associated with oxidative rancidity by consumers (Campo et al., 2006). This was set as the threshold level for oxidation analysis.

Internal Subjective Cooked Color and Cooked a* values

There was a significant ($P < 0.05$) interaction of pull day × fat percentage for internal subjective cooked color shown in Table 7. The 93/7 patties were consistently browner internally after cooking than the 73/27 patties throughout each pull day. Additionally, the 93/7 patties had browner internal cooked color compared to the 81/19 patties on d 1. The 73/27 patties had significantly ($P < 0.05$) pinker cooked internal meat color than all other patties packed on d 8 and d 15.

There was a significant interaction ($P < 0.05$) of pull day × fat percentages for a* values after cooking patties (Table 7). Grobbel (2008) found that product packaged in CO-MAP had higher internal a* values post cooking than product not packaging in MAP. On pull d 1 and 15 fat percentages 81/19 and 73/27 were significantly ($P < 0.05$) redder than the 93/7 patties post cooking. Additionally, on pull d 8, the 73/27 patties were significantly ($P < 0.05$) redder internally than the 93/7 patties. On pull d 8 and 15 the 81/19 and 73/7 patties were similar in cooked interior a* values, as expected since they were similar in fat percentage determined by FOSS FoodScan™. During previous unpublished research conducted by Hearn et. al, denatured metmyoglobin had an average a* value of 15, this leads us to believe metmyoglobin was formed on the interior of the patties prior to cooking.

Cook Loss Percentage

There was a significant interaction ($P < 0.05$) of pull day × fat percentage for cook loss percentage shown in Table 7. Both 81/19 and 73/27 patties had significantly more cook loss than the 93/7 MAP on pull d 8. Troutt et al. (1992) found that patties with less fat had lower cook losses. On pull d 15, 81/19 patties had significantly higher cook loss than all other patties on that pull day. These results are similar to findings of others who reported increased cooking loss from ground beef patties with higher fat percentages (Troutt et al, 1992, Cross et al. 1980). Our 81/19 and 73/27 ground beef was similar when analytical fat percentages were measured so 81/19 having the highest cook loss on pull d 15 and similar to 73/27 on other days was expected.

CONCLUSION

Meat color is one of the most important visual attributes consumers use when purchasing fresh beef. Myoglobin in the form of oxymyoglobin (OxyMb) and carboxymyoglobin (COMb) results in a bright cherry-red color, desired by consumers. The use of MAP technologies can be used to improve meat color. The use of CO, specifically in MAP, can be utilized to extend display color brightness and reduce surface discoloration. With the use of CO, COMb can form which denatures slower during the cooking process than OxyMb; thus, persistent pinking can occur in cooked meat.

All CO-MAP patties had formed COMb upon putting into retail display, regardless of pull day or fat percentage. CO-MAP will reduce the amount of discoloration within patties. Patties with higher amount of lean will discolor less than patties with increased amount of fat. The leaner patties have redder color throughout display compared to patties with higher fat contents. It is suspected by the end of retail, exposure to oxygen caused the issue of pinking to decrease, since oxymyoglobin was forming.

Ground beef with more fat will have more pinking after cooking to proper endpoint temperature than patties with lower fat content. With consumer confusion on pinking within meat products post cooking, further research should be conducted to better understand the relationship of fat content within ground beef and dark storage times resulting in persistent pinking.

Table 1. Means[1] of proximate analysis measurements for protein, fat, and moisture in ground beef chubs from three different fat percentages

Lean/Fat Percentage	Protein %	Fat %	Moisture %
93/7	20	8	72
81/19	16	21	63
73/27	17	22	61

[1] Averages of three composite samples

Table 2. Least square means for pH after 3 d in retail display from 3 pull days (1, 8, 15 d in dark storage) of ground beef patties in MAP[1] packaging (n = 27 master bags; 81 patties in display)

Lean/Fat Percentage	Pull Day		
	1	8	15
93/7	5.70[a]	5.43[bc]	5.24[d]
81/19	5.70[a]	5.38[c]	5.43[bc]
73/27	5.48[bc]	5.47[bc]	5.51[b]
SEM[2]	0.04	0.04	0.04

[1]MAP = Modified atmosphere packaging
[2]SEM = Standard error of the mean
[a-d] Least square means that do not share a common subscript are significantly different ($P < 0.05$)

Table 3. Least square means for display color[1] during 3 d in retail display from all pull days (1, 8, 15 d in dark storage) of ground beef patties in MAP[2] packaging (n = 27 master bags; 81 patties in display)

Lean/Fat Percentage	Retail Day		
	1	2	3
93/7	5.1[c]	6.4[b]	7.3[a]
81/19	2.7[fg]	2.6[g]	4.0[d]
73/27	3.4[ef]	3.1[fg]	3.9[de]
SEM[3]	0.22	0.22	0.22

[1]Display color: 1 = very light red; 5 = bright red; 8 = dark red
[2]MAP = Modified atmosphere packaging
[3]SEM = Standard error of the mean
[a-g] Least square means that do not share a common subscript are significantly different ($P < 0.05$)

Table 4. Least square means for surface discoloration[1] during 3 d in retail display from all pull days (1, 8, 15 d in dark storage) of ground beef patties in MAP[2] packaging (n = 27 master bags; 81 patties in display)

Lean/Fat Percentage	Retail Day		
	1	2	3
93/7	1.0^{d}	1.1^{cd}	3.1^{b}
81/19	1.0^{d}	1.7^{bcd}	5.1^{a}
73/27	1.0^{d}	1.9^{bc}	4.9^{a}
SEM[3]	0.18	0.18	0.18

[1]Surface discoloration: 1 = no discoloration (0%); 3 = small discoloration (21-40%); 6 = extensive discoloration (81-100%)
[2]MAP = Modified atmosphere packaging
[3]SEM = Standard error of the mean
[a-d] Least square means that do not share a common subscript are significantly different ($P < 0.05$)

Table 5. Least square means for L* values during 3 d in retail from all pull days (1, 8, 15 d in dark storage) of ground beef patties in MAP[1] packaging (n = 27 master bags; 81 patties in display)

L* values			
Retail Day	1 56.8^{a}	2 53.4^{b}	3 53.1^{b}
Lean/Fat Percentage	93/7 49.6^{c}	81/19 56.6^{b}	73/27 57.1^{a}
SEM[2]	0.19	0.19	0.19

[1]MAP = Modified atmosphere packaging
[2]SEM = Standard error of the mean
[a-c] Least square means that do not share a common subscript are significantly different ($P < 0.05$) within each row (retail day, lean/fat percentage)

Table 6. Least square means for a* values during 3 d in retail display from all pull days (1, 8, 15 d in dark storage) of ground beef patties in MAP[1] packaging (n = 27 master bags; 81 patties in display)

Lean/Fat Percentage	a* values		
	Retail Day		
	1	2	3
93/7	37.4[a]	29.7[c]	22.2[e]
81/19	35.2[b]	27.7[d]	14.9[f]
73/27	34.4[b]	27.5[d]	15.2[f]
SEM[2]	0.66	0.66	0.66

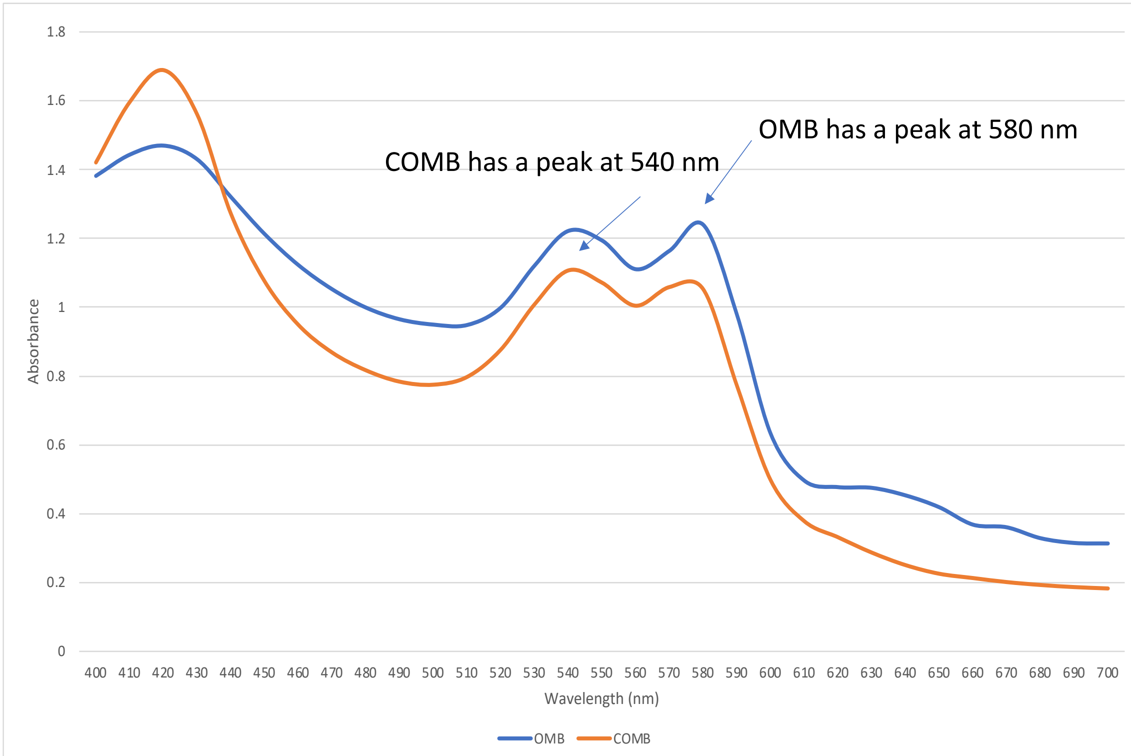
[1]MAP = Modified atmosphere packaging
[2]SEM = Standard error of the mean
[a-f]Least square means that do not share a common subscript are significantly different ($P < 0.05$)

Table 7. Least square means for internal cooked color[1], cook loss %, and cooked a* values after 3 d in retail display from 3 pull d (1, 8, 15 d in dark storage) of ground beef patties in MAP[2] packaging (n = 27 master bags; 81 patties in display) cooked to 71°C

Pull Day	Lean/Fat Percentage	Internal cooked color	Cooked a* values	Cook loss %
1	93/7	6.5^{a}	12.4^{ef}	37.7^{a}
	81/19	3.6^{c}	24.6^{a}	33.2^{bc}
	73/27	4.1^{bc}	19.6^{b}	26.4^{d}
8	93/7	5.8^{a}	12.7^{ef}	31.2^{c}
	81/19	5.6^{a}	14.5^{def}	37.2^{a}
	73/27	3.9^{c}	17.6^{bcd}	36.0^{ab}
15	93/7	6.8^{a}	11.5^{f}	33.5^{bc}
	81/19	5.5^{ab}	15.7^{cde}	37.8^{a}
	73/27	3.8^{c}	18.9^{bc}	33.9^{bc}
SEM[3]		0.50	1.38	1.12

[1]Internal cooked color: 1 = very red; 7 = tan/ brown
[2]MAP = Modified atmosphere packaging
[3]SEM = Standard error of the mean
[a-f]Least square means that do not share a common subscript are significantly different ($P < 0.05$) within column of a trait (internal cooked color, cooked a* values, and cook loss %)

Figure 1. Myoglobin Classification as shown by peaks in absorbance across wavelengths



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APPENDICES

Display Discoloration	
1	Very light red or grayish-pink
2	Moderately light red or grayish-pink
3	Light red or grayish-pink
4	Slightly bright red or grayish-pink
5	Bright red or grayish-pink
6	Slightly dark red or grayish-pink
7	Moderately dark red or grayish-pink
8	Dark red or grayish-pink

Surface Discoloration	
1	No discoloration, 0%
2	Slight discoloration, 1-20%
3	Small discoloration, 21-40%
4	Modest discoloration, 41- 60%
5	Moderate discoloration, 61- 80%
6	Extensive discoloration, 81-100%

Internal Cooked Color	
1	very red
2	slightly red
3	pink
4	slightly pink
5	pinkish- gray
6	grayish tan/ brown
7	tan/ brown

VITA

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Master of Science

Thesis: EVALUATING THE OCCURRENCE OF PERSISTENT PINKING IN GROUND BEEF PATTIES OF DIFFERING FAT PERCENTAGES PACKAGED IN CARBON MONOXIDE MODIFIED ATMOSPHERE PACKAGES

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