EFFECTS OF DIFFERING CARBON DIOXIDE LEVELS ON MEAT COLOR IN MODIFIED ATMOSPHERE PACKAGING

By

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EFFECTS OF DIFFERING CARBON DIOXIDE

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Abstract: The objective of this study was to assess the effect of different carbon dioxide (CO_2) concentration gas flushes in master packages on the display life of fresh beef. Twenty-five USDA Low Choice ribeye rolls (IMPS 112A) from carcasses in program G-87B (USDA-AMS; live specification – Angus) were collected from Creekstone Farms in Arkansas City, KS. Each sub-primal was wet aged for 12 d. After aging, each sub-primal was opened and over sprayed with a SYNTRx 3300 solution. Ribeye rolls were sliced 2.54 cm thick and placed onto foam trays (n = 108 trays) with an absorbent pad and over-wrapped with perforated polyolefin film. Trays were randomly assigned to either a 10%, 20% or 30% CO_2 concentration master bag (n = 27 master bags; n = 9/CO_2 concentration). Three master bags, containing four trays, for each CO_2 concentration (10%, 20% and 30%) were randomly selected for opening after 10, 15, 18 d of dark storage, and the trays were placed in a display case for retail display evaluation for 5 d. Headspace analysis was conducted before each master bags were opened, and objective color measurements were collected on d 0 - 5 of retail display. Muscle color, surface discoloration and overall acceptability were analyzed daily by a trained panel (n = 6). The results of this study showed steaks from the 10% CO_2 concentration darkened and discolored more ($P < 0.05$) rapidly than steaks from either the 20 or 30% CO_2 concentrations. Additionally, steaks from master bags flushed with the 10% CO_2 concentration were the only steaks in display to reach unacceptable levels and discrimination by consumers. Steaks from master bags flushed with 20 or 30% CO_2 concentration performed comparably ($P > 0.05$) with minimal differences. The 20% CO_2 concentration master bag flush outperformed the 10% CO_2 concentration in all aspects of the study, as well as surpassed or equaled the 30% CO_2 concentration. Thus, it is recommended 20% CO_2 concentration gas flush of master bags could be utilized by the industry to reduce CO_2 use.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The most important factor influencing consumers purchasing decision of meat is color (Mancini and Hunt, 2005). Consumers associate a bright cherry-red color in beef with wholesomeness and freshness (Suman and Joseph, 2013). As time on display increases the bright cherry-red color diminishes and discoloration (MetMb) increases (Mancini and Hunt, 2005). Metmyoglobin formation and discoloration depends on many exogenous factors including muscle, temperature and oxygen partial pressure (Mancini and Hunt, 2005). Reducing the residual oxygen levels in packages can help extend shelf-life and limit discoloration. Residual oxygen levels must be less than 0.15% to prevent the formation of MetMb (Mancini and Hunt, 2005).

Modified atmosphere packaging (MAP) removes or replaces the gaseous environment surrounding a product before sealing the package (McMillin, 2008). An example of MAP is an aerobic technology with the use of CO_2, N_2 and CO (CO-MAP). Tri-gas CO-MAP is effective in stabilizing color as the CO binds to the myoglobin and forms COMb (Sørheim et al., 1999). Additionally, oxygen absorbers (better known as scavengers) can be utilized to ensure dissolved oxygen is removed throughout storage (Cichello, 2015). Oxygen scavengers work by reacting with oxygen through the oxidation of iron. This iron is usually stored in a sachet and added to the master bag directly (Cichello, 2015). Within a few hours of packaging the oxygen scavengers should rapidly decrease the residual oxygen within the mother bag and continue to keep it reduced for up to 21 d in storage (Arteaga Custode et al., 2017). Case ready packaging can be found in 60% of the meat case (Belcher, 2006).

With the COVID-19 pandemic, ethanol production rapidly decreased with fewer people driving and demanding fuel; thus, ethanol byproducts also decreased in availability including CO_2 needed for MAP (Bandoim, 2020). Therefore, the objective of this study was to investigate the effect of varying carbon dioxide levels on the color stability of ribeye steaks in low-oxygen, tri-gas master packages with oxygen scavengers.

CHAPTER II

REVIEW OF LITERATURE

Meat Color

The most important factor influencing consumers purchasing decision of meat is color (Mancini and Hunt, 2005). Consumers associate a bright cherry-red color in beef with wholesomeness and freshness (Suman and Joseph, 2013). The sarcoplasmic heme protein, Myoglobin (Mb), is responsible for the red color of meat (Suman and Joseph, 2013). Mb can exist in 4 redox states: deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), carboxymyoglobin (COMb) and metmyoglobin (MetMb; Suman and Joseph, 2013). Deoxymyoglobin is a purplish-red color that occurs when no ligand is bound to the 6^{th} site of the central heme iron of myoglobin (Mancini and Hunt, 2005). Oxygenation occurs when Mb is exposed to oxygen and diatomic oxygen binds to the 6^{th} site (Mancini and Hunt, 2005). After oxygenation OxyMb is formed and produces a bright cherry-red color (Suman and Joseph, 2013). OxyMb, or the bright cherry-red color, is what consumers strongly prefer (Mancini and Hunt, 2005). Furthermore, both DeoxyMb and OxyMb have central iron in the ferrous state (Mancini and Hunt, 2005). Following extended exposure to oxygen, MetMb is formed with a change of the iron valence state to ferric and water bound to the 6^{th} site. This form of Mb is incapable of biding oxygen (Suman and Joseph, 2013). The formation of MetMb causes meat discoloration which can result in revenue loss costing the United States an estimated $3 billion and the global market $14.2 billion annually (Maia Research Analysis, 2020). When MetMb reaches 20% coverage, meat is discounted and after reaching 40% MetMb meat is completely rejected by consumers (McMillin, 2008). MetMb formation and discoloration depends on many exogenous factors including muscle, temperature and oxygen partial pressure (Mancini and Hunt, 2005). A muscle's ability to reduce the MetMb formation can result in a more color stable product (McKenna et al., 2005). The longissimus thoracis, the muscle comprising a majority of the ribeye steak, can be considered a color stable muscle compared to the psoas major, the muscle making up filet steaks, which is considered color liable (McKenna et al., 2005). The ribeye steaks had deeper oxygen penetration and higher a* values throughout the display period, allowing them to have a longer consumer acceptability (McKenna et al., 2005). Further, the oxidation of DeoxyMb is more rapid than the oxidation of OxyMb. With high levels of oxygen, oxidation occurs slower and can better reduce MetMb formation than packaging with low levels of oxygen. Oxymyoglobin is oxidized at oxygen levels of 4% while DeoxyMb is oxidized at levels of 0.5-1% oxygen (Jakobsen, 2002). Understanding these factors is an important area of research in meat science and has led to various packaging technologies to help limit oxidation, combat discoloration and prolong shelf-life.

Shelf-Life

The time from packaging to final use by the consumer is considered the shelf-life of a product. The shelf-life is important for the meat industry and for consumers alike

(McMillin, 2008). The major influencers of shelf-life are very similar to the factors that influence meat color, which is why the two work synonymously. The factors include the initial product, gas mixture, packaging and temperature (McMillin, 2008). Display life occurs once the product is placed in the retail case for consumer purchase. As shelf-life and display life increase the color decreases through the mechanisms mentioned previously (Holman et al., 2017). With the need for transportation to retail stores across the country, meat shelf-life becomes an important factor to ensure the consumer receives a safe high quality product.

Case Ready Packaging

Beginning in the 2000's the need for a centralized packaging system became evident (McMillin, 2008). In the past, primals would be sent to the retail store in a vacuum package bag and then be portioned in-house. During this time, meat was displayed in polyvinyl chloride film wrapped over oxygen permeable trays (McMillin, 2008). Consumers began to become accustomed to the bright cherry-red color of meat from this packaging technique. After a shortage in skilled labor, an increase in competition between retail chains and the need for longer shelf times packaging shifted (Cole Jr. 1986). Individual cutting at retail stores moved to case-ready operations. Case-ready can be defined as the fabrication and packaging in a non-retail facility for display at retail with little to no manipulation of individual packaging (McMillin, 2008). Case ready packaging can be found in 60% of the meat case (Belcher, 2006). Centralized technology has many advantages including efficiency and increased food safety (Jeyamkondan et al., 2000). By using machinery to package in master bags there is limited human handling which can reduce the threat of some microbial contamination and increase the safety of the meat packaged (Jeyamkondan et al., 2000). In regards to improved efficiency when the primal does not have to be sent to the retailer with all the external fat and bone intact, it reduces the waste at cutting and allows for higher yields (Jeyamkondan et al., 2000).

Modified Atmosphere Packaging

An example of these case-ready technologies developed to prolong the shelf-life of fresh meats and to meet new consumer demands is Modified Atmosphere Packaging (MAP). Modified atmosphere packaging works to remove or replace the gaseous environment surrounding a product before sealing the package (McMillin, 2008). Some examples of MAP are vacuum packaging, controlled atmosphere packaging (CAP) and active MAP (McMillin, 2008). Under vacuum packaging in an anerobic environment meat stays in the DeoxyMb state revealing a purple color. While this method is very advantageous for delaying microbial spoilage, most consumers prefer the appearance of bright cherry-red oxygenated beef at retail (John et al., 2005). With consumers preferring a tray over-wrapped with polyvinyl chloride (PVC), another packaging technology was implemented. Master bag packaging is the current industry norm. Master pack is when trays are packaged in traditional PVC and then placed in a larger master bag with multiple other trays. Once the master bag reaches the retail store, employees simply remove the trays and place them on display with minimal labor. The low oxygen MAP in the master bags allows other gases to bind to the myoglobin and reduce oxidation until removal from the package. Thus, the color-stability is prolonged in master bag MAP (Limbo et al., 2013).

The 3 main gases used in MAP are carbon dioxide, nitrogen and carbon monoxide (Arvanitoyannis, 2012). Carbon Dioxide is utilized as a fungistatic and bacteriostatic. The solubility of carbon dioxide increases with an increase in temperature, therefore in lower temperatures carbon dioxide is a more effective antimicrobial (Arvanitoyannis, 2012). Nitrogen's function in MAP is to prevent bag collapse since it has low solubility in water (Arvanitoyannis, 2012). Carbon monoxide has been used since approval in CO-MAP as it forms a stable carboxymyoglobin. However, it poses a threat during the packaging process as it is highly flammable and toxic (Arvanitoyannis, 2012).

High Oxygen MAP

Beginning in the early 2000's the most common form of MAP with fresh meat was high oxygen MAP (Eilert, 2005). High oxygen environments can saturate the environment and cause an increase in prolonged red color (Suman and Joseph, 2013). With the use of high oxygen MAP Top Sirloin steaks had a bright cherry-red color throughout the 14 day study as opposed to a typical 3-7 d in PVC packaging (Cornforth, 2008). However, there are some major concerns with high oxygen packaging. One of the most common is increased lipid oxidation and rancid flavors (Ozlem Kizilirmak Esmer, 2010). While the rancid flavors are an obvious concern, lipid oxidation can also lead to color instability as oxidation releases aldehydes that lead to Mb oxidation (Suman and Joseph, 2013). Additionally, a food safety concern posed by high oxygen MAP is premature browning (John et al., 2005). High oxygen MAP ground beef can appear fully cooked, or fully brown, at temperature far below the safe internal temperature (Cornforth, 2008). With these concerns in mind, the industry shifted to a low-oxygen MAP alternative.

Low oxygen MAP

Another MAP application is an aerobic technology with the use of carbon dioxide, nitrogen and carbon monoxide (CO-MAP). Carbon monoxide MAP is effective in stabilizing color as the CO binds to the myoglobin and forms COMb (Sørheim et al., 1999). In 2002, the FDA approved the use of 0.4% carbon monoxide in MAP systems (John et al., 2005). With this level of CO shelf-life can be increased to 21 d rather than the 14 d of high oxygen MAP (Suman and Joseph, 2013). Additionally, CO-MAP has been shown to correct the oxidation issue present in a high oxygen system. Moreover, another issue resolved by excluding oxygen is premature browning. Even so, one of the major disadvantages to CO-MAP is persistent pinking, or masking spoilage due to enhanced color stability (Cornforth, 2008).

Most MAP gas flushes include 30% carbon dioxide, 0.4% carbon monoxide and balanced with nitrogen (Arvanitoyannis, 2012). Carbon dioxide is included at levels between 20-30% because at levels over 40% the master has a higher tendency to collapse as the meat will absorb much of the carbon dioxide. In addition, carbon dioxide at levels lower than 15% does not have a satisfactory reduction in microbial growth posing a food safety concern (McMillin, 2008). Moreover, the addition of higher carbon dioxide levels led to more deflation than 20% (Arteaga Custode et al., 2017). Carbon monoxide is included at a limit of 0.4% as it was set by the FDA due to the health concerns of the employees handling the products (Suman and Joseph, 2013). Also, carbon monoxide used at 2% lead consumers to believe the color was artificially and not acceptable for purchase (Sørheim et al., 1999). Finally, the level of nitrogen is solely used to balance the mixture and to create an anaerobic environment by displacing oxygen. This in turn, reduces microbial growth and allows for proper fill in the master bag (Arvanitoyannis, 2012).

Oxygen Scavenger Technology

Even with low-oxygen packaging, there is still a need for further intervention to ensure residual oxygen stays low. Oxygen absorbers are the key to ensuring dissolved oxygen is removed throughout display (Cichello, 2015). The reason removing oxygen is so important in MAP is to prevent browning of meat. Residual oxygen levels must be less than 0.15% to prevent the formation of MetMb (Mancini and Hunt, 2005). Oxygen scavengers work by reacting with oxygen through the oxidation of iron. This iron is usually stored in a sachet and added to the master bag directly (Cichello, 2015). Ferrous iron oxide used in most scavengers become active when exposed to oxygen and begin absorbing the residual oxygen (Cichello, 2015). The scavengers should be able to remove 1-1.5% per hour in order to reduce the oxygen by 45 min to ensure re-bloom after re-exposure to oxygen (McMillin, 2008). The potential to re-bloom is increased when an oxygen scavenger is used. In low-oxygen MAP without sachet scavengers, steaks did not bloom as well or remain as color stable for 42 d compared to master bags with scavengers (Beggan, 2005). Within a few hours of packaging the oxygen scavengers should rapidly drop the residual oxygen and continue to keep oxygen reduced for up to 21 d in storage (Arteaga Custode et al., 2017). The use of an oxygen scavenger helps maintain the Mb in the DeoxyMb state during storage and allows the product to convert to OxyMb when reaching retail (Arteaga Custode et al., 2017). Furthermore, the a* values of meat were higher (redder) than those master bags without scavengers (Arteaga Custode et al., 2017). This improvement becomes apparent between 3-7 d of storage, allowing the product to be held longer in a color stable state (Beggan, 2006). Steaks that were packaged with oxygen scavengers and had a reduction in oxygen consumption during storage had a deeper level of oxymyoglobin upon exposure to oxygen than those without (Isdell, 2003). This is important in display life because MetMb starts from the center of meat and rises to the surface (Mancini and Hunt, 2005).

A potential disadvantage of oxygen scavengers is the need for oxygen to free-flow around the sachet for the iron to bind. Without carbon dioxide being generated, there may be bag collapse when oxygen is absorbed (McMillin, 2008). In addition to the iron scavengers absorbing oxygen, the scavengers will absorb some of the carbon dioxide as well. Moreover, increasing the amount of time in a master pack also increases the amount of carbon dioxide absorbed and could result in bag collapse as well (Arteaga Custode et al., 2017).

COVID-19 Impact

With the COVID-19 pandemic, ethanol production rapidly decreased with fewer people driving and demanding fuel (Bandoim, 2020). With a decrease in ethanol production, byproducts including carbon dioxide also decreased in availability (Bandoim, 2020). About 30% of all the carbon dioxide in the United States comes as a by-product of ethanol production (NAMI, 2020). Moreover, due to the transportation costs of the carbon dioxide a higher proportion of the carbon dioxide used in the Midwest (60-65%) is from ethanol production (NAMI, 2020). Of the carbon dioxide produced 98% of it is food grade, so there is not a large need to separate food grade from other carbon dioxide (NAMI, 2020). Reports have found carbon dioxide has dropped 20% since 2020 and without intervention could continue to a 50% decrease (Bandoim, 2020). In April of

2020, NAMI wrote a letter to vice-president Mike Pence asking for intervention in order to combat the CO_2 shortage (Bandoim, 2020). The coalition that wrote the letter includes the National Pork Board, North American Meat Institute and the National Cattlemen's Beef Association. In addition to many professional organizations recognizing the need for a reduction in carbon dioxide use, retail companies have also reached out to research universities to inquire about potential quality issues if packaging technologies used reduced levels of carbon dioxide in MAP.

After a year of the pandemic, the carbon dioxide shortage did not reach the low that was predicted, but the industry is still feeling the effects (Southard, 2021). Even without government intervention the meat industry will still have to monitor the carbon dioxide supply to ensure no detrimental consequences effect meat distribution (Southard, 2021).

Conclusions

Since meat color is so instrumental in consumer purchasing, preserving the acceptable bright cherry-red color is essential in meat packaging. Through the use of packaging technologies such as MAP and oxygen scavengers, an environment can be created to prolong the shelf-life of fresh meat. By using color stable muscles such as the longissimus thoracic, companies can also increase the length of time with acceptable color. However, with the impact of COVID-19, the amount of carbon dioxide used in MAP packaging needs to be considered. Therefore, the objective of this study was to investigate the effect of varying carbon dioxide levels on the color stability of ribeye steaks in a traditional low-oxygen MAP with oxygen scavengers.

CHAPTER III

EFFECTS OF DIFFERING CARBON DIOXIDE LEVELS ON MEAT COLOR IN MODIFIED ATMOSPHERE PACKAING

ABSTRACT

The objective of this study was to assess the effect of different carbon dioxide (CO_2) concentration gas flushes in master packages on the display life of fresh beef. Twenty-five USDA Low Choice ribeye rolls (IMPS 112A) from carcasses in program G-87B (USDA-AMS; live specification – Angus) were collected from Creekstone Farms in Arkansas City, KS. Each sub-primal was wet aged for 12 d. After aging, each sub-primal was opened and over sprayed with a SYNTRx 3300 solution. Ribeye rolls were sliced 2.54 cm thick and placed onto foam trays (n = 108) with an absorbent pad and over-wrapped with perforated polyolefin film. Trays were randomly assigned to either a 10%, 20% or 30% CO_2 concentration master bag (n = 27 master bags; n = 9/CO_2 concentration). Three master bags, containing 4 trays, for each CO_2 concentration (10%, 20% and 30%) were randomly selected for opening after 10, 15, 18 d of dark storage, and the trays were placed in a display case for retail display evaluation for 5 d. Headspace analysis was conducted before master bags were opened, and objective color measurements were collected on d 0 - 5 of retail display. Muscle color, surface

discoloration and overall acceptability were analyzed daily by a trained panel (n = 6). The results of this study showed steaks from the 10% CO_2 concentration darkened and discolored more (P < 0.05) rapidly than steaks from either the 20 or 30% CO_2 concentrations. Additionally, steaks from master bags flushed with the 10% CO_2 concentration were the only steaks in display to reach unacceptable levels and discrimination by consumers. Steaks from master bags flushed with 20 or 30% CO_2 concentration performed comparably (P > 0.05) with minimal differences. The 20% CO_2 concentration master bag flush outperformed the 10% CO_2 concentration in all aspects of the study, as well as surpassed or equaled the 30% CO_2 concentration. Thus, it is recommended 20% CO_2 concentration gas flush of master bags could be utilized by the industry to reduce CO_2 use.

INTRODUCTION

The most important factor influencing consumers purchasing decision of meat is color (Mancini and Hunt, 2005). Consumers associate a bright cherry-red color in beef with wholesomeness and freshness (Suman and Joseph, 2013). As time on display increases the bright cherry red color will diminish and discoloration (MetMb) increases (Mancini and Hunt, 2005). Metmyoglobin formation and discoloration depends on many exogenous factors including muscle, temperature and oxygen partial pressure (Mancini and Hunt, 2005). Reducing the residual oxygen levels in packages can help extend shelf-life and limit discoloration. Residual oxygen levels must be less than 0.15% to prevent the formation of MetMb (Mancini and Hunt, 2005).

Modified atmosphere packaging (MAP) works to remove or replace the gaseous environment surrounding a product before sealing the package (McMillin, 2008). An example of MAP is an aerobic technology with the use of carbon dioxide, nitrogen and carbon monoxide

(CO-MAP). Tri-gas CO-MAP is effective in stabilizing color as the CO binds to the myoglobin and forms COMb (Sørheim et al., 1999). Additionally, Oxygen absorbers (better known as scavengers) can be utilized to ensure dissolved oxygen is removed throughout storage (Cichello, 2015). Oxygen scavengers work by reacting with oxygen through the oxidation of iron. This iron is usually stored in a sachet and added to the master bag directly (Cichello, 2015). Within a few hours of packaging the oxygen scavengers should rapidly drop the residual oxygen and continue to keep it reduced for up to 21 d in storage (Arteaga Custode et al., 2017).

With the COVID-19 pandemic, ethanol production rapidly decreased with fewer people driving and demanding fuel; thus, ethanol byproducts also decreased in availability including carbon dioxide needed for MAP (Bandoim, 2020). Therefore, the objective of this study was to investigate the effect of varying carbon dioxide levels on the color stability of ribeye steaks in low-oxygen, tri-gas master packages with oxygen scavengers.

MATERIALS AND METHODS

Product Collection

Twenty-five USDA Low Choice ribeye rolls (IMPS 112A) from carcasses in program G-87B (USDA-AMS; live specification – Angus) were collected from Creekstone Farms in Arkansas City, KS with the packaging date established as d 0. 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, each sub-primal was wet aged for 12 d at an average of 0.05°C ± 1.0°C. After aging, each sub-primal was opened and over sprayed with a SYNTRx 3300 solution (Synergy Technologies, Inc., Shreveport, LA, USA) at a 2% dilution. After 1 min drying time, the ribeye rolls were sliced using a meat slicer (Bizerba USA INC., Piscataway, NJ) at 2.54 cm thick starting at the posterior end discarding the face steak. Once sliced, the steaks were placed in pairs of adjacent steaks (n = 216 steaks; 108 pairs) onto foam trays (n = 108 trays; Pactiv 63P906SP1- 9.75 x 7.625 x .95, Pactiv advanced packaging solutions, Lake Forest, IL, USA) with an absorbent pad and over-wrapped with perforated polyolefin film (PVC; Berry Omnifilm, Berry Global, Evansville, IN, USA). After PVC packaging, the steak pairs on trays were randomly assigned either a 30% (Airgas NI FGCD30CM415A; 0.42%CO, 30.01%CO_2, balance N_2), 20% (Airgas NI FGCD20CM415A; 0.37%CO, 19.97%CO_2, balance N_2) or 10% (Airgas NI FGCD10CM415A; 0.39%CO, 9.997%CO_2, balance N_2) carbon dioxide concentration master bag (n = 27 master bags; n = 9/CO_2 concentration) flushed with M-Tek CORR-VAC® MARK-III (M-Tek Corporation, Elgin, IL, USA). The trays were packaged four per master bag with one scavenger (Multisorb FreshPax® CR). Master bags (Bemis, Master beef z-0300, 22.75 in x 29 in) were held in dark storage in Meat 65126 totes (Tosca®, Atlanta, GA, USA) at 0.05°C ± 1.0°C until their respective pull d (10, 15, or 18 d) for retail display.

Headspace Analysis

Three additional master bags per CO_2 concentration were packaged and assigned to headspace analysis. O_2, CO_2 and CO were evaluated at 0, 6, 24, 30, and 48 h using a Mocon PAC CHECK® Model 333 Triple Gas Analyzer (Minneapolis, MN, USA). Additionally, on each pull day, each master bag was analyzed, and the measurements were recorded.

Retail Display

On each pull day (10,15, and 18) 9 master bags per CO_2 concentration were removed from dark storage. One tray per master bag was randomly selected for initial microbial growth. The remaining packages were used for retail display and given a random number to ensure traceability to master bag. The product was then photographed at 0 and 60 min after opening. Each sample was placed in a Hussmann IM1SL retail case set at 2.0°C ± 2.0°C. All samples were displayed in retail cases lit with Philips LED T8 Lamps (model number 9290011240B-453597) manufactured in Niles, OH. Both retail lights and ceiling lights in the retail room, remained on for 24 h/d throughout the study. Each day during the retail display, visual and instrumental color measurements were recorded, photographs were taken once daily, and packages were rotated to minimize case variation. At the end of display, one tray per master bag was randomly selected and evaluated for final microbial growth.

Visual Color Analysis

Visual muscle color, surface discoloration and overall acceptability were evaluated by a six-member trained panel once daily. Panelists were trained using base pictures for reference on muscle color, percent discoloration and acceptability. Muscle color was evaluated on a scale of 1 to 8, with 1 being extremely bright cherry red and 8 being extremely dark 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%). Overall acceptability was determined on a 3-point scale, with 1 being acceptable and 3 being unacceptable.

Instrumental Color Analysis

Instrumental color was measured quantitatively with a portable, reflected-color measurement spectrophotometer. Measurements were taken on day 0 of display at 0 and 60 min as well as every day throughout retail display. Three readings of each package were taken each time at random locations with the HunterLab MiniScan® EZ 4500L (2.5-cm aperture, illuminant A, and 10° standard observer angle; Reston, VA, USA), and readings were averaged. Lightness, white to black is measured by L*, the higher the value, the lighter (or whiter) the product. A positive a* value represents red color, and a negative value represents a green color. A positive b* value measures yellow and a negative value represents blue.

Microbiology

Total Aerobic Plate Count (TPC) was obtained from a composite sample across both steaks from one tray for each concentration at the beginning and end of retail display time at Oklahoma State University. For TPC analysis, 10 g samples from each product and treatment were homogenized in a sterile stomacher bag containing 90 mL of sterile 0.1% peptone water and pummeled for 30 seconds at 230 rpm using a Stomacher-400. Total plate counts were determined by plating 1 mL of the sample homogenate on 3M™ Petrifilm™ Aerobic Count Plate (St. Paul, MN, USA) with the respective decimal dilutions. Plates were incubated for 48 h at 37°C before counting and reporting the TPC per cm^2. Plates were counted according to the 3M™ Petrifilm™ Aerobic Count Plate Interpretation Guide.

Statistical Analysis

A completely randomized design was used to evaluate the effects of an CO_2 concentration in a master bag packaging system in extended dark storage on retail color of beef. The experimental unit was the master bag (*n* = 27) which were randomly assigned to various CO_2

concentrations. Simple means were calculated for headspace analysis. All other data were analyzed using PROC GLIMMIX of SAS 9.4 (SAS Institute Inc., Cary, North Carolina), where main effects were pull day, retail day and CO_2 concentration 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

Oxygen Depletion/Headspace Analysis

Residual oxygen in master bags initially increased from h 0 to 6 for the 20 and 30% CO_2 concentrations while 10% saw a slight decrease (Figure 1). According to McMillin, a scavenger should be able to reduce oxygen by 1-1.5% per hour (McMillin, 2008). From h 6 to 24, master bags from all three CO_2 concentrations decreased dramatically in percentage O_2 (Figure 1). Further, from h 24 to 48 master bags from all three CO_2 concentrations decreased in percentage O_2 until the 20 and 30% reached 0.0% residual oxygen (Figure 1). The master bags flushed with 10% CO_2 contained more O_2 over the entire 48 h period and never decreased below the goal of less than 0.15% residual oxygen (Figure 1). It is important to reduce O_2 in master bags to delay discoloration caused by MetMb formation (Mancini and Hunt, 2005). Means of headspace gases in the master bags (including leakers) used for retail display are presented in Table 1. The 20 and 30% CO_2 concentration master bags maintained levels below 0.05% O_2 until pull d 15, while the 10% CO_2 concentration bags on average never reached O_2 below 0.05% for any pull day. Oxygen scavengers should hold residual oxygen below 0.05% in master packages up to 21 d in dark storage (Arteaga Custode et al., 2017). The master bag flushed with 10% CO_2 concentration had a leaker each pull day and bags with 20% CO_2 had one leaker on pull d 15 while the bags with 30% CO_2 concentration had no leakers for the duration of the study. The elevated residual oxygen level in the bags flushed with 10% CO_2 could result in an increase in MetMb formation (Jakobsen, 2002). The limited CO_2 in the master bags flushed with the 10% concentration could have led to bag collapse and account for the higher tendency of leakers.

Muscle Color

There was a significant pull day × retail day interaction ($P < 0.05$). For every retail day, muscle color of steaks increased from extremely bright cherry-red to moderately dark red for all CO_2 concentrations, as expected (Table 2). Steaks that discolor more than consumer acceptability levels will be discounted or discriminated (Smith, 2000). Moreover on pull d 10, all steaks started with a more desirable ($P < 0.05$) muscle score than pull d 15 or 18 until retail d 3 when the scores were consistent ($P > 0.05$) for all pull d (Table 2). Ribeiro et al. (2016) found over a 72 h period desirable red color of steaks remained following dark storage, which is consistent with our results. On pull d 10, muscle color of steaks increased ($P < 0.05$) from retail d 0 to 3 and then plateaued with similar ($P > 0.05$) muscle scores display d 3 to 5 (Table 2). Following a similar trend, on pull d 15, muscle color increased ($P < 0.05$) from retail d 0 to 2 then steadied until retail d 5 where the highest scores ($P < 0.05$) were recorded (Table 2). Previous research has noted over time in retail display muscle color decreased (Isdell, 1999; Harlan, 2011; Kinsey, 2011; Perry, 2018).

Additionally, the interaction for retail day × CO_2 concentration was significant ($P < 0.05$). For the 10% CO_2 concentration, muscle color darkened ($P < 0.05$) between retail d 0 and 1 and again between retail d 2 and 3; however, muscle color was similar ($P > 0.05$) on retail d

3,4 and 5 (Table 3). In the 30% CO_2 concentration muscle color increased ($P < 0.05$) from retail d 0 to 1, from retail d 2 to 3 and from 4 to 5 (Table 3). Steaks from master bags with 20% concentration were brightest ($P < 0.05$) on d 0, then became darker ($P < 0.05$) on d 1 and even darker ($P < 0.05$) on d 2 where they remained similar on d 3 but darker ($P < 0.05$) on d 4 and 5. In the 30% CO_2 concentration muscle color increased ($P < 0.05$) from retail day 0 to 1, from retail d 2 to 3 and 4 was darker on d 5 compared to d 3 (Table 3). Similarly, Stetzer et al. (2006) found steaks packaged in modified atmosphere packages with CO had high redness scores until 14 d of storage. As retail display increases at each storage day, muscle color scores decrease and the bright cherry red color diminishes (Harlan, 2011). On retail d 0,1 and 3, the steaks from master bags in 20% CO_2 concentration had a more ($P < 0.05$) desirable muscle color than the steaks packaged in 10% CO_2 concentration master bags (Table 3). The 20% CO_2 concentration maintained acceptable color longer ($P < 0.05$) than the 10% CO_2 concentration, having a brighter cherry red color until retail d 4 (Table 3). Steaks from master bags flushed with 30% CO_2 concentration had similar muscle color ($P > 0.05$) scores to both the 10 and 20% CO_2 concentration steaks for all retail d.

Surface Discoloration

There was a significant ($P < 0.05$) three-way interaction of pull day × retail day × CO_2 concentration shown in Table 4. Discoloration of steaks occurs from extended exposure to residual oxygen and as a result the formation of MetMb occurs giving meat a brown appearance (Suman and Joseph, 2013). Consumers will discount a product at 20% discoloration and completely reject the product when discoloration reaches 40% (McMillin, 2008). Only steaks from the 10% CO_2 concentration on pull d 18 retail d 5 reached 20% discoloration ($P < 0.05$), and thus would have been discounted. Discoloration and MetMb formation can occur on a steaks surface within 7 d of cutting (Madhavi and Carpenter, 1993). For pull d 10, steaks from the master bags flushed with 10% CO_2 exhibited more ($P < 0.05$) discoloration on retail d 4 and 5 than the steaks flushed with 20 or 30% CO_2. Discoloration was similar ($P > 0.05$) for all CO_2 concentrations on pull d 15 for all retail d. On pull d 18 steaks from master bags flushed with 10% CO_2 concentration discolored ($P < 0.05$) more than the steaks from bags flushed with 20% CO_2 concentration. Steaks from master bags flushed with 20 and 30% CO_2 concentration had similar ($P > 0.05$) discoloration for the duration of the study. The percentage of discoloration visible increased ($P < 0.05$) with time in dark storage and retail display. Results from this study are in agreement with researchers who noted steaks from master bags with higher residual oxygen levels had more discoloration, as residual oxygen increases more MetMb (discoloration) is formed (Mancini and Hunt, 2005). The steaks flushed with 10% CO_2 had a higher tendency for leakers, which could be the main cause of increased surface discoloration. Moreover, Hunt et al. (2004) found an increase in storage time of steaks resulted in an increase in percentages of discoloration, which was similar to results from the present study. Without the use of oxygen scavengers levels of residual oxygen remain higher in master packages and percentages of irreversible discoloration also increase (Venturini et al., 2006); thus, master packages in this study, as well as virtually all steaks packed in master bags in the marketplace contain scavenger.

Overall Acceptability

There was a significant ($P < 0.05$) three-way interaction of pull day × retail day × CO_2 concentration shown in Table 5. Consumers prefer a bright cherry red product with no discoloration; therefore, visual appearance is the primary limiting factor in purchasing decisions (Smith, 2000). Modified atmosphere packaging has been shown to increase overall acceptability and prolong this acceptability for more d of steaks in retail display (Kinsey, 2011). For the pull d

10, steaks from the 20 and 30% CO_2 concentration master bags had similar ($P > 0.05$) acceptability throughout retail display; however, steaks from master bags flushed with 10% CO_2 reached lower acceptability levels ($P < 0.05$) on retail d 5. For pull d 15, steaks from master bags flushed with all CO_2 concentrations were similar ($P > 0.05$) for the duration of the retail display. For pull d 18, the steaks packaged with 10% CO_2 were less acceptable ($P < 0.05$) from retail d 0 to 4 compared to the steaks from master bags with 20 and 30% CO_2 concentrations. Steaks in bags flushed with 20 and 30% CO_2 maintained similar ($P > 0.05$) levels of acceptability for the entirety of the study. Muscle color and discoloration percentages predict overall acceptability. Studies showing steaks with higher muscle scores and discoloration percentages leads to decreased acceptability parallels the findings of this study (Isdell, 2003; Smith, 2000).

L* values

There was a significant interaction ($P < 0.05$) of pull day × retail day for L* values. Lightness decreased ($P < 0.05$) over display time for steaks from all CO_2 concentration master bags (Table 6). Steaks from pull d 18 retail d 0 had the lightest ($P < 0.05$) color and pull d 10 retail day 4 had the darkest (Table 6). Steaks pulled from dark storage d 10 remained the lightest ($P < 0.05$) during display and did not decrease in lightness until retail d 2; while steaks pulled on d15 and 18 both saw decreases ($P < 0.05$) from retail d 0 to 1 (Table 6). Perry (2018) also observed a decrease in lightness values (L*) of steaks over time in dark storage and then retail display. Additionally, Jayasingh et al. (2001) observed over time a drop in L* values from steaks packaged in modified atmosphere packaging with CO.

a* values

The was a significant interaction (P < 0.05) of pull day × retail day. The effect of CO_2 concentration (P < 0.05) is not shown in tabular form but can be summarized as the steaks from packages with 20% CO_2 concentration having higher (P < 0.05) a* values than both 10 and 30% concentrations, however a* values from steaks in master bags flushed with 10 and 30% CO_2 were not significantly (P > 0.05) different from one another. Hunt et al. (2004) reported that a decrease in a* values will occur during display of steaks packaged in modified atmosphere packaging with CO.

Steaks flushed from all three CO_2 concentrations held in dark storage longer and on display longer had lower (P < 0.05) the a* values were resulting in a less red appearance (Table 7). After extended exposure to oxygen, regardless of CO_2 concentration, other researchers found steaks had lower a* values (Isdell, 1999; Perry, 2018). Steaks in dark storage for 15 d maintained redness scores the longest (P < 0.05) without a decrease until retail d 4. Other researchers also showed the a* of steaks packaged in modified atmospheres remained constant in storage for up to 15 d (Lagerstedt et al., 2011). However, steaks in dark storage the longest (18 d) decreased in a* value the most rapidly (P < 0.05) with a decrease on retail d 1 (Table 7). Additionally, steaks from pull d 10 had the most correction of redness through MetMb reducing activity as shown by an increase (P < 0.05) in redness from retail d 3 to 4 after a decrease from retail d 1 to 2 (Table 7). The longissimus dorsi muscle is considered a color stable muscle and has the ability to reduce MetMb (McKenna et al., 2005). Additionally, meat typically decreases in a* by retail display 4 (Arteaga Custode et al., 2017). Holman et al. (2017) reported that consumers set 14.5 as the level of acceptability for a* values of steaks; thus, all steaks displayed in this study regardless of CO_2 concentration, dark storage time or retail display time maintained acceptable a* values.

Total Plate Count

Bacteria counts were too few to count at the first dilution in all treatments; well below any indicators of bacterial spoilage and therefore it not shown in tabular form. SYNTRx 3300 has been shown to reduce bacteria 0.3 to 0.5 log CFU/cm^2 (Geornaras et al., 2012). Steaks fabricated from whole muscle should have sterile surface; thus, proper sanitation of equipment, including slicers, packagers and knives, along with proper handling should avoid introduction of bacteria to the surface (McSharry et al., 2021).

CONCLUSION

The most important factor affecting consumer meat purchasing decisions is color. Therefore, color and maintaining acceptable color is of utmost importance in the retail sector of the meat industry. The use of MAP technologies can be used to improve meat color. As shown in this study, different levels of carbon dioxide in a tri-gas MAP and the use of oxygen scavengers can extend muscle color brightness, reduce or prevent surface discoloration, as well as improve acceptability and objective color measures. The 20% CO_2 concentration master bag flush outperformed the 10% CO_2 concentration in all aspects of the study, as well as surpassed or equaled the 30% CO_2 concentration. This knowledge allows case ready facilities to potentially decrease the carbon dioxide needed and help to combat the national shortage of CO_2 due to COVID-19. Additionally, the food safety interventions outlined in this study provided a safe product for the entirety of the study. Further research areas include various products with less color stability including ground beef or steaks with color liable muscles.

Table 1. Means of headspace[1] gas concentration readings of oxygen, carbon dioxide and carbon monoxide in master bags[2] containing four overwrapped packages of ribeye steaks in dark storage for 10, 15, or 18 d (n = 27 master bags).

		Gas		
Pull Day	Concentration	O_2	CO_2	CO
	10%	6.50*	7.05	0.20
10	20%	0.00	20.05	0.35
	30%	0.01	28.49	0.37
	10%	5.17*	6.75	0.22
15	20%	6.41*	14.63	0.24
	30%	0.51	26.85	0.33
	10%	5.32*	9.78	0.24
18	20%	0.43	21.27	0.31
	30%	0.57	29.20	0.32

[1] Averages of the three bags pulled
[2] 10% mixture (0.39%CO, 9.997%CO_2, balance N_2); 20% mixture (0.37%CO, 19.97%CO_2, balance N_2) or 30% mixture (0.42%CO, 30.01%CO_2, balance N_2)
*Four leakers were identified

Table 2. Least square means for muscle color[1] for 5 d in retail display from 3 pull d (10, 15, or 18 d in dark storage) of ribeye steaks (*n* = 27 master bags; 81 packages in display) from master bags[2] with 10%, 20% or 30% CO_2 concentration.

Pull day	0	1	2	3	4	5
10	2.33[i]	3.21[h]	3.65[g]	4.49[cd]	4.65[bc]	4.59[bc]
15	3.77[fg]	4.21[de]	4.51[c]	4.72[bc]	4.90[ab]	5.23[a]
18	3.89[efg]	4.02[ef]	4.42[cd]	4.45[cd]	4.59[bc]	4.89[ab]
SEM[3]	0.12	0.12	0.12	0.12	0.12	0.12

[1] Muscle Color: 1 = extremely bright cherry red; 4 = slightly bright cherry red; 8 = extremely dark red

[2] 10% mixture (0.39%CO, 9.997%CO_2, balance N_2); 20% mixture (0.37%CO, 19.97%CO_2, balance N_2) or 30% mixture (0.42%CO, 30.01%CO_2, balance N_2)

[3]SEM = Standard error of the mean

[a-i] Least square means that do not share a common subscript are significantly different ($P < 0.05$)

Table 3. Least square means for muscle color[1] from 3 CO_2 concentrations (10, 20 and 30%) in master bags[2] of ribeye steaks displayed for 5 d (*n* = 27 master bags; 81 packages in display) for all pull d (10, 15 and 18 d in dark storage).

CO_2 concentration	0	1	2	3	4	5
10%	3.54^{gh}	4.11^{ef}	4.41^{cde}	4.73^{ab}	4.90^{ab}	5.03^{a}
20%	3.12^{i}	3.55^{gh}	4.10^{ef}	4.36^{de}	4.55^{bcd}	4.72^{abc}
30%	3.33^{hi}	3.78^{fg}	4.07^{ef}	4.57^{bcd}	4.70^{abcd}	4.96^{a}
SEM[3]	0.12	0.12	0.12	0.12	0.12	0.12

[1] Muscle Color: 1 = extremely bright cherry red;4 = slightly bright cherry red; 8= extremely dark red

[2] 10% mixture (0.39%CO, 9.997%CO_2, balance N_2); 20% mixture (0.37%CO, 19.97%CO_2, balance N_2) or 30% mixture (0.42%CO, 30.01%CO_2, balance N_2)

[3]SEM = Standard error of the mean

[a-i] 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] of ribeye steaks displayed for 5 d from three pull d (10, 15, or 18 d in dark storage) of master bags[2] flushed with 10,20, or 30% CO_2 concentration (n = 27 master bags; 81 packages in display).

		Retail day					
Pull Day	CO^2	0	1	2	3	4	5
10	10%	1.00^{m}	1.04^{m}	1.24^{ijklm}	$1.76^{defghijklm}$	$1.87^{cdefghijkl}$	2.35^{abcde}
	20%	1.00^{m}	1.00^{m}	1.00^{m}	1.02^{m}	1.11^{klm}	$1.35^{ghijklm}$
	30%	1.00^{m}	1.00^{m}	1.00^{m}	1.19^{jklm}	1.11^{klm}	$1.31^{ghijklm}$
15	10%	$1.31^{ghijklm}$	$1.57^{efghijklm}$	$1.46^{fghijklm}$	$1.91^{cdefghijk}$	$2.06^{bcdefghi}$	2.35^{abcde}
	20%	$1.41^{ghijklm}$	$1.59^{efghijklm}$	$1.65^{efghijklm}$	$1.91^{cdefghijk}$	$2.02^{bcdefghij}$	2.48^{abcd}
	30%	1.11^{klm}	$1.41^{ghijklm}$	1.28^{hijklm}	$1.68^{defghijklm}$	$1.94^{cdefghijk}$	2.33^{abcde}
18	10%	2.15^{bcdefg}	2.26^{bcdef}	2.35^{abcde}	2.50^{abcd}	2.83^{ab}	3.09^{a}
	20%	1.02^{m}	1.04^{lm}	1.15^{klm}	$1.39^{ghijklm}$	$1.56^{efghijklm}$	2.15^{bcdefg}
	30%	1.15^{klm}	1.24^{ijklm}	$1.54^{efghijklm}$	$1.78^{defghijklm}$	$2.09^{bcdefgh}$	2.70^{abc}
SEM[3]		0.30	0.30	0.30	0.30	0.30	0.30

[1] Surface Discoloration: 1 = no discoloration (0%); 3 = small discoloration (21-40%); 6 = extensive discoloration (81-100%)

[2] 10% mixture (0.39%CO, 9.997%CO_2, balance N_2); 20% mixture (0.37%CO, 19.97%CO_2, balance N_2) or 30% mixture (0.42%CO, 30.01%CO_2, balance N_2)

[3]SEM = Standard error of the mean

[a-m] Least square means that do not share a common subscript are significantly different (P < 0.05)

Table 5. Least square means for overall acceptability[1] of ribeye steaks displayed for 5 d from three pull d (10, 15, or 18 d in dark storage) of master bags[2] flushed with 10, 20, or 30% CO_2 concentration (n = 27 master bags; 81 packages in display).

		Retail day					
Pull Day	CO_2	0	1	2	3	4	5
10	10%	1.04^{jk}	1.15^{fghijk}	1.19^{efghijk}	1.37^{bcdefghijk}	1.50^{abcdefgh}	1.69^{abc}
	20%	1.00^{k}	1.00^{k}	1.04^{jk}	1.00^{k}	1.07^{hijk}	1.06^{ijk}
	30%	1.00^{k}	1.00^{k}	1.02^{jk}	1.02^{jk}	1.00^{k}	1.06^{ijk}
15	10%	1.06^{ijk}	1.17^{efghijk}	1.15^{fghijk}	1.22^{efghijk}	1.30^{cdefghijk}	1.74^{ab}
	20%	1.15^{fghijk}	1.24^{defghijk}	1.30^{cdefghijk}	1.41^{abcdefghijk}	1.44^{abcdefghij}	1.59^{abcde}
	30%	1.02^{jk}	1.15^{fghijk}	1.09^{hijk}	1.19^{efghijk}	1.26^{cdefghijk}	1.57^{abcdef}
18	10%	1.48^{abcdefghi}	1.54^{abcdefg}	1.67^{abcd}	1.67^{abcd}	1.69^{abc}	1.74^{ab}
	20%	1.00^{k}	1.00^{k}	1.00^{k}	1.06^{ijk}	1.11^{ghijk}	1.41^{abcdefghijk}
	30%	1.00^{k}	1.04^{jk}	1.07^{hijk}	1.13^{ghijk}	1.30^{cdefghijk}	1.81^{a}
SEM[3]		0.15	0.15	0.15	0.15	0.15	0.15

[1] Overall acceptability: 1 = acceptable; 3 = unacceptable
[2] 10% mixture (0.39%CO, 9.997%CO_2, balance N_2); 20% mixture (0.37%CO, 19.97%CO_2, balance N_2) or 30% mixture (0.42%CO, 30.01%CO_2, balance N_2)
[3]SEM = Standard error of the mean
[a-k] Least square means that do not share a common subscript are significantly different (P < 0.05)

Table 6. Least square means for L*[1] from ribeye steaks in master packages[2] in dark storage for 10, 15, or 18 d displayed for 5 d (n = 27 master bags; 81 packages in display) from all CO_2 concentrations (10, 20 or 30%)

Pull day	0	1	2	3	4	5
10	46.82^{bcdefg}	47.23^{bcde}	44.71^{h}	46.02^{efg}	43.79^{i}	45.78^{fgh}
15	47.76^{bc}	46.47^{defg}	46.37^{defg}	46.90^{bcdef}	45.45^{gh}	45.51^{gh}
18	50.53^{a}	48.21^{b}	47.49^{bcd}	46.75^{cdefg}	46.66^{cdefg}	45.99^{efgh}
SEM[3]	0.51	0.51	0.51	0.51	0.51	0.51

[1] L* values: higher values indicate lighter color
[2] 10% mixture (0.39% CO, 9.997% CO_2, balance N_2); 20% mixture (0.37% CO, 19.97% CO_2, balance N_2) or 30% mixture (0.42% CO, 30.01% CO_2, balance N_2)
[3]SEM = Standard error of the mean
[a-i] Least square means that do not share a common subscript are significantly different (P < 0.05)

Table 7. Least square means for a*[1] from ribeye steaks in master packages[2] in dark storage for 10, 15, or 18 d displayed for 5 d (n = 27 master bags; 81 packages in display) from all CO_2 concentrations (10, 20 or 30%)

Pull day	0	1	2	3	4	5
10	34.01^{a}	32.86^{bc}	29.55^{efgh}	29.75^{efgh}	32.08^{bcd}	28.32^{ghij}
15	31.22^{cde}	29.97^{defg}	28.76^{fghi}	28.95^{fgh}	27.27^{ij}	26.81^{ij}
18	33.61^{ab}	31.63^{cde}	30.29^{defg}	28.91^{fghi}	27.72^{hij}	26.33^{j}
SEM[3]	0.78	0.78	0.78	0.78	0.78	0.78

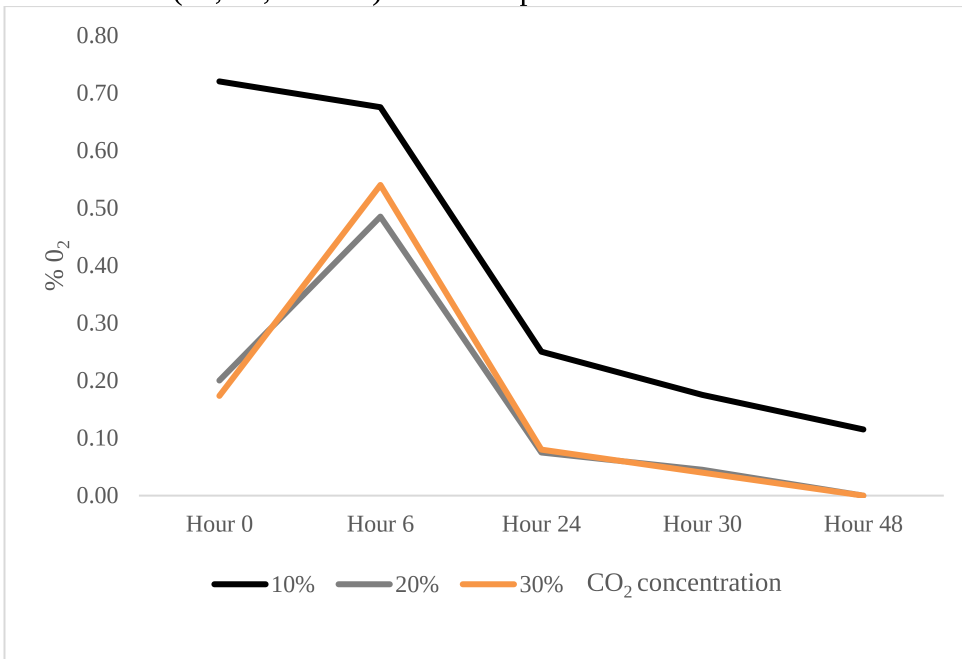
[1] a* values: higher values indicate redder color

[2] 10% mixture (0.39% CO, 9.997% CO_2, balance N_2); 20% mixture (0.37% CO, 19.97% CO_2, balance N_2) or 30% mixture (0.42% CO, 30.01% CO_2, balance N_2)

[3]SEM = Standard error of the mean

[a-j] Least square means that do not share a common subscript are significantly different (P < 0.05)

Figure 1. Oxygen depletion curve as averages from master bags flushed with varying CO_2 concentrations (10, 20, or 30%) over 48 h period



REFERENCES

Arteaga Custode, I. S., J. A. Campbell, J. R. Cassar, and E. W. Mills. 2017. Oxygen Scavengers affect Gas Mixture and Color Stability of Master Packed Ground Beef. Meat and muscle biology 1(1):181-191. doi: 10.22175/mmb2017.04.0025

Arvanitoyannis, I. 2012. Modified Atmosphere and active packaging technologies. CRC Press.

Bandoim, L. 2020. Suprising shortage of carbon dioxide threatens food and beverage industries Forbes.

Beggan, M., Allen, P,. & Butler, F. 2006. Oxygen scavenger effect on the development of metmyoglobin on beefsteaks during early low-oxygen storage. Journal of Muscle Foods (17):381-397.

Beggan, M., Allen, P,. & Butler, F. . 2005. The use of micro-perforated lidding film in low-oxygen storage of beef steaks. Journal of Muscle Foods (16):103-116.

Belcher, J. N. 2006. Industrial packaging developments for the global meat market. Meat science 74(1):143-148. doi: 10.1016/j.meatsci.2006.04.031

Cichello, S. A. 2015. Oxygen absorbers in food preservation: a review. Journal of food science and technology 52(4):1889-1895. doi: 10.1007/s13197-014-1265-2

Cole Jr, A. B. 1986. Retail packaging systems for fresh red meat cuts. In: 39th reciprocal meat conference, Champaign, Illinois, USA. p 106-111.

Cornforth, D., M.C. Hunt. 2008. Low-oxygen packaging of fresh meat with carbon dioxide. Meat Quality, microbiology, and safety, AMSA White Paper Series. American Meat Science Association, Savoy, Illinois. p. 1-10.

Eilert, S. J. 2005. New packaging technologies for the 21st century. Meat science 71(1):122-127. doi: 10.1016/j.meatsci.2005.04.003

Geornaras, I., H. Yang, G. Moschonas, M. C. Nunelly, K. E. Belk, K. K. Nightingale, D. R. Woerner, G. C. Smith, and J. N. Sofos. 2012. Efficacy of Chemical Interventions against Escherichia coli O157:H7 and Multidrug-Resistant and Antibiotic-Susceptible Salmonella on Inoculated Beef Trimmings. Journal of Food Protection 75(11):1960-1967.

Harlan, T. 2011. Influence of oxygen scavenger technology on retail stability of fresh beef in tri-gas packaging, Oklaoma State University, Stillwater, OK.

Holman, B. W. B., R. J. van de Ven, Y. Mao, C. E. O. Coombs, and D. L. Hopkins. 2017. Using instrumental (CIE and reflectance) measures to predict consumers' acceptance of beef colour. Meat science 127:57-62. doi: 10.1016/j.meatsci.2017.01.005

Hunt, M. C., R. A. Mancini, K. A. Hachmeister, D. H. Kropf, M. Merriman, G. Lduca, and G. Milliken. 2004. Carbon Monoxide in Modified Atmosphere Packaging Affects Color, Shelf Life, and Microorganisms of Beef Steaks and Ground Beef. Journal of food science 69(1):FCT45-FCT52. doi: 10.1111/j.1365-2621.2004.tb17854.x

Isdell, E., P. Allen, A. Doherty, and F. Butler. 1999. Colour stability of six beef muscles stored in a modified atmosphere mother pack system with oxygen scavengers. International Journal of Food Science & Technology (34):71-80.

Isdell, E., P. Allen, A. Doherty, and F. Butler. 2003. Effect of packaging cycle on the colour stability of six beef muscles stored in a modified atmosphere mother pack system with oxygen scavengers. International Journal of Food Science & Technology (38):623-632.

Jakobsen, M. a. G. B. 2002. The use of CO2 in packaging of fresh red meats and its effect on chemical quality changes in the meat: A review. Journal of Muscle Foods (13):143-168.

Jayasingh, P., D. P. Cornforth, C. E. Carpenter, and D. Whittier. 2001. Evaluation of carbon monoxide treatment in modified atmosphere packaging or vacuum packaging to increase color stability of fresh beef. Meat Science 59(3):317-324. doi: https://doi.org/10.1016/S0309-1740(01)00086-9

Jeyamkondan, S., D. S. Jayas, and R. A. Holley. 2000. Review of Centralized Packaging Systems for Distribution of Retail-Ready Meat. Journal of Food Protection 63(6):796-806. doi: 10.4315/0362-028x-63.6.796

John, L., D. Cornforth, C. E. Carpenter, O. Sorheim, B. C. Pettee, and D. R. Whittier. 2005. Color and thiobarbituric acid values of cooked top sirloin steaks packaged in modified atmospheres of 80% oxygen, or 0.4% carbon monoxide, or vacuum. Meat science 69(3):441-449. doi: 10.1016/j.meatsci.2004.08.013

Kinsey, R. 2011. Effects of modified atmosphere packaging on retail color stability in fresh beef, Oklahoma State University, Stillwater, OK.

Lagerstedt, Å., K. Lundström, and G. Lindahl. 2011. Influence of vacuum or high-oxygen modified atmosphere packaging on quality of beef M. longissimus dorsi steaks after different ageing times. Meat Science 87(2):101-106. doi: https://doi.org/10.1016/j.meatsci.2010.08.010

Limbo, S., E. Uboldi, A. Adobati, S. Iametti, F. Bonomi, E. Mascheroni, S. Santagostino, T. H. Powers, L. Franzetti, and L. Piergiovanni. 2013. Shelf life of case-ready beef steaks (Semitendinosus muscle) stored in oxygen-depleted master bag system with oxygen scavengers and CO2/N2 modified atmosphere packaging. Meat Science 93(3):477-484. doi: https://doi.org/10.1016/j.meatsci.2012.10.009

Madhavi, D. L., and C. E. Carpenter. 1993. Aging and Processing Affect Color, Metmyoglobin Reductase and Oxygen Consumption of Beef Muscles. Journal of Food Science 58(5):939-942. (https://doi.org/10.1111/j.1365-2621.1993.tb06083.x) doi: https://doi.org/10.1111/j.1365-2621.1993.tb06083.x

Maia Research Analysis, G. 2020. Meat wastage at stores and losses due to discoloration of meat 2015-2020.

Mancini, R. A., and M. C. Hunt. 2005. Current research in meat color. Meat science 71(1):100-121. doi: 10.1016/j.meatsci.2005.03.003

McKenna, D. R., P. D. Mies, B. E. Baird, K. D. Pfeiffer, J. W. Ellebracht, and J. W. Savell. 2005. Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. Meat science 70(4):665-682. doi: 10.1016/j.meatsci.2005.02.016

McMillin, K. W. 2008. Where is MAP Going? A review and future potential of modified atmosphere packaging for meat. Meat science 80(1):43-65. doi: 10.1016/j.meatsci.2008.05.028

McSharry, S., L. Koolman, P. Whyte, and D. Bolton. 2021. The microbiology of beef from carcass chilling through primal storage to retail steaks. Current Research in Food Science 4:150-162. doi: https://doi.org/10.1016/j.crfs.2021.03.002

NAMI. 2020. Availability of carbon dioxide for use in the meat industry, North American Meat Institute.

Ozlem Kizilirmak Esmer, R. I., Nurcan Degirmencioglu, Ali Degirmencioglu. 2010. The effects of modified atmosphere gas composition on microbiological criteria, color

and oxidation values of minced beef meat. Meat Science (88):221-226.

Perry, M. 2018. Influence of oxygen scavenger technology on retail stability of fresh beef in tri-gas master packaging, Oklahoma State University, Stillwater, OK.

Ribeiro, F. A., M. A. Almeida, J. S. D. S. Pinto, K. A. Guimarães, E. S. Villa, A. C. Venturini, and C. J. C. Castillo. 2016. Shelf-life of case-ready steak, stew and ground beef (Longissimus dorsi m.) in modified atmosphere packaging. Meat Science 112:124. doi: https://doi.org/10.1016/j.meatsci.2015.08.057

Smith, G. B., KE. Sofos, JN. Tatum, JD. Williams, SN. 2000. Economic Implications of improved color stability in beef. In: C. F. EA Decker, CJ Lopez-Bote, editor, Antioxidants in Muscle Foods: Nutritional Strategies to Improve Quality. Wiley Intersci, New York. p. 397-426.

Sørheim, O., H. Nissen, and T. Nesbakken. 1999. The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. Meat science 52(2):157-164. doi: 10.1016/S0309-1740(98)00163-6

Southard, L. 2021. In-plant operations: Averting a crisis Meat and Poultry.

Stetzer, A. J., R. A. Wickland, D. D. Paulson, E. M. Tucker, B. J. Macfarlane, and M. S. Brewer. 2006. Effect of carbon monoxide and high oxygen modified atmosphere packaging (MAP) on quality characteristics of beef strip steaks. Journal of Muscle Foods (18):56-66.

Suman, S. P., and P. Joseph. 2013. Myoglobin Chemistry and Meat Color. Annual review of food science and technology 4(1):79-99. doi: 10.1146/annurev-food-030212-182623

Venturini, A. C., C. J. C. Contreras, C. I. G. L. Sarantópoulos, and N. D. M. Villanueva. 2006. The Effects of Residual Oxygen on the Storage Life of Retail-Ready Fresh Beef Steaks Masterpackaged Under a CO2 Atmosphere. Journal of Food Science 71(7):S560-S566. (https://doi.org/10.1111/j.1750-3841.2006.00126.x) doi: https://doi.org/10.1111/j.1750-3841.2006.00126.x

APPENDICES

Multisorb (Walmart) Steak Color Score Panel

Evaluator: **Date:** **Time:**

Muscle Color (MC)	Surface Discoloration (SD)	Overall Accepability (OA)
1 Extremely Bright Cherry Red	**1** No Discoloration **(0%)**	**1** Acceptable
2 Bright Cherry Red	**2** Minimal Discoloration **(1-20%)**	**2** Discounted
3 Moderately Cherry Red	**3** Small Discoloration **(21-40%)**	**3** Unacceptable
4 Slightly bright Cherry Red	**4** Modest Discoloration **(41-60%)**	
5 Slightly Dark Cherry Red	**5** Moderate Discoloration **(61-80%)**	
6 Moderately Dark Red	**6** Extensive Discoloration **(81-100%)**	
7 Dark Red		
8 Extremely Dark Red		

ID	MD	SD	OA	ID	MD	SD	OA
1				19			
2				20			
3				21			
4				22			
5				23			
6				24			
7				25			
8				26			
9				27			
10				28			
11				29			
12				30			
13				31			
14				32			
15				33			
16				34			
17				35			
18				36			

VITA

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