

INFLUENCE OF OXYGEN SCAVENGER
TECHNOLOGY ON RETAIL STABILITY OF FRESH
BEEF IN TRI-GAS MASTER BAG PACKAGING

By

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Title of Study: INFLUENCE OF OXYGEN SCAVENGER TECHNOLOGY ON
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Abstract: The objective of this study was to evaluate the effects of three types of O₂ scavengers on color stability in a retail environment. Five different products were each placed into a master bag (n = 45) with either a control, test-A, or test-B scavenger. Products included: top sirloin steaks, top round steaks, ground beef (GB) patties (85% lean 15% fat GB 0.45 kg loaf (90% lean 10% fat), and GB 1.36 kg loaf (73% lean 27% fat). On d 5, 8 and 11 (ground product) and d 5, 8 and 15 (whole muscle product) of dark storage, one master bag from each treatment was randomly pulled and assigned to retail display. Headspace analysis was conducted before the product was removed from the master bag to prevent the use of a leaking package in the study. Visual color and objective color measurements (L* and a*) were collected on d 1 - 4 of display. Muscle color, display color and surface discoloration were all analyzed by a trained panel (n = 6). Total plate count was conducted on d 1 and d 4 of display. The results of this study showed minimal significant differences between scavenger treatments and one scavenger did not have a consistent advantage over the others. Top sirloin steaks, top round steaks, GB patties and GB 73/27 loaves maintained acceptable visual and instrumental color values throughout all of retail display for all scavenger types. The only product that reached levels of discrimination by consumers were the 90/10 loaves stored with the control and test-A scavengers due to amount of surface discoloration. Therefore, these results conclude the test O₂ scavengers performed comparable to the control.

TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION.....	1
II. REVIEW OF LITERATURE.....	3
Meat Color	3
Packaging.....	5
Case-Ready Packaging.....	5
Carbon Monoxide MAP Packaging.....	6
Overwrap Packaging.....	8
Active Packaging.....	9
Oxygen Scavenger Technology.....	9
Shelf Life.....	12
Conclusion	13
III. INFLUENCE OF OXYGEN SCAVENGER TECHNOLOGY ON RETAIL STABILITY OF FRESH BEEF IN TRI-GAS MASTER BAG PACKAGING... 14	
Abstract.....	14
Introduction.....	15
Materials and Methods.....	16
Product Collection	16
Storage	17
Headspace Analysis	17
Retail Display	17
Tray Assignments	18
Visual Color Analysis.....	18
Instrumental Color Analysis.....	19
Total Aerobic Plate Count.....	19
Statistical Analysis.....	20
Results and Discussion	20
Top Sirloin Steaks.....	20
Top Round Steaks.....	21
Ground Beef Patties.....	22
Ground Beef 90/10 Loaves	23

Chapter	Page
Ground Beef 73/27 Loaves	23
Total Aerobic Plate Count.....	24
Conclusion	24
LITERATURE CITED	32
APPENDICES	36

LIST OF TABLES

Table	Page
Table 1. Trays utilized for retail display per product (n = 63).....	26
Table 2. Least squares means for retail display of top sirloin steaks with scavenger ¹ as main effect (n = 9 master bags).....	27
Table 3. Least squares means for retail display of top round steaks with scavenger ¹ as main effect (n = 9 master bags).....	28
Table 4. Least squares means for retail display of ground beef patties with scavenger ¹ as main effect (n = 9 master bags).....	29
Table 5. Least squares means for retail display of ground beef 90/10 loaves with scavenger ¹ as main effect (n = 9 master bags).....	30
Table 6. Least squares means for retail display of ground beef 73/27 loaves with scavenger ¹ as main effect (n = 9 master bags).....	31

CHAPTER I

INTRODUCTION

Meat color is extremely influential in purchasing decisions, as consumers associate a bright-red color with freshness. As time in the retail case increases, meat will change color and surface discoloration will start to appear which consumers find undesirable. This formation of metmyoglobin depends on the retail stability of a product and can be decreased through the use of technological advancements in meat packaging. One of these advancements is the use of a type of case-ready packaging which includes a master bag, oxygen scavenger and individual retail-ready trays. Case-ready packaging started to become popular in the late 1990's and was included in a National Meat Case Study in 2002 occupying 49% of the fresh meat case (Sealed Air, 2016). The presence of case-ready packaging continues to increase in the retail sector fresh meat case with a jump from 66% in 2010 to 76% in 2015 (North American Meat Institute, 2016).

Master bag packaging helps to improve the shelf life of fresh beef through the use of an oxygen scavenger. In a low-oxygen master bag, meat is stored with a minimal residual concentration of O₂ (0.5 to 1.0%) which can cause browning that is not able to be reversed (Nassu et al., 2010). An oxygen scavenger will decrease the residual oxygen in the headspace of a package due to the iron oxide compounds present. These compounds are able to reduce oxygen levels to below 0.01% (Cruz et al., 2012) which helps prevent the formation of

metmyoglobin. Arteaga Custode et al. (2017), Uboldi et al. (2014) and Isdell et al. (1999) all found product stored with an oxygen scavenger resulted in a brighter more cherry-red color and longer shelf life.

CHAPTER II

REVIEW OF LITERATURE

Meat Color

Meat color is one of the most important aspects of consumer acceptability of meat. This is because when beef was first displayed in a package, it was in an air-permeable retail package which allowed beef to be bloomed to a bright cherry-red color that consumers associated with freshness (McMillin, 2008). Since a consumer is unable to evaluate odor or texture without opening the package, color remains a main influence on consumers purchasing decisions. After an extended amount of time, discoloration of beef can be seen on the surface of the product which consumers tend to associate with a decrease in product quality and safety (Mancini and Hunt, 2005). Discoloration has been found to cost the U.S. meat industry more than one billion dollars a year (Smith et al., 2000).

One way to quantitatively measure meat color is through the use of CIE L*a*b* color values. The a* value is the best way to predict consumer acceptance of beef color since consumers associate a bright cherry-red color with freshness in beef. Holman et al. (2017) found that 14.5 was the minimum a* value to be considered acceptable with a 95% acceptance rate from consumers. These values will decrease as storage and display time increase (Arteaga Custode et al., 2017; English et al., 2016; Hunt et al., 2004). The main pigment in meat color is myoglobin which is a water-soluble protein. There are four chemical

forms of myoglobin: deoxymyoglobin, oxymyoglobin, carboxymyoglobin and metmyoglobin. Deoxymyoglobin contains iron in the reduced state and produces a dark purplish-red or purplish-pink color. Deoxymyoglobin is commonly associated with vacuum packaged beef and meat before cutting. Oxymyoglobin forms in the presence of oxygen resulting in a bright cherry-red color. Oxymyoglobin will penetrate further below the meat's surface as time exposed to oxygen increases. Carboxymyoglobin forms in the presence of carbon monoxide and produces a bright cherry-red color in the absence of oxygen. Metmyoglobin is the oxidized form of myoglobin and results in a tan to brown color and discoloration. Even though consumers are only able to visually see surface metmyoglobin in the grocery store, subsurface metmyoglobin plays an important role in beef color. Subsurface metmyoglobin is located between superficial oxymyoglobin and interior deoxymyoglobin (Mancini and Hunt, 2005). Over time, subsurface metmyoglobin will thicken and rise upwards (Mancini and Hunt, 2005). Oxygen partial pressure, temperature, pH, meat's reducing activity and microbial growth will all play a role in the formation of metmyoglobin (Mancini and Hunt, 2005).

Myoglobin oxidation occurs quicker at lower O₂ levels due to the oxidation of deoxymyoglobin being faster than the oxidation of oxymyoglobin (Jakobsen and Bertelsen, 2002). At 0.0% O₂, deoxymyoglobin is the dominating form (Jakobsen and Bertelsen, 2002). This changes to metmyoglobin at 0.5 - 1.0% O₂ and oxymyoglobin at 4.0% O₂ (Jakobsen and Bertelsen, 2002). In order to prevent discoloration of beef, O₂ levels must be less than 0.1 - 0.15% (Jakobsen and Bertelsen, 2002). This can be done through modifications in meat packaging.

Packaging

In meat packaging, three trends regularly arise: product safety, an increase in case-ready packaging, and consumers who want fresh, high-quality and convenient products (Nassu et al., 2010). When considering storage of fresh beef, the main concerns are color, lipid oxidation and microbial growth (Esmer et al., 2011). Since fresh meat is very perishable, packaging is an extremely important factor, and type of packaging will greatly affect the microbial population. Packaging allows fresh meat to be protected from the environment therefore, increasing the preservation of the product. The main causes for deterioration of meat are microbial spoilage, moisture loss, color change and oxidative rancidity (Bell, 2001). Through proper packaging, shelf life of the product can be extended.

Case-Ready Packaging: Over the years, packaging and consumer demands have shifted, resulting in a change from the butcher at your local grocery store cutting and packaging your meat to a majority of case-ready or centralized packaging being displayed in retail cases. Case-ready packaging allows labor to be eliminated at the retail store for cutting, trimming and overwrapping allowing human handling to be reduced therefore improving food safety (Nassu et al., 2010). Also, retailers no longer have to purchase whole sides of carcasses or whole primals, instead they can individually select the retail cuts which sell the best in their market. This results in the reduction of fabrication and packaging costs for the retail sector (John et al., 2005) and allows for inventory to be more effectively managed (North American Meat Institute, 2016). In order to be successful, case-ready packaging must be able to maintain the shelf life of a product and also include retail ready trays that will visibly display the product in the oxymyoglobin state and be pre-priced and labeled (Nassu et al., 2010). Since chilled meat with a long storage life is in high demand, case-ready

packaging is an efficient way to meet that need (Jeyamkondan et al., 2000). One of the ways this is done is by utilizing a master bag, a type of modified atmosphere packaging, which will contain individual case-ready retail packages and aims to prevent bloom until display and delay bacterial growth.

Carbon Monoxide MAP Packaging: Modified atmosphere packaging (MAP) is a type of packaging that slows respiration rates, extends shelf life and affects the sensory quality of a product by changing the gaseous environment surrounding a food product enclosed in high-barrier material (Nassu et al., 2010). This gaseous environment is referred to as headspace. There are different gas mixtures used in MAP depending upon product type, the expected shelf life of the product, and the desires of the processor and consumer (Arvanitoyannis, 2012). Microbial growth, the products' sensitivity to O₂ and CO₂, and need for color stability will ultimately decide which type of MAP is used (Arvanitoyannis, 2012). There are two main types of MAP: high-oxygen MAP (HiOx-MAP) and carbon monoxide MAP (CO-MAP). High-oxygen MAP will contain 60 - 80% O₂ and is used to maintain the bright cherry-red color of beef but quickens lipid oxidation and the growth of off-flavors and odors (Hunt et al. 2004). Carbon monoxide MAP contains 20 - 40% CO₂ and less than 0.4% of CO and is used to inhibit microbial growth.

The master bag, a type of CO-MAP, extends shelf life through a mixture of gasses including carbon dioxide (CO₂), nitrogen (N₂) and carbon monoxide (CO). Carbon dioxide is a colorless gas with bacteriostatic and fungistatic properties (Arvanitoyannis, 2012). Carbon dioxide allows myoglobin to remain in its reduced form while also reducing the growth of aerobic spoilage bacteria (Isdell et al., 1999). Nitrogen aids in the prevention of microbial growth helping prevent aerobic spoilage but anaerobic bacteria growth may still occur

(Arvanitoyannis, 2012). Nitrogen also helps stop the master bag from collapsing which can occur due to the absorption of CO₂ by meat during storage (Nassu et al., 2010). The U.S. approved the use of CO as a MAP gas in fresh meat distribution in 2002 and in fresh meat packaging in 2004 (Cornforth and Hunt, 2008). The use of CO in headspace gas leads to the formation of carboxymyoglobin. Since CO is very toxic and flammable, its level in packaging must be less than 0.4% (FSIS, 2019). If the low-oxygen MAP did not contain CO, issues with meat re-blooming would arise (Hunt et al., 2004).

Using CO-MAP has many advantages in the meat industry. There is a significant increase in color stability due to microbial plate counts being below spoilage levels, this stability maintains for 28 d for ground beef and 35 d for steak and roasts (Cornforth and Hunt, 2008). Growth of spoilage organisms and pathogenic bacteria are decreased as well because of the anaerobic environment, refrigeration and use of CO₂ (Cornforth and Hunt, 2008). After 2 d of display time, Uboldi et al. (2015) found that ground beef patties that had been stored in a master bag had lower microbial growth compared to those that were never stored in a master bag due to the presence of carbon dioxide in headspace gas. John et al. (2005) found that premature browning and increased oxidation were prevented through the use of CO-MAP. John et al. (2005) also reported that steaks packaged in CO-MAP better maintained a bright cherry-red color, higher a* values, throughout a 21 d storage period than those packaged in HiOx-MAP and vacuum packaging.

However, there are disadvantages to using CO-MAP. The use of CO in food packaging has a negative connotation due to it being a hazardous gas (Cornforth and Hunt, 2008). Also, meat packaged in CO-MAP for an extended period of time may appear bright cherry-red but could be masking high levels of bacterial growth and spoilage (Cornforth and

Hunt, 2008; Jayasingh et al., 2001). Fresh meat in CO-MAP has a shelf life of 21 d (Suman and Joseph, 2013). Carbon monoxide MAP significantly improves stability of beef color but may conceal spoilage due to the desired cherry-red color lasting longer than the microbiological shelf life of the product (Suman and Joseph, 2013). The anaerobic conditions combined with increased levels of CO₂ in CO-MAP allow aerobic spoilage microflora growth to be slowed but supports the growth of lactic acid bacteria (Esmer et al., 2011).

The success of a master bag to lengthen shelf life and ensure blooming will depend on many factors including the cut of meat and quality, the ratio of carbon dioxide to product mass, and master bag dimensions (Uboldi et al., 2014). However, the most important factors are residual oxygen and the permeability of the primary packaging (Uboldi et al., 2014). If oxygen is not removed as quickly as possible from the headspace, permanent discoloration can occur during storage. Oxygen permeability of the film for the case-ready packages is important for proper blooming of the product. Since the individual retail packages are removed from the master bag before display, an anoxic atmosphere in the bag must be achieved in order to prevent oxidation and bacterial growth (Arteaga Custode et al., 2017).

Overwrap Packaging: As long as a product is packaged with a high O₂ transmission rate, blooming will follow when the tray is removed from the master bag (Isdell et al., 1999). To ensure a high O₂ transmission rate, overwrap packaging is used which places a product in a foam tray with a soaker pad. Both the tray and product are then covered by a clear, elastic, oxygen-permeable film. Advantages to using overwrap packaging are transparency, their ability to be a barrier to water vapor transmission, and their selectivity in gas permeability (Arvanitoyannis, 2012).

Polyvinyl chloride (PVC) is a type of overwrap packaging widely used in the U.S. for beef because it allows beef to be sold in desired color conditions (bright cherry-red).

Polyvinyl chloride packaging's film and equipment is also inexpensive and easy to use. The film used for PVC packaging is thin and easily heat-sealable. However, PVC often leads to leaky packages due to the film being easily punctured or torn. Another disadvantage is PVC has a short display life due to its exposure to oxygen increasing aerobic bacteria growth which results in a more rapid formation of metmyoglobin (Cornforth and Hunt, 2008). Steaks typically have a shelf life of 5 to 7 d when packaged in PVC but this is decreased for ground beef products to 3 d (Cornforth and Hunt, 2008; Uboldi et al., 2015).

Active Packaging

A newer opportunity in packaging is active packaging (AP) which focuses on improving the shelf life, safety and sensory properties of a product by changing its packaging conditions (Nassu et al., 2010). Active packaging allows fresh products to better meet consumer demands of quality and safety. Active packaging is able to extend shelf life while also maintaining nutritional quality of the product and reducing pathogenic and spoilage microorganism growth (Arvanitoyannis, 2012).

Oxygen Scavenger Technology: An oxygen (O₂) scavenger is a type of AP used to decrease residual oxygen in meat packaging to help extend the shelf life of product. An O₂ scavenger must not only be able to absorb oxygen but also be safe for humans due to its use in food. Most O₂ scavengers will be in a sachet form which contains iron based powders that will oxidize when exposed to oxygen and moisture (Cruz et al., 2012). When meat is stored in the residual concentration of O₂ (0.5 to 1.0%) browning will occur that is not able to be

reversed (Nassu et al., 2010). Due to the compounds in a scavenger, O₂ levels are able to be reduced to below 0.01% (Cruz et al., 2012), therefore, slowing meat discoloration.

There are a few requirements for an O₂ scavenger to be successful. Packaging with a high oxygen barrier is needed so the scavenger does not become saturated and lose its ability to trap oxygen (Cruz et al., 2012). Also, heat sealing for packaging must be done correctly to ensure air will not enter the package through the seal (Cruz et al., 2012). Lastly, the right size and type of scavenger must be used. The type of oxygen scavenger used will depend on many different factors including the amount of O₂ in the headspace, initially trapped in the food and transferred from surrounding air into the package during storage as well as the type of food, water activity and desired shelf life of the product (Cruz et al., 2012). Iron-based O₂ scavengers will absorb oxygen and carbon dioxide in MAP packaging but will stop once all of the oxygen is depleted from the headspace (Arteaga Custode et al., 2017). During storage, an effective O₂ scavenger is able to keep myoglobin in its reduced state (deoxymyoglobin) which will allow meat to bloom to oxymyoglobin once removed from the master bag (Arteaga Custode et al., 2017).

Arteaga Custode et al. (2017) found the oxygen concentration in a master bag is affected by headspace gas, storage time, presence of meat, and the use of an oxygen scavenger. The study used two different gas mixtures: 80% N₂/20% CO₂ and 70% N₂/30% CO₂. An average O₂ concentration of 0.02% was recorded throughout the 28 d storage period. The initial O₂ concentration in the master bags was 3.54% but O₂ scavengers were able to reduce O₂ concentration quickly in MAP packaging after a few hours of storage. Arteaga Custode et al. (2017) also found ground beef stored without an O₂ scavenger resulted in lower a* values when compared to product with a scavenger resulting in a shorter shelf life. Ground

beef stored without an oxygen scavenger experienced lower levels of oxymyoglobin and higher levels of metmyoglobin during display especially as storage time increased when compared to ground beef stored with an O₂ scavenger (Arteaga Custode et al., 2017). Oxymyoglobin did decrease by d 4 of display with the use of a scavenger but color stability was still improved for ground beef when compared to master bags that did not contain an oxygen scavenger (Arteaga Custode et al., 2017). By d 14 of storage, CO₂ was reduced to very low levels due to the inclusion of oxygen scavengers; therefore, removing the ability of CO₂ to reduce microbial growth (Arteaga Custode et al., 2017). Also, by d 14 the ground beef stored without an O₂ scavenger in its master bag was deemed unsuitable for display due to color resulting from an oxygen concentration of 3.79% in the headspace gas (Arteaga Custode et al., 2017).

Isdell et al. (1999) found the oxygen level in master bags and trays with O₂ scavengers were significantly lower than those without. Isdell et al. (1999) also observed after 2 weeks of storage the steaks packaged with O₂ scavengers bloomed to a bright cherry-red color initially; whereas, those without the scavengers did not bloom due to residual O₂ content in the master bag which resulted in the formation of metmyoglobin. Uboldi et al. (2014) discovered oxygen scavengers were able to rapidly reduce the levels of residual O₂ to lower than 0.08% during the first 10 h of packaging in the master bag allowing myoglobin to be effectively reduced to deoxymyoglobin over the storage period. Uboldi et al. (2014) also found the master bags without a scavenger exhibited an O₂ concentration of 1.5% which caused irreversible effects on meat color resulting in brown hues.

Shelf Life

Shelf life is the time period between packaging and final use by the consumer and is very important for the retail market of the meat industry. Shelf life is ultimately influenced by the product, gas mixture, packaging, headspace, storage temperature and additives (McMillin, 2008). Simply, shelf life is the time until the product is no longer suitable for consumption due to spoilage.

The case life or display life of a product begins once it is placed in display and is described as the time until it starts to change color. This change of color from bright cherry-red to a tan brown does not always indicate spoilage but is not desirable for consumers. In PVC packaging, discoloration will start to occur due to the formation of metmyoglobin even when bacterial numbers are low (Cornforth and Hunt, 2008). Consumers will discriminate against meat with 20% surface metmyoglobin (MacDougall, 1982) and will not purchase meat once that percentage has surpassed 40% (Greene et al., 1971).

Meat will start to bloom immediately after being exposed to oxygen to form oxymyoglobin. Wulf and Wise (1999) found the a^* value will stabilize after 78 min of bloom time. As time in the display case increases, the surface layer of oxymyoglobin will progressively become thicker as it moves deeper from the surface. Discoloration will start to occur once the reducing mechanisms of the muscle start nearing depletion. This will happen once the oxygen supply does not favor deoxymyoglobin or oxymyoglobin and a third layer of pigment will develop between deoxymyoglobin and myoglobin called metmyoglobin. This layer will result in a brown color which decreases consumer acceptance of the product and will increase as display time increases (Holman et al., 2017). To prevent metmyoglobin

formation, meat needs to be in a greatly aerobic (more than 15% oxygen) or entirely anoxic (less than 0.1% oxygen) environment (Bell, 2001).

In retail display, ground beef is more perishable than steaks due to exposure to atmospheric oxygen during the grinding process therefore, further enabling oxidative and degradative reactions (Uboldi et al., 2015). Ground beef patties stored in master bags will discolor quicker than patties immediately displayed after packaging due to the life of the product. When storage time increases, the oxygen consumption rate of the tissue is decreased resulting in a higher oxygen availability for protein and lipid oxidation allowing metmyoglobin to increase (Uboldi et al., 2015). Whole muscle product packaged in a low oxygen master bag system will have an expected shelf life of 28 - 35 d and case life of 2 - 7 d. Ground product packaged in a low oxygen master bag system will have an expected shelf life 25 - 30 d and case life of 2 - 3 d (Delmore, 2009).

Conclusion

Due to the cost associated with the loss of product from discoloration, shelf life must be improved for meat products. With the use of an oxygen scavenger in meat packaging, residual O₂ has been reduced in packaging systems and color stability has been increased. Studies have shown the master bag system with the use of an oxygen scavenger is an effective choice for increasing shelf life of meat, allowing meat to be cut and packaged at a case-ready facility.

CHAPTER III

INFLUENCE OF OXYGEN SCAVENGER TECHNOLOGY ON RETAIL STABILITY OF FRESH BEEF IN TRI-GAS MASTER BAG PACKAGING

Abstract

The objective of this study was to evaluate the effects of three types of O₂ scavengers on color stability in a retail environment. Five different products were each placed into a master bag (n = 45) with either a control, test-A, or test-B scavenger. Products included: top sirloin steaks, top round steaks, ground beef (GB) patties (85% lean 15% fat), GB 0.45 kg loaf (90% lean 10% fat), and GB 1.36 kg loaf (73% lean 27% fat). On d 5, 8 and 11 (ground product) and d 5, 8 and 15 (whole muscle product) of dark storage, one master bag from each treatment was randomly pulled and assigned to retail display. Headspace analysis was conducted before the product was removed from the master bag to prevent the use of a leaking package in the study. Visual color and objective color measurements (L* and a*) were collected on d 1 - 4 of display. Muscle color, display color and surface discoloration were all analyzed by a trained panel (n = 6). Total plate count was conducted on d 1 and d 4 of display. The results of this study showed minimal significant differences between scavenger treatments and one scavenger did not have a consistent advantage over the others. Top sirloin steaks, top round steaks, GB patties and GB 73/27 loaves maintained acceptable visual and instrumental color values throughout all of retail display for all scavenger types. The only product that reached levels of discrimination by consumers were the 90/10 loaves

stored with the control and test-A scavengers due to amount of surface discoloration. Therefore, these results conclude the test O₂ scavengers performed comparable to the control.

Introduction

Since consumers associate a bright-red color with freshness, meat color is extremely influential in purchasing decisions. As time in the retail case increases, meat will change color and surface discoloration will start to appear which consumers find undesirable. This formation of metmyoglobin depends on the retail stability of a product and can be decreased through the use of technological advancements in meat packaging. One of these advancements is the use of a type of case-ready packaging which includes a master bag, oxygen scavenger and individual retail-ready trays.

Master bag packaging helps to improve the shelf life of fresh beef through the use of an oxygen scavenger. In a low-oxygen master bag, meat is stored with a minimal residual concentration of O₂ (0.5 to 1.0%) which can cause browning that is not able to be reversed (Nassu et al., 2010). An oxygen scavenger will decrease the residual oxygen in the headspace of a package due to the iron oxide compounds present. These compounds are able to reduce oxygen levels to below 0.01% (Cruz et al., 2012) which helps to prevent the formation of metmyoglobin. Arteaga Custode et al. (2017), Uboldi et al. (2014) and Isdell et al. (1999) all found product stored with an oxygen scavenger resulted in a brighter more cherry-red color and longer shelf life.

Oxygen scavenger use in master bag packaging has been proven to extend shelf life of fresh beef. Due to the importance of retail stability, research and development of O₂ scavenger technology continues to improve in order to meet consumer demands. Retail stability of meat is extremely important because the more stable a product is in the display

case, the less likely that product will discolor. Discoloration has been found to cost the U.S. meat industry more than one billion dollars a year (Smith et al., 2000). Therefore, the purpose of this study was to compare the effects of test O₂ scavengers to a control O₂ scavenger on color stability in the retail case for top sirloin steaks, top round steaks, ground beef patties, ground beef 90/10 loaves and ground beef 73/27 loaves.

Materials and Methods

Product Collection: Product was collected at National Beef in Hummels Wharf, PA with the packaging date established as d 0. Five different products (Table 1) were packaged for this study, two different whole muscle products and three different ground beef products: top sirloin steaks, top round steaks (thin), ground beef (GB) patties (85% lean 15% fat), GB 0.45 kg loaf (90% lean 10% fat), and GB 1.36 kg loaf (73% lean 27% fat). The top sirloin steaks, top round steaks, GB 90/10 loaves, GB 73/27 loaves were placed on a black foam tray, and the GB patties were stacked and placed on a black foam tray with 4 patties. After the products were placed on a foam tray, they were overwrapped with a perforated film. All master bags were packaged in a flow wrap machine (OMORI Machinery Co., Nishikata, Koshigaya-shi, Saitama, Japan). Two top sirloin steak trays, two top round steak trays, three patty trays, three 90/10 loaf trays and two 73/27 loaf trays were placed a master bag with one of the three oxygen scavengers (Multisorb Filtration Group, Buffalo, NY, USA): control (FreshPax®CR), test-A (FreshPax®CR Gen5) or test-B (FreshPax®CR Gen5). The difference between the scavengers cannot be disclosed as it is considered proprietary information. All products were gas flushed with tri-gas (30% CO₂, 0.4% CO, balance N₂). Five master bags for display evaluation per product were collected (n = 75 master bags). Product was transported through freight to Oklahoma State University (Stillwater, OK) with

the average ambient temperature of $0.05^{\circ}\text{C} \pm 1.32^{\circ}\text{C}$ during transport measured with an EasyLog EL-USB-2-LCD data logger (Lascar Electronics, Erie, PA, USA).

Storage: Once the product arrived at OSU, boxes were placed in a refrigerated room in dark storage. Product was stored at an average of $1.66^{\circ}\text{C} \pm 0.14^{\circ}\text{C}$ for 5, 8, 11 (ground product only) and 5, 8, 15 (whole muscle product only) d post packaging measured with an EasyLog EL-USB-2-LCD data logger (Lascar Electronics, Erie, PA, USA).

Headspace Analysis: All master bags were analyzed using a Mocon PAC CHECK® Model 333 Triple Gas Analyzer (Minneapolis, MN, USA) to determine the percent O₂, CO₂ and CO present in the headspace at the time of removal from dark storage. On each pull day, the headspace was measured for each master bag and recorded. If a master bag was found to be a leaker, the product was not used in retail display. Product included in the pulls had to have an oxygen reading less than 0.10%. Isdell et al. (1999) found 0.10% to be the maximum level of O₂ to be allowed in the headspace and recommended 0.04% at a storage temperature of 2°C. All of the product included in the retail display had an acceptable oxygen concentration based on the levels established (< 0.10%).

Retail Display: After designated cold storage time, one master bag from each scavenger treatment was randomly removed from dark storage and subjected to retail display. Each sample was placed in a Hussmann IM1SL retail case set at 2.0°C that ran a defrost cycle approximately every 6 h for 15 to 20 min. The average temperature in the display cases throughout the study was $3.31^{\circ}\text{C} \pm 0.61^{\circ}\text{C}$ measured with a LogTag® SRIL-8 Dry Ice “Probe-less” Temperature Recorder. All samples were displayed in retail cases lighted with Philips LED T8 Lamps (model number 9290011240B-453597) manufactured in Niles, OH.

Both retail lights and ceiling lights within the retail room remained on throughout the study. Packages were randomly rotated daily throughout display.

Tray Assignments: On each pull (d 5, 8, 11 and 15), one tray per treatment (n = 15) was randomly selected for initial microbial growth. The remaining packages (n = 21 trays pull 5 and 8; n = 15 trays pull 11; n = 6 trays pull 15) were used for retail display and given a random number. During retail display, color measurements were taken once daily for 4 d of display. At the end of display, one tray per treatment (n = 15) was randomly selected from the packages to be evaluated for a final microbial growth count.

Visual Color Analysis: A trained panel (n = 6) evaluated visual color once daily throughout retail display. All panelists were subjected to take and pass the Farnsworth Munsell 100-hue test. Panelists were trained for visual color using the AMSA Meat Color Measurement Guidelines (2012). Visual muscle color and surface discoloration were evaluated for the whole muscle product (top sirloin and top round steaks). Visual display color and surface discoloration were evaluated for the GB products (patties, 90/10 loaves and 73/27 loaves). Muscle color was evaluated on a 7-point scale (1 = extremely bright cherry red, 7 = extremely dark red). Display color was evaluated on a 8-point scale (1 = very bright red, 8 = tan or brown). Surface discoloration was evaluated on a 7-point scale (1 = no discoloration [0%], 7 = extensive discoloration [81-100%]).

The value of acceptability for muscle color for whole muscle product was set at 4.5 and any product with a slightly dark red color (Appendix A) or value of 5 or higher was deemed unacceptable. The value of acceptability for display color for ground product was set at 5.5 and any product with dark red or tannish-red color (Appendix B) or a value of 6 or higher was deemed unacceptable. MacDougall (1982) found consumers will discriminate

against meat with 20% of surface metmyoglobin and will not purchase the product once surface discoloration reaches 41% (Greene et al., 1971). A color score value of 3 represented the 11 - 20% range and a value of 5 was deemed as unacceptable due to it indicating 41 - 60% of surface discoloration (Appendix B).

Instrumental Color Analysis: Instrumental color was measured quantitatively with a portable, reflected-color measurement spectrophotometer, the HunterLab MiniScan® EZ 4500L (2.5-cm aperture, illuminant A, and 10° standard observer angle; Reston, VA, USA). Measurements were taken every day throughout retail display. Two readings per tray were taken of every product. The readings were randomly taken from the sample's surface each day. CIE L* and a* color values were collected. L* measures brightness, the higher the value, the brighter the product. A positive a* value represents red color with a negative value representing a green color. The instrument was standardized before each use with white and black standard plates.

Total Aerobic Plate Count: Total Aerobic Plate Count (APC) was obtained from one tray of each treatment at the beginning and end of retail display time. For APC 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 sec at 230 rpm using a Stomacher-400. Aerobic plate count was 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 hr at 37°C before counting and reporting the APC per cm². 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 O₂ scavenger master bag packaging system in extended dark storage on retail color of beef. The experimental unit was the master bag (n = 45) which were randomly assigned to a scavenger treatment. Due to limited numbers from restrictions in retail display space, data were analyzed only by scavenger treatment and not by pull day. Least squares means were calculated using PROC GLM of SAS (SAS Institute Inc., Cary, North Carolina), where the main effect (scavenger treatment) was included in the model and evaluated by retail day. When a significant F-test was identified ($P < 0.05$), least squares means were separated using a pairwise t-test (PDIF option).

For APC, least squares means were calculated using PROC GLM of SAS, where main effects and significant three-way and two-way interactions were included in the model. Main effects were defined as dark storage days (pull), display day, and treatment. The interactions were evaluated for all the main effects. If the interactions were not significant ($P > 0.05$), then a final model was analyzed with treatment, display day and dark storage days as main effects.

Results and Discussion

Top Sirloin Steaks: During retail display, no significant differences ($P > 0.05$) were found in top sirloin steaks between scavenger in muscle color, surface discoloration or L* on any retail day (Table 2). However, on d 1 top sirloin steaks from master bags (MB) with the test-B scavenger measured a higher a* value ($P < 0.05$) than steaks in MB with the test-A scavenger indicating more redness (Table 2). Both of these scavengers were not significantly different ($P > 0.05$) from top sirloin steaks in the MB with the control scavenger. On retail d 2, 3 and 4, no other differences ($P > 0.05$) were seen between scavenger treatment for a*. In a

previous industry study, top sirloin steaks did not display any differences between scavenger treatment but did reach levels of unacceptability by d 4 of display after 7, 14 and 18 d of dark storage (Perry and Cassens, 2018).

Isdell et al. (1999) found the two main muscles in top sirloin steaks, *Gluteus medius* (GM) and *Biceps femoris* (BF), did not perform effectively in a mother bag system with an O₂ scavenger. However, in this study top sirloin steaks remained relatively stable during all of retail display for all three pulls. There was a difference in the gas atmosphere between this study and Isdell et al. (1999). Master bags in this study were flushed with a tri-gas containing 30% CO₂, 0.4% CO and a balance of N₂. The master bags in Isdell et al. (1999) contained 50% CO₂ and 50% N₂. The inclusion of CO in this study was important for the re-blooming of product once removed from the master bag and could explain why the GM and BF performed poorly in Isdell et al. (1999). The use of CO as a MAP gas was not approved in the U.S. for fresh meat distribution until 2002 and for fresh meat packaging until 2004 (Cornforth and Hunt, 2008).

Top Round Steaks: On retail display d 4, top round steaks from MB with the control scavenger exhibited a more cherry-red color ($P < 0.05$) than those from MB with the test-A and -B scavengers (Table 3). There were no differences ($P > 0.05$) found in muscle color of top round steaks between scavenger treatment on d 1, 2 and 3 of display. Top round steaks stored with the test-B scavenger displayed the most surface discoloration ($P < 0.05$) on every day of display (Table 3) but did not reach the established level of discrimination. On d 2, top round steaks from the MB with the control scavenger exhibited the least discoloration ($P < 0.05$) but on d 1, 3 and 4 were not statistically different ($P > 0.05$) than steaks included in MB with the test-A scavenger. Top round steaks did not exhibit any differences between

scavenger for L* value ($P > 0.05$). On d 3 of display, top round steaks stored with the control scavenger displayed a higher a* value ($P < 0.05$) than steaks from MB with the test-B scavenger (Table 3). However, both of these scavengers were similar ($P > 0.05$) to top round steaks from MB with the test-A scavenger. Top round steaks on retail d 1, 2 and 4 did not show any differences ($P > 0.05$) in scavenger treatment. Isdell et al. (1999) found the muscle in top round steaks, the *Semimembranosus* (SM), was a more color stable muscle than the GM and BF. Hunt et al. (2004) found case life color to be improved for the SM, which has intermediate to low color stability, due to the inclusion of CO during storage in a master bag.

Ground Beef Patties: No significant differences ($P > 0.05$) were found between scavenger on any retail day for GB patties in display color, surface discoloration and L* (Table 4). There was a difference in a* value on d 2 of display; patties packaged with the test-B scavenger indicated more redness ($P < 0.05$) than those in MB with the control and test-A scavengers (Table 4). On retail d 1, 3 and 4, no other differences ($P > 0.05$) were seen between scavengers for a* value. For patties, Uboldi et al. (2015) found the ideal dark storage time in CO-MAP master bag conditions was 8 to 10 d. The ground beef patties in this study were stored in a master bag for up to 11 d in dark storage. However, the patties did not reach close to a level of unacceptability for any parameters. This is expected to have been due to the use of CO and an O₂ scavenger in the master bag which both help to extend shelf life. Hunt et al. (2004) found 80/20 ground beef stored in a master bag with CO to have more color stability during display than GB displayed in traditional overwrap packaging that was only exposed to atmospheric oxygen with no dark storage. Perry and Cassens (2018) kept patties in dark storage for up to 18 d and did not reach unacceptable levels for display color and surface discoloration for 5 d of display. However, patties in Cassens (2018) were rated unacceptable after 13 d of dark storage and only 2 d of display.

Ground Beef 90/10 Loaves: Throughout each day of retail display there were no differences ($P > 0.05$) seen in display color or a^* value for GB 90/10 loaves between scavenger types (Table 5). On retail d 4, surface discoloration and L^* value showed significant differences ($P < 0.05$) between scavenger (Table 5). Loaves with the control scavenger in the MB showed more surface metmyoglobin formation ($P < 0.05$) than those packaged with the test-B scavenger but both were similar ($P > 0.05$) to 90/10 loaves included in MB with the test-A scavenger. 90/10 loaves packaged in the MB with the control scavenger exhibited a higher L^* value ($P < 0.05$) than those included with the test-A and -B scavengers indicating more brightness. There were no other differences ($P > 0.05$) found for surface discoloration and L^* value on d 1, 2 and 3.

Arteaga Custode et al. (2017) found a^* values in ground beef to decrease by d 4 of display and that as dark storage in master bags increased, redness was reduced. Retail d 4 for 90/10 loaves had a greater decline in a^* value for loaves originating from the MB with the control and test-A scavengers. Delmore (2009) expected ground product packaged in a low oxygen master bag system should have a shelf life of 25 - 30 d and case life of 2 - 3 d. The expected case life was found to be accurate for 90/10 loaves as those stored with the control and test-A scavengers reached discrimination levels by consumers on d 4 of display due to surface discoloration. Similar findings were seen in Cassens (2018) where 0.45 kg loaves (85/15) reached unacceptable levels after 13 d of dark storage and on d 3 of display.

Ground Beef 73/27 Loaves: Display color, L^* and a^* values did not reveal any differences ($P > 0.05$) on any retail day between scavenger treatment for 73/27 loaves (Table 6). The inclusion of CO in packaging has been shown to provide a stable red color throughout display (Cornforth and Hunt, 2008). On d 2, 73/27 loaves packaged with the

control scavenger exhibited more surface discoloration ($P < 0.05$) than those included in MB with the test-A and -B scavengers (Table 6). However, no other differences ($P > 0.05$) were found between scavenger treatments on d 1, 3 and 4. Two previous industry partner studies with similar product, 1.36 kg (80/20) and 2.04 kg (73/27) loaves, found no differences between scavenger treatment for display color and surface discoloration (Cassens, 2018; Perry and Cassens, 2018). Similar to this study, Perry and Cassens (2018) did not have any 2.04 kg loaves reach unacceptable levels throughout all of retail display. However, Cassens (2018) found 1.36 kg loaves reached the unacceptable line by d 3 of display after 13 d of dark storage. This could have been caused by the longer period in dark storage.

Total Aerobic Plate Count: None of the counts for APC were to the level of spoilage (log 6 CFU/ g). Cornforth and Hunt (2008) reported whole muscle product and ground beef's microbial plate counts packaged with CO may remain under spoilage levels for as long as 35 d and 28 d, respectively. There were significant differences ($P < 0.05$) observed but only as pull day and retail day increased. This was expected because as shelf life increased, an increase in APC was anticipated; thus the data was not presented in tabular form. Hunt et al. (2004) was concerned with the possibility of the use of CO in the master bag masking spoilage in beef. However, Hunt et al. (2004) did not find aerobic microbial growth to the levels of spoilage, similar to the present study.

Conclusion

Shelf life has continued to improve through technological advancements in meat packaging and this is being done cheaper and more effective than ever before. The results of this study showed minimal differences between scavenger treatments and one scavenger did not have a consistent advantage over the others. Top sirloin steaks, top round steaks, GB

patties and GB 73/27 loaves maintained acceptable visual and instrumental color values throughout all of retail display. The only product that reached levels of discrimination by consumers were the 90/10 loaves stored with the control and test-A scavengers due to amount of surface discoloration. Even so, each scavenger was deemed acceptable in maintaining product shelf life. Therefore, based upon these findings, the test oxygen scavengers performed comparable to the control.

Table 1. Trays utilized for retail display per product (n = 63)

Product	Product per Tray	Trays per Master Bag	Trays included in Retail Display per Pull
Top sirloin steak	1 steak	2 trays	1 tray
Top round steak (thin)	4 - 7 steaks	2 trays	1 tray
Ground beef 85/15	4 patties	3 trays	2 trays
Ground beef 90/10	0.45 kg loaf	3 trays	2 trays
Ground beef 73/27	1.36 kg loaf	2 trays	1 tray

Table 2. Least squares means for retail display of top sirloin steaks with scavenger¹ as main effect (n = 9 master bags)

	Retail Day ²			
	1	2	3	4
Muscle Color³				
Control	1.06	2.11	3.28	3.81
Test-A	1.14	2.11	3.08	3.61
Test-B	1.17	2.08	3.44	4.08
SEM ⁷	0.08	0.14	0.19	0.16
Surface Discoloration⁴				
Control	1.00	1.00	1.33	1.83
Test-A	1.00	1.00	1.44	1.78
Test-B	1.00	1.00	1.50	2.00
SEM ⁷	0.00	0.00	0.15	0.19
L* value⁵				
Control	45.37	43.76	43.31	41.03
Test-A	45.57	43.55	42.58	41.16
Test-B	44.03	42.55	41.92	42.70
SEM ⁷	0.84	1.03	1.42	0.81
a* value⁶				
Control	36.10 ^{ab}	28.92	26.13	24.40
Test-A	34.50 ^b	29.63	27.56	24.83
Test-B	37.36 ^a	31.54	26.05	23.32
SEM ⁷	0.71	1.01	0.92	0.89

¹Scavenger types: control (FreshPax®CR); test-A (FreshPax®CR Gen5); test-B (FreshPax®CR Gen5)

²Retail day: represents each day of retail display- 1, 2, 3 and 4

³Muscle color: 1 = extremely bright cherry red; 7 = extremely dark red

⁴Surface discoloration: 1 = no discoloration (0%); 7 = extensive discoloration (81 – 100%)

⁵L* value: measures brightness- a higher value indicates more brightness

⁶a* value: measures redness- a higher value indicates more red color

⁷SEM: Standard error of the mean

^{a-b}Least squares means within a column within a parameter with different letters are significantly different ($P < 0.05$)

Table 3. Least squares means for retail display of top round steaks with scavenger¹ as main effect (n = 9 master bags)

	Retail Day ²			
	1	2	3	4
Muscle Color³				
Control	1.42	2.19	2.58	3.00 ^b
Test-A	1.72	2.56	3.03	3.58 ^a
Test-B	1.42	2.11	2.97	3.61 ^a
SEM ⁷	0.15	0.20	0.17	0.19
Surface Discoloration⁴				
Control	1.06 ^b	1.06 ^c	1.11 ^b	1.28 ^b
Test-A	1.33 ^b	1.33 ^b	1.33 ^b	1.56 ^b
Test-B	1.83 ^a	1.94 ^a	2.72 ^a	2.67 ^a
SEM ⁷	0.10	0.09	0.13	0.13
L* value⁵				
Control	46.66	47.31	46.22	46.01
Test-A	46.47	44.13	43.83	43.96
Test-B	51.87	47.48	48.06	44.51
SEM ⁷	1.78	1.53	1.88	1.85
a* value⁶				
Control	33.23	31.87	29.88 ^a	28.05
Test-A	32.98	30.19	28.48 ^{ab}	26.84
Test-B	32.14	30.30	26.50 ^b	26.01
SEM ⁷	1.00	0.87	0.71	0.88

¹Scavenger types: control (FreshPax®CR); test-A (FreshPax®CR Gen5); test-B (FreshPax®CR Gen5)

²Retail day: represents each day of retail display- 1, 2, 3 and 4

³Muscle color: 1 = extremely bright cherry red; 7 = extremely dark red

⁴Surface discoloration: 1 = no discoloration (0%); 7 = extensive discoloration (81 – 100%)

⁵L* value: measures brightness- a higher value indicates more brightness

⁶a* value: measures redness- a higher value indicates more red color

⁷SEM: Standard error of the mean

^{a-c}Least squares means within a column within a parameter with different letters are significantly different ($P < 0.05$)

Table 4. Least squares means for retail display of ground beef patties with scavenger¹ as main effect (n = 9 master bags)

	Retail Day ²			
	1	2	3	4
Display Color³				
Control	1.24	1.71	2.47	2.89
Test-A	1.17	1.72	2.47	2.92
Test-B	1.14	1.63	2.44	2.88
SEM ⁷	0.06	0.09	0.11	0.13
Surface Discoloration⁴				
Control	1.14	1.19	1.25	1.28
Test-A	1.14	1.22	1.31	1.25
Test-B	1.19	1.14	1.19	1.19
SEM ⁷	0.06	0.07	0.07	0.07
L* value⁵				
Control	55.14	55.03	54.04	52.45
Test-A	54.88	54.26	53.30	51.85
Test-B	55.33	53.92	53.27	51.65
SEM ⁷	0.34	0.35	0.28	0.47
a* value⁶				
Control	34.05	31.13 ^b	29.78	29.40
Test-A	34.61	31.61 ^b	29.53	29.03
Test-B	35.01	33.42 ^a	30.37	29.76
SEM ⁷	0.43	0.43	0.41	0.63

¹Scavenger types: control (FreshPax®CR); test-A (FreshPax®CR Gen5); test-B (FreshPax®CR Gen5)

²Retail day: represents each day of retail display- 1, 2, 3 and 4

³Display color: 1 = very bright red; 8 = tan or brown

⁴Surface discoloration: 1 = no discoloration (0%); 7 = extensive discoloration (81 – 100%)

⁵L* value: measures brightness- a higher value indicates more brightness

⁶a* value: measures redness- a higher value indicates more red color

⁷SEM: Standard error of the mean

^{a-b}Least squares means within a column within a parameter with different letters are significantly different ($P < 0.05$)

Table 5. Least squares means for retail display of ground beef 90/10 loaves with scavenger¹ as main effect (n = 9 master bags)

	Retail Day ²			
	1	2	3	4
Display Color³				
Control	1.29	2.04	3.10	4.90
Test-A	1.25	1.79	3.10	4.31
Test-B	1.29	1.88	3.08	3.92
SEM ⁷	0.07	0.10	0.18	0.30
Surface Discoloration⁴				
Control	1.03	1.33	2.33	4.28 ^a
Test-A	1.00	1.28	2.14	3.58 ^{ab}
Test-B	1.00	1.14	1.94	2.78 ^b
SEM ⁷	0.02	0.07	0.17	0.35
L* value⁵				
Control	56.17	53.81	52.13	52.59 ^a
Test-A	55.07	52.61	51.28	50.24 ^b
Test-B	55.40	52.99	51.96	50.88 ^b
SEM ⁷	0.46	0.58	0.41	0.54
a* value⁶				
Control	33.87	31.37	26.73	19.81
Test-A	34.81	32.27	28.03	23.24
Test-B	34.45	31.08	28.31	25.16
SEM ⁷	0.37	0.49	1.12	1.77

¹Scavenger types: control (FreshPax®CR); test-A (FreshPax®CR Gen5); test-B (FreshPax®CR Gen5)

²Retail day: represents each day of retail display- 1, 2, 3 and 4

³Display color: 1 = very bright red; 8 = tan or brown

⁴Surface discoloration: 1 = no discoloration (0%); 7 = extensive discoloration (81 – 100%)

⁵L* value: measures brightness- a higher value indicates more brightness

⁶a* value: measures redness- a higher value indicates more red color

⁷SEM: Standard error of the mean

^{a-b}Least squares means within a column within a parameter with different letters are significantly different ($P < 0.05$)

Table 6. Least squares means for retail display of ground beef 73/27 loaves with scavenger¹ as main effect (n = 9 master bags)

	Retail Day ²			
	1	2	3	4
Display Color³				
Control	1.39	1.97	2.44	2.75
Test-A	1.53	2.22	2.47	2.92
Test-B	1.53	2.25	2.58	2.97
SEM ⁷	0.14	0.18	0.17	0.11
Surface Discoloration⁴				
Control	1.00	1.78 ^a	1.56	2.11
Test-A	1.00	1.17 ^b	1.67	2.06
Test-B	1.00	1.39 ^b	1.72	2.06
SEM ⁷	0.00	0.10	0.12	0.11
L* value⁵				
Control	58.32	57.35	54.96	55.05
Test-A	58.26	56.80	56.67	54.72
Test-B	58.53	56.44	54.96	56.11
SEM ⁷	0.78	0.84	0.58	0.97
a* value⁶				
Control	33.49	31.01	30.06	27.89
Test-A	33.75	30.47	29.08	26.91
Test-B	33.24	31.04	29.48	26.54
SEM ⁷	0.50	0.64	0.35	0.73

¹Scavenger types: control (FreshPax®CR); test-A (FreshPax®CR Gen5); test-B (FreshPax®CR Gen5)

²Retail day: represents each day of retail display- 1, 2, 3 and 4

³Display color: 1 = very bright red; 8 = tan or brown

⁴Surface discoloration: 1 = no discoloration (0%); 7 = extensive discoloration (81 – 100%)

⁵L* value: measures brightness- a higher value indicates more brightness

⁷SEM: Standard error of the mean

⁶a* value: measures redness- a higher value indicates more red color

^{a-b}Least squares means within a column within a parameter with different letters are significantly different ($P < 0.05$)

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APPENDICES

Appendix A

Steak Visual Color Scoring Scales

Muscle Color (MC)	Description
1	Extremely Bright Cherry Red
2	Bright Cherry Red
3	Moderately Cherry Red
4	Cherry Red
5	Slightly Dark Red
6	Moderately Dark Red
7	Extremely Dark Red

Surface Discoloration (SD)	Description
1	No Discoloration (0%)
2	Minimal Discoloration (1-10%)
3	Slight Discoloration (11-20%)
4	Small Discoloration (21-40%)
5	Modest Discoloration (41-60%)
6	Moderate Discoloration (61-80%)
7	Extreme Discoloration (81-100%)

Appendix B

Ground Beef Visual Color Scoring Scales

Display Color (DC)	Description
1	Very Bright Red
2	Bright Red
3	Dull Red
4	Slightly Dark Red
5	Moderately Dark Red
6	Dark Red or Tannish-Red
7	Dark Reddish-Tan
8	Tan or Brown

Surface Discoloration (SD)	Description
1	No Discoloration (0%)
2	Minimal Discoloration (1-10%)
3	Slight Discoloration (11-20%)
4	Small Discoloration (21-40%)
5	Modest Discoloration (41-60%)
6	Moderate Discoloration (61-80%)
7	Extreme Discoloration (81-100%)

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