Extending the Shelf-life of Beef Steaks Using Acerola Cherry Powder and Rosemary Extract in Order to Remain Competitive in the International Market

A Thesis

Presented in Partial Fulfillment of the Requirements for the

Degree of Master of Science

with a

Major in Animal Science

in the

College of Graduate Studies

University of Idaho

by

Jessie B. Van Buren

Major Professor: Michael Colle, Ph.D.

Committee Members: Phillip Bass, Ph.D.; James Church, M.S.

Department Administrator: Robert Collier, Ph.D.

May 2021

Authorization to Submit Thesis

This thesis of Jessie B. Van Buren, submitted for the degree of Master of Science with a Major in Animal Science and titled "Extending the Shelf-life of Beef Steaks Using Acerola Cherry Powder and Rosemary Extract in Order to Remain Competitive in the International Market" has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____ Date: _____
Michael Colle, Ph.D.

Committee Members: _____ Date: _____
Phillip Bass, Ph.D.

_____ Date: _____
James Church, M.S.

Department Administrator: _____ Date: _____
Robert Collier, Ph.D.

Abstract

Improvements in retail shelf-life of exported beef will help with merchandising and increase the competitiveness of US beef products in the global market. The objectives of these studies were to 1) determine the effect of the topical application of 0.05% acerola cherry powder (C) and 0.10% rosemary extract (R) from various suppliers (1, 2, 3) on beef chuck roll and bone-in short rib steak retail shelf-life and 2) determine the effect of the topical application of 0.05%-0.10% acerola cherry powder and 0.10%-0.20% rosemary extract in combination on beef chuck roll and bone-in short rib steak retail shelf-life. Chuck roll steaks treated with R1 and R2 had a brighter oxygenated lean color than untreated control steaks, while C2 and R2 treated steaks were more uniform than untreated control steaks. R3 improved short rib bone marrow redness and yellowness, and C1 brightened short rib steak oxygenated lean color. When combining antioxidants, chuck roll steaks had improved redness and higher concentrated combinations delayed lipid oxidation. In short rib steaks, antioxidant combinations delayed lipid oxidation and improved oxygenated lean color. Applying topical antioxidants can improve retail shelf-life stability of beef chuck rolls and bone-in short ribs aged for an extended period.

Acknowledgements

First, I would like to thank Dr. Michael Colle, my major professor, for encouraging me to step outside my comfort zone and for mentoring me in the lab as an undergraduate and now as a graduate student. I would also like to thank my committee members, Dr. Phil Bass and Jim Church, for reminding me to always keep the big picture in mind and ensuring research was beneficial and applicable to the cattle and meat industries. Thank you to James Nasados and Kacie Hoffman for your patience and assistance while I was working and learning in the meat lab.

Thank you to my fellow meat science graduate students, Brianna Buseman, Tanya Weber, Jessica Lancaster, Jaxon Smart, and Kayleen Oliver, for your support and guidance during my projects and presentations and for your constant friendship. I would also like to thank the undergraduate students in the Meat Science Lab and the research technicians for your assistance running TBARS for what felt like forever and evaluating color in the cooler so early in the morning. I would like to thank the faculty, staff, and students at the University of Idaho that I have been fortunate enough to call family throughout my time here. The knowledge I gained and experiences I had are irreplaceable.

Funded by The Beef Checkoff. We gratefully acknowledge financial support of this research by the Idaho Beef Council. This work is/was supported by the USDA National Institute of Food and Agriculture, Hatch project W4177 Enhancing the Competitiveness and Value of U.S. Beef Support was also received from the Idaho Experiment Station. Additionally, we appreciate AB Foods for assisting us in procuring product. We are also thankful for the facilities and personnel at the University of Idaho Vandal Brand Meats Lab that made this research possible.

Dedication

Thank you to my family, especially my mom and dad, for your constant love and support throughout school. You prioritized my education above all else, taught me to never stop learning, and inspired my passion for the beef industry.

Lastly, thank you to Dakota for having unlimited patience and always being my rock.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

A	Acerola cherry powder
AMSA	American Meat Science Association
C	Control
C1, C2, C3	Acerola cherry powder 1, 2, 3
FAS	Foreign Agricultural Service
IMPS	Institutional Meat Purchase Specifications
M1, M2, M3, M4	Mixture of antioxidants
MMb	Metmyoglobin
MRA	Metmyoglobin reducing activity
OC	Oxygen consumption
OMb	Oxymyoglobin
R	Rosemary extract
R1, R2, R3	Rosemary extract 1, 2, 3
SV	*Serratus ventralis*
TBARS	Thiobarbituric acid reactive substances
US	United States
USDA	United States Department of Agriculture
USMEF	United States Meat Export Federation

Chapter 1: Review of Literature

Beef Exports

In 2019, 14.1% of United States (US) beef was exported, totaling over $7.4 billion and contributing $308.74 per head. The Republic of Korea was found to be the US beef industry's largest growing market with a 6% increase in value and volume compared to 2018. Additionally, since 2010 the value of the beef exports has more than tripled while the volume has doubled, showing increased value per pound. Over half of Korea's beef imports came from the US. After the introduction of the United States – Korea Free Trade Agreement in 2012, Korean consumption (annual per capita) of beef increased from 22 to 27.5 pounds in 2019 with over one-third of their consumption coming from US beef (USMEF, 2020). The agreement has decreased the cost of exports by decreasing tariffs and other obstacles for US producers. Additionally, the level of beef production in Korea does not support the quickly increasing demand from consumers, and, therefore, must be supplemented with imports. US beef is in high demand in Korea due to its higher degree of marbling and competitive price (USDA – FAS, 2020). In 2020, chilled beef exports grew 29% higher than in 2019, totaling a value of $554 million. Additionally, compared to 2019, in 2020 US beef increased its Korean market share by 2.5% in both chilled beef and total beef (USMEF, 2021). Even though 2019 and 2020 brought economic problems on a global scale, trade values in Korea are expected to continue to increase due to growing household incomes (USDA – FAS, 2020). Beef cuts that are commonly exported to Korea include bone-in short ribs (IMPS 123A) and chuck rolls (IMPS 116A). A major challenge for the beef export market (especially the Korean market) is the transport time of the product. This time is often greater than 28 days and results in poor retail shelf-life of the product, often only 1 day. Korean

retailers have reported concerns to the United States Meat Export Federation regarding discoloration of US beef steaks in their retail case (USMEF, 2020).

Shelf-life

Retail shelf-life of beef encompasses a number of factors including color, lipid oxidation, microbial growth, and fluid loss. Color is the most important determining factor for consumers when purchasing a beef product (Mancini and Hunt, 2005). Lipid oxidation is correlated with myoglobin oxidation and, therefore, has an influence on color (Lawrie and Ledward, 2006). Additionally, lipid oxidation will lead to off flavors and odors of the product (Tarladgis et al., 1960; Greene and Cumuze, 1981). Microbial growth can lead to spoilage including off flavors and odors but can also oxidize meat pigments causing discoloration (Lawrie and Ledward, 2006).

In the United States, product is wet aged in a vacuum package on average for 25.9 days prior to being fabricated for retail display (Martinez et al., 2015). Aging times vary from six to 102 days but aging longer than 26 days would be considered extended aging (Martinez et al., 2015). A benefit of extended aging is the increase in tenderness (Suman et al., 2014). However, previous research has shown extended aging can cause shelf-life issues including color, lipid oxidation, metmyoglobin reducing activity, and oxygen consumption (Colle et al., 2015; 2016; English et al., 2016).

Color

Mancini and Hunt (2005) and Suman and Joseph (2013) have reviewed the chemistry of meat color. Myoglobin is an iron containing, oxygen binding protein in muscles largely responsible for meat color. When beef is first cut, it is dark red or purple in color. At this

time, the myoglobin is in a deoxymyoglobin state, due to the lack of oxygen bound. After being exposed to air, oxygen binds to myoglobin and the beef blooms into a bright cherry red color, which is the oxymyoglobin state and is most appealing to consumers. The rate of bloom depends on pH, temperature, and oxygen levels. Over time, oxygen steals an electron from the iron in myoglobin and the steak enters an oxidized state called metmyoglobin becoming brown in color. Myoglobin oxidation results in steak discoloration (AMSA, 2012). Consumers associate discoloration with the level of freshness of meat, and, therefore, color is the leading factor in meat purchasing decisions (Mancini and Hunt, 2005). Metals can increase the number of oxygen radicals and the rate of oxidation and browning. Antemortem factors that also influence color include: genetics, sex, age, diet, season, and stress (AMSA, 2012). Postmortem factors, such as extended aging, also influence color. *Longissimus lumborum* steaks aged longer than 14 days darkened and were less red throughout retail display (Colle et al., 2015; 2016). Additionally, as *Longissimus lumborum* and *Gluteus medius* aging periods increased, oxygenated lean color darkened and color uniformity lessened, while browning and discoloration increased (Colle et al., 2015; 2016).

Reducing metmyoglobin is a key step in delaying discoloration and extending shelf-life (AMSA, 2012). Metmyoglobin can be reduced from the ferric state to the ferrous state by reducing enzymes. However, this process requires NADH, which unfortunately is constantly decreasing in postmortem muscle (Sammel et al., 2002). Mitochondrial oxygen consumption rate also decreases with aging leading to a reduction in NADH production, an essential factor for reducing metmyoglobin (Mancini & Ramanathan, 2014). Extended aging also decreases metmyoglobin reducing activity and pH (English et al., 2016). Decreases in

mitochondrial function and metmyoglobin reducing activity leads to steak discoloration issues (McKenna et al., 2005).

Gill (1996) suggested that when bone-in products are cut, red blood cells within the marrow are disturbed resulting in a release of hemoglobin that gathers at the surface of the exposed bone marrow. Lanari et al. (1995) suggested that hemoglobin and myoglobin, both iron containing proteins, have similar color states. When iron is oxidized from ferrous (2+) to ferric (3+), the protein enters a methemoglobin state similar to metmyoglobin. This results in the unpleasant, blackened bone appearance. Additionally, Gill (1996) suggested that when bone-in beef cuts are stored or aged for a longer period of time, the bone blackens faster during retail display.

Objective color is measured using a mechanical instrument, and subjective color consists of trained evaluators scoring meat. Objective color is measured in three dimensions, L* (lightness), a* (redness), and b* (yellowness; Mancini et al., 2005). The redness of beef can be used to predict the consumers' acceptance. When a* is greater than 14.5, beef is considered acceptable (Holman et al., 2017). Interestingly, similar results were found in lamb, with consumer acceptance thresholds at a* greater than 14.5. Additionally, lamb with L* values less than 44 were considered unacceptably dark to consumers (Khliji et al., 2010). In bone marrow, a* is the best way to evaluate color stability (Mancini et al., 2004). Subjective color can include a variety of categories including: oxygenated lean color (1 = extremely bright cherry red, 8 = extremely dark red), amount of browning (1 = no evidence of browning, 6 = dark brown), discoloration (1 = none, 5 = extreme), surface discoloration or percent metmyoglobin (1 = no discoloration 0%, 6 = extensive discoloration 81-100%), and surface color uniformity (1 = uniform or no two-toning, 5 = extreme two-toning). Bone

marrow color can also be measured subjectively (1 = bright reddish-pink to red, 7 = black discoloration; AMSA, 2012). Subjective color is commonly scored by 2 or more evaluators (Colle et al., 2015; 2016).

Color stability can also be measured using biochemical processes. First, the level of activity of reducing metmyoglobin to oxymyoglobin can be measured. Meat samples are treated with sodium nitrite for 20 minutes to accelerate the production of metmyoglobin. Samples are vacuum packaged to eliminate oxygen permeability. Color reflectance is measured immediately following packaging and two hours later to determine the percent change in metmyoglobin. If a sample has a high level of metmyoglobin reducing activity, the sample will contain a lower percentage of metmyoglobin following the two hours. Enzymatic activity of metmyoglobin reductase can also be measured using a fast assay involving ferrocyanide and homogenized samples. Although the enzymatic method is faster, measuring reducing activity by comparing two time points (initial and two hours later) is beneficial in determining shelf-life stability. Color stability can also be measured by looking at oxygen consumption. For this analysis, samples are placed in a refrigerated environment and allowed to bloom for two hours. Samples are vacuum packaged and color reflectance is measured immediately and twenty minutes later to determine the percent change in oxymyoglobin. Samples that have smaller changes in oxymyoglobin have lower enzymatic consumption of oxygen due to respiration and are more shelf stable. (AMSA, 2012)

Color stability varies between muscles, as well. A majority of beef bone-in short ribs and chuck rolls, cuts commonly exported to Korea, are made up of the *Serratus ventralis* (SV) muscle. A study done by McKenna et al. (2005) looked at measuring the characteristics of different muscles including the SV, which was classified to have “low” color stability due

to its rapid rate of discoloration during retail display. Additionally, the SV has a quickly decreasing a* (redness), and low metmyoglobin reducing activity. Whereas the *Longissimus dorsi* was classified as having "high" color stability. Another study by Von Seggern et al. (2005) evaluated differences between muscles in the chuck. Muscles evaluated that can be found in a chuck roll include: *Complexus*, *Longissimus dorsi*, *Multifidus/Spinalis dorsi*, *Rhomboideus*, SV, and *Splenius*. After being exposed to oxygen for an hour, the *Longissimus dorsi* was similar in lightness (L*) to the *Complexus*, *Rhomboideus*, SV, and *Splenius*. The *Multifidus/Spinalis dorsi* was significantly darker than the rest of the muscles. Additionally, the *Longissimus dorsi* was similar in redness (a*) to the *Complexus*, *Multifidus/Spinalis dorsi*, and SV. The *Rhomboideus* and *Splenius* were significantly less red than the rest of the muscles. The number of muscles present in chuck roll steaks causes a uniformity challenge due to the varying color stabilities of the individual muscles.

Muscle pH influences steak color and varies between muscles. A lower pH often results in lighter colored meat due to increased light reflectance on the steak surface. A higher pH is associated with a darker color due to increased water holding capacity in proteins (Lawrie and Ledward, 2006). The *Splenius* has a low pH (5.55), whereas the SV and *Multifidus/Spinalis dorsi* have a high pH (5.92 and 5.97, respectively) compared to the remainder of the muscles in the chuck roll that have a moderate pH (5.69-5.76; McKenna et al., 2005; Von Seggern et al., 2005). Therefore, the SV in short ribs and chuck rolls is generally darker than the *Longissimus lumborum* found in rib steaks due to its higher pH.

Rapid discoloration of short rib and chuck roll steaks has reduced the shelf-life to around one day. Due to the steak discoloration challenges, Korean retailers have been hesitant to purchase more US originating chuck roll and short rib steaks than could be sold in

one day. Unfortunately, this means that if retailers run out of steaks in the early afternoon, they would be unable to restock for the dinner rush, a lost opportunity for US beef sales (USMEF, 2020).

Lipid Oxidation

Lipids found in meat marbling and within cell membranes auto-oxidize after being exposed to oxygen. Lipids lose a hydrogen, become free radicals, and further oxidation produces peroxides (Murray et al., 2006). These products cause off-flavors and odors, which can be detected by consumers at thiobarbituric acid reactive substance (TBARS) values greater than 1.0 (Tarladgis et al., 1960; Greene and Cumuze, 1981). Oxidation can be accelerated by high temperatures, metals, and light (Lawrie and Ledward, 2006; Murray et al., 2006). Lipid oxidation increased with longer aging periods in the *Gluteus medius*, *Biceps femoris*, *Semimembranosus*, and *Longissimus lumborum* (Colle et al., 2015; 2016; Mancini & Ramanathan, 2014). Rate of oxidation varies by muscle. For example, the SV has been found to have delayed lipid oxidation during retail display (McKenna et al., 2005). Increased lipid oxidation leads to a faster rate of discoloration, or oxidation of myoglobin, and a shorter shelf-life at the retail case (Lawrie and Ledward, 2006).

Lipid oxidation is measured two ways in meat products. Both include using thiobarbituic acid reactive substances during which thiobarbituric acid reacts with malonaldehyde, a byproduct of lipid oxidation, resulting in a pink chromogen that can be measured using a spectrophotometer. The first method is called the “rapid, wet method.” This method utilizes a hot water bath to accelerate the reaction and is a relatively quick method but can result in incorrect measurements. The acid can react with sugars within the oxidized meat to produce a yellow chromogen, resulting in an incorrect evaluation of further

oxidized meat than the pink chromogen samples. The second method that avoids this issue is the "distillation method." The sample is heated until steam containing isolated malonaldehyde is produced. Acid is added to the collected distillate to change the color of the oxidized samples. Unfortunately, this method requires more time and is, therefore, utilized less frequently. (AMSA, 2012)

Microbial Growth

Throughout the carcass fabrication process, steps are put in place to reduce the microbial load. Steps include a carcass lactic acid bath and/or a hot water rinse, as well as proper sanitation of people, knives, tables, and other tools prior to use (Lawrie and Ledward, 2006). Unfortunately, the application of lactic acid or acetic acid can lower the pH of the meat to the point where myoglobin oxidation is stimulated leading to a decrease in redness during retail display (Mancini and Hunt, 2005). Microbes can lead to a myriad of shelf-life issues from discoloration to off flavors to lethal toxins (Lawrie and Ledward, 2006). Depending on the microorganism, some require environments with increased oxidation and others require increased reducing activity, and the microbes can manipulate the environment to match their needs (Aberle et al., 2012). Microbes can oxidize oxymyoglobin to metmyoglobin, bind oxymyoglobin to hydrogen sulfide to produce a green pigment, or breakdown myoglobin to yellow or green pigments. Other pigments include pink, black, white, and blue-green. Off odors are produced by breaking down proteins, whereas off flavors are produced by degrading sugars (Lawrie and Ledward, 2006). As aging time increases, the load of aerobic microbes increases, which can lead to a faster rate of discoloration (Colle et al., 2015; 2016).

Microbial load can be analyzed through five common steps. First a sample is taken from the meat typically by swabbing the surface. Next samples are diluted and then plated in a dish containing a growth medium. Plates are incubated to allow for microbial growth and finally counted. A quicker test, enzyme-linked immunosorbent assay (ELISA), has been developed for detecting certain hazardous microbes, such as Salmonella and Listeria. Additionally, with the advancement of technology, microbial DNA can be analyzed to identify specific species (Aberle et al., 2012).

Fluid Loss

Postmortem, a carcass continues to generate energy as long as glycogen and NADH are available. However, due to a lack of oxygen present, cells produce energy anaerobically. A buildup of lactic acid within the meat causes the pH to decrease. As a carcass nears the meat isoelectric point (5.2), proteins denature resulting in a lessened capacity to hold water. This water is evaporated or drips off the carcass. Furthermore, when meat is cut, cell disruption results in more fluid losses. Processors work to avoid the loss of product due to fluid loss and the resulting loss in profit. Additionally, at the retail counter accumulation of fluid in packages can be unsightly to customers and result in a drier cooked product for the consumer. (Lawrie and Ledward, 2006)

Factors antemortem and postmortem can influence fluid loss. Genetics, muscle type, stress, and marbling also play a role in fluid losses. Stress in Type II muscle fibers prior to harvest can result in increased fluid loss and meat referred to as Pale, Soft, and Exudative, or PSE. Interestingly, stress in Type I muscle fibers decreases fluid loss and results in a Dark, Firm, and Dry, or DFD, meat. Postmortem processing and cooler conditions can influence the loss, as well. Higher cooler temperatures or slow freezing times denatures proteins and

decreases water holding capacity. As aging time increases, fluid loss decreases during retail display. This is hypothesized to be due to an increase in the net charge of proteins after releasing calcium (2+) and absorbing potassium (1+). Unfortunately, the common fabrication technique of cutting meat perpendicular to the muscle fiber direction increases fluid loss. Other retail display practices that influence fluid loss include tightness of overwrapping and weight placed on the surface of the meat. (Lawrie and Ledward, 2006)

Fluid loss can be measured during aging as storage losses or during retail display. Fluid loss in each stage can be measured similarly. An initial weight is taken prior to storage or display, and a final weight is taken at the end. The percent fluid loss is calculated using a basic percent change formula (Colle et al., 2016).

Antioxidants

One strategy to improve shelf-life is the use of antioxidants as either a nutrition supplement or post-harvest treatment (Faustman et al., 1989; Lanari et al., 1995; Liu et al., 1996; Mancini et al., 2004; Ismail et al., 2009; Kim et al., 2013; Gómez et al., 2016; Zhang et al., 2016). Internationally, US beef has been at a disadvantage to competition such as Australian beef, which has a longer shelf-life (> 3 days) due to its shorter transport time (USMEF, 2020). Additionally, Australian beef has higher levels of antioxidants already present in the product due to their feeding practices. Antioxidants can work to delay lipid oxidation or myoglobin oxidation to stabilize color and lipids (Murray et al., 2006). Adding Vitamin E to Holstein steers' diets decreased lipid oxidation and metmyoglobin accumulation in ground sirloin after six days of retail display (Faustman et al., 1989). Supplementing α-tocopherol in cattle diets has been shown to increase redness (a*) and decrease yellowness (b*) of the *Longissimus lumborum*, *Semimembranosus*, and *Gluteus*

medius (Liu et al., 1996). Supplementing Vitamin E in swine diets has also been shown to improve lumbar vertebrae color stability as well as the *Longissimus lumborum* (Lanari et al., 1995). Currently, in US beef production systems Vitamin E is not commonly included in cattle diets.

A possible solution to keep US beef competitive in the market is the application of antioxidants to the surface of the steaks. Metal chelators and radical scavenging antioxidants can prevent the creation of metmyoglobin and, therefore, browning (AMSA, 2012). The synthetic Vitamin C antioxidant, ascorbic acid, delayed the decrease in redness of ground beef throughout retail display (Ismail et al., 2009). High concentrations of ascorbic acid (1.5% and 2.5%) delayed the graying of bone marrow in lumbar vertebrae from 1 day to 3 days (Mancini et al., 2004). In the meat industry, ground product has been mixed with synthetic versions of antioxidants, but recently there has been a push by domestic and international consumers to utilize naturally occurring antioxidants (Oswell et al., 2018). Natural plant extracts, such as butterbur and broccoli, have acted as antioxidants and improved the shelf-life of fresh beef patties, including lipid oxidation and fresh meat color (Kim et al., 2013). When grape seed extract was incorporated into ground beef patties, lipid oxidation during retail display was delayed (Gómez et al., 2016). Additionally, in raw ground beef, mulberry leaf extract maintains retail color and decreased lipid oxidation (Zhang et al., 2016). It was recently found that topical application of acerola cherry powder and rosemary extract improved bone marrow color in beef bone-in short ribs and surface discoloration in beef chuck rolls (Van Buren et al., 2020; 2021).

Antioxidants containing Vitamin C or ascorbic acid, such as acerola cherry powder, are chelators and oxygen scavengers. Ascorbic acid extends meat shelf-life by delaying

myoglobin oxidation. Oxidation occurs when oxygen steals an electron from iron bound to oxymyoglobin making the meat enter a metmyoglobin state. Ascorbic acid prevents this step by acting as an oxygen radical quencher. Additionally, ascorbic acid works as a reducing agent to reverse the oxidation of metals, such as iron (Murray et al., 2006). However, in low concentrations, ascorbic acid acts like a meat pro-oxidant by reducing iron from the ferric to ferrous state, a powerful pro-oxidant (Buettner and Jerkiewicz, 1996).

Antioxidants containing Vitamin E or phenolic compounds similar to tocopherol, such as rosemary extract, are radical quenchers that work to delay lipid oxidation and extend shelf-life. Tocopherols react with lipid peroxide radicals created through the peroxidation of fatty acids. The products, tocopheroxyl radicals, are less reactive and damaging. However, in high concentrations Vitamin E can also act as a pro-oxidant due to a tocopheroxyl radical's ability to enter deeper into a cell (Murray et al., 2006).

Utilizing Vitamin C and E antioxidants in combination can increase their effectiveness. Vitamin C reduces tocopheroxyl radicals back to tocopherol to be reused. The products, monodehydroascorbate radicals, become ascorbate, a nonradical (Murray et al., 2006). Applying combinations of antioxidants (0.05% ascorbic acid + 0.10% rosemary extract) to *Longissimus lumborum* in a retail setting, decreased browning and brightened oxygenated lean color (Colle et al., 2019). When comparing antioxidant combinations, a mixture of rosemary (500 ppm) and Vitamin C (500 ppm) was found to be the most effective at increasing the shelf-life of *Longissimus dorsi* beef steaks packaged in a modified atmosphere. Comparisons were made against Vitamin C, taurine + Vitamin C, and α-tocopherol + Vitamin C. The shelf-life was extended by 10 days due to the delay in lipid and myoglobin oxidation (Djenane et al., 2002).

Summary

Beef exports contribute to US beef producer profits, and, therefore, it is imperative to maintain a highly desirable product for international retailers. Aging beef for an extended period of time during shipping has resulted in decreased shelf-life stability in Korean retail displays. Shelf-life components include steak color and oxidation of lipids, as discoloration and off flavors and odors are unappealing to consumers. Finding a possible solution for eliminating these shelf-life issues is a top priority for the United States Meat Export Federation. Commercial utilization of antioxidants in ground product and recent research topically applying antioxidants to steaks demonstrates the availability of possible solutions.

The objectives of this research were to 1) determine the effect of the topical application of 0.05% acerola cherry powder (C) and 0.10% rosemary extract (R) from various suppliers (1, 2, 3) on beef chuck roll and bone-in short rib steak retail shelf-life and 2) determine the effect of the topical application of 0.05%-0.10% acerola cherry powder and 0.10%-0.20% rosemary extract in combination on beef chuck roll and bone-in short rib steak retail shelf-life. We hypothesized that utilizing acerola cherry powder and rosemary extract in combination at higher concentrations would improve the color and lipid oxidation, important shelf-life components, of beef aged for an extended period of time.

References

Aberle, E. D., J. C. Forrest, D. E. Gerrard, and E. W. Mills. 2012. 5th ed. Kendall Hunt Publishing Company. Dubuque, IA.

American Meat Science Association. 2012. Meat color measurement guidelines. 2nd ed. Am. Meat Sci. Assoc. Chicago, IL.

Buettner, G. R., and B. A. Jurkiewicz. 1996. Catalytic metals, ascorbate and free radicals: Combinations to avoid. Radiat. Res. 145:532-541. doi:10.2307/3579271.

Colle, M. C., R. P. Richard, K. M. Killinger, J. C. Bohlscheid, A. R. Gray, W. I. Loucks, R. N. Day, A. S. Cochran, J. A. Nasados, and M. E. Doumit. 2015. Influence of extended aging on beef quality characteristics and sensory perception of steaks from the gluteus medius and longissimus lumborum. Meat Sci. 110:32–39. doi:10.1016/j.meatsci.2015.06.013.

Colle, M. C., R. P. Richard, K. M. Killinger, J. C. Bohlscheid, A. R. Gray, W. I. Loucks, R. N. Day, A. S. Cochran, J. A. Nasados, and M. E. Doumit. 2016. Influence of extended aging on beef quality characteristics and sensory perception of steaks from the biceps femoris and semimembranosus. Meat Sci. 119:110–117. doi:10.1016/j.meatsci.2016.04.028.

Colle, M. C., R. P. Richard, M. C. Colle, W. I. Loucks, G. K. Murdoch, P. D. Bass, C. J. Williams, and M. E. Doumit. 2019. Retail display properties and consumer perception of extended aged beef topically treated with ascorbic acid and rosemary extract. Meat and Muscle Biol. 3(1):42–50. doi:10.22175/mmb2018.05.0011.

Djenane, D., A. Sánchez-Escalante, J. A. Beltrán, and P. Roncalés. 2002. Ability of α-tocopherol, taurine and rosemary, in combination with vitamin C, to increase the oxidative stability of beef steaks packaged in modified atmosphere. Food Chem. 76:407-415. doi:10.1016/S0308-8146(01)00286-2.

English, A. R., G. G. Mafi, D. L. VanOverbeke, and R. Ramanathan. 2016. Effects of extended aging and modified atmospheric packaging on beef top loin steak color. J. Anim. Sci. 94:1727–1737. doi:10.2527/jas.2015-0149.

Faustman, C., R. G. Cassens, D. M. Schaefer, D. R. Buege, S. N. Williams, and K. K. Scheller. 1989. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. J. Food Sci. 54(4):858–862. doi:10.1111/j.1365-2621.1989.tb07899.x.

Gill, C. O. 1996. Extending the storage life of raw chilled meats. Meat Sci. 43:99-109. doi: 10.1016/0309-1740(96)00058-7.

Gómez, I., M. J. Beriain, J. A. Mendizabal, C. Realini, and A. Purroy. 2016. Shelf life of ground beef enriched with omega-3 and/or conjugated linoleic acid and use of grape seed extract to inhibit lipid oxidation. Food Sci. & Nutrition. 4(1):67-79. doi:10.1002/fsn3.251.

Greene, B. E., and T. H. Cumuze. 1981. Relationship between TBA numbers and inexperienced panelists' assessment of oxidized flavor in cooked beef. J. Food Sci. 47:52–54, 58. doi:10.1111/j.1365-2621.1982.tb11025.x.

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 Sci. 127:57–62. doi:10.1016/j.meatsci.2017.01.005.

Ismail, H. A., E. J. Lee, K. Y. Ko, H. D. Paik, and D. U. Ahn. 2009. Effect of antioxidant application methods on the color, lipid oxidation, and volatiles of irradiated ground beef. J. Food Sci. 74(1):25-32. doi:10.1111/j.1750-3841.2008.00991.x.

Khliji, S., R. van de Ven, T. A. Lamb, M. Lanza, and D. L. Hopkins. 2010. Relationship between consumer ranking of lamb colour and objective measures of colour. Meat Sci. 85(2):224-229. doi:10.1016/j.meatsci.2010.01.002.

Kim, S. J., S. C. Min, H. J. Shin, Y. J. Lee, A. R. Cho, S. Y. Kim, and J. Han. 2013. Evaluation of the antioxidant activities and nutritional properties of ten edible plant extracts and their application to fresh ground beef. Meat Sci. 93:715-722. doi:10.1016/j.meatsci.2012.11.029.

Lanari, M. C., D. M. Schaefer, and K. K. Scheller. 1995. Dietary vitamin E supplementation and discoloration of pork bone and muscle following modified atmosphere packaging. Meat Sci. 41(3):237–250. doi:10.1016/0309-1740(95)00006-7.

Lawrie, R. A., and D. A. Ledward. 2006. Lawrie's Meat Science. 7th Ed. Woodhead Publishing Limited. Cambridge, England.

Liu, Q., K. K. Scheller, S. C. Arp, D. M. Schaefer, and M. Frigg. 1996. Color coordinates for assessment of dietary vitamin E effects on beef color stability. J. Anim. Sci. 74:106-116. doi:10.2527/1996.741106x.

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

Mancini, R. A., and R. Ramanathan. 2014. Effects of postmortem storage time on color and mitochondria in beef. Meat Sci. 98:65-70. doi:10.1016/j.meatsci.2014.007.

Mancini, R. A., M. C. Hunt, K. A. Hachmeister, D. H. Kropf, and D. E. Johnson. 2004. Ascorbic acid minimizes vertebrae discoloration. Meat Sci. 68(3):339-345. doi:10.1016/j.meatsci.2004.03.017.

Martinez, H. A., A. N. Arnold, J. C. Brooks, C. C. Carr, K. B. Gehring, D. B. Griffin, D. S. Hale, G. G. Mafi, D. D. Johnson, C. L. Lorenzen, R. J. Maddock, R. K. Miller, D. L. VanOverbeke, B. E. Wasser, and J. W. Savell. 2017. National beef tenderness survey–2015: Palatability and shear force assessments of retail and foodservice beef. Meat and Muscle Biol. 1:138-148. doi:10.22175/mmb2017.05.0028.

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 Sci. 70:665–682. doi:10.1016/j.meatsci.2005.02.016.

Murray, R. K., D. K. Granner, and V. W. Rodwell. 2006. Harper's Illustrated Biochemistry. 27th Ed. McGraw-Hill Companies, Inc. New York, NY.

Oswell, N. J., H. Thippareddi, and R. B. Pegg. 2018. Practical use of natural antioxidants in meat products in the U.S.: A review. Meat Sci. 145:469-479. doi:10.1016/j.meatsci.2018.07.020.

Sammel, L. M., M. C. Hunt, D. H. Kropf, K. A. Hachmeister, and D. E. Johnson. 2002. Comparison of assays for metmyoglobin reducing ability in beef inside and outside semimembranosus muscle. J. of Food Sci. 67:978-984. doi:10.1111/j.1365-2621.2002.tb09439.x.

Suman, S. P., and P. Joseph. 2013. Myoglobin chemistry and meat color. Annual Review of Food Sci. and Tech. 4(1):79–99. doi:10.1146/annurev-food-030212-182623.

Suman, S. P., M. C. Hunt, M. N. Nair, and G. Rentfrow. 2014. Improving beef color stability: Practical strategies and underlying mechanisms. Meat Sci. 98(3):490-504. doi:10.1016/j.meatsci.2014.06.032.

Tarladgis, B. G., B. M. Watts, and M. T. Younathan. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37:44–48. doi:10.1007/BF02630824.

USDA – FAS. 2020. 2019 United States Agricultural Export Yearbook: South Korea 2019 Export Highlights. https://www.fas.usda.gov/south-korea-2019-export-highlights. (Accessed 4 January 2021.)

USMEF. 2020. U.S. Meat Export Federation Annual Report 2019. https://www.usmef.org/downloads/2019-USMEF-Annual-Report.pdf. (Accessed 22 January 2020.)

USMEF. 2021. U.S. Meat Export Federation Annual Report 2020. https://www.usmef.org/downloads/2020-USMEF-Annual-Report.pdf. (Accessed 14 January 2021.)

Van Buren, J. B., B. J. Buseman, T. M. Weber, J. A. Nasados, J. M. Lancaster, J. H. Smart, P. D. Bass, and M. J. Colle. 2020. Extending the shelf-life of beef chuck roll steaks using acerola cherry powder and rosemary extract. Proc. 2020 International Congress of Meat Science and Technology and Reciprocal Meat Conf., 120.

Van Buren, J. B., B. J. Buseman, T. M. Weber, J. A. Nasados, J. M. Lancaster, J. H. Smart, P. D. Bass, and M. J. Colle. 2021. Extending the shelf-life of beef bone-in short rib steaks using acerola cherry powder and rosemary extract. Proc. 2021 American Society of Animal Science Midwest Section Meeting.

Von Seggern, D. D., C. R. Calkins, D. D. Johnson, J. E. Brickler, and B. L. Gwartney. 2005. Muscle profiling: Characterizing the muscles of the beef chuck and round. Meat Sci. 71:39–51. doi:10.1016/j.meatsci.2005.04.010.

Zhang, X., D. Li, Q. Meng, C. He, and L. Ren. 2016. Effect of mulberry leaf extracts on color, lipid oxidation, antioxidant enzyme activities and oxidative breakdown products of raw ground beef during refrigerated storage. J. Food Quality. 39:159-170. doi:10.1111/jfq.12187.

Chapter 2: Extending the shelf-life of beef chuck roll steaks using acerola cherry powder and rosemary extract

Submitted to *Meat and Muscle Biology*

Abstract

Improvements in retail shelf-life of exported beef will help with merchandising and increase competitiveness in the worldwide market for US beef products. The objective of this study was to determine the effect of the topical application of acerola cherry powder and rosemary extract from various suppliers on beef chuck roll steak retail shelf-life, focusing on color stability and lipid oxidation. Beef chuck rolls (IMPS 116A; N = 8) from USDA Choice carcasses were purchased from a commercial harvest facility and aged (0°C) for 28 d post-fabrication. Following aging, steaks were cut 1.02 cm-thick and systematically assigned to a treatment based on subprimal location. Treatments included: untreated control (C), topically sprayed (2ml) with an acerola cherry powder solution (0.05%) from one of three suppliers (C1, C2, C3), or topically sprayed (2ml) with a rosemary extract solution (0.10%) from one of three suppliers (R1, R2, R3). Half of the steaks were assigned by location to d 0 lipid oxidation, metmyoglobin reducing activity, and oxygen consumption rate; the remaining steaks were assigned to 4 days of retail display followed by d 4 lipid oxidation and metmyoglobin reducing activity. Throughout retail display, objective and subjective color was measured at h 1, h 7, and every 12 hours afterward until h 91. There was an interaction observed between retail display time and treatment ($P = 0.018$) for a* (redness). In general, throughout retail display redness decreased, but steaks treated with C3 were numerically the

reddest treatment at h 19, 43, and 55. Steaks treated with R1 and R2 had a brighter oxygenated lean color ($P = 0.002$) than control steaks, while C2 and R2 treated steaks were more uniform ($P = 0.036$) than control steaks. Applying topical antioxidants can improve retail shelf-life stability of beef chuck rolls aged for an extended period.

Keywords: antioxidants, extended aging, shelf-life, beef

Introduction

According to the United States (US) Meat Export Federation (USMEF), in 2019, 14.1% of US beef was exported, contributing over $7.4 billion of revenue, averaging $308.74 per head. The Republic of Korea (South Korea) was identified to be the US beef industry's largest growing export market due to growing household incomes and insufficient beef production (USMEF, 2020; 2021; USDA – FAS, 2020).

Currently, it takes a minimum of 28 d to ship chilled beef from the US to South Korea (USMEF, 2020). Colle et. al (2015; 2016) determined extended aging can cause product quality issues, such as increased lipid oxidation, darkened lean color (L*), and reduced redness (a*) throughout retail display. Extended aging also has been shown to decrease metmyoglobin reducing activity and pH (English et al., 2016), as well as mitochondrial oxygen consumption rate (Mancini and Ramanathan, 2014). Further, much of the chuck roll steak contains the *Serratus ventralis* (SV) muscle, which has been classified as having low color stability (McKenna et al., 2005).

A potential solution to improve US beef shelf-life is the application of antioxidants to the surface of the steaks. Determining a method to improve the color stability of US beef in

international markets requiring lengthy shipping could provide the opportunity for increased international demand for US beef exports, and producer profits. The objective of this study was to determine the effect of the topical application of acerola cherry powder and rosemary extract from various suppliers on beef chuck roll steak shelf-life including color and lipid oxidation.

Methods and Materials

Product preparation

Beef chuck rolls (IMPS 116A; N = 8) from USDA Choice carcasses were purchased from a commercial harvest facility and transported under refrigeration (3.5 h; 4°C) to the University of Idaho Meat Laboratory. Subprimals were wet-aged at 0°C for 28 d to simulate trans-Pacific shipping time prior to being prepared for retail display. Following aging, chuck rolls were thinly cut into 1.02 cm-thick steaks (N = 126) to mimic South Korean retail steak fabrication style. Steaks were equally assigned based on location within each subprimal to a treatment group: untreated control (C), topically sprayed (2 ml) with a 0.05% acerola cherry powder solution from one of three commercially available suppliers [C1 (Fortium® A Dry, Kemin Industries, Des Moines, IA), C2 (Micro Ingredients Acerola Cherries Organic Vitamin C Powder, Montclair, CA), or C3 (Acerola Cherry Powder, Pure Organic Ingredients, American Fork, UT)], or topically sprayed (2 ml) with a 0.10% rosemary extract solution from one of three commercially available suppliers [R1 (Fortium® R10 Dry, Kemin Industries, Des Moines, IA), R2 (Herbalox® Rosemary Extract, Kalsec, Kalamazoo, MI), or R3 (GUARDIAN® Rosemary Extract 08, Danisco, Madison, WI)]. Half of the steaks were

then assigned by location to d 0 lipid oxidation, metmyoglobin reducing activity (MRA), and oxygen consumption (OC) rate; the remaining steaks were assigned to 4 days of retail display followed by d 4 lipid oxidation and MRA. Steaks were displayed in a retail display room at 2°C. The display room was equipped with natural white 4000 W lights, and the average light intensity was 849 lux (Fisherbrand Traceable Dual-Range Light Meter, Fisher Scientific, Waltham, MA).

Retail fluid loss

Following topical application on d 0, retail display steaks were weighed, placed in white foam trays (CKF Inc. #88142, Langley, BC, Canada), and overwrapped with an oxygen permeable PVC film (oxygen transmission rate: 1,450 cc/645 cm^2 per 24 h; water vapor transmission rate: 17.0 g/645 cm^2 per 24 h; Koch Industries, Inc. #7500-3815, Wichita, KS). After 4 d of retail display, steaks were removed from the retail packaging and re-weighed to determine retail fluid loss. Percent retail fluid loss was calculated using the following equation:

$$\%\, Fluid\ Loss = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} \times 100\%$$

Retail color

Retail display steaks were allowed to bloom for 60 minutes, and then two objective color measurements per steak were obtained on the SV muscle using a Nix Pro 2 Sensor (Nix Sensor Ltd., Hamilton, Ontario, Canada). The Nix Pro 2 Sensor was equipped with a 14 mm-diameter measuring area and a 2° standard observer. The instrument was set to Illuminant D_{65} and Commission International de l'Eclairage (CIE) L* (lightness), a*

(redness), and b* (yellowness) values were recorded. After the initial reading on d 0 of retail display, color was measured a second time 6 h later. Subsequent color measurements were taken twice daily (06:00 and 18:00) on d 1, 2, and 3, and once (06:00) on d 4. Oxygenated lean color, discoloration, surface discoloration, color uniformity, and amount of browning were measured twice daily by six subjective evaluators following American Meat Science Association guidelines (AMSA, 2012). To avoid potential effects due to display location, steaks were rotated in the retail environment daily.

Metmyoglobin reducing activity

Nitric oxide MRA was measured after treatment on d 0 and on d 4 of retail display on the SV muscle within the chuck roll steak following protocols outlined in Section XI of the Meat Color Measurement Guidelines (AMSA, 2012). Color measurements were obtained using a Hunter MiniScan EZ (Restin, Virginia) equipped with a 25 mm-diameter measuring area and a 10° standard observer. The instrument was set to Illuminant A and reflectance from 400 to 700 nm was recorded. Calibration occurred by measuring against black and white calibration tiles prior to measuring color. The percentage of metmyoglobin (MMb) was calculated following equations in Section XI of the Meat Color Measurements Guidelines (AMSA, 2012). MRA was calculated as follows:

$$MRA = \left[\frac{Initial\ \%\ MMb - Final\ \%\ MMb}{Initial\ \%\ MMb}\right] \times 100$$

Oxygen consumption

Oxygen consumption was measured on the SV muscle within the chuck roll steak after treatment on d 0 following protocols outlined in the Meat Color Measurement

Guidelines (AMSA, 2012). Color measurements were taken using a Hunter MiniScan EZ (Restin, Virginia) equipped with a 25 mm-diameter measuring area and a 10° standard observer. The instrument was set to illuminant A and reflectance from 400 to 700 nm was recorded. Calibration occurred by measuring against black and white calibration tiles prior to measuring color. The percentage of oxymyoglobin (OMb) was calculated following equations in Section XI of the Meat Color Measurements Guidelines (AMSA, 2012). OC was calculated as follows:

$$OC = \left[\frac{Initial\ \%\ OMb - Final\ \%\ OMb}{Initial\ \%\ OMb}\right] \times 100$$

Lipid oxidation

Thiobarbituric acid reactive substances (TBARS) were analyzed in duplicate on d 0 after treatment and on d 4 of retail display following the protocol in Section XI, Appendix O of the Meat Color Measurement Guidelines (AMSA, 2012). Samples were excised from the SV muscle within the chuck roll steak avoiding the steak edge and large pieces of fat and connective tissue and weighed 1 g.

Statistical analysis

Data was collected on 8 subprimals and 63 steaks per antioxidant treatment-retail display time combination. Data were analyzed using mixed model analysis of variance. Antioxidant treatments, retail display time, and their interaction were assumed as fixed effects. In this factorial design, there were six antioxidant treatments and one control treatment. MRA and lipid oxidation were evaluated at two retail display time points, whereas color was analyzed at nine retail display time points. Retail display time was

considered a repeated measure modeled as a compound symmetric correlation structure. Subjective color data were averaged based on retail display time, evaluator, and antioxidant treatment prior to analysis, where evaluator was considered a random block effect in a randomized complete block design. Objective color data were analyzed with subprimal and steak location within subprimal as randomized block effects. Treatment least square means differences were assessed through pair-wise comparisons for significant effects. Significance was determined at $P < 0.05$. All statistical analyses were conducted using SAS V 9.4 (SAS Inc., Cary, NC).

Results and Discussion

Retail fluid loss

Antioxidants did not impact fluid loss in the steaks throughout retail display (Table 2.1; $P = 0.372$) similar to Colle et al. (2019). The overall average retail display fluid loss was 2.21%, which is slightly higher than steaks from *Longissimus lumborum*, *Gluteus medius*, *Biceps femoris*, and *Semimembranosus* aged for an extended period of time observed in previous studies (Colle et al., 2015; 2016; 2019). Cutting the steaks thinner (1.02 cm) than traditional fabrication (2.54 cm) may have increased fluid loss, as greater surface area has been associated with increased fluid loss (Aberle et al., 2012).

Retail objective color

An interaction between retail display time and antioxidant treatment was not observed for L* ($P = 0.607$) or b* ($P = 0.591$), but there was an interaction between treatment and time for a* ($P = 0.018$; Table 2.2). In general, across all treatments, steak redness declined, or

became less red, over time of retail display. This is consistent with Gómez et al. (2016) who found redness decreased over retail display in ground beef patties regardless of antioxidant treatments. Additionally, McKenna et al. (2005) found that redness declined during retail display in the SV muscle. At 1 h, 31 h and 91 h of retail display, a* values did not differ between treatments. The control steaks were the least red treatment at h 7. C3 treated steaks were numerically the reddest treatment at h 19, 43, and 55. Additionally, C3 treated steaks were not statistically less red than the control steaks at any time during retail display. Because of the ability of ascorbic acid, the active ingredient of acerola cherry powder, to donate electrons to prevent the myoglobin oxidation process (Buettner and Jerkiewicz, 1996) and based on previous research of ascorbic acid in ground beef (Ismail et al., 2009), it was anticipated that acerola cherry powder would improve redness more than rosemary extract treated steaks and control steaks. All treatments throughout the entire retail display had redness values greater than 14.5, which is the threshold for consumers' acceptability (Holman et al., 2017). Antioxidant treatments differed in L* ($P < 0.001$) but not b* ($P = 0.186$; Table 2.1). Steaks treated with C3 were darker (0.79 L* units) than the control steaks, which would likely be noticeable by customers at a retail case. Steak L* ($P < 0.001$) and b* ($P < 0.001$) values differed over time of retail display (Table 2.3). Steaks were lightest 19 h into retail display and darkest after 43 h. Steaks were yellowest between 1 and 7 h of retail display. This observation is consistent with previous research where aged steaks darkened and declined in yellowness during retail display (Colle et al., 2015; 2016).

Retail subjective color

Antioxidant treatments did influence chuck roll steak oxygenated lean color ($P = 0.002$), surface discoloration ($P = 0.027$), and steak uniformity ($P = 0.036$; Table 2.1).

Interestingly, R1 and R2 treated steaks had the brightest oxygenated lean color, outperforming the acerola cherry powder antioxidants. It has been found that in small amounts, ascorbic acid, found in acerola cherry powder, can act as a pro-oxidant of meat by reducing ferric iron to a strong pro-oxidant, ferrous iron (Buettner and Jerkiewicz, 1996). Steaks treated with C2 and R2 were more uniform, or had less two-toning, than the control steaks. Chuck roll steaks contain many muscle groups each with differing discoloration rates including: *Complexus*, *Longissimus dorsi*, *Multifidus/Spinalis dorsi*, *Rhomboideus*, SV, and *Splenius*. As previously stated, the SV is characterized as a "low color stability" muscle, whereas the *Longissimus thoracis* muscle is considered "high color stability" (McKenna et al., 2005). Unfortunately, since the uniformity improvements between treatments are so minimal numerically, customers would not be able to detect a difference. There was not a difference between treatments in amount of browning ($P = 0.362$) or discoloration ($P = 0.194$; Table 1). Throughout retail display, oxygenated lean color ($P < 0.001$), amount of browning ($P < 0.001$), discoloration ($P < 0.001$), surface discoloration ($P < 0.001$), and uniformity ($P < 0.001$) values differed (Table 2.3). Steaks had the brightest oxygenated lean color and were most uniform in color at the initial evaluation. Steaks had the least amount of browning, discoloration, and surface discoloration at the initial evaluation and after 7 h of retail display. For all subjective color measurements, steaks had the least favorable evaluation after 4 d of retail display. Throughout retail display, lean color brightness and color uniformity decreased, whereas amount of browning, discoloration, and surface discoloration increased. This observation is consistent with previous research where aged steaks discolored during retail display (Colle et al., 2015; 2016; English et al., 2016).

Metmyoglobin reducing activity

There was an interaction observed between retail display and treatment in MRA ($P < 0.001$; Figure 2.1). On d 0 of retail display, steaks treated with C1, C2, C3, R2, and R3 all had a higher, or more desirable, MRA than the control steaks. However, on d 4 antioxidant treated steaks did not have improved MRA compared to the control steaks. Measuring MRA daily throughout retail display could determine the number of days that antioxidant treated steaks had superior MRA. As expected, all treatments decreased in MRA from d 0 to d 4. MRA decreases over time due to decreasing NADH levels postmortem and causes increased levels of metmyoglobin (Sammel et al., 2002; AMSA, 2012). Additionally, as aging time increases, initial MRA decreases creating further challenges (English et al., 2016). Finding a solution to increase MRA on d 4 would improve color stability of meat by delaying the onset of browning.

Oxygen consumption

Treatments did not impact OC ($P = 0.053$; Table 2.1). The ability to maintain moderate OC throughout retail display could stabilize color by regenerating NADH levels for MRA without reducing the oxygen partial pressure at the surface (Sammel et al., 2002).

Lipid oxidation

No retail display by treatment interaction ($P = 0.829$) was observed. Furthermore, a difference in lipid oxidation between treatments ($P = 0.276$) was not observed (Table 2.1). Similar results were found in the *Semimembranosus* and *Longissimus lumborum* when ascorbic acid and rosemary extract were topically applied (Colle et al., 2019). Other previous research has shown a delay in lipid oxidation; however, in these studies the

antioxidants were mixed into a ground product (Ismail et al., 2009; Kim et al., 2013; Gómez et al., 2016; Zhang et al., 2016). Previously, adding vitamin E to cattle diets decreased lipid oxidation in ground beef after 6 d of retail display (Faustman et al., 1989). Vitamin E works to delay lipid oxidation by quenching radicals created by oxidizing fatty acids (Murray et al., 2006). Increasing the penetration depth of antioxidants into steaks could delay the oxidation of lipids below the steak surface without having to adjust US feeding strategies. Additionally, decreasing lipid oxidation would prevent myoglobin oxidation and, thus, steak discoloration (Mancini and Hunt, 2005). As anticipated, lipid oxidation increased ($P <$ 0.001) from d 0 to d 4 of retail display (0.31 to 1.00 mg malondialdegyde/kg meat, respectively) similar to previous research (Kim et al., 2013; Colle et al., 2019). The lipid oxidation threshold for the ability of consumers to detect off flavors in meat is TBARS > 1.0 (Tarladgis et al., 1960; Greene and Cumuze, 1981). In the current study on d 4 of retail display the control steaks and steaks treated with C1, C3, R1, and R2, were above the lipid oxidation threshold with values of 1.09, 1.01, 1.05, 1.03, and 1.07, respectively. Further research is needed to collect consumer sensory data to determine if off-flavors are detectable under the experimental conditions of the present study.

Conclusion

Antioxidant treatments of acerola cherry powder and rosemary extract improved color brightness and uniformity during the retail display of steaks cut from beef chuck rolls aged 28 d post fabrication. Simple topical applications at a retail meat merchandising setting following extended aging, either due to lengthy storage or transportation duration, can extend the shelf-life stability of fresh beef short ribs. Further research is needed to determine if

utilizing antioxidants in combination with one another will further extend shelf-life. Additional investigation of using acerola cherry powder and rosemary extract in higher concentrations may further delay myoglobin and lipid oxidation.

Acknowledgements

This research was funded by The Beef Checkoff. We gratefully acknowledge financial support from Checkoff dollars provided by the Idaho Beef Council. Support for this research project was also provided by the Idaho Agriculture Experiment Station. We are also appreciative for the personnel at the University of Idaho Vandal Brand Meats Lab that made this research possible.

Literature Cited

Aberle, E. D., J. C. Forrest, D. E. Gerrard, and E. W. Mills. 2012. Principles of Meat Science. 5th ed. Kendall Hunt Publ. Co., Dubuque, IA.

American Meat Science Association. 2012. Meat color measurement guidelines. 2nd ed. Am. Meat Sci. Assoc., Chicago, IL.

Buettner, G. R., and B. A. Jurkiewicz. 1996. Catalytic metals, ascorbate and free radicals: Combinations to avoid. Radiat. Res. 145:532-541. doi:10.2307/3579271.

Colle, M. C., R. P. Richard, K. M. Killinger, J. C. Bohlscheid, A. R. Gray, W. I. Loucks, R. N. Day, A. S. Cochran, J. A. Nasados, and M. E. Doumit. 2015. Influence of extended aging on beef quality characteristics and sensory perception of steaks from the gluteus medius and longissimus lumborum. Meat Sci. 110:32–39. doi:10.1016/j.meatsci.2015.06.013.

Colle, M. C., R. P. Richard, K. M. Killinger, J. C. Bohlscheid, A. R. Gray, W. I. Loucks, R. N. Day, A. S. Cochran, J. A. Nasados, and M. E. Doumit. 2016. Influence of extended aging on beef quality characteristics and sensory perception of steaks from the biceps femoris and semimembranosus. Meat Sci. 119:110–117. doi:10.1016/j.meatsci.2016.04.028.

Colle, M. C., R. P. Richard, M. C. Colle, W. I. Loucks, G. K. Murdoch, P. D. Bass, C. J. Williams, and M. E. Doumit. 2019. Retail display properties and consumer perception of extended aged beef topically treated with ascorbic acid and rosemary extract. Meat and Muscle Biol. 3(1):42–50. doi:10.22175/mmb2018.05.0011.

English, A. R., G. G. Mafi, D. L. VanOverbeke, and R. Ramanathan. 2016. Effects of extended aging and modified atmospheric packaging on beef top loin steak color. J. Anim. Sci. 94:1727–1737. doi:10.2527/jas.2015-0149.

Faustman, C., R. G. Cassens, D. M. Schaefer, D. R. Buege, S. N. Williams, and K. K. Scheller. 1989. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. J. Food Sci. 54(4):858–862. doi:10.1111/j.1365-2621.1989.tb07899.x.

Gómez, I., M. J. Beriain, J. A. Mendizabal, C. Realini, and A. Purroy. 2016. Shelf life of ground beef enriched with omega-3 and/or conjugated linoleic acid and use of grape seed extract to inhibit lipid oxidation. Food Sci. & Nutrition. 4(1):67-79. doi:10.1002/fsn3.251.

Greene, B. E., and T. H. Cumuze. 1981. Relationship between TBA numbers and inexperienced panelists' assessment of oxidized flavor in cooked beef. J. Food Sci. 47:52–54, 58. doi:10.1111/j.1365-2621.1982.tb11025.x.

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 Sci. 127:57–62. doi:10.1016/j.meatsci.2017.01.005.

Ismail, H. A., E. J. Lee, K. Y. Ko, H. D. Paik, and D. U. Ahn. 2009. Effect of antioxidant application methods on the color, lipid oxidation, and volatiles of irradiated ground beef. J. Food Sci. 74(1):25-32. doi:10.1111/j.1750-3841.2008.00991.x.

Kim, S. J., S. C. Min, H. J. Shin, Y. J. Lee, A. R. Cho, S. Y. Kim, and J. Han. 2013. Evaluation of the antioxidant activities and nutritional properties of ten edible plant extracts and their application to fresh ground beef. Meat Sci. 93:715-722. doi:10.1016/j.meatsci.2012.11.029.

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

Mancini, R. A., and R. Ramanathan. 2014. Effects of postmortem storage time on color and mitochondria in beef. Meat Sci. 98:65-70. doi:10.1016/j.meatsci.2014.007.

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 Sci. 70:665–682. doi:10.1016/j.meatsci.2005.02.016.

Murray, R. K., D. K. Granner, and V. W. Rodwell. 2006. Harper's Illustrated Biochemistry. 27th Ed. McGraw-Hill Companies, Inc. New York, NY.

Sammel, L. M., M. C. Hunt, D. H. Kropf, K. A. Hachmeister, and D. E. Johnson. 2002. Comparison of assays for metmyoglobin reducing ability in beef inside and outside semimembranosus muscle. J. of Food Sci. 67:978-984. doi:10.1111/j.1365-2621.2002.tb09439.x.

Tarladgis, B. G., B. M. Watts, and M. T. Younathan. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37:44–48. doi:10.1007/BF02630824.

USDA – FAS. 2020. 2019 United States Agricultural Export Yearbook: South Korea 2019 Export Highlights. https://www.fas.usda.gov/south-korea-2019-export-highlights. (Accessed 4 January 2021.)

USMEF. 2020. U.S. Meat Export Federation Annual Report 2019. https://www.usmef.org/downloads/2019-USMEF-Annual-Report.pdf. (Accessed 22 January 2020.)

USMEF. 2021. U.S. Meat Export Federation Annual Report 2020. https://www.usmef.org/downloads/2020-USMEF-Annual-Report.pdf. (Accessed 14 January 2021.)

Zhang, X., D. Li, Q. Meng, C. He, and L. Ren. 2016. Effect of mulberry leaf extracts on color, lipid oxidation, antioxidant enzyme activities and oxidative breakdown products of raw ground beef during refrigerated storage. J. Food Quality. 39:159-170. doi:10.1111/jfq.12187.

Table 2.1. Estimated mean effects of topical antioxidant treatment on chuck roll steak fluid loss, color, oxygen consumption, and lipid oxidation (N = 63)

	Topical Antioxidant Treatment[1]							
Trait	Control	C1	C2	C3	R1	R2	R3	SEM
Retail fluid loss, %	1.00	1.55	4.72	1.23	1.52	3.99	1.28	1.50
L*	39.20^{ab}	38.87^{bc}	38.90^{bc}	38.41^{c}	39.95^{a}	39.79^{a}	38.86^{bc}	0.98
b*	13.57	14.05	13.77	14.11	14.23	14.25	13.89	0.30
Oxygenated lean color[2]	5.3^{a}	5.3^{a}	5.3^{ab}	5.3^{a}	5.2^{c}	5.2^{bc}	5.4^{a}	0.3
Amount of browning[3]	2.7	2.7	2.7	2.7	2.7	2.7	2.6	0.2
Discoloration[4]	2.6	2.5	2.6	2.6	2.5	2.6	2.5	0.1
Surface discoloration[5]	2.8^{abc}	2.7^{bc}	2.7^{abc}	2.9^{a}	2.7^{bc}	2.8^{ab}	2.7^{c}	0.1
Color uniformity[6]	2.7^{a}	2.7^{abc}	2.6^{c}	2.7^{ab}	2.7^{a}	2.6^{bc}	2.7^{ab}	0.1
Oxygen consumption, %	63.30	67.93	58.33	66.17	65.12	66.79	61.62	4.00
Lipid oxidation[7]	0.71	0.65	0.61	0.69	0.68	0.70	0.54	0.08

[abc]Within a row, means without a common superscript differ ($P < 0.05$)

[1]Treatments included: an untreated control (C), topically sprayed (2ml) with a 0.05% acerola cherry powder solution from one of three suppliers (C1, C2, or C3), or topically sprayed (2ml) with a 0.10% rosemary extract solution from one of three suppliers (R1, R2, or R3)

[2]Oxygenated lean color scale – 1 = extremely bright cherry-red, 8 = extremely dark red

[3]Amount of browning scale – 1 = no evidence of browning, 6 = dark brown

[4]Discoloration scale – 1 = none, 5 = extreme

[5]Surface discoloration scale – 1 = no discoloration (0%), 6 = extensive discoloration (81-100%)

[6]Color uniformity scale – 1 = uniform no two-toning, 5 = extreme two-toning

[7]mg malondialdehyde/kg meat

Table 2.2. Topical antioxidant treatment x retail display time on chuck roll steak a* values (redness; N = 63; SEM = 0.79)

Time of Retail Display (hours)	Topical Antioxidant Treatment[1]						
	Control	C1	C2	C3	R1	R2	R3
1	$23.09^{a,u}$	$22.29^{a,u}$	$23.61^{a,u}$	$22.15^{a,uv}$	$23.80^{a,u}$	$23.69^{a,u}$	$23.78^{a,uv}$
7	$22.21^{b,uv}$	$23.08^{ab,u}$	$23.68^{ab,u}$	$23.89^{ab,u}$	$25.00^{a,u}$	$23.44^{ab,u}$	$24.22^{a,u}$
19	$21.05^{a,vw}$	$18.86^{b,vw}$	$20.35^{ab,v}$	$22.24^{a,uv}$	$20.37^{ab,v}$	$21.96^{a,uv}$	$22.18^{a,vw}$
31	$21.70^{a,uvw}$	$19.96^{a,vw}$	$20.53^{a,v}$	$19.67^{a,wx}$	$20.49^{a,v}$	$20.97^{a,v}$	$20.75^{a,w}$
43	$19.80^{ab,wx}$	$18.32^{ab,wx}$	$19.21^{ab,vw}$	$20.41^{a,vw}$	$19.61^{ab,v}$	$18.19^{b,w}$	$18.63^{ab,x}$
55	$18.14^{ab,xy}$	$16.16^{bc,y}$	$15.88^{c,y}$	$18.35^{a,xy}$	$16.73^{abc,w}$	$17.10^{abc,wx}$	$16.07^{c,z}$
67	$16.94^{abc,yz}$	$16.84^{abc,xy}$	$17.76^{a,wxy}$	$17.40^{ab,yz}$	$15.31^{bc,w}$	$15.30^{c,x}$	$18.22^{a,xy}$
79	$16.03^{ab,z}$	$15.90^{ab,y}$	$17.97^{a,wx}$	$16.19^{ab,z}$	$16.74^{ab,w}$	$15.64^{b,x}$	$16.37^{ab,y}$
91	$16.75^{a,yz}$	$16.44^{a,xy}$	$16.06^{a,xy}$	$15.43^{a,z}$	$14.81^{a,w}$	$16.34^{a,wx}$	$16.46^{a,y}$

[abc]Within a row, means without a common superscript differ ($P < 0.05$)

[u-z]Within a column, means without a common superscript differ ($P < 0.05$)

[1]Treatments included: an untreated control (C), topically sprayed (2ml) with a 0.05% acerola cherry powder solution from one of three suppliers (C1, C2, or C3), or topically sprayed (2ml) with a 0.10% rosemary extract solution from one of three suppliers (R1, R2, or R3)

Table 2.3. Estimated mean effects of retail display time on chuck roll steak color (N = 63)

	Time of Retail Display (hours)									
Trait	1	7	19	31	43	55	67	79	91	SEM
L*	39.31^{bc}	39.19^{bc}	40.72^{a}	39.16^{bcd}	38.22^{e}	39.78^{b}	38.68^{cde}	38.31^{de}	38.88^{cde}	0.99
b*	15.73^{a}	15.25^{a}	15.09^{ab}	14.02^{c}	13.95^{c}	14.59^{bc}	12.76^{d}	12.27^{d}	12.14^{d}	0.32
Oxygenated lean color[1]	4.1^{a}	4.3^{b}	4.9^{c}	5.3^{d}	5.4^{d}	5.6^{e}	5.9^{f}	6.0^{fg}	6.1^{g}	0.3
Amount of browning[2]	1.0^{a}	1.0^{a}	1.8^{b}	2.2^{c}	2.8^{d}	3.3^{e}	3.7^{f}	4.0^{g}	4.3^{h}	0.2
Discoloration[3]	1.0^{a}	1.0^{a}	1.5^{b}	2.0^{c}	2.5^{d}	3.1^{e}	3.6^{f}	4.0^{g}	4.3^{h}	0.1
Surface discoloration[4]	1.0^{a}	1.0^{a}	1.7^{b}	2.1^{c}	2.8^{d}	3.5^{e}	3.8^{f}	4.3^{g}	4.5^{h}	0.1
Color uniformity[5]	1.5^{a}	1.7^{b}	2.2^{c}	2.3^{c}	2.6^{d}	3.0^{e}	3.3^{f}	3.7^{g}	3.9^{h}	0.1

[a-h]Within a row, means without a common superscript differ ($P < 0.05$)

[1]Oxygenated lean color scale – 1 = extremely bright cherry-red, 8 = extremely dark red

[2]Amount of browning scale – 1 = no evidence of browning, 6 = dark brown

[3]Discoloration scale – 1 = none, 5 = extreme

[4]Surface discoloration scale – 1 = no discoloration (0%), 6 = extensive discoloration (81-100%)

[5]Color uniformity scale – 1 = uniform no two-toning, 5 = extreme two-toning

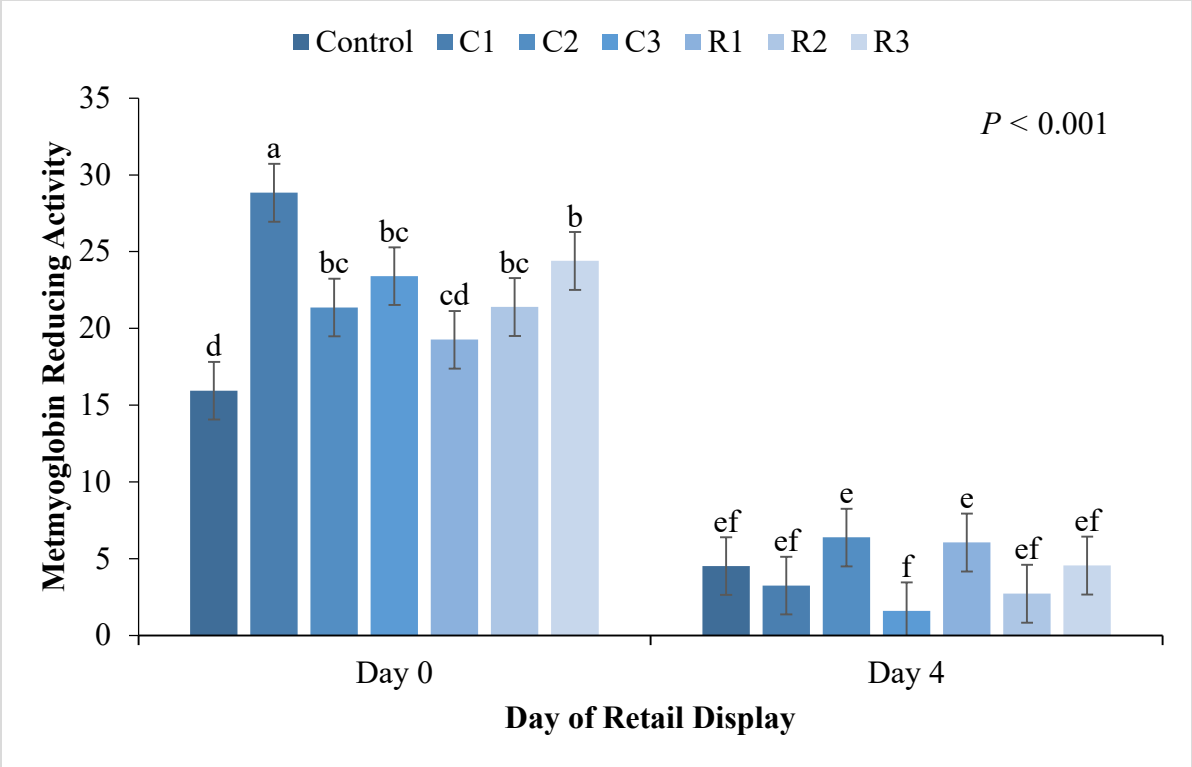

Figure 2.1. Metmyoglobin reducing activity (MRA) values for antioxidant treatment by retail display time for chuck roll steaks (N = 63). Each steak was randomly assigned based on location to be an untreated control (C), topically sprayed (2ml) with a 0.05% acerola cherry powder solution from one of three suppliers (C1, C2, or C3), or topically sprayed (2ml) with a 0.10% rosemary extract solution from one of three suppliers (R1, R2, or R3). Steaks were overwrapped with an oxygen permeable PVC film and displayed in a retail display room at 2°C for 4 d. MRA was determined on d 0 and d 4 of retail display for the control and each antioxidant treatment with the following equation: MRA = [(Initial % metmyoglobin – Final % metmyoglobin) ÷ Initial % metmyoglobin] × 100. Values are shown as least square means ± SE. $^{a\text{-}f}$Means without a common superscript differ ($P < 0.05$).

Chapter 3: Extending the shelf-life of beef bone-in short rib steaks using acerola cherry powder and rosemary extract

Submitted to *Meat and Muscle Biology*

Abstract

Improving the retail shelf-life of steaks from beef bone-in short ribs, an item commonly exported, will improve salability of beef in international markets and therefore improve potential profits for producers. The objective of this study was to determine the effect of the topical application of acerola cherry powder and rosemary extract from various suppliers on beef bone-in short rib shelf-life regarding color stability and lipid oxidation. Beef bone-in short ribs (IMPS 123A; N = 18) from USDA Choice carcasses were purchased from a commercial harvest facility and aged (0°C) for 28 d post-fabrication. Following aging, 1.02 cm-thick steaks were cut perpendicular to the rib bones and systematically assigned to a treatment based on steak location within the subprimal. Treatments included: untreated control (C), topically sprayed (2ml) with an acerola cherry powder solution (0.05%) from one of three suppliers (C1, C2, C3), or topically sprayed (2ml) with a rosemary extract solution (0.10%) from one of three suppliers (R1, R2, R3). Half of the steaks were assigned by location to d 0 lipid oxidation, metmyoglobin reducing activity (MRA), and oxygen consumption; the remaining steaks were assigned to 4 days of retail display followed by d 4 lipid oxidation and MRA. Throughout retail display, objective and subjective color was measured at h 1, h 7, and every 12 hours afterward until h 91. Steaks treated with antioxidants C1, C2, C3, and R3 were a brighter red than control steaks in terms of subjective

oxygenated lean color ($P < 0.001$). There was an interaction ($P = 0.028$) between day of retail display and MRA. Steaks treated with C3 and R2 antioxidants did not change in MRA between d 0 and 4 ($P = 0.620$, $P = 0.428$, respectively). Applying topical antioxidants can improve shelf-life of beef bone-in short ribs aged for an extended period.

Keywords: antioxidants, extended aging, shelf-life, beef

Introduction

According to the United States (US) Meat Export Federation (USMEF), in 2019, 14.1% of US beef was exported, contributing over $7.4 billion of revenue, averaging $308.74 per head. The Republic of Korea (South Korea) was identified to be the US beef industry's largest growing export market due to growing household incomes and insufficient beef production (USMEF, 2020; 2021; USDA – FAS, 2020).

Currently, it takes a minimum of 28 d to ship chilled beef from the US to South Korea (USMEF, 2020). Colle et. al (2015; 2016) determined extended aging can cause product quality issues, such as increased lipid oxidation, darkened lean color (L*), and reduced redness (a*) throughout retail display. Extended aging also has been shown to decrease metmyoglobin reducing activity and pH (English et al., 2016), as well as mitochondrial oxygen consumption rate (Mancini and Ramanathan, 2014). Additionally, Gill (1996) suggested that when bone-in beef cuts stored or aged for a longer period of time are cut for retail display, the bone blackens faster than unaged bones. Further, the lean tissue of a short rib steak contains the *Serratus ventralis* muscle, which has been classified as having low color stability (McKenna et al., 2005).

A potential solution to improve US beef shelf-life is the application of antioxidants to the surface of the steaks. Determining a method to improve the color stability of US beef in international markets requiring lengthy shipping could provide the opportunity for increased international demand for US beef exports, and producer profits. The objective of this study was to determine the effect of the topical application of acerola cherry powder and rosemary extract from various suppliers on beef bone-in short rib steak shelf-life including color and lipid oxidation.

Methods and Materials

Product preparation

Beef bone-in short ribs (IMPS 123A; N = 18) from USDA Choice carcasses were purchased from a commercial harvest facility and transported under refrigeration (3.5 h; 4°C) to the University of Idaho Meat Laboratory. The subprimals were wet-aged at 0°C for 28 d to simulate trans-Pacific shipping time prior to being prepared for retail display. Following aging, short ribs were cut perpendicular to the bone into 1.02 cm-thick steaks (N = 126) to mimic South Korean retail steak fabrication style of bone-in short ribs. Steaks were equally assigned based on location within each subprimal to a treatment group: untreated control (C), topically sprayed (2 ml) with a 0.05% acerola cherry powder solution from one of three commercially available suppliers [C1 (Fortium® A Dry, Kemin Industries, Des Moines, IA), C2 (Micro Ingredients Acerola Cherries Organic Vitamin C Powder, Montclair, CA), or C3 (Acerola Cherry Powder, Pure Organic Ingredients, American Fork, UT)], or topically sprayed (2 ml) with a 0.10% rosemary extract solution from one of three commercially

available suppliers [R1 (Fortium® R10 Dry, Kemin Industries, Des Moines, IA), R2 (Herbalox® Rosemary Extract, Kalsec, Kalamazoo, MI), or R3 (GUARDIAN® Rosemary Extract 08, Danisco, Madison, WI)]. Half of the steaks were assigned by steak location within subprimal to d 0 lipid oxidation, metmyoglobin reducing activity (MRA), and oxygen consumption (OC) rate; the remaining steaks were assigned to 4 days of retail display followed by d 4 lipid oxidation and MRA. Steaks were displayed in a retail display room at 2°C. The display room was equipped with natural white 4000 W lights, and the average light intensity was 849 lux (Fisherbrand Traceable Dual-Range Light Meter, Fisher Scientific, Waltham, MA).

Retail fluid loss

Following treatment, steaks were weighed, placed in white foam trays (CKF Inc. #88142, Langley, BC, Canada), and overwrapped with an oxygen permeable PVC film (oxygen transmission rate: 1,450 cc/645 cm^2 per 24 h; water vapor transmission rate: 17.0 g/645 cm^2 per 24 h; Koch Industries, Inc. #7500-3815; Wichita, KS). Following 4 days of retail display, steaks were removed from the retail packaging and re-weighed to determine retail moisture loss. Percent retail fluid loss was calculated using the following equation:

$$\%\ Fluid\ Loss = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} \times 100\%$$

Retail color

Retail display steaks were allowed to bloom for 60 minutes, and then two objective color measurements per steak were obtained using a Nix Pro 2 Sensor (Nix Sensor Ltd., Hamilton, Ontario, Canada). Two objective color measurements were also taken on the bone

marrow of the short ribs. The Nix Pro 2 Sensor was equipped with a 14 mm-diameter measuring area and a 2° standard observer. The instrument was set to Illuminant D_{65} and Commission International de l'Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) values were recorded. After the initial reading on d 0 of retail display, color was measured a second time 6 h later. Subsequent color measurements were taken twice daily (06:00 and 18:00) on d 1, 2, and 3, and once (06:00) on d 4. Oxygenated lean color, discoloration, surface discoloration, bone marrow color, color uniformity, and amount of browning were measured twice daily by four subjective evaluators following American Meat Science Association guidelines (AMSA, 2012). To avoid potential effects due to display location, steaks were rotated in the retail environment daily.

Metmyoglobin reducing activity

Nitric oxide MRA was measured after treatment on d 0 and on d 4 of retail display following protocols outlined in Section XI of the Meat Color Measurement Guidelines (AMSA, 2012). Color measurements were obtained using a Hunter MiniScan EZ (Restin, Virginia) equipped with a 25 mm-diameter measuring area and a 10° standard observer. The instrument was set to Illuminant A and reflectance from 400 to 700 nm was recorded. Calibration occurred by measuring against black and white calibration tiles prior to measuring color. The percentage of metmyoglobin (MMb) was calculated following equations in Section XI of the Meat Color Measurements Guidelines (AMSA, 2012). MRA was calculated as follows:

$$MRA = \left[\frac{Initial\ \%\ MMb - Final\ \%\ MMb}{Initial\ \%\ MMb}\right] \times 100$$

Oxygen consumption

Oxygen consumption was measured after treatment on d 0 following protocols outlined in the Meat Color Measurement Guidelines (AMSA, 2012). Color measurements were taken using a Hunter MiniScan EZ (Restin, Virginia) equipped with a 25 mm-diameter measuring area and a 10° standard observer. The instrument was set to illuminant A and reflectance from 400 to 700 nm was recorded. Calibration occurred by measuring against black and white calibration tiles prior to measuring color. The percentage of oxymyoglobin (OMb) was calculated following equations in Section XI of the Meat Color Measurements Guidelines (AMSA, 2012). OC was calculated as follows:

$$OC = \left[\frac{Initial\ \%\ OMb - Final\ \%\ OMb}{Initial\ \%\ OMb}\right] \times 100$$

Lipid oxidation

Thiobarbituric acid reactive substances (TBARS) were analyzed in duplicate on d 0 after treatment and on d 4 of retail display following the protocol in Section XI, Appendix O of the Meat Color Measurement Guidelines (AMSA, 2012). Samples weighed 1 g and were cut to avoid the steak edge and large pieces of fat and connective tissue.

Statistical analysis

Data was collected on 18 subprimals and 63 steaks per antioxidant treatment-retail display time combination. Data were analyzed using mixed model analysis of variance. Antioxidant treatments, retail display time, and their interaction were assumed as fixed effects. In this factorial design, there were six antioxidant treatments and one control treatment. MRA and lipid oxidation were evaluated at two retail display time points,

whereas color was analyzed at nine retail display time points. Retail display time was considered a repeated measure modeled as a compound symmetric correlation structure. Subjective color data were averaged based on retail display time, evaluator, and antioxidant treatment prior to analysis, where evaluator was considered a random block effect in a randomized complete block design. Objective color data were analyzed with subprimal and steak location within subprimal as randomized block effects. Treatment least square means differences were assessed through pair-wise comparisons for significant effects. Significance was determined at $P < 0.05$. All statistical analyses were conducted using SAS V 9.4 (SAS Inc., Cary, NC).

Results and Discussion

Retail fluid loss

The control and R1 treated steaks lost the most fluid over retail display ($P = 0.024$; Table 1). The remaining antioxidant treatments (C1, C2, C3, R2, and R3) all had decreased fluid loss compared to the control steaks. Loss of fluid during retail display results in a drier cooked product for the consumer (Lawrie and Ledward, 2006). More research is needed to determine the relationship between topical antioxidant treatments and retail fluid loss. Future research utilizing a positive control treatment of 2 ml water topically applied to steaks could assist in determining the relationship.

Retail objective color

An interaction between retail display time and treatment was not observed in bone marrow L*, a*, or b* ($P = 0.936$, $P = 0.246$, and $P = 0.277$, respectively). However,

antioxidant treatments differed in L*, a*, and b* ($P < 0.001$, $P = 0.001$, $P = 0.004$, respectively; Table 3.1). Interestingly, steaks treated with R3 had darker and redder bone marrow than the control steaks. Additionally, all rosemary extract treated steaks (R1, R2, and R3) had more yellow bone marrow than the control steaks. Ascorbic acid, the active ingredient of acerola cherry powder, has the ability to donate electrons to prevent the myoglobin oxidation process (Buettner and Jerkiewicz, 1996), which is similar to hemoglobin oxidation (Lanari et al., 1995). Based on previous research of ascorbic acid on vertebrae (Mancini et al., 2004), it was anticipated that acerola cherry powder would improve redness more than rosemary extract treated bone marrow and untreated bone marrow (C). However, differences in bone marrow redness between treatments did not occur possibly due to the lower concentrations of acerola cherry powder used compared to research by Mancini et al. (2004) who used higher concentrations of ascorbic acid. Utilizing higher acerola cherry powder concentrations in future research could improve redness as lower concentrations of ascorbic acid act as a meat pro-oxidant by reducing iron from the ferric to ferrous state, a powerful pro-oxidant (Buettner and Jerkiewicz, 1996). Throughout retail display, bone marrow differed in L*, a*, and b* ($P = 0.020$, $P < 0.001$, $P < 0.001$, respectively; Table 3.2). Lightness of the bone marrow fluctuated throughout retail display, whereas redness and yellowness decreased over time. The decline in bone marrow color after being exposed to oxygen was anticipated due to the oxidation of iron within the hemoglobin (Gill, 1996; Lanari et al., 1995).

When evaluating the lean tissue of the short ribs, an interaction between retail display time and treatment was not observed for L*, a*, or b* ($P = 0.354$, $P = 0.925$, and $P = 0.366$, respectively). Additionally, there was not a difference in L*, a*, or b* between treatments (P

= 0.445, $P = 0.412$, and $P = 0.855$, respectively; Table 3.1). However, it is important to note all treatments had redness values greater than 14.5, which is the threshold for consumers' acceptability (Holman et al., 2017). Throughout retail display, L*, a*, and b* values did differ ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively; Table 3.2). Initial readings at 1 h were the darkest, reddest, and yellowest. Over time, steaks lightened and decreased in redness and yellowness. This observation is consistent with previous research where aged steaks declined in redness and yellowness during retail display (Colle et al., 2015; 2016). Throughout retail display, steaks maintained redness above the acceptability threshold, greater than 14.5 (Holman et al., 2017).

Retail subjective color

Color uniformity ($P < 0.001$) and oxygenated lean color ($P < 0.001$) differed between treatments (Table 3.1). The R2 treated steaks were less uniform than the control steaks. Steaks treated with antioxidants C1, C2, C3, and R3 had a brighter oxygenated lean color than the control steaks. This supports the findings that ascorbic acid found in acerola cherry powder extends shelf-life by delaying myoglobin oxidation (Buettner and Jerkiewicz, 1996). Interestingly, these results were not reflected when evaluating a* or objective redness. Antioxidants did not impact amount of browning ($P = 0.338$), discoloration ($P = 0.162$), surface discoloration ($P = 0.289$), or bone marrow color ($P = 0.859$; Table 3.1). Throughout retail display, there were changes in oxygenated lean color ($P < 0.001$), amount of browning ($P < 0.001$), discoloration ($P < 0.001$), surface discoloration ($P < 0.001$), color uniformity ($P < 0.001$), and bone marrow color ($P < 0.001$; Table 3.2). Oxygenated lean color, color uniformity, and bone marrow color had the lowest score, or most desirable appearance, at the initial evaluation at 1 h. Until 55 h of retail display, steaks maintained a score of 1 in amount

of browning, discoloration, and surface discoloration (no browning, no discoloration, and 0% metmyoglobin, respectively). Across all subjective color categories, the final evaluation at 91 h resulted in the highest (least desirable) score. This observation is consistent with previous research where aged steaks discolored during retail display (Colle et al., 2015; 2016; English et al., 2016).

Metmyoglobin reducing activity

There was an interaction between retail display and treatment ($P = 0.028$; Figure 3.1). None of the antioxidant treated steaks were significantly different than the control steaks on d 0 or d 4. Within treatment, MRA decreased from d 0 to d 4 for the control steaks and steaks treated with C1, C2, R1, and R3. Steaks treated with C3 and R2 did not differ in MRA from d 0 to d 4 of retail display. MRA decreases over time due to decreasing NADH levels postmortem and causes increased levels of metmyoglobin (Sammel et al., 2002; AMSA, 2012). Additionally, as aging time increases, initial MRA decreases creating further color stability challenges (English et al., 2016). Utilizing antioxidants to increase MRA on d 4 could improve color stability of meat by delaying the onset of browning.

Oxygen consumption

Treatments did not impact OC ($P = 0.224$; Table 3.1). The ability to maintain moderate OC throughout retail display could stabilize color by regenerating NADH levels for MRA without reducing the oxygen partial pressure at the surface (Sammel et al., 2002).

Lipid oxidation

There was not an interaction between day of retail display and treatment ($P = 0.191$) or a treatment influence on lipid oxidation ($P = 0.323$; Table 3.1). Similar results were found in the *Semimembranosus* and *Longissimus lumborum* when ascorbic acid and rosemary extract were topically applied (Colle et al., 2019). Other previous research has shown a delay in lipid oxidation; however, in these studies the antioxidants were mixed into a ground product (Ismail et al., 2009; Kim et al., 2013; Gómez et al., 2016; Zhang et al., 2016). Previously, adding vitamin E to cattle diets decreased lipid oxidation in ground beef after 6 d of retail display (Faustman et al., 1989). Vitamin E works to delay lipid oxidation by quenching free radicals created by oxidizing fatty acids (Murray et al., 2006). Increasing the penetration depth of antioxidants into steaks could delay the oxidation of lipids below the steak surface without having to adjust US feeding strategies. Additionally, decreasing lipid oxidation would prevent myoglobin oxidation and, thus, steak discoloration (Mancini and Hunt, 2005). As anticipated, lipid oxidation increased ($P < 0.001$) from d 0 to d 4 of retail display (0.15 to 0.78 mg malondialdegyde/kg meat, respectively) similar to previous research (Kim et al., 2013; Colle et al., 2019). The lipid oxidation threshold for the ability of consumers to detect off flavors in meat is TBARS > 1.0 (Tarladgis et al., 1960; Greene and Cumuze, 1981). It is important to note that in this study on d 4 of retail display none of the treatments were above the lipid oxidation threshold.

Conclusion

Antioxidant treatments of acerola cherry powder and rosemary extract improved bone marrow redness and oxygenated lean color during the retail display of steaks cut from beef bone-in short ribs aged 28 d post fabrication. Simple topical applications at a retail meat merchandising setting following extended aging, either due to lengthy storage or transportation duration, can extend the shelf-life stability of fresh beef bone-in short ribs. Further research is needed to determine if utilizing antioxidants in combination with one another will further extend shelf-life. Additional investigation of using acerola cherry powder and rosemary extract in higher concentrations may further delay myoglobin and lipid oxidation.

Acknowledgements

This research was funded by The Beef Checkoff. We gratefully acknowledge financial support from Checkoff dollars provided by the Idaho Beef Council. Support for this research project was also provided by the Idaho Agriculture Experiment Station. We are also appreciative for the personnel at the University of Idaho Vandal Brand Meats Lab that made this research possible.

Literature Cited

American Meat Science Association. 2012. Meat color measurement guidelines. 2nd ed. Am. Meat Sci. Assoc., Chicago, IL.

Buettner, G. R., and B. A. Jurkiewicz. 1996. Catalytic metals, ascorbate and free radicals: Combinations to avoid. Radiat. Res. 145:532-541. doi:10.2307/3579271.

Colle, M. C., R. P. Richard, K. M. Killinger, J. C. Bohlscheid, A. R. Gray, W. I. Loucks, R. N. Day, A. S. Cochran, J. A. Nasados, and M. E. Doumit. 2015. Influence of extended aging on beef quality characteristics and sensory perception of steaks from the gluteus medius and longissimus lumborum. Meat Sci. 110:32–39. doi:10.1016/j.meatsci.2015.06.013.

Colle, M. C., R. P. Richard, K. M. Killinger, J. C. Bohlscheid, A. R. Gray, W. I. Loucks, R. N. Day, A. S. Cochran, J. A. Nasados, and M. E. Doumit. 2016. Influence of extended aging on beef quality characteristics and sensory perception of steaks from the biceps femoris and semimembranosus. Meat Sci. 119:110–117. doi:10.1016/j.meatsci.2016.04.028.

Colle, M. C., R. P. Richard, M. C. Colle, W. I. Loucks, G. K. Murdoch, P. D. Bass, C. J. Williams, and M. E. Doumit. 2019. Retail display properties and consumer perception of extended aged beef topically treated with ascorbic acid and rosemary extract. Meat and Muscle Biol. 3(1):42–50. doi:10.22175/mmb2018.05.0011.

English, A. R., G. G. Mafi, D. L. VanOverbeke, and R. Ramanathan. 2016. Effects of extended aging and modified atmospheric packaging on beef top loin steak color. J. Anim. Sci. 94:1727–1737. doi:10.2527/jas.2015-0149.

Faustman, C., R. G. Cassens, D. M. Schaefer, D. R. Buege, S. N. Williams, and K. K. Scheller. 1989. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. J. Food Sci. 54(4):858–862. doi:10.1111/j.1365-2621.1989.tb07899.x.

Gill, C. O. 1996. Extending the storage life of raw chilled meats. Meat Sci. 43:99-109. doi:10.1016/0309-1740(96)00058-7.

Gómez, I., M. J. Beriain, J. A. Mendizabal, C. Realini, and A. Purroy. 2016. Shelf life of ground beef enriched with omega-3 and/or conjugated linoleic acid and use of grape seed extract to inhibit lipid oxidation. Food Sci. & Nutrition. 4(1):67-79. doi:10.1002/fsn3.251.

Greene, B. E., and T. H. Cumuze. 1981. Relationship between TBA numbers and inexperienced panelists' assessment of oxidized flavor in cooked beef. J. Food Sci. 47:52–54, 58. doi:10.1111/j.1365-2621.1982.tb11025.x.

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 Sci. 127:57–62. doi:10.1016/j.meatsci.2017.01.005.

Ismail, H. A., E. J. Lee, K. Y. Ko, H. D. Paik, and D. U. Ahn. 2009. Effect of antioxidant application methods on the color, lipid oxidation, and volatiles of irradiated ground beef. J. Food Sci. 74(1):25-32. doi:10.1111/j.1750-3841.2008.00991.x.

Kim, S. J., S. C. Min, H. J. Shin, Y. J. Lee, A. R. Cho, S. Y. Kim, and J. Han. 2013. Evaluation of the antioxidant activities and nutritional properties of ten edible plant

extracts and their application to fresh ground beef. Meat Sci. 93:715-722. doi:10.1016/j.meatsci.2012.11.029.

Lanari, M. C., D. M. Schaefer, and K. K. Scheller. 1995. Dietary vitamin E supplementation and discoloration of pork bone and muscle following modified atmosphere packaging. Meat Sci. 41(3):237–250. doi:10.1016/0309-1740(95)00006-7.

Lawrie, R. A., and D. A. Ledward. 2006. Lawrie's Meat Science. 7th Ed. Woodhead Publishing Limited. Cambridge, England.

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

Mancini, R. A., and R. Ramanathan. 2014. Effects of postmortem storage time on color and mitochondria in beef. Meat Sci. 98:65-70. doi:10.1016/j.meatsci.2014.007.

Mancini, R. A., M. C. Hunt, K. A. Hachmeister, D. H. Kropf, and D. E. Johnson. 2004. Ascorbic acid minimizes vertebrae discoloration. Meat Sci. 68(3):339-345. doi:10.1016/j.meatsci.2004.03.017.

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 Sci. 70:665–682. doi:10.1016/j.meatsci.2005.02.016.

Murray, R. K., D. K. Granner, and V. W. Rodwell. 2006. Harper's Illustrated Biochemistry. 27th Ed. McGraw-Hill Companies, Inc. New York, NY.

Sammel, L. M., M. C. Hunt, D. H. Kropf, K. A. Hachmeister, and D. E. Johnson. 2002. Comparison of assays for metmyoglobin reducing ability in beef inside and outside

semimembranosus muscle. J. of Food Sci. 67:978-984. doi:10.1111/j.1365-2621.2002.tb09439.x.

Tarladgis, B. G., B. M. Watts, and M. T. Younathan. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37:44–48. doi:10.1007/BF02630824.

USDA – FAS. 2020. 2019 United States Agricultural Export Yearbook: South Korea 2019 Export Highlights. https://www.fas.usda.gov/south-korea-2019-export-highlights. (Accessed 4 January 2021.)

USMEF. 2020. U.S. Meat Export Federation Annual Report 2019. https://www.usmef.org/downloads/2019-USMEF-Annual-Report.pdf. (Accessed 22 January 2020.)

USMEF. 2021. U.S. Meat Export Federation Annual Report 2020. https://www.usmef.org/downloads/2020-USMEF-Annual-Report.pdf. (Accessed 14 January 2021.)

Zhang, X., D. Li, Q. Meng, C. He, and L. Ren. 2016. Effect of mulberry leaf extracts on color, lipid oxidation, antioxidant enzyme activities and oxidative breakdown products of raw ground beef during refrigerated storage. J. Food Quality. 39:159-170. doi:10.1111/jfq.12187.

Table 3.1. Estimated mean effects of topical antioxidant treatment on bone-in short rib steak fluid loss, color, oxygen consumption, and lipid oxidation (N = 63)

	Topical Antioxidant Treatment[1]							
Trait	Control	C1	C2	C3	R1	R2	R3	SEM
Retail fluid loss, %	3.35^{a}	0.62^{b}	0.40^{b}	0.15^{b}	1.54^{ab}	0.52^{b}	0.61^{b}	0.80
Bone marrow L*	45.39^{abc}	46.28^{a}	45.21^{bcd}	44.75^{cd}	46.13^{ab}	46.08^{ab}	44.11^{d}	0.92
Bone marrow a*	14.54^{b}	14.20^{b}	14.76^{b}	14.07^{b}	14.16^{b}	14.30^{b}	15.52^{a}	0.37
Bone marrow b*	10.00^{c}	10.57^{bc}	10.56^{bc}	10.57^{bc}	10.69^{b}	10.75^{b}	11.35^{a}	0.25
Lean L*	41.11	41.22	41.06	42.08	41.22	40.68	40.90	0.68
Lean a*	20.49	21.47	21.36	21.11	20.49	20.83	21.10	0.38
Lean b*	13.17	13.90	13.88	13.41	13.60	13.68	13.80	0.41
Oxygenated lean color[2]	4.6^{a}	4.1^{c}	4.1^{bc}	4.2^{b}	4.5^{a}	4.5^{a}	4.2^{b}	0.2
Amount of browning[3]	1.1	1.1	1.1	1.1	1.1	1.1	1.1	0.1
Discoloration[4]	1.1	1.1	1.1	1.1	1.1	1.1	1.1	0.1
Surface discoloration[5]	1.1	1.1	1.1	1.1	1.1	1.1	1.1	0.1
Color uniformity[6]	1.3^{bc}	1.3^{bc}	1.3^{c}	1.3^{bc}	1.4^{b}	1.4^{a}	1.3^{bc}	0.1
Bone marrow color[7]	3.4	3.4	3.5	3.6	3.3	3.2	3.3	0.5
Oxygen consumption, %	48.78	55.64	52.16	43.49	58.04	55.74	60.68	6.00
Lipid oxidation[8]	0.50	0.53	0.51	0.47	0.42	0.48	0.35	0.07

[abc]Within a row, means without a common superscript differ ($P < 0.05$)

[1]Treatments included: an untreated control (C), topically sprayed (2ml) with a 0.05% acerola cherry powder solution from one of three suppliers (C1, C2, or C3), or topically sprayed (2ml) with a 0.10% rosemary extract solution from one of three suppliers (R1, R2, or R3)

[2]Oxygenated lean color scale – 1 = extremely bright cherry-red, 8 = extremely dark red

[3]Amount of browning scale – 1 = no evidence of browning, 6 = dark brown

[4]Discoloration scale – 1 = none, 5 = extreme

[5]Surface discoloration scale – 1 = no discoloration (0%), 6 = extensive discoloration (81-100%)

[6]Color uniformity scale – 1 = uniform no two-toning, 5 = extreme two-toning

[7]Bone marrow color scale – 1 = bright reddish-pink to red, 7 = black

[8]mg malondialdehyde/kg meat

Table 3.2. Estimated mean effects of retail display time on bone-in short rib steak color (N = 63)

	Time of Retail Display (hours)									
Trait	1	7	19	31	43	55	67	79	91	SEM
Bone marrow L*	46.11^{ab}	45.56^{abcd}	44.83^{cd}	45.16^{bcd}	45.67^{abc}	46.52^{a}	45.25^{bcd}	45.23^{bcd}	44.45^{d}	0.92
Bone marrow a*	20.01^{a}	17.01^{b}	13.52^{cde}	14.18^{c}	13.64^{cd}	13.21^{def}	12.75^{f}	13.46^{cdef}	12.79^{ef}	0.38
Bone marrow b*	14.06^{a}	11.46^{b}	10.56^{c}	9.51^{d}	9.96^{cd}	10.00^{cd}	9.53^{d}	10.60^{c}	10.10^{cd}	0.27
Lean L*	39.79^{cd}	39.46^{d}	40.55^{bc}	40.89^{b}	43.03^{a}	41.14^{b}	41.06^{b}	43.34^{a}	41.39^{b}	0.64
Lean a*	21.77^{a}	20.47^{c}	22.00^{a}	21.56^{ab}	20.85^{bc}	20.68^{c}	20.78^{bc}	20.18^{c}	20.52^{c}	0.34
Lean b*	15.25^{a}	11.59^{f}	14.89^{ab}	13.12^{de}	14.37^{bc}	12.81^{e}	13.77^{cd}	14.27^{bc}	12.62^{e}	0.33
Oxygenated lean color[1]	3.5^{f}	3.7^{e}	4.0^{c}	4.1^{d}	4.4^{c}	4.6^{bc}	4.7^{b}	4.9^{a}	5.0^{a}	0.2
Amount of browning[2]	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{e}	1.2^{b}	1.2^{b}	1.3^{a}	0.1
Discoloration[3]	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{e}	1.2^{b}	1.2^{b}	1.3^{a}	0.1
Surface discoloration[4]	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{e}	1.2^{b}	1.3^{b}	1.4^{a}	0.1
Color uniformity[5]	1.1^{g}	1.0^{g}	1.2^{f}	1.2^{ef}	1.3^{de}	1.4^{cd}	1.5^{bc}	1.6^{b}	1.7^{a}	0.2
Bone marrow color[6]	1.9^{e}	2.8^{d}	3.5^{c}	3.3^{c}	3.4^{c}	3.4^{c}	3.9^{b}	4.0^{b}	4.5^{a}	0.5

[a-h]Within a row, means without a common superscript differ ($P < 0.05$)

[1]Oxygenated lean color scale – 1 = extremely bright cherry-red, 8 = extremely dark red

[2]Amount of browning scale – 1 = no evidence of browning, 6 = dark brown

[3]Discoloration scale – 1 = none, 5 = extreme

[4]Surface discoloration scale – 1 = no discoloration (0%), 6 = extensive discoloration (81-100%)

[5]Color uniformity scale – 1 = uniform no two-toning, 5 = extreme two-toning

[6]Bone marrow color scale – 1 = bright reddish-pink to red, 7 = black

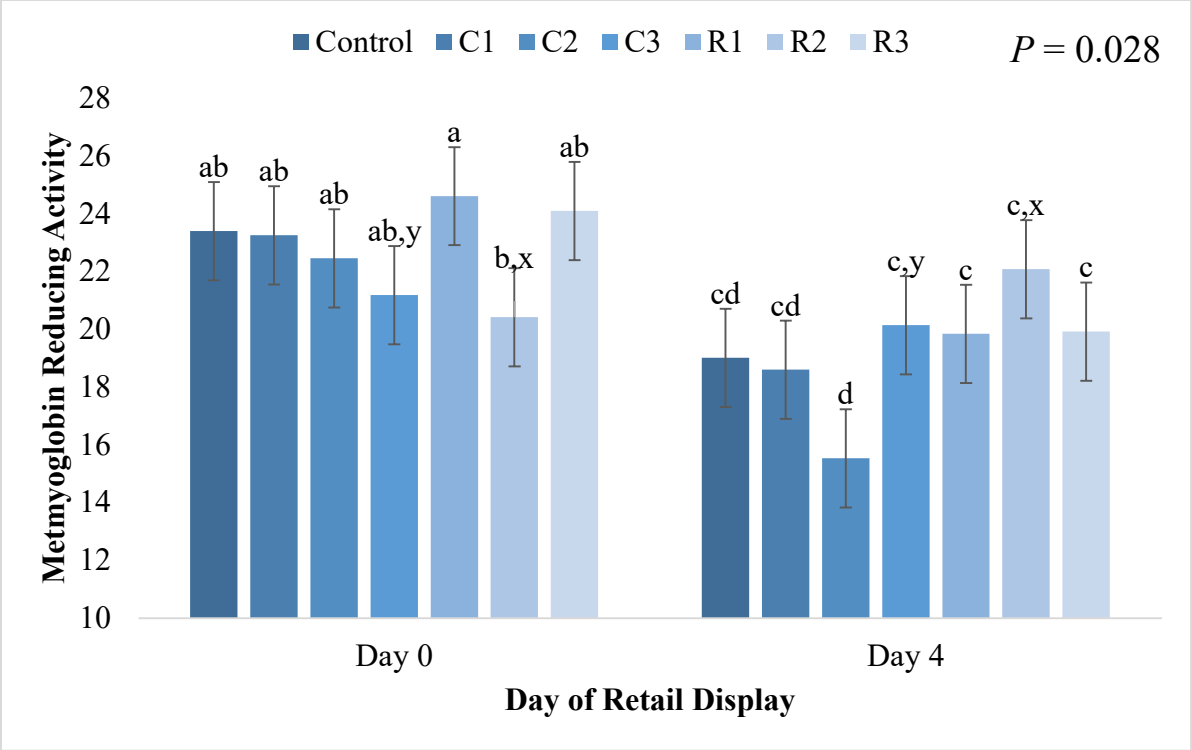

Figure 3.1. Metmyoglobin reducing activity (MRA) values for antioxidant treatment by retail display time for bone-in short rib steaks (N = 63). Each steak was randomly assigned based on location to be an untreated control (C), topically sprayed (2ml) with a 0.05% acerola cherry powder solution from one of three suppliers (C1, C2, or C3), or topically sprayed (2ml) with a 0.10% rosemary extract solution from one of three suppliers (R1, R2, or R3). Steaks were overwrapped with an oxygen permeable PVC film and displayed in a retail display room at 2°C for 4 d. MRA was determined on d 0 and d 4 of retail display for the control and each antioxidant treatment with the following equation: MRA = [(Initial % metmyoglobin – Final % metmyoglobin) ÷ Initial % metmyoglobin] × 100. Values are shown as least square means ± SE. $^{a\text{-}d}$Within a day, means without a common superscript differ ($P < 0.05$). ^{xy}Within a treatment, means without a common superscript differ ($P < 0.05$).

Chapter 4: Extending the shelf-life of beef chuck roll and bone-in short rib steaks using combinations of acerola cherry powder and rosemary extract

Abstract

Improving the retail shelf-life of steaks from beef chuck rolls and bone-in short ribs, items commonly exported, will improve salability of beef in international markets and therefore improve potential profits for producers. The objective of this study was to determine the effect of the topical application of acerola cherry powder and rosemary extract in combination on beef chuck roll and bone-in short rib shelf-life with regard to color stability and lipid oxidation. Beef chuck rolls (IMPS 116A; N = 9) and bone-in short ribs (IMPS 123A; N = 18) from USDA Choice carcasses were purchased from a commercial harvest facility and aged (0°C) for 28 d post-fabrication. Following aging, 1.02 cm-thick steaks were cut (perpendicular to the rib bones on the short rib steaks) and systematically assigned to a treatment based on steak location within the subprimal. Treatments included: untreated control (C) or topically sprayed (2 ml) with a treatment of an acerola cherry powder solution (0.05% A), rosemary extract solution (0.10% R), or mixture of the acerola cherry powder and rosemary extract (M1 = 0.05% A + 0.1% R; M2 = 0.1% A + 0.1% R; M3 = 0.05% A + 0.2% R; M4 = 0.1% A + 0.2% R). Half of the steaks were assigned by location to d 0 lipid oxidation, metmyoglobin reducing activity (MRA), and oxygen consumption; the remaining steaks were assigned to 4 days of retail display followed by d 4 lipid oxidation and MRA. Throughout retail display, chuck roll steak objective and subjective color was measured at h 1, h 18, and every 12 hours afterward until h 90. Short rib steak color was measured once daily for 4 days. Chuck roll steaks treated with A, M2, and M3, were redder (higher a*) than

the untreated (C) steaks ($P = 0.001$), and treating steaks with M2, M3, and M4 decreased lipid oxidation ($P = 0.004$). On d 0 and d 4, untreated bone marrow was less red than all of the antioxidant treated bone marrow ($P = 0.011$), and steaks treated with M4 did not increase in lipid oxidation from d 0 to d 4 ($P < 0.001$). Applying topical antioxidants improves shelf-life stability of beef chuck rolls and bone-in short ribs aged for an extended period.

Keywords: antioxidants, extended aging, shelf-life, beef

Introduction

According to the United States (US) Meat Export Federation (USMEF), in 2019, US beef exports contributed over $7.4 billion of revenue, averaging $308.74 per head (USMEF, 2020). The Republic of Korea (South Korea) was identified as the US beef industry's largest growing export market with a 29% increase in chilled US beef purchases in 2020 (USMEF, 2021; USDA – FAS, 2020).

Currently, it takes a minimum of 28 d to ship chilled beef from the US to South Korea (USMEF, 2020). Colle et. al (2015; 2016) determined extended aging increases lipid oxidation and reduces lean color redness throughout retail display. Extended aging also decreases metmyoglobin reducing activity and pH (English et al., 2016), as well as mitochondrial oxygen consumption rate (Mancini and Ramanathan, 2014). Additionally, Gill (1996) suggested that when bone-in cuts are aged for a longer period of time, the bone blackens faster than unaged bones during retail display. Further, the lean tissue of chuck roll and short rib steaks contains the *Serratus ventralis* (SV) muscle, which has been classified as having low color stability (McKenna et al., 2005).

A potential solution to improve US beef shelf-life is the application of antioxidants to the surface of the steaks (Colle et al., 2019; Van Buren et al., 2021a; 2021b). Determining a method to improve the color stability of US beef in international markets requiring lengthy shipping could provide the opportunity for increased international demand for US beef exports and producer profits. The objective of this study was to determine the effect of the topical application of acerola cherry powder and rosemary extract in combination on beef chuck roll and bone-in short rib steak shelf-life including color and lipid oxidation.

Methods and Materials

Product preparation

Beef chuck rolls (IMPS 116A; N = 9) and bone-in short ribs (IMPS 123A; N = 18) from USDA Choice carcasses were purchased from a commercial harvest facility and transported under refrigeration (4 h; 4°C) to the University of Idaho Meat Laboratory. The subprimals were wet-aged at 0°C for 28 d to simulate trans-Pacific shipping time prior to being prepared for retail display. Following aging, chuck rolls and short ribs (perpendicular to the bone) were cut into 1.02 cm-thick steaks (N = 126 and N = 126, respectively) to mimic South Korean retail steak fabrication style. Steaks were equally assigned based on location within each subprimal to a treatment group. Treatments included: untreated control (C) or topically sprayed (2 ml) with an acerola cherry powder solution (0.05% A), rosemary extract solution (0.10% R), or mixture of acerola cherry powder and rosemary extract (M1 = 0.05% A + 0.1% R; M2 = 0.1% A + 0.1% R; M3 = 0.05% A + 0.2% R; M4 = 0.1% A + 0.2% R). Half of the steaks were assigned by steak location within subprimal to d 0 lipid oxidation,

metmyoglobin reducing activity (MRA), and oxygen consumption (OC) rate; the remaining steaks were assigned to 4 days of retail display followed by d 4 lipid oxidation and MRA. Steaks were displayed in a retail display room at 2°C. The display room was equipped with natural white 4000 W lights, and the average light intensity was 849 lux (Fisherbrand Traceable Dual-Range Light Meter, Fisher Scientific, Waltham, MA).

Retail fluid loss

Following treatment, steaks were weighed, placed in white foam trays (CKF Inc. #88142, Langley, BC, Canada), and overwrapped with an oxygen permeable PVC film (oxygen transmission rate: 1,450 cc/645 cm^2 per 24 h; water vapor transmission rate: 17.0 g/645 cm^2 per 24 h; Koch Industries, Inc. #7500-3815; Wichita, KS). Following 4 days of retail display, steaks were removed from the retail packaging and re-weighed to determine retail moisture loss. Percent retail fluid loss was calculated using the following equation:

$$\%\ Fluid\ Loss = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} \times 100\%$$

Retail color

Retail display steaks were allowed to bloom for 60 minutes, and then two objective color measurements per steak were obtained on the SV using a Nix Pro 2 Sensor (Nix Sensor Ltd., Hamilton, Ontario, Canada). Two objective color measurements were also taken on the bone marrow of the short ribs. The Nix Pro 2 Sensor was equipped with a 14 mm-diameter measuring area and a 2° standard observer. The instrument was set to Illuminant D_{65} and Commission International de l'Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) values were recorded. Oxygenated lean color, amount of browning,

discoloration, surface discoloration, color uniformity, and bone marrow color were measured by five subjective evaluators following American Meat Science Association guidelines (AMSA, 2012). Color was evaluated on short rib steaks once daily throughout the 4 d retail display. After the initial chuck roll steak readings at h 1 of retail display, subsequent chuck roll color measurements were taken twice daily (06:00 and 18:00) on d 1, 2, and 3, and once (06:00) on d 4. To avoid potential effects due to display location, steaks were rotated in the retail environment daily.

Metmyoglobin reducing activity

Nitric oxide MRA was measured on the SV after treatment on d 0 and on d 4 of retail display following protocols outlined in Section XI of the Meat Color Measurement Guidelines (AMSA, 2012). Color measurements were obtained using a Hunter MiniScan EZ (Restin, Virginia) equipped with a 25 mm-diameter measuring area and a 10° standard observer. The instrument was set to Illuminant A and reflectance from 400 to 700 nm was recorded. Calibration occurred by measuring against black and white calibration tiles prior to measuring color. The percentage of metmyoglobin (MMb) was calculated following equations in Section XI of the Meat Color Measurements Guidelines (AMSA, 2012). MRA was calculated as follows:

$$MRA = \left[\frac{Initial\ \%\ MMb - Final\ \%\ MMb}{Initial\ \%\ MMb}\right] \times 100$$

Oxygen consumption

Oxygen consumption was measured on the SV after treatment on d 0 following protocols outlined in the Meat Color Measurement Guidelines (AMSA, 2012). Color

measurements were taken using a Hunter MiniScan EZ (Restin, Virginia) equipped with a 25 mm-diameter measuring area and a 10° standard observer. The instrument was set to Illuminant A and reflectance from 400 to 700 nm was recorded. Calibration occurred by measuring against black and white calibration tiles prior to measuring color. The percentage of oxymyoglobin (OMb) was calculated following equations in Section XI of the Meat Color Measurements Guidelines (AMSA, 2012). OC was calculated as follows:

$$OC = \left[\frac{Initial\ \%\ OMb - Final\ \%\ OMb}{Initial\ \%\ OMb}\right] \times 100$$

Lipid oxidation

Thiobarbituric acid reactive substances (TBARS) were analyzed in duplicate on d 0 after treatment and on d 4 of retail display following the protocol in Section XI, Appendix O of the Meat Color Measurement Guidelines (AMSA, 2012). Samples weighed 1 g and were cut from the SV avoiding the steak edge, large pieces of fat, and connective tissue.

Statistical analysis

Data was collected on 63 steaks per antioxidant treatment-retail display time combination. Data were analyzed using mixed model analysis of variance. Antioxidant treatments, retail display time, and their interaction were assumed as fixed effects. In this factorial design, there were six antioxidant treatments and one control treatment. MRA and lipid oxidation were evaluated at two retail display time points, whereas color was analyzed at five or eight retail display time points. Retail display time was considered a repeated measure modeled as a compound symmetric correlation structure. Subjective color data were averaged based on retail display time, evaluator, and antioxidant treatment prior to analysis,

where evaluator was considered a random block effect in a randomized complete block design. Objective color data were analyzed with subprimal and steak location within subprimal as randomized block effects. Treatment least square means differences were assessed through pair-wise comparisons for significant effects. Significance was determined at $P < 0.05$. All statistical analyses were conducted using SAS V 9.4 (SAS Inc., Cary, NC).

Results and Discussion

Retail fluid loss

In the chuck roll steaks, there was not a difference between treatments for fluid loss ($P = 0.316$; Table 4.1). However, in the short rib steaks, M1, M3, and M4 treated steaks had the least fluid loss, but did not differ from untreated (C) steaks ($P = 0.034$; Table 4.2). Loss of fluid during retail display results in a drier cooked product for the consumer (Lawrie and Ledward, 2006). These results are similar to previous research, in which antioxidants did not impact chuck roll steak fluid loss but did impact short rib steak fluid loss (Van Buren et al., 2021a; 2021b). More research is needed to determine the relationship between topical antioxidant treatments and retail fluid loss in bone-in steaks. Treating steaks with 2 ml of topically applied water could assist in determining the relationship.

Retail objective color

When evaluating the chuck roll steaks, an interaction between retail display time and treatment was not observed for L*, a*, or b* ($P = 0.587$, $P = 0.898$, and $P = 0.335$, respectively). Steaks treated with M2 and M3 had the darkest (lowest L*) lean tissue numerically, but none of the antioxidant treatments differed from the untreated (C) steaks (*P*

= 0.012; Table 4.1). Steaks treated with A, M2, and M3, were redder (higher a*) than the untreated (C) steaks and R3 treated steaks ($P = 0.001$; Table 4.1). Ascorbic acid, the active ingredient of acerola cherry powder, donates electrons to prevent the myoglobin oxidation process (Buettner and Jerkiewicz, 1996). Based on previous research of ascorbic acid in ground beef (Ismail et al., 2009), it was anticipated that acerola cherry powder would improve redness more than rosemary extract treated steaks and control steaks. All treatments had redness values greater than 14.5, which is the threshold for consumers' acceptability (Holman et al., 2017). Steaks treated with A had higher b* values or were more yellow than the untreated control steaks ($P = 0.034$; Table 4.1). Lightness fluctuated throughout retail display ($P = 0.022$; Table 4.3). Steaks were reddest and yellowest at the initial evaluation at h 1, but over time, steaks decreased in redness and yellowness ($P < 0.001$, and $P < 0.001$, respectively; Table 4.3). This observation is consistent with previous research where aged steaks declined in redness and yellowness during retail display (Colle et al., 2015; 2016; Van Buren et al., 2021b). Throughout retail display, steaks maintained redness above the acceptability threshold, a* greater than 14.5 (Holman et al., 2017).

An interaction between retail display time and treatment was observed in bone marrow L* ($P = 0.038$; Table 4.4). Untreated bone marrow did not change in lightness from d 0 to d 4. All antioxidant treated bone marrow were darkest on d 4. Untreated bone marrow was similar in lightness to A and M3 treated bone marrow throughout the entire retail display. However, on d 4 bone marrow treated with M2, M4, and R were darker than the untreated bone marrow. Previous research found rosemary extract treated rib bone marrow was darker than untreated bone marrow (Van Buren et al., 2021a).

An interaction between retail display time and treatment was also observed in bone marrow a* ($P = 0.011$; Table 4.4). Across all treatments, bone marrow was the reddest on d 0. On d 0 and d 4, untreated bone marrow was less red than all of the antioxidant treated bone marrow. Bone marrow treated with M2 was consistently one of the reddest treatments every day of retail display. Ascorbic acid prevents myoglobin oxidation (Buettner and Jerkiewicz, 1996), which is similar to hemoglobin oxidation (Lanari et al., 1995). Based on previous research of ascorbic acid on vertebrae (Mancini et al., 2004), it was anticipated that acerola cherry powder would improve redness more than rosemary extract treatments and untreated bone marrow. However, in this study lower concentrations (0.05%) of acerola cherry powder could have reduced bone marrow redness due to the ability of ascorbic acid to act as a meat pro-oxidant (Buettner and Jerkiewicz, 1996). Previous research has also seen an inability of acerola cherry powder to function as an antioxidant in rib bone marrow at low concentrations (0.05%; Van Buren et al., 2021a).

An interaction between retail display time and treatment was not observed in bone marrow b* ($P = 0.232$). Additionally, antioxidant treatments did not differ in b* ($P = 0.065$; Table 4.2). Throughout retail display, bone marrow differed in b* ($P = 0.022$; Table 4.5). Yellowness decreased over time similar to previous research (Van Buren et al., 2021a). The decline in bone marrow color after being exposed to oxygen was anticipated due to the oxidation of iron within the hemoglobin (Lanari et al., 1995; Gill, 1996).

When evaluating the lean tissue of the short ribs, an interaction between retail display time and treatment was observed for a* ($P = 0.009$; Table 4.4). On d 0 there was not a difference in redness between treatments. All of the treatments except steaks treated with M3 decreased in redness throughout retail display. This observation is consistent with

previous research where aged steaks declined in redness during retail display (Colle et al., 2015; 2016). The untreated control steaks and steaks treated with M4 were consistently two of the reddest steaks each day of retail, but numerically M4 treated steaks were redder than the control steaks every day. All treatments had redness values greater than the consumers acceptability threshold throughout retail display (Holman et al., 2017). An interaction between retail display time and treatment was not observed for L* or b* ($P = 0.932$ and $P = 0.574$, respectively). Additionally, there was not a difference in L* or b* between treatments ($P = 0.409$ and $P = 0.214$, respectively; Table 4.2). However, lightness and yellowness of the steaks varied throughout retail display ($P < 0.001$ and $P < 0.001$, respectively; Table 4.5).

Retail subjective color

In the chuck rolls, steaks treated with M4 and R had a brighter oxygenated lean color than the untreated control steaks or steaks treated with A ($P = 0.044$; Table 4.1). These results were not reflected when evaluating a*, or objective redness, but is similar to previous research applying rosemary extract to chuck roll steaks (Van Buren et al., 2021b). Antioxidant treated steaks did not differ in discoloration from the untreated control steaks, but all treatments containing rosemary extract had less discoloration than the A treated steaks ($P = 0.029$; Table 4.1). This does not support the findings that ascorbic acid prevents steak discoloration by delaying myoglobin oxidation (Buettner and Jerkiewicz, 1996). Antioxidants did not impact amount of browning ($P = 0.149$), surface discoloration ($P = 0.531$), or color uniformity ($P = 0.061$; Table 4.1). Throughout retail display, there were changes in oxygenated lean color ($P < 0.001$), amount of browning ($P < 0.001$), discoloration ($P < 0.001$), surface discoloration ($P < 0.001$), and color uniformity ($P < 0.001$; Table 4.3). All subjective color categories had the most desirable appearance on d 0 and declined in

desirability throughout retail display, which is consistent with previous research where aged steaks discolored during retail display (Colle et al., 2015; 2016; English et al., 2016; Van Buren et al., 2021b).

When evaluating the short rib steaks, steaks treated with M2, M3, M4, and R had a brighter oxygenated lean color than the untreated control steaks ($P = 0.028$; Table 4.2). Interestingly, M3 treated steaks were the brightest oxygenated lean numerically and maintained a* values, or objective redness, over the 4 d retail display. Additionally, A and M1 treated steaks did not differ from the untreated control steaks in lean color. This does not support previous short rib acerola cherry powder applications (Van Buren et al., 2021a) or the findings that ascorbic acid extends shelf-life by delaying myoglobin oxidation (Buettner and Jerkiewicz, 1996). Interestingly, these results were not reflected when evaluating lean tissue a* or objective redness. None of the antioxidant treatments had less browning or less discoloration than the untreated control steaks ($P = 0.001$ and $P = 0.041$, respectively; Table 4.2). Bone marrow treated with M1, M2, M3, and R had less discoloration than the untreated control steaks ($P = 0.016$; Table 4.2). This differs from previous research that did not see an improvement in bone marrow discoloration when treated with antioxidants likely due to the higher concentrations and combinations used in the current study (Van Buren et al., 2021a). Treatments did not differ in surface discoloration ($P = 0.071$) or color uniformity ($P = 0.332$; Table 4.2). Throughout retail display, there were changes in oxygenated lean color ($P < 0.001$), amount of browning ($P < 0.001$), discoloration ($P < 0.001$), surface discoloration ($P < 0.001$), color uniformity ($P < 0.001$), and bone marrow color ($P < 0.001$; Table 4.5). Oxygenated lean color and bone marrow color had the lowest score, or most desirable appearance, on d 0. For 1 d of retail display, steaks maintained the lowest score in amount of

browning, discoloration, surface discoloration, and color uniformity. Across all subjective color categories, values increased to less desirable scores throughout retail display. In previous research aged steaks discolored during retail display (Colle et al., 2015; 2016; English et al., 2016; Van Buren et al., 2021a).

Metmyoglobin reducing activity

In the chuck roll and short rib steaks, there was not an interaction between retail display and treatment ($P = 0.248$, $P = 0.216$, respectively). Additionally, treatments did not differ in MRA in short rib steaks ($P = 0.315$; Table 4.2). However, chuck roll steaks treated with M4 had higher MRA than any other antioxidant treated steaks or untreated steaks ($P = 0.027$; Table 4.1). Increasing MRA improves meat color stability by delaying browning by reducing metmyoglobin to oxymyoglobin (Sammel et al., 2002). Additionally, as aging time increases, initial MRA decreases creating further color stability challenges (English et al., 2016). In the chuck roll steaks, there was a decrease in MRA from d 0 to d 4 ($P < 0.001$; Table 4.6). MRA decreases over time due to decreasing NADH levels postmortem and causes increased levels of metmyoglobin (Sammel et al., 2002; AMSA, 2012). Unexpectedly, MRA in the short rib steaks increased from d 0 to d 4 ($P < 0.001$; Table 4.6). The increase was only approximately 5%.

Oxygen consumption

In the chuck roll steaks oxygen consumption was highest in steaks treated with R ($P = 0.007$; Table 4.1). OC did not differ between treatments in the short rib steaks ($P = 0.570$; Table 4.2) similar to previous research (Van Buren et al., 2021a). The ability to maintain

moderate OC throughout retail display could stabilize color by regenerating NADH levels for MRA without reducing the oxygen partial pressure at the surface (Sammel et al., 2002).

Lipid oxidation

In the chuck roll steaks, there was not an interaction between day of retail display and treatment ($P = 0.572$), but treating steaks with M2, M3, and M4 decreased lipid oxidation ($P = 0.004$; Table 4.1). The ability to decrease lipid oxidation prevents myoglobin oxidation and, thus, steak discoloration (Mancini and Hunt, 2005). Other previous research mixing antioxidants into a ground product has shown a delay in lipid oxidation (Ismail et al., 2009; Kim et al., 2013; Gómez et al., 2016; Zhang et al., 2016). However, when ascorbic acid and rosemary extract were topically applied on the *Semimembranosus* and *Longissimus lumborum*, lipid oxidation did not change (Colle et al., 2019). This difference between studies may be due to the use of higher concentrations of antioxidants in the present experiment. As anticipated, lipid oxidation increased ($P < 0.001$; Table 4.6) from d 0 to d 4 of retail display similar to previous research (Kim et al., 2013; Colle et al., 2019). The lipid oxidation threshold for the ability of consumers to detect off flavors in meat is TBARS > 1.0 (Tarladgis et al., 1960; Greene and Cumuze, 1981). In this study on d 4 of retail display all of the treatments were above the lipid oxidation threshold.

In the short rib steaks, there was an interaction for lipid oxidation between day of retail display and treatment ($P < 0.001$; Figure 4.1). Treatments did not differ in TBARS values on d 0. Steaks treated with M4 did not increase in lipid oxidation from d 0 to d 4, whereas the remaining treatments did increase. On d 4, steaks treated with M1, M3, and M4 all had less lipid oxidation than the untreated control steaks. Additionally, steaks treated with

A had greater lipid oxidation than the untreated control steaks. All treatments were less than the lipid oxidation threshold on d 4 (Tarladgis et al., 1960; Greene and Cumuze, 1981). Previously, adding vitamin E, or tocopherols, to cattle diets decreased lipid oxidation in ground beef after 6 d of retail display (Faustman et al., 1989). Applying antioxidant mixtures containing rosemary extract on steaks could delay the oxidation of lipids without having to adjust US feeding strategies. Rosemary extract contains tocopherols and works to delay lipid oxidation by quenching free radicals created by oxidizing fatty acids (Murray et al., 2006). Although acerola cherry powder increased lipid oxidation, when used with rosemary extract, ascorbic acid can reduce tocopherol radicals to be reused (Murray et al., 2006).

Conclusion

Antioxidant treatments of acerola cherry powder and rosemary extract improved chuck roll and bone-in short rib steak lipid oxidation and redness during retail display from beef aged 28 d post fabrication. Simple topical applications at a retail meat merchandising setting following extended aging due to prolonged storage or transportation time, can extend the shelf-life stability of chilled beef. Additional investigation of using acerola cherry powder and rosemary extract on low color stability muscles or frozen beef may provide more opportunities for extending beef shelf-life.

Acknowledgements

This research was funded by The Beef Checkoff. We gratefully acknowledge financial support from Checkoff dollars provided by the Idaho Beef Council. Support for

this research project was also provided by the Idaho Agriculture Experiment Station. We are also appreciative for the personnel at the University of Idaho Vandal Brand Meats Lab that made this research possible.

Literature Cited

American Meat Science Association. 2012. Meat color measurement guidelines. 2nd ed. Am. Meat Sci. Assoc. Chicago, IL.

Buettner, G. R., and B. A. Jurkiewicz. 1996. Catalytic metals, ascorbate and free radicals: Combinations to avoid. Radiat. Res. 145:532-541. doi:10.2307/3579271.

Colle, M. C., R. P. Richard, K. M. Killinger, J. C. Bohlscheid, A. R. Gray, W. I. Loucks, R. N. Day, A. S. Cochran, J. A. Nasados, and M. E. Doumit. 2015. Influence of extended aging on beef quality characteristics and sensory perception of steaks from the gluteus medius and longissimus lumborum. Meat Sci. 110:32–39. doi:10.1016/j.meatsci.2015.06.013.

Colle, M. C., R. P. Richard, K. M. Killinger, J. C. Bohlscheid, A. R. Gray, W. I. Loucks, R. N. Day, A. S. Cochran, J. A. Nasados, and M. E. Doumit. 2016. Influence of extended aging on beef quality characteristics and sensory perception of steaks from the biceps femoris and semimembranosus. Meat Sci. 119:110–117. doi:10.1016/j.meatsci.2016.04.028.

Colle, M. C., R. P. Richard, M. C. Colle, W. I. Loucks, G. K. Murdoch, P. D. Bass, C. J. Williams, and M. E. Doumit. 2019. Retail display properties and consumer perception of extended aged beef topically treated with ascorbic acid and rosemary extract. Meat and Muscle Biol. 3(1):42–50. doi:10.22175/mmb2018.05.0011.

English, A. R., G. G. Mafi, D. L. VanOverbeke, and R. Ramanathan. 2016. Effects of extended aging and modified atmospheric packaging on beef top loin steak color. J. Anim. Sci. 94:1727–1737. doi:10.2527/jas.2015-0149.

Faustman, C., R. G. Cassens, D. M. Schaefer, D. R. Buege, S. N. Williams, and K. K. Scheller. 1989. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. J. Food Sci. 54(4):858–862. doi:10.1111/j.1365-2621.1989.tb07899.x.

Gill, C. O. 1996. Extending the storage life of raw chilled meats. Meat Sci. 43:99-109. doi: 10.1016/0309-1740(96)00058-7.

Gómez, I., M. J. Beriain, J. A. Mendizabal, C. Realini, and A. Purroy. 2016. Shelf life of ground beef enriched with omega-3 and/or conjugated linoleic acid and use of grape seed extract to inhibit lipid oxidation. Food Sci. & Nutrition. 4(1):67-79. doi:10.1002/fsn3.251.

Greene, B. E., and T. H. Cumuze. 1981. Relationship between TBA numbers and inexperienced panelists' assessment of oxidized flavor in cooked beef. J. Food Sci. 47:52–54, 58. doi:10.1111/j.1365-2621.1982.tb11025.x.

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 Sci. 127:57–62. doi:10.1016/j.meatsci.2017.01.005.

Ismail, H. A., E. J. Lee, K. Y. Ko, H. D. Paik, and D. U. Ahn. 2009. Effect of antioxidant application methods on the color, lipid oxidation, and volatiles of irradiated ground beef. J. Food Sci. 74(1):25-32. doi:10.1111/j.1750-3841.2008.00991.x.

Kim, S. J., S. C. Min, H. J. Shin, Y. J. Lee, A. R. Cho, S. Y. Kim, and J. Han. 2013. Evaluation of the antioxidant activities and nutritional properties of ten edible plant

extracts and their application to fresh ground beef. Meat Sci. 93:715-722. doi:10.1016/j.meatsci.2012.11.029.

Lanari, M. C., D. M. Schaefer, and K. K. Scheller. 1995. Dietary vitamin E supplementation and discoloration of pork bone and muscle following modified atmosphere packaging. Meat Sci. 41(3):237–250. doi:10.1016/0309-1740(95)00006-7.

Lawrie, R. A., and D. A. Ledward. 2006. Lawrie's Meat Science. 7th Ed. Woodhead Publishing Limited. Cambridge, England.

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

Mancini, R. A., and R. Ramanathan. 2014. Effects of postmortem storage time on color and mitochondria in beef. Meat Sci. 98:65-70. doi:10.1016/j.meatsci.2014.007.

Mancini, R. A., M. C. Hunt, K. A. Hachmeister, D. H. Kropf, and D. E. Johnson. 2004. Ascorbic acid minimizes vertebrae discoloration. Meat Sci. 68(3):339-345. doi:10.1016/j.meatsci.2004.03.017.

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 Sci. 70:665–682. doi:10.1016/j.meatsci.2005.02.016.

Murray, R. K., D. K. Granner, and V. W. Rodwell. 2006. Harper's Illustrated Biochemistry. 27th Ed. McGraw-Hill Companies, Inc. New York, NY.

Sammel, L. M., M. C. Hunt, D. H. Kropf, K. A. Hachmeister, and D. E. Johnson. 2002. Comparison of assays for metmyoglobin reducing ability in beef inside and outside

semimembranosus muscle. J. of Food Sci. 67:978-984. doi:10.1111/j.1365-2621.2002.tb09439.x.

Tarladgis, B. G., B. M. Watts, and M. T. Younathan. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37:44–48. doi:10.1007/BF02630824.

USDA – FAS. 2020. 2019 United States Agricultural Export Yearbook: South Korea 2019 Export Highlights. https://www.fas.usda.gov/south-korea-2019-export-highlights. (Accessed 4 January 2021.)

USMEF. 2020. U.S. Meat Export Federation Annual Report 2019. https://www.usmef.org/downloads/2019-USMEF-Annual-Report.pdf. (Accessed 22 January 2020.)

USMEF. 2021. U.S. Meat Export Federation Annual Report 2020. https://www.usmef.org/downloads/2020-USMEF-Annual-Report.pdf. (Accessed 14 January 2021.)

Van Buren, J. B., B. J. Buseman, T. M. Weber, J. A. Nasados, J. M. Lancaster, J. H. Smart, P. D. Bass, and M. J. Colle. 2021a. Extending the shelf-life of beef bone-in short rib steaks using acerola cherry powder and rosemary extract. Meat and Muscle Biol. Submitted.

Van Buren, J. B., B. J. Buseman, T. M. Weber, J. A. Nasados, J. M. Lancaster, J. H. Smart, P. D. Bass, and M. J. Colle. 2021b. Extending the shelf-life of beef chuck roll steaks using acerola cherry powder and rosemary extract. Meat and Muscle Biol. Submitted.

Zhang, X., D. Li, Q. Meng, C. He, and L. Ren. 2016. Effect of mulberry leaf extracts on color, lipid oxidation, antioxidant enzyme activities and oxidative breakdown products of raw ground beef during refrigerated storage. J. Food Quality. 39:159-170. doi:10.1111/jfq.12187.

Table 4.1. Estimated mean effects of topical antioxidant treatment on chuck roll steak fluid loss, color, metmyoglobin reducing activity, oxygen consumption, and lipid oxidation (N = 63)

	Topical Antioxidant Treatment[1]							
Trait	Control	A	M1	M2	M3	M4	R	SEM
Retail fluid loss, %	1.96	3.63	4.97	1.88	2.14	1.83	3.99	1.15
L*	38.10^{abc}	38.25^{ab}	38.31^{ab}	37.59^{bc}	37.27^{c}	38.82^{a}	38.23^{ab}	0.59
a*	19.99^{b}	20.83^{a}	20.38^{ab}	20.78^{a}	21.26^{a}	20.27^{ab}	19.99^{b}	0.35
b*	14.06^{bc}	14.81^{a}	14.26^{abc}	13.95^{c}	14.59^{ab}	14.10^{bc}	14.08^{bc}	0.23
Oxygenated lean color[2]	4.9^{a}	5.0^{a}	4.9^{ab}	4.9^{ab}	4.8^{ab}	4.7^{b}	4.7^{b}	0.4
Amount of browning[3]	2.6	2.8	2.7	2.6	2.6	2.6	2.6	0.3
Discoloration[4]	2.3^{ab}	2.4^{a}	2.3^{b}	2.3^{b}	2.3^{b}	2.3^{b}	2.3^{b}	0.3
Surface discoloration[5]	2.5	2.5	2.4	2.4	2.4	2.4	2.5	0.3
Color uniformity[6]	2.6	2.8	2.7	2.6	2.6	2.6	2.7	0.1
Metmyoglobin reducing activity, %	9.30^{b}	9.67^{b}	9.50^{b}	9.53^{b}	9.10^{b}	12.44^{a}	8.55^{b}	1.00
Oxygen consumption, %	50.88^{bc}	60.62^{ab}	52.46^{bc}	53.75^{bc}	47.16^{c}	57.24^{ab}	65.47^{a}	4.00
Lipid oxidation[7]	0.85^{a}	0.86^{a}	0.76^{ab}	0.71^{b}	0.67^{b}	0.72^{b}	0.77^{ab}	0.07

[abc]Within a row, means without a common superscript differ ($P < 0.05$)

[1]Treatments included: an untreated control, topically sprayed (2 ml) with a 0.05% acerola cherry powder solution (A), topically sprayed (2 ml) with a 0.10% rosemary extract solution (R), or topically sprayed (2 ml) with a mixture of the acerola cherry powder and rosemary extract (M1 = 0.05% A + 0.1% R; M2 = 0.1% A + 0.1% R; M3 = 0.05% A + 0.2% R; M4 = 0.1% A + 0.2% R)

[2]Oxygenated lean color scale – 1 = extremely bright cherry-red, 8 = extremely dark red

[3]Amount of browning scale – 1 = no evidence of browning, 6 = dark brown

[4]Discoloration scale – 1 = none, 5 = extreme

[5]Surface discoloration scale – 1 = no discoloration (0%), 6 = extensive discoloration (81-100%)

[6]Color uniformity scale – 1 = uniform no two-toning, 5 = extreme two-toning

[7]mg malondialdehyde/kg meat

Table 4.2. Estimated mean effects of topical antioxidant treatment on bone-in short rib steak fluid loss, color, metmyoglobin reducing activity, and oxygen consumption (N = 63)

	Topical Antioxidant Treatment[1]							
Trait	Control	A	M1	M2	M3	M4	R	SEM
Retail fluid loss, %	1.50^{abc}	1.52^{abc}	0.43^{bc}	2.58^{a}	0.46^{bc}	-0.08^{c}	2.10^{ab}	0.70
Bone marrow b*	11.00	10.88	11.83	11.92	12.35	11.62	13.06	0.65
Lean L*	39.08	38.28	38.74	38.78	38.51	38.27	37.94	0.74
Lean b*	14.80	14.62	14.85	14.80	15.75	15.17	15.35	0.39
Oxygenated lean color[2]	4.6^{a}	4.4^{ab}	4.3^{ab}	4.2^{bc}	4.0^{c}	4.1^{c}	4.3^{bc}	0.5
Amount of browning[3]	1.5^{cd}	1.6^{ab}	1.7^{a}	1.6^{bc}	1.5^{cd}	1.5^{d}	1.5^{cd}	0.1
Discoloration[4]	1.4^{abc}	1.5^{a}	1.5^{ab}	1.5^{ab}	1.4^{bc}	1.4^{c}	1.4^{abc}	0.1
Surface discoloration[5]	1.4	1.6	1.5	1.5	1.4	1.4	1.4	0.1
Color uniformity[6]	1.6	1.7	1.7	1.7	1.6	1.6	1.6	0.1
Bone marrow color[7]	3.5^{a}	3.4^{abc}	3.3^{cd}	3.4^{bcd}	3.4^{bcd}	3.5^{ab}	3.3^{d}	0.3
Metmyoglobin reducing activity, %	19.57	19.84	20.79	20.96	20.77	21.43	19.31	0.80
Oxygen consumption, %	70.06	65.68	64.01	65.82	67.11	67.97	63.39	3.20

[abc]Within a row, means without a common superscript differ ($P < 0.05$)

[1]Treatments included: an untreated control (C), topically sprayed (2 ml) with a 0.05% acerola cherry powder solution (A), topically sprayed (2 ml) with a 0.10% rosemary extract solution (R), or topically sprayed (2 ml) with a mixture of the acerola cherry powder and rosemary extract (M1 = 0.05% A + 0.1% R; M2 = 0.1% A + 0.1% R; M3 = 0.05% A + 0.2% R; M4 = 0.1% A + 0.2% R)

[2]Oxygenated lean color scale – 1 = extremely bright cherry-red, 8 = extremely dark red

[3]Amount of browning scale – 1 = no evidence of browning, 6 = dark brown

[4]Discoloration scale – 1 = none, 5 = extreme

[5]Surface discoloration scale – 1 = no discoloration (0%), 6 = extensive discoloration (81-100%)

[6]Color uniformity scale – 1 = uniform no two-toning, 5 = extreme two-toning

[7]Bone marrow color scale – 1 = bright reddish-pink to red, 7 = black

Table 4.3. Estimated mean effects of retail display time on chuck roll steak color (N = 63)

	Time of Retail Display (hours)								
Trait	1	18	30	42	54	66	78	90	SEM
L*	39.79^{cd}	39.46^{d}	40.55^{bc}	40.89^{b}	43.03^{a}	41.14^{b}	41.06^{b}	43.34^{a}	0.64
a*	21.77^{a}	20.47^{c}	22.00^{a}	21.56^{ab}	20.85^{bc}	20.68^{c}	20.78^{bc}	20.18^{c}	0.34
b*	15.25^{a}	11.59^{f}	14.89^{ab}	13.12^{de}	14.37^{bc}	12.81^{e}	13.77^{cd}	14.27^{bc}	0.33
Oxygenated lean color[1]	3.5^{f}	3.7^{e}	4.0^{c}	4.1^{d}	4.4^{c}	4.6^{bc}	4.7^{b}	4.9^{a}	0.2
Amount of browning[2]	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{e}	1.2^{b}	1.2^{b}	0.1
Discoloration[3]	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{e}	1.2^{b}	1.2^{b}	0.1
Surface discoloration[4]	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{c}	1.0^{e}	1.2^{b}	1.3^{b}	0.1
Color uniformity[5]	1.1^{g}	1.0^{g}	1.2^{f}	1.2^{ef}	1.3^{de}	1.4^{cd}	1.5^{bc}	1.6^{b}	0.2

[a-h]Within a row, means without a common superscript differ ($P < 0.05$)

[1]Oxygenated lean color scale – 1 = extremely bright cherry-red, 8 = extremely dark red

[2]Amount of browning scale – 1 = no evidence of browning, 6 = dark brown

[3]Discoloration scale – 1 = none, 5 = extreme

[4]Surface discoloration scale – 1 = no discoloration (0%), 6 = extensive discoloration (81-100%)

[5]Color uniformity scale – 1 = uniform no two-toning, 5 = extreme two-toning

Table 4.4. Estimated mean effects of topical antioxidant treatment and retail display time on bone-in short rib steak color (N = 63)

Trait	Day of Display	Topical Antioxidant Treatment[1]							
		Control	A	M1	M2	M3	M4	R	SEM
Bone marrow L*	0	$44.47^{ab,w}$	$43.47^{b,wx}$	$46.09^{a,w}$	$45.13^{ab,w}$	$43.77^{ab,w}$	$43.60^{ab,w}$	$43.22^{b,w}$	1.15
	1	$45.30^{a,w}$	$45.51^{a,w}$	$43.42^{ab,x}$	$42.40^{b,x}$	$45.01^{b,w}$	$43.03^{ab,w}$	$44.05^{ab,w}$	
	2	$45.58^{ab,w}$	$43.89^{abc,wx}$	$42.00^{c,xy}$	$44.63^{abc,wx}$	$45.76^{abc,w}$	$42.89^{a,w}$	$45.56^{ab,w}$	
	3	$45.11^{a,w}$	$44.27^{ab,wx}$	$43.17^{ab,xy}$	$45.05^{a,wx}$	$44.28^{a,w}$	$42.08^{ab,w}$	$44.59^{ab,w}$	
	4	$43.64^{a,w}$	$41.81^{ab,x}$	$40.72^{bc,y}$	$38.86^{c,y}$	$41.12^{c,x}$	$38.98^{a,x}$	$38.88^{c,x}$	
Bone marrow a*	0	$16.23^{c,w}$	$17.97^{b,w}$	$18.26^{b,w}$	$19.98^{a,w}$	$20.04^{a,w}$	$18.99^{ab,w}$	$19.28^{ab,w}$	0.58
	1	$13.28^{b,xy}$	$13.30^{b,xy}$	$15.19^{a,x}$	$16.08^{a,x}$	$15.11^{a,x}$	$15.61^{a,x}$	$15.56^{a,x}$	
	2	$13.68^{ab,w}$	$12.26^{b,y}$	$13.81^{a,xy}$	$14.24^{a,y}$	$13.85^{a,x}$	$13.81^{ab,y}$	$13.77^{ab,yz}$	
	3	$13.92^{a,w}$	$12.91^{a,xy}$	$13.38^{a,y}$	$14.40^{a,y}$	$13.81^{a,x}$	$13.90^{a,y}$	$12.96^{a,z}$	
	4	$11.97^{c,y}$	$14.24^{b,x}$	$14.56^{b,xy}$	$16.21^{b,x}$	$13.87^{b,x}$	$15.08^{ab,xy}$	$14.79^{ab,xy}$	
Lean a*	0	$22.86^{a,wxy}$	$23.38^{a,w}$	$22.50^{a,w}$	$22.04^{a,w}$	$23.91^{a,w}$	$24.24^{a,w}$	$22.55^{a,w}$	0.93
	1	$23.84^{a,w}$	$22.20^{ab,wx}$	$23.06^{ab,w}$	$21.36^{b,w}$	$22.68^{ab,w}$	$24.04^{a,wx}$	$23.68^{ab,w}$	
	2	$23.02^{ab,wx}$	$20.90^{b,xy}$	$22.37^{b,w}$	$21.97^{b,w}$	$22.59^{b,w}$	$24.88^{a,w}$	$22.03^{ab,w}$	
	3	$20.65^{a,y}$	$21.81^{a,wxy}$	$22.56^{a,w}$	$22.97^{a,w}$	$21.84^{a,w}$	$21.12^{a,y}$	$22.36^{a,w}$	
	4	$21.54^{ab,xy}$	$19.70^{bc,y}$	$19.55^{bc,x}$	$19.04^{c,x}$	$23.50^{a,w}$	$21.74^{ab,xy}$	$18.16^{c,x}$	

[abc]Within a trait and day, means without a common superscript differ ($P < 0.05$)

[xyz]Within a trait and treatment, means without a common superscript differ ($P < 0.05$)

[1]Treatments included: an untreated control (C), topically sprayed (2 ml) with a 0.05% acerola cherry powder solution (A), topically sprayed (2 ml) with a 0.10% rosemary extract solution (R), or topically sprayed (2 ml) with a mixture of the acerola cherry powder and rosemary extract (M1 = 0.05% A + 0.1% R; M2 = 0.1% A + 0.1% R; M3 = 0.05% A + 0.2% R; M4 = 0.1% A + 0.2% R)

Table 4.5. Estimated mean effects of retail display time on bone-in short rib steak color (N = 63)

	Day of Retail Display					
Trait	0	1	2	3	4	SEM
Bone marrow b*	13.11[a]	11.29[b]	11.47[b]	11.51[b]	11.67[b]	0.57
Lean L*	38.39[bc]	37.70[c]	38.96[b]	39.84[a]	37.68[c]	0.70
Lean b*	16.15[a]	14.64[c]	15.10[bc]	15.57[ab]	13.77[d]	0.33
Oxygenated lean color[1]	3.3[b]	4.2[a]	4.6[a]	4.5[a]	4.7[a]	0.48
Amount of browning[2]	1.0[c]	1.1[c]	1.7[b]	1.8[b]	2.2[a]	0.16
Discoloration[3]	1.0[c]	1.1[c]	1.6[b]	1.7[b]	1.9[a]	0.14
Surface discoloration[4]	1.0[c]	1.1[c]	1.6[b]	1.7[b]	1.9[a]	0.14
Color uniformity[5]	1.1[d]	1.2[d]	1.8[c]	2.0[b]	2.2[a]	0.08
Bone marrow color[6]	2.2[c]	3.4[b]	3.7[a]	3.7[a]	4.0[a]	0.31

[a-h]Within a row, means without a common superscript differ ($P < 0.05$)
[1]Oxygenated lean color scale – 1 = extremely bright cherry-red, 8 = extremely dark red
[2]Amount of browning scale – 1 = no evidence of browning, 6 = dark brown
[3]Discoloration scale – 1 = none, 5 = extreme
[4]Surface discoloration scale – 1 = no discoloration (0%), 6 = extensive discoloration (81-100%)
[5]Color uniformity scale – 1 = uniform no two-toning, 5 = extreme two-toning
[6]Bone marrow color scale – 1 = bright reddish-pink to red, 7 = black

Table 4.6. Estimated mean effects of retail display time on steak metmyoglobin reducing activity and lipid oxidation (N = 63)

	Day of Retail Display		
Trait	0	4	SEM
Metmyoglobin reducing activity, %			
Chuck roll	15.71[a]	3.75[b]	1.29
Bone-in short rib	16.60[b]	24.16[a]	0.59
Lipid oxidation[1]			
Chuck roll	0.36[b]	1.17[a]	0.07

[a-h]Within a row, means without a common superscript differ ($P < 0.05$)

[1]mg malondialdehyde/kg meat

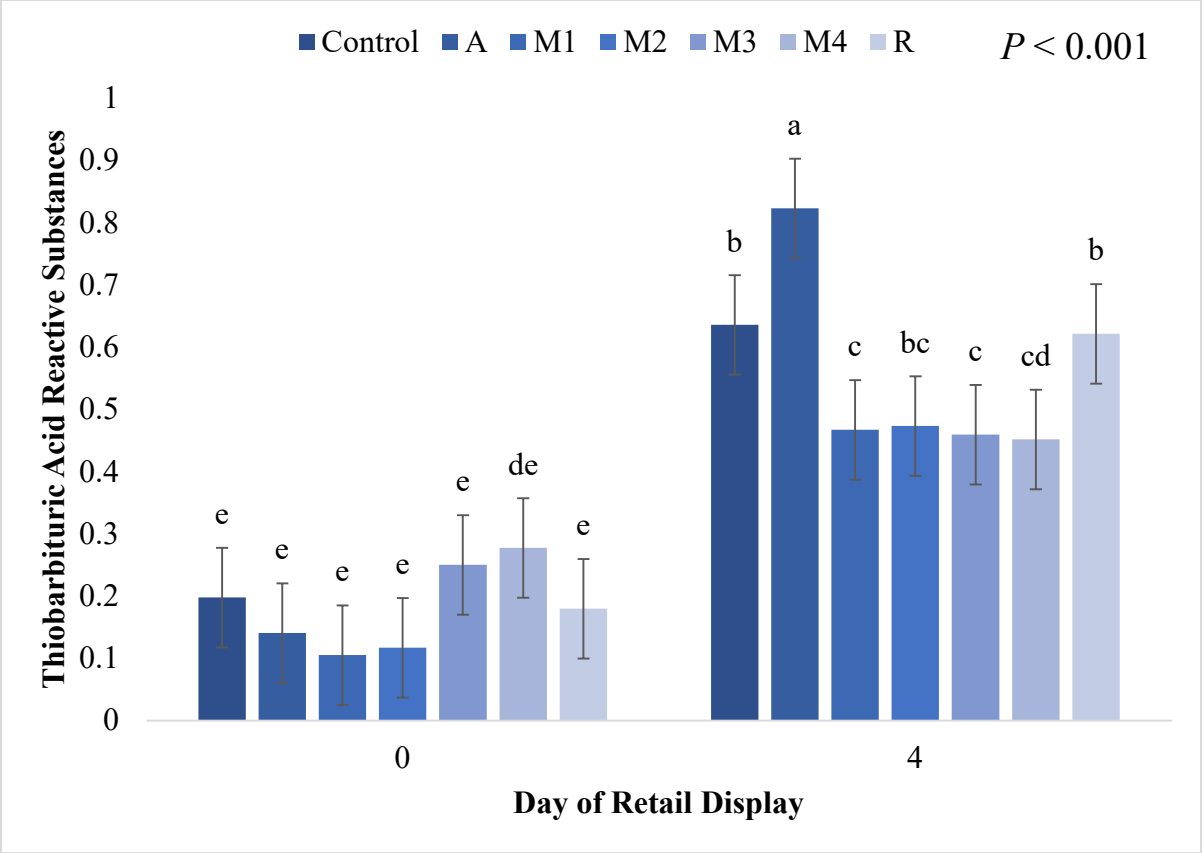

Figure 4.1. Thiobarbituric acid reactive substances (TBARS) values for antioxidant treatment by retail display time for bone-in short rib steaks (N = 63). Each steak was randomly assigned based on location to be an untreated control (C), topically sprayed (2 ml) with a 0.05% acerola cherry powder solution (A), topically sprayed (2 ml) with a 0.10% rosemary extract solution (R), or topically sprayed (2 ml) with a mixture of the acerola cherry powder and rosemary extract (M1 = 0.05% A + 0.1% R; M2 = 0.1% A + 0.1% R; M3 = 0.05% A + 0.2% R; M4 = 0.1% A + 0.2% R). Steaks were overwrapped with an oxygen permeable PVC film and displayed in a retail display room at 2°C for 4 d. TBARS were measured on d 0 and d 4 of retail display for the control and each antioxidant treatment. Values are shown as least square means ± SE. $^{a\text{-}e}$Means without a common superscript differ ($P < 0.05$).

Appendix A - TBARS for oxidative rancidity – rapid, wet method

Adapted from Appendix O: TBARS for Oxidative Rancidity (AMSA, 2012)

Principle: In the presence of thiobarbituric acid (TBA), malonaldehyde and other aldehyde products of lipid oxidation (TBA reactive substances; TBARS) form pink chromogens with maximum absorbance at 532-535 nm. However, in the presence of interfering sugars, a yellow chromagen forms, which can be avoided using the distillation method (Tarladgis, 1960).

Reagents:

1. TBA stock solution - 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25N HCl.
2. Stock solutions (100 mL) are sufficient for 20 individual tests. Stock solution may be stored at room temperature in the dark (foil-wrapped container).

Procedure:

1. Finely chop or mince a portion of the product of interest. Weigh out duplicate 0.25 g samples.
2. Add 1.25 ml TBA stock solution to each sample, giving a dilution factor of 6. Mix well.
3. Heat samples 10 min in boiling water in loosely capped 2.0 ml eppendorf tubes Caution: tightly capped tubes may burst during heating. Positive samples turn pink during heating.
4. Cool tubes in tap water.
5. Centrifuge at 5,000 × g for 10 min to obtain a clear supernatant.
6. Carefully pipette 200 µl of the supernatant to a 96 well plate. Take care that the solution remains clear.
7. Measure supernatant absorbance at 532 nm against a blank that contains all the reagents minus the meat.
8. Calculate the TBA value expressed as ppm malonaldehyde, using 1.56×10^5/M/cm as the extinction coefficient of the pink TBA chromogen (Sinnhuber and Yu, 1958), as follows:
   TBARS number (mg MDA/kg) = sample A_{532} × (1 M TBA chromagen/156,000) × [(1mole/L/M] × (0.003 L/0.5 g meat) × (72.07 g MDA/mole MDA) × 1000 mg/g) × 1000 g/kg)
   or
   TBARS value (ppm) = sample A_{532} × 2.77

References:

Buege, J.A. and Aust, S.D. 1978. Microsomal lipid peroxidation. Methods in Enzymology 52:302-304.

Sinnhuber, R.O. and Yu, T.C. 1958. 2-Thiobarbituric acid acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. Food Technology 12(1):9-12.

Appendix B – Metmyoglobin reducing capacity of intact or ground meat

Adapted from Appendix J (AMSA, 2012)

Principle: Surface pigments are initially oxidized to MMb by soaking the sample slice in a dilute sodium nitrite solution for 20 minutes. The steak sample (1.02 cm-thick) is vacuum packaged, and surface % MMb is monitored for 2 hours at 30°C by measuring reflectance *K/S* ratios (572/525 nm). Sample reducing ability is defined as the percentage decrease in surface MMb concentration during the incubation period. The decline in MMb is assumed to reflect the tissue's ability to reduce ferric heme iron.

Reagent:

1. 0.3% (w/w) sodium nitrite solution: Tare a large beaker, and weigh 3.0 g $NaNO_2$ into the beaker and add distilled water to 1000 g. Make fresh daily. Incubate at room temperature.

Procedure:

1. Remove a 3 cm × 3 cm × 2 cm sample of muscle tissue with no visible fat or connective tissue. For ground meat, use a similar sized sample that has been uniformly packed together to help avoid crumbling when the sample is immersed.
2. Be sure to orient sample to identify which surface will be evaluated later. This surface may be fresh cut or the surface that was displayed.
3. Submerge sample in 0.3% $NaNO_2$ solution for 20 minutes at room temperature to induce MMb formation. Ground samples can be placed on a small screen to help lower and raise the cube with minimal crumbling.
4. Remove sample from beaker, and blot to remove excess solution. Retain the 3-dimensional shape as much as possible and place the surface for evaluation up in an impermeable bag and vacuum package (a good, uniform vacuum). The vacuum may slightly flatten or round the samples.
5. Scan immediately for reflectance from 400 to 700 nm to determine the initial amount of MMb formed on the surface. Maintain surface integrity.
6. Place sample in an incubator at 30°C, and rescan after 2 hours to determine the remaining amount of MMb.

Calculations:

%MMb = [*K/S*572 ÷ *K/S*525 (for 100% DMb)] − [*K/S*572 ÷ *K/S*525 (sample)] ÷ [*K/S*572 ÷ *K/S*525 (for 100% DMb)] − [*K/S*572 ÷ *K/S*525 (for 100% MMb)][× 100]

MRA (% of MMb reduced) = [(Initial %MMb − Final %MMb) ÷ Initial %MMb] × 100

Or

Use the initial MMb formed as an indicator of MRA (see note below).

Notes: Some authors (McKenna et al., 2005; Mancini et al., 2008) indicate that the initial amount of MMb formed by oxidation in sodium nitrite solution is a good indicator of sample MRA. However, King et al. (2011) found that percentage reduction was better than the initial amount of MMb formed. Thus, it is best to collect and statistically analyze both the initial amount of MMb formed, and the percentage of MMb reduced over the incubation time.

References:

King, D. A., S. D. Shackelford, A. B. Rodriguez, and T. L. Wheeler. 2011. Effect of time of measurement on the relationship between metmyoglobin reducing activity and oxygen consumption to instrumental measures of beef longissimus color stability. Meat Sci. 87:26–32.

Mancini, R. A., M. Seyfert, and M. C. Hunt. 2008. Effects of data expression, sample location, and oxygen partial pressure on initial nitric oxide metmyoglobin formation and metmyoglobin-reducing-activity measurement in beef muscle. Meat Sci. 79:244–251.

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 Sci. 70:665–682.

Mohan, A., S. Muthukrishnan, M. C. Hunt, T. J. Barstow, and T. A. Houser. 2010d. Kinetics of metmyoglobin reduction by malate dehydrogenase. J. Agric. Food Chem. 58:6994–7000.

Raines, C. R., M. C. Hunt, and J. A. Unruh. 2010. Contributions of muscles of various color stabilities to the overall color stability of ground beef. J. Food Sci. 75:C85–C89.

Sammel, L. M., M. C. Hunt, D. H. Kropf, K. A. Hachmeister, C. L. Kastner, and D. E. Johnson. 2002b. Influence of chemical characteristics of beef inside and outside semimembranosus on color traits. J. Food Sci. 67:1323–1330.

Watts, B. M., J. Kendrick, M. W. Zipser, B. Hutchins, and B. Saleh. 1966. Enzymatic reducing pathways in meat. J. Food Sci. 32:855–862.

Appendix C – Oxygen consumption of intact muscle or ground meat

Adapted from Appendix I (AMSA, 2012)

Principle: Freshly cut meat slices are oxygenated (allowed to bloom) for a standardized time and temperature and then vacuum packaged. The decline in OMb due to enzyme respiration is measured as an indicator of the tissue's ability to consume oxygen. Reflectance spectra over the range 400 to 700 nm are recorded immediately and a second time after 20 minutes in a water bath or incubator kept at 25°C. Oxymyoglobin levels are calculated using the ratio of the reflectance at 610 and 525 nm after *K/S* transformation as described in Section IX. Higher *K/S*610/*K/S*525 ratios indicate higher OMb levels. Oxygen consumption (OC) is reported as the difference in percentage from the first and last measurements.

Equipment and Supplies:

1. Vacuum packaging machine
2. PVC film
3. Highly oxygen-impermeable vacuum bags (O_2 permeability ≤0.6 g O_2/625 cm^2/24 hours at 0°C)
4. Spectrometer that can scan and record surface reflectance from 400 to 700 nm (see Section IX)

Procedure:

1. All samples to be assayed must be the same temperature, 4°C, for instance. Otherwise, oxygen consumption will be faster for samples at warmer temperatures and bloom development (oxygenation) will be less; it will be slower for those at colder temperatures and bloom development will be more.
2. Keep all samples at 2 to 4°C to help ensure uniform oxygenation. For intact, whole muscle, use a sharp knife to remove a 3 cm × 3 cm × 2 cm sample with minimal visible fat or connective tissue. For ground samples, prepare a comparable sized cube that has been uniformly packed. Again, the visible fat level should be typical of the lean portion of the sample. Avoid dull knives that disrupt surface structure. Also avoid excessive handling and pressing of the blooming surface of ground product (see Madhavi and Carpenter, 1993).
3. If the surface is not a fresh cut, then just before starting the bloom step, remove a thin surface layer to expose fresh tissue.
4. Cover the freshly cut surface with a small piece of oxygen-permeable film to avoid drying. Keep the film (polyvinyl chloride film is commonly used) in one, smooth layer to ensure uniform exposure of the surface to air. Make note of the film's oxygen permeability.
5. Bloom for 2 hours at 2 to 4°C (or some other standardized time). Take care to keep all samples at the same temperature during this step because blooming is very temperature dependent.
6. After bloom, remove the PVC film and place the sample in a pouch with very low oxygen permeability. Quickly vacuum package with high vacuum; keep the vacuum uniform from sample to sample.

7. IMMEDIATELY scan the surface of the sample for reflectance from 400 to 700 nm to determine the initial %OMb. The spectrophotometer must be calibrated through the vacuum bag film.
8. To speed up oxygen consumption, use an incubator or water bath at 25°C. Re-scan the same surface after 20 minutes (or some standardized time appropriate to the meat being used).

Calculations:
%OMb = [*K/S*610 ÷ *K/S*525 (for 100% DMb)] − [*K/S*610 ÷ *K/S*525 (sample)] ÷ [*K/S*610 ÷ *K/S*525 (for 100% DMb)] − [*K/S*610 ÷ *K/S*525 (for 100% OMb)][× 100]
Oxygen consumption = [(Initial %OMb − Ending %OMb) ÷ Initial %OMb][× 100]

Notes: Madhavi and Carpenter (1993) described a reflectance procedure for measuring oxygen consumption (OC), using a spectrophotometer with reflectance attachment to measure surface OMb levels of vacuum packed samples initially, and at 5-minute intervals (20 minutes total) at 4°C. Samples were smaller (2.5 × 2.5 × 0.5 cm) to fit in the sample port of the reflectance unit. Relative concentration of OMb was calculated using the method of Krzywicki (1979). However, that method was modified by Tang et al. (2004) and their revised wavelengths are recommended (see Section IX). OC was expressed as percentage of time-zero surface OMb consumed during 10 minutes in vacuum. Mancini, Hunt and Kropf (2003) reported a method using reflectance at 610 nm to directly determine OMb. This is possible because OMb has its unique reflectance at 610 while 610 is isobestic for both DMb and MMb (see Section IX for further discussion of meat surface reflectance measurements and calculation of K/S ratios). This method has been used successfully (see King et al., 2011). Some research has reported an actual "rate of oxygen consumption" using percentage changes of OMb per unit of time. This is more laborious and time consuming. With a large number of samples, "oxygen consumption" is often calculated as the "average percentage reduction of OMb" relative to the initial level of OMb formed on the sample. The time for deoxygenation of the sample must be standardized. Usually, 20 minutes is sufficient to detect sample differences.

References:
King, D. A., S. D. Shackelford, A. B. Rodriguez, and T. L. Wheeler. 2011. Effect of time of measurement on the relationship between metmyoglobin reducing activity and oxygen 102 96 consumption to instrumental measures of beef longissimus color stability. Meat Sci. 87:26–32.

Madhavi, D. L., and C. E. Carpenter. 1993. Aging and processing affect color, metmyoglobin reductase and oxygen consumption of beef muscles. J. Food Sci. 58:939–942.

Mancini, R. A., M. C. Hunt, and D. H. Kropf. 2003. Reflectance at 610 nanometers estimates oxymyoglobin content on the surface of ground beef. Meat Sci. 64:157–162.

Mohan, A., M. C. Hunt, T. J. Barstow, T. A. Houser, and D. M. Hueber. 2010a. Nearinfrared oximetry of three postrigor skeletal muscles for following myoglobin redox forms. Food Chem. 123:456–464.

Tang, J., C. Faustman, and T. A. Hoagland. 2004. Krzywicki revisited, equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. J. Food Sci. 69:C717–C720.