EFFECTS OF ROSEMARY AND GREEN TEA ANTIOXIDANTS ON GROUND BEEF PATTIES IN TRADITIONAL AND MODIFIED ATMOSPHERE PACKAGING

By

LAURA ELAINA YODER

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EFFECTS OF ROSEMARY AND GREEN TEA ANTIOXIDANTS ON GROUND BEEF PATTIES IN TRADITIONAL AND MODIFIED ATMOSPHERE PACKAGING

Thesis Approved:

Dr. Gretchen Mafi

Thesis Adviser

Dr. Ranjith Ramanathan

Dr. Deborah VanOverbeke

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The great Dr. Seuss once wrote: "So be sure when you step. Step with care and great tact and remember that Life's a Great Balancing Act." This quote struck a chord with me as I have truly lived to see every part of this quote come to life in my short time at Oklahoma State. Moving away from my home and family was exciting and nerve wracking all at the same time, but quickly Oklahoma State became more of a family than strangers I had just met. Many people have played a hand in making my time here at OSU the adventure that it has been, and no amount of 'thank yous' will be sufficient.

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Abstract:
Consumers purchase ground beef more than any other beef product in the U.S. with 88% of consumers preferring traditional foam trays with overwrap (PVC), however PVC has a very limited shelf life. Utilizing gases like carbon monoxide (CO) and antioxidants like rosemary and green tea have been shown to extend shelf-life. The objective of this study was to analyze the effects of rosemary and green tea in ground beef in PVC and modified atmosphere packaging (MAP). Four treatments were used in this study: control, 2500 ppm rosemary, 300 ppm green tea, and 2500 rosemary + 300 ppm green tea. Patties from each treatment (n = 42) were randomly packaged into 1 of 3 types: PVC, MAP, or master packages (MP), MAP and MP flushed with 0.4% CO, 69.6% N_2, and 30% CO. Patties in PVC and MAP were put directly into simulated retail display for 7 d and MP were put in dark storage for 7 d, then removed and put in cases for 7 d of display. Objective and subjective color were measured, along with lipid oxidation and trained sensory panel measurements. As expected, patties in MAP had significantly ($P < 0.05$) lower lipid oxidation values and were significantly higher ($P < 0.05$) in all color values than patties in PVC after display. In MAP green tea significantly ($P < 0.05$) improved a* and chroma values on d 6 of retail display and had lower surface discoloration scores from d 3 to 6 compared to rosemary + green tea treatment. In MP patties, green tea statistically ($P < 0.05$) improved L* values, display color and surface discoloration scores compared to the combination treatment. However, green tea did not significantly (P > 0.05) improve values when compared to rosemary for these parameters. Trained taste panelists could not detect a difference between control and green tea patties ($P > 0.05$) for the green-hay attribute, however rosemary was highly detectable in the green-hay attribute.

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CHAPTER I

INTRODUCTION

According to research, consumers relate color of meat to freshness and consider fresh beef as a bright red color, any deviation they consider to be unacceptable (Bechtel et al., 1986). Consumer purchasing decisions are more often influenced by color than any other meat quality factor, because consumers correlate freshness and wholesomeness with color. It has been found that nearly 15% of retail beef is discounted due to discoloration, which results in $1 billion lost in annual revenue (Smith et al., 2000). Metmyoglobin is the pigment responsible for discoloration of meat products at almost every step of the meat processing chain and is a primary factor to off-put consumers at a retail case (Ramanathan et al., 2019). Oxidation of meat can cause deterioration of color, increase off flavors, and shorten shelf-life (Falowo et al., 2014).

Zink (1997) discussed the desire of consumers to use less traditional chemical preservatives, thus allowing less flexibility to produce a product with a comparable shelf-life. The food industry is utilizing technologies like innovative packaging practices and new ingredients to meet the consumer demands. The research on 'clean label' has increased significantly in the last 20 years. Asioli et al. (2017) reported in "Food Technology Magazine" the term 'clean label' was cited only two times in 2000, however has increased to 77 times in 2016, clearly indicating an interest and importance of the research. Thus, this project was designed to evaluate the use of plant derived antioxidants in combination with differing packaging types to achieve a longer shelf life of a ground beef product.

CHAPTER II

REVIEW OF LITERATURE

Meat Color

The primary protein responsible for meat color is myoglobin (Mb) with other heme proteins playing a role including hemoglobin and cytochrome c. Meat color can vary due to numerous reasons including but not limited to: species, age, sex, and muscle type. Myoglobin is a water-soluble sarcoplasmic protein with an iron centered-porphyrin ring, or a heme ring, and a globular protein portion, globulin (Aberle, 2012; Mancini and Hunt, 2005). The iron in the heme group can exist in the reduced (ferrous, Fe^{2+}) or oxidized (ferric, Fe^{3+}) states. The heme ring can form six bonds, four of them being to pyrrole nitrogens and the fifth to histidine (Suman and Joseph, 2013). The sixth and final site can bind to various ligands. Variation of the sixth binding site and charge of the heme-iron dictates the color of meat. Due to the variations, there are several primary chemical forms of Mb are responsible for meat color (Mancini and Hunt, 2005).

Deoxymyoglobin (DeoxyMb) occurs when no ligand is present and bonding on the sixth site and the heme iron is in Fe^{2+}. Typically, this is observed in vacuum type packaging or immediately after cutting meat prior to atmospheric exposure and is purplish-red in color. Oxygenation begins even when very little oxygen is present for Mb to bind with at the sixth binding site, turning DeoxyMb to oxymyoglobin (OxyMb). Once oxygenation occurs in beef with normal pH (approximately 5.6), meat will take the typical bright cherry-red color. This time is commonly referred to as bloom or bloom time. As the amount of oxygen exposure increases, the penetration of oxygen into a muscle will continue beneath the surface of the meat. The ability of oxygen to penetrate into meat can also depend on the temperature, pH and oxygen partial pressure along with other biological factors (Mancini and Hunt, 2005). Myoglobin serves as an oxygen source for mitochondria to be used for energy production and oxidative phosphorylation, and when needed, oxymyoglobin transfers its oxygen to the mitochondria (Ramanathan et al., 2019; Wittenberg and Wittenberg, 1989). Metmyoglobin (MetMb) can occur when either ferric or ferrous iron are oxidized and is responsible for browning or discoloration of meat (Ramanathan et al., 2019). Metmyoglobin is bound to water over oxygen in the sixth binding site due to its affinity to water (Suman and Joseph, 2013). Metmyoglobin primarily is reveled below the surface of a product where the partial pressure is lowest and moves towards the surface of a product and is typically brown in color (Mancini and Hunt, 2005). Numerous catalysts including but not limited to: oxygen partial pressure, temperature, pH, microbial growth and light, can engage the oxidation process (Aberle, 2012; Suman and Joseph, 2013).

Due to recent interest and growing use in the meat industry, carboxymyoglobin (COMb) has been of special interest to many researchers. Carbon monoxide is able to bind to Mb in the sixth position, which forms a very bright cherry-red color (Mancini and Hunt, 2005). It has been shown DeoxyMb is able to convert to COMb easier, when compared to OxyMb or MetMb, however when exposed to atmospheric free CO, the bound CO molecule will dissociate from Mb (Mancini and Hunt, 2005). In 2002 the United States Food and Drug Administration added CO to the Generally Recognized as Safe (GRAS) status to be utilized in modified atmospheric packaging (MAP) up to 0.4% (FDA, 2002).

Color Stability

In a proteomic approach to better understand color stability of specific beef muscles, Joseph et al. (2012) found antioxidant and chaperone proteins are over abundant in beef muscles that are considered color-stable, and they help protect myoglobin from oxidation to help improve color stability. Antioxidants have been shown to promote color stability on both whole muscle cuts and further processed meats by providing a favorable environment for Mb by limiting oxidation of the heme-iron (Suman and Joseph, 2013). According to Ramanathan et al. (2009) mitochondria can out-compete Mb for oxygen that remains in meat post-mortem, which results in the deoxygenated pigment thus resulting in dark colored meat.

Antioxidants can improve Mb stability, however in the presence of pro-oxidants Mb is destabilized and vulnerable to oxidation. Lipid oxidation can produce reactive byproducts such as ketones and aldehydes that can jeopardize color stability. In many studies, it has been shown feeding vitamin E prior to slaughter can increase color stability and decrease lipid oxidation of beef and lamb (Suman and Joseph, 2013). Suman et al. (2007) found beef Mb was more susceptible to lipid oxidation-induced oxidation than pork Mb when using mass spectrometry and proteomic tests and continued to describe how the proximal histidine favors aldehyde adduction, which explains why lipid oxidation-induced Mb oxidation is more extensive in beef than pork. Yin et al. (2011) suggests the occurrence of lipid oxidation of both mammalian and avian Mb directly correlates to the number of histidines present. Previously, research was conducted studying lipid oxidation and color stability analyzing only OxyMb, however after 2004 with the approval of CO modified atmosphere packaging (MAP) in the fresh meat retail case, the stability of COMb and effects of lipid oxidation became important to evaluate in research studies. Carboxymyoglobin has been known to be more stable in redox form than OxyMb, however Joseph et al. (2010) found with mass spectrometric studies COMb and OxyMb undergo lipid oxidation-induced oxidation similarly.

Lipid Oxidation

Lipid oxidation can severely lower the quality of a product and is one of the main factors that limits acceptability of meat and meat products. Lipid oxidation can occur in any meat product, however it is more likely to occur in products altered from their original state, like ground products. Meat mincing, grinding and chopping are examples of processes that disrupt the muscle cell membranes and exposes them to pro-oxidant substances which accelerate oxidation and deterioration of a product. Several catalysts are typically associated with the promotion of oxidation in meat products including light, gases, temperature and pH. Lipid oxidation occurs in three phases, initiation, propagation and termination; and is more likely to occur in the presence of unsaturated fatty acids (UFA) (Morrissey et al., 1998). Lipid oxidation produces off-flavors by the compounds aldehydes and ketones, which result in rancidity flavor or warmed-over flavor (WOF).

The first step during the initiation phase is the removal or loss of a hydrogen (H^+) ion or a hydroxyl group (OH) from a carbon in a fatty acid chain (R) by a catalyst, which ultimately is the loss of an electron. Due to polyunsaturated fatty acids (PUFA) having multiple double-bonds, they are more likely to be targeted. The free H^+ combines with a free radical (R•), which is a compound that lacks one or more paired electrons, to become RH, and then can be catalyzed by a hydroxyl radical (HO•), however the affinity for two H^+ ions and one Oxygen (O) ion to be stable together, the reaction products are one R• and a water ion (Morrissey et al., 1998).

$$RH + HO\bullet \rightarrow R\bullet + H_2O$$

The free radical reacts quickly with O_2 to form a peroxyl radical (ROO•), thus begins the propagation phase (Manat, 2008)

$$R\bullet + O_2 \rightarrow ROO\bullet$$

Due to ROO• being more highly oxidized than R• or the fatty acid, it will continuously oxidize other FA which creates a chain reaction that produces a hydroperoxide (ROOH) and another free radical, as seen below, which is when the off-flavors begin as ROOH is the chemical formula for both aldehydes and ketones (Morrissey et al., 1998). Morrissey et al. (1998) continues to describe how free-radicals can react with several other ions present including Fe^{2+} and Cu^{+} to create more ROO•.

$$ROO\bullet + RH \rightarrow ROOH + R\bullet$$

Manat (2008) and Morrissey et al. (1998) continue to describe the final phase of termination as the reactions seen below and is the combination of free radicals and stabilization of non-radical end products.

$$R\bullet + R\bullet \rightarrow R\text{-}R$$

$$R\bullet + ROO\bullet \rightarrow ROOR$$

$$ROO\bullet + ROO\bullet \rightarrow ROOR + O_2$$

Antioxidants and organic compounds have been used as an injection or topical spray onto whole muscle products and in product formulation of further processed meat products to help reduce the occurrence of oxidation. Bouarab-Chibane et al. (2017) found the inclusion of several natural plant extracts and powders retards lipid oxidation in ground beef patties to less than 1 mg/kg^{-1} in 12 days of refrigerated storage. Additional research has been conducted evaluating the addition of several natural antioxidants into meat systems to retard lipid oxidation.

Plant antioxidants added to meat

Antioxidants are often used in further processed products to limit the amount of lipid and protein oxidation that occurs in the meat system. Oxidation can impact the overall color, flavor, odor and texture and nutritional value of a meat product. Antioxidants combat oxidation by being electron donors to the proteins and lipids that lose electrons to free radicals during oxidation.

Many comminuted and processed meats contain antioxidants due to the number of catalysts introduced during their production. Antioxidants contain compounds like flavonoids, phenols, carotenoids and tocopherols, which are actually scavenging for the free radicals (Oswell et al., 2018). Oswell et al. (2018) continues to discuss how the free hydroxy groups on flavonoids is what allows them to scavenge free radicals. Recently, consumer demand has shifted to include natural, fresh, and healthier products, including the added ingredients used in meat products. This shift has driven research to find natural replacements for typical industry used antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG), among others, respectively. Although antioxidants that are an oleoresin, essential oil, or extract that is from a spice or fruit, can be labeled on a product as 'spices', 'flavoring', or 'natural flavoring' among many others, other synthetic antioxidants like BHA and BHT have to be labeled as their common name (9 CFR 317, FSIS Directive 7120.).

Rosemary Research

Rosemary is a common natural antioxidant used in replacement of synthetic antioxidants like BHA, BHT and PG. There have been numerous studies conducted to report the success of the use of rosemary as an antioxidant in meat systems. Chang et al. (1977) found rosemary inhibited oxidation in animal fats as well as a blend of BHA, BHT, PG and citric acid, and was superior in preventing oxidation in vegetable oils. One study showed lipid oxidation was lowest and myoglobin oxidation was lower than controls when rosemary was added to ground beef patties; thus it would extend shelf life in the retail case (Balentine et al., 2006). When combined with n-3 fish oil to a ground beef system, rosemary was found to inhibit lipid oxidation along with BHA (Lee et al., 2005). Bouarab-Chibane et al. (2017) discussed how they and other researchers found there is a significant correlation to how well a plant derived antioxidant scavenges free radicals and their total phenolic content. Lee et al. (2005) explained how several researchers have discovered rosemary has several phenolic compounds with the ability to scavenge free radicals including: carnosic acid, carnosol, rosmarinic acid, rosmanol and rosmaridiphenol. Another study confirmed the addition of rosemary with vitamin C was best at inhibiting lipid oxidation in beef steaks (Djenane et al., 2002).

Additionally, it has been shown rosemary extract in combination with MAP packaging, lowered the lipid oxidation levels in ground beef after 24 days in refrigerated storage (Sánchez-Escalante et al., 2003). Rosemary is useful in reducing the occurrence of lipid oxidation, as shown by many previous studies, but also has been shown to protect fading of color and limit production of surface discoloration and MetMb production. Sánchez-Escalante et al. (2003) found the addition of rosemary to ground beef patties, did not decrease redness (a*) values as much as other plant extract treatments and preserved the a* value between 8 to 20 days of storage, while oregano only limited fading until day 12. Additionally, Sánchez-Escalante, et al. (2003) continued by saying rosemary in addition to ascorbic acid exhibited a dramatic inhibition on the formation of MetMb over 20 days storage time, and when added alone, rosemary was second in reducing MetMb formation, with rosemary + ascorbic acid combination being the best, respectively. Sánchez-Escalante et al. (2003) reported rosemary combined with vitamin C was effective in the delayed formation of MetMb. Djenane et al. (2002) also found that when combined with n-3 fish oil, rosemary had the highest a* (redness) values on days 4 and 6 of storage. When steaks were topically sprayed with a rosemary and vitamin C combination, steaks had a significantly higher a* value than those without the spray in several lighting and storage conditions, including dark storage (Djenane et al., 2003). Djenane et al. (2003) showed the rosemary and vitamin C mixture significantly inhibited off-odor formation and discoloration, especially when displayed under UV-free lights.

Martínez et al. (2006) found pork sausages with the combined addition of rosemary and ascorbic acid had the highest a* values over a 20-day dark storage period compared to sausages with the addition of borage and green tea when combined with ascorbic acid. Additionally, Martínez et al. (2006) found rosemary sausages inhibited lipid oxidation through the entire 20-day dark storage period. Martínez et al. (2006) inferred rosemary has the capability of reducing lipid and myoglobin oxidation, whereas green tea and borage only inhibited lipid oxidation but had no significant effects on the formation of MetMb. Schilling et al. (2018) found after 7 and 14 days of storage, the combination of rosemary at 2500 ppm and green tea at 300 ppm in pork sausage inhibited lipid oxidation the best compared to rosemary and green tea at differing combination levels, respectively.

McBride et al. (2007) found the addition of rosemary to ground beef at different concentrations had different sensory attributes. The addition of rosemary at 1% had adverse effects as samples were shown to have the highest WOF scores compared to patties with other levels of rosemary incorporated. There was no significant difference between control samples and those with the addition of rosemary at 0.1% and 0.25% for any sensory attributes tested.

Green Tea Research

After 12 days in dark storage, pork sausage with varying levels of green tea had the highest a* values compared to sausages with control, borage and pu-erh tea additives, but dropped significantly by d 16. Additionally, the sausages with green tea added had very low TBARS values at day 12 (Martínez et al., 2006). However, by d 16 no matter the concentration of green tea added, sausages had reached well above 1 mg/kg^{-1} of malonaldehyde, which Tarladgis et al. (1960) considered the threshold to detect rancidity for trained sensory panelists. When combined with ascorbic acid, green tea did not reduce lipid oxidation alone in pork sausages (Martínez et al., 2006). Rababah et al. (2011) found in ground goat meat kept in dark storage, green tea had the least significant effect on both color and lipid oxidation, with both the lowest a* and highest TBARS values, compared to ground goat with the addition of grape seed extract, TBHQ, and control. It was reported that green tea significantly reduced TBARS values in ground beef patties kept in dark storage for 10 days. Additionally, it was shown green tea singly was effective in reducing TBARS values, however when combined with NaCl, TBARS values were lower than green tea alone, showing NaCl can have synergistic effects (Tang et al., 2001).

Liu et al. (2015) reported green tea significantly slowed discoloration of ground beef patties when compared to patties with added BHA and control patties over 8 days of storage, however was less effective than carnosine, grape seed extract and vitamin E. Additionally, patties with green tea showed more lipid stability compared to control patties over the same 8-day storage period and had a similar antioxidant effect of BHA (Liu et al., 2015). After 3 and 5 days of storage, green tea had lower TBARS values than BHT at several added concentrations and similar values to black tea extract at several concentrations (Jayawardana et al., 2019). Due to the TBARS values of all of the green tea concentrations being similar to black tea and above BHT after 5 days of storage, the lowest concentration of 0.05% green tea extract was selected for further testing. During sensory testing there was no significant differences detected in color, odor, texture, juiciness, taste or overall acceptability when comparing patties with added black tea extracts at 0.05% and 0.3%, green tea extract at 0.05%, and control patties (Jayawardana, et al., 2019). Jo et al. (2003) showed both irradiated and non-irradiated freeze-dried green tea added to ground pork had significantly higher a* values than control pork patties. Additionally, there was no differences in odor, taste and tenderness between the two green tea concentrations in ground beef patties, but were higher than control patties (Jo et al., 2003). When added to ground beef in combination with sulfite, green tea kept a stable Chroma value compared to control patties of no added ingredients and sulfite only (Bañón et al., 2007). Additionally, Bañón et al. (2007) found trained sensory panelists detected rancid flavors in ground beef patties with sulfite added after 3 and 6 days of display, however did not detect rancid flavors in patties with sulfite and green tea added after the same display period; which was consistent with lower TBARS values compared to the sulfite patties.

Meat Packaging

The shapes and sizes of meat packages are numerous, however most of them fall into a few categories including: over-wrap (OW) or air-permeable, modified atmosphere packaging (MAP), and vacuum (VP). Different packaging types can have different characteristics that influence consumer acceptability of the product. Air-permeable packaging is typically done with polyvinyl chloride (PVC), which is air-permeable film with pores which allow oxygen to move in and out through the package, which causes OxyMb formation (McMillin, 2017). Another common packaging type is MAP, which in meat processing has several gas blends available to use including oxygen, which typically contains around 80% O_2 but can be up to 100%; Carbon dioxide (limited to 30% CO_2); Carbon monoxide (limited to 0.4% CO); and Nitrogen (limited to 69.5% N_2). Each blend of gases has different advantages to consider when producing a product and can create the desired environment for meat products (McMillin, 2008). Oxymyoglobin is produced when in high oxygen (HiOx) packaging, however it will eventually oxidize and the surface will turn into MetMb (McMillin, 2017). Elevated O_2 levels in MAP extends the OxyMb state, however under prolonged high oxygen conditions, lipid oxidation is stimulated. On the other hand, when under CO_2, color stability is prolonged and microbial growth is obstructed (Brody, 1989). CO_2 and CO both produce COMb which is much more stable than OxyMb, in addition, CO_2 is used to prevent microbial growth (McMillin, 2017). Nitrogen is often used to flush for VP or with other gases to prevent the MAP package from collapsing and as filler as it does not react with the Mb (McMillin, 2017).

Overwrap with PVC continues to be the most commonly found packaging type in the retail case due to the desired red color. When MAP packaging became more widely used in the industry PVC OW declined. However, with the introduction of master packages (MP), OW returned to the favored packaging type. Master packages, also known as master packs, mother packs and mother bags, allow fresh meat to be packaged in PVC OW trays, then placed in a large bag which is flushed with a gas blend and sealed. Master packages combine the desired color attributes of MAP with the consumer preference of OW trays. Increase use of MP has decreased the use of MAP in the retail case; it is now third most common packaging type in the industry behind PVC and VP, respectively (Bolton, 2013). Bolton (2019) discussed how MAP seems to have found its niche in the meat packaging industry with ground beef and turkey, however it only makes up roughly 12% of the packaging in the retail space. Overwrap has seen a decline in use from 43% to 34% in the last eight years (Bolton, 2019). When asked, 88% of consumers purchased ground beef in OW packaging, with chub packages of ground beef the second most popular (Salvage, 2014).

O'Keeffe and Hood (1980) showed exposure to O_2 at a low partial pressure (~4mm) can result in discoloration of meat in the irreversible formation of MMb. Modified atmospheric packaging can offer added elements to increase color stability and shelf life of meat. The use of CO at less than 1% promotes and allows the formation of a stable COMb pigment where CO is bound to myoglobin in the sixth binding site. This can increase the shelf life and color stability due to the increased affinity of Mb to CO (Sørheim et al., 1999). Wilkinson et al. (2006) found the use of 0.4% CO increased Chroma values and decreased hue values of pork loins in MP when compared to MP with 100% CO_2. Venturini et al. (2014) looked at MP with high (99.8%) and moderate (60%) concentrations of CO_2 in the gas blend with three steak cuts and ground beef inside each MP over a 7, 14, 21, and 28-day shelf life. After opening each MP, steaks and ground beef were placed into a retail coffin with color measurements taken after being exposed to atmospheric oxygen for 1, 24 and 48 hours. The day the MP was opened did not have an effect on L* or a* values of either gas blend treatment. Only days 14 and 21 of the high CO_2 gas blend were statistically different than all other days of both gas blends for discoloration and overall appearance. After day 28 ground beef samples bloomed for 1 and 24 hours had no significant difference in a*, b*, visual color, discoloration or overall appearance scores in both gas blends, however had significantly lower scores in all categories for both blends except L* values in the moderate CO_2 blend (Venturini, et al., 2014).

Oxygen scavenging can be advantageous when products are in the presence of high O_2 levels and light to reduce oxidation (Suppakul et al., 2003). When the anoxic state is desired in a MP, oxygen scavengers are included in the package prior to flushing with gas and sealing (Santagostino et al., 2011). Uboldi et al. (2014) found after immediately closing MP with oxygen scavengers inside the average residual oxygen content was about 1.5%; however, after 10 hours, oxygen scavengers were able to reduce the amount of free oxygen to below the detectable level of the device used, of 0.08% and were able to keep it at or below that level for the 7 days of refrigerated storage.

Conclusion

Many studies have been conducted to better understand meat color and color stability of beef products. Inclusion of antioxidants is well known to extend the shelf life of meat products, however moving to a more natural label creates questions on how effective they can be when compared to the industry standard of synthetic antioxidants, and how they will affect the color and color stability of the products in a retail case. The type of packaging has been shown to affect the shelf life of ground beef, however the combination of different packaging types with natural antioxidants has not been extensively evaluated. Therefore, the objective of this study is to understand the effects on color, oxidation, and sensory attributes with the addition of two natural antioxidants and 3 packaging types in ground beef.

CHAPTER III

EFFECTS OF ROSEMARY AND GREEN TEA ANTIOXIDANTS ON GROUND BEEF PATTIES IN MUTLIPLE PACKAGING TYPES

ABSTRACT

Consumers purchase ground beef more than any other beef product in the U.S. with 88% of consumers preferring traditional foam trays with overwrap (PVC), however PVC has a very limited shelf life. Utilizing gases like carbon monoxide (CO) and antioxidants like rosemary and green tea have been shown to extend shelf-life. The objective of this study was to analyze the effects of rosemary and green tea in ground beef in PVC and modified atmosphere packaging (MAP). Four treatments were used in this study: control, 2500 ppm rosemary, 300 ppm green tea, and 2500 rosemary + 300 ppm green tea. Patties from each treatment (n = 42) were randomly packaged into 1 of 3 types: PVC, MAP, or master packages (MP), MAP and MP flushed with 0.4% CO, 69.6% N_2, and 30% CO. Patties in PVC and MAP were put directly into simulated retail display for 7 d and MP were put in dark storage for 7 d, then removed and put in cases for 7 d of display. Objective and subjective color were measured, along with lipid oxidation and trained sensory panel measurements. As expected, patties in MAP had significantly ($P < 0.05$) lower lipid oxidation values and were significantly higher ($P < 0.05$) in all color values than patties in PVC after display. In MAP green tea significantly ($P < 0.05$) improved a* and chroma values on d 6 of retail display and had lower surface discoloration scores from d 3 to 6 compared to rosemary + green tea treatment. In MP patties, green tea statistically ($P <$

0.05) improved L* values, display color and surface discoloration scores compared to the combination treatment. However, green tea did not significantly ($P > 0.05$) improve values when compared to rosemary for these parameters. Trained taste panelists could not detect a difference between control and green tea patties ($P > 0.05$) for the green-hay attribute, however rosemary was highly detectable in the green-hay attribute.

INTRODUCTION

According to research, consumers relate color of meat to freshness and consider fresh meat as a bright red color, any deviation they consider to be unacceptable (Bechtel et al., 1986). Consumer purchasing decisions are more often influenced by color than any other meat quality factor, because consumers correlate freshness and wholesomeness with color. It has been found that nearly 15% of retail beef is discounted due to surface discoloration, which results in $1 billion lost in annual revenue (Smith et al., 2000). Metmyoglobin is the pigment that is responsible for browning and discoloration of meat products at almost every step of the meat processing chain and is a primary factor to off-put consumers at a retail case (Ramanathan et al., 2019). Oxidantion is a major cause of the quality of meat products to lower as it can promote detterioration of color, promote off flavors, and shorten shelf-life (Falowo et al., 2014).

Antioxidants can be found in many plants and their ability to reduce oxidation can be better than commonly found synthetic antioxidants (Oswell et al., 2018). According to the Code of Federal Regulations, ingredients do not have to be labeled individually instead as ‘spices’, ‘essential oil’, or ‘flavor’, which can make consumers percive the product to be more natural, rosemary and green tea both fall into those categories (Oswell et al., 2018). Both rosemary (Balentine et al., 2006; Chang et al., 1977; Lee et al., 2005;) and green tea (Jayawardana et al., 2019; Rababah et al., 2011; Lie et al., 2015) have been shown to reduce lipid oxidation in numerous studies. Additionally, rosemary and green tea have been shown to improve color stability in different packaging types. (Jo et al., 2003; Bañon et al., 2007; Sánchez-Escalante et al., 2003) Packaging type also has been shown to have an effect on lipid and protein oxidation levels. Traditional PVC is oxygen permiable, which can speed up the oxidation time, however

MAP is flushed with different gas contents to improve shelf stability with the reduction of oxygen and addition of CO and CO_2 (McMillin, 2017). Sørheim et al. (1999) showed that the use of CO up to 1% can increase the shelf life and color stability of meat due to the affinity of Mb to CO. Thus, the objective of this study was to evaluate the effects of rosemary and green tea in ground beef patties in traditional and modified atmosphere packaging to achieve a longer shelf life.

METHODOLOGY

Raw material preparation, proximate analysis and pH

Two chuck rolls (IMPS #116A) from USDA Low Choice carcasses were selected at random 72 h postmortem from a commercial processing facility, vacuum packaged and transported to the Robert M. Kerr Food and Agricultural Products Center at Oklahoma State University. Chuck rolls were removed and pH was measured to ensure no differences and averaged 5.67 and 5.68, respectively. Chuck rolls were ground with a 9 mm plate, then finely ground with a 3 mm plate utilizing a Biro mixer grinder (Model AFMG-24, Biro Manufacturing Company, Marblehead, OH) to homogenize the sample. Proximate analyses were measured to calculate the percentage of protein, fat and moisture of the composite meat sample. Protein, fat and moisture contents ranged from 20.6 to 21.4%, 11.8 to 13.5%, and 65.4 to 66.3%, respectively. The ground beef sample was measured using NIR with AOAC (2007.04) approved near infrared spectrophotometer (FoodScan Lab Analyzer, Serial No. 91753206, Foss, NIR systems Inc., Slangerupgade, Denmark, 2014). As shown in Figure 3.1, samples of the ground chuck were weighed to form 115 g patties and were randomly assigned to 1 of 4 treatment groups. Rosemary (RSM), green tea (GT) and rosemary combined with green tea (R+G) were added to randomly assigned samples and hand mixed for 15 s. Rosemary was added at 2500 ppm, green tea at 300 ppm, and rosemary and green tea at 2500 ppm + 300 ppm, respectively. In total, 168 patties were formed with an Adjust-A-Burger patty press. Control (C) patties were hand mixed for 15 s with no added ingredients. Antioxidant levels were based on results on Schilling et al. (2018). All ground beef not utilized for patties, proximate analysis or initial lipid oxidation testing was vacuum packaged and frozen for trained sensory panel testing.

Packaging and storage

Forty-eight patties, 12 per-treatment, were randomly assigned to PVC (traditional oxygen permeable polyvinyl chloride) and MAP (modified atmospheric packaging; 0.4% CO, 69.6% N_2, and 30% CO_2); and 72 patties, 18 patties per treatment, were assigned to MP (PVC inside package flushed with 0.4% CO, 69.6% N_2, and 30% CO). Amount of patties differ in packaging types to ensure consistent number (n = 6) for each pull day test (d 3, 6, 9, 12). White PVC trays (NoviPro; 16.5 cm x 21.75 cm x 3.22 cm) were obtained from NoviPax (Oak Brook, IL), white MAP trays (Rock-Tenn DuraFresh™ rigid trays) were obtained from Cryovac Sealed Air (Duncan, SC), and MP (68.5 cm x 45.7 cm) were obtained from Amcor Flexibles North America (Yuba City, CA). Patties assigned to PVC or MP were placed into PVC trays with absorbent pads and over-wrapped with a PVC film (15,500–16,275 cm^3 $O_2/m_2/24$ h at 23 °C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film, Koch Supplies, Kansas City, MO). After over-wrap, patties assigned to MP were then placed inside a MP along with a FreshPax© oxygen scavenger (Multisorb Technologies, Buffalo, NY) and flushed with a tri-gas blend (0.4% CO, 69.6% N_2, and 30% CO) for 10 s and heat sealed with a double chamber packaging machine (C500, Multivac, Wolfertschweden, Germany), each antioxidant treatment and a control package was represented in each MP. Patties assigned to MAP utilized a multi-layer barrier film (LID 1050 film) obtained from Cryovac Sealed Air (Duncan, SC) flushed with the same tri-gas blend (0.4% CO, 69.6% N_2, and 30% CO_2) and was sealed with a Mondini semi-automatic tray-sealing machine (Model

CV/VG-5, G. Mondini S.P.A. Cologne, Italy). All gas was certified food grade and obtained from Stillwater Steel and Welding Supply (Stillwater, OK). The percentage of O_2, CO_2, and CO was obtained utilizing a headspace analyzer (Bridge 900131 O_2/CO_2/CO, Illinois Instruments, Ingleside, IL) of MAP and MP, flushed with gas and sealed immediately prior to packaging patties in their respective packaging type.

Retail Display

After packaging, MAP and PVC packages were placed in two coffin-style retail display cases (Model: M1-8EB, Hussmann, Bridgeton, MO) set at 4ºC ± 1, under continuous LED lighting (Philips LED lamps; 12 watts, 48 inches, color temperature = 3,500°K; Phillips, China), for 7 d and rotated daily within each case, to minimize variance due to temperature or light intensity caused by location in the cases. Master packages were kept in dark storage conditions at 4ºC ±1 for 7 d. Temperature was monitored continuously in retail cases and dark storage to ensure fluctuation out of range did not occur by utilizing 6 temperature LogTag readers (LogTag TRIX-8 Temperature Data Recorder; MicroDAQ, Contoocook, NH). Prior to opening each MP, a headspace analyzer was utilized to obtain the percentage of O_2, CO_2, and CO after dark storage time. After opening, trays were placed inside the coffin-style retail cases for 7 d.

Instrumental Color, visual color panel and lipid oxidation

After packaging in PVC or MAP, instrumental color was measured on the surface of all patties using a HunterLab MiniScan XE Plus spectrophotometer (Model 45/0 LAV, 2.5-cm diameter aperture, illuminant A, 10° observer; HunterLab, Reston, VA). Measurements were recorded at two different locations on each surface and utilized CIE L*, a*, b* and chroma values. Instrumental color was measured every 24 h on d 0, 1, 2, 3, 4, 5, and 6, in two different locations on each patty during display period. Initial instrumental color measurements were taken on patties in MP on d 0 prior to being placed in dark storage, however did not have instrumental color measured during dark storage period. After 7 d dark storage period, patties were removed and instrumental color was measured at two different locations, then placed in retail cases for 7 d. Instrumental color measurements were taken every 24 h on d 6, 7, 8, 9, 10, 11, and 12 during display.

A trained panel (n = 6) evaluated visual color every 24 h. Each panelist was tested with the Farnsworth Munsell 100-hue test and successfully passed. Prior to serving on the panel, panelists were trained according to AMSA (2012) guidelines. Panelist evaluated each patty every 24 h of display and scored the display color (DC) on an 8-point scale (1 = very light red, 2 = moderately light red, 3 = light red, 4 = slightly bright red, 5 = bright red, 6 = slightly dark red, 7 = moderately dark red, 8 = dark red) and surface discoloration (SD) on a 7-point scale (1 = no discoloration 0% (Metmyoglobin), 2 = minimal discoloration 1-10%, 3 = slight discoloration 11-20%, 4 = small discoloration 21-40%, 5 = modest discoloration 41-60%, 6 = moderate discoloration 61-80%, 7 = extensive discoloration 81-100%). Scales based on AMSA (2012) guidelines, however, no guidelines were presented for ground meat in MAP packaging, so the same scale was used on all patties, regardless of packaging type. Patties that were randomly assigned for removal for lipid oxidation tests had instrumental and visual color measurements taken prior to removal from individual packaging.

Lipid oxidation was measured on d 0, from a composite sample of the raw ground beef representing initial lipid oxidation of the raw material. On d 3, 6, 9, and 12, six patties from each treatment x packaging type that were represented in simulated retail display, were randomly selected and removed for lipid oxidation tests. A modified procedure of Witte et al. (1970) was utilized. Three grams of each patty were blended with 20% TCA solution for 10 s using a Waring commercial blender (Model 33BL7; New Hartford, CT) and filtered through a Whatman 42 filter paper into 125 mL Erlenmeyer flasks. One mL of filtrate was combined with 1 mL of TBA solution into test tube and place into 100°C water bath for 10 min. Samples were removed from the water bath and cool at room temperature for 5 min. Absorbance of samples was measured at 532 nm using a Shimadzu UV-2600 PC spectrophotometer. Lipid oxidation results were reported as mg malonaldehyde/kg meat (Section XI, AMSA, 2012).

Trained Sensory Panel

Taste panels were conducted on samples from all treatment groups. Panelists were trained to evaluate green-hay flavor, rancid flavor, and fatty flavor prior to serving on the panel according to the standards of AMSA (2016). Eight panelists were seated for all 3 sessions and evaluated 8 samples per session. Patties were made immediately prior to cooking, following the same procedure as described above and assigned a random 3-digit code. Ground beef was thawed at 4ºC for approximately 24 h. All patties were cooked on a George Foreman Grilling Machine (Model GRP99, Lake Forest IL) to an internal temperature of 74ºC. After cooking, patties were cut according to AMSA (2016), placed in a sample cup labeled with their corresponding code and placed in a warmer to maintain temperature throughout the sensory evaluation time. Panelists were seated at individual booths under red lighting and were provided deionized water and salt-free crackers to cleanse their palette between samples. Samples were served in pairs with 4 pairs served at each panel session. Panelists were asked to evaluate green-hay, rancid, and fatty flavor attributes on a 3-point scale (1 = not detectable, 2 = slightly detectable, 3 = strongly detectable).

Statistical Analysis

A 3 x 4 factorial design was used to evaluate the effects of the 3 packaging types on the 4 treatment types. For proximate analysis and pH, means were generated using the PROC MEANS procedure of SAS (SAS). Instrumental color (L*, a*, and Chroma), lipid oxidation, visual color panel and trained sensory panel were analyzed using the MIXED procedure of SAS. Fixed variables included antioxidant treatment and day; when packaged in PVC and MAP, packaging type also was included as a fixed variable. L*, a*, and chroma values, mg malonaldehyde/kg, pull day, display color and surface discoloration, and flavor scores served as the dependent variables; and panelist served as the random effect. For all models, least squares means and SE were generated. When a significant F-test was identified ($P < 0.05$), least squares means were separated using a pairwise t-test (PDIFF option).

RESULTS AND DISCUSSION

Instrumental Color Measurements

Polyvinylchloride Overwrap and Modified Atmosphere Packaging

After 7 d in simulated retail display there was a packaging x day ($P < 0.0001$) interaction for L* values (Figure 3.1). Patties in MAP were not statistically different from d 0 to d 6 ($P = 0.196$). While patties in PVC were significantly ($P < 0.0001$) lighter than patties in MAP on d 0, by d 3 patties in PVC were significantly ($P < 0.0001$) darker than patties. Rogers et al. (2014) found similar values between ground beef patties in PVC and MAP compared to this study. As expected, L* values of patties in PVC packages decreased from d 0 to d 6 ($P < 0.0001$). These findings are consistent with Suman et al. (2010), who found ground beef patties decreased in L* values over a 6 d shelf life. Additionally, there was a treatment ($P = 0.0082$) effect for L* values (Figure 3.2). Patties with rosemary + green tea treatment were significantly ($P < 0.05$) darker than control patties, however not significantly darker than rosemary ($P = 0.0544$) or green tea ($P = 0.1559$). Balentine et al. (2006) found when adding rosemary after the final grind negatively impacts L* values after 8 d of storage in PVC. Bouarab-Chibane et al. (2017) found similar results as this study that green tea had similar L* values in patties without green tea leaves added after 8 d of storage.

There was a packaging x treatment x day ($P < 0.0001$) interaction for a* values for patties in PVC and MAP packaging (Table 3.1). On d 0, all patties in PVC had significantly ($P < 0.05$) higher a* values than all patties in MAP, regardless of treatment. However, this may be due to the conversion of oxymyoglobin to carboxymyoglobin through deoxymyoglobin (AMSA, 2012). By d 6 patties in MAP, regardless of treatment, had significantly ($P < 0.05$) higher a* values than patties in PVC. This aligns with Rogers et al. (2014) which found after 5 d control patties in PVC had lower a* values but patties in MAP with CO did not decrease in a* values. However, of the patties in MAP on d 6, those with green tea had numerically the highest a* value and were significantly ($P < 0.05$) higher than patties with rosemary + green tea treatment, but not different than other treatments ($P > 0.05$). By the end of display period, patties with the rosemary treatment in PVC had significantly ($P < 0.05$) higher a* values than any other patties in PVC, however a* values were not as high as those of patties in MAP. These findings are consistent with Luño et al. (1998) which found ground beef patties in MAP had acceptable a* values after 28 d of storage. Lee et al. (2005) also found after 6 d of display, patties in PVC with rosemary treatment had significantly higher a* values than control patties. Redness (a*) values of patties in MAP on d 6 in this study align with values from Jeong and Claus (2010) after the same simulated retail display time. Patties kept in high O_2 MAP with added green tea leaves were shown to have significantly lower a* values than control (Bouarab-Chibane et al., 2017). While this does not match the findings found of patties in MAP with green tea in this study, this may be due an oxygen content that was more similar to PVC, which did have lower a* values when green tea was added compared to control.

Chroma values had a treatment x packaging x day ($P < 0.0001$) interaction for patties in PVC and MAP (Table 3.2). No matter the treatment, on d 0 patties in MAP were significantly ($P < 0.05$) darker than patties in PVC. These findings are supported by Jeong and Claus (2010) which also found only on d 0 patties in MAP had lower chroma values than patties in PVC, then every day following patties in MAP had higher chroma values than patties in PVC. On d 3, no patties in MAP were significantly ($P > 0.05$) different from each other, however all were significantly ($P < 0.05$) more intense in color than on d 0. Adverse effects occurred with patties in

PVC, as all of them were significantly ($P < 0.05$) less intense in color on d 3, no matter the treatment. Nonetheless, control and green tea patties in PVC had significantly ($P < 0.05$) higher chroma values than patties with rosemary and rosemary + green tea treatments on d 3. On d 6, patties with rosemary treatment in PVC ($P < 0.05$) and significantly increased in chroma values from d 3. Control and green tea patties in MAP on d 6 were significantly ($P < 0.05$) more intense in color. On d 6, patties with green tea in MAP had significantly ($P < 0.05$) higher chroma values than rosemary + green tea patties.

Master Packages

Patties in master packages had a treatment x day ($P < 0.001$) interaction for L* values as shown in Figure 3.3. On d 0 and d 6 patties from each treatment were not significantly ($P > 0.05$) different from each other, however, on d 6 they all had significantly ($P < 0.05$) higher L* values than other days. Jayasingh et al. (2001) and Lavieri and Williams (2014) both found that patties in MP after a week increased in L* values from d 0, which is consistent with this study. By d 9, all patties L* values had significantly ($P < 0.05$) decreased from d 6. Green tea patties L* values were significantly ($P < 0.05$) higher than control patties, but neither were significantly ($P > 0.05$) different from the rosemary or rosemary + green tea patties on d 9. From d 9 to d 12, rosemary was the only treatment that did not significantly ($P > 0.05$) decrease in L* values. On d 12, patties with green tea antioxidants had statistically ($P < 0.05$) similar L* values with control and rosemary patties, however control and rosemary were statistically ($P < 0.05$) different. Rosemary + green tea had numerically the lowest L* values on d 12, but were not significantly ($P > 0.05$) different from control patties.

Patties in MP experienced a treatment x day ($P < 0.0001$) interaction for a* values as shown in Figure 3.4. On d 0 regardless of treatment, no patties had different ($P > 0.05$) a* values. After 7 d of dark storage, that trend continued as no patties were significantly ($P > 0.05$) redder than others, however green tea and rosemary + green tea treated patties had significantly ($P < 0.05$) lower values from d 0. This contradicts what De Santos et al. (2007) and Lavieri and Williams (2014) showed the longer ground beef and pork in MP the higher the a* value. Watts et al. (1978) concluded CO is absorbed by ground beef in packaging then exposed to atmospheric gases has a half-life of 3 days, which coincides with the results in this study, as patties on d 9 had significantly ($P < 0.05$) lower a* values than on d 6. On d 9 both rosemary and control patties had significantly ($P < 0.05$) lower a* values than green tea and rosemary + green tea patties which were not significantly ($P > 0.05$) different, however, rosemary patties were significantly ($P < 0.05$) darker red than control. Interestingly, on d 12, patties with the rosemary treatment significantly ($P < 0.05$) increased in a* values from d 9 and were not statistically different ($P > 0.05$) from any other patties. All patties on d 12 had similar ($P > 0.05$) a* values. In addition, patties with rosemary treatment were the only patties to significantly ($P < 0.05$) change from d 9 to d 12. After 6 d in MP, Jeong and Claus (2010) found after ground beef patties were immediately removed had much lower a* values than were found in this study, regardless of antioxidant additive, however they did not include an oxygen scavenger in the MP.

Similar to a* values, patties in MP had a treatment x day ($P < 0.0001$) interaction for chroma values (Figure 3.5). On d 0, patties had similar ($P > 0.05$) chroma values. However, after 6 d in dark storage, all patties significantly ($P < 0.05$) decreased in chroma values. On d 9, similar to a* trends, patties with rosemary antioxidants had significantly ($P < 0.05$) lower chroma values than all other patties. Green tea and rosemary + green tea treated patties had significantly ($P < 0.05$) higher values than the other two treatments on d 9. On d 12 control, green tea, and rosemary + green tea patties all had statistically ($P > 0.05$) similar chroma values to d 9. Rosemary treated patties values significantly ($P < 0.05$) increased from d 9 to d 12, similar to a*. It was found that patties stored in MP then put in display showed a similar decrease in a* values of patties not in

MP over the same amount of display time, concluding that CO in MP can extend the life of ground beef as it would have the same retail display shelf-life (Hunt et al., 2004).

Visual color analysis

Polyvinylchloride Overwrap and Modified Atmosphere Packaging

There was a packaging x day ($P < 0.0001$) interaction for display color values (Figure 3.6). On d 0, patties in PVC packaging were significantly ($P < 0.0001$) brighter than patties in MAP. However, by d 2, patties in MAP were not significantly ($P > 0.05$) different in display color from patties in PVC. From d 4 to d 6 patties in MAP did not significantly ($P > 0.05$) change, however patties in PVC significantly ($P < 0.05$) increased in display color values. A trained color panel found after 5 d of dark storage, ground beef patties in PVC were significantly ($P < 0.05$) darker than patties in MAP (Rogers et al., 2014).

Surface discoloration had a packaging x treatment x day ($P < 0.0001$) interaction (Table 3.3). On d 0, there was no discoloration on any patties no matter the treatment or packaging type. By d 3, patties with rosemary treatment in PVC had a significantly ($P < 0.0001$) greater surface discoloration score than any other treatments. From d 0 to d 3, patties in MAP did not significantly ($P > 0.05$) change in surface discoloration values, no matter the treatment. By d 6, patties in PVC had significantly ($P < 0.001$) discolored compared to patties in MAP, no matter the treatment. Arvanitoyannis and Stratakos (2012) discussed it has been found many times over how CO in MAP can contribute to color stability due to COMb formation, which is consistent with the findings in this study. Control and green tea patties in PVC on d 6 had the highest discoloration values and were significantly ($P < 0.05$) more discolored than patties with rosemary and rosemary + green tea treatments. While dramatically lower in surface discoloration scores, patties in MAP had increased discoloration values numerically, however only patties with the rosemary + green tea treatment were significantly ($P < 0.05$) different from d 3 to d 6. Sánchez-Escalante et al. (2003) reported rosemary inhibited MetMb formation in ground beef patties compared to control patties for 20 d in MAP.

Master Packages

For both display color ($P < 0.0001$) and surface discoloration ($P < 0.0001$), there was a treatment x day interaction that occurred for patties in MP with results shown in Table 3.4. From d 6 – 12 values significantly ($P < 0.05$) increased, no matter the treatment for both display color and surface discoloration. Both display color and surface discoloration had no significant ($P > 0.05$) differences between all treatment types on d 6. Hunt et al. (2004) found after 21 d in MP and 1 d in retail display panelists scored the display color of ground beef patties similarly to patties on d 1 of patties in traditional PVC not stored in MP, which is similar to the findings in this study. On d 9, patties with green tea and rosemary + green tea had significantly ($P < 0.05$) lower display color values than control and rosemary patties. However, Greene et al. (1971) established 40% MetMb causes rejection of meat products by consumers, thus d 9 values of surface discoloration would indicate they may have been rejected. On d 12, green tea patties display color values were statistically ($P < 0.05$) lower than rosemary + green tea patties. On d 12, green tea patties were significantly ($P < 0.05$) less discolored than rosemary + green tea patties. Luño et al. (2000) found patties in MP showed no discoloration after 10 d inside MP, and did not show any signs of discoloration until d 15 in MP. Additionally, it was found that rosemary and ascorbic acid improved a* values in high oxygen MP, no matter the light source (Djenane et al., 2003).

Lipid Oxidation Analysis

Polyvinylchloride Overwrap and Modified Atmosphere Packaging

A significant packaging x treatment interaction ($P < 0.0001$) occurred for lipid oxidation analysis (Table 3.5). In both packaging types, patties with an antioxidant addition had significantly lower lipid oxidation values compared control patties in PVC ($P < 0.0001$) and MAP ($P < 0.05$) packaging. Patties with green tea and rosemary + green tea in PVC had significantly lower values than patties with rosemary added singly ($P < 0.0001$). These results align with Schilling et al. (2018), as they found the combination of rosemary + green tea inhibited lipid oxidation better than rosemary. However, in this study, green tea when added singly, inhibited lipid oxidation as well as rosemary + green tea, where Schilling et al. (2018) did not find this to be the case. While not statistically different, it is to note that in MAP packaging, patties with rosemary + green tea had the least lipid oxidation, followed by green tea and rosemary patties. In high oxygen MAP, Sánchez-Escalante et al. (2003) found rosemary significantly inhibited lipid oxidation from occurring in ground beef compared to control, however in the current study, rosemary and control were not significantly different.

There was a packaging x day interaction ($P < 0.0001$) for lipid oxidation analysis (Figure 3.7). On both d 3 of display and d 6, patties in MAP packaging had significantly ($P < 0.0001$) lower lipid oxidation values than those in PVC packaging. Patties in MAP did not significantly change from d 3 to d 6 ($P = 0.0649$), nor were they significantly different from lipid oxidation values measured on d 0 from the composite same of ground chuck ($P < 0.05$). Values of patties in PVC increased significantly ($P < 0.05$) from d 0 composite sample values on both d 3 and d6. Interestingly, values from patties in PVC were significantly lower ($P = 0.0026$) from d 3 to d 6. Bouarab-Chibane et al. (2017) also found ground beef patties with green tea had significantly lower lipid oxidation values than control with in high oxygen MAP.

Master Packages

Patties packaged in MP had a significant ($P < 0.05$) treatment x day interaction for lipid oxidation values (Figure 3.8). On d 6, control patty values were significantly ($P < 0.05$) higher than values from d 0, however patties with antioxidants were not significantly ($P > 0.05$) different than lipid oxidation values on d 0. Lavieri and Williams (2014) found after 7 d in MP patties did not significantly increase in lipid oxidation values, which was not found in this study. After 7 d of dark storage, control patties had significantly ($P < 0.05$) higher values than patties with antioxidant treatments. Additionally on d 6, no treatments were statistically ($P > 0.05$) different from another. The same trend continued on d 9, with control patties having significantly ($P < 0.05$) higher values than all antioxidant treatments and with no antioxidant treatments statistically differing from one another. However, all patties, no matter the treatment had significantly ($P < 0.05$) higher values lipid oxidation values than on d 6. Interestingly, on d 12, patties with green tea and rosemary + green tea treatment values statistically ($P < 0.05$) decreased from d 9 and were similar to the values of green tea on d 6. However, on d 12, control and rosemary patties were not statistically ($P > 0.05$) different from d 9 to d 12. After 20 d in MP, Sánchez-Escalante et al., (2001) found beef steaks with a rosemary treatment had significantly lower lipid oxidation values than control steaks or steaks treated with other antioxidants, which is similar to the findings in this study.

Trained Sensory Analysis

Results of treatment effects on sensory traits are displayed in Table 3.6. Panelists detected no differences between any treatments and control for fatty flavor ($P = 0.6427$) or rancid flavor ($P = 0.3152$). However, panelists did detect a difference in the green-hay flavor ($P < 0.0001$). Patties with rosemary were reported to have a significantly ($P < 0.0001$) more detectable green hay flavor than all other treatments. Rosemary + green tea treated patties were statistically ($P < 0.0001$) more detectable for the green-hay attribute than green tea and control patties. Panelists detected no significant ($P < 0.0001$) differences for green-hay flavor between control and green tea patties. Jayawardana et al. (2019) found consumers could not taste a difference in overall flavor between sausages with green tea and without, which is similar to the findings from this study. Bouarab-Chibane et al. (2017) found different results when using a discrimination test between green tea and control patties, as a majority of the consumers rated the patties with a spinach-like taste, however this is probably due to using whole green tea leaves, rather than an extract.

CONCLUSION

Ground beef is the most consumed beef product in the United States, however the shelf life is lower than other beef products due to the grinding process. Improving shelf life will reduce food waste, along with allow retailers to keep ground beef on the shelf longer. Utilizing MAP and MP packaging can improve the shelf life duration of ground beef and other meat products due to the formation of CarboxyMb. The addition of green tea improved subjective color measurements and L* values, however not other objective color measurements. The addition of green tea and rosemary to ground beef products did not improve shelf life from control patties. Panelists not being able to detect the green tea additive is positive, as this can be utilized in other meat products that prefer a label friendly antioxidant, but not impact the flavor.

Table 3.1. Least squares means for *a** values[1] (packaging x treatment x display time interaction) of ground beef patties displayed for 7 d

			Display, d		
Parameter	Packaging[2]	Treatment[3]	0	3	6
*a** values	PVC	Control	32.02^{a,u}	21.14^{b,w}	19.42^{b,x}
SE = 0.44		Rosemary	31.58^{a,u}	17.66^{c,x}	22.49^{b,w}
		Green Tea	32.50^{a,u}	20.71^{b,v}	18.95^{c,x}
		Rosemary + Green Tea	31.48^{a,u}	19.37^{b,w}	19.02^{b,x}
	MAP	Control	22.01^{c,w}	26.80^{b,u}	28.44^{a,uv}
		Rosemary	22.54^{b,vw}	26.77^{a,u}	28.50^{a,uv}
		Green Tea	22.68^{c,vw}	27.48^{b,u}	29.52^{a,u}
		Rosemary + Green Tea	23.29^{b,v}	26.78^{a,u}	27.64^{a,v}

[1] A greater *a** value represents redder meat
[2] PVC = polyvinyl chloride overwrap; MAP = modified atmosphere packaging (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide)
[3] treatments include: control, rosemary 2500 ppm, green tea 300 ppm, and rosemary 2500 + green tea 300 ppm
[a–c] within a row, least squares means without a common superscript differ ($P < 0.05$)
[u-x] within a column, least squares means without a common superscript differ ($P < 0.05$)

Table 3.2. Least Squares Means for chroma values[1] (packaging x treatment x day interaction) of ground beef patties displayed for 7 d

			Display d		
Parameter	Packaging[2]	Treatment[3]	0	3	6
Chroma	PVC	Control	40.59[a,uv]	27.76[b,v]	25.99[c,x]
SE = 0.45		Rosemary	39.88[a,v]	24.22[c,x]	28.37[b,w]
		Green Tea	41.25[a,u]	27.25[b,v]	25.26[c,x]
		Rosemary + Green tea	39.90[a,v]	25.82[b,w]	25.92[b,x]
	MAP	Control	25.98[c,x]	31.44[b,u]	33.17[a,uv]
		Rosemary	26.77[b,wx]	31.62[a,u]	33.26[a,uv]
		Green Tea	26.82[c,wx]	32.32[b,u]	34.35[a,u]
		Rosemary + Green tea	27.66[b,w]	31.38[a,u]	32.35[a,v]

[1] CIE a^* and b^* values were used to calculate chroma [$\sqrt{(a^{*2} + b^{*2})}$]

[2] PVC = polyvinyl chloride overwrap; MAP = modified atmosphere packaging (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide)

[3] Treatments include: control, rosemary 2500 ppm, green tea 300 ppm, and rosemary 2500 + green tea 300 ppm

[a–c] Within a row, least squares means without a common superscript differ ($P < 0.05$)

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

Table 3.3. Least squares means for surface discoloration values[1] (packaging x treatment x display time interaction) of ground beef displayed for 7 d

Parameter	Packaging[2]	Treatment[3]	Display d		
			0	3	6
Surface Discoloration	PVC	Control	1.00[c]	2.37[b,v]	5.14[a,u]
SE = 0.19		Rosemary	1.00[b]	4.21[a,u]	4.40[a,v]
		Green Tea	1.00[c]	2.56[b,v]	5.56[a,u]
		Rosemary + Green Tea	1.00[c]	2.73[b,v]	4.53[a,v]
	MAP	Control	1.00	1.00[w]	1.19[w]
		Rosemary	1.00	1.19[w]	1.39[w]
		Green Tea	1.00	1.00[w]	1.14[w]
		Rosemary + Green Tea	1.00[b]	1.00[b,w]	1.57[a,w]

[1] A lower surface discoloration indicates less discoloration and Metmyoglobin formation (1 = no discoloration (0% Metmyoglobin), 7 = extensive discoloration (81-100%)

[2] PVC = polyvinyl chloride overwrap; MAP = modified atmosphere packaging (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide)

[3] Treatments include: control, rosemary 2500 ppm, green tea 300 ppm, and rosemary 2500 + green tea 300 ppm

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

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

Table 3.4. Least squares means of display color and surface discoloration values[1] (treatment x day interaction) of ground beef patties in master packages[2] in simulated retail display for 7 d after dark storage

		Display d		
Parameter	Treatment[3]	6	9	12
Display Color	Control	2.21[c]	4.37[b,u]	6.39[a,uv]
SE = 0.29	Rosemary	2.01[c]	5.12[b,u]	6.35[a,uv]
	Green Tea	1.91[c]	3.86[b,v]	6.09[a,v]
	Rosemary + Green Tea	1.99[c]	3.88[b,v]	6.71[a,u]
Surface Discoloration	Control	1.01[c]	3.03[b,u]	4.92[a,uv]
SE = 0.28	Rosemary	1.01[c]	3.17[b,u]	4.92[a,uv]
	Green Tea	1.00[c]	2.02[b,uv]	4.78[a,v]
	Rosemary + Green Tea	1.06[c]	2.17[b,uv]	5.28[a,u]

[1] A lower display color score indicates a brighter color (1 = very light red, 7 = dark red); a lower surface discoloration indicates less discoloration and Metmyoglobin formation (1 = no discoloration (0% Metmyoglobin), 7 = extensive discoloration (81-100%)

[2] Master packages (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide)

[3] Treatments included control, Rosemary 2500 ppm, green tea 300 ppm, and rosemary 2500 + green tea 300 ppm

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

[u,v] Within a column of the same parameter, least squares means without a common superscript differ ($P < 0.05$)

Table 3.5. Least Squares Means of packaging[1] x treatment[2] interaction for lipid oxidation[3] values

Parameter		Packaging	
	Treatment	MAP	PVC
SE = 0.01	Control	0.36[c]	0.59[a]
	Rosemary	0.32[cde]	0.46[b]
	Green Tea	0.31[de]	0.35[cd]
	Rosemary + Green Tea	0.30[e]	0.35[cd]

[1] PVC = polyvinyl chloride overwrap; MAP = modified atmosphere packaging (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide)

[2] Treatments include: control, rosemary 2500 ppm, green tea 300 ppm, and rosemary 2500 + green tea 300 ppm

[3] Values are shown as mg malonaldehyde/kg meat

[a-e] Within rows and columns, least squares means without a common super script differ ($P < 0.05$)

Table 3.6. Least squares means of sensory analysis traits of ground beef patties with four treatments[1]

	Attribute	*P* – value	Treatment	
SE = 0.09	Green-Hay	< 0.0001	Control	1.02[c]
			Rosemary	1.86[a]
			Green Tea	1.00[c]
			Rosemary + Green Tea	1.50[b]
	Fatty	0.64		
	Rancid	0.32		

[1] Treatments include: control, rosemary 2500 ppm, green tea 300 ppm, and rosemary 2500 + green tea 300 ppm

[a-c] Least squares means values differ without a common subscript ($P < 0.05$)

Figure 3.1. Flow chart of patties by treatment and packaging.

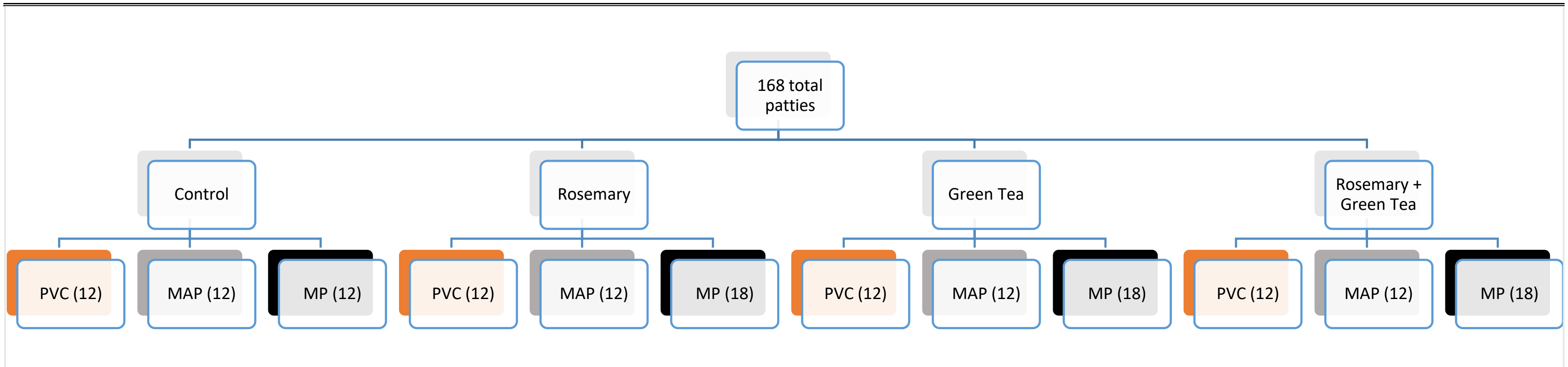
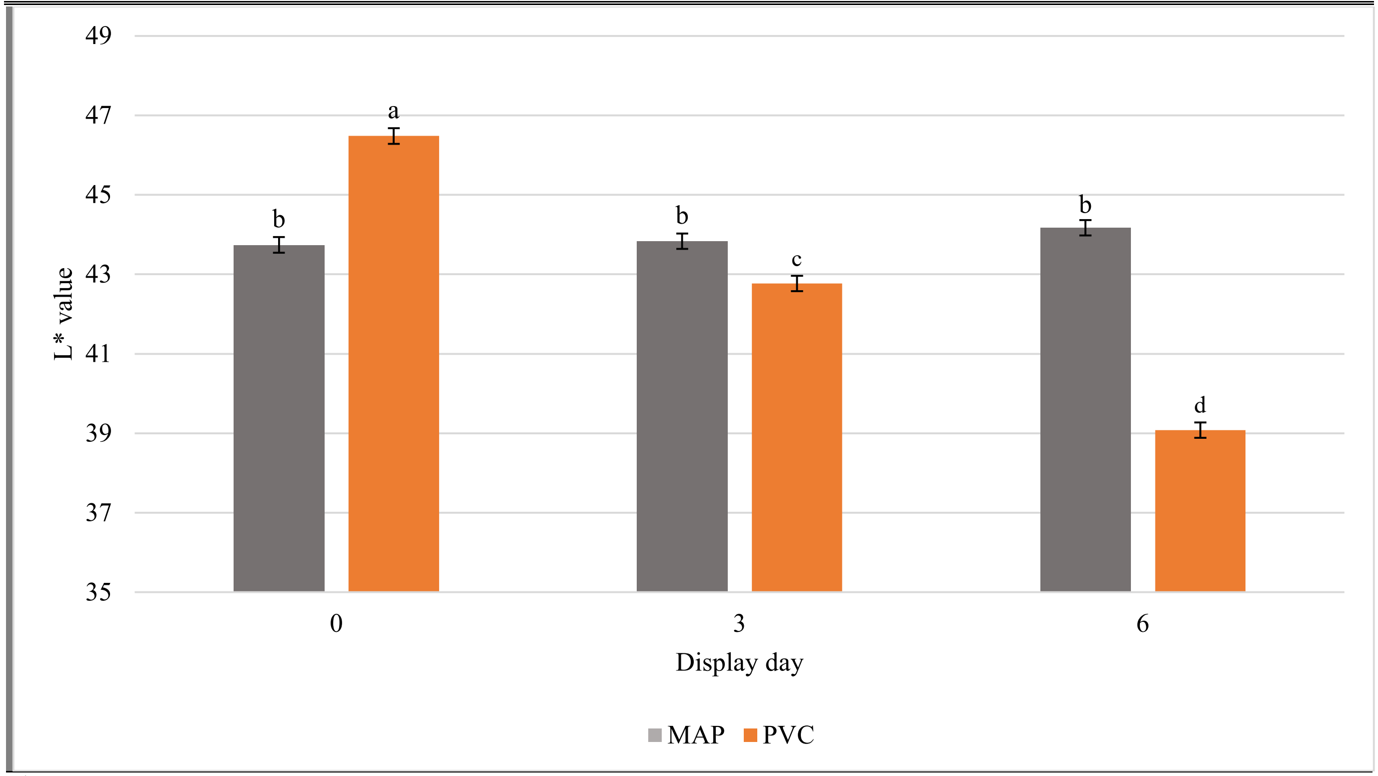


Figure 3.2. Least squares means for L* values with a packaging[1] x display day interaction of ground beef patties displayed for 7 d in simulated retail display

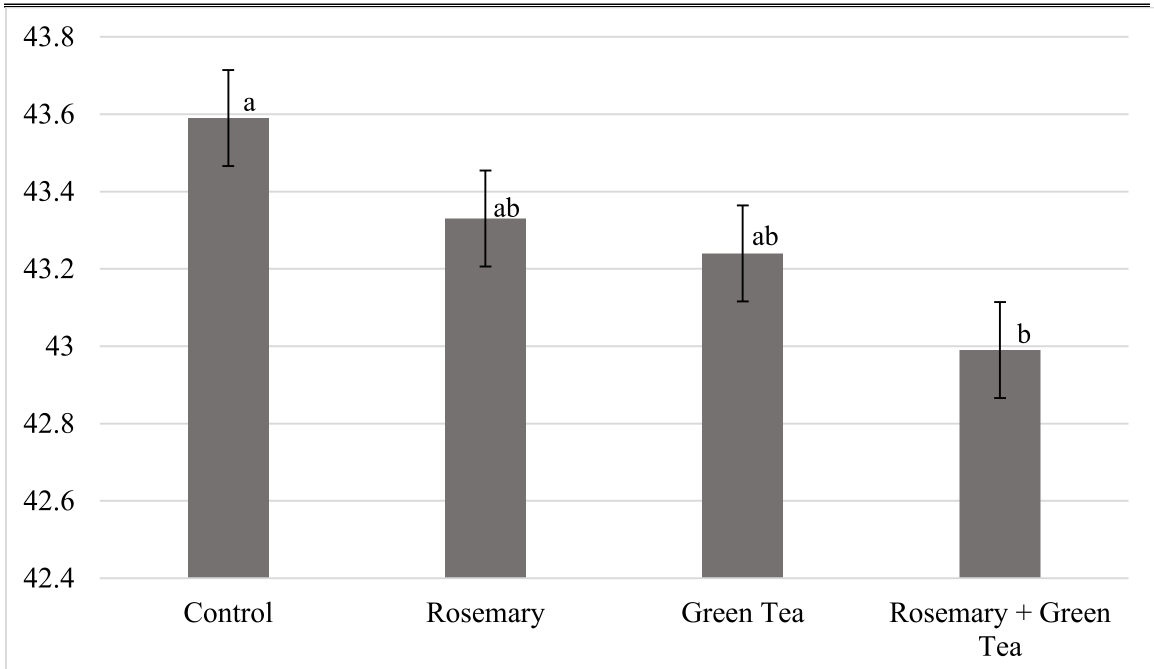


[1] PVC = polyvinyl chloride overwrap; MAP = modified atmosphere packaging (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide)

[a – d] Least squares means values differ without a common subscript ($P < 0.05$)

Standard error bars are indicated for overall packaging x day interaction (SE = 0.19)

Figure 3.3. Least squares means for L* values on ground beef patties displayed for 7 d in simulated retail display with a treatment[1] effect

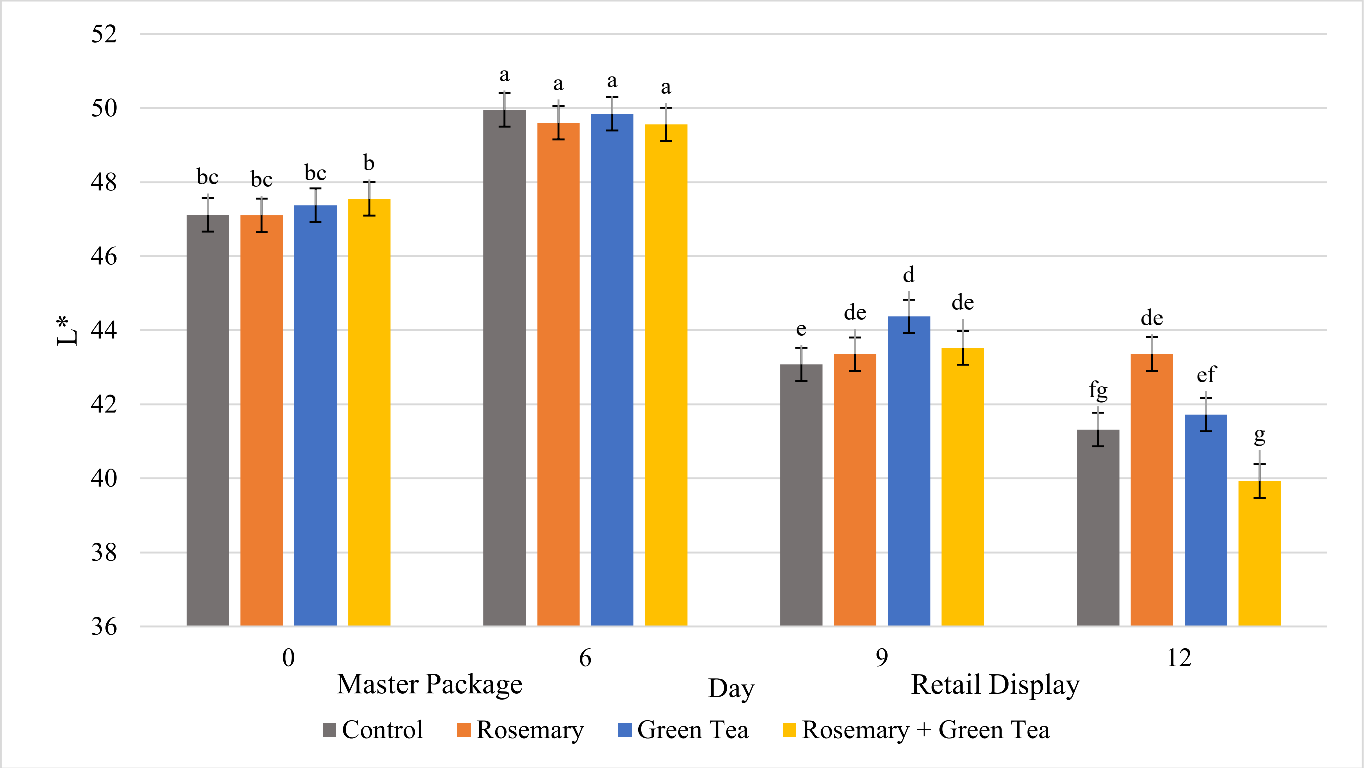


[1] Treatments include: control, rosemary 2500 ppm, green tea 300 ppm, and rosemary 2500 + green tea 300 ppm

[a,b] Least squares means values differ without a common subscript ($P < 0.05$)

Standard error bars are indicated for overall packaging x day interaction (SE = 0.12)

Figure 3.4. Least squares means for L* values with a treatment[1] x display day interaction of ground beef patties in dark storage master packages[2] for 7 d then in simulated retail display for 7 d



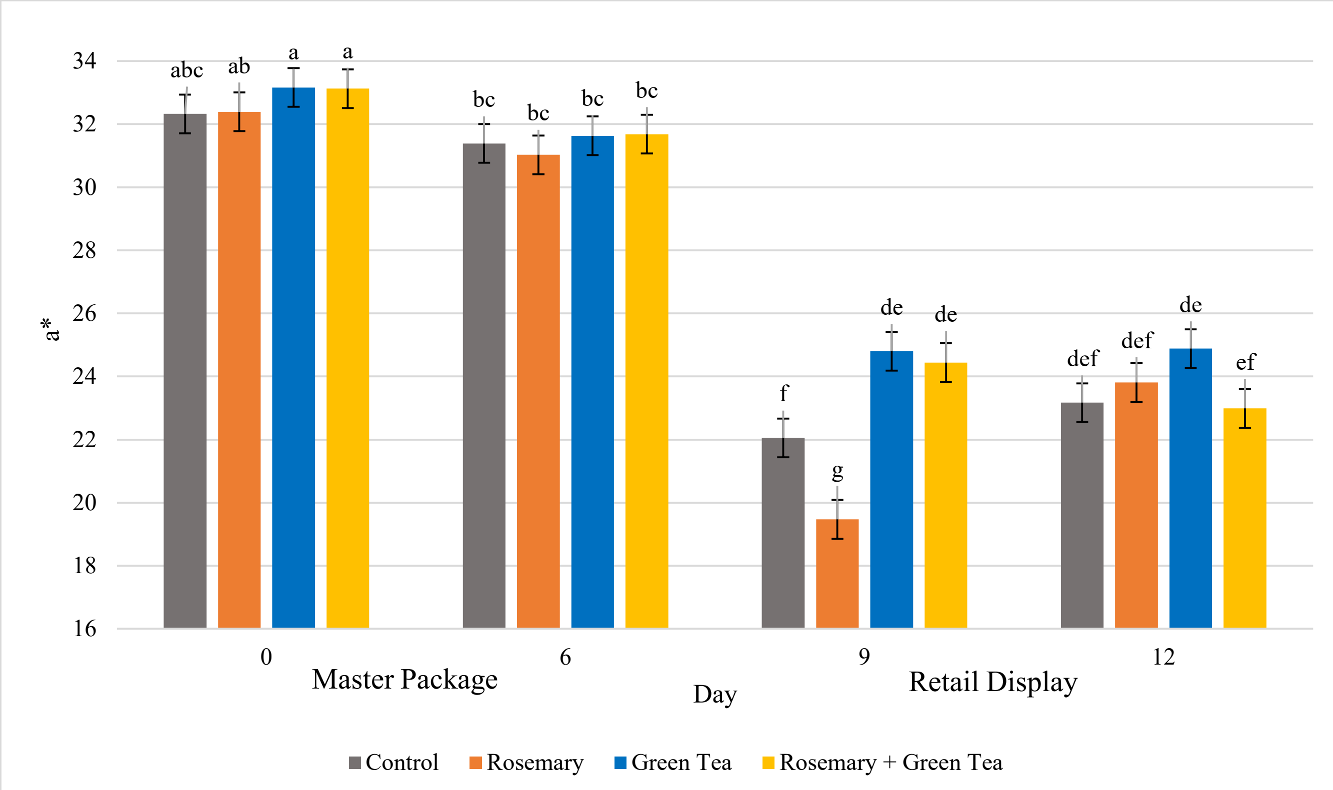
[1] Treatments include: control, rosemary 2500 ppm, green tea 300 ppm, and rosemary 2500 + green tea 300 ppm

[2] Master packages (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide)

[a – g] Least squares means values differ without a common subscript ($P < 0.05$)

Standard error bars are indicated for overall packaging x day interaction (SE = 0.45)

Figure 3.5. Least squares means of treatment[1] x day interaction for a* values of ground beef patties in master packages[2] in dark storage for 7 d then in simulated retail display for 7 d



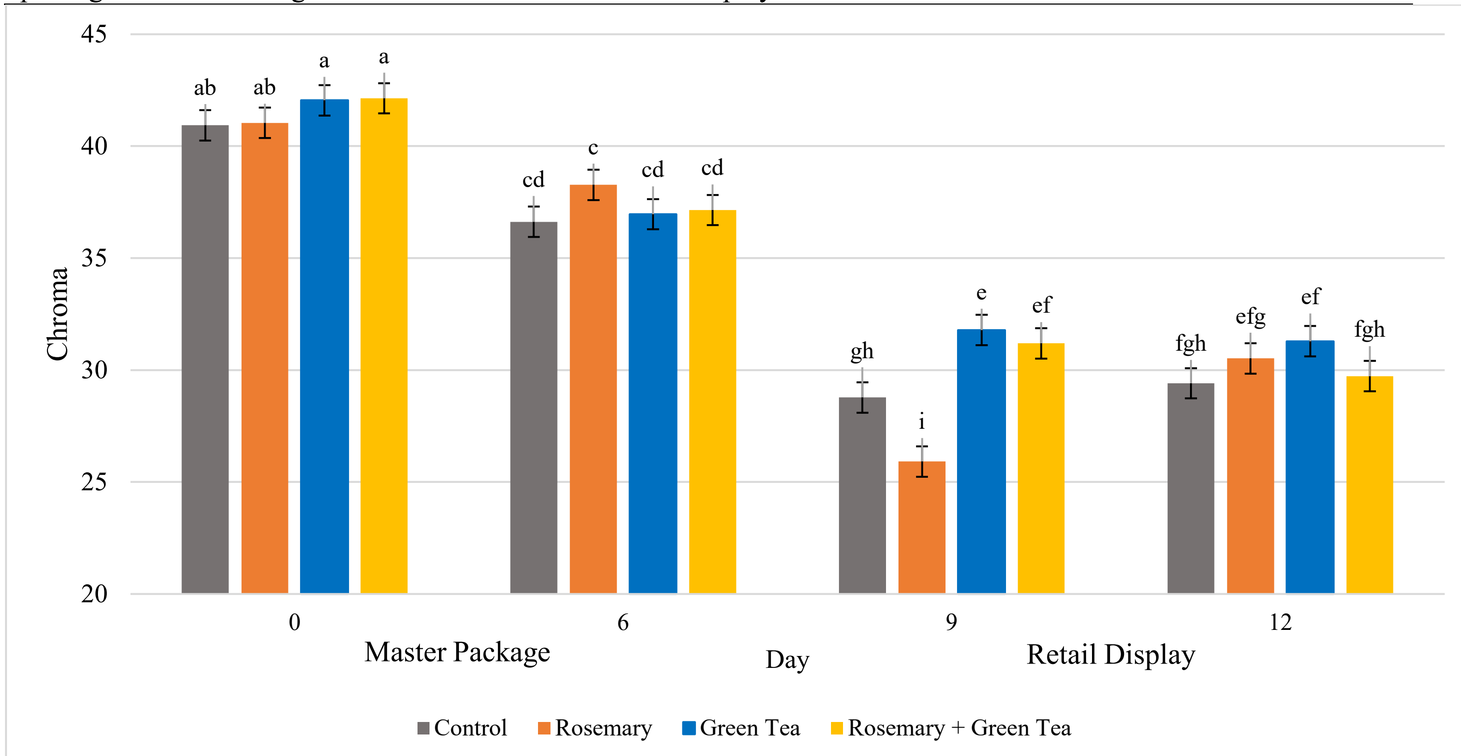
[1] Treatments include: control, rosemary 2500 ppm, green tea 300 ppm, and rosemary 2500 + green tea 300 ppm

[2] Master packages (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide)

[a – g] Least squares means without a common superscript differ ($P < 0.05$)

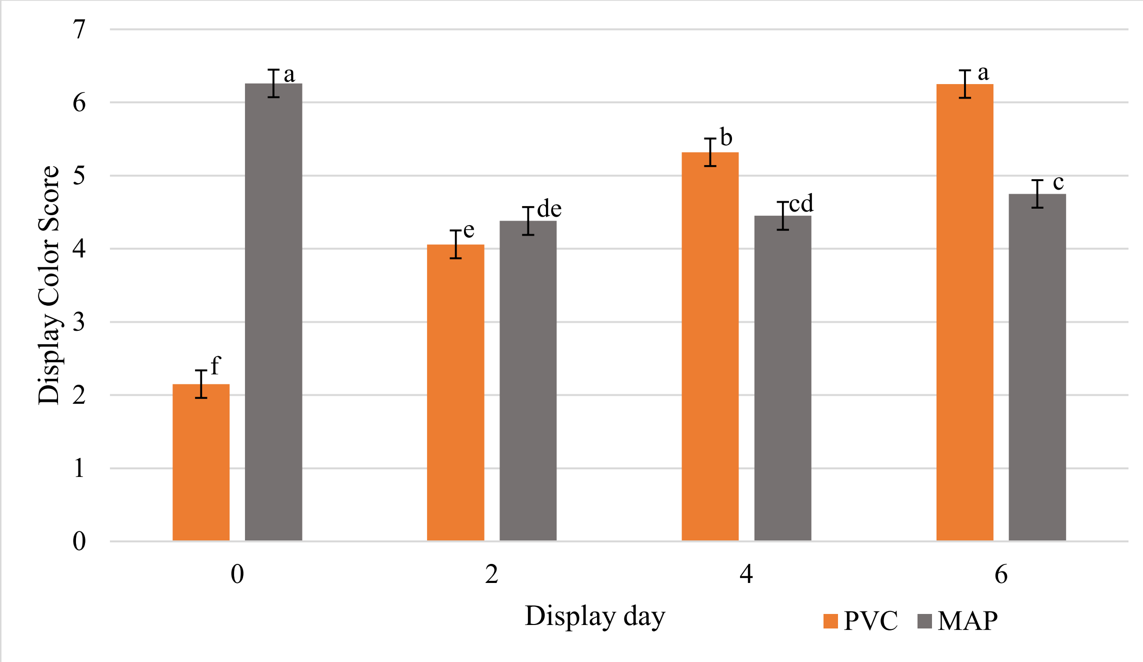
Standard error bars are indicated for overall packaging x day interaction (SE = 0.62)

Figure 3.6. Least squares means of a treatment[1] x day interaction for chroma[2] values of ground beef patties in master packages[3] in dark storage for 7 d then in simulated retail display for 7 d



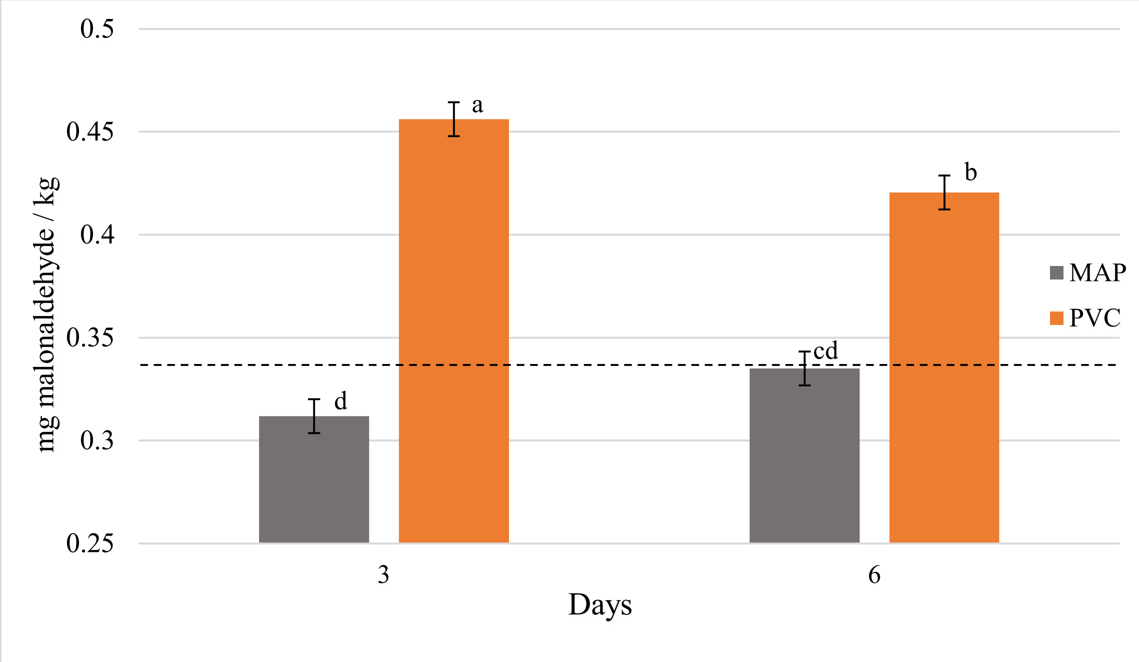
[1] Treatments include: control, rosemary 2500 ppm, green tea 300 ppm, and rosemary 2500 + green tea 300 ppm
[2] CIE a^* and b^* values were used to calculate chroma [$\sqrt{(a^{*2} + b^{*2})}$]
[3] Master packages (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide)
[a – i] Least squares means without a common superscript differ ($P < 0.05$)
Standard error bars are indicated for overall packaging x day interaction (SE = 0.68)

Figure 3.7. Least squares means for display color values[1] (packaging[2] x day interaction) of ground beef displayed for 7 d



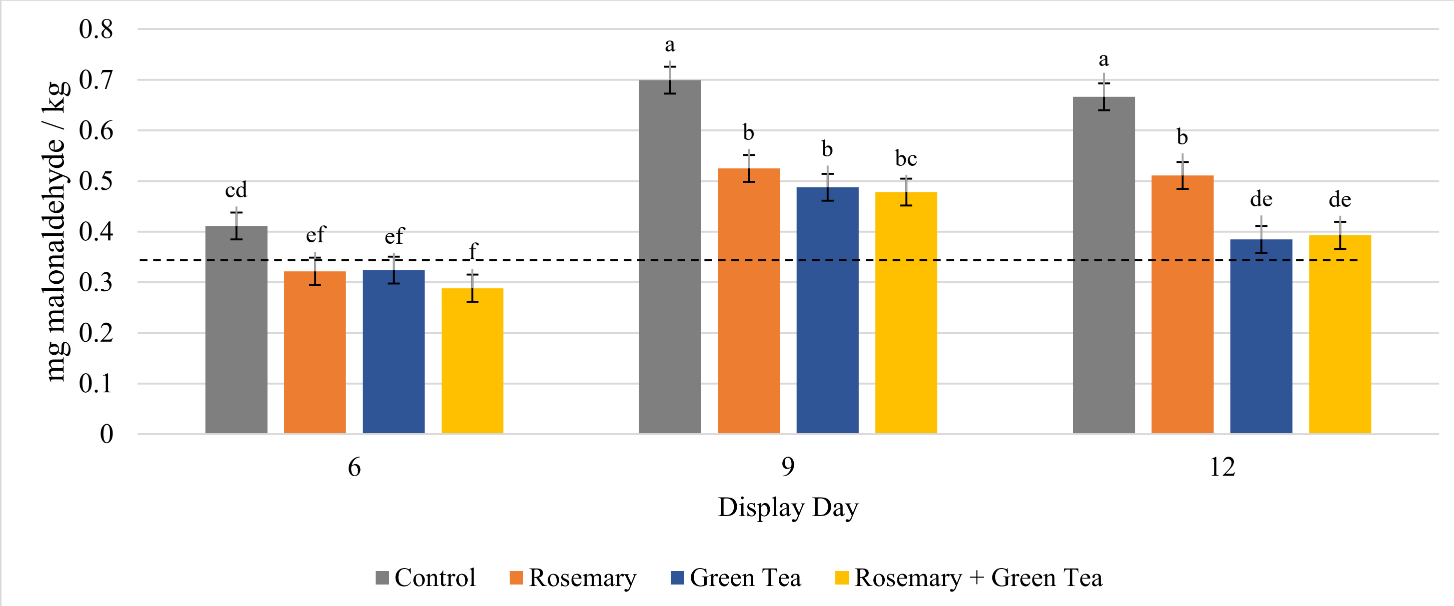
[1] A lower display color score indicates a brighter color (1 = very light red, 7 = dark red)
[2] PVC = polyvinyl chloride overwrap; MAP = modified atmosphere packaging (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide)
[a – f] Least squares means values differ without a common subscript ($P < 0.05$)
Standard error bars are indicated for overall packaging x day interaction (SE = 0.19)

Figure 3.8. Least squares means of packaging[1] x day interaction for lipid oxidation[2] values of ground beef patties in simulated retail display for 7 d



[1] PVC = polyvinyl chloride overwrap; MAP = modified atmosphere packaging (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide)
[2] Values are shown as mg malonaldehyde/kg meat
[a-d] Least squares means values differ without a common subscript ($P < 0.05$)
Dashed line indicates least squares means value of composite sample tested on d 0 in no packaging (LS means = 0.336)
Standard error bars are indicated for overall packaging x day interaction (SE = 0.008)

Figure 3.9. Least squares means of treatment[1] x day interaction for lipid oxidation[2] values of ground beef patties in dark storage master packages[3] for 7 d then in simulated retail display for 7 d



[1] Treatments include: control, rosemary 2500 ppm, green tea 300 ppm, and rosemary 2500 + green tea 300 ppm
[2] Values are shown as mg malonaldehyde/kg meat
[3] Master packages (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide)
[a – f] Least squares means values differ without a common subscript ($P < 0.05$)
Dashed line indicates least squares means value of composite sample tested on d 0 in no packaging (LS means = 0.336)
Standard error bars are indicated for overall packaging x day interaction (SE = 0.03)

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APPENDICES

Date: ___________________ **Name:____________________**

Session Number: __________ **Sample #:_________________**

Directions:

Today, you will be tasting samples of beef. Prior to tasting each sample, please take a bit of the crackers, followed by a sip of water both that are provided for you. After tasting each sample, please mark in the box representing your answer for each of the following questions. Please take a bite of cracker and sip of water between tasting each sample.

1. Indicate by placing a mark in the box the **Green-Hay** of the beef sample.
2.

☐	☐	☐
Not Detectable		Strongly Detectable

3. Indicate by placing a mark in the box the **Rancid Flavor** of the beef sample.

☐	☐	☐
Not Detectable		Strongly Detectable

4. Indicate by placing a mark in the box the **Fatty Flavor** of the beef sample.

☐	☐	☐
Not Detectable		Strongly Detectable

Ground beef retail color panel

Evaluator: Date: Time:

Display Color (DC)	Surface Discoloration (SD)
1 Very light red	1 No discoloration **(0%)**
2 Moderately light red	2 Minimal discoloration **(1-10%)**
3 Light red	3 Slight discoloration **(11-20%)**
4 Slightly bright red	4 Small discoloration **(21-40%)**
5 Bright red	5 Modest Discoloration **(41-60%)**
6 Slightly dark red	6 Moderate Discoloration **(61-80%)**
7 Moderatly dark red	7 Extensive Discoloration **(81-100%)**
8 Dark red	

ID	DC	SD	ID	DC	SD	ID	DC	SD
1			21			41		
2			22			42		
3			23			43		
4			24			44		
5			25			45		
6			26			46		
7			27			47		
8			28			48		
9			29			49		
10			30			50		
11			31			51		
12			32			52		
13			33			53		
14			34			54		
15			35			55		
16			36			56		
17			37			57		
18			38			58		
19			39			59		
20			40			60		

VITA

Laura Elaina Yoder

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF ROSEMARY AND GREEN TEA ANTIOXIDANTS ON GROUND BEEF PATTIES IN TRADITIONAL AND MODIFIED ATMOSPHERE PACKAGING

Major Field: Animal Science

Biographical:

Education:

Completed the requirements for the Master of Science in Animal Science at Oklahoma State University, Stillwater, Oklahoma in May, 2020.

Completed the requirements for the Bachelor of Science in Agriculture Education – Communications at Iowa State University, Ames, Iowa in 2018.

Experience: Graduate Research and Teaching Assistant, 2018 – 2020; Tyson Foods Research and Development Intern, 2019; American Meat Science Association Youth Programs Intern, 2018; Iowa State University Meat Judging Coach 2017; Tyson Fresh Meats Food Safety and Quality Assurance, 2016;

Professional Memberships: American Meat Science Association, Institute of Food Technologists, Poultry Science Association