

EFFECTS OF ENHANCEMENT ON BEEF
LONGISSIMUS COLOR

By

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EFFECTS OF ENHANCEMENT ON BEEF
LONGISSIMUS COLOR

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Abstract: Aging can improve beef palatability and eating experience. However, extended aging negatively impacts meat color and oxidative stability. The overall goal of the current research was to determine the effect of enhancement, followed by aging on beef *longissimus* color. Eleven boneless Choice strip loins (*longissimus lumborum*) were collected 3 d postmortem, divided into 3 equal sections, and assigned to one of the three enhancements (control, 0.5% lactate and 0.2% rosemary). The loin sections were enhanced and wet-aged for 14 d at 3°C. After aging, each loin section was cut into 2 cm thick steaks and overwrapped with PVC film and kept for 6 d of the retail display. Surface color, lipid oxidation, oxygen consumption, metmyoglobin reducing activity, and microbial counts were measured on 0, 3, and 6 d of the retail display. Throughout the display, loins enhanced with rosemary had greater ($P < 0.05$) L* values than lactate and control. Loins enhanced with lactate had greater ($P < 0.05$) a*, chroma, and ratio (630/580) nm during the display time. The lactate had the greatest ultimate pH ($P < 0.05$). By 6 d of the retail display, the loins treated either with lactate or rosemary had lower ($P < 0.05$) lipid oxidation when compared with control. Lactate had lower ($P < 0.05$) OC on 3 and 6 d of the display than control and rosemary. Metmyoglobin reducing activity was greater ($P < 0.05$) in lactate on 6 d of the display than other enhancements. The lactate and rosemary had lower ($P < 0.05$) aerobic bacteria count than control during the display. However, lactate and rosemary did not have a significant effect on lactic acid bacteria growth. The current research indicates that lactate-enhancement followed by aging has the potential to limit the discoloration of steaks during the retail display.

Keywords: postmortem aging, color stability, meat color, *longissimus*, beef

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CHAPTER I

INTRODUCTION

The myoglobin form and red saturation are important sensory perceptions that determine consumer purchasing decisions. Retaining the fresh meat color by minimizing myoglobin oxidation is critical to maximize the economic benefits related to the retailing of fresh beef. Previous research noted that the US beef industry loses approximately \$1 billion annually due to the discoloration (Smith et al., 2000).

The meat industry utilizes postmortem aging as a value-addition process. Aging of beef can impact meat quality by various biochemical pathways (Yaun et al., 2018). However, extended aging can negatively affect meat color stability when repackaged and displayed under retail display conditions (Kim, Frandsen & Rosenvold, 2011). The aging mechanisms regulating the color stability of muscles are not fully understood. However, studies have shown that NADH, mitochondrial content, and metabolites involved in color stability decrease with the aging period (Tang et al., 2005; Ramanathan et al., 2011).

Lactate is approved as generally recognized as safe (GRAS) by Food, Drug, and Administration (FDA), and is commonly used as an antimicrobial in meat and meat products. Lactate also has shown to improve meat color stability (Brewer et al., 1991;

Papadopoulos et al., 1991; Vote et al., 2000; Anuj et al., 2014). Rosemary has been used as a natural antioxidant due to its ability to minimize myoglobin and lipid oxidation (Sanchez et al., 2001; Seydim et al., 2006; Baker et al., 2013). Although previous research has noted that lactate and rosemary can improve color stability, no research has determined the effect of the lactate and rosemary enhancement, followed by aging on beef color. Therefore, the objective of the present study was to determine the effect of the lactate and rosemary enhancement, followed by aging on the quality of the beef *longissimus* during the simulated retail display.

CHAPTER II

LITERATURE REVIEW

Meat Color

Consumers associate a bright-red color with wholesomeness since they cannot evaluate the odor or texture at the point of sale. Hence any deviation from red color could be adverse to the marketing of fresh beef (Faustman & Cassens, 1990). In meat, the myoglobin is the main protein responsible for meat color, and the redox form of the heme group within the myoglobin and the ligand bound to the myoglobin determines meat color (AMSA, 2012).

Myoglobin is a cytoplasmic hemoprotein that consists of a globin and heme-binding domain (Ordway & Garry, 2004). According to Ordway & Garry (2004), the backbone of myoglobin is composed of eight α -helices wrapped around a central pocket containing the heme group. Various ligands such as oxygen and water can bind with the heme group. The myoglobin concentration in an animal depends on various factors such as age, muscle types, and species (Harvey, 2007).

The ligand present at the 6th binding site and the iron valency determine the meat color. The myoglobin forms which exist in fresh meat are deoxymyoglobin (DMb), oxymyoglobin (OMb), and the oxidized ferric form, metmyoglobin (MMb) (Zhu et al., 2009; AMSA, 2012). During the retail display of fresh meat, the interconversion among

the forms is a dynamic process. The deoxygenation of OMb to DMb can occur under low oxygen partial pressure, and the reversible reaction may occur immediately if the oxygen reunites with DMb (O’Keeffe & Hood, 1982). Thermodynamically, the conversion of OMb to MMb is unlikely; thus, deoxygenation of OMb to DMb occurs first, followed by rapid oxidation of DMb to MMb (AMSA, 2012).

Oxy-myoglobin is the pigment responsible for producing a bright-red color of fresh meat, and it is formed in the presence of oxygen when it binds with the 6th coordination of the heme within the myoglobin (King & Whyte, 2006; AMSA, 2012). This conversion of DMb to OMb is called oxygenation or “blooming.”

Deoxy-myoglobin is formed when the oxygen level is very low and is typically seen on fresh-cut meat or in anaerobic packaging (King & Whyte, 2006). There is no ligand bound with the 6th coordination site of the heme within DMb state. The color of DMb is purplish-red and is less stable than OMb as the presence of oxygen stabilizes the myoglobin (O’Keeffe & Hood, 1982).

Myoglobin in the ferric state is called MMb. The spontaneous conversion of DMb to MMb is referred to as autoxidation, and in this state, the meat becomes brown (Tofteskov, Hansen & Bailey, 2017). Metmyoglobin is favored at a low concentration of oxygen (about 1 to 2% oxygen) (AMSA, 2012) and water is the ligand at the 6th coordination site of the heme within the myoglobin (Young & West, 2005).

Factors affecting meat color

Pre-harvest factors such as gender, age, breed, genetics, diet, feeding, and proper handling of livestock at packing plants can influence myoglobin chemistry (Mancini &

Hunt, 2005; King et al., 2009; Hocquette et al., 2012; Grandin, 2015). Several studies have evaluated the impact of vitamin E as an antioxidant in animal feeds to prevent lipid oxidation (Faustman, Cassens, Schaefer, Buege & Scheller, 1989; Arnold, Scheller et al., 1992). These studies reported supplementation of vitamin E minimized lipid oxidation and enhanced beef color. Lipid oxidation is conducive to MMb formation; therefore, it induces fresh meat discoloration (Faustman & Cassens, 1990; Faustman, Sun, Mancini & Suman, 2010).

Researches have suggested that color stability is muscle-specific (McKenna et al., 2005; King, Shackelford, Rodriguez & Wheeler, 2011). Seyfert et al. (2007) studied different bovine muscle and reported that *longissimus lumborum* (LL) is more color stable than *psoas major* (PM). The sarcoplasmic protein of LL has a high level of antioxidant proteins such as thioredoxin, peroxiredoxin-2, peptide methionine sulfoxide reductase, and chaperone than PM (Suman, Hunt, Nair, & Rentfrow, 2014). Abraham et al. (2017) compared metabolite profile differences between beef LL and PM, and they reported differences in metabolites concentration between muscle types.

Various post-harvest techniques has been practiced by the meat industry to improve quality and food safety. Temperature is an important variable during storage, which influences meat color (Hopkins, Lamb, Kerr, Van de Ven & Ponnampalam, 2013). Li et al. (2017) reported that ovine muscles kept at - 0.8°C for 10 d demonstrated more color stability compared with those kept at 4°C.

Bacteria growth can affect meat discoloration. The competition between aerobic bacteria during its exponential growth with myoglobin for the oxygen induces MMb formation (Walker, 1980; Seideman et al., 1983). Robach & Costilow (1961) reported

species of aerobic bacteria such as *Pseudomonas* and *Achromobacter faciens* inoculated on steaks surface caused meat discoloration by reducing the oxygen tension to the meat surface, while facultative anaerobe *Lactobacillus plantarum* did not cause MMb formation.

Another factor affecting meat color is the design of packaging. There are many options for packaging chilled raw meat, which can provide several benefits that go beyond the function of food protection. Air-permeable packaging allows the O₂ from the atmosphere to bind to myoglobin to form OMb, which is viewed as a red color until the reducing capacity of the meat is exhausted and the MMb form predominates (McMillin, 2017). Vacuum packaging removes ambient air by enforcement of negative pressure, which can enhance the shelf-life of the product due to the decrease in the O₂ level inside the package. Modified atmosphere packaging plays an important role in chilled raw beef, and it has increasingly been marketed by the beef industry in case-ready meat products (Suman et al., 2014). Modified atmosphere packaging is defined as the removal of atmosphere gases inside the package and replacing them with a desired gaseous environment, which may influence myoglobin redox chemistry and meat color (Manu-Tawiah, 1992; McMillin, 2017).

Postmortem aging

Postmortem aging is a value-addition process to enhance meat quality, and it has been used by the meat industry to improve tenderness and flavor development (Kim et al., 2018). Calpains and cathepsins degrade cytoskeletal myofibrillar proteins, resulting in improved tenderness (Setyabrata & Kim, 2019). Wyrwicz et al. (2016) reported significant differences in WBSF values were noted in beef aged for 14 days. Kim, Kemp

& Samuelsson (2016) identified tryptophan, phenylalanine, valine, tyrosine, glutamate, isoleucine and leucine were more abundant in dry-aged than wet-aged beef.

Aging and meat color

The impact of aging on meat color and color stability has not been completely studied. Mancini (2012) reported increased aging time improved color intensity by supplying myoglobin with oxygen. King et al. (2012) reported color stability diminished with a prolonged aging period. Meat color stability during postmortem aging also depends on the rate of myoglobin oxidation, which is also influenced by lipid oxidation (Joseph et al., 2010). These oxidation reactions generate free radicals and secondary oxidation products such as aldehydes (e.g., nonenal, malonaldehyde, 4-hydroxy-2-nonenal, and hexenal), ketones and epoxides, which can stimulate myoglobin oxidation (Faustman et al., 2010).

Oxygen consumption

Meat color intensity and stability are influenced by mitochondrial activity via two mechanisms: oxygen consumption (OC) and metmyoglobin reduction activity (Mancini & Ramanathan, 2014). Oxygen consumption originated from mitochondria respiration will compete for O₂, hence with less O₂ available for myoglobin oxygenation, the initial red color decreases (Mancini & Ramanathan, 2014). Atkinson & Follet (1973) studied the color stability of beef, lamb and pork. They reported OC is inversely related to rate of discoloration; beef, with the lowest oxygen uptake and most extended display shelf-life, and lamb, with the greatest oxygen uptake and shortest display shelf-life. Investigation on

labile and stable muscle (e.g., *psoas major* and *longissimus*, respectively) pointed out that labile muscle had greater OC and mitochondria content postmortem than color stable muscles (Lanari & Cassens, 1991; Madhavi & Carpenter, 1993).

Metmyoglobin reduction activity

The ability of postmortem muscle to regenerate ferrous myoglobin from ferric myoglobin is referred to as metmyoglobin reducing activity (MRA). It can occur by three different pathways: enzymatic, non-enzymatic, and mitochondria-mediated. Elroy et al. (2015) compared the non-enzymatic reduction in-vitro of bovine, porcine and equine MMb at pH 5.6 and 7.4, and they concluded that non-enzymatic MRA is species- and pH-dependent. Chen, Yu, Han, Zhang & Guo (2018) conducted a study in-vitro about the effect of the products of lipid oxidation (4-hydroxy-2-nonenal (HNE) and hexanal) on MRA of bovine muscle, and they reported that HNE and hexanal caused damage to the mitochondrial structure and also decreased electrons-mediate responsible for the MRA.

Metmyoglobin reducing activity requires NADH as an electron donor, an enzyme such as NADH-dependent cytochrome b₅ reductase as an electron carrier, and cytochrome b₅ as an electron acceptor (Elroy et al., 2015). The function of NADH-cytochrome b₅ reductase is to transfer two electrons from NADH to two molecules of cytochrome b₅, and then its reduced form transfers the electrons to acceptors, including MMb (Shirabe et al., 1992).

Role of substrate in meat color

As postmortem time increases, the nicotinamide adenine dinucleotide hydride (NADH) level decreases. Researchers have studied the effect of the various substrates such as lactate, pyruvate, malate, and succinate on NADH level (Kim et al., 2009). Researchers have demonstrated enhancement with lactate in whole beef cuts via injection and in ground beef patties has improved color stability. However, lactate darkened meat color (Knock et al., 2006; Ramanathan, Manini & Konda, 2008). Watts, Kendrick, Zipser, Hutchins & Saleh (1966) reported increased MRA in ground beef patties due to the LDH activity, which can regenerate NADH level. Kim et al. (2006) carried out a study on the examination of the interaction of lactate and LDH with MRA in-vitro, and they reported that LDH plays a role in lactate-mediate stabilization of meat color via increased LDH activity due to its conversion to pyruvate and the concomitant regeneration of NADH. Other studies pointed out that lactate can provide antioxidant capacity, increase the reducing activity and increase NADH concentration, which can provide a conducive environment to keep the myoglobin form in its reducing ferrous form (Kim, Keeton, Smith, Berghman & Savell, 2009). Tang et al. (2005) showed succinate could increase metmyoglobin reduction activity via mitochondria-mediated. Hence, succinate has the potential to be used as an ingredient to improve fresh meat color stability. Pyruvate improved color stability due to its antioxidant effect on muscle lipids (Ramanathan, Mancini & Dady, 2011).

Antioxidant

According to the National Cancer Institute (2017), an antioxidant is a substance that protects cells from undesirable free radicals. The oxidative stress in organisms causes damages in membrane lipids, proteins, and DNA, which is responsible for chronic disorders such as cancer, heart disease, stroke, and inflammation. (Lim et al., 2019). The cellular defense system and the external source of antioxidants are important to decay the oxidation reaction in the organism (Chan & Chan, 2015).

The autoxidation process occurs when carbon-based compounds are degraded to form peroxy radicals, followed by reaction with oxygen to produce more peroxy and free radicals (William et al., 1980; Kolakowska, 2003). Phenolic antioxidants are effective in limiting the oxidation process by chelating peroxy radicals (Makahleh, Saad & Bari, 2015).

Lipid oxidation is the primary food spoilage process leading to off-flavor, odors, and undesirable compounds (e.g., aldehydes, ketones, and organic acids), causing quality and nutritional value loss (Tian et al., 2013; Makahleh, Saad & Bari, 2015). Several synthetic compounds have been used in a variety of food products. The common antioxidants are tertiary-butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, octyl gallate, and dodecyl gallate (Shah et al., 2014). The natural source of antioxidants can be found in fruits and vegetables, which are rich in compounds such as tocopherols, beta-carotene, and ascorbic acid (Makahleh, Saad & Bari, 2015).

Role of antioxidant in meat

Meat products, due to their composition (e.g., prooxidant ingredients) and mechanical processing, are highly susceptible to oxidation (Lee et al., 2005; Chandrasekar & Shahidi, 2015). Lipoperoxidation in meat is governed by several factors: free iron and myoglobin present in the muscle, polyunsaturated fatty acids, and α -tocopherol are the main targets of oxidative rancidity (Lima, Fernandes, Simionato, Oliveira & Silva, 2015).

Serpen, Gokmen & Fogliano (2012) studied the antioxidant capacity of beef, chicken, pork, and fish upon thermal treatment, and they reported the level of antioxidant capacity of these samples varied. Chicken showed the highest level, followed by pork, beef, and fish. Vasilatos & Savvaidis (2013) investigated the use of rosemary and chitosan in fresh turkey meat in vacuum packaging stored at 2°C. The samples treated with 0.25% of rosemary oil, and 1.5% of chitosan, resulted in acceptable organoleptic quality. Similarly, a study carried out by Riznar et al. (2006) about the effect of the rosemary as an antioxidant and on antimicrobial capacity in chicken frankfurters under three storage temperature of 4, 12 and 25°C demonstrated there was no difference in flavor between control and rosemary treatments. Also, the rosemary formulation of chicken frankfurter had more antioxidant and antimicrobial capacity when compared with control during storage.

Rosemary

Rosemary (*Rosmarinus officinalis* L.) belongs to the family Laminaceae, it is a woody green shrub indigenous to the Mediterranean. However, nowadays, it is cultivated

as an ornamental plant around the globe (Berdahl & McKeague, 2015). Rosemary has a long history of culinary and medicinal usage. In 1977, methods were developed for extracting the antioxidant from rosemary and reducing the flavor of their extracts. These discoveries were important for the use of rosemary extract as a preservative ingredient, while maintaining the flavor characteristics of foods (Chang, 1977). Rosemary produces a variety of antioxidant phenolic compounds such as carnosic acid, carnosol, methyl carnosate, rosmanol, epirosmanol, isorosmanol, 12-O-methyl 1 carnosic acid, Rosmanol-7-ethyl ether, Dimethoxy-rosmanol, rosmadial, Rosmariquinone (Miltirone), Rosmaridiphenol, and Rosmarinic acid (Richheimer et al., 1996; Berdahl & McKeague, 2015).

Some extracting methods are (1) solvent extraction (extracting the dried ground herb with food-grade solvents such as hexane, methanol, ethanol, and acetone), (2) mechanical extraction (process enforces the use of oil-soluble and pressure), and (3) supercritical carbon dioxide extraction (the antioxidant is liberated from the biomass by rigorous control of pressure and temperature) (Berdahl & McKeague, 2015). Commercially, rosemary extract is available in oil-soluble, water dispersible, water-soluble, and powder extracts. In meat and meat products, the recommended level of 0.2% will not cause flavor issues (Berdahl & McKeague, 2015).

We hypothesized that enhancing beef with lactate or rosemary will improve color during the retail display. Therefore, the objective of the current study was to investigate the use of the buffered lactate and rosemary enhancement, followed by aging on *longissimus* steak quality.

CHAPTER III

MATERIAL AND METHODS

Raw material and processing

Eleven boneless Choice beef strip loins (IMPS #180; A skeletal maturity and less than 30 months based on dentition) were purchased from Creekstone Farms in Arkansas City, KS 3 d postmortem in January. The strip loins were vacuum packaged and transported on ice to the Robert M. Kerr Food and Agricultural Products Center at Oklahoma State University, Stillwater, OK.

Each loin was divided into three equal sections. Each section within a loin was assigned randomly to one of the three enhancements: C = control (no-enhancement negative control), BL = 0.5% of buffered lactate, and RO = 0.2% of rosemary in the final product. Each strip loin section was weighed, individually pumped by product weight with enhancement solution to 2.5% and 6% of green weight, buffered lactate, and rosemary, respectively, using a handle held multi-needle injector and reweigh to record injection levels. The brine solutions were kept overnight at 4°C.

The injector was drained and thoroughly flushed between the enhancement solutions. The pumped loin sections were massaged manually for 30 s to distribute the solutions homogeneously. The sections were vacuum-sealed in 11 x 22 cm, 3-mil high barrier Cryovac vacuum bags (Sealed Air, St. Louis, MO), and stored in the dark at 3 ±

1°C for 14 d. After 14 d aging, the loin sections were unpackaged and sliced into 2 cm thick steak (n = 3 steaks per loin section x 11 loins x 3 enhancement = 99 steaks) using a meat slicer (Bizerba USA Inc., Piscataway, NJ). The steaks were packaged using polyvinyl chloride overwrap (PVC) with soaker pads (Cryovac tray brand) utilizing a single roll film wrap machine (Winholt WHSS-1, 115V; Winholt, Woodbury, NY) and assigned for 6 d of the simulated retail display.

Retail display

The steaks were stored under continuous fluorescent lighting (Philips fluorescent lamps; 12 watts, 121.9 cm, color temperature = 3,500 °K; Phillips, China) in white open-top display cases at $3 \pm 1^\circ\text{C}$ for 6 d. Light intensity ranged from 710-1150 lx (Extech Instruments Corporation, Waltham, MA). The temperature was monitored continuously by 3 temperature log tag readers (Log Tag TRIX-8 Temperature Data Recorder; MicroDAQ, Contoocook, NH). The packages were rotated daily to minimize within case location effect from front to back and side to side during the display.

Muscle pH and proximate composition

One steak from each loin section (n = 99 steaks) was measured for pH after the aging period on 0, 3, and 6 d of the display. The pH was determined using a Hanna solids pH meter (Hanna model HI 99163, Woonsocket, Rhode Island, USA) connected to pH probe at 3 random locations. The pH meter was standardized at pH 4 and 7. The chemical composition of the 3 d postmortem strip loins was determined using near-infrared spectrophotometer (FOSS Food Scan 78,800, Dedicated Analytical Solutions,

DK-3400 Hillerod, Denmark), and data processing was determined using ISIscan™ Software.

Surface color measurements

The CIE values (L^* , a^* , and b^*) on the surface of the same assigned packaged steaks from each loin section ($n = 33$ steaks) were measured at 3 random locations using a HunterLab Miniscan XE Plus spectrophotometer (2.5 cm aperture, Illuminant A, and 10° standard observer angle; HunterLab Associates, Reston, VA, USA). The CIE values were used to calculate chroma $[(a^{*2}+b^{*2})^{1/2}]$ and hue angle $[\arctang(a^*/b^*)]$. The wavelengths between 600 and 700 nm were used to calculate the discoloration ratio (630/580) nm.

Metmyoglobin reducing activity

Sample from one steak from each loin section ($n = 99$ steaks) was measured for MRA after the aging period on 0, 3, and 6 d of the display. The sample dimension of $3 \times 3 \times 2 \text{ cm}^3$ were submerged in a 0.3% solution of sodium nitrate (Sigma Aldrich, St. Louis, MO) for 20 min at room temperature, to facilitate MMb formation, and then removed, blotted with a paper towel, vacuum packaged (Prime Source Vacuum Pouches, 4 mil, $27 \times 20 \text{ cm}$; Koch Supplies Inc., Kansas City, MO) and measured at 2 random locations using HunterLab Miniscan XE Plus spectrophotometer to determine the pre-incubation percentage of MMb. The samples were incubated at 30°C for 2 h to induce MMb reduction. Thereafter, the samples were removed from the incubator and rescanned to determine the percentage of remaining surface MMb.

The reflectance from 400-700 nm were used to calculate % MMb according to the AMSA (2012), equation 1.

$$\% \text{Metmyoglobin} = [1.395 - \left(\frac{A_{572}-A_{700}}{A_{525}-A_{700}}\right)] * 100 \quad (1)$$

The following equation (2) was used to calculate MRA:

$$\text{MRA} = (\% \text{ surface MMb pre-incubation} - \% \text{ surface MMb post-incubation}) \quad (2)$$

Oxygen consumption

Another section of the steak (n = 99 steaks) with the same dimensions as the MRA sample was used for OC on 0, 3, and 6 d of the display. The samples were cut parallel to the surface to expose fresh tissue and allowed to bloom for 30 min at 4°C under PVC, thereafter, vacuum packaged and measured at 2 random locations using a HunterLab Miniscan. Then, incubated at 30°C to induce the conversion of OMb to DMb. The surface color readings of incubated samples were taken at 30 and 45 min.

The reflectance from 400-700 nm were used to calculate % OMb according to the AMSA (2012), equation 3 and 4.

$$\% \text{Deoxymyoglobin} = \left\{ 2.375 * \left[1 - \left(\frac{A_{473}-A_{700}}{A_{525}-A_{700}} \right) \right] \right\} * 100 \quad (3)$$

$$\% \text{Oxymyoglobin} = 100 - (\% \text{Metmyoglobin} + \% \text{Deoxymyoglobin}) \quad (4)$$

The following equation (5) was used to calculate OC:

$$\text{OC} = \% \text{ OMb prior-incubation} - \% \text{ OMb post-incubation} \quad (5)$$

Lipid oxidation

The lipid oxidation products (n = 72 steaks) were measured in duplicate as 2-thiobarbituric acid reactive substances (TBARS) values on 0, 3, and 6 d of the display

(Witte et al., 1970). Three grams from the steaks' surface of each loin section were mixed with 27 mL of 11% trichloroacetic acid solution (TCA). The mixture was homogenized using a laboratory blender for 20 s. Homogenate was filtered through a Whatman No. 42 filter paper (54 cm diameter, GE Whatman; Sigma Aldrich, St. Louis, MO). One mL of the supernatant was vortexed with 1 mL of 20 mM thiobarbituric acid (TBA) solution and incubated at 95°C in a water bath for 10 min. The samples were then cooled for 15 min at room temperature, and 1 mL of cooled filtrate was utilized to measure absorbance at 532 nm using a Shimadzu UV-2600 PC spectrophotometer (Shimadzu Inc., Columbia, MD). The blank sample for the spectrophotometer consisted of 1 mL TCA solution and 1 mL TBA solution. The TBARS values were expressed as mg malonaldehyde/kg of meat.

Microbiological analysis

Ten grams from the steak's surface of each loin section (n = 99 steaks) were aseptically placed into a sterile stomacher bag and added 90 mL of peptone water (0.1% diluted). The mixture was then macerated for 1 min at 225 rpm using a stomacher (Stomacher 400 Lab Blender, Seward Laboratory System Inc., London, UK). Homogenate was then decimal serial diluted in 0.1% peptone water and used for enumeration of microorganisms on 0, 3, and 6 d of the retail display.

Aerobic plate counts (APC) were determined by inoculating 1 mL of the homogenized samples, at selected dilutions factor, onto 3M™ Petrifilm™ aerobic count plate (3M Microbiology products, ST. Paul, MN, USA). The Petrifilms were then incubated for 48 h at 37°C. Lactic acid bacteria counts (LAB) were determined as described above for APC, except that the Petrifilms type used was 3M™ Petrifilm™

lactic acid bacteria (3M Microbiology products, ST. Paul, MN, USA) and the Petrifilms were incubated for 48 h at 30°C.

Statistical Analysis

The experimental design was a split-plot (n = 11 replications). In the whole-plot, each loin was divided into three equal sections and randomly assigned to one of the three enhancement (control, lactate, and rosemary). Following aging, each loin section was cut into three steaks (sub-plot experimental unit) and randomly assigned to three display time (0, 3, and 6 days). All data were analyzed using the General Linear Model Procedure (PROC GLM) of the Statistic Analysis System software (SAS Institute, Inc., 1990). The fixed effects include enhancement, display time, and their interaction. For instrumental color measurement (CIE values, chroma, hue angle, and ratio (630/580) nm), the repeated option was used (the readings were taken on steaks assigned for the day 6 of display). Microbial counts were transformed to base 10 logarithms of colony-forming units (CFU) per gram of beef samples. Least significant differences were applied to compare the means, and PDIFF was used for statistical significance level at 5%.

CHAPTER IV

RESULTS

Muscle pH and proximate composition

The proximate composition of the 11 loins prior to enhancement was 23.51% protein, 4.36% fat, and 71.89% moisture. The average enhancement-levels were 5.96 and 2.9% for rosemary and buffered lactate, respectively. The pH average of the 11 loins previous to enhancement was 5.53 (Table 1), which is normal for meat without Pale, Soft and Exudative (PSE) or Dark, Firm and Dry (DFD) defect (Gasperlin et al., 2000). Therefore, strip loins were appropriate for this study. There was a significant display time and enhancement interaction for pH ($P = 0.01$; Table 1). Loins enhanced with lactate and rosemary had a more stable pH. The lactate-enhancement had a greater pH ($P < 0.05$) than other enhancements during the display time.

Simulated retail display

A display time and enhancement interaction ($P < 0.05$) existed for a^* , chroma, hue angle and ratio (630/580) nm (Table 2). Loins enhanced with lactate had greater a^* values as of 2 d of display. There was a significant difference in a^* values between lactate and other enhancements on 3, 4 and 6 d of the display. Changes in a^* values between the first and last day of the display were 14.91, 11.14, 13.58, for control, lactate,

and rosemary, respectively; a greater change represents less color stability during the display, therefore, more discoloration. Loins enhanced with lactate sustained a^* values between 2 and 3 d ($P = 0.09$), and 3 and 4 d ($P = 0.06$) of the display, while loins enhanced with rosemary and control decreased ($P < 0.0001$) a^* values as display time progressed.

A greater chroma value indicates a more intense red color. The lactate-enhancement had greater chroma values ($P < 0.05$) on 3, 4, and 6 d of the display than other enhancements. The hue angle indicates shifts in color over time; a greater value denotes more discoloration. The rosemary-enhancement had greater hue angle than lactate-enhancement until 4 d of the display and differed from control on 3 d of display ($P < 0.05$; Table 2). A greater ratio (630/580) nm indicates more redness due to either OMb or DMb, and a ratio of 1.0 or less denotes MMb form. Loins enhanced with lactate had a greater ratio during the display ($P < 0.05$). Loins enhanced with rosemary differed from control on 0 and 2 d of the display ($P < 0.05$).

There was a main effect of enhancement for L^* values ($P < 0.0001$; Figure 2). Throughout the display, loins enhanced with rosemary were lighter (greater L^* values; $P < 0.05$) than other enhancements. Loins enhanced with lactate and control did not differ from each other in L^* values on 6 d of display ($P = 0.41$).

Metmyoglobin reduction activity (MRA)

There was a significant display time and enhancement interaction for MRA ($P = 0.007$; Table 3). At the beginning of display, lactate-enhancement had the greatest MRA, and it was different from rosemary ($P = 0.008$) and similar to control ($P = 0.07$). On day

3 of the display, all enhancements had similar MRA ($P > 0.05$). On day 6 of display, loins enhanced with lactate had a greater MRA than control and rosemary ($P < 0.0001$).

Oxygen consumption (OC)

There was a significant display time and enhancement interaction for OC ($P = 0.003$; Table 3). At the beginning of display, all enhancements had similar OC ($P > 0.05$). As retail display progressed, the loins enhanced with lactate had a lower OC ($P < 0.05$) compared with control and rosemary. The rosemary did not differ ($P > 0.05$) from control during the display time. For all enhancements, OC decreased from day 0 to day 3 of the display ($P < 0.0001$). When compared day 3 to day 6 of the display, lactate and rosemary maintained the OC at the same level ($P = 0.09$).

Lipid oxidation

There was a significant display time and enhancement interaction for lipid oxidation ($P = 0.05$; Table 4). The amount of TBARS values are presented as mg malonaldehyde per kg of beef (mg MDA/kg of beef). The lactate and rosemary enhancement had the same ($P > 0.05$) antioxidant effectiveness in the beef strip loins during the display. It was observed that rosemary and lactate reduced TBARS values by 30 and 25%, respectively, compared with control, which had TBARS value of 1.48 on 6 d of display.

Microbial counts

The microbial counts average of the 11 loins previous to enhancement were 0.67 and 1.67 for LAB and APC, respectively. The microbial analysis was accomplished to confirm that the discoloration of the strip loins was not caused by a high initial microbial population (Roback & Costilow, 1961; Walker, 1980; Seideman et al., 1983).

There was a main effect of enhancement for aerobic plate ($P = 0.0002$; Figure 1). During the retail display, the loins enhanced with lactate and rosemary did not differ from each other ($P = 0.67$), and they had lower counts than control. As retail display progressed, the population of aerobic bacteria increased for all enhancements. There was a main effect of display time for lactic acid bacteria ($P = 0.0001$; results not shown). The lactate, rosemary, and control enhancement did not differ from each other ($P > 0.05$) during the display. The lactic acid bacteria growth was not different between day 0 and day 3 of the display ($P = 0.88$).

CHAPTER V

DISCUSSION

Color measurements

Studies have reported extend aged loins may negatively impact the meat color stability and display shelf-life (Madhavi et al., 1993; Vitale et al., 2014; Ma et al., 2017). The present study is in agreement with Mancini et al. (2004a), Ramanathan (2008), and Mancini et al. (2009), where lactate sustained a^* values of the beef strip loins during retail display. Although rosemary and lactate enhancement were similar in a^* values during the first two days of the display, the red color of the rosemary-enhancement deteriorated more in the remaining days of the display than the lactate-enhancement.

The greater L^* values observed in loins enhanced with rosemary than in control and lactate may be due to the greater percentage of green weight (possibly due to water content) in rosemary-enhancement, which increases the reflectance of light, hence increases L^* values (Rahman, 2007). The loins enhanced with buffered lactate were visually not darkened.

Studies have shown that lactate darkening effect can be due to physical and biochemical pathways (Kim et al., 2006; Ramanathan et al., 2008; Mancini et al., 2009; Ramanathan et al., 2009; Ramanathan et al., 2010). The lactate induced effects on darkening may be due to mitochondria utilizing lactate, increasing cellular respiration,

and increasing oxygen consumption, which leads to decrease myoglobin oxygenation (Ramanathan et al., 2008; Ramanathan et al., 2013). In the present study, the humectant properties of the lactate may have contributed to decreasing water activity and reflectance of light, which decreases the L* values (Houtsma, 1996).

The chroma and ratio values had similar trends, where loins enhanced with lactate had greater values than other enhancements. It supports previous studies about the capacity of the lactate in improving the color stability of fresh meat. The mechanism of the lactate increasing the color stability of muscle may be due to the production of NADH via LDH activity. This process helps the MMb conversion to DMb, and then, the DMb can be oxygenated to form OMb (Lawrence et al., 2004; Kim et al., 2006).

Lipid oxidation

The TBARS values observed at the end of the display were below 2.0 mg MDA/kg of beef, which was reported by Greene & Cumuze (1981) to be the limit for the perception of rancid flavor in the beef. Rosemary and lactate enhancement had the same ($P > 0.05$) antioxidant capacity during the display. The loins enhanced with lactate and rosemary did not have their TBARS values statistically increased between 0 and 3 d of the display. Rosemary has been reported to possess an antioxidative capacity due to certain compounds such as carnosol, rosmanol, rosmariquinone and rosmaridiphenol, which can be more effective than synthetic antioxidant such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) (Houlihan et al., 1984; Nakatani et al., 1984; Barbut et al., 1985). Phenolic compounds present in rosemary can decrease oxidation of OMb, thus prolong the color shelf-life (Loliger, 1983). The antioxidative

activity of the lactate may be due to the interaction of the complex formed lactate-Fe⁺³ or lactate-Fe⁺² (Seydim et al., 2006). The TBARS values in this study are in agreement with Seydim et al. (2006), where the use of 0.2% of rosemary or 2% of lactate was effective delaying lipid oxidation in ground meat patties. Lee et al. (2005) and Sánchez-Escalante et al. (2001) reported that rosemary was effective in inhibiting lipid oxidation in ground beef patties. McBride et al. (2006) reported that rosemary treatment was more effective in inhibiting lipid oxidation in ground beef patties under aerobic packaging than BHA and BHT treatments. Maca et al. (1999) reported that lipid oxidation decreased as lactate levels increased from 1% to 4% in beef top rounds. MacVann & Nnanna (1991) reported that TBARS values decreased in raw beef with the addition of 1 and 2% of lactate. Sallam & Samejima (2004) reported refrigerated ground beef patties treated with 3% of sodium lactate decreased the lipid oxidation.

MRA and OC

Lactate-enhancement may serve as a glycolytic substrate to form reducing equivalents such as NADH for MRA (Mancini et al., 2004). The hydrogen from lactate is transferred to NAD⁺ to form NADH through the lactate dehydrogenase (LDH) activity, an endogenous enzyme that removes a hydrogen atom from lactate (Kim et al., 2005). The results of the present study are in agreement with Kim et al. (2006), where steak enhanced with 2.5% of sodium lactate had more than 2.5 times as much MRA on day 14 of the display as control steak. Mancini et al. (2006) reported that beef strip loins enhanced with 2% of potassium lactate improved MRA.

It is unlikely that the addition of rosemary replenishes the NADH pool. However, the rosemary may play a role in color stability through its antioxidant capacity (Martin, 2014). Further, a byproduct from lipid oxidation such as 4-hydroxy-2-nonenal (HNE) can decrease the LDH activity to regenerate NADH for MRA (Ramanathan et al., 2014). The results of this present study are in accordance with Sood (2018), who studied the effect of rosemary and oregano enhancement on beef strip loin. The author reported that the oregano treatment had a greater MRA, and the rosemary treatment did not differ statistically from the control during the retail display. Colle et al. (2019) reported that beef strip loins aged for 14 days, followed by rosemary enhancement did not differ from control in MRA.

The OC refers to oxygen consumed mainly by mitochondria and enzymes (Ramanathan et al., 2019). The level of the OC must be moderate; otherwise, if it is too high, the bloom capacity of the meat decreases, and if it is too low, the metmyoglobin level increases (Mancini & Hunt, 2005). On day 3 of display, the low level of the OC in the lactate-enhancement may have helped the myoglobin to bind with the oxygen available, which allowed the myoglobin to maintain its reduced form (McKenna et al., 2005). The rosemary and control enhancement had a greater level of the OC, supporting the assumption that greater oxygen consumption decreases the oxygen availability to the myoglobin.

Microbiological analysis

At the end of display, the microbial counts were lower than 7 log CFU/g, the spoilage limit for chilled meat (ICMSF, 1986). In the present study, the lactate and

rosemary enhancement had the same ($P = 0.67$) capacity to limit the growth of APC. The antimicrobial mechanism of the lactate may be due to its water activity lowering effect. In addition, the lactate has a low pKa value, at which its un-dissociated form passes through the walls of bacteria and alter their metabolism (Jensen et al., 2003; Theobald, 2015). Some compounds present in rosemary extract can have an antimicrobial effect such as phenolic compounds, abietane diterpenes, carnosol and ursolic acid (Cuvelier et al., 1996; Campo et al., 2000).

The addition of the lactate decreased counts of APC in refrigerated ground beef (Eckert et al., 1997), in fresh pork sausage (Brewer et al., 1991; O' Connor et al., 1993), and in cooked beef (Miller & Acuff, 1994). The use of rosemary decreased the microbial population in frozen ground beef patties (Gahrue et al., 2017), in beef steaks during the refrigerated period (Djenane et al., 2002), and in frozen ground lamb patties (Baker et al., 2013).

In chill vacuum-packaged beef, LAB are capable of growing (Egan, 1983; Krockel, 2013). We hypothesized that the extend wet-aging created a favorable condition for anaerobic bacteria to grow, achieving their log-phase during the display. Although lactate and rosemary have antimicrobial proprieties, the concentration used in this study was not enough to achieve the minimal inhibitory concentration against LAB. Brewer et al. (1993) and Sameshina et al. (1997) documented that pork sausage in vacuum packaging treated with 1 and 2% of sodium lactate was effective against LAB. Papadopoulos et al. (1991) reported that LAB were the predominant microbial population in beef treated with sodium lactate in vacuum packaging.

CHAPTER VI

CONCLUSION

The results of the present study demonstrated beef strip loins enhanced with buffered lactate prior to wet-aging for 14 d prolonged the color stability during the retail display without darkening the steak's surface, while the rosemary-enhancement had a labile color, deteriorating as retail display time progressed. In addition, 0.5% of buffered lactate and 0.2% of rosemary showed a preservation capacity against lipid oxidation and aerobic bacteria. However, the antimicrobial activity was not observed against lactic acid bacteria. The industry needs to produce meat with shelf-life long enough to fulfill logistics, retail sales, and consumer demands; therefore, characterizing the glycolytic substrates that play a role in color stability will help to develop processing strategies to mitigate industry economic losses due to the meat discoloration. In future studies, it would be informative to investigate the effect of the buffered lactate- and rosemary-enhancement in metabolites involved in extending the color stability of aged *longissimus* muscle.

Table 1. Effect of lactate and rosemary on pH of the beef strip loins during the simulated retail display (n = 99)

Parameter	Average pH loins 3 d postmortem	Enhancements ¹	Storage display (day)		
			0	3	6
pH	5.53	Control	5.34 ^a	5.58 ^{de}	5.54 ^{cd}
SE = 0.02		BLA	5.43 ^b	5.68 ^f	5.69 ^f
		RO	5.49 ^c	5.62 ^e	5.62 ^e

^{a-f} Least square means with different letters are different ($P < 0.05$).

¹ BLA = buffered lactate, RO = rosemary, Control = no-enhancement.

Table 2. Instrumental color of the beef strip loins during the simulated retail display (n = 33)

Parameters ¹	Enhancements ²	Storage display (day)				
		0	2	3	4	6
a* SE = 0.54	Control	33.05 ^h	29.52 ^g	26.73 ^{de}	22.28 ^{bc}	18.14 ^a
	BLA	33.14 ^h	30.36 ^g	29.07 ^{fg}	27.62 ^{ef}	22.00 ^b
	RO	33.14 ^h	29.06 ^{fg}	25.26 ^d	23.61 ^c	19.56 ^a
Chroma SE = 0.63	Control	41.60 ⁱ	37.37 ^h	34.10 ^{ef}	29.03 ^c	23.70 ^a
	BLA	41.81 ⁱ	38.16 ^h	36.50 ^{gh}	34.77 ^{fg}	28.50 ^c
	RO	42.19 ⁱ	37.30 ^h	32.94 ^e	31.01 ^d	25.58 ^b
Hue angle SE = 0.42	Control	37.36 ^a	37.81 ^{ab}	38.31 ^{ab}	40.00 ^{cd}	39.87 ^{cd}
	BLA	37.54 ^a	37.28 ^a	37.22 ^a	37.48 ^a	40.35 ^d
	RO	38.21 ^{ab}	38.85 ^{bc}	40.5 ^d	40.53 ^d	39.91 ^{cd}
Ratio (630/580) nm SE = 0.14	Control	6.87 ⁱ	4.86 ^{ef}	3.84 ^d	3.08 ^b	2.44 ^a
	BLA	7.08 ⁱ	5.46 ^g	4.76 ^{fg}	4.76 ^{ef}	3.38 ^{bc}
	RO	6.31 ^h	4.51 ^e	3.28 ^c	3.29 ^{bc}	2.60 ^a

^{a-i} Least square means with different letter within a parameter are different ($P < 0.05$).

¹ a* (redness); greater values mean more red color, chroma (intensity); greater values mean more intense red color, hue angle (discoloration); greater values mean more discoloration, R (630/580) nm; larger ratios mean more OMb or DMb. Refer to text for chroma and hue angle equation.

² BLA = buffered lactate, RO = rosemary, Control = no-enhancement.

Table 3. Effects of lactate and rosemary on OC and MRA of the beef strip loins during the simulated retail display (n = 99)

Parameter ¹	Enhancements ²	Storage display (day)		
		0	3	6
OC SE = 2.19	Control	41.63 ^{de}	27.06 ^b	36.28 ^{cd}
	BLA	39.26 ^{de}	16.76 ^a	16.77 ^a
	RO	42.62 ^e	25.9 ^b	31.15 ^{bc}
MRA SE = 5.3	Control	78.49 ^{bc}	25.4 ^a	21.65 ^a
	BLA	91.61 ^c	34.42 ^a	66.11 ^b
	RO	71.94 ^b	23.09 ^a	32.26 ^a

^{a-e} Least square means with different letter within a parameter are different ($P < 0.05$).

¹ OC = oxygen consumption, MRA = metmyoglobin reducing activity. Refer to text for OC and MRA calculation.

² BLA = buffered lactate, RO = rosemary, Control = no-enhancement.

Table 4. Effect of lactate and rosemary on lipid oxidation of the beef strip loins during the simulated retail display (n = 72)

Parameter ¹	Average TBARS loins 3 d postmortem	Enhancements ²	Storage display (day)		
			0	3	6
TBARS SE = 0.01	0.35	Control	0.41 ^a	0.76 ^b	1.48 ^d
		BLA	0.50 ^a	0.52 ^{ab}	1.10 ^c
		RO	0.42 ^a	0.52 ^{ab}	1.04 ^c

^{a-d} Least square means with different letter are different ($P < 0.05$).

¹ TBARS = Thiobarbituric acid reactive substances.

² BLA = buffered lactate, RO = rosemary, Control = no-enhancement.

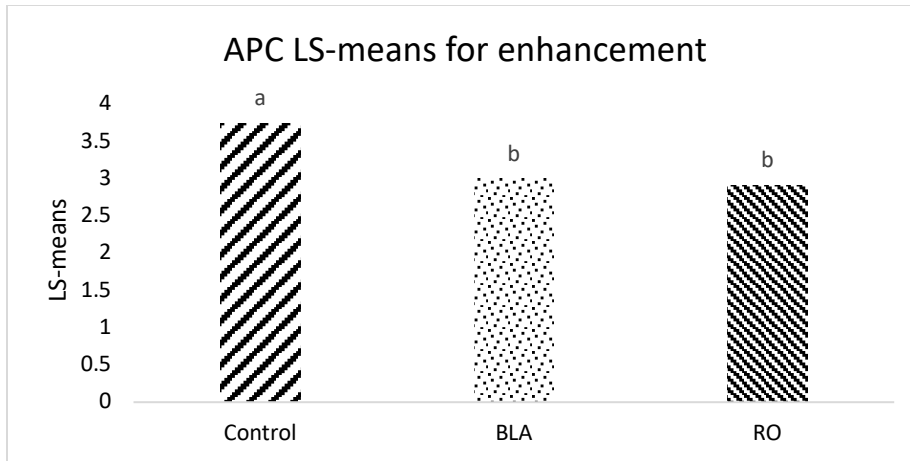


Figure 1: Main effect of enhancement for aerobic plate counts of the beef strip loins during the simulated retail display (n = 99). SE = 0.14, BLA = buffered lactate, RO = rosemary, Control = no-enhancement.

^{a-b} Least square means with different letters are different ($P < 0.05$).

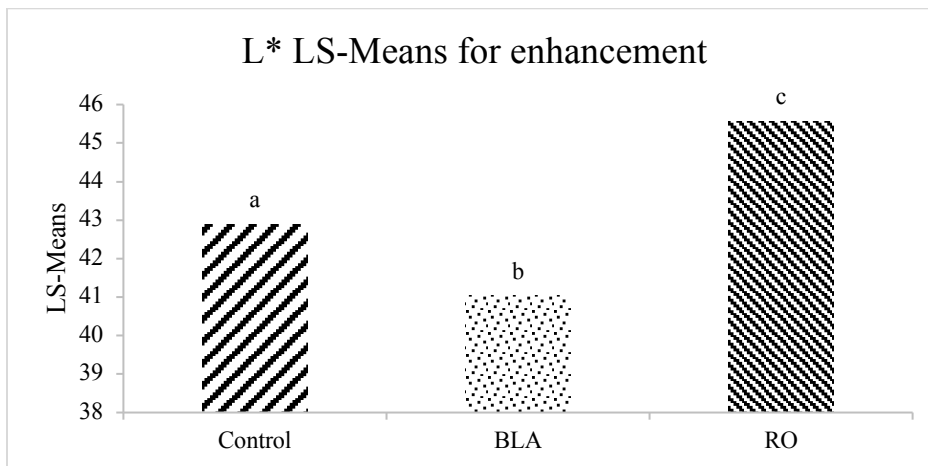


Figure 2: Main effect of enhancement for L* value of the beef strip loins during the simulated retail display (n = 33). SE = 0.22, BLA = buffered lactate, RO = rosemary, Control = no-enhancement.

^{a-c} Least square means with different letters are different ($P < 0.05$).

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