STRATEGIES TO EXTEND THE SHELF LIFE OF AEROBICALLY STORED BEEF

TRIMMINGS

A Dissertation

by

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ABSTRACT

Shelf life for fresh beef trimmings is often determined qualitatively based on color, lipid oxidation, microbial growth, and off odors and flavors. The first study was conducted to determine the number of storage days needed to reach the end of shelf life and to understand the interrelationships of spoilage mechanisms. Beef bottom round flats (*Biceps femoris*; BF) and chuck clod hearts (*Triceps brachii*; TB) were sliced to simulate trimmings. Trimmings were stored aerobically, at 5℃ under fluorescent lighting for 12 days. Objective color, pH, microbial plate counts, and volatile compounds were measured on days 0, 3, 6, 9, and 12. The data showed that 6 storage days is the end of shelf life for color and 9 storage days is the end of shelf life for microbial and organoleptic spoilage. The second study was conducted to evaluate the efficacy of selected ingredients to extend the shelf life of aerobically stored beef trimmings. USDA select BF and TB were sliced to simulate trimmings and stored aerobically for 6 or 9 days. Samples were treated with ingredients selected to extend shelf life by addressing color, microbial spoilage, or off odor and flavor development. Samples treated with reduced nicotinamide adenine dinucleotide (NADH) and sodium erythorbate (NaE) had greater ($P < 0.05$) a* (redness) values (12.76 and 11.47 respectively) compared to day 6 control samples (7.93) and decreased ($P < 0.05$) percent metmyoglobin (MMb) (29.96 and 37.34 %, respectively) compared to day 6 control samples (57.02%) measured 2 hours post treatment. Color measurements taken 48 hours post treatment indicated that NaE treated TB samples retained improved a* value (9.48)

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and MMb % (41.17%) versus NADH treated TB samples (a* 7.82; MMb 51.72%).
Cetylpyridinium chloride (0.5%) treated beef trimmings had lower ($P < 0.05$) aerobic
mesophile plate counts (7.4 $\log_{10}$ CFU/cm^2) than day 9 control samples (8.0 $\log_{10}$
CFU/cm^2). No significant differences ($P > 0.05$) were observed in volatile compound
groups between treatments. However, beef trimmings treated with sodium bicarbonate
(NaB) on storage day 9, had roughly 15 times less aldehydes present than day 9 control
samples. Treatment solutions NaE, CPC, and NaB all performed better than other
treatments for improving color, and reducing microbial counts and off odor volatiles.
These treatment solutions can be used in combination to extend the shelf life of beef
trimmings if applied near the end of shelf life.

DEDICATION

To my late grandmother, Patsy Yeater, my prayer warrior.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

I.I Introduction

Shelf life of beef trimmings can generally be defined as the length of storage time before the trimmings are no longer suitable for their intended use. Shelf life of fresh beef is a function of maintaining acceptable color, low microbial growth, and undetectable off odors and flavors including those from lipid oxidation. The length of shelf life is dependent on many intrinsic and extrinsic factors. Intrinsic factors such as proximate composition and pH (*1-3*) can influence shelf life and may be difficult to control in beef trimmings, especially in large lots of commingled trimmings coming from different animals. Thus controlling extrinsic factors such as packaging atmosphere (*4-6*) and storage temperature (*7-9*) are crucial in maintaining consistent shelf life. Many strategies for extending the shelf life of fresh beef steaks (*6, 10-12*) and ground beef (*13-16*) have been investigated. These studies have focused on the application of interventions at the beginning of storage to improve or sustain color, microbiological, and/or organoleptic shelf life. Little attention has been paid to the application of interventions after the onset of quality deterioration. Therefore an opportunity exists to extend the shelf life of fresh beef trimmings by addressing quality changes in the areas of color, lipid oxidation, microbial growth, and off odors or flavors.

This study is aimed at extending the shelf life of beef trimmings. The primary objective of this research was to evaluate the efficacy of selected ingredients to extend the shelf life of beef trimmings by attacking each mechanism (color, microbial spoilage,

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off odors and flavors) individually when applied at or near the end of shelf life. In order to properly apply treatments, a study was first conducted to determine the end of shelf life for beef trimmings stored in a model system. The initial study also helped us to understand the mechanisms and interrelationships that cause spoilage in aerobically stored fresh beef trimmings.

I.II Fresh Meat Shelf Life

The shelf life of fresh meat is the duration of storage before the meat can no longer be used for its intended purpose. Spoilage is the end of shelf life for most fresh meat and can be manifested as unacceptable color, odor, microbial growth, or any combination of these. The time it takes for meat to be considered spoiled and thus at the end of its shelf life depends on a number of intrinsic and extrinsic factors.

I.II.I Relationship of Spoilage and Intrinsic Food Properties. Factors such as meat source, fat and moisture content, pH, and time post mortem are all intrinsic properties that affect meat shelf life (*17*). In many cases, information regarding each of these factors is unknown to meat processors especially with respect to the use of large quantities of commingled trimmings. (i.e., 2000 lb combo bins). Controlling the extrinsic factors becomes very important in extending shelf life of fresh meat because controlling intrinsic factors may be difficult. Trimmings may be purchased with a specified fat content, however muscle pH and meat source are likely to vary. The time post mortem of the trimmings often depends on transit times, which may be than 2-3 days for some processing facilities.

I.II.II Relationship of Spoilage and Extrinsic Food Properties. Storage temperature, storage duration, and packaging are the most important extrinsic factors that contribute to the length of fresh meat trimming shelf life. Temperature can affect the rate of metmyoglobin accumulation, lipid oxidation, and microbial growth (*7, 18-21*). Storage duration is important because even with optimal storage conditions fresh meat will eventually reach the end of shelf life. Over time, metmyoglobin reducing ability will be depleted, spoilage microorganisms will grow beyond 7.0 $\log_{10}$ CFU/g, and off odors will develop due to lipid oxidation and microbial growth. Packaging can control the exposure of meat to oxygen and light both of which can accelerate spoilage and reduce shelf life (*19*). Vacuum packaging will extend shelf life (*16, 19*) however this is not feasible or economical for large quantities (i.e., 2000 lb combo bins) of fresh meat trimmings. These mechanisms will be explained in subsequent sections however it is important to identify the many factors contributing to the spoilage of fresh meat trimmings.

I.III Meat Color

Color is one of the most important factors in consumer decision making when purchasing fresh meat and meat products (*22, 23*). Consumers use discoloration as an indicator of wholesomeness although color stability, or the ability to maintain acceptable color, varies between different muscles and packaging systems (*24*). For example *Psoas major* steaks may reach 50% metmyoglobin (brown discoloration) by day 3 of retail storage (O_2 permeable polyvinyl chloride (PVC) overwrap, 1℃ , exposed to fluorescent lighting), while *Longissimus lumborum* steaks may not reach the same amount of

discoloration until 7 days of storage (*25*). These observed differences in color and color stability are influenced by both pre-harvest and post-harvest factors.

   I.III.I Pre Harvest Factors Affecting Meat Color. Genetic factors such as breed type and lineage influence color stability. King et al. (*26*) conducted a study investigating the role of animal genetics on animal to animal variation in color stability using steers (n= 464) representing the 7 most common beef breeds. The study found that animal breed was not significant (P > 0.05) for a* (redness), b* (yellowness), and chroma (indicates vividness) of *Longissimus thoracis* steaks when measured on day 0 of simulated retail display (O_2 permeable PVC overwrap, 1℃ , exposed to fluorescent lighting) (*26*). Breed was however significant (P < 0.05) for a*, b*, and chroma (indicates vividness) when measured on day 6 of simulated retail display. These data indicate that breed has less of an impact on initial color than on color stability during aerobic storage. Limited research has been done to identify genomic quantitative loci responsible for genetic variation between animals in terms of color attributes. De Koning et al. (*27*) identified four sex specific chromosomes (SSC) 2, 3, 4, and 13, with genetic markers influencing instrumental color scores in pork meat. Variation in the position of the genetic marker on SSC 13 was shown to influence L* (lightness) by 4.3-23.5 units. Variation in the position of the genetic marker on SSC 2 and 3 were shown to influence a* (redness) by 1.23-2.9 units. Variation in the position of the genetic marker on SSC 4 was reported to influence b* (yellowness) by 1.8-2.5 units (*27*). Gutiérrez-gil et al. (*28*) identified a genetic marker that significantly affected heme

pigments (hemoglobin and myoglobin) on chromosome BTA6 in beef meat. However, no genetic markers were identified for instrumental color attributes.

Feeding regimen also influences color stability of fresh meats, specifically when antioxidants are added to the diet. Vitamin E supplementation has been shown to improve meat color stability by increasing the meat's ability to reduce metmyoglobin (MMb; oxidized myoglobin exhibiting a brown color) to the more favorable oxymyoglobin (myoglobin exhibiting bright cherry red color). The meat's inherent ability to reduce MMb is one of the main factors determining the color stability of meat. Chan et al. (*29*) reported lower ($P < 0.05$) percentage of MMb for *Psoas major*, *Gluteus medius*, and *Longissimus lomborum* from Holstein steers fed vitamin E (2,000 mg/ day for 122 days) compared to the same muscles from control Holstein steers, over a 12 day (O_2 permeable PVC overwrap, 4℃ , exposed to fluorescent lighting) storage period. In a study of Charolais cattle, finishing mode (grass or grain finished) was shown to have a significant effect on a* values and MMb accumulation for l*ongissimus dorsi* steaks of heifers on day 6 of storage (O_2 permeable PVC overwrap, 4℃ , stored in darkness) (*30*).

Environmental factors such as stress can impact fresh meat color and color stability (*31*). Animals that endure long-term stress, such as genetic stress susceptibility, before slaughter experience depleted muscle glycogen stores which result in decreased accumulation of lactic acid in the muscle during rigor mortis. This results in higher ultimate pH (6.0 or higher) and manifests itself as dark, firm, and dry meat (DFD)(*32*). Additionally, exposure to short term stress such as fighting with other animals or an unfamiliar environment before slaughter can result in rapid glycolysis and the release of

the hormone epinephrine early post mortem. These lead to an elevated level of lactic

acid in post rigor muscle and a lower ultimate pH (5.2 or lower) resulting in pale, soft,

and exudative meat (PSE)(*33*). PSE meat has a lighter color due to the denaturation of

myoglobin during the chilling process (period of low pH and high temperature) and the

change in light reflection on the meats surface caused by low pH. PSE meat not only

exhibits a less acceptable color but also has less color stability than normal (pH 5.6-5.8)

meat. PSE meat is however, not commonly observed in beef (*34*).

I.III.II Differences in Biochemical Properties of Different Primal Cuts.

Extensive research has been conducted to profile different muscles in terms of their

individual tenderness and color stability. Researchers at the University of Nebraska-

Lincoln (Lincoln, NE) conducted the bovine myology and muscle profiling study which

has laid the foundation for the understanding of biochemical differences between bovine

muscles (*35*). Similarly, a National Pork Check Off funded study was conducted by

researchers at Iowa State University (Ames, IA), Michigan State University (Lansing,

MI), and the University of Wisconsin (Madison, WI) to profile porcine muscles. This

study determined differences in pH, water holding capacity, objective color and flavor of

25 pork muscles (*36*). Behrends (*37*) identified differences in color stability, MMb

accumulation, and metmyoglobin reducing ability (MRA) between nine bovine muscles.

The study reported that *Longissiums lumborum* and *Longissiums thoracis* maintained

their redness (a*) over 7 storage days (O$_2$ permeable PVC overwrap, 4℃ , exposed to

fluorescent lighting), while the other 7 muscles measured decreased in a* (*37*).

McKenna (*38*) investigated the causes of the different color stabilities of 19 bovine

muscles, concluding that color stability is ultimately a response to the relationship between a muscle's oxygen consumption rate and its MRA.

 I.III.III Post Harvest Factors Affecting Meat Color. Following harvest many factors can influence meat color and color stability. During the conversion of muscle to meat, chilling rate and the rate of pH decline can have an impact on the color of meat (*10, 39, 40*). Storage conditions such as packaging, temperature, and exposure to light can increase or decrease color stability. Packaging atmosphere (aerobic or anaerobic) influences the color of meat by altering the presence and concentration of oxygen (*5, 6, 41*). Packaging system also impacts the color stability of meat during storage as exposure to low partial oxygen pressure accelerates the formation of metmyoglobin (*4, 42, 43*). Storage temperature influences the rate of discoloration in fresh meat (*7-9*). Hood (*9*) reported that *Longissimus dorsi* steaks stored (O_2 permeable PVC overwrap, exposed to fluorescent light) at 10°C for 96 hours had 2-5 times the discoloration of *Longissimus dorsi* steaks stored at 0°C for 96 hours. Exposure to light can cause photo oxidation of myoglobin resulting in discoloration of the meat surface (*9, 22, 44*).

 I.III.IV Myoglobin and Oxygen Consumption. Myoglobin is a water soluble, sarcoplasmic protein made up of a globular portion and a prosthetic heme group (**Figure 1.1**). The heme iron is located at the center of a porphyrin ring and has 6 binding sites. Four of the binding sites are bound to nitrogen atoms in the porphyrin ring structure, the
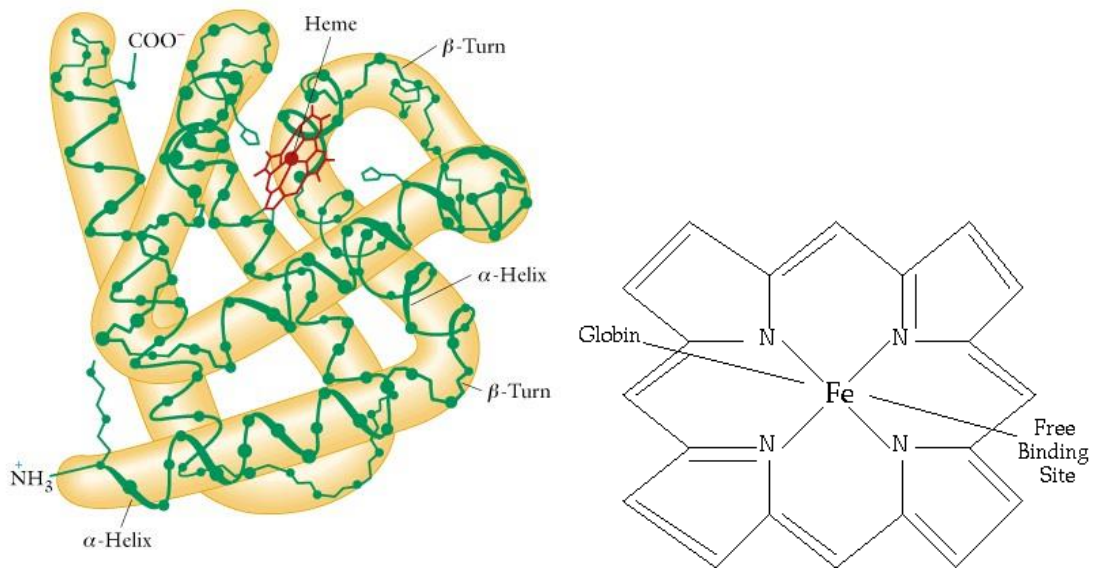
Figure 1-1. Myoglobin Structure. (Left) Myoglobin Tertiary Structure (*45*) and (Right) Simplified Porphyrin Ring Structure (*46*).

fifth binding site is bound to the proximal histidine-93 of the globular protein, and the sixth binding site is considered the free binding site (*47*). In living muscle myoglobin is responsible for the storage of O_2, for utilization via oxidative phosphorylation. Myoglobin can be found in many different chemical states in meat, however the three states common to fresh meat are oxymyoglobin (bright, cherry red muscle color in beef, grayish pink color in pork), deoxymyoglobin (purple muscle color), and metmyoglobin (brown muscle color). There are two factors that primarily dictate the state of myoglobin, the redox state of the heme iron and the molecule bound to the sixth binding site of the heme iron. When the heme iron is oxidized it becomes ferric iron or Fe^{3+} and the myoglobin turns the meat to a brown color. Reduced heme iron is ferrous iron or Fe^{2+} and in this state depending on the availability of oxygen the myoglobin will either be deoxymyoglobin (no oxygen bound, resulting in purple muscle color) or oxymyoglobin (O_2 bound to 6th ligand, resulting in bright muscle color). Under aerobic conditions, myoglobin undergoes autoxidation (to metmyoglobin) and subsequent reduction (to deoxymyoglobin) and oxygenation (to oxymyoglobin) (*24, 47*). Oxygen consumption rate (OCR) varies between different muscles (*37, 38*), likely due to residual mitochondrial activity differences in post mortem muscles (*47*). OCR has been shown to have an inverse relationship with color stability. O'Keefe and Hood (*8*) found that muscles with higher OCR tended to have the most discoloration (poor color stability). Renerre and Labas (*48*) reported that muscles with high color stability, such as the *Tensor faciae latae*, possessed a low OCR while muscles with poor color stability, such as the *Diaphragma* possessed a high OCR. As oxygen is consumed, myoglobin is being

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oxidized and subsequently reduced by endogenous MRA until the MRA has been

exhausted. Thus, lower OCR will prolong color shelf life.

   I.III.V Metmyoglobin Reducing Ability/ Activity. The mechanisms that reduce

myoglobin from the oxidized *met* state to the reduced *oxy* state has been referred to as

metmyoglobin reducing activity (MRA), which Bekhit and Faustman (*49*) reviewed

comprehensively. It is well documented that reduction of metmyoglobin (MMB), can

take place both enzymatically and non-enzymatically, and both systems use available

reduced nicotinamide adenosine dinucleotide (NADH) to reduce MMB (*47, 50, 51*).

Enzymatic reduction primarily uses the enzyme cytochrome b_5 reductase which is found

in the mitochondria to reduce MMB (*52*). This enzyme system requires cytochrome b_5 as

an electron mediator and NADH as a cofactor. Non-enzymatic reduction of MMB uses

an electron donor such as NADH to directly reduce the heme iron from the ferric to the

ferrous state (*1, 53, 54*).

   Atkinson and Follet (*55*) proposed that color stability is dependent on the ratio of

a specific muscle's MRA to OCR rather than the amount of either MRA or OCR

observed in a specific muscle. Muscles with high OCR can have good color stability if

they possess enough MRA. Muscles with low MRA therefore, can have good color

stability if they possess low amounts of OCR. McKenna et al. (*38*) also offered this

explanation, providing examples of *Tensor fasciae latae*, *Adductor,* and *Semitendinosus*.

Adductor exhibited low OCR (1.32 % increase in CO_2 on storage day 2) but very low

MRA (19.1 % MMb reduced on storage day 5) resulting in significantly lower ($P < 0.05$)

a* on storage (O_2 permeable PVC overwrap, 2℃ , exposed to fluorescent lighting) day 5

(17.92) than on storage day 1 (20.39), evidence of poor color stability. In contrast, *Tensor fasciae latae* had an OCR (1.40 % increase in CO_2 on storage day 2) similar to the *Adductor* but a relatively higher MRA (45.90% MMb reduced on storage day 5) resulting in no change ($P > 0.05$) in a* values from storage day 1 (18.60) to storage day 5 (18.67), indicative of good color stability. The *Semitendinosus* possessed a high OCR (1.82 % increase in CO_2 on storage day 2) but also had a high enough MRA (52.80% MMb reduced on storage day 5) to provide good color stability. Good color stability was indicated by no change ($P > 0.05$) in a* values from storage day 1 (21.64) to storage day 5 (21.19)(*38*). Increasing the MRA of meat by adding reducing equivalents may have the potential to extend color shelf life of fresh meat.

> *I.III.VI Postmortem Chilling Effects*. Chilling rate impacts color and color stability due to the effect of abnormal post mortem protein denaturation. As the muscle pH declines while muscle temperature is declining at a slower than normal rate, conditions are optimal (pH < 6.0, Temperature > 30℃) for protein denaturation including myoglobin (*40*). This is especially true for muscles like the beef *semimembranosus*, a large muscle located on the inside of the round, which cools more slowly. This muscle often exhibits a two tone color as the most interior portion of the muscle is paler and less color stable (*10, 39*).

> *I.III.VII pH Effect*. Meat pH that is much higher or lower than the normal pH (5.6-5.8) will exhibit a darker (high pH) or lighter (low pH) surface color. The change in pH alters the charge of amino acid side chains which modifies the space between muscle fibers, ultimately changing the reflection of light on the meat surface (*21*). pH can also

influence color stability, as low ultimate pH is favorable for the development of MMb and thus low pH muscles have lower color stability (*1*). Ledward et al. (*3*), found that *Semimembranosus* steaks with high pH (> 5.8) developed less (20%) MMb at 80 hours of storage (O2 permeable PVC overwrap, 1℃, exposed to fluorescent lighting) than *Semimembranosus* steaks (40% MMb) with normal pH (5.6) indicating greater color stability for high pH meat. Hutchison et al. (*56*) found pH to significantly impact the efficiency of MRA in vitro, as MRA was greater at pH 7.4 than 6.4 or 5.7. The pH of meat is an important factor in determining shelf life, as it has a direct impact on not only the color of the meat but also the color stability of the muscle.

      I.III.VIII Lactate Dehydrogenase and Meat Color. In muscle, lactate is converted to pyruvate via the enzyme lactate dehydrogenase (LDH), which generates reduced nicotinamide adenine dinucleotide (NADH). As previously discussed, NADH is utilized in both the enzymatic and non-enzymatic reduction of MMb. Kim et al.(*57*) proposed that the increase in NADH seen in lactate enhanced beef was the mechanism causing an increase in color stability. They reported that *Longissimus lumborum* steaks enhanced with 2.5% potassium lactate, had a higher (P < 0.05) NADH concentration (1.4 ug/mL) than the non-enhanced control (0.9 ug/mL) after 14 days of storage (80% O$_2$ modified atmosphere package, 2℃, exposed to fluorescent lighting on days 9-14)(*57*). Kim et al. (*25*) later verified the role of LDH in MRA of beef. Their study showed that *Longissimus lumborum* steaks injected with 180 mM oxamate (LDH inhibitor) had a lower (P < 0.05) a* value, saturation index (an indication of vividness), and higher (P < 0.05) hue angle (indicator of redness), than the untreated control samples on day 10 of

storage (O2 permeable PVC overwrap, 1℃ , exposed to fluorescent lighting)(*25*).

Sodium and potassium lactates, which are commonly used as antimicrobials in processed

meat products, have been shown to extend shelf life of fresh meat harnessing the LDH

pathway (*42, 58-60*). Lactate enhancement is an option to deliver NADH (reducing

equivalents) in order to bolster MRA of meat and extend color shelf life.

I.III.IX *Packaging of Fresh Meat*. Packaging systems also dictate the

concentration of O_2 in the packaging atmosphere which impacts both color and color

stability of fresh meat (*4-6, 10, 21*). Packaging materials vary in their O_2 permeability

which is measured as oxygen transmission rate (OTR). PVC overwrap typically has a

high OTR while vacuum pouches and top films for modified atmosphere packaging

(MAP) typically have a low OTR. Fresh meat stored in vacuum packaging (O_2

impermeable film) will exhibit dark red/ purple color as the myoglobin is in the deoxy

state. However, once the meat is exposed to oxygen the meat will "bloom" and

myoglobin will return to the oxygenated state. Meat stored aerobically either in combo

bins or in trays overwrapped with oxygen permeable film (typically PVC) will exhibit

bright red color (oxymyoglobin) until MRA is depleted at which time the meat will

appear brown (metmyoglobin), indicative of MMb accumulation. Modified atmosphere

packaging (MAP) is a commonly used system for fresh meat in which meat is stored in a

container with a gas impermeable film (low OTR) and the headspace is filled with a gas

mixture which may be comprised of oxygen, carbon dioxide, carbon monoxide, and/or

nitrogen. High oxygen (usually 80% or greater O_2) MAP can be used to maintain color

stability in fresh meat. In a MAP packaging system, there is little chance for low partial

oxygen pressure to exist, resulting in MRA being depleted more slowly than in an aerobic packaging system, where oxidative stress is caused by low partial oxygen pressure (*6*). Low partial oxygen pressure is a low oxygen pressure from roughly 4 -10 mm Hg, an atmosphere that is highly oxidizing (*1*). To deter microbial spoilage when using high oxygen MAP, carbon dioxide can be used in addition to the oxygen (*21*). Carbon monoxide (CO) has been used to fix the bright cherry red color. Myoglobin with CO gas bound is known as carboxymyoglobin, which is much more stable than oxymyoglobin because the heme iron has a higher affinity for CO than for O_2 (*5*). The use of CO for fresh meat has received some criticism as the stable color may mask microbial spoilage thus appearing attractive (bright red color) while having unacceptable odor and microbial counts (*41*). Packaging can extend the color shelf life of fresh meat; however vacuum packaging and MAP are not realistic options from a cost and labor perspective for the packaging of large quantities of fresh meat trimmings.

I.III.X Color Measurement Methods. Objective color can be measured using a colorimeter or spectrophotometer. These devices scan the meat surface for either tristimulus color values or spectral reflectance values. The concept of tristimulus values (XYZ) was introduced by the Commission Internationale de I'Eclariage (CIE) in the 1930's and has since been modified into a uniform color space, known as CIE LAB scale (*61, 62*). The L* value is the measure of lightness based on a 100 point scale ranging from 0 (absolute darkness) to 100 (absolute lightness). The a * value is the measure of redness, measured on a 120 point scale ranging from -60 (green) to +60 (red). The b* value is the measure of yellowness, measured on a 120 point scale ranging

from -60 (blue) to +60 (yellow). All colors fall somewhere in the 3 dimensional color space. Color measurements can be taken using different illuminants and observers which both influence the results. Common illuminants used for color measurement are illuminant A (incandescent, tungsten-filament lighting, 2875 K color temperature), illuminant C (average north sky daylight, 6774 K), and illuminant D_{65} (noon daylight, 6500 K). Colorimeters commonly use either a 2° or 10° observer, which is the angle at which the reflected light is captured. The American Meat Science Association (AMSA) recommends the use of illuminant A (emphasizes the proportion of red wavelengths) and a 10° observer (captures a larger portion of the sample than the 2° observer) for measuring fresh meat color (62). It is important to report which illuminant and observer was used for objective color measurement when reporting research findings. Additional color attributes can be calculated using the L*, a*, b* color scores. Hue angle (HA), which can indicate redness, can be calculated as HA = $\tan^{-1}$(b*/a*) (62). Saturation index (SI), which is a measure of vividness or color intensity can be calculated as SI $=\sqrt{a^{*2} + b^{*2}}$ (62). HA and SI are both related to concentration of OMb (63).Measuring reflectance is useful as these data simulate how humans perceive color. Spectral reflectance data can be used to quantify myoglobin states. There are two common methods of utilizing reflectance data. One uses selected wavelengths which are then modified by a correction factor to determine DMB and MMB and then calculate OMB (64). The other method, first proposed by Stewart et al. (65) uses reflectance data to calculate K/S ratios at the isobestic wavelengths of myoglobin states. Both methods are useful in determining myoglobin states and are non-invasive measurements so repeated

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measurements on the same sample are possible. MRA can be determined in a number of ways. Total reducing ability (TRA) originally described by Lee et al. (*66*), is not specific to myoglobin. Meat samples are homogenized and mixed with potassium ferricyanide ($K_3[Fe(CN)_6]$) for 1 hr to oxidize the myoglobin. Ammonium sulfate ($[NH_4]_2SO_4$), lead acetate ($Pb[C_2H_3O_2]_2$), distilled water and trichloroacetic acid ($C_2HCl_3O_2$)are added and then the sample is filtered, transferred to a cuvette and absorbance is read at 420 nm (*66*). Aerobic reducing ability (ARA) uses 3x2x1.27 cm^2 meat samples, exposed to 1% O_2 atmosphere for 24 hours and then reflectance is measured (400-700nm) to determine percentage of myoglobin states. Samples are then allowed to reduce for 24 hours at atmospheric oxygen (21%) (*67*). Nitric oxide myoglobin reducing ability (NORA) is a method similar to ARA with the exception that meat samples are oxidized by submerging them in 0.3% sodium nitrite ($NaNO_2$) solution for 20-30 min and vacuum packaged. Packages are scanned for reflectance (400-700nm), incubated at 30℃ for 2 hours and scanned for reflectance again (400-700nm) (*39, 51*). The results from the NORA method can be reported in a number of ways either reporting the initial percentage of metmyoglobin after oxidation, the final percentage of metmyoglobin after reduction, or the change between the two (*68, 69*). The metmyoglobin reducing activity assay is a more complex method that measures metmyoglobin reductase enzyme activity. In this method, a muscle homogenate is prepared and mixed with exogenous metmyoglobin (oxidized by potassium ferricyanide) and exogenous NADH. The subsequent reduction is monitored with a spectrophotometer over 3 mins (*70*). Sammel et al. (*39*) conducted a study in which they

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determined reducing ability with several currently used methods (TRA, ARA, NORA, MRA) and determined that ARA correlated the strongest with measures of color stability (r=-0.58 for visual color; r=0.56 for a*) over 6 storage days (O_2 permeable PVC overwrap, 0℃ , exposed to fluorescent lighting), compared to other reducing ability measurement methods.

In addition to measuring reducing ability, measuring oxygen consumption rate (OCR) and oxygen penetration depth (OPD) can be useful in characterizing color stability. OCR has been described differently in the literature (*37-39, 68*), however with the exception of King et al. (*68*) the procedure involved placing a meat sample in a high oxygen atmosphere and measuring the headspace O_2 concentration before and after a set storage time. King et al. (*68*) allowed samples to oxygenate then vacuum packaged the samples. The packages were scanned for reflectance, incubated for 30 min in a 30℃ water bath, and scanned again for reflectance to determine the OMb % (*68*). The OCR was calculated as the percentage of OMb prior to incubation minus the percent OMb after incubation. OPD was described by McKenna et al. (*38*) and again by Behrends (*37*) briefly 1 cm thick meat samples were placed between two glass plates, overwrapped with oxygen permeable film, and the depth of the oxymyoglobin layer was measured each sampling day (1-7) with calipers (*37, 38*).

I.IV Meat Flavor

Desirable cooked meat flavor is what is typically identified as the inherent flavor of meat (*71*). Odors and flavors associated with spoilage can predominate meat flavor. These odors and flavors are volatile aromatic compounds that can be perceived as sweet

and fruity aromas, rancid, or putrid odors (*17, 72-75*). Volatile compounds are formed

during the breakdown of fresh meat or during cooking. Rancidity is the flavor

associated with the breakdown of lipids, which commonly occurs due to oxidation in

meat (*17*). Some spoilage organisms possess a lipase enzyme that will liberate fatty acid

chains from the glycerol backbone of a triacylglycerol or diacylglycerols, which is

known as hydrolytic rancidity (*76*).

      I. IV.I Mechanisms of Meat Flavor. Most of the flavor attributes associated

with meat (meaty, beefy, brown roasted) are found exclusively in cooked meat as

heating is required to produce these aromas (*77*). Meat flavor is determined by a

combination of detection of basic tastes by the gustatory senses of the tongue, the

detection of aromas by the olfactory bulb, and somatosensory perception by the

trigeminal nerves (*77*). The main flavors in meat are driven by volatile aromatic

compounds resulting from the Maillard reaction, lipid thermal degradation, or a

combination of the two (*75*). Maillard reaction, commonly referred to as non-enzymatic

browning, is the reaction of an amino acid and a reducing sugar catalyzed by heat.

Amino compounds condense with a carbonyl group of the reducing sugar which

produces a glycosylamine (*77*). The Strecker degradation, a component of the Maillard

reaction, is the deamination and decarboxylation of amino acids while the carbonyl

compounds formed previously becomes an aminoketone or an amino alcohol (*71*).

Lipid thermal degradation generates hundreds of volatile compounds that tend to impact

meat flavor more than products of Maillard reactions except in meats that are cooked

directly on a high heat source (*71*). In this reaction, neutral triglycerides and polar

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phospholipids are disassembled, releasing fatty acids. These fatty acids are modified to form aldehydes, ketones, alcohols, which resemble the fatty acid they were produced from partially explaining species flavor differences (*77*). The volatile compounds produced by both the Maillard reaction and lipid thermal degradation impact beef flavor, and in some cases they interact with one another (*71*). Some of the products of lipid thermal degradation can undergo the steps of the Maillard reaction generating new volatile compounds (*77*).

There are some meat flavor attributes associated with raw meat. Many of the commonly identified flavors are perceived as negative flavors such as bloody, metallic, and salty (*78*). The mechanism of lipid thermal degradation and lipid oxidation are actually similar (fatty acids are modified to form different compounds) and likewise the volatile compounds produced are similar (*71*). The aromas in oxidized beef have been described as rancid off flavors while the aromas of lipid thermal degradation are described as favorable. Kerth and Miller (*77*) stated that during cooking the chemical reactions of lipid thermal degradation happens much faster than lipid oxidation. The reaction rate possibly allows for more favorable aromas to develop in cooked beef that is not developed in oxidized beef. The ability to reduce the amount of volatile compounds contributing to off odors and flavors seems to be a necessary step in extending the shelf life of fresh meat.

I. IV.II Flavor Measurement Methods. Sensory evaluation is commonly used to detect or identify off odors and flavors in meat products (*77*). Many different methods of sensory analysis may be used to quantify off odors and flavors of meat such as

descriptive testing, discriminative testing, and consumer sensory testing (*79*).

Descriptive sensory testing utilizes trained sensory panelists (usually 6-10) to quantify

flavor attributes found in a flavor lexicon (*80*). While descriptive sensory data is useful

in determining the presence and quantities of specific flavors in meat, it does not indicate

acceptability of the sample or how well samples are liked. Discriminative testing is a

form of consumer sensory analysis that compares samples and determines if consumers

can differentiate between the samples. Consumer sensory panels are used to determine

how well consumers like a product or if the sample is considered acceptable, typically

consumer panels survey a large number of test subjects. More recently the use of gas

chromatography and mass spectrometry have been used to identify and quantify specific

volatile compounds responsible for the off odors and flavors (*77, 81-83*). Data from this

type of analysis is often paired with sensory analysis to explain relationships between

specific volatile compounds and the presence of off odors or flavors.

I.V Lipid Oxidation

  I.V.I Mechanisms of Lipid Oxidation. Oxidation has three main steps:

initiation, propagation, and termination. Initiation of lipid oxidation occurs when a

hydrogen is abstracted from fatty acid by a free radical forming a lipid radical (*74*). Free

radicals are typically reactive oxygen species such as singlet oxygen ($^1O_2$) and

superoxide radicals ($\cdot O_2H$), hydroxyl radicals (OH), or oxidized metals (such as ferric

iron, Fe^{3+})(*84*). Lipid radicals are very unstable and react with molecular oxygen to form

lipid peroxyl radicals. Once lipid peroxyl radicals are formed, they continue to react

with other fatty acids forming more lipid radicals and lipid hydroperoxides; this is

known as propagation (*17*). Lipid hydroperoxides are the product of lipid oxidation, and

typically are converted to more stable compounds like aldehydes and ketones (*85*).

Termination occurs when two radicals react with each other to form a stable by product

or when antioxidants react with the radicals to form stable compounds (*17*). Without the

addition of antioxidants, termination occurs after there are far too many radicals formed

to avoid a negative impact on the product. In fresh meats, it is thought that the primary

lipids subjected to lipid oxidation are polar phospholipids, despite only accounting for

1% of all lipids which are found in membranes due to the presence of mostly unsaturated

fatty acids (*86*). Lipid oxidation is manifested as off odors and flavors, so the most

appropriate intervention in fresh meat spoilage may be to address the off odors and

flavors rather than lipid oxidation.

     I.V.II Mechanisms of Antioxidants. Antioxidants can act in three ways to

prevent oxidation in food products. They can scavenge free radicals, chelate metals, and

quench singlet oxygen Antioxidants scavenge free radicals by donating a hydrogen to

form a stable compound and an antioxidant radical (*87*). While still a radical compound,

the antioxidant radical is much more stable than a free radical or a lipid radical. Metal

chelating is the binding of metals in the food product by antioxidants which inhibits the

metals from initiating oxidation (*85*). Quenching singlet oxygen is the energy transfer of

an electron from an antioxidant to singlet oxygen, transforming it to its native state,

triplet oxygen (*88*). Antioxidants are not likely to react with lipid radicals directly

however because the reaction of molecular oxygen with lipid radicals is very

thermodynamically favorable and thus happens almost instantly (*74*).

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I.V.III Methods of Quantifying Lipid Oxidation. Thiobarbituric acid reactive

substances (TBARS) is a tried and true method for determining lipid oxidation. A steam

distillation method (*89*) for the determination of TBARS in meat products was

developed in the 1960's and since many variants have emerged (*90*). All of the

distillation methods quantify malonaldehyde (MDA) in samples, which is a secondary

peroxidation product. Distillates are mixed with 2- thiobarbituric acid and heated to

form a stable MDA-TBA complex. The complex is a stable hemichrome that exhibits a

pink-red color. Absorbance of the complex is measured and compared to the absorbance

of known values of MDA. TBARS are reported as mg MDA/ kg of meat. An alternate

extraction method for TBARS was developed (*91*) in which trichloroacetic acid (TCA)

is added to meat samples to liberate lipid peroxidation products. As with the distillation

method, the extraction method has seen many variations (*92, 93*). Ultimately all TBARS

methods quantify MDA as an estimation of the total lipid oxidation products present in

the sample. Fernandez et al. (*94*) provided a good review of several TBARS

methodologies.

Beyond TBARS, peroxide value, gas chromatography and mass spectrometry,

and trained sensory panels have all been used to quantify lipid oxidation. Devasagayum

et al. (*95*) and Barriuso et al. (*96*) reviewed methods for quantifying lipid peroxidation

products. They reported that gas chromatography and mass spectrometry (GC-MS) is a

precise, highly repeatable method. Barriuso et al. (*96*) stated that monitoring the levels

of hexanal and pentanal as markers of oxidation in food products. Devasagayum et al.

(*95*) suggested that estimation of isoprostanes derived from the oxidation of arachidonic

acid is a viable method for quantifying lipid oxidation. GC-MS is more expensive

relative to other common methods of lipid oxidation determination, though it provides

increased precision and repeatability.

I.VI Microbial Spoilage

All foods can be subject to spoilage by a wide range of spoilage microorganisms.

These organisms can be found on products due to contamination pre harvest, during

harvest, further processing, or even post lethality contamination in the case of ready to

eat products (*19*). It is an important distinction to separate the potentially harmful

pathogenic bacteria and the quality deteriorating spoilage bacteria. Fresh raw meat,

stored aerobically under refrigerated conditions has a predominate microbiota consisting

of *Pseudomonas* spp., *Moraxella* spp., and *Acinetobacter* spp. (*2, 19, 20, 73*). These

organisms will grow on the surface of intact meat and trimmings typically resulting in

the development of off odors and the formation of slime. Off odors do not typically

occur until microbial counts reach 10^7 CFU/cm^2 and the organisms have metabolized all

available simple carbohydrates and begun metabolizing amino acids (*2*). Slime

formation occurs once the counts are high enough (10^8 CFU/cm^2 or higher) that

colonies coalesce into a slime layer as well as the secretion of polysaccharides from

carbohydrates (*97*). Initial load of microorganisms at the onset of storage is one of the

biggest factors influencing growth, although meat pH, storage temperature, and

packaging atmosphere all impact survival and growth of spoilage microorganisms (*2,*

19).

I.VI.I Antimicrobial Interventions. Antimicrobial interventions can be either

bacteriostatic or bactericidal in nature. Bacteriostatic products will prevent the growth

of organisms and though some reduction of existing microbial load can occur, they tend

to be minimal (< 3 log cycles). These type solutions are effective for post lethality

applications on cooked meats but are less useful on fresh meat trimmings. Bactericidal

products will kill organisms on the surface of the meat and in some cases will offer a

residual bacteriostatic effect as well. Antimicrobials utilizing different chemistries are

needed to address different types of organisms. For example, Gram-negative bacteria

have an outer membrane and Gram-positive bacteria do not have an outer membrane,

and thus the mechanisms of attack against the two types differ. Organic acids have

proven to be very effective against Gram-negative bacteria as they can diffuse across the

cell membrane in the non-dissociated state and dissociate inside the cell, which lowers

the intracellular pH and disrupts the organism (*98-101*). Gram-positive bacteria are

more resistant to organic acids because they have a thicker cell wall (*100*). In the case of

fresh trimmings, an antimicrobial that can both reduce the load of spoilage

microorganisms as well as prevent the growth of additional microorganisms is

particularly useful.

In the case of spoilage organisms that have formed a slime, or an

exopolysaccharide structure, the enzyme saccharidase can be used to enzymatically

cleave the complex carbohydrate into smaller units which can be easily removed (*102*).

I.VI.II Methods of Quantifying Spoilage Organisms. Spoilage organisms are

quantified generally via the aerobic plate count (APC). The APC method allows for any

aerobic bacteria colony to develop on a non-selective medium such as tryptic soy agar

(TSA) (*103*). Meat surfaces are swabbed or aseptically excised, then pulverized in a

sterile diluent. Serial dilutions are carried out from the sample to reach a dilution that

will yield a countable number of colony forming units (CFU) typically 25-250 CFU

(*104*). A small aliquot from the dilutions are then plated onto the medium and are

incubated to allow for growth. Incubation temperatures and durations are chosen based

on the type of organism being quantified. Psychrotrophic bacteria will be incubated at

7℃ for 10 days (*105*) and mesophilic bacteria will be incubated at 35-37℃ 24-48 hours.

More recently APC Petrifilms ® (3M Food Safety, St. Paul, MN) have been used to

quantify APC and are considered an equivalent medium for AOAC antimicrobial

efficacy methods (*106*). Petrifilms are a dry, ready to use plating medium consisting of a

rehydratable film and a clear overlay film. It is generally accepted that meat products

are spoiled when APC reaches 10^7 CFU/cm^2 which can be reached in as little as 3-5

days depending on the storage conditions and the initial load of organisms (*2*).

I.VII Interactions Between Mechanisms of Spoilage

     I.VII.I Myoglobin and Lipid Oxidation. There is a strong evidence of a link

between lipid oxidation and myoglobin oxidation (*107-110*). The formation of lipid

radicals has been shown to increase the rate of oxidation of myoglobin from the oxy

state to the met state (*108*). Likewise the accumulation of metmyoglobin (Fe^{3+}) can

increase the rate of lipid oxidation as the oxidized heme iron will catalyze the free

radical formation of lipids (*109*). In a study comparing beef, pork, and poultry the

concentration of unsaturated fatty acids was shown to explain less of the differences in

TBA values between species than heme iron content (*111*). This relationship is likely more noticeable in ground product due to the increased surface area and mechanical destruction of membranes which may increase the likelihood of lipid oxidation in fresh meats.

   I.VII.II Microbial Spoilage and Off Odors and Flavors. Spoilage microorganisms utilize the carbohydrates, proteins, and lipids available in meat as a food source. The byproducts of spoilage bacteria metabolism include compounds such as fatty acids, alcohols, and ketones that emit fruity and/ or sweet odors (*72*). Pseudomonads preferentially metabolize simple carbohydrates, followed by the metabolism of free amino acids (which are available due to previous proteolysis) leaving behind foul smelling compounds such as putrescine and cadaverine (*112*). Additionally, some spoilage organisms use lipolytic enzymes to break down complex lipids, accelerating oxidation and producing rancid odors. The relationship between microbial growth and off odors/ flavors has been reported in the literature. Crowley et al. (*16*) reported sensory panel scores indicating off odors on minced beef once *Pseudomonas* counts reached 88 $\log_{10}$ CFU/g. Putrescine concentration was shown to increase drastically once total aerobic viable counts rose above 8.0 $\log_{10}$ CFU/cm^2 (*112*).

   I.VII.III Lipid Oxidation and Off Odors and Flavors. The main outcome of lipid oxidation is rancidity, which produces an undesirable odor and flavor in meat. In cooked meat, the off flavors from rancidity are generally referred to as warmed over flavor (WOF). St. Angelo et al. (*82*), reported that TBARS values correlated highly (R=0.80) with sensory scores for WOF in fresh cooked beef samples. In a beef patty

study comparing TBARS values to trained sensory panel data, control patties with no antioxidant showed both higher TBARS values and off odor intensity scores than beef patties made with added antioxidants (*113*). While this study did not specifically indicate a causative relationship between lipid oxidation and off odor sensory scores it is certainly a plausible explanation. The volatile compounds causing WOF are mainly aldehydes and ketones that are developed in the meat from the breakdown of lipid hydroperoxide compounds formed during lipid oxidation (*82*). Many of these compounds have been identified using gas chromatography and mass spectrometry as drivers of rancid off odors in meat (*83*). Hexanal concentration was shown to be highly correlated with WOF sensory scores (R= 0.80) and TBARS values (R=0.92) on fresh beef samples (*82*). To better understand the amounts of these compounds needed for rancid off odors to be detected, a study was conducted in which pentanal, hexanal, hexanal, heptanal, octenal, and decadienal were added separately at known concentrations to individual raw beef samples (*114*). The samples were then evaluated by a trained panel which found the detection odor threshold for rancidity to be as low as 0.23 ppm for heptanal and as high as 7.87 ppm for hexanal (*114*).

I.VIII Ingredients Used to Enhance Meat Color, Reduce Lipid Oxidation, and Reduce Microbial Growth

I.VIII.I Sodium Lactate. Sodium lactate is the sodium salt of lactic acid. Commercially available preparations are manufactured from the microbial fermentation of sugars. Sodium lactate is generally recognized as safe (GRAS) by the FDA and permitted for use in meat and poultry products at a rate of up to 4.8% (*115*). Lactates

prepared with different cations such as potassium or calcium are also commercially available. These lactates are especially useful in low sodium products. Sodium lactate is a commonly used antimicrobial in ready to eat (RTE) meat products such as frankfurters as it is effective in controlling *Listeria monocytogenes* (*116-118*). The lactate contains the mostly ineffective anion form, though when used at a sufficiently high concentration, the available undissociated form (lactic acid) is able to move into the microorganism through the cell membrane and dissociate, disrupting the cell. Additionally, the salt content may reduce the water activity enough to inhibit microorganisms. Previous research has shown that injection with a lactate solution improved color and increased color stability over storage time in fresh beef (*12, 25*). The improvement in color stability is thought to be due to an increase in the pool of NADH generated by the conversion of lactate to pyruvate by the endogenous lactate dehydrogenase enzyme (*57*).

   I.VIII.II Reduced Nicotinamide Adenosine Dinucleotide. NADH is a cofactor and electron carrier/donor that is involved in many biochemical reactions of the body (*119*). This compound can be found in both the reduced and oxidized form in many cells of the body. NADH is available commercially as a lab grade chemical which is very costly and thus it has not been utilized as a food additive. NADH has been used experimentally as a treatment for Parkinson's disease (*120*). NADH is currently available in the U.S. as a dietary supplement marketed as ENADA (5 mg daily dose, Birkmayer Nutraceuticals, Las Vegas, NV).

I.VIII.III Sodium Erythorbate. Sodium erythorbate is the sodium salt of erythorbic acid, which is an isomer of ascorbic acid. Sodium erythorbate is commonly used as a cure accelerator in cured meat products as it provides necessary reducing potential for the conversion of nitrite to nitric oxide. In cured meats it can be used at levels up to 547 ppm and is required at the level of 547 ppm in injected or tumbled bacon products (*121*). Due to the high heat cooking method commonly used to prepare bacon there is a slight risk of nitrosamine formation if residual nitrite is present in bacon; the addition of sodium erythorbate ensures the complete reduction of the added nitrite to nitric oxide. Additionally erythorbic acid is allowed up to 400 ppm as a color stabilizer in ground beef, though it must be listed on the ingredient statement (*115*). Sodium erythorbate used in a cocktail with trisodium phosphate and MAP increased shelf life of beef steaks (1-2 days) and ground beef patties (1-3 days) compared to untreated control samples (*122*). Sodium erythorbate was reported to delay discoloration in ground beef patties as effectively as erythorbic acid (*123*). Sodium erythorbate and ascorbic acid have been shown to prevent bone discoloration in bone-in retail cuts (*124, 125*).

I.VIII.IV Sodium Carbonate and Sodium Bicarbonate. The sodium salts of carbonic acid have many uses in food production. Sodium carbonate and bicarbonate are approved for use in pH adjustment in eggs, brines, and fresh beef/pork cuts, as a denuding agent for tripe (potassium carbonate or calcium carbonate), or in combination with an acid to generate CO_2 in packaging (*115*). Carbonate or bicarbonates have been investigated as an injectable solution to address quality issues in fresh meat. Studies have evaluated the efficacy of postmortem sodium bicarbonate injection at improving

the quality of PSE pork (*126, 127*). Kaufman et al. (*126*) reported that early postmortem

injection with sodium bicarbonate (0.2-0.4 M) can reduce drip loss in PSE pork without

negative flavor implications. Wynveen et al. (*127*) reported that postmortem injection

(18 min) of PSE pork with sodium bicarbonate (0.3 M) was effective in reducing

postmortem pH decline and improved pork quality. Sodium bicarbonate has been

investigated as an alkaline component of marinades and potentially a replacement for

sodium phosphates in pork and poultry (*128-130*). Sindelar et al. (*131, 132*) conducted

research on the use of sodium bicarbonate solutions in fresh meats to help overcome the

off flavors associated with sow taint in pork loins. These studies found that sow loins

injected with 0.35 M sodium bicarbonate and 0.5% sodium tripolyphosphate at a 15%

pump had consumer sensory values similar to non-tainted commodity loins (*132*).

  I.VIII.V Sodium Metasilicate. Sodium metasilicate, pentahydrate (NaMS) is a

GRAS substance allowed for use as a direct food additive (*133*). SMS is very alkaline

(pH 12.5 in 1% solution) and is soluble in water up to 80 g/ 100 g water at 35℃ . NaMS

has long been thought to dissolve as readily as sodium chloride (*134*). It can be used at

the rate of 2% on beef primals, subprimals, and trimmings as an antimicrobial agent

(*115*). NaMS has shown comparable effectiveness compared to lactic acid and trisodium

phosphate in controlling *Escherichia coli* O157:H7 in vitro (*135*). When used as an

antimicrobial, NaMS is considered a processing aid and is not required to be labeled on

meat and poultry products. NaMS was evaluated in ground beef patties to determine the

effects on quality over shelf life (*136*). Beef patties made from NaMS (4 %) treated

trimmings had lower TBARS values, off odors, and off flavors, as well as improved

color scores compared to non-treated control and other antimicrobials over 7 days of storage (*136*) .

   I.VIII.VI Lactic Acid. Lactic acid is a commonly used antimicrobial on meat products. Lactic acid spray is used for decontamination of hot carcasses during slaughter as it is effective in reducing *E. coli* O157:H7 (*98-100*). Lactic acid has also been shown to be effective at reducing numbers of pathogenic bacteria and APC on beef trimmings (*137-139*). Lactic acid is approved for use as an antimicrobial up to 5% solution on beef carcasses, sub primals, and trimmings and does not need to be labeled (*115*). Its mode of action is disruption of the microbial cell by diffusion across the cell membrane and dissociating inside the cell (*101*). Ground beef from trimmings treated with lactic acid (2 %) had darker color than control ground beef and no off odor development over 42 day refrigerated storage in chubs (*137*).

   I.VIII.VII Combination of Citric Acid, Hydrochloric Acid, and Water. This combination of acids is currently approved for the use as an antimicrobial on beef carcasses, parts, or trim at a pH of 0.5-2.0, regardless of the concentration used as long as the pH is within the allowable range (*115*). This antimicrobial may be applied as a spray or dip with a minimum contact time of 2 to 5 seconds. The use of the antimicrobial is considered a processing aid and is not required to be labeled (*115*). Kalchayanand et al. (*140*) reported a reduction in APC of 1.8 $\log_{10}$ CFU/cm^2 on beef flank (*Cutaneous trunci*) that were inoculated (3.0 $\log_{10}$ CFU/cm^2 of nine strain *E. coli* cocktail) pre-rigor and spray treated with CL (2.0%, pH 1.8).

I.VIII.VIII Cetylpyridinium Chloride (CPC). CPC is a quaternary ammonia compound that is used commercially in many antiseptic products. CPC is currently only approved for use as an antimicrobial on raw poultry carcasses or parts (*115*). Despite not being approved for use on beef, CPC has been shown to effectively reduce microbial loads on fresh beef. Lim and Mustapha (*141*) found 0.5% CPC to significantly reduce counts of *E. coli* O157:H7, *L. monocytogenes*, and *Staphylococcus aureus* on fresh beef over 14 days of storage. CPC used at 0.5% was also shown to effectively reduce *L. monocytogenes* and *S. aureus* on fresh beef over 8 days of storage. CPC (0.5% solution) reduced APC counts by 0.6 $\log_{10}$ CFU/g on fresh beef trimmings (*142*). Ground beef patties made from CPC (0.5% solution) treated trimmings where shown to not be impacted with respect to color attributes or TBARS over 7 days of retail storage; sensory off odors were lower on day 7 for CPC treated patties than for control (*14*).

I.VIII.IX Buffered Vinegar. Sodium diacetate, a blend of acetic acid and sodium acetate (the sodium salt of acetic acid), is a commonly used antimicrobial for RTE meats and poultry (*116, 143, 144*). Sodium diacetate has been shown to inhibit aerobic microbe growth in ground beef stored at 5℃ for 8 days (*143*). In an effort to develop more "clean label" or "natural" antimicrobials buffered vinegar (essentially sodium diacetate) was developed. Buffered vinegar is prepared by first neutralizing the acetic acid in vinegar with a base salt, generating acetate and then mixing the neutralized vinegar with regular vinegar (pH ~ 2.4). The result is a blend of acetic acid and acetate that has slightly more acetate and thus a higher pH than acetic acid (*145*). Additionally the buffered vinegar product does not impart the same odor and flavor that vinegar

would. Many commercially available preparations of buffered vinegar are made with cultured sugars (*145*). These products are permitted for use in meat products up to 4.8% of the formulation and are labeled as their common names, such as "cultured cane sugar" and "vinegar" (*115*).

I.IX Literature Summary

The shelf life of fresh meat is influenced by both intrinsic and extrinsic factors. Many intrinsic factors are difficult to control in meat and therefore extrinsic factors like storage temperature, packaging atmosphere and duration of storage must be controlled to maximize shelf life. Shelf life is characterized by meat color, microbial growth, and the development of off odors/flavors. Meat color is one of the most important factors in consumer purchasing decisions of meat. Meat color can be influenced by genetics, diet, and the animal's pre harvest environment. Meat color is determined by the abundance of myoglobin and the redox state of its heme iron. Myoglobin is found in the deoxygenated state (dark purple color) when no oxygen in present. In the presence of oxygen, myoglobin will become oxygenated (bright red color in beef), forming oxymyoglobin. Oxidative stress, such as low partial pressure of O_2, results in the formation of metmyoglobin (MMb) formation (brown color) and must be reduced by metmyoglobin reducing ability (MRA) to become oxygenated again. MRA is a function of the availability of NADH in postmortem muscle, which has been shown to reduce metmyoglobin both enzymatically and non-enzymatically. Meat color and color stability have been shown to vary between muscles due to the inherent MRA and oxygen consumption rates. Oxygen consumption rate is the rate at which muscle metabolizes

oxygen likely by mitochondrial activity. Chilling rate and post mortem pH affect meat color and color stability. Meat with a higher than normal pH (>5.8) will exhibit a darker color and resist the development of MMb. Lactate dehydrogenase (LDH) is an enzyme in muscle that generates NADH via the conversion of L-lactate to pyruvate. Lactate enhancement of fresh meat has been shown to increase the color shelf life during retail display. Packaging systems like vacuum packaging, modified atmosphere packaging (MAP) and high oxygen MAP can extend the color shelf life of fresh meat by reducing oxidative stress. Meat flavor is generally thought of as the desirable flavors in cooked meat. These flavors are developed during cooking from the Maillard reaction and lipid thermal degradation. There are hundreds of volatile compounds that contribute the meat flavor. Meat flavor can be dominated by off flavors caused by oxidation and microbial growth. Lipid oxidation is caused by the presence of free radicals which can be generated from oxygen, heat, or light. Once lipid oxidation is initiated, it is rapidly propagated, until termination occurs. Lipid radicals are capable of oxidizing other lipids to form lipid hydroperoxides which are the first stable products of lipid oxidation. Lipid oxidation products are mainly aldehydes and ketones that contribute to the oxidized flavor in meat. Microbial spoilage of aerobically stored fresh meat is predominately from aerobic organisms such as *Pseudomonas* and *Acinetobacter*. If spoilage organisms are allowed to grow unchecked, they will manifest in slime formation and off odor development. These mechanisms of spoilage are interrelated as is the case where an increase in lipid oxidation will promote the oxidation of myoglobin. Microbial spoilage and lipid oxidation can lead to the development of off odors/flavors. Microbial growth

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can increase the pH of the muscle which in turn influences the color and color stability of the meat. These relationships make it necessary to address shelf life in fresh meat holistically rather than focusing on only one mechanism. Many ingredients have been used to extend the shelf life of fresh meat, in most cases applying the intervention at the onset of storage. These same ingredients may be useful in the extension of shelf life by applying them at or near the end of shelf life.

CHAPTER II

INVESTIGATION OF MECHANISTIC INTERRELATIONSHIPS CAUSING

SPOILAGE OF AEROBICALLY STORED BEEF TRIMMINGS

II.I Overview

Shelf life for fresh beef trimmings is often determined qualitatively based on color, lipid oxidation, microbial growth, and off odors and flavors. This study was conducted to determine the number of storage days needed to reach the end of shelf life and to understand the interrelationships of spoilage mechanisms. Beef bottom round flats (*Biceps femoris*; BF) and chuck clod hearts (*Triceps brachii*; TB) were sliced to simulate trimmings. Trimmings were stored aerobically, at 5℃ under fluorescent lighting for 12 days. Objective color, pH, microbial plate counts, and volatile compounds were measured on days 0, 3, 6, 9, and 12. L* (lightness) values decreased from day 0 (40.74) to day 12 (30.63). Saturation index (SI) values decreased ($P < 0.05$) from day 0 (27.09) to day 12 (11.92). Percent metmyoglobin (MMb) was lowest on day 0 (19.98%) and highest on day 6 (38.75%) and were only similar ($P > 0.05$) on days 9 and 12. a* (redness) values for both muscles decreased ($P < 0.05$) from day 0 (BF 20.84; TB 20.75) to day 12 (BF 8.83; TB 6.93). pH increased ($P < 0.05$) for both TB and BF from day 0 (BF 5.51; TB 5.63) to day 12 (BF 6.62; TB 6.79). Relative metmyoglobin reducing ability declined over storage time though values on days 0 (56.99 %) and 3 (64.57%) were higher ($P < 0.05$) than on days 6 (40.12%) and 9 (29.07%). Of the headspace volatiles, only total alcohols increased significantly ($P < 0.05$) from 57,791 on day 0 to 265,152 on day 12. Aerobic mesophile (AM) and psychrotroph (AP) counts

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both increased ($P < 0.05$) from day 0 to day 12, reaching 7.0 $\log_{10}$ CFU/cm^2 by day 9.

The data suggest that 6 storage days indicates the end of shelf life for color and 9 storage

days indicates the end of shelf life for microbial and organoleptic spoilage

II.II Introduction

Shelf life of beef trimmings can generally be defined as the length of storage

time before the trimmings are no longer suitable for their intended use. Shelf life is a

quality concern more than a safety issue, and can be characterized by color, lipid

oxidation, microbial growth, and off odors or flavors. In many cases shelf life is

evaluated on a qualitative basis rather than a quantitative basis. Large beef processors

likely monitor shelf life quantitatively although these data are not published and are used

internally to determine the shelf life guarantee for a particular product. For example, a

processor may guarantee beef trimmings to be suitable for use for up to 5 days. In some

cases, transportation may take 3 days giving a further processor only 2 days of the

guaranteed shelf life to use the product. An opportunity exists to extend the shelf life of

fresh beef trimmings by addressing quality changes in the areas of color, lipid oxidation,

microbial growth, and off odors or flavors.

The length of shelf life for beef trimmings is influenced by both intrinsic and

extrinsic factors. Intrinsic factors such as proximate composition and pH (*1-3*) can

influence shelf life and may be difficult to control in beef trimmings, especially in large

lots of comingled trimmings coming from different animals. Thus controlling extrinsic

factors such as packaging atmosphere (*4-6*) and storage temperature (*7-9*) are more

easily controlled and are crucial in maintaining consistent shelf life. Due to the variable

nature of the intrinsic and extrinsic factors influencing the shelf life of beef trimmings, one cannot accurately predict the length of shelf life for all beef trimmings based on data from one production system.

This study is the first phase of a larger study aimed at extending the shelf life of beef trimmings. In order to appropriately apply interventions to extend shelf life, determining the end of shelf life for beef trimmings stored in a model system was conducted. The objectives of this study were to: (1) determine the number of storage days at which aerobically-stored beef trimmings reach the end of shelf life, and (2) understand the mechanisms and interrelationships that cause spoilage in aerobically stored fresh beef trimmings.

II.III Materials and Methods

II.III.I Sample Collection and Preparation. Vacuum-packaged USDA Select beef bottom round flats (IMPS 171B, *Biceps femoris,* BF) and USDA Select beef chuck clod hearts (IMPS 114E, *Triceps brachii,* TB) were purchased from a local processor and cut into 1.27 cm slices using a commercial gravity feed slicer (Model SE 12, Bizerba, Piscataway, NJ), to simulate trimmings. Trimmings were stored aerobically at $5 \pm 2°C$ on linear low density polyethylene (LLDPE) lined plastic trays. Trays were covered with 1 mil LLDPE sheeting (Oxygen Transmission Rate: 5000 $cc/m^2/24$ h at 25°C and 0% R.H.) and exposed to cool white fluorescent light (40 watt CW Plus, Phillips Lighting Co, Somerset, NJ) at an intensity of 150-350 lux. Samples for analysis were collected on days 0, 3, 6, 9, and 12 of aerobic storage. On sampling days color was measured in triplicate and samples were frozen (-80°C) for chemical analysis (proximate

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on day 0 only, pH on all sampling days). Thirty g samples were collected for gas

chromatography mass spectrometry analysis. Five 10 cm^2 samples were aseptically

excised and used for microbial analysis.

II.III.II Proximate Composition and pH. Previously frozen samples were

thawed, trimmed of visible fat and connective tissue and frozen in liquid nitrogen.

Samples were then powdered using a waring blender (Model 33BL79, Waring

Commercial, New Hartford, CT). Powdered samples were used to determine proximate

composition (moisture [AOAC 985.14] and fat [AOAC 985.15] using CEM Smart

System (CEM Corp., Matthews, NC) and protein [AOAC 992.15] using a nitrogen

analyzer (F528, Leco Corp., St. Joseph, MI)). Ten grams of powdered sample was

blended with 90 mL of distilled, deionized water for pH determination using a glass

probe (VWR Symphony Red Tip Reference Probe, VWR International Radnor, PA and

benchtop pH meter (VWR Symphony 810, VWR International).

II.III.IV Objective Color. Samples were evaluated for L*(lightness), a* (red to

green), and b* (yellow to blue) color scores as well as spectral reflectance using a

HunterLab Miniscan XE plus (3.18 cm aperture and 10° standard observer; Hunter

Associates Laboratory, Inc., Reston, VA). The spectrophotometer was standardized

using white and black standard tiles. Illuminant D 65 was used for L*, a*, b* color

values and illuminant A was used for spectral reflectance. Additionally a* and b* values

were used to calculate saturation index (saturation index $= \sqrt{a^{*2} + b^{*2}}$) and hue angle

(hue angle $= \tan^{-1}(b^*/a^*)$) which is reported as degrees. Spectral reflectance data (474

nm, 525 nm, 572 nm, 610 nm, and 700 nm) was used to calculate percentage of

myoglobin states (*62, 64*).

      II.III.V Metmyoglobin Reducing Ability Determination. In order to determine

both inherent metmyoglobin reducing ability (MRA) and potential MRA (given

exogenous reduced nicotinamide adenine dinucleotide; NADH) the nitric oxide

metmyoglobin reducing ability (NORA) method was used (*62*) as well as a new

modified NORA method utilizing added NADH. The first experiment evaluated the

effect of meat particle size (chopped or intact), method (NORA or modified NORA), and

storage day on MRA values. For this experiment, 3 x 3 cm^2 squares were cut from

samples on storage days 1 and 7, and the remaining samples were chopped to roughly 5

mm and a 50 g sub sample was taken. One chopped and one intact sample on each day

were treated with NADH (Sigma Aldrich Co., St. Louis, MO) by pipetting 1ml of 70mM

NADH solution per 50 g of meat, on the light exposed surface, and allowed a 30 min

contact time. An additional 2 samples of both intact and chopped were left untreated as

the control samples. Samples were submerged (light exposed surface down) in 0.3%

solution of sodium nitrite (NaNO$_2$, Mallinckrodt, Inc., Paris, KY) for 20 min at room

temperature (21℃). Samples were then blotted dry and vacuum-packaged. Samples

were scanned with HunterLab Miniscan XE plus (Hunter Associates Laboratory) to

determine initial % metmyoglobin (MMb) calculated from reflectance data (*62, 64*) and

then incubated at 30°C for 120 min. Post reduction % MMb was determined as

previously stated. Data were reported as % initial metmyoglobin formation (IMF), %

post reduction metmyoglobin formation (PRMF), absolute MRA (AMRA), and relative

MRA (RMRA). Data reported as IMF are the percentage of myoglobin in the met state immediately following oxidation in 0.3% $NaNO_2$. Data reported as PRMF are the percentage of myoglobin in the metmyoglobin state immediately following 120 min anaerobic incubation at 30℃. Data reported as AMRA are the absolute reduction in MMb percentage during incubation and is calculated as IMF-PRMF. Data reported as RMRA are the relative percent of MMb reduced during incubation and is calculated as ((IMF-PRMF)/IMF) X 100.

Experiment 2 evaluated the effects of method (NORA or modified NORA), muscle, and storage day. Treatment with NADH and NORA sampling was performed as previously described. Muscles used were USDA select bottom round flats and USDA select clod hearts (BF and TB), and all samples were left intact. Both BF and TB were used to evaluate the two methods of measuring MRA on muscles with differing amounts of inherent MRA. McKenna et al. (*38*) found BF to have less MRA (RMRA determined by NORA method) than TB over 5 days of storage. Samples were measured on storage days 0, 3, 6, and 9 to evaluate the two methods on samples with increasing MMb %.

II.III.VI Gas Chromatography/Mass Spectrometry. Previously frozen samples (30 g) were placed into glass jars (486 mL) with a 1.6 mm-thick Teflon piece under the metal lid and then placed in a water bath at 70°C for 45 min. The jars were then removed from the water bath and set at room temperature (21℃), where sample headspace were collected with a solid-phase micro-extraction (SPME) Portable Field Sampler (Supelco 504831, 75 μm Carboxen/ polydimethylsiloxane, Sigma-Aldrich Co.). The headspace above each meat sample in the glass jar was collected for 2 hr on the

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SPME. Upon completion of collection, SPME were injected in the GC injection port, where the sample was desorbed at 280°C for 3 min. The sample was then loaded onto the multi-dimensional gas chromatograph into the first column (30 m x 0.53 mm ID/ BPX5 [5% phenyl polysilphenylene-siloxane] x 0.5 μm, SGE Analytical Sciences, Austin, TX) then to the second column (30 m x 0.53 mm ID [BP20- polyethylene glycol] x 0.50 μm, SGE Analytical Sciences). The temperature started at 40°C and increased at a rate of 7° C /min until reaching 260°C. The gas chromatography column was then split at a three-way valve with one column going to the mass spectrometer (Agilent Technologies 5975 series MSD, Santa Clara, CA) and one column going to each of the two sniff ports, which were heated to a temperature of 115° C, and fitted with glass nose pieces. The sniff ports and software for determining flavor and aroma are a part of the AromaTrax program (MicroAnalytics-Aromatrax, Round Rock, TX). A 3 point external standard curve was run using known concentrations of 1, 3 dichlorobenzene. This standard was used to estimate concentrations (ppm) from total ion count peak area data. Data from GC/MS was used to identify and quantify volatiles associated with spoilage and rancidity. The estimated concentrations were compared to published odor thresholds of volatile compounds associated with off flavors in meat.

II.III.VII Microbiological Analysis. Aseptically excised samples (five 10 cm^2 disks) were transferred to stomacher bags containing 100 mL of 0.1% (w/v) peptone water (PW; Becton, Dickinson and Co., Sparks, MD) and hand pummeled for 1 min. One mL of sample was aseptically transferred into a sterile tube containing 9 mL 0.1% (w/v) PW (Becton, Dickinson and Co.) or deMan, Rogosa, and Sharpe broth (MRS;

Becton, Dickinson and Co.). Serial dilutions were prepared and aseptically plated onto

three sets of Petrifilms (3M® Microbiology, St. Paul, MN). One set of aerobic count

(AC) films were incubated for 48 hours at 35°C before enumeration to quantify aerobic

mesophiles (AM). One set of AC petrifilms were incubated for 7 days at 7°C to quantify

aerobic psychrotrophs (AP). From the MRS broth, which is best suited for the recovery

and growth of lactic acid bacteria, a set of AC Petrifilms were plated and incubated for 7

days at 7°C to select for aerobic psychrotophic lactic acid bacteria (APLAB).

     II.III.VIII Experimental Design. The overall experiment was designed as a 2

(muscle) by 5 (storage day) factorial and was replicated 3 times. MRA experiment 1

was a 2 (method) by 2 (particle size) by 2 (storage day) factorial and MRA experiment 2

(muscle) by 2 (method) by 4 (storage day) factorial, both replicated 3 times. Data were

analyzed by ANOVA using the SAS v9.3 (SAS Institute, Inc., Cary, NC) GLM

procedure with $\alpha = 0.05$. Fixed effects for color attributes, pH, volatile compounds, and

microbial growth were muscle and days of storage. In MRA experiment 1, fixed effects

were method, particle size, and storage day. In MRA experiment 2, fixed effects were

method, muscle, and storage day. Least squares means were calculated for significant

main effects and interactions, then significant differences were determined using

Fisher's protected LSD. Pearson's chi squared test was used to determine correlations

between measured parameters with an alpha of 0.05.

II.IV Results and Discussion

   II.IV.I Proximate Composition and pH. Moisture, fat, and protein proximate

analysis were conducted on day 0 samples only. TB had significantly higher (P < 0.001)

moisture content (73.48%) than BF (70.85%). Conversely BF had significantly higher

(P < 0.001) fat (6.05%) and protein (18.90%) content than did TB (3.76%, 17.87%).

Von Seggern et al. (*146*), reported higher moisture content for TB (73.23%) than BF

(71.61%) as well as a lower fat content for TB (5.65%) than for BF (6.86%). Their

study profiled 39 beef muscles from carcasses spanning two quality grades, five yield

grades, and carcass weights ranging from 250- 491 kg (*146*). There was a significant

muscle by storage day interaction (P = 0.0001) for pH (**Table 2-1**), although on all

storage days TB possessed a higher pH than BF. The pH of TB samples increased from

5.63 on storage day 0 to 6.79 on storage day 12, while BF increased from 5.51 on

storage day 0 to 6.62 on storage day 12. The greatest numerical pH difference between

TB and BF was observed on storage day 6, 5.96 and 5.63 respectively. Higher pH

values for TB compared to BF have been reported by Von Seggern et al. (*146*) and

McKenna et al. (*38, 146*). Behrends (*37*) reported an increase in pH of beef samples

stored aerobically from 5.45 (day 1) to 5.50 (day 7) (*37*). Bala et al. (*147*) observed an

increase in pH from 5.5 to 6.6 for beef short loin samples, inoculated with 2.2 $\log_{10}$

CFU/cm^2 *Pseudomonas fragi*, over a 20 day storage period .

Table 2-1. Least Squares Means for Storage Day by Muscle Interaction of pH of Aerobically Stored Beef Trimmings.

Muscle	Day 0	Day 3	Day 6	Day 9	Day 12	SEM[1]
Biceps Femoris	5.51[dy]	5.53[cdy]	5.63[cy]	6.21[by]	6.62[ay]	0.025
Triceps Brachii	5.63[dx]	5.65[dx]	5.96[cx]	6.44[bx]	6.79[ax]	

[abcd] LSMeans within a row with different superscripts are significantly different (P < 0.05)

[xy] LSMeans within a column with different superscripts are significantly different (P < 0.05)

[1]-Standard error of the mean

While the samples were inoculated with aerobic spoilage bacteria, the initial load was not higher than what would be expected on fresh beef.

   II.IV.II Objective Color. Least squares means for significant main effects of color attributes can be seen in **Table 2-2**. L* or lightness values, differed (P < 0.05) between muscles as BF exhibited higher L* values (37.01) than did TB (35.30). Von Seggern et al. (*146*), observed the same relationship for L* value between BF (41.38) and TB (39.47). However, their study only measured objective color on day 0 and not over a longer storage period which could explain the generally higher L* values reported (*146*). L* values decreased (P < 0.05) from 40.74 on day 0 to 30.64 on day 12. b* values were lower for TB (12.32) than for BF (13.61) indicating that BF samples had a more yellow tint to their color. b* values decreased from day 0 (17.33) to day 12 (8.87). Saturation index (SI) was higher for BF (19.45) than for TB (18.19) across all storage days. The SI values decreased from 27.09 on storage day 0 to 11.92 on storage day 12. Percent MMb was highest on day 6 (38.75%), increasing from 19.98% on day 0 and decreasing to 30.77% on day 12. Percent oxymyoglobin (OMb) was highest on day 0 (80.01%) and lowest on day 6 (59.94%). The apparent auto reduction of the myoglobin pigment after day 6 could be attributed to microbial growth or increased pH (*67, 148*). There was a significant muscle by storage day interaction for a*, hue angle (HA), and percent deoxymyoglobin (DMb) (**Table 2-3**). a* values for both BF and TB decreased (P < 0.05) over 12 storage days. a* values have been observed to decrease over storage time in numerous aerobic storage studies (*25, 37-39, 149*). On storage day 12 BF had a

Table 2-2. Least Squares Means for Significant Main Effects Muscle and Storage Day of Color Measurements of Aerobically Stored Beef Trimmings.

	L*[1]	b*[2]	Saturation Index[3]	% MMb[4]	% OMb[5]
Muscle					
Biceps Femoris	37.01[a]	13.61[a]	19.45[a]	--	--
Triceps Brachii	35.30[b]	12.32[b]	18.19[b]	--	--
SEM[6]	0.269	0.142	0.195	--	--
Storage Day					
Day 0	40.74[a]	17.33[a]	27.09[a]	19.98[d]	80.01[a]
Day 3	41.03[a]	14.45[b]	21.03[b]	35.32[b]	63.30[c]
Day 6	36.26[b]	13.18[c]	17.65[c]	38.75[a]	59.94[d]
Day 9	32.14[c]	10.98[d]	16.41[d]	31.48[c]	68.24[b]
Day 12	30.63[c]	8.87[e]	11.92[e]	30.77[c]	69.15[b]
SEM	0.427	0.224	0.309	0.786	0.835

[abcde] LSMeans within a main effect and column with different superscripts are significantly different ($P < 0.05$)

[1]- L* is a color value (0-100) measuring lightness (100) to darkness (0)

[2]- b* is a color value representing yellow to blue; Positive values indicate yellowness

[3]- Saturation index $= \sqrt{a^{*2} + b^{*2}}$, measure of vividness

[4]- Percentage of myoglobin in the met state (Fe^{3+}; Empty 6th ligand)

[5]- Percentage of myoglobin in the oxy state (Fe^{2+}; O_2 bound to 6th ligand)

[6]- Standard Error of the Mean

Table 2-3. Least Squares Means for Storage Day by Muscle Interaction of Color Measurements of Aerobically Stored Beef Trimmings.

	Day 0	Day 3	Day 6	Day 9	Day 12	SEM[1]
***a*[2]**						
Biceps Femoris	20.84[a]	15.09[b]	11.25[c]	12.85[c]	8.83[dx]	0.388
Triceps Brachii	20.75[a]	15.31[b]	11.92[c]	11.44[c]	6.93[dy]	
***Hue Angle*[3]**						
Biceps Femoris	40.81[c]	44.83[b]	50.58[ax]	43.11[bc]	47.71[ab]	0.862
Triceps Brachii	39.09[c]	43.11[b]	47.13[ay]	41.38[bc]	50.58[a]	
***% Deoxymyoglobin*[4]**						
Biceps Femoris	0.00	0.67	0.66	0.32	0.09	0.338
Triceps Brachii	0.00[b]	2.10[a]	1.95[a]	0.25[b]	0.05[b]	

[abc] LSMeans within a row with different superscripts are significantly different (P < 0.05)

[xy] LSMeans for each parameter within a column with different superscripts are significantly different (P < 0.05)

[1]-Standard error of the mean

[2]- a* is a color space value representing red to green; Positive values indicate redness

[3]- Hue angle = $\tan^{-1}(b*/a*)$, expressed in degrees

[4]- Percentage of myoglobin in the deoxy state (Fe^{2+}; nothing bound to 6th ligand)

significantly higher a* value (8.83) than TB (6.93). McKenna et al. (*38*), observed

higher a* values over 5 days of aerobic storage for TB compared to BF, although not

significantly different. Hue Angle (HA) values increased from day 0 to day 6, decreased

on day 9 and rose again on day 12 for both muscles. Only on day 6 were HA values

greater for BF (50.58) than for TB (47.13).As HA values increase from 0, the color is

perceived as being less red. King et al. (*68*) and Sammel et al. (*39*) both found HA to

increase over 5 days of storage which is in agreement with the present study. Few

studies have stored meat samples aerobically beyond 5 days providing little evidence to

confirm or dispute the reduction in HA beyond day 6 of storage. Percentage DMb was

low for both muscles relative to the values of OMb and MMb. Due to the aerobic

storage conditions, it was expected that DMb % on the surface of the meat samples

would be close to zero. The need for myoglobin to transition through DMb during a

cycle of oxidation and subsequent reduction and oxygenation could explain the sporadic

occurrence of DMb observed in this study (*24*). As MMb % was the highest on day 6, as

well as significant reductions in L*, a*, b*, and SI values from storage day 3 to storage

day 6 were observed, 6 days of aerobic storage indicates the end of color shelf life for

fresh beef trimmings stored in this system.

 II.IV.III MRA Experiment 1. Particle size did not have a significant interaction

with method for initial MMb formation, post reduction MMb formation, or relative

MRA. The use of the modified NORA method resulted in increased relative MRA, and

decreased IMF and PRMF (**Table 2-4**). Absolute MRA however was higher in chopped

Table 2-4. Least Squares Means for Method Effect on Metmyoglobin Reducing Ability Measurements.

	RMRA[1]	IMF[2]	PRMF[3]
	(%)	(%)	(%)
Modified NORA	56.23[a]	50.51[b]	21.85[b]
NORA[4]	46.37[b]	59.51[a]	32.30[a]
SEM[5]	2.386	0.738	1.515

[ab] LSMeans within a row with different superscripts are significantly different ($P < 0.05$)

[1]- Relative percentage of metmyoglobin reduced during anaerobic incubation at 30℃, calculated as ((IMF-PRMF)/IMF) X 100

[2]- Initial metmyoglobin formation, Percentage of myoglobin in the met state following oxidation in 0.3% $NaNO_2$

[3]- Post reduction metmyoglobin formation, Percentage of myoglobin in the met state following 120 min anaerobic incubation at 30℃

[4]- Nitric Oxide Reducing Ability

samples when modified NORA was used (32.55%) compared to NORA (27.47%) while

intact samples using modified NORA had numerically lower (P > 0.05) AMRA

(24.77%) than did intact NORA samples (27.24%). This interaction can be explained by

the way AMRA is calculated. The IMF is not taken into account, thus samples with

lower IMF will inherently have lower AMRA. There was as significant storage day by

particle size interaction for all measured attributes. Both RMRA and AMRA were

higher for intact samples on storage day 1 but lower for intact samples on storage day 7.

Neither IMF nor PRMF was different between particle sizes on storage day 1, but both

were different between particle sizes on storage day 7. Chopped samples were chopped

on each sampling day; therefore on storage day 7, sample subsurface was exposed in

chopped samples. Subsurface was not exposed to the same oxygen concentration as the

surface and thus did not exhaust MRA. Mancini et al. (*69*), evaluated the effect of

sampling location (surface or subsurface) on MRA values, and found that on day 6 of

storage (O_2 permeable PVC overwrap, 2℃ , exposed to fluorescent lighting) all muscles

had significantly (P < 0.05) greater IMF values for surface than subsurface and less (P <

0.05) PRMF on the subsurface than surface. The lack of a method by particle size

interaction for IMF, PRMF, and RMRA suggests that chopping samples is not necessary

to determine the potential MRA of a meat sample using the modified NORA method.

 II.IV.IV MRA Experiment 2. The MRA values can be seen in **Table 2-5**.

Modified NORA method yielded lower IMF and PRMF values than NORA on all

storage days. The RMRA values were higher for modified NORA on all days, though

Table 2-5. Least Squares Means for Storage Day by Method Interaction of Four Metmyoglobin Reducing Ability Parameters.

		Day 0	Day 3	Day 6	Day 9	SEM[1]
Initial Metmyoglobin Formation[2]						
(IMF)	Modified NORA	47.08[by]	47.04[by]	43.27[cy]	49.81[ay]	0.597
	NORA	54.07[x]	53.40[x]	55.34[x]	55.82[x]	
Post Reduction Metmyoglobin Formation[3]						
(PRMF)	Modified NORA	20.12[bc]	16.61[cy]	25.71[by]	35.48[ay]	1.342
	NORA	23.88[b]	24.07[bx]	47.86[ax]	50.88[ax]	
Relative Metmyoglobin Reducing Ability[4]						
(RMRA)	Modified NORA	56.99[a]	64.57[a]	40.12[bx]	29.07[bx]	2.618
	NORA	55.47[a]	54.87[a]	13.52[by]	8.76[by]	

[abc] LSMeans within a row with different superscripts are significantly different ($P < 0.05$)

[xy] LSMeans for each parameter within a column with different superscripts are significantly different ($P < 0.05$)

[1]-Standard error of the mean

[2]- Percentage of myoglobin in the met state immediately following oxidation in 0.3% $NaNO_2$

[3]- Percentage of myoglobin in the met state immediately following 120 min anaerobic incubation at 30°C

[4]- Relative percentage of metmyoglobin reduced during anaerobic incubation at 30°C, calculated as ((IMF-PRMF)/IMF) X 100

only significantly higher than NORA on days 6 (40.12 %, 13.52%) and 9 (29.07%, 8.76%). Sammel et al. (*39*), showed generally higher values for MMb reductase assay (uses added NADH) than NORA values on *Semimembranosus* samples stored over 5 days. King et al. (*68*), found a similar decline in RMRA and increase in IMF and PRMF values from day 0 to day 6 of storage on *Longissimus thoracis* samples. A significant muscle by storage day effect was observed for RMRA. Both muscles generally declined in RMRA from storage day 0 to storage day 9, though no differences were observed between days 0 and 3. Only on storage day 6 was RMRA higher for TB (32.61%) than BF. (21.03%). McKenna et al. (*38*), observed higher RMRA values on day 5 for TB (34.20 %) than for BF (29.60%), these values were not significantly different likely due to the large variation of the 19 muscles evaluated (17.70 % - 55.70 % on storage day 5). The differences in reducing ability between different muscles can be attributed to differences in OCR and the inherent ability of each muscle to replenish NADH over storage time (*38, 68, 150*). The higher values of MRA attributes observed when using the modified NORA method indicates that the meat samples possess the capacity to reduce MMb if sufficient reducing equivalents are available. These data indicate that meat with unsatisfactory color could potentially regain acceptable color if provided with the required reducing equivalents.

Simple Pearson correlation coefficients show that RMRA values determined via NORA are more highly correlated with MMb accumulation (r = -0.44, P < 0.001) than values determined via MNORA (r = -0.24, P = 0.0187). Additionally a * values were more highly correlated with RMRA from NORA (r = 0.71, P < 0.001) than from

MNORA (r = 0.45, P < 0.001). Sammel et al. (*39*), found similar correlations as NORA correlated better than MMb reductase assay for both MMb accumulation (r = -0.49, r = -0.09) and a* values (r = 0.40, r = 0.14). Methods that add NADH like modified NORA and MMb reductase assay do not correlate well with measures of inherent color stability because these methods are not measuring inherent reducing ability.

    II.IV.V Gas Chromatography-Mass Spectrometry. Volatile compounds were grouped into functional groups (acids, alcohols, aldehydes, alkanes, alkenes, ketones, furans, amides, and amines) to be analyzed. Due to the nature of reactivity, groups of compounds were seen as a better indication of what is present on a given storage day than individual compounds. Volatile compounds that have been used as an indication of spoilage or lipid oxidation including acetoin, dimethyl sulfide, dimethyl disulfide, pentanal, and butanal were identified in this study (*83, 151*). Volatile compound data are reported as area under the curve and can be seen in **Table 2-6.** Total alcohols increased from day 0 (57,791) to day 12 (265,152). TB had more total ketones (360,009) than did BF (163,089). While not significant, there was a large numerical difference between day 6 and day 9 for alcohols (57,833; 158,773), aldehydes (14,923; 38,325) alkenes (99,951; 126,607), and ketones (112,501; 213,262). Detectable odor thresholds for aldehydes in fresh beef have been established ranging from 0.23 ppm (heptanal) to 7.87 ppm (hexanal) (*114*). Using the external standard curve to estimate concentration (ppm) of total aldehydes in this study, day 6 was estimated at 0.096 ppm and day 9 was estimated at 0.34 ppm. Based on these estimations aldehydes were below any reported odor threshold on day 6 but in the range of detectable odor thresholds on day 9. The data

Table 2-6. Least Squares Means for Storage Day and Muscle of Volatile Groups of Aerobically Stored Beef Trimmings.

	Storage Day						Muscle			P-Values		
	Day 0	Day 3	Day 6	Day 9	Day 12	SEM	*B. Femoris*	*T. Brachii*	SEM	Day	Muscle	Day X Muscle
Total Acid	9,616[b]	284,309[a]	47,237[b]	62,393[b]	271,850[a]	56,566	101,776	168,386	35,776	0.0039	0.2029	0.0421
Total Alcohol	57,791[b]	82,515[b]	57,833[b]	158,773[ab]	265,152[a]	52,750	149,924	96,501	33,362	0.0474	0.2709	0.8953
Total Aldehyde	18,735	2,798	14,923	38,325	13,004	16,052	4,597	30,517	10,152	0.6282	0.0861	0.6084
Total Alkane	15,523[b]	76,847[a]	6,430[b]	8,395[b]	40,580[b]	11,685	41,163[a]	17,947[b]	7,390	0.0016	0.0380	0.0014
Total Alkene	172,483	10,3527	99,951	126,607	116,830	44,369	109,348	138,411	28,062	0.7835	0.4725	0.1404
Total Ketone	367,402	239,538	112,501	213,262	375,041	91,333	163,089[b]	360,009[a]	57,764	0.2480	0.0257	0.2582
Total Furan	0	0	0	2,039	0	912	0	816	577	0.4307	0.3293	0.4307
Total Amide	424[b]	4,174[ab]	0[b]	695[b]	32,470[a]	10,325	10,647	4,458	6,530	0.1596	0.5104	0.9099
Total Amine	0	0	0	0	738	330	295	0	209	0.4307	0.3293	0.4307

[ab] – LSMeans within a main effect and row with different superscripts are significantly different (P < 0.05)

suggests that day 9 of aerobic storage in this study indicates the end of organoleptic shelf life based on the accumulation of alcohols, aldehydes, and ketones by storage day 9. The storage day by muscle interaction was significant for both total acids (P = 0.0421) and total alkanes (P = 0.0014) (**Table 2-7**). Total acids were only greater (P < 0.05) on day 12 for TB (467,984) compared to BF (75,718). Alkanes were present in the lowest number for both muscles on day 6 of storage, though no significant differences between storage days were observed for TB.

II.IV.VI Microbial Growth. Both AM and AP were observed in greater numbers on TB (6.0 and 7.5 $\log_{10}$ CFU/ cm^2 respectively) than on BF (5.6 and 6.5 $\log_{10}$ CFU/ cm^2 respectively). **Figure 2-1** shows the growth of both AM and AP over 12 storage days. An increase for AM was observed from 3.4 $\log_{10}$ CFU/ cm^2 (day 0) to 7.9 $\log_{10}$ CFU/ cm^2 (day 12) while AP increased from 5.1 $\log_{10}$ CFU/ cm^2 (day 0) to 9.1 $\log_{10}$ CFU/ cm^2 (day 12). Both classes of microorganisms reached 7.0 $\log_{10}$ CFU/ cm^2 by storage day 9. No APLAB were detected above the limit of detection (1 CFU/ cm^2) on any storage day for either muscle. Growth of 7.0 $\log_{10}$ CFU/ cm^2 for APC is generally the threshold of microbial spoilage as APC growth beyond 7.0 $\log_{10}$ CFU/ cm^2 will likely result in pronounced off odors and flavors, growth beyond 8-8.5 $\log_{10}$ CFU/ cm^2 can manifest slime formation on the meat surface (*97*). Based on these data, 9 days of storage would indicate the end of shelf life in terms of microbial growth for fresh beef trimmings stored in this system.

II.IV.VII Pearson Correlation Coefficients. Simple Pearson correlation coefficients can be found in **Table 2-8.** Muscle pH was highly correlated with microbial

Table 2-7. Least Squares Means for Storage Day by Muscle Interaction of Total Acids and Total Alkanes of Aerobically Stored Beef Trimmings.

	Day 0	Day 3	Day 6	Day 9	Day 12	SEM[1]
Total Acid						
B. Femoris	$9,379^b$	$346,359^a$	$4,601^b$	$72,823^b$	$75,718^{by}$	$79,997$
T. Brachii	$9,854^b$	$222,258^b$	$89,873^b$	$51,963^b$	$467,984^{ax}$	
Total Alkane						
B. Femoris	$3,635^c$	$139,607^{ax}$	0^c	$7,820^{bc}$	$54,753^b$	$16,525$
T. Brachii	$27,410$	$14,088^y$	$12,861$	$8,971$	$26,406$	

^{abc} LSMeans within a row with different superscripts are significantly different ($P < 0.05$)
^{xy} LSMeans for each parameter within a column with different superscripts are significantly different ($P < 0.05$)
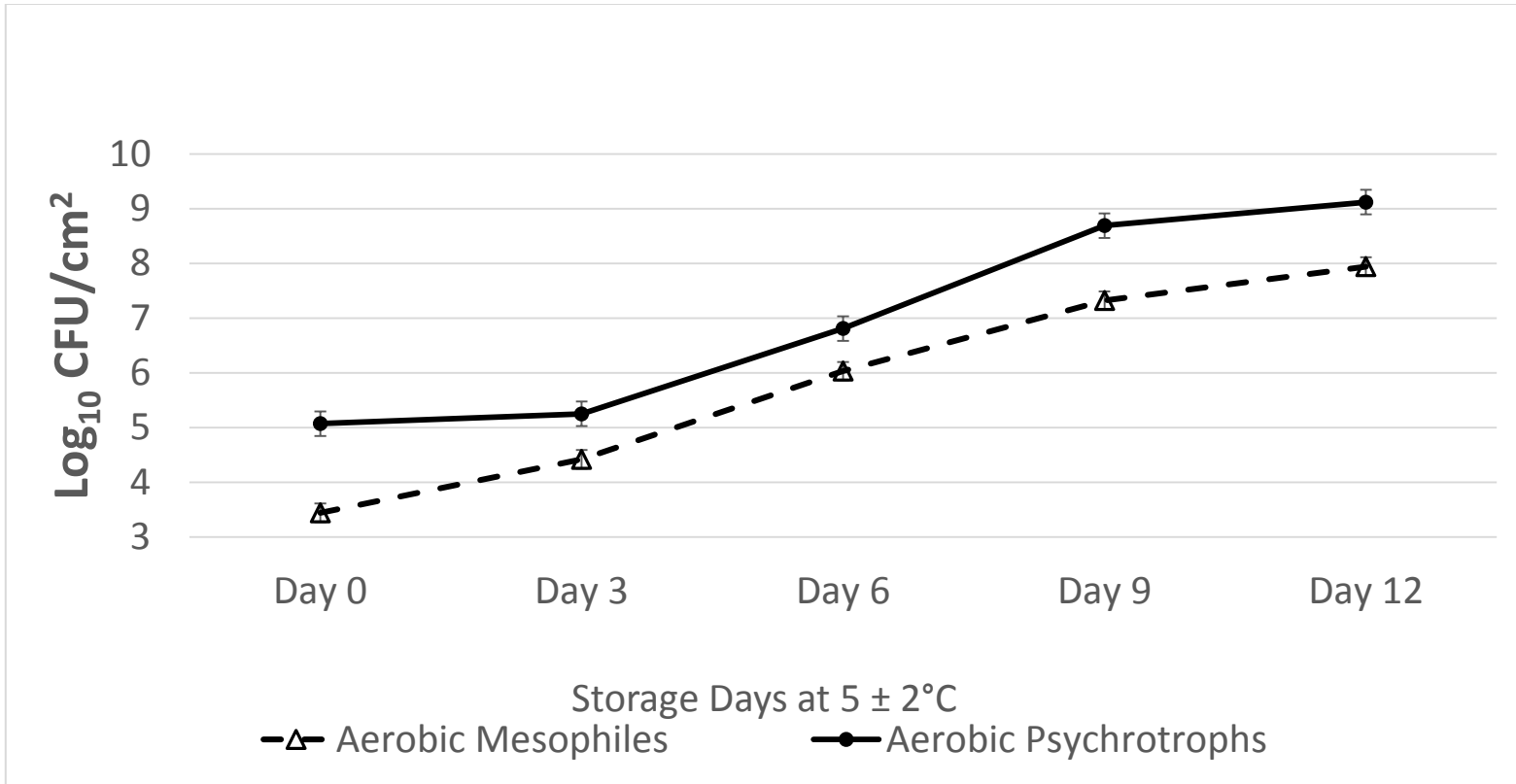
Figure 2-1. Least Squares Means of Growth for Aerobic Mesophile and Aerobic Psychrotroph Microorganisms Over 12

Storage Days on Fresh Beef Trimmings. Samples were stored aerobically at 5°C under fluorescent lighting (150-300 lux).

Error bars denote the standard error of the mean for each organism type (AM SEM = 0.170; AP SEM = 0.225).

Table 2-8. Simple Pearson Correlation Coefficients for Color Attributes, Microbial Growth, and Volatile Groups of Aerobically Stored Beef Trimmings.

	Aerobic Mesophiles	Aerobic Psychrotrophs	L*	a*	b*	Hue Angle	Saturation Index	% Met myoglobin	% Deoxymyoglobin	% Oxymyoglobin	Total Ketones	Total Alkanes	Total Alkenes	Total Aldehydes	Total Alcohols	Total Acids
pH	0.86¥	0.84¥	-0.83¥	-0.74¥	-0.86¥	0.29	-0.81¥	0.10	-0.25	-0.07	0.16	-0.01	-0.09	0.17	0.54†	0.25
Total Acids	0.14	0.17	-0.11	-0.35	-0.32	0.33	-0.35	0.18	-0.02	-0.17	0.12	0.05	0.33	0.03	0.29	
Total Alcohols	0.52†	0.58¥	-0.48†	-0.46*	-0.54†	0.14	-0.51†	0.13	-0.24	-0.09	0.50†	0.11	0.31	0.44*		
Total Aldehydes	0.15	0.22	-0.14	-0.07	-0.11	-0.02	-0.09	0.01	-0.16	0.01	0.31	-0.07	0.66¥			
Total Alkenes	-0.07	0.07	0.19	0.20	0.21	-0.07	0.21	-0.90¥	-0.30	0.12	0.56†	0.30				
Total Alkanes	-0.13	-0.13	0.32	0.29	0.12	0.09	0.06	0.07	-0.21	-0.04	0.23					
Total Ketones	0.01	0.15	-0.05	0.02	-0.08	-0.12	-0.02	-0.13	-0.11	0.13						
% Oxymyoglobin	-0.33	-0.27	0.08	0.60¥	0.33	-0.61¥	0.50†	-0.99¥	-0.53†							
% Deoxymyoglobin	-0.09	-0.17	0.04	-0.11	-0.07	0.05	-0.10	0.44*								
% Metmyoglobin	0.36	0.31	-0.09	-0.61¥	-0.35	0.64¥	-0.51†									
Saturation Index	-0.88¥	-0.76¥	0.81¥	0.98¥	0.96¥	-0.58¥										
Hue Angle	0.42*	0.29	-0.09	-0.73	-0.34											
b*	-0.86¥	-0.78¥	0.91¥	0.88¥												
a*	-0.84¥	-0.71¥	0.70¥													
L*	-0.74¥	-0.78¥														
Aerobic Psychrotrophs	0.88¥															

* -Correlation is significant (P < 0.05)

†- Correlation is very significant (P < 0.01)

¥- Correlation is extremely significant (P < 0.001)

growth and objective color attributes (L*, a*, b*). pH and microbial growth both

increased over storage period. The increase in pH can be attributed to the microbial

production of ammonia, producing a more favorable environment for microbial growth

(*147*). Muscle pH influences water holding capacity and ultimately objective color, as

observed with PSE and DFD meat. AM and AP were very highly correlated (P < 0.001)

with objective color attributes and SI. The utilization of oxygen at the meat surface by

aerobic microorganisms can influence the availability oxygen and initiate reducing

conditions (*148*). As objective color is in part a function myoglobin state, this

significant correlation is expected. Percent OMb and percent MMb were highly

correlated (P < 0.001) with a* (r = 0.60, r = -0.61) and HA (r = -0.61, r = 0.64), both

indicators of redness. Saturation Index (SI) was correlated with OMb (P = 0.0053, r =

0.50) and MMb (P = 0.0037, r = -0.51). Total alcohols were the only volatile group that

correlated significantly with other measured attributes. Alcohols were significantly

correlated (P < 0.001) with aerobic psychrotrophic bacteria (r = 0.58).

II.V Conclusions

The findings of this study suggest that 6 days of aerobic storage results in the end

of color shelf life, 9 days of storage results in the end of microbial and organoleptic shelf

for fresh beef trimmings stored in this system. There is evidence of interrelationships of

these mechanisms as seen in the correlation data. Additionally, the MRA data indicate

that meat with unsatisfactory color could potentially regain acceptable color if provided

with the required reducing equivalents. Based on the results of this study, selected

ingredients will be applied to improve color at 6 days of storage and reduce microbial

plate counts and off odors/flavors at 9 days of storage in this model system.

CHAPTER III

EFFICACY OF SELECTED INGREDIENTS AT EXTENDING THE SHELF LIFE OF

AEROBICALLY STORED BEEF TRIMMINGS

III.I Overview

This study was conducted to evaluate the efficacy of selected ingredients to

extend the shelf life of aerobically stored beef trimmings. USDA select beef bottom

round flats (*Biceps femoris;* BF) and beef chuck clod hearts (*Triceps brachii;* TB) were

sliced to simulate trimmings and stored aerobically at 5°C for 6 or 9 days. Samples were

treated with ingredients selected to extend shelf life by addressing color, microbial

spoilage, or off odor and flavor development. Samples treated with reduced

nicotinamide adenine dinucleotide (NADH) and sodium erythorbate (NaE) had greater

($P < 0.05$) a* (redness) values (12.76 and 11.47 respectively) and decreased ($P < 0.05$)

metmyoglobin (MMb) % (29.96 and 37.34 % respectively) compared to day 6 control

samples (a* 7.93; MMb 57.02%) measured 2 hr post treatment. Color measurements

taken 48 hr post treatment indicated that NaE treated TB samples retained the apparent

improvement in a* (9.48) and MMb % (41.17%) better than NADH treated TB samples

(a* 7.82; MMb 51.72%). Cetylpyridinium chloride (0.5%) treated beef trimmings had

lower ($P < 0.05$) aerobic mesophile plate counts (7.4 $\log_{10}$ CFU/cm^2) than day 9 control

samples (8.0 $\log_{10}$ CFU/cm^2). No significant differences were observed in volatile

compound groups between treatments however beef trimmings treated with sodium

bicarbonate had roughly 15 times less aldehydes present than day 9 control samples.

III.II Introduction

      Shelf life of fresh beef is a function of sustaining acceptable color, low microbial spoilage, and undetectable off odors and flavors including those from lipid oxidation. The length of shelf life is dependent on many intrinsic and extrinsic factors. Research has been conducted on extending the shelf life of fresh beef steaks (*6, 10-12*) and ground beef (*13-16*). The studies have focused on the application of interventions to increase color, microbiological, and organoleptic shelf life at the beginning of shelf life. While many of these findings have translated to practical applications for shelf life extension, little attention has been paid to the application of interventions after the onset of quality deterioration.

      This project is aimed at developing strategies to extend the shelf life of beef trimmings at or near the end of shelf life. Previously conducted research indicated that the end of shelf life for beef trimmings stored aerobically in a model system was 6 days for color and 9 days for microbial spoilage and off odor/flavor development. Thus the objectives of this study were to evaluate the efficacy of selected ingredients to extend the shelf life of beef trimmings stored for 9 or 6 days, by attacking each mechanism (color, microbial spoilage, off odors and flavors) individually.

III.III Materials and Methods

      III.III.I Chemicals. Sodium carbonate (Na_2CO_3; Anhydrous, ACS reagent grade >99.5%), sodium bicarbonate ($NaHCO_3$; Anhydrous, ACS reagent grade >99.5%), sodium metasilicate pentahydrate ($Na_2SiO_3 \cdot 5H_2O$; >95%) and reduced nicotinamide adenine dinucleotide ($C_{21}H_{27}N_7O_{14}P_2$; NADH; grade II disodium salt, >98%) were

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purchased from Sigma Aldrich (Milwaukee, WI). Sodium erythorbate ($C_6H_7NaO_6$; Zhenzhou Tuoyang Bioengineering CO., Henan Province, China) was purchased from a local food ingredient supplier. Sodium L-lactate ($C_3H_5NaO_3$; Purasal S, 60%), lactic acid ($C_3H_6O_3$; Purac FCC, 88%), and buffered vinegar (Verdad Powder N6) were procured from Corbion Purac (Purac Biochem, Gorinchem, Netherlands). Cetylpyridinium chloride ($C_{21}H_{38}ClN$; Cecure™) and a combination of hydrochloric acid (HCl), citric acid ($C_6H_8O_7$), and water (Citrilow™) were procured from Safe Foods Corp. (Little Rock, AR).

III.III.II Sample Collection and Preparation. Vacuum-packaged USDA Select beef bottom round flats (IMPS 171B, *Biceps femoris,* BF) and USDA Select beef chuck clod hearts (IMPS 114E, *Triceps brachii,* TB) were purchased from a local processor and cut into 1.27 cm slices using a commercial gravity feed slicer (Model SE 12, Bizerba, Piscataway, NJ), to simulate trimmings. Trimmings were stored aerobically at $5 \pm 2°C$ on linear low density polyethylene (LLDPE) lined plastic trays. Trays were covered with 1 mil LLDPE sheeting (Oxygen Transmission Rate: 5,000 $cc/m^2/24$ h at 25°C and 0% R.H.) and exposed to cool white fluorescent light (40 watt CW Plus, Phillips Lighting Co, Somerset, NJ) at an intensity of 150-350 lux. Samples treated to improve color were stored for 6 days prior to treatment and samples treated to improve microbial spoilage, off odors, and off flavors were stored for 9 days prior to treatment.

III.III.III Sample Treatment. Treatment solutions for off odors and flavors were distilled deionized water (DI), sodium bicarbonate (NaB), sodium carbonate (NaC), sodium metasilicate (NaMS), sodium metasilicate with sodium bicarbonate

(NaMS/NaB), and sodium metasilicate with sodium carbonate (NaMS/NaC). Treatment

solutions for microbial spoilage were distilled deionized water (DI), lactic acid (LA),

Citrilow ™ (CL, a combination of hydrochloric acid, citric acid, and water),

cetylpyridinium chloride (CPC), and buffered vinegar (BV). Treatment solutions for

color were distilled deionized water (DI), NADH, sodium erythorbate (NaE), sodium

lactate (NaL), NADH with sodium lactate (NADH/NaL), and sodium erythorbate with

sodium lactate (NaE/NaL). A day 0 control and an untreated day 9 (6 for color) were

also used for sample analysis.

Treatment solutions were prepared by dissolving dry or liquid concentrate into

distilled deionized water. If heat was needed to completely dissolve a compound, it was

heated and allowed to cool back to room temperature (21℃) before application to the

beef trim as a treatment. **Table 3-1** shows the treatment solutions, concentrations, pH,

and any preparation notes. Meat samples were treated with solutions (except NADH) by

submerging in treatment solution for 30 s with agitation. Immediately following

treatment, excess solution was removed by placing the sample in a manual, centrifugally

driven spinning basket and hand spun for 30 s. The NADH solution (0.1M) was applied

by pipetting 1ml/50 g to light exposed surface. Meat samples treated for off odors and

flavors were allowed to rest for 1 hr at 5℃ before a 30-40 g sample was collected and

frozen (-80℃) until analyzed for volatile compounds. Meat samples treated for

microbial spoilage were allowed to rest for 1 hr at 5℃ before 5 10 cm^2 disks were

Table 3-1. Treatment Solutions, Concentrations, pH and Preparation Notes

Treatment Solution	Mechanism	Concentration	pH (Mean $\pm$ S.D.)	Preparation Notes
Sodium Carbonate	Off Odors/ Flavors	1.125 M	11.83 $\pm$ 0.08	119.22 g / 1 L water, mixed at 25℃
Sodium Bicarbonate	Off Odors/ Flavors	1.425 M	8.06 $\pm$ 0.06	119.72 g / 1 L water, mixed at 35℃
Sodium Metasilicate	Off Odors/ Flavors	0.28 M	13.09 $\pm$ 0.17	60 g/ 1 L water, mixed at 25℃
Sodium Metasilicate/ Sodium Carbonate	Off Odors/ Flavors	0.28 M/ 1.125 M	12.80 $\pm$ 0.07	Dissolved sodium carbonate as described then add sodium metasilicate as described
Sodium Metasilicate/ Sodium Bicarbonate	Off Odors/ Flavors	0.28 M/ 1.425 M	9.56 $\pm$ 0.08	Dissolved sodium bicarbonate as described then add sodium metasilicate as described
Lactic Acid	Microbial Spoilage	5.00 %	2.03 $\pm$ 0.09	57 mL / 1 L water, mixed at 21℃checked concentration with sodium hydroxide titration kit
Citrilow [1]	Microbial Spoilage	5.00 %	1.05 $\pm$ 0.01	50 mL / 1 L water, mixed at 21℃, adjusted solution to pH target 1.05
Cetylpyridinium Chloride	Microbial Spoilage	0.50 %	4.22 $\pm$ 0.40	12.5 mL / 1 L water, mixed at 21℃ checked concentration with titration kit[3]
Buffered Vinegar	Microbial Spoilage	15.00 %	5.93 $\pm$ 0.08	150 g / 1 L water, mixed at 21℃
NADH [2]	Color	0.1 M	-	70.94 g/ 1 L water, mixed at 21℃, prepared immediately before use
Sodium Erythorbate	Color	0.1 M	7.03 $\pm$ 0.15	20 g/ 1 L water, mixed at 21℃
Sodium Lactate	Color	4.46 M	7.88 $\pm$ 0.16	Used stock 60% solution

[1]- Combination of hydrochloric acid, citric acid, and water
[2]- Reduced nicotinamide adenine dinucleotide
[3]- Titration kit supplied by Safe Foods Corporation (Little Rock, AR)

aseptically removed from the light exposed surface and transported to the microbiology lab for plating. Meat samples treated for color were allowed to rest for 2 hr at 5℃ before being evaluated for objective color, myoglobin states, and surface pH. Meat samples treated for color were evaluated for objective color, state of myoglobin, and surface pH again after 48 hr at 5℃.

III.III.IV Proximate Composition and pH. Previously frozen samples were thawed and homogenized in a food processor (Custom 14, Cuisinart Corp., East Windsor, NJ). Homogenized samples were used to determine proximate composition (moisture [AOAC 985.14] and fat [AOAC 985.15] using CEM Smart System (CEM Corp., Matthews, NC) and protein [AOAC 992.15] using a nitrogen analyzer (F528, Leco Corp., St. Joseph, MI)). Ten g of powdered sample was blended with 90 mL of distilled deionized water for pH determination using a glass probe (VWR Symphony Red Tip Reference Probe, VWR International Radnor, PA and benchtop pH meter (VWR Symphony 810, VWR International).

III.III.V Objective Color. Samples treated to improve color, were evaluated for L*(lightness), a* (red to green), and b* (yellow to blue) color scores as well as spectral reflectance using a Hunter lab Miniscan XE plus (3.18 cm aperture and 10° standard observer; Hunter Associates Laboratory, Inc., Reston, VA). The spectrophotometer was standardized using white and black standard tiles. Illuminant D_{65} was used for L*, a*, b* color values and illuminant A was used for spectral reflectance (*62*). Additionally a* and b* values were used to calculate saturation index (saturation index $=\sqrt{a^{*2} + b^{*2}}$) and hue angle (hue angle $= \tan^{-1}(b*/a*)$) which is reported as degrees. Spectral

reflectance data (474 nm, 525 nm, 572 nm, 610 nm, and 700 nm) was used to calculate percentage of myoglobin states (*62, 64*). Following both 2 and 48 hr post treatment objective color measurements, surface pH was determined using a flat surface probe (VWR Symphony Flat Surface pH Probe, VWR International) and benchtop pH meter (VWR Symphony 810, VWR International).

 III.III.VI Microbiological Analysis. Aseptically excised samples (five 10 cm^2 disks) removed from light-exposed surfaces of beef trimmings treated to reduce microbial counts, were transferred to stomacher bags containing 100 mL of 0.1% (w/v) peptone water (PW; Becton, Dickinson and Co., Sparks, MD) and hand pummeled for 1 min. One mL of sample was aseptically transferred into a sterile tube containing 9 mL 0.1% (w/v) PW (Becton, Dickinson and Co.). Serial dilutions were prepared and aseptically plated onto two sets of Petrifilms ® (3M® Microbiology, St. Paul, MN)(*103*). One set of aerobic count (AC) films were incubated for 48 hr at 35°C before enumeration to quantify aerobic mesophiles (AM). One set of AC Petrifilms were incubated for 7 days at 7°C to quantify aerobic psychrotrophs (AP)(*105*)

 III.III.VII Gas Chromatography/Mass Spectrometry. Previously frozen samples (30-40 g) treated to improve off odors and flavors, were placed into glass jars (486 mL) with a 1.6 mm-thick Teflon piece under the metal lid and then placed in a water bath at 70°C for 45 min. The jars were then removed from the water bath and set at room temperature (21°C) , where sample headspace was collected with a solid-phase micro-extraction (SPME) portable field sampler (Supelco 504831, 75 µm carboxen/ polydimethylsiloxane, Sigma-Aldrich Co., St. Louis, MO). The headspace above each

meat sample in the glass jar was collected for 2 hr on the SPME. Upon completion of collection, SPME were injected in the GC injection port, where the sample was desorbed at 280°C for 3 min. The sample was then loaded onto the multi-dimensional gas chromatograph into the first column (30 m x 0.53 mm ID/ BPX5 [5% phenyl polysilphenylene-siloxane] x 0.5 μm, SGE Analytical Sciences, Austin, TX), then to the second column (30 m x 0.53 mm ID [BP20- polyethylene glycol] x 0.50 μm, SGE Analytical Sciences). The temperature started at 40°C and increased at a rate of 7° C /min until reaching 260°C. The GC column was then split at a three-way valve with one column going to the mass spectrometer (Agilent Technologies 5975 series MSD, Santa Clara, CA) and one column going to each of the two sniff ports, which were heated to a temperature of 115° C, and fitted with glass nose pieces. The sniff ports and software for determining flavor and aroma are a part of the AromaTrax program (MicroAnalytics-Aromatrax, Round Rock, TX). The GC-MS data were reported as area under the curve and represent total ion count. A 3-point external standard curve (1, 3 dichlorobenzene) was run to estimate concentration of volatiles from area data. The GC-MS data were used to identify and quantify volatiles associated with spoilage and rancidity.

III.III.VIII Experimental Design. Data were analyzed by ANOVA using the SAS v9.3 (SAS Institute, Inc., Cary, NC) GLM procedure with $\alpha = 0.05$. Fixed effects for color attributes, pH, volatile compounds, and microbial growth were muscle and treatment. Least squares means were calculated for significant main effects and their interaction, then significant differences were determined using the PDIFF function of SAS.

III.IV Results and Discussion

Moisture (72.45-74.00 %), fat (4.74-6.22 %), and protein (21.14-21.49 %) contents were constant ($P > 0.05$) across all experiments. Day 0 pH values were higher ($P < 0.05$) for TB samples (5.56-5.63) than for BF samples (5.46-5.56) for beef trimmings used in all experiments. No differences in pH were observed between beef trimming samples from the same muscle across all three experiments. **Table 3-2** shows the pickup percentages from treatment with solutions. The pickup percentages across all treatments (-0.21 – 2.96 %) varied depending on the specific treatment solution. The NaL treatment showed a negative pickup (%) indicating the beef trimming samples lost weight during treatment. This treatment was applied as a 60% solution, which has a high ionic strength. Moisture was potentially drawn out of the meat samples and into the solution do to the hygroscopic nature of a 60% NaL solution.

III.IV.I Beef Trimmings Treated for Color. Least squares means for treatment effect on 2 hr post treatment color attributes can be found in **Table 3-3**. Two hour post treatment a* values of samples treated with reduced nicotinamide adenine dinucleotide (NADH) or sodium erythorbate (NaE) were greater than day 6 control samples and samples treated with distilled, deionized water (DI) or sodium lactate (NaL).

Table 3-2. Least Squares Means of Pickup Percentages for All Treatment Solutions.

Treatment Solution Off Odors and Flavors	Pickup (%)[3]
Distilled Deionized Water	0.37
Sodium Carbonate	0.11
Sodium Bicarbonate	0.16
Sodium Metasilicate	2.05
Sodium Metasilicate/Sodium Carbonate	0.17
Sodium Metasilicate/Sodium Bicarbonate	2.11
Standard Error of the Mean	0.115
Microbial Spoilage	**Pickup (%)**
Distilled Deionized Water	0.72
Lactic Acid	1.61
Citrilow ™ [1]	2.96
Cetylpyridinium Chloride	0.40
Buffered Vinegar	0.66
Standard Error of the Mean	0.297
Color	**Pickup (%)**
Distilled Deionized Water	0.47
NADH [2]	1.76
Sodium Erythorbate	0.51
Sodium Lactate	-0.21
Sodium Lactate/ NADH	1.66
Sodium Lactate/ Sodium Erythorbate	0.84
Standard Error of the Mean	0.125

[1]- Combination of hydrochloric acid, citric acid, and water

[2]- Reduced nicotinamide adenine dinucleotide

[3]-Pickup (%) calculated as (post treatment weight –Initial weight)/ initial weight X 100

Table 3-3. Least Squares Means for Treatment Effect on Color Attributes Measured 2 Hours Post Treatment of Beef Trimmings Treated to Improve Color.

	L*[3]	a*[4]	b*[5]	Hue Angle[6]	Saturation Index[7]	MMb (%)[8]
Day 0 Control	38.65[a]	19.63[a]	15.34[a]	37.81[d]	24.93[a]	24.62[d]
Day 6 Control	37.28[ab]	7.93[d]	12.46[de]	57.86[a]	14.87[e]	57.02[a]
Distilled Deionized Water	38.22[a]	7.55[d]	12.60[d]	59.00[a]	14.77[ef]	57.65[a]
NADH[1]	38.13[ab]	12.76[b]	13.97[b]	47.55[bc]	18.97[b]	29.96[c]
Sodium Erythorbate	37.92[ab]	11.29[c]	13.49[bc]	49.84[b]	17.62[c]	37.43[b]
Sodium Lactate	36.29[b]	7.50[d]	11.42[f]	56.71[a]	13.78[f]	54.81[a]
Sodium Lactate/ NADH	35.54[b]	11.47[c]	11.69[ef]	45.83[c]	16.41[d]	30.80[c]
Sodium Lactate/ Sodium Erythorbate	36.94[ab]	11.58[c]	13.05[cd]	48.69[bc]	17.47[c]	36.05[b]
SEM[2]	0.434	0.253	0.202	0.802	0.240	1.138

[abcdef] LSMeans within a column with different superscripts are significantly ($P < 0.05$) different

[1]-Reduced Nicotinamide Adenine Dinucleotide

[2]-Standard Error of the Mean

[3]-L* is a color value (0-100) measuring lightness (100) to darkness (0)

[4]-a* is a color space value representing red to green; Positive values indicate redness

[5]-b* is a color value representing yellow to blue; Positive values indicate yellowness

[6]-Hue angle = $\tan^{-1}(b*/a*)$, expressed in degrees

[7]-Saturation index = $\sqrt{a*^2 + b*^2}$, measure of vividness

[8]-Percentage of myoglobin in the met state (Fe^{3+}; Empty 6th ligand)

Only samples treated with NaL had 2 hr post treatment L* values (NaL 36.29; NaL/NADH 35.54) lower (P < 0.05) than the day 0 control samples (38.65). All treated samples had b* values greater than the day 0 control samples; b* values of samples treated with NaE, NADH, and NaL/NaE were greater (P < 0.05) than day 6 control and DI samples. Hue angle (HA) values were highest for day 6 control (57.86), DI (59.00), and NaL (56.71) treated samples. The HA values for samples treated with NADH (47.55), NaE (49.84), NaL/NADH (45.83), and NaL/NaE (48.59) were lower (P < 0.05) than day 6 control, DI, and NaL. Saturation index (SI), an indication of vividness was higher for day 0 control (24.93) than day 6 control and all treated samples. The NADH treated samples had a SI value of 18.97 which was higher (P < 0.05) than all other treatments. Samples treated with NaL had the lowest SI value (13.78) similar to DI (14.77) treated samples. After 2 hr, MMb (%) was higher for all treated samples than day 0 control samples (24.62 %) Samples treated with NADH (29.96 %) and NaL/NADH (30.80 %) had MMb (%) values numerically closest to the day 0 control samples. There was a significant muscle by treatment interaction for OMb and DMb percentages (**Table 3-4**). Samples from the BF had generally higher percentage OMb, although no values were significantly higher between muscles treated with the same solution. The NADH and NaL/NADH treated samples for both muscles were not different (NADH: 67.18 %, TB; 70.74 %, BF) (NaL/NADH: 66.56 %, TB; 69.60 %, BF) compared to the day 0 controls (73.93 %, TB; 75.02 %, BF). There were no differences in DMb between the day 0 control and any treatments on BF samples measured 2 hr post treatment. The DMb % was highest for TB samples treated with NaL (5.79 %) which

Table 3-4. Least Squares Means for Muscle by Treatment Effect on Color Attributes Measured 2 Hours Post Treatment of Beef Trimmings Treated to Improve Color.

	OMb (%)[3]		DMb (%)[4]	
	T. Brachii	*B. Femoris*	*T. Brachii*	*B. Femoris*
Day 0 Control	73.93[a]	75.02[a]	1.67[b]	0.14
Day 6 Control	38.22[c]	44.15[c]	2.03[b]	1.56
Distilled Deionized Water	37.52[c]	44.04[c]	2.50[ab]	0.64
NADH[1]	67.18[ab]	70.74[ab]	2.16[b]	0.00
Sodium Erythorbate	63.69[b]	61.03[b]	0.07[b]	0.35
Sodium Lactate	37.55[c]	45.64[c]	5.79[ax]	1.40[y]
Sodium Lactate/ NADH	66.56[ab]	69.60[ab]	2.14[b]	0.10
Sodium Lactate/ Sodium Erythorbate	63.52[b]	62.14[b]	1.09[b]	1.15
SEM[2]	1.783	1.783	0.673	0.673

[abc] LSMeans within a column with different superscripts are significantly ($P < 0.05$) different

[xy] LSMeans within a parameter in the same row with different superscripts are significantly ($P < 0.05$) different

[1]- Reduced Nicotinamide Adenine Dinucleotide

[2]- Standard Error of the Mean

[3]- Percentage of myoglobin in the oxy state (Fe^{2+}; O_2 bound to 6th ligand)

[4]- Percentage of myoglobin in the deoxy state (Fe^{2+}; empty 6th ligand)

could be indicative of the reduction of MMb % by NADH produced through the conversion of lactate to pyruvate. There is an apparent improvement in color observed in samples treated with NADH and NaE compared to the day 6 control and DI water treatments. While these values are not similar to day 0 control these data indicate an improvement in color (redness, HA, SI, and OMb %) which is promising for shelf life extension. Least squares means of treatment effect on 48 hr L*, OMb %, and DMb % can be found in **Table 3-5**. Only NaL/NADH (36.17) and NaL/NaE (36.47) had lower (P < 0.05) L* values than day 0 control (38.65). Samples treated with NaE had the highest OMb percentage after 48 hr (56.39 %), which was higher than all other treatments except NaL/NaE (53.24%) although nothing was similar to day 0 control (74.47 %). Muscle by treatment interaction least squares means for 48 hr color attributes and MMb % are shown in **Table 3-6**. The highest treated a* value for TB was NaE (9.48) which was only higher (P < 0.05) than day 6 control (6.56) and NaL (6.48). The highest 48 hr a* values for BF were NaL/NaE (11.26) and NaE (10.22) although these values only differed (P < 0.05) from day 0 control (19.69). The NaE treated TB samples had the lowest HA value (51.66) of treated samples, however all treated samples were significantly lower (P < 0.05) than the day 0 control (37.39). Day 6 control and treated BF samples' HA values ranged from 49.05 to 57.88 but were only different from the day 0 control (38.70). SI values were greater (P < 0.05) for BF samples treated with NaL (16.01) compared to the TB samples treated with NaL (13.65). Of the samples treated with NaL/NaE, BF samples had higher (P < 0.05) SI values (17.21) than TB samples (14.72). The MMb % was lowest for NaE treated samples of TB (41.17) and NaL/NaE

Table 3-5. Least Squares Means for Treatment Effect on Color Attributes Measured 48 Hours Post Treatment of Beef Trimmings Treated to Improve Color

	L*[3]	OMb (%)[4]	DMb (%)[5]
Day 0 Control	38.65[a]	74.47[a]	0.91[b]
Day 6 Control	36.40[ab]	44.74[c]	0.61[b]
Distilled Deionized Water	36.88[ab]	44.83[c]	0.51[b]
NADH[1]	38.03[ab]	46.71[c]	0.73[b]
Sodium Erythorbate	36.51[ab]	56.39[b]	1.36[ab]
Sodium Lactate	36.78[ab]	42.69[c]	1.40[ab]
Sodium Lactate/ NADH	36.17[b]	45.39[c]	3.57[a]
Sodium Lactate/ Sodium Erythorbate	36.47[ab]	53.24[bc]	2.13[ab]
SEM[2]	0.511	1.837	0.557

[abc] LSMeans within a column with different superscripts are significantly ($P < 0.05$) different

[1]- Reduced Nicotinamide Adenine Dinucleotide

[2]- Standard Error of the Mean

[3]- L* is a color value (0-100) measuring lightness (100) to darkness (0)

[4]- Percentage of myoglobin in the oxy state (Fe^{2+}; O_2 bound to 6th ligand)

[5]- Percentage of myoglobin in the deoxy state (Fe^{2+}; empty 6th ligand)

Table 3-6. Least Squares Means for Muscle by Treatment Effect on Color Attributes Measured 48 Hours Post Treatment of Beef Trimmings Treated to Improve Color.

	a^{*3}		b^{*4}		Hue Angle[5]		Saturation Index[6]		MMb (%)[7]	
	TB[8]	BF[9]	TB	BF	TB	BF	TB	BF	TB	BF
Day 0 Control	19.57[a]	19.69[a]	14.92[a]	15.76[a]	37.39[c]	38.70[b]	24.62[a]	25.23[a]	24.40[c]	24.83[c]
Day 6 Control	6.56[c]	8.47[b]	13.33[ab]	12.86[b]	63.75[a]	57.29[a]	14.94[b]	15.65[bc]	54.82[a]	54.46[a]
Distilled Deionized Water	6.83[bc]	8.80[b]	13.27[ab]	13.53[b]	62.90[a]	57.55[a]	15.08[b]	16.49[bc]	55.50[a]	53.83[a]
NADH[1]	7.82[bc]	8.92[b]	12.59[b]	13.66[b]	58.29[ab]	57.32[a]	15.10[b]	16.69[bc]	51.72[ab]	53.39[ab]
Sodium Erythorbate	9.48[b]	10.22[b]	11.23[b]	13.34[b]	51.66[b]	52.55[a]	15.28[b]	16.99[bc]	41.17[b]	43.33[ab]
Sodium Lactate	6.48[c]	8.39[b]	11.92[b]	13.25[b]	61.55[ab]	57.88[a]	13.65[by]	16.01[bcx]	58.30[a]	53.52[a]
Sodium Lactate/ NADH	7.84[bc]	8.66[b]	11.84[b]	12.28[b]	57.25[ab]	55.31[a]	14.40[b]	15.42[c]	51.00[ab]	51.07[ab]
Sodium Lactate/ Sodium Erythorbate	8.54[bc]	11.26[b]	11.89[b]	12.90[b]	54.65[ab]	49.05[a]	14.72[by]	17.21[bx]	49.36[ab]	39.89[b]
SEM[2]	0.591		0.349		2.100		0.356		2.780	

[abc] LSMeans within a column with different superscripts are significantly (P < 0.05) different

[xy] LSMeans within a parameter in the same row with different superscripts are significantly (P < 0.05) different

[1]- Reduced Nicotinamide Adenine Dinucleotide

[2]- Standard Error of the Mean

[3]- a* is a color space value representing red to green; Positive values indicate redness

[4]-b* is a color value representing yellow to blue; Positive values indicate yellowness

[5]- Hue angle = $\tan^{-1}(b^*/a^*)$, expressed in degrees

[6]-Saturation index = $\sqrt{a^{*2} + b^{*2}}$, measure of vividness

[7]-Percentage of myoglobin in the met state (Fe^{3+}; Empty 6th ligand)

[8]-*Triceps brachii*

[9]-*Biceps femoris*

treated samples of BF (39.89). In both cases these values are lower than the day 6

control for each muscle respectively (54.82% TB, 54.46 % BF). However no treated

samples for either muscle showed 48 hr MMb % values similar to the day 0 control

(24.40% TB, 24.83 % BF)

Color attributes measured at 2 and 48 hr were not compared statistically, though

the improvement in color attributes (a*, HA, SI, OMb %) generally declined from 2 hr to

48 hr. The NaE treated samples maintained their color improvement longer, apparent by

higher values at 48 hr. These data indicate that the effect of color improvement does not

last 48 hr post treatment. However, an increase in color shelf life by even 24 hours may

be of benefit to processors utilizing beef trimmings.

Surface pH measurements did not differ (P > 0.05) between day 0 control and treated

samples (**Table 3-7**). Treated samples were numerically higher than untreated control at

2 hr and 48 hr. Surface pH was higher for samples 48 hr post treatment than 2 hr post

treatment. Increased pH could be a contributing factor to improvement of color

attributes (*21*) as higher pH is less favorable for the formation of MMb (*3*) and more

favorable for metmyoglobin reducing activity (*56*). Samples treated with NaL had

numerically higher pH compared to the control samples and showed no improvement in

color attributes compared to control samples. The ineffectiveness of the NaL treatment

could be due to the negative solution pickup. Shivas et al. (*13*) reported greater (P <

0.05) a* values and lower MMb % for ground beef treated with 0.05% ascorbic acid

(14.32 and 45.31%) compared to the untreated control (8.78 and 80.12%)

Table 3-7. Least Squares Means of Treatment Effect for Surface pH taken at 2 and 48 hours Post Treatment on Beef Trimmings Treated for Color.

	2 Hour	48 Hour
Day 0 Control	5.66	5.66
Day 6 Control	5.71	5.85
Distilled Deionized Water	5.62	5.94
NADH[1]	5.79	6.03
Sodium Erythorbate	5.79	6.22
Sodium Lactate	5.81	5.91
Sodium Lactate/ NADH	5.83	6.06
Sodium Lactate/ Sodium Erythorbate	5.76	5.94
SEM[2]	0.062	0.118

[1]- Reduced Nicotinamide Adenine Dinucleotide

[2]- Standard Error of the Mean

after 10 days of simulated retail display (O_2 permeable PVC film, 2℃ , exposed to

fluorescent lighting). Ground beef treated with ascorbic acid (0.05%) also exhibited

higher beef flavor intensity and lower TBA values than untreated control (*13*). Mancini

et al. (*124*) reported a similar effect on surface redness (a*) of bone-in *Longissimus*

lumborum steaks treated with the same concentration (0.5-1.5%) of either ascorbic acid

or NaE. Manu-Tawiah et al. (*122*) found that ground beef treated with a mixture of NaE

(0.05%) citric acid (0.025%) and tetra sodium pyrophosphate (0.3%) exhibited higher (P

< 0.05) a* values (10.7) than untreated control samples (7.0) (*122*). The treated ground

beef samples maintained a bright red color for 1-3 days longer during simulated retail

storage (O_2 permeable PVC film, 2℃ , exposed to fluorescent lighting) than untreated

control (*122*). Suman et al. (*42*) reported greater (P < 0.05) a* values for ground beef

patties treated with potassium lactate (KL; 2.5%) compared to the untreated control

(21.5 and 18.7 respectively) when packaged with O_2 permeable PVC film for 4 days at

2℃. Kim (*152*) reported greater a* values for KL (2.5%) enhanced *Semimembranosus*

steaks (24.0) compared to untreated control steaks (20.5) after 14 days of simulated retail

storage (O_2 impermeable film, flushed with 80% O_2,2℃ , exposed to fluorescent

lighting). The increased redness achieved with lactate enhancement is likely due to the

generation of NADH following conversion of lactate to pyruvate (*25*). The improved

color attributes (a*, HA, SI, OMb %) observed in this study for trimmings treated with

NADH are consistent with the findings of other research involving lactate enhancement.

The present study however did not see color improvements in samples treated with NaL.

Seyfert et al. (*58*) did not report higher (P <0.05) a* or SI values for lactate enhanced

(2.5% NaL and 2.5% KL) ground beef patties over three storage (O_2 permeable PVC

film, 2.5℃, exposed to fluorescent lighting) days. A potential cause could be that the

treatment of trimmings after post mortem NADH pool has been exhausted resulted in a

pronounced increase MMb accumulation which did not allow enough NADH to

accumulate before being utilized. All previous work has focused on lactate enhancement

prior to storage, allowing NADH to be generated via lactate to pyruvate during entire

storage period. Seyfert et al. (*58*), Kim (*152*), and Suman et al.(*42*) observed an initial

decrease in L* values in meat treated with lactate, consistent with the current findings,

which can be explained by the increase in pH and the increased ionic strength of the

lactate salt increasing water holding capacity and reducing the reflection of light on the

meat surface.

The improvement in color attributes in beef trimmings treated with NaE,

especially the ability to maintain increased values up to 48 hr makes NaE the optimal

solution in this study, for improving color of beef trimmings at or near the end of shelf

life. NADH treatment was more effective than NaE at 2 hr post treatment however the

effects were depleted by 48 hr and NADH costs roughly $75 per gram. The NADH

treatment solution used in this study would cost 55 cents per pound of treated meat

which is not likely economical on a large scale.

III.IV.II Beef Trimmings Treated for Microbial Spoilage. Aerobic plate

counts for aerobic mesophiles (AM) (**Table 3-8**) for all treatments were numerically

lower than the day 9 control (8.0 $\log_{10}$ CFU/cm^2) although only cetylpyridinium chloride

Table 3-8. Least Squares Means for Treatment Effect for Aerobic Mesophile Plate Counts of Beef Trimmings Treated for Microbial Spoilage.

	Aerobic Mesophiles Log$_{10}$ CFU/cm^2
Day 0 Control	3.4[c]
Day 9 Control	8.0[a]
Distilled Deionized Water	7.9[ab]
Lactic Acid	7.6[ab]
Citrilow ™[1]	7.8[ab]
Cetylpyridinium Chloride	7.4[b]
Buffered Vinegar	7.0[ab]
SEM[2]	0.12

[abc] LSMeans with different superscript are significantly different (P < 0.05)

[1]- Combination of hydrochloric acid, citric acid, and water

[2]- Standard error of the mean

(CPC) showed significantly lower AM counts (7.4 $\log_{10}$ CFU/cm^2). All counts were significantly higher on day 9 than day 0 control (3.4 $\log_{10}$ CFU/cm^2). A significant muscle by treatment effect was observed for aerobic psychrotroph (AP) plate counts (**Table 3-9**). No treatment showed AP counts lower ($P < 0.05$) than day 9 control (8.1 $\log_{10}$ CFU/cm^2) for *Triceps brachii* (TB) muscle samples, though CPC was numerically the lowest (7.7 $\log_{10}$ CFU/cm^2). *Biceps femoris* (BF) muscle samples treated with CPC had the lowest AP values (7.1 $\log_{10}$ CFU/cm^2) which was lower than day 9 control (8.0 $\log_{10}$ CFU/cm^2) but not different from other treatments. Kalchayanand et al. (*140*) reported a reduction in aerobic plate counts (APC) of 1.8 $\log_{10}$ CFU/cm^2 on beef flank (*Cutaneous trunci*) that were inoculated (3.0 $\log_{10}$ CFU/cm^2 of nine strain *E. coli* cocktail) pre-rigor and spray treated with CL (2.0%, pH 1.8). Stelzleni et al. (*15*) found that ground beef patties made with beef trimmings treated with buffered vinegar (BV; 2.5% in final product) showed lower ($P < 0.01$) AP counts (3.0 $\log_{10}$ CFU/g) than untreated control samples (7.0 $\log_{10}$ CFU/g) after five days of simulated retail display (O$_2$ permeable PVC overwrap, 2℃ , exposed to fluorescent lighting). In the present study however, Citrilow (CL) and BV treated samples did not exhibit lower microbial counts (AM or AP) than day 9 control samples. Pohlman et al. (*142*) found that treatment of inoculated (cocktail of 10^7 *Escherichia coli* and *Salmonella enterica serovar Typhimurium*) beef trimmings with CPC (0.5%) resulted in a reduction of APC by 0.6 $\log_{10}$ CFU/g. Ground beef made from trimmings treated with CPC (0.5%) had APC on day 7 of storage (O$_2$ permeable PVC overwrap, 4℃, exposed to fluorescent lighting) of 6.6 $\log_{10}$ CFU/g which was not different ($P < 0.05$) from APCs

Table 3-9. Least Squares Means for Muscle by Treatment Effect for Aerobic Psychrotroph Plate Counts[1] of Beef Trimmings Treated for Microbial Spoilage.

	T. Brachii	B. Femoris
Day 0 Control	2.9[b]	3.6[c]
Day 9 Control	8.1[a]	8.0[a]
Distilled Deionized Water	7.9[a]	7.8[ab]
Lactic Acid	7.7[a]	7.6[ab]
Citrilow ™ [2]	7.8[a]	7.9[ab]
Cetylpyridinium Chloride	7.7[a]	7.1[b]
Buffered Vinegar	7.8[a]	7.9[ab]
SEM[3]	0.15	

[abc] LSMeans within a column with different superscript are significantly different (P < 0.05)

[1]- Plate counts reported as Log_{10} CFU/cm^2

[2]- Combination of hydrochloric acid, citric acid, and water

[3]- Standard error of the mean

on day 0 (6.7 Log$_{10}$ CFU/g) (*142*). Stivarius et al. (*139*) found that treatment of

inoculated (cocktail of 10^7 *E. coli* and *Salmonella Typhimurium*) beef trimmings with

lactic acid (5.0%) resulted in a reduction of APCs of 0.6 log$_{10}$ CFU/g. Treated samples

in the current study exhibited plate counts lower than the day 9 control by levels

consistent with the findings of other researchers (*139, 142*). While this study did not

investigate residual effect of antimicrobial treatments at preventing the growth of AM or

AP, it is likely that growth would not occur if storage continued after treatment.

While the treatment of beef trimmings with antimicrobials is beneficial in

reducing the numbers of microorganisms present, some treatments may result in

unintended color or organoleptic issues. Dias-Morse et al. (*153*) reported that ground

beef generated from beef trimmings treated with CL (15%) showed lower (P < 0.05) a*

and saturation index (SI) values and greater hue angle (HA) values than untreated

control ground beef after 2 days of simulated retail display (O$_2$ permeable PVC

overwrap, 4℃, exposed to fluorescent lighting). Stelzleni et al. (*15*) showed that ground

beef patties made with beef trimmings treated with BV (2.5% in final product) did not

differ (P > 0.05) from untreated control patties in color attributes (a*, HA, SI) or TBA

number over seven days of simulated retail display (O$_2$ permeable PVC overwrap, 2℃ ,

exposed to fluorescent lighting). Pohlman et al. (*142*) found that CPC (0.5 %) treatment

of beef trimmings did not result in development of off odors determined by sensory

panelists and resulted in increased (P < 0.05) a* values (19.09) compared to the

untreated control (18.08). Jiminez-Villareal et al. (*14*) showed that ground beef patties

generated from beef trimmings treated with lactic acid (LA; 2%) had higher (P < 0.05)

percentage of discoloration (determined by visual appraisal) than untreated control patties on storage (O_2 permeable PVC overwrap, 4℃, exposed to fluorescent lighting) days 1-3. The same study found that beef patties generated from beef trimmings treated with CPC (0.5%) showed a*, HA, and SI values similar to the untreated control over 7 storage days (*14*). Stivarius et al. (*139*) found ground beef made from trimmings treated with LA (5.0%) had lower ($P < 0.05$) a* and SI values than untreated control ground beef on days 0, 1,and 7 of simulated retail storage (O_2 permeable PVC overwrap, 4℃, exposed to fluorescent lighting).

Beef trim samples treated with CPC had lower AP and AM plate counts than day 9 control samples in the present study. Previous research indicates that CPC treatment can prevent microbial growth for up to 7 days of storage after treatment (*142*). These data suggest that CPC is the best choice of antimicrobial for extending shelf life by treating beef trimmings at or near the end of shelf life. Treatment with CPC was also shown by several researchers to maintain or improve color attributes and did not result in off odor or off flavor development (*14, 142*).

III.IV.III Beef Trimmings Treated for Off Odors/Flavors. Acids, alcohols, aldehydes, and ketones were all higher in day 9 control samples than day 0 control samples, though not significantly (**Table 3-10**). Aldehydes were lowest for samples treated with sodium bicarbonate (NaB; 99,776) and sodium metasilicate with sodium bicarbonate (NaMS/NaB; 170,826) even lower than day 0 control (193,619). Day 9

Table 3-10. Least Squares Means of Treatment Effect for Volatile Groups of Beef Trimmings Treated for Off Odors and Flavors.

	Acids	Alcohols	Aldehydes	Alkanes	Alkenes	Ketones	Sulfides
Day 0 Control	20,610	25,671	193,619	12,841	0	74,459	15,221
Day 9 Control	114,198	132,4753	1,699,705	11,682	9,261	633,389	2,872
Distilled Deionized Water	12,206	701,664	840,512	3,293	0	12,786	0
Sodium Carbonate	16,907	2,347,121	354,193	40,391	36,364	1,027,796	0
Sodium Bicarbonate	132,921	771,645	99,776	30,208	6,787	589,773	626
Sodium Metasilicate	75,068	987,391	420,776	44,950	3,639	411,696	0
Sodium Metasilicate/Sodium Carbonate	7,250	633,741	259,082	13,170	13,413	552,787	0
Sodium Metasilicate/Sodium Bicarbonate	21,380	1,030,940	170,826	51,350	0	744,800	3,792
SEM[1]	53,135	549,251	498,075	21,151	13,745	402,186	3,517

[1]- Standard Error of the Mean

control samples (1,699,705) had roughly 15 times higher aldehydes total ion count than

samples treated with NaB (99,776). Based on the external standard curve, estimated

aldehyde concentration (ppm) for day 9 control samples is 173.94 ppm and 0.96 ppm for

samples treated with NaB. This decrease in aldehydes likely indicates that the NaB

treatment would reduce lipid oxidation products. Sindelar et al. (*131*) reported no

difference in thiobarbituric acid reactive substances (TBARS) values between control

pork loins and sow-tainted pork loins treated with NaB (0.35-0.70 M) and sodium

tripolyphosphate (STP; 0.25- 0.50%). The same study showed NaB treated (0.63 M)

sow-tainted pork loins to have sour aftertaste scores (determined by trained sensory

panel) similar to non-sow tainted control pork loins (*131*). Sindelar et al. (*132*) showed

that sow-tainted pork loins treated with NaB (0.35 M) and STP (0.50 %) had consumer

flavor (5.87) and overall acceptability (5.70) scores similar to the non-sow-tainted

control pork loins (6.14 and 5.97 respectively). Sodium metasilicate (NaMS) treatment

alone resulted in numerically lower aldehydes (420,776) compared to day 9 control

samples (1,699,705) but not as low as NaMS/NaB treatment (170,826). Quilo et al.

(*136*) found that ground beef patties made from beef trimmings treated with NaMS

(4.0%) had lower ($P < 0.05$) sensory off odor scores than untreated control patties on

storage (O_2 permeable PVC overwrap, 4℃ , exposed to fluorescent lighting) days 0-3,

and a similar sensory off odor scores on storage day. The same study also reported

lower ($P < 0.05$) TBARS numbers for NaMS treated (4.0%) ground beef patties

compared to untreated control patties on storage days 0, 3, and 7 (*136*).

III.V Conclusions

Sodium erythorbate (NaE), cetylpyridinium chloride (CPC), and sodium bicarbonate (NaB) all performed better than other treatment solutions for improving color, reducing microbial numbers, and improving odor/flavor, respectively. Treatment with NaE showed improvement in objective color and reduction of MMb % at both 2 and 48 hr after treatment. Treatment with CPC achieved the greatest reduction in both AM and AP plate counts and has been shown to maintain or improve color while not affecting organoleptic properties. Though no significant reductions in volatile compound groups occurred, NaB exhibited the lowest numerical value for aldehyde volatiles. These solutions have the potential to extend the shelf life of aerobically stored beef trimmings if applied at or near the end of shelf life.

CHAPTER IV

CONCLUSIONS

Color shelf life was determined to end after 6 days of aerobic storage in this model system because of the high amount of discoloration observed. Microbial growth reached 7.0 $\log_{10}$ CFU/cm^2 and off odor volatile compounds increased by storage day 9, indicating the end of microbial and organoleptic shelf for fresh beef trimmings stored in this system. Additionally the MRA data indicated that meat with unsatisfactory color could potentially regain acceptable color if provided with required reducing equivalents. This information was used to apply interventions aimed at extending shelf life in the second study. Data from the second study indicated that sodium erythorbate (NaE), cetylpyridinium chloride (CPC), and sodium bicarbonate (NaB) all performed better than other treatment solutions for reducing color and microbial deterioration and improving odor/flavor, respectively. Treatment with NaE showed improvement in objective color and reduction of MMb % at both 2 and 48 hr after treatment. Treatment with CPC achieved the greatest reduction in both AM and AP plate counts and has been shown to maintain or improve color while not affecting organoleptic properties. Although no significant reductions in volatile compound groups occurred, NaB exhibited the lowest numerical value for aldehyde volatiles. This information can be used by industry to develop strategies for the extension of shelf life of beef trimmings.

CHAPTER V

FUTURE RESEARCH

Future studies should focus on the use of sodium erythorbate (400 ppm), cetylpyridinium chloride (0.5%), and sodium bicarbonate (1.125 M) in combination to holistically address the mechanisms of spoilage to extend the shelf life of beef trimmings. An experiment should be conducted investigating the best way to apply all three solutions either in one mixture or in a series of applications. All solutions should be applied on day 6 of aerobic storage as that is the day this research indicated as the end of color shelf life. The order of application should be evaluated as one solution may reduce the efficacy of the next.

Additionally investigation of the shelf life of beef trimmings after treatment with this solution system should be conducted to determine how long the effect of these treatments will last. A study using treated beef trimmings to make a product may be helpful in determining if this treatment impacts the functional properties of the myofibrillar proteins. Lastly if allowable a sensory study should be conducted to evaluate the organoleptic acceptability of these products produced with treated beef trimmings.

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APPENDIX A

COOLER STORAGE AND SETUP

The food cooler in KLCT 329 was used for storage of beef trimmings. The cooler was set at 5℃ for the duration of the study. Temperature was monitored each day both by the thermometer on the outside of the cooler and by a separate temperature probe inside the cooler. Fluorescent lights were hung in the cooler roughly 1 meter above the samples and were positioned to make the light intensity uniform throughout the cooler. The light intensity target was 150-300 lux and was measured at 4 locations in the cooler on each day of the study. Samples were stored on white plastic platters which were lined with LLDPE plastic sheeting. Samples were covered with the same sheeting to prevent dehydration though every effort was made to prevent the sheeting touching the meat samples.

APPENDIX B

POWDERING SAMPLES FOR ANALYSIS

1) Previously frozen samples were thawed until meat could be cut

2) Meat samples were hand cut into small cubes ½ inch or smaller

3) Sample was placed into a wire straining basket and lowered into a container of liquid nitrogen

4) Samples were submerged for 30 sec or until liquid nitrogen stopped bubbling

5) Frozen sample pieces were transferred to a stainless steel waring blender and blended until a homogenous powder was formed

6) Powdered samples were transferred to a whirl pack back and stored frozen until analysis.

APPENDIX C

LECO F-528 RAPID NITROGEN/PROTEIN ANALYSIS

Performing Leak Checks

(Prior to running any samples)

> 1. Press "Diagnostics", then press "Leak Check", Select either "Oxygen Leak Check" or "Helium Leak Check". (Both leak checks should be performed).
>
> 2. If leak check is ok, continue on to analysis. If leak check does not pass, refer to instrument manual.

Running Blanks

> 1. Press "Analyze" then press "Start" to open the sample chamber, press "Start" again to close the chamber and begin blank.
>
> 2. Run blanks until protein reading is near zero (0.012 or -0.012), approximately 5 blanks
>
> 3. Check the S.D. of blanks by pressing "Results", select at least 3 blanks by highlighting blanks and pressing "Select", then press "Menu" and select "Statistics". The S.D. should be ≤ 0.03.
>
> 4. Calculate blank by pressing "Calibrate", select at least 3 blanks by highlighting blanks and pressing "Select", then press "Menu" and select "Calculate Blank", press "Exit"

Running Standards

(Performed before new project, after bottles are changed, after maintenance)

> 1. Weigh ~.3500 grams of standard (EDTA) in tin foil cups, record weights (Need at least 5).
>
> 2. Press "Analyze", enter the weight, press "Select" twice, then enter Sample ID.

3. Place standard in opening, then press "Start" to open the sample chamber, press "Start" again to close the chamber and begin analysis.

4. The first few standards will be conditioning standards, do not use for calibration.

5. Check the S.D. (or RSD) of standards by pressing "Results", select at least 3 standards by highlighting blanks and pressing "Select", then press "Menu" and select "Statistics". The target S.D. is on the certificate of analysis with the standard.

6. Calculate calibration by pressing "Calibrate", select at least 3 standards by highlighting standards and pressing "Select", then press "Menu" and select "Calculate Calibration", enter Nitrogen Standard value found on certificate of analysis with the standard, press "Select", press "Yes", press " Exit".

7. Recalculate by pressing "Menu" on calibration screen, press "Recalculate", press "Recalculate Today", press "Exit".

Running Samples

1. Weigh ~.3000 grams of sample in tin foil cups, record weights (done in triplicate).

2. Press "Analyze", enter the weight, press "Select" twice, then enter Sample ID.

3. Place sample in opening, then press "Start" to open the sample chamber, press "Start" again to close the chamber and begin analysis.

4. Record % Protein from screen.

APPENDIX D

CEM SMART SYSTEM MOISTURE AND SMART TRAC FAT ANALYSIS

Analyzing Samples

     1. Frozen meats should be thawed to room temperature before analysis.

     2. Meat samples should be finely ground or homogenized in a food processor to as smooth a consistency as possible. Grind at least 50 grams per sample to be representative of the batch.

     3. Select appropriate program based on best description of sample using key pad.

     4. Place two sample pads in SMART SYSTEM SOLIDS/MOISTURE Analyzer microwave chamber on the balance and push "TARE". Tare weight will be automatically recorded.

     5. With the Teflon coated spatula, spread appropriate weight of homogenized sample evenly on the center of one of the square pads.

NOTE: Sample size should be in the range given on the screen.
NOTE: Raw samples should be spread in a square pattern, cooked samples should be spread in a circle pattern.

     6. Cover sample with other tared square sample pad.

     7. Place sample pads on balance, close lid carefully, shut the lid latch.

     8. Press "Ready", watch screen during drying. Power level may be adjusted at this time if temperature of sample increases too rapidly. Sample should not be burned.

     9. After drying, remove the sample and roll in TRAC FILM. Place roll into plastic sample sleeve and compress, insert sleeve into NMR chamber.

     10. Read Moisture and Fat from Screen, using "Results" option on menu.

Samples should be run in triplicate, with +/- .5% S.D. between triplicates.

APPENDIX E

MEAT PH MEASUREMENT PROCEDURE

Equipment:

Blender
Pint Jars
pH meter with pH electrode
Stir plate
Magnetic stir bars
Reagents:
Distilled water
Buffer, pH 4.0 and pH 7.0

Procedure:

1. Place approximately 10 g of the frozen powdered sample into a pint jar.

2. Add 90 g distilled water to the pint jar, attach blender blade, o-ring, and screw

cap. Blend on high speed for 15 to 20 seconds to make a smooth slurry.

3. Place a magnetic stir bar in the bottom of the jar and place on stir plate. Stir

plate should be moderately agitating the sample (~200 RPM) when the probe is

lowered into the sample jar.

4. Measure the pH of this slurry with a pH meter that has been calibrated with

two standard buffer solutions. One buffer at pH = 7.0 and the other (either 4 or

10) having a pH value near that of the final.

5. The electrode should be placed in the stirred slurry for about 30 seconds to

allow the electrode to equilibrate.

6. Press read to begin pH measurement. "Stable" will appear when reading is

finished. Record the pH of the slurry after the electrode has stabilized.

6. Do NOT leave the pH probe in the meat slurry. Remove the pH probe from the slurry and wash it thoroughly with distilled water. Be sure to gently wipe all fat and connective tissue from the probe.

7. Always store the pH probe in CLEAN distilled water or pH 7 buffer. NEVER let the bulb dry out.

APPENDIX F

HUNTER LAB MINI SCAN XE PLUS STANDARD OPERATING PROCEDURES

** Always handle the black and white standardization plates with care. Do not scratch or chip them.

1. Plug Mini Scan into electrical outlet.

2. Wrap PVC overwrap over aperture insuring a smooth, tight fit. Also wrap the black and white standardization plates with PVC overwrap. Make sure there are no air bubbles or wrinkles on the surface of the plates where the readings will be taken.

3. Wipe the black plate with a Kimwipe to insure it is clean and place the black plate on the circle of the calibration tile holder.

4. Place the Mini Scan on the calibration tile holder so the two rubber feet are in the two holes of the holder and the aperture is centered on the black plate. The aperture should fit flatly on the black plate to insure that there is no interference when taking readings.

5. Push the lightning bolt key on the Mini Scan to turn the unit on.

6. Make sure that the XYZ values on the screen correspond to the XYZ values listed on the back of the white plate.

7. You are now ready to standardize the unit. Press the lightning bolt key and the Mini Scan will read the black plate.

8. When the reading is complete, the screen will indicate that the machine is ready to read the white plate.

9.Remove the black plate from the calibration tile holder and replace it with the

white plate. Wipe the white plate with a Kimwipe. Make sure that the

aperture of the Mini Scan sits flatly on the white plate.

10. Press the lightning bolt key to read the white plate.

11. Press the lightning bolt key three times and the MiniScan will be ready to

read the first sample.

Procedure to Record L* a* b* Color Scores

12. Use left and right arrow keys to select the appropriate setup.

13. (For my research Daylight Color was used with a 10 degree observer)

14. Position the aperture of the Mini Scan on the part of the meat sample to

be tested. Be sure that the aperture fits flatly on the meat but do not apply

pressure. The spot to be tested should be representative of the steak

muscle tissue. There should not be a lot of connective tissue, seam fat or

subcutaneous fat where the color reading is taken.

15. To take a reading, press the lightning bolt key.

16. Record the L*a*b* values and press the lightning bolt key again to take a

second reading of exactly the same spot on the meat sample.

17. Record the L*a*b* values and take a third reading by pressing the

lightning bolt key.

18. Record the L*a*b* values.

19. The Mini Scan is now ready to read the next sample. Repeat the

process. Before taking readings on the second meat sample, make sure

that the PVC overwrap covering the aperture is clean and free of fat or

anything that might interfere with a clean reading.

Procedure to Record Spectral Reflectance Data

20.   Use left and right arrow keys to select the appropriate setup.

(For my research, Spectral Data was used with illuminant A and a 10 degree observer)

Note: Spectral data reports the reflectance from 400 – 700 nm every 10 nm in 5 columns

with an anchor value at the top of each column.

400 nm	470 nm	540 nm	610 nm	680 nm
Value	Value	Value	Value	Value
Value	Value	Value	Value	Value
Value	Value	Value	Value	Value
Value	Value	Value	Value	
Value	Value	Value	Value	
Value	Value	Value	Value	
Value	Value	Value	Value	

21. Position the aperture of the Mini Scan on the part of the meat sample to be

tested. Be sure that the aperture fits flatly on the meat but do not apply

pressure. The spot to be tested should be representative of the steak muscle

tissue. There should not be a lot of connective tissue, seam fat or

subcutaneous fat where the color reading is taken.

22. To take a reading, press the lightning bolt key.

23. Record the reflectance values from 400-700 nm and press the lightning bolt key again to take a second reading of the meat sample.

24. Record the reflectance values from 400-700 nm and take a third reading by pressing the lightning bolt key.

25. Record the reflectance values from 400-700 nm.

26. The Mini Scan is now ready to read the next sample. Repeat the process. Before taking readings on the second meat sample, make sure that the PVC overwrap covering the aperture is clean and free of fat or anything that might interfere with a clean reading.

27. When all readings are complete, unplug it from the electrical source.

Be sure that the Mini Scan is clean and that the aperture is clean before putting the machine away

APPENDIX G

MRA DETERMINATION WITH NITRIC OXIDE REDUCING ABILITY

Solutions:

0.3% NaNO₂ – weigh 3.0 g $NaNO_2$ into beaker and add distilled water to 1000 g

Procedure:

1. Cut 3x3 CM^2 squares from each sample slice.

2. Submerge two samples from each tray in 0.3% $NaNO_2$ solution for 20 min at room temperature to oxidize myoglobin.

3. Submerge samples in 0.3% $NaNO_2$ solution for 20 min at room temperature.

4. Blot samples dry and vacuum package in high barrier vacuum bags.

5. Measure reflectance at 400-700 nm with hunter colorimeter (standardized with black and white tiles covered with vacuum bag).

6. Incubate at 30℃ for 120 minutes to induce reduction of metmyoglobin.

7. Measure reflectance at 400-700 nm.

Note: Data will be reported as Initial metmyoglobin formation (IMF) (% MMb in the sample before incubation), post reduction metmyoglobin formation (PRMF) (% MMb in the sample after incubation), absolute metmyoglobin reducing ability (ARMA) (difference between IMF and PRMF), and relative metmyoglobin reducing ability (RMRA) (IMF-PRMF/IMF x 100).

Procedure modified from Protocol J. "Metmyoglobin Reducing Capacity of Intact or Ground Meat" in *Section XI: Details of Analytical Analyses Related to Meat Color* of *Meat Color Measurement Guidelines* (AMSA, 2012b).

Metmyoglobin percentage at both 0 and 120 minutes is calculated according to Protocol D. "Calculating Myoglobin Forms via Selected Wavelengths" in *Section IX: Equations*

for Quantifying Myoglobin Redox Forms in Fresh Meat of *Meat Color Measurement Guidelines* (AMSA, 2012a).

Protocol D. is adapted from Krzywicki (1979) in which reflex attenuance (A) is used instead of reflectance (R), where A=Log 1/R at any given wavelength. The equation for percent MMb is as follows:

$$\%MMb= (1.395-[(A572-A700)/(A525-A700)])\text{x } 100$$

.

APPENDIX H

MRA DETERMINATION WITH MODIFIED NITRIC OXIDE REDUCING ABILITY

Solutions:

0.3% NaNO$_2$ – weigh 3.0 g NaNO$_2$ into beaker and add distilled water to 1000 g

70 mM NADH- weigh 0.496 g NADH into beaker and add distilled water to 10 g
(*Make only as much as needed*)

Procedure:

1. Purchase 3 select bottom round flats and 3 select chuck clod hearts.

2. Slice flats and clod hearts to 12.7mm and place on lined trays in 329 cooler, cover with LLDPE Sheeting (same as preliminary study).

3. Take two Day 0 slices from each (6) tray and remove two 3x3 CM^2 squares from each slice. (Will have 4 squares of each flat and clod heart, 24 squares in total).

4. Submerge two samples from each tray in 0.3% NaNO$_2$ solution for 20 min at room temperature to oxidize myoglobin.

6. Add 1 ml(per 50 g of sample) of 70 mM NADH solution to remaining 2 samples from each tray, store at 4℃ for 30 min then submerge samples in 0.3% NaNO$_2$ solution for 20 min at room temperature.

(i.e. 35g intact sample will be treated with 0.7 ml of 70 mM NADH solution)

7. Blot samples dry and vacuum package in high barrier vacuum bags.

8. Measure reflectance at 400-700 nm with hunter colorimeter (standardized with black and white tiles covered with vacuum bag).

9. Incubate at 30℃ for 120 minutes to induce reduction of metmyoglobin.

10. Measure reflectance at 400-700 nm.

11. Repeat steps 3-10 on days 3,6, and 9.

Note: Data will be reported as Initial metmyoglobin formation (IMF) (% MMb in the

sample before incubation), post reduction metmyoglobin formation (PRMF) (% MMb in

the sample after incubation), absolute metmyoglobin reducing ability (ARMA)

(difference between IMF and PRMF), and relative metmyoglobin reducing ability

(RMRA) (IMF-PRMF/IMF x 100).

Procedure modified from Protocol J. "Metmyoglobin Reducing Capacity of Intact or

Ground Meat" in *Section XI: Details of Analytical Analyses Related to Meat Color* of

Meat Color Measurement Guidelines (AMSA, 2012b).

Metmyoglobin percentage at both 0 and 120 minutes is calculated according to Protocol

D. "Calculating Myoglobin Forms via Selected Wavelengths" in *Section IX: Equations

for Quantifying Myoglobin Redox Forms in Fresh Meat* of *Meat Color Measurement

Guidelines* (AMSA, 2012a).

Protocol D. is adapted from Krzywicki (1979) in which reflex attenuance (A) is used

instead of reflectance (R), where A=Log 1/R at any given wavelength. The equation for

percent MMb is as follows:

$$\textbf{\%MMb= (1.395-[(A572-A700)/(A525-A700)])x 100}$$

.

APPENDIX I

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY PROTOCOL

1. Sample should be wrapped in aluminum foil and stored at -80℃ until analysis

2. Record weight of samples, place into 486 mL jar and cover with Teflon lid.

3. Place the jars in a 70℃ water bath for 45 min

4. After 45 min remove from water bath and insert the SPME

5. Collect volatiles for 2 hours on the SPME

6. Remove SPMES and store (covered with aluminum foil) at -80℃ until analysis

NOTE: ENSURE THAT FILAMENT IS NOT OUTSIDE OF METAL SHEATH WHEN INSERTING OR REMOVING SPME

7. On the computer program for GC/MS, load method (Beef) and wait for temperature to reach 40℃

8. Click on the sample ID arrow, enter sample name, and click "ok and run method"

9. When prompted, click the start button on the GC/MS

10. Check that Status is "RUN" and that light is on inside GC/MS

11. Insert SPME into injection port and lower filament (desorption occurs in first 3 minutes)

12. During run, click * on aroma trax program when an aromatic event begins

13. Click on the 0-100 scale when aromatic event ends

14. Integrate the data by first opening the appropriate chromatogram in the GC/MS data analysis program

15. Click Chromatogram, then "Select Integrator", Select "RTE"

16. Click Chromatogram, then "percent report"

17. Click Chromatogram, then "Integrate"

18. Click Spectrum, then "library search report", choose "screen"

19. Open results.csv file to view the integrated data

External Standard Curve

1. Make diluted solutions of 1,3 dichlorbenzene bracketing the expected concentration in your samples

2. Place 100 uL of diluted sample into 486 mL jar

3. Collect headspace volatiles with SPME for 2 hr at room temperature

4. Run SPME on Beef method

5. Run regression analysis of ppm and area

6. This data is only to estimate the concentration of volatiles

Statistical Note

Samples that did not result in any quality peaks were entered as 0 for all volatiles. Only a truly missing data point would be entered as ".". This approach prevented any statistical differences in phase II volatile data but was the appropriate analysis.

APPENDIX J

DATA FROM CHAPTER II

INVESTIGATION OF MECHANISTIC INTERRELATIONSHIPS CAUSING

SPOILAGE OF AEROBICALLY STORED BEEF TRIMMINGS
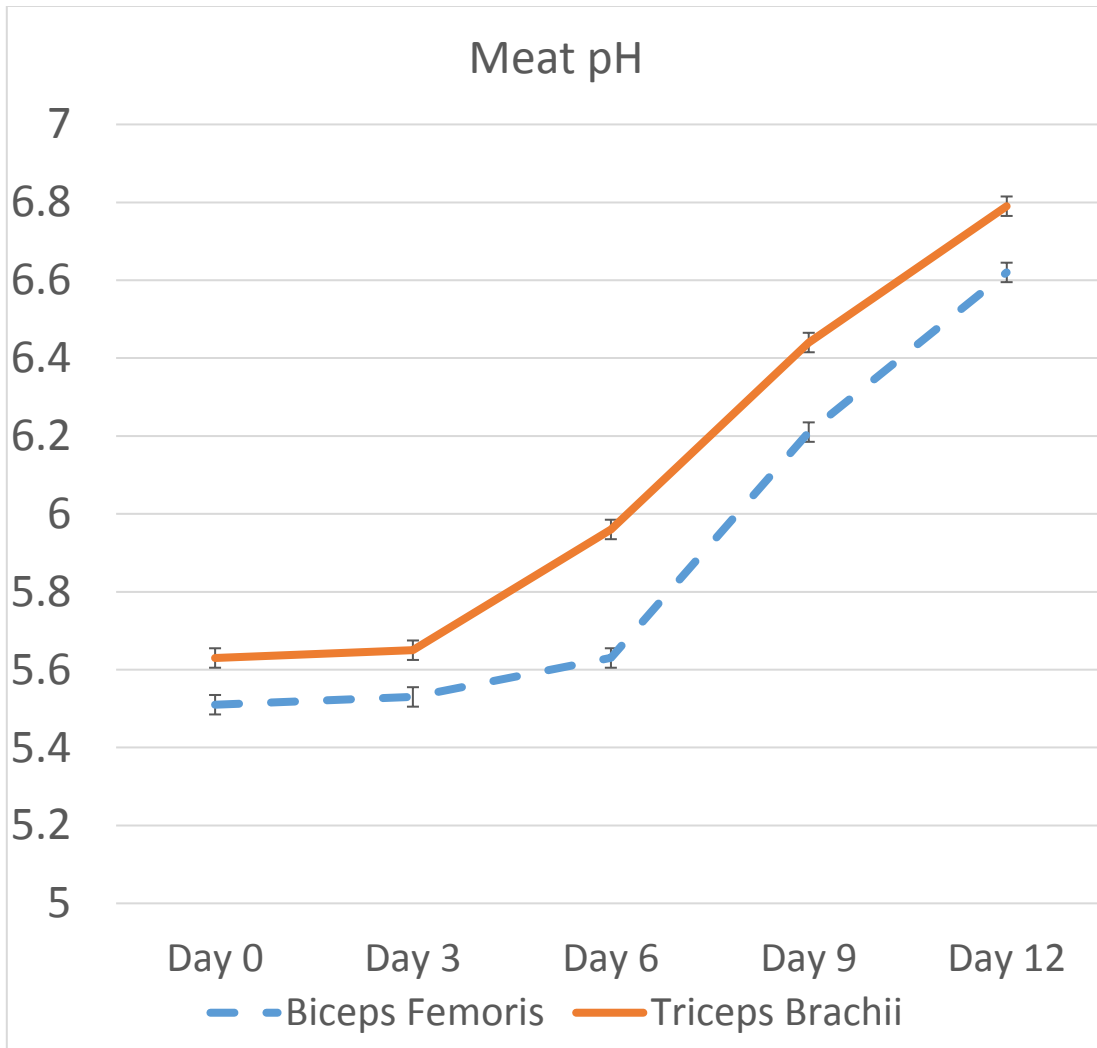
Figure J-1. Least Squares Means of Muscle by Storage Day Interaction for Meat pH.
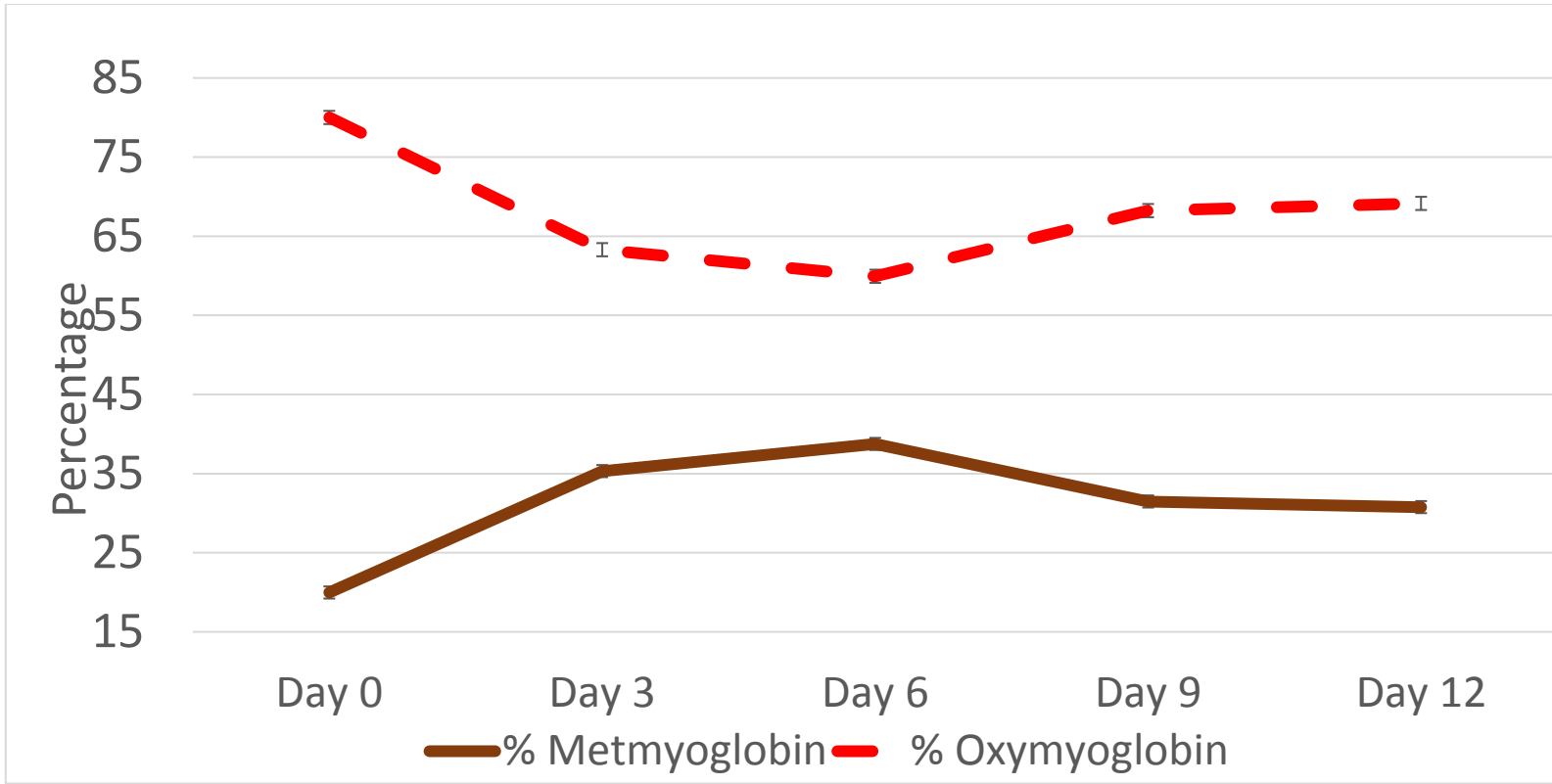
Figure J-2. Least Squares Means of Storage Day Effect for Myoglobin States.
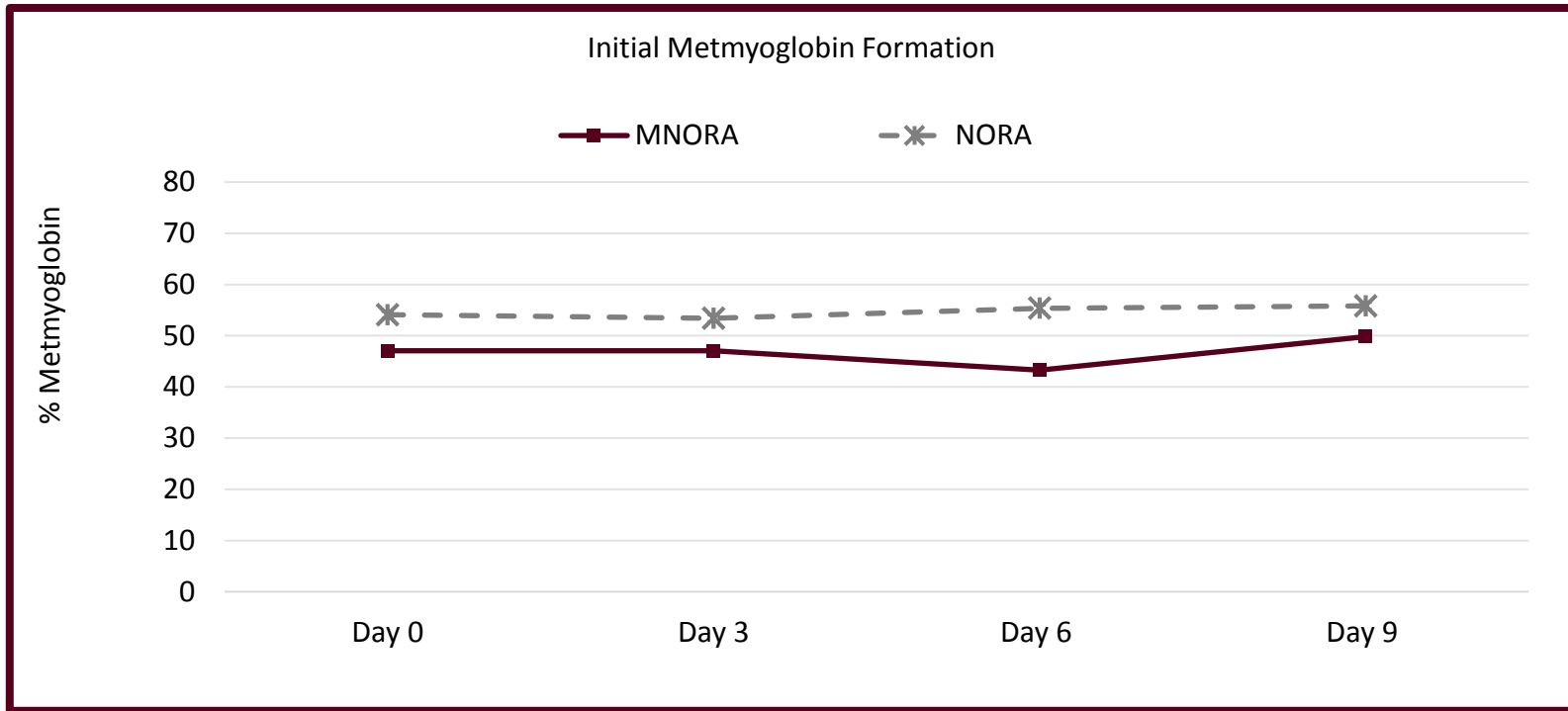
Figure J-3. Least Squares Means for Method by Storage Day Interaction of Initial Metmyoglobin Formation.
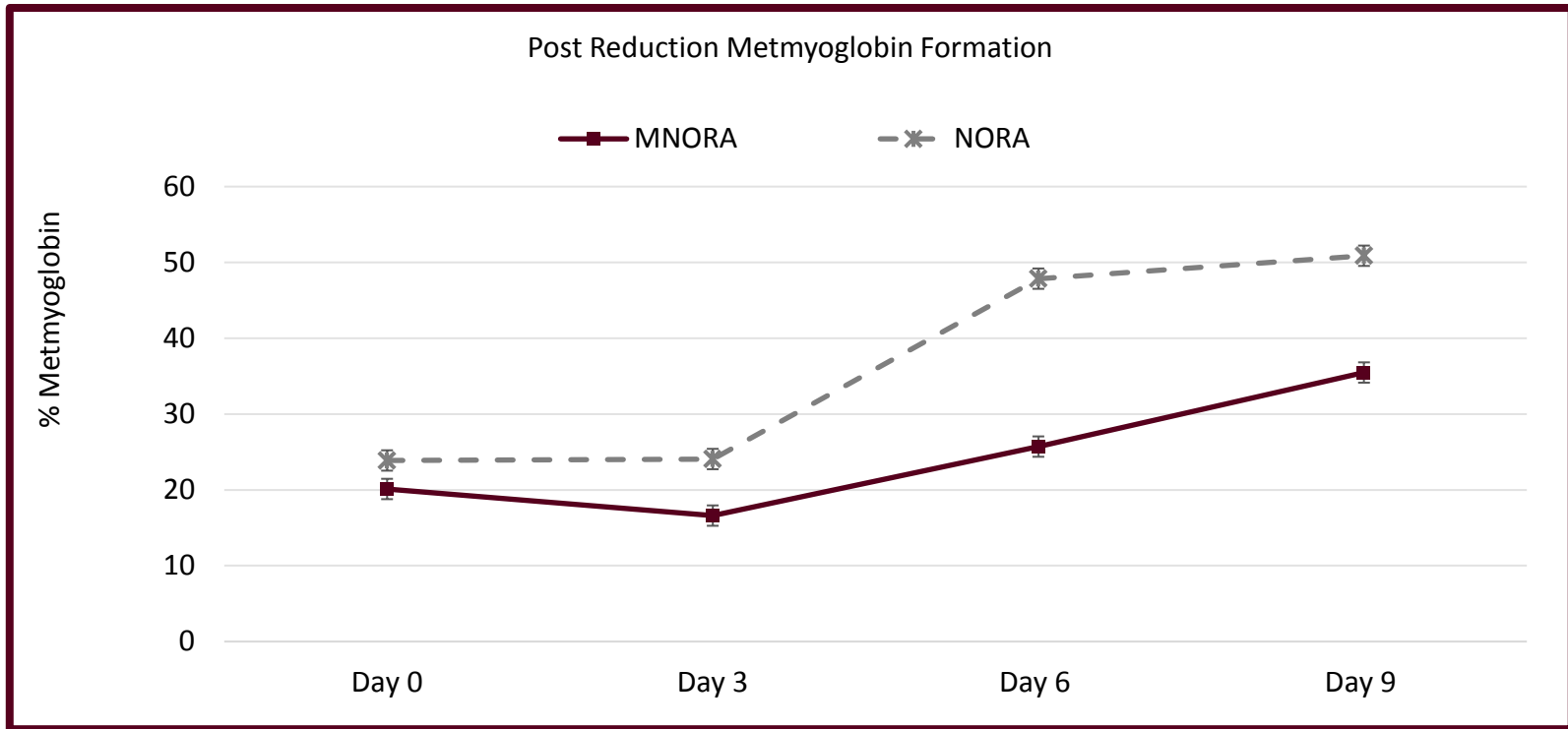
Figure J-5 Least Squares Means for Method by Storage Day Interaction of Post Reduction Metmyoglobin Formation.
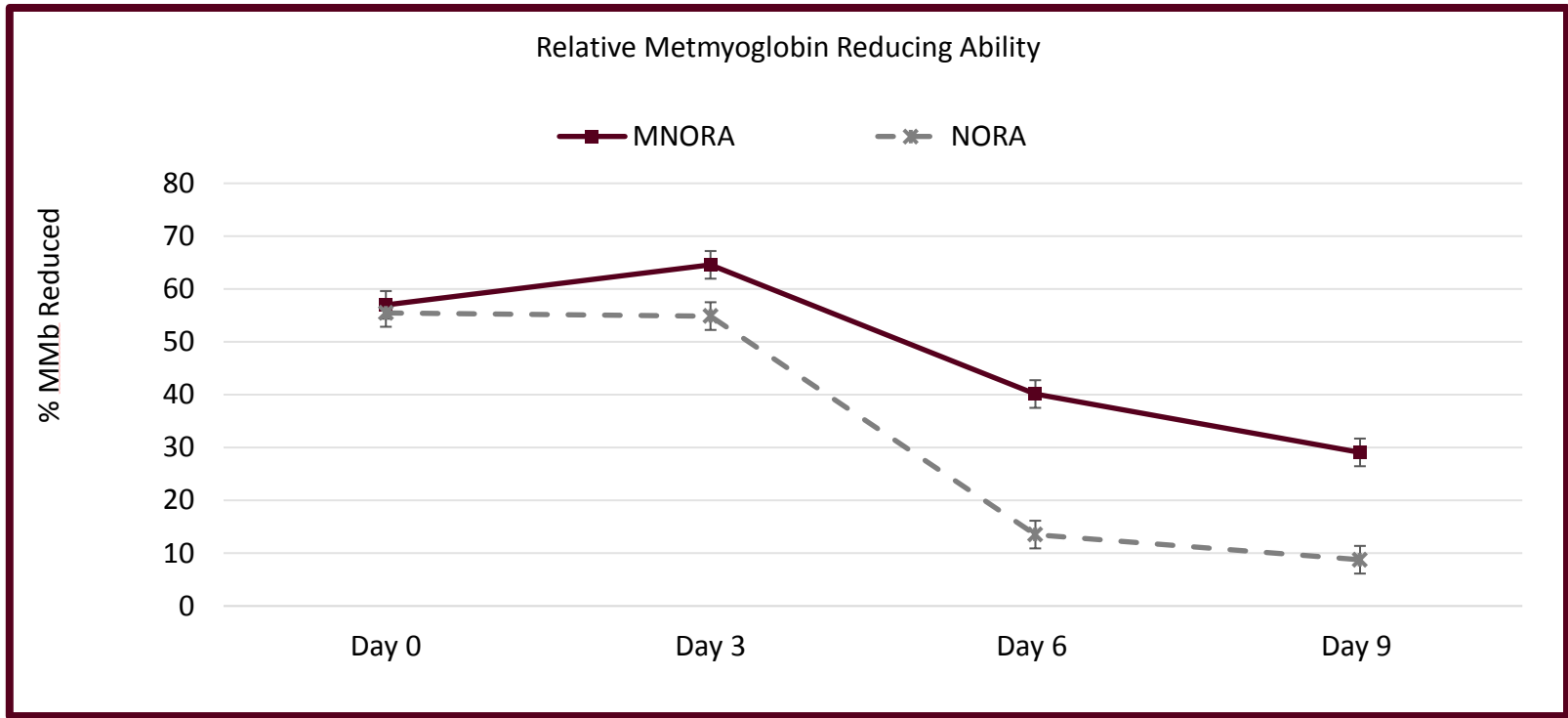
Figure J-6 Least Squares Means for Method by Storage Day Interaction of Relative Metmyoglobin Reducing Ability.
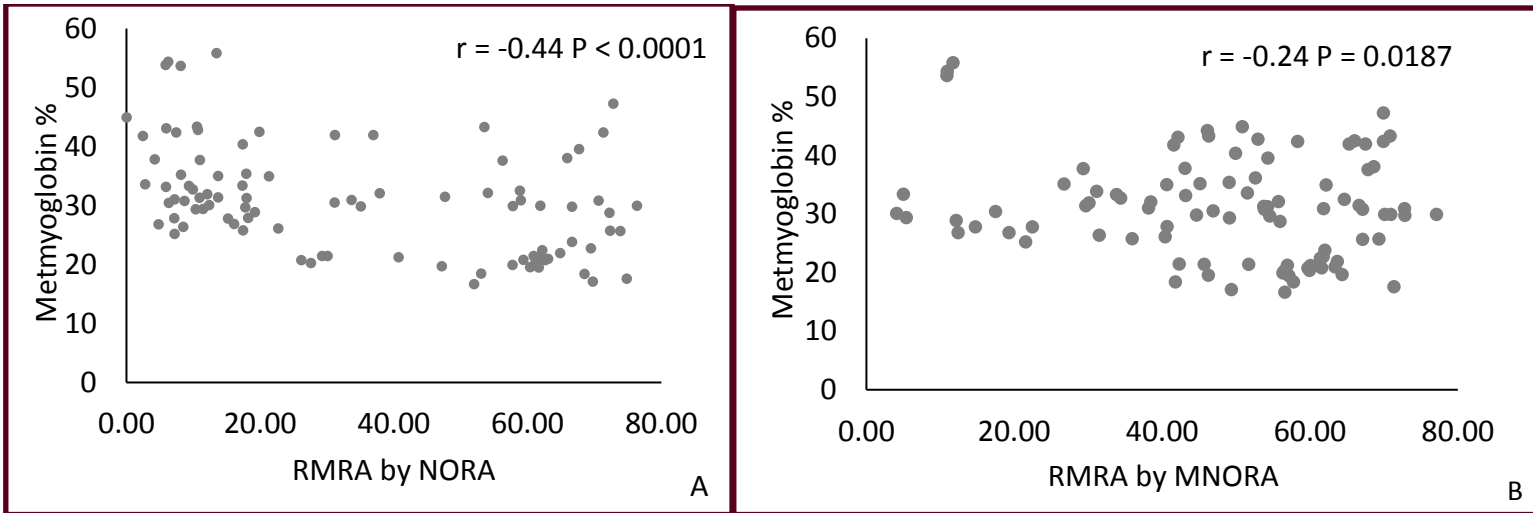
Figure J-7. Pearson Correlation Scatter Plot for Metmyoglobin % and RMRA determined by NORA (A) and RMRA determined by MNORA (B)..
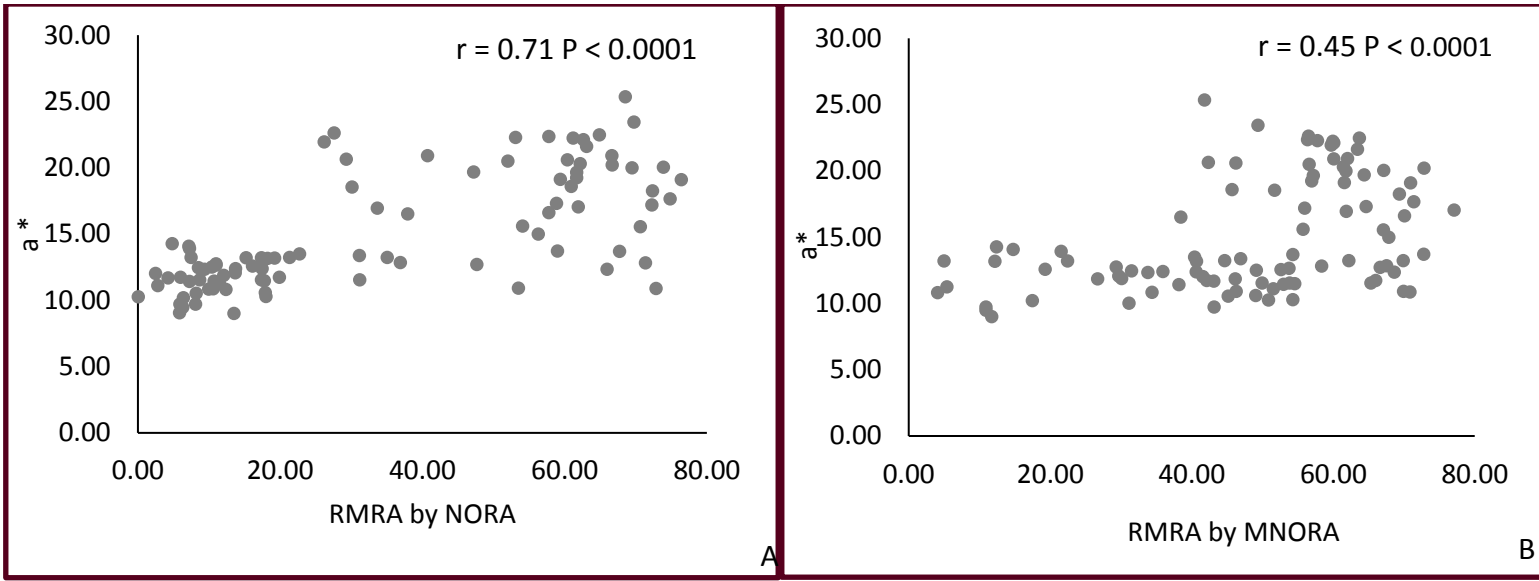
Figure J-8. Pearson Correlation Scatter Plot for a* Values and RMRA determined by NORA (A) and RMRA determined by

MNORA (B)..
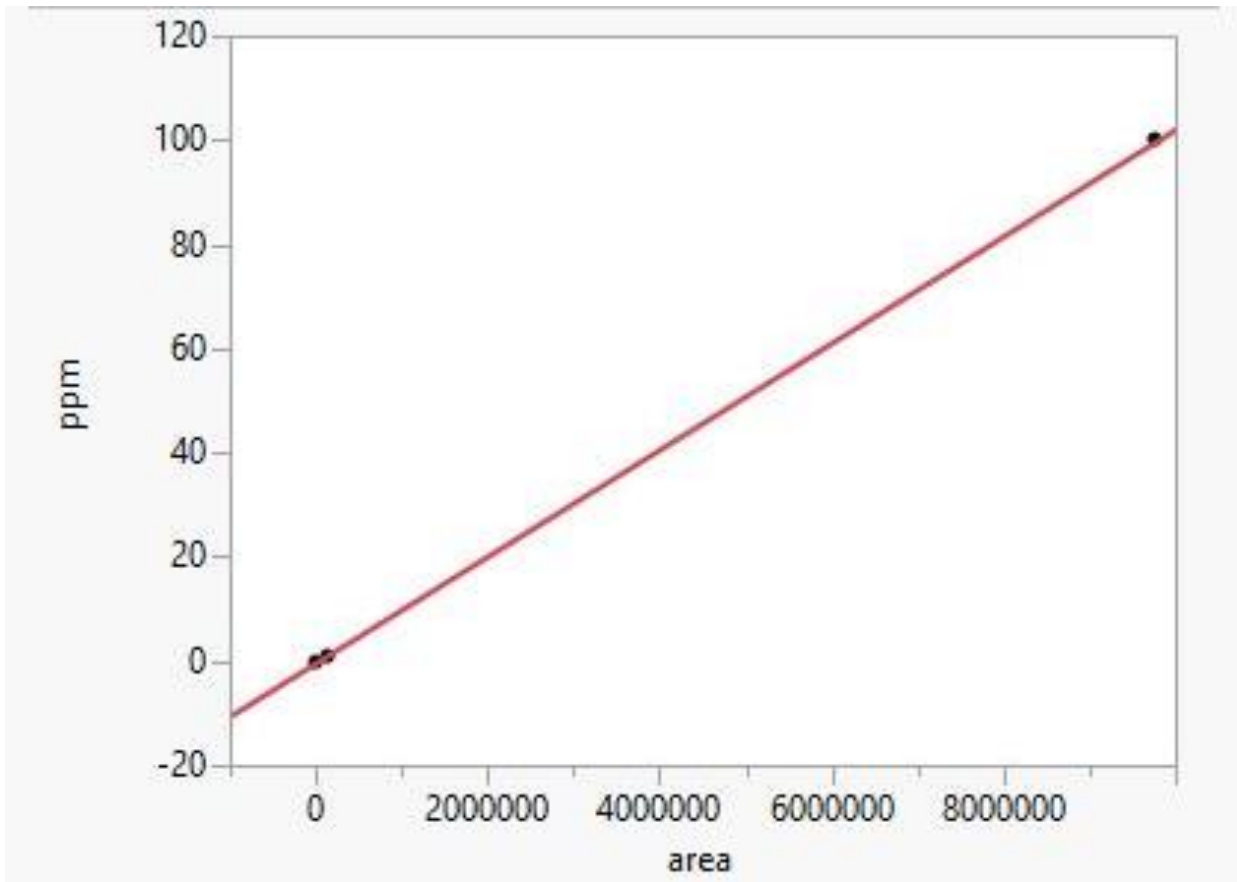
Figure J-9. External Standard Curve for 1,3 Dichlorobenzene. X axis is area (total ion count) from GC-MS and Y axis is concentration (ppm) of standard samples. Standard curve was used to estimate concentration of volatile compounds. 100,000 area equals an estimated 1 ppm.

Storage Day 0

Storage Day 3

Storage Day 6

Storage Day 9

Storage Day 12

Figure J-10. *Triceps brachii* over 12 days of aerobic storage at 5℃ under fluorescent lighting.

Figure J-11. Post incubation samples for MRA using NORA (top) method and MNORA (bottom) method.

APPENDIX K

DATA FROM CHAPTER III

EFFICACY OF SELECTED INGREDIENTS AT EXTENDING THE SHELF LIFE OF

AEROBICALLY STORED BEEF TRIMMINGS
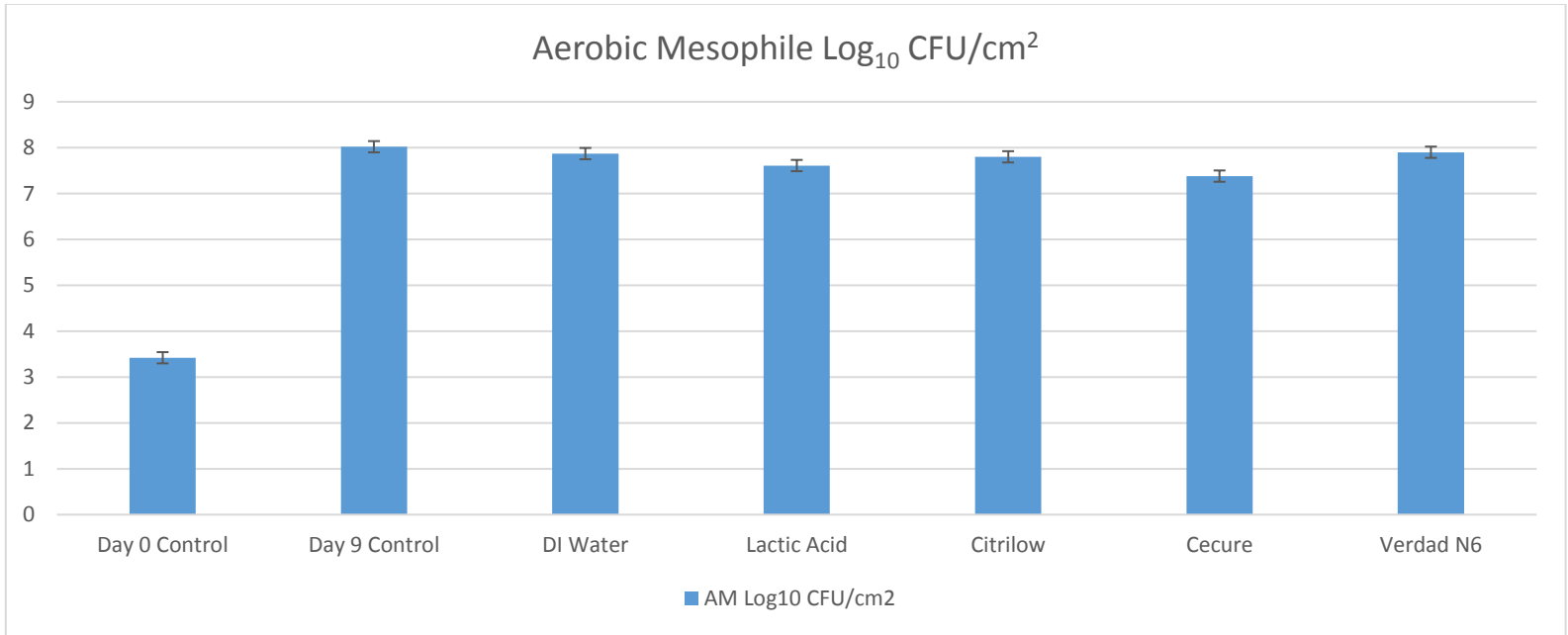
Figure K-1. Least Squares Means for Treatment Effect on Aerobic Mesophile Plate Counts..
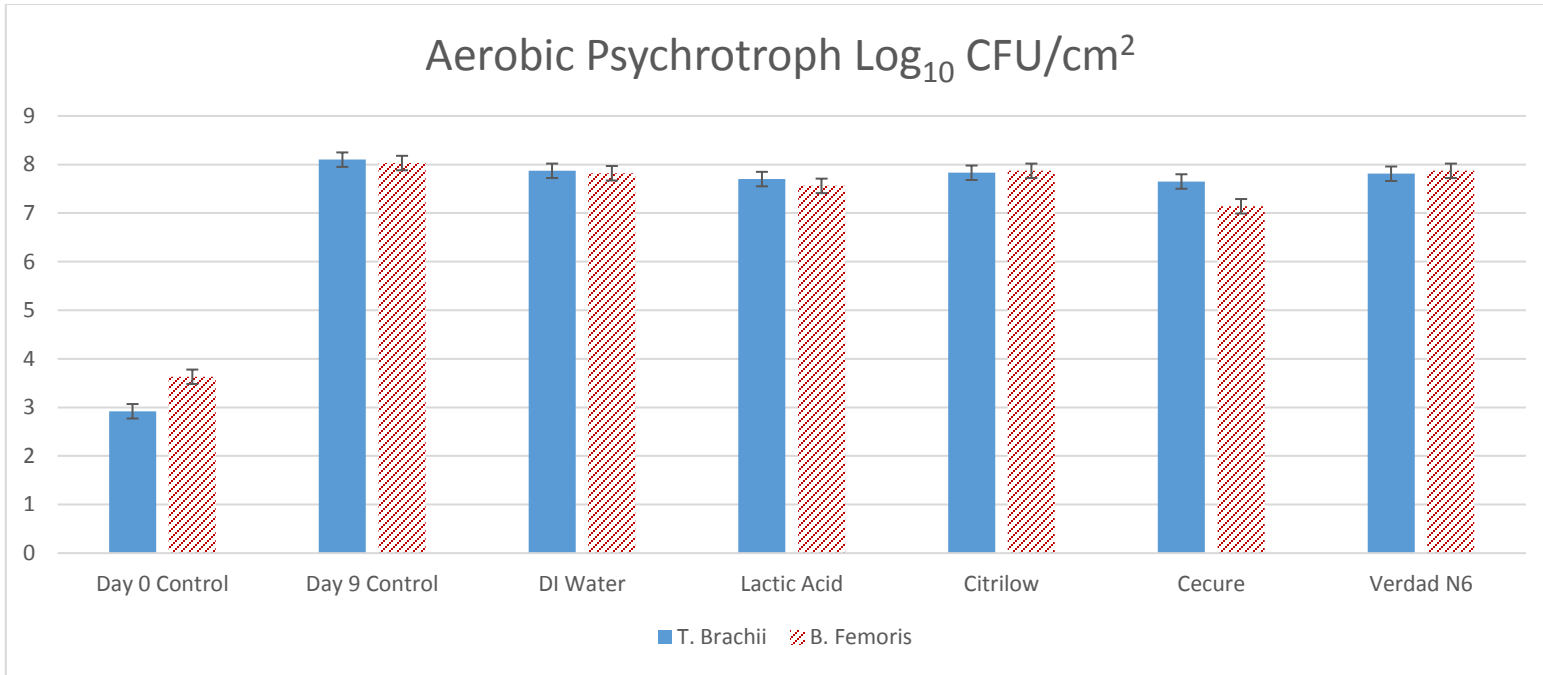
Figure K-2. Least Squares Means for Muscle by Treatment Interaction on Aerobic Psychrotroph Plate Counts.
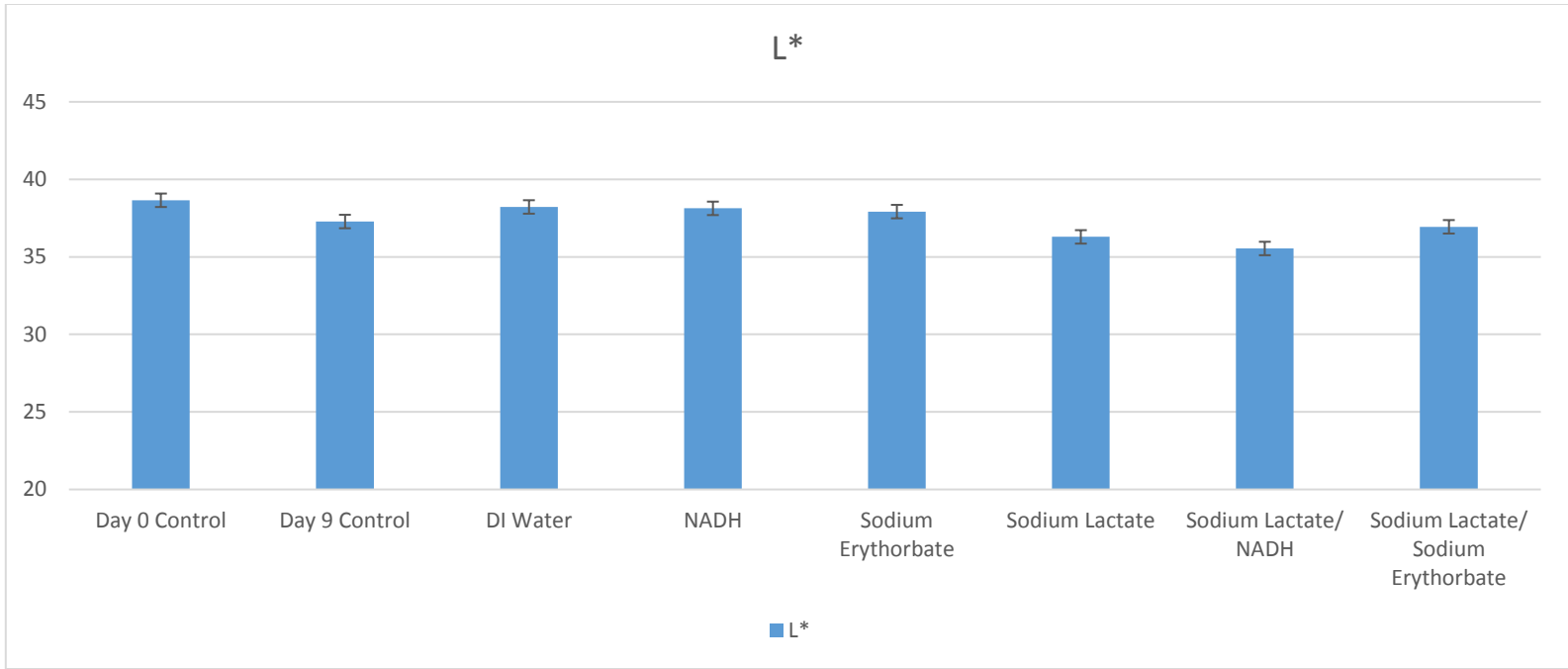
Figure K-3. Least Squares Means for Treatment Effect on L* Values of Treated Beef Trimmings Measured at 2 hours Post Treatment.
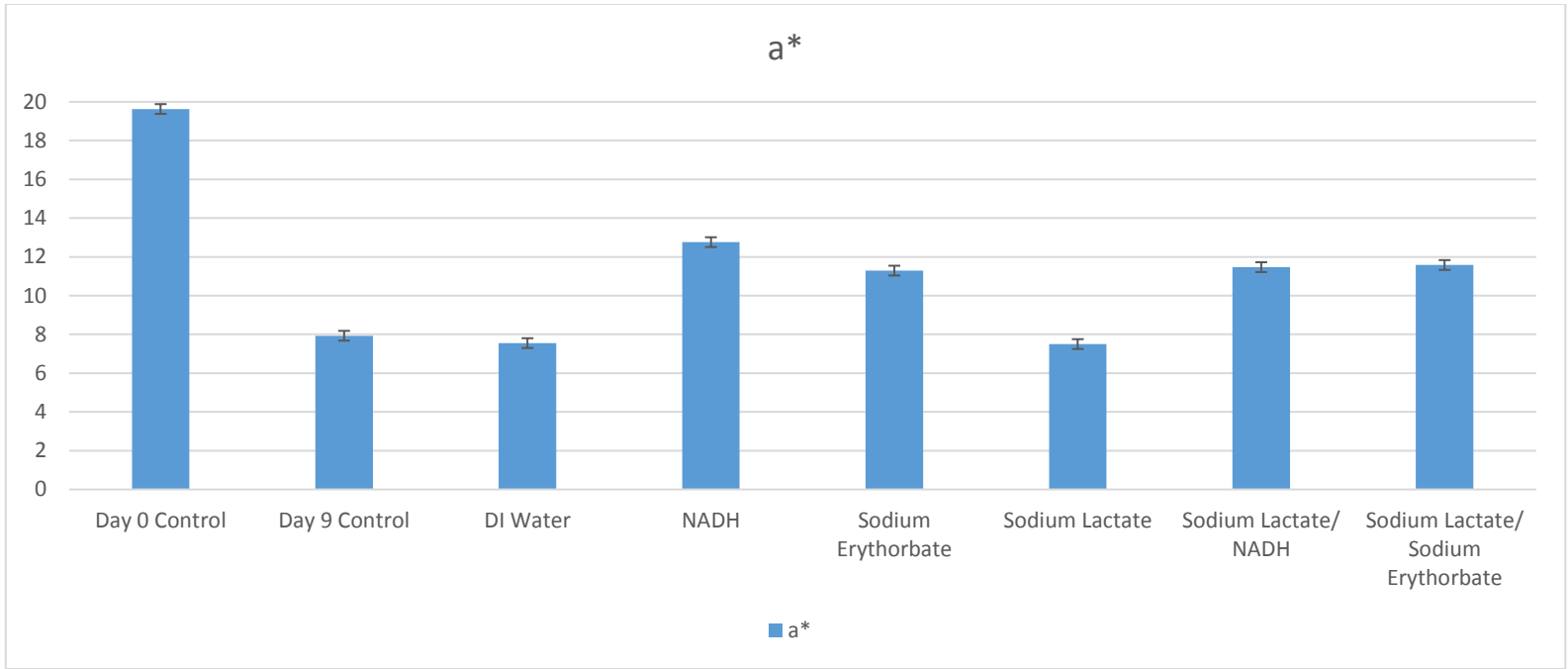
Figure K-4. Least Squares Means for Treatment Effect on a* Values of Treated Beef Trimmings Measured at 2 hours Post Treatment.
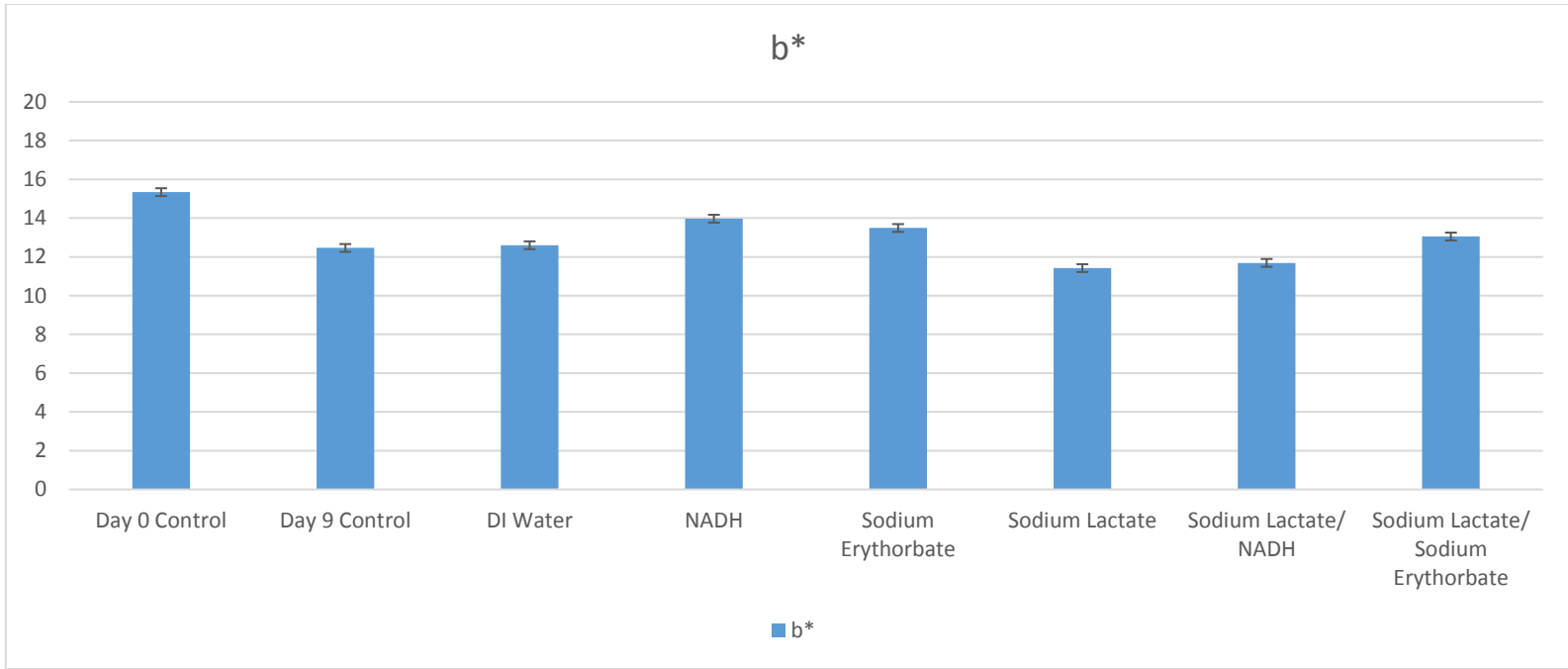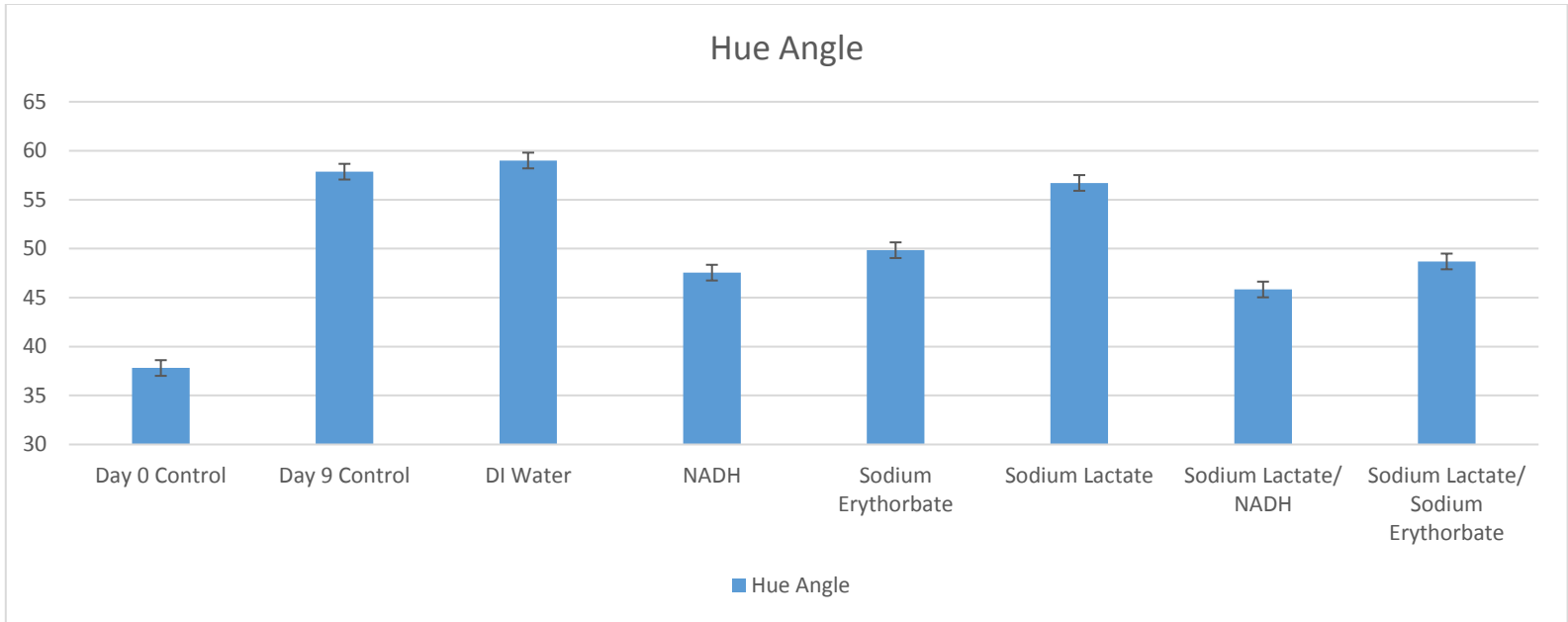
Figure K-5. Least Squares Means for Treatment Effect on b* Values of Treated Beef Trimmings Measured at 2 hours Post

Treatment.

Figure K-6. Least Squares Means for Treatment Effect on Hue Angle Values of Treated Beef Trimmings Measured at 2 hours
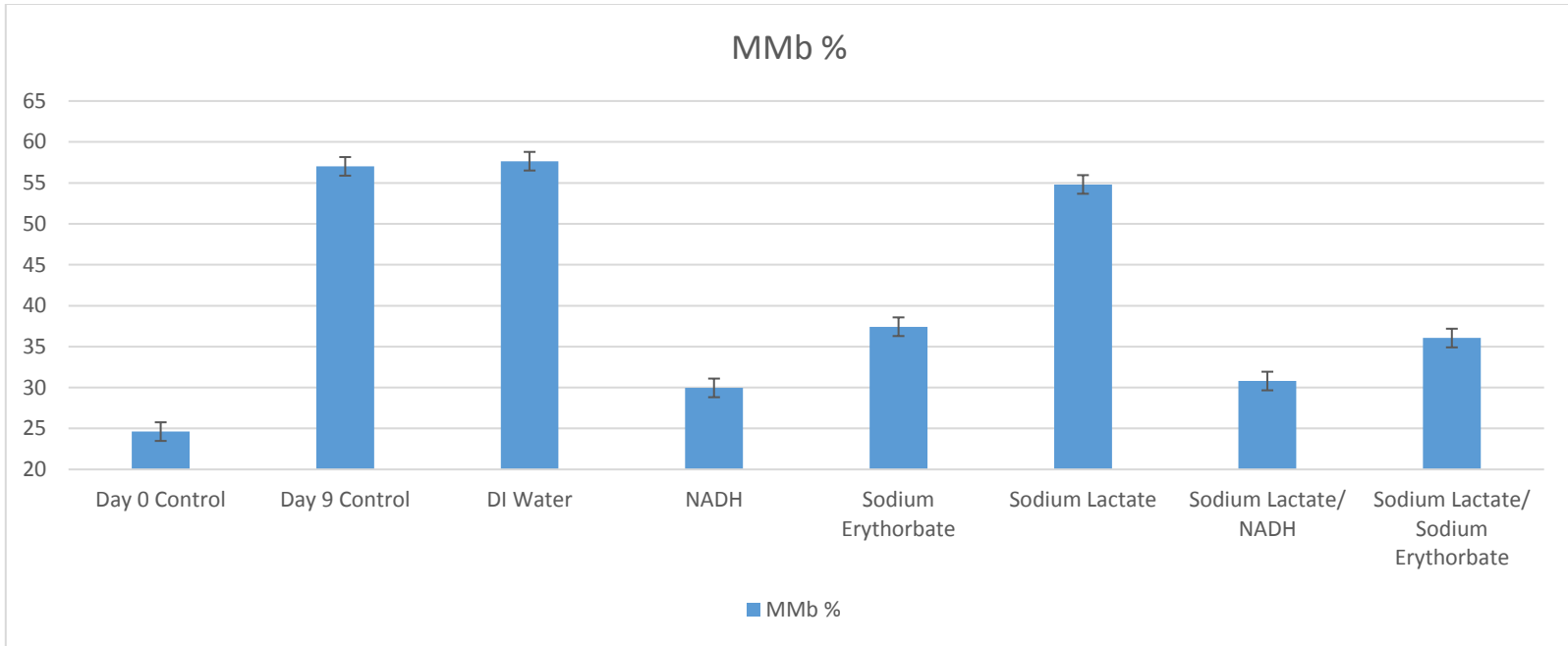
Post Treatment

Figure K-7. Least Squares Means for Treatment Effect on Metmyoglobin Percentage of Treated Beef Trimmings Measured at 2 hours Post Treatment.
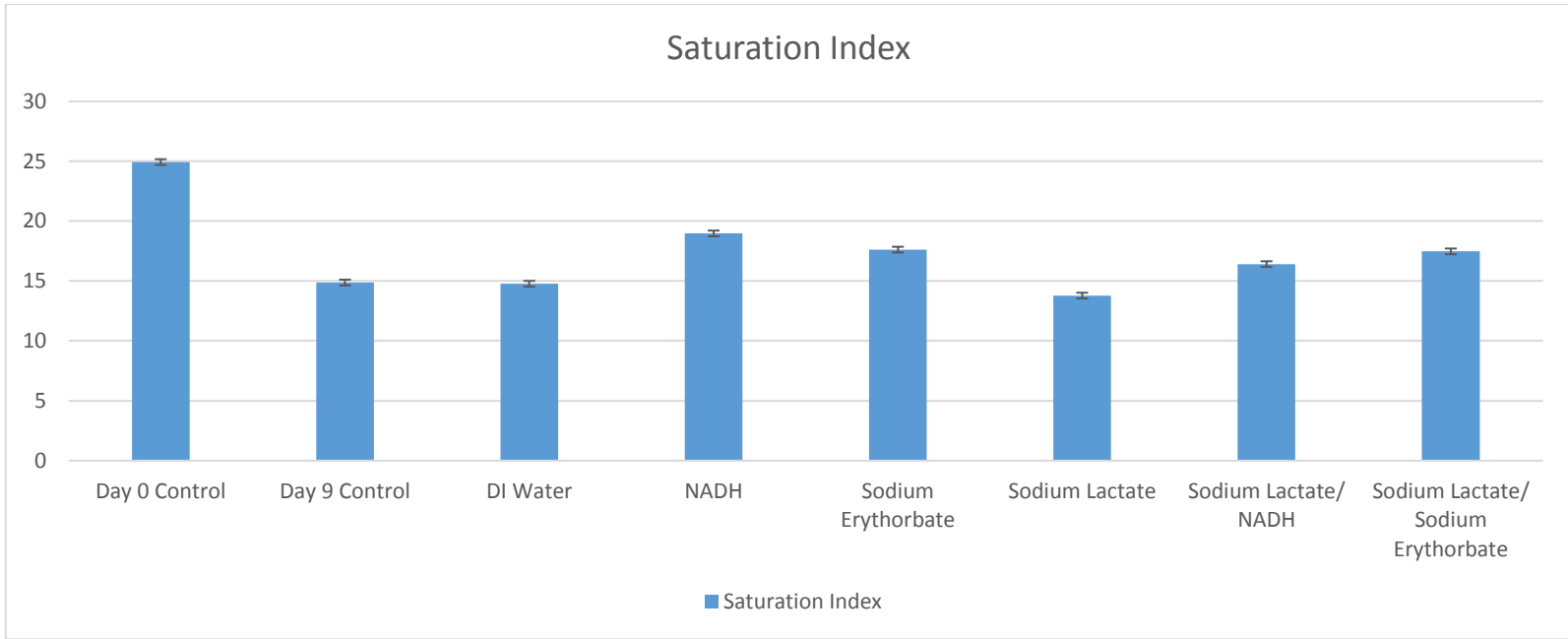
Figure K-8. Least Squares Means for Treatment Effect on Saturation Index Values of Treated Beef Trimmings Measured at 2 hours Post Treatment.
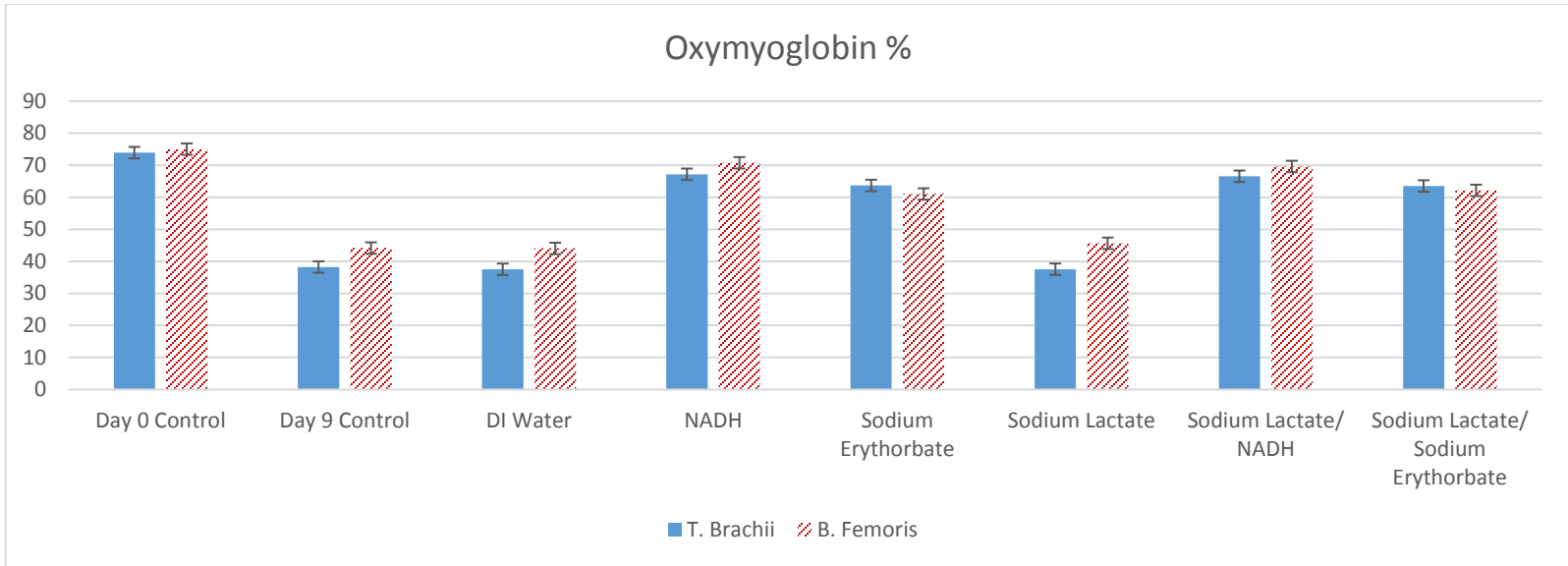
Figure K-9. Least Squares Means for Muscle by Treatment Interaction for Oxymyoglobin Percentage of Treated Beef

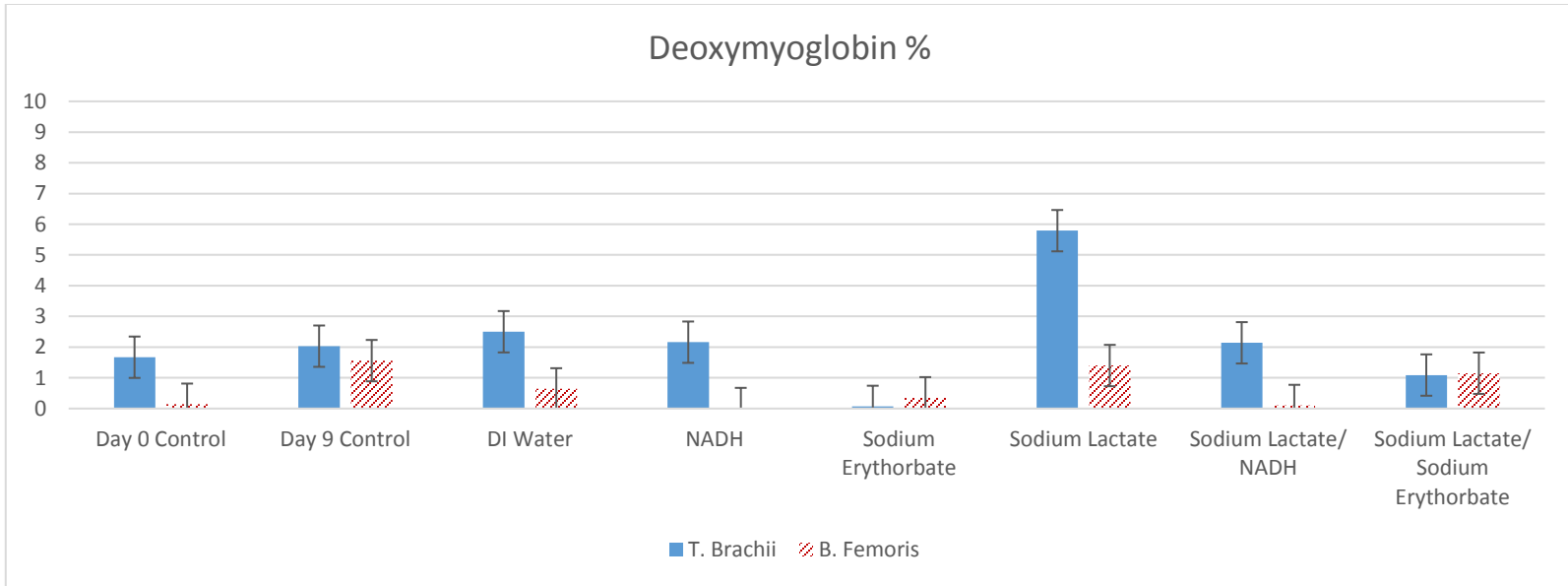Trimmings Measured at 2 hours Post Treatment.

Figure K-10. Least Squares Means for Muscle by Treatment Interaction for Deoxymyoglobin Percentage of Treated Beef Trimmings Measured at 2 hours Post Treatment.

	Pre Treatment	2 hr Post Treatment	24 hr Post Treatment	48 hr Post Treatment
DI Water				
NADH				
Sodium Erythorbate				

Figure K-10. *Triceps Brachii* treated with different solutions to improve color taken over 48 hours.

| | Pre Treatment | 2 hr Post Treatment | 24 hr Post Treatment | 48 hr Post Treatment |

Figure K-11. *Triceps Brachii* treated with different solutions to improve color taken over 48 hours.