DEVELOPMENT OF A PROCESSING AID CONTAINING SODIUM LACTATE, SODIUM ERYTHORBATE AND SODIUM BICARBONATE APPLIED TO BEEF TRIMMINGS TO ASSESS THEIR IMPACT ON QUALITY OF GROUND BEEF PATTIES

A Dissertation

by

CHI-HENG WU

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Chair of Committee,	Wesley N. Osburn
Committee Members,	Chris Kerth
	Roland R. Kaunas
	Thomas M. Taylor
Head of Department,	Cliff Lamb

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ABSTRACT

A significant portion of fresh beef trimmings are distributed aerobically under refrigeration from large beef fabrication plants to further processors to use as the raw material source for products such as beef patties. Time from fabrication to arrival at further processing facilities may be anywhere from 0 to 5 days, or longer due to inclement weather conditions. This variation impacts the overall condition (color, odor/flavor/spoilage) of these trimmings destined for further processing, i.e, beef pattie manufacture. The economic loss due to meat discoloration was estimated to be 1 billion/year. Most researches utilized single ingredient to address a specific problem, such as myoglobin oxidation, lipid peroxidation, etc. Hence, we proposed to develop a processing aid containing multiple selected ingredients to resolve the myoglobin oxidation, lipid peroxidation, off odor, and microbial spoilage which reduce the shelf life of aerobically stored beef trimmings. Phase 1 and 2 were conducted to investigate the effect of sodium lactate (NaL), potassium lactate (KL), sodium erythorbate (NaE), and sodium bicarbonate (NaB) on fresh beef trimmings for ground beef production within and exceeding federal regulations. For phase 1 and 2, a full factorial design was used to generate treatment combinations containing NaL (0.1 to 1.5 M), NaE (1 mM to 0.6 M), and NaB (0.1 to 1.5 M) with water used as a control in both studies. Based on phase 1 and 2, a 2^3 central composite response surface design (RSM), which generated 15 treatment combinations containing NaL (0.1 to 1.5 M), NaE (0.1 to 0.6 M), and NaB (0.1 to 1.5 M) with water used as a control was used to

optimize the concentration level of treatment combinations. All phases used the same the procedure for treatment preparation, sample preparation, treatment application, sample collection and analysis. Beef trimmings fabricated from 14-day-postmortem carcasses were aerobically stored for 6 days at 5 ^{o}C. After storage, beef trimmings were coarse ground, treated with various treatments, reground, and formed into beef patties wrapped with oxygen permeable films. The patties were stored under simulated retail conditions: 5°C, cool white fluorescent light (200 to 300 lux) and analyzed at day 0, 3, 6, and 9 of storage to assess the effectiveness of each treatment in preventing further quality deterioration. Objective color (L*, a*, and b*), 2-thiobarbituric acid (TBA) determinations, GC-MS for off-odor assessment and aerobic plate counts (APC) were measured. The treated beef patties with NaB (1.5 M), NaE (0.1 to 0.8 M) and NaL (1 M) had the greatest effect on enhancing the color stability, reducing the lipid peroxidation and controlling the microbial spoilage compared to the control upon storage day 6 for phase 1 and 2, respectively. Based on these results, we optimized the concentration levels of NaL, NaE, and NaB which were active ingredients using response surface methodology (RSM) to develop a processing aid. The treated beef patties showed improved a* values, MMb formations, TBA values and total ion counts of hexanal compared to the control upon storage day 6 ($P < 0.05$). Based on the results, a* and TBA values were used to conduct multivariate RSM analyses for day 3 and 6. The predicted value of hexanal was 0 for all treatments. Multivariate RSM was conducted to overlap the contour plots of predicted a* and TBA

values at day 3 and 6 to better approximate the optimal ingredient concentrations for a* values. The optimum concentration ranges of solutions based on this analysis were NaL (0.43 to 0.57 M), NaE (0.35 M) and NaB (1 M) with predicted a* values > 11 and TBA values < 0.52. Results of the research suggested that a combination of NaL, NaE and NaB (0.43 to 0.57, 0.35 and 1 M, respectively) could be applied into ground beef patties generated from aerobically 6-days-stored (5 ^{o}C) beef trimmings to improve color stability, reduce lipid oxidation and mitigate off-odor upon 6 days storage with retail display condition.

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The GC-MS analysis for Chapter II and III was provided by Professor Chris Kerth and conducted by Kayley Wall.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Economic impact of fresh meat color and its discoloration

The gross value of global beef production is 241 billion US dollars per year (FAO, 2014). The discoloration of beef raw materials/retail products leads to economic loss, estimated to be more than $1 billion annually in the United States (Smith *et al.*, 2000). Color and its stability dominantly affect the marketability of beef because it is the first quality attribute evaluated by the consumer during purchase (Faustman & Cassens, 1990; Troy & Kerry, 2010). When consumers are choosing fresh retail beef products, they expect to see either a bright-cherry-red color (oxymyoglobin) or purplish-red color (deoxymyoglobin) of vacuum-packaged beef as indicators of product freshness and wholesomeness. Discolored (brown; metmyoglobin) beef products are often sold at discounted prices or if the discoloration is extensive it is discarded. Therefore, fresh meat color and its ability to maintain color (color stability) are the most important quality attributes of meat affecting consumer purchase decisions (Troy & Kerry, 2010; Font-i-Furnols & Guerrero, 2014).

Although vacuum or modified atmosphere packaging (MAP) of fresh meat can aid in alleviating meat discoloration, a significant portion of raw materials (fresh beef trimmings), for further processing into bulk or ground beef patties, is placed in plastic lined cardboard bins designed to hold approximately 1000 kg of product. A plastic

overwrap covers the combo bin to prevent hazards from coming into contact with the product during refrigerated transport. Under these conditions beef trimmings are exposed to oxygen during transport and storage until used for product manufacture. The time from fabrication to arrival at further processing facilities may be anywhere from 0 to 5 days or longer due to inclement weather conditions. This variation impacts the overall condition (color, odor, flavor, and spoilage) of these trimmings destined for further processing, i.e., beef patty manufacture. Processors, through inventory control, must decide whether combo bins of aerobically-stored fresh beef trimmings can be used, should be diverted to cooked products, discarded, or returned. If used to manufacture fresh beef product such as beef patties, they are packaged either fresh (MAP packaging) or frozen (boxed) and transported to distribution centers/retailers before being placed into retail counters for purchase. Fresh beef trimmings of selected beef muscles begin to exhibit signs of deterioration color, odors and increased microbial spoilage at 6 days of aerobic storage (Yeater, 2016). Trimmings that arrive at a further processing plant 5 to 6 days after fabrication and are processed into patties may exhibit decreased shelf life during retail display and may be discounted or discarded earlier due to poor color or odor (Yeater, 2016). Hence, there is a need for research to address this issue.

1.2 Myoglobin

1.2.1 The functionality and structure of myoglobin in muscle

Myoglobin, a water-soluble protein, is located within the muscle and determines the color of meat. It consists of 154 amino acids with a molecular weight of 17 kDa and is responsible for transporting oxygen to muscle in living organisms. Myoglobin not only enhances the diffusion of oxygen from the extracellular space to the mitochondria but also provides oxygen influx to mitochondria under low oxygen partial pressure (Bekhit & Faustman, 2005; Wittenberg & Wittenberg, 1989).

sThe three-dimension structure of myoglobin was first identified by X-ray crystallography with the resolution at 6 Å (Kendrew *et al.*, 1958). The structure of myoglobin consists of 8 α-helices that surround a central pocket heme prosthetic group containing an iron atom (Suman & Joseph, 2013; Khoshouei *et al.*, 2017; Phillips, 1980; Figure I-1) . The iron atom is bound within the center of the heme group by four of the iron atom's six coordination sites, the 5^{th} site of iron attaches to a proximal histidine residue (His-93) and the 6^{th} site provides reversible binding to ligands, including water, oxygen, etc. (Lehninger *et al.*, 2013).

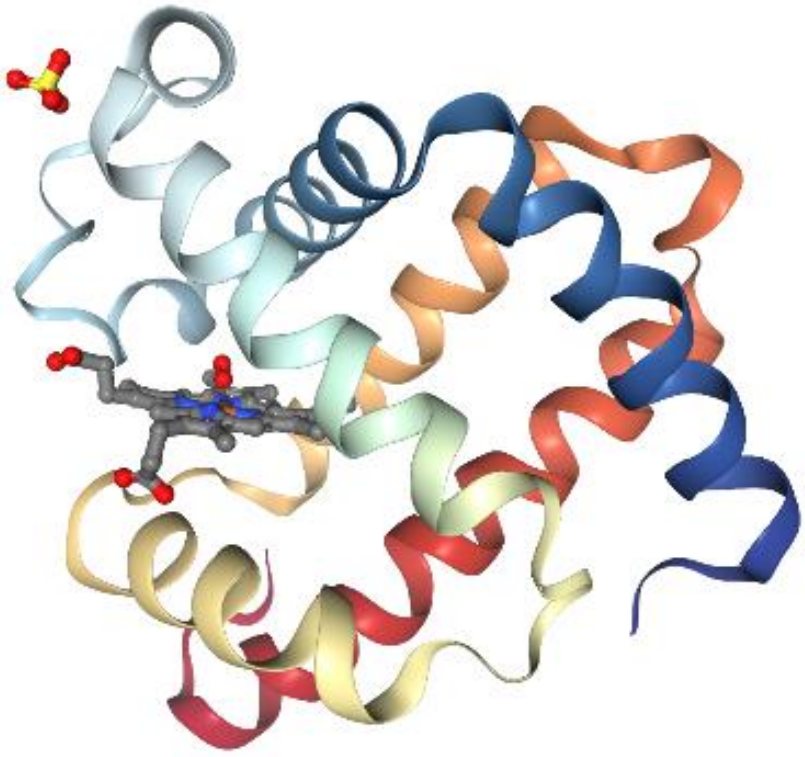

Figure I-1: Structure of oxymyoglobin
The figure is generated through biological macromolecular structures enabling breakthroughs in research and education protein data bank (1MBO; Phillips, 1980). The 8 α-helices surround a central pocket heme prosthetic group containing an iron atom which binds an oxygen molecule.

1.2.2 The mechanism of meat color

Meat color is predominantly determined via a dynamic conformational change between three major chemical forms of myoglobin, deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb; Faustman & Cassens, 1990; AMSA, 2012). Basically, the color of meat is due to the light scattering and light absorption properties of different forms of myoglobin. The color of beef containing abundant amounts of DMb, OMd and MMb is purplish, bright cherry-red and brown, respectively.

The dynamic conformation change of myoglobin involves the valence state of iron atom and the interaction between the iron atom with other ligands.

The color of vacuum-packaged (oxygen removed, oxygen impermeable film) fresh meat is a dark purplish color due to the abundance of DMb at the exposed fresh meat surface as a result of a low oxygen tension environment (<1.4 mm Hg). Deoxymyoglobin contains ferrous iron (Fe^{2+}) with a vacant binding site (no ligand attached; Suman *et al.*, 2014), and results in a dark purplish color on the surface of fresh meat. After removing the vacuum package, the blooming effect will change the meat color from purplish color into a cherry-red color. The OMb is generated in the blooming effect when diatomic oxygen from the atmosphere occupies the 6^{th} site of ferrous iron in the DMb (path 1 in Figure I-2). The blooming effect (myoglobin oxygenation) is affected by partial pressures of oxygen, time, temperature, pH, and competition for oxygen by mitochondria (Suman & Joseph, 2013). The deoxygenation of myoglobin is favored under low-oxygen partial which is mostly caused by the oxygen consumption of mitochondria. Theoretically, DMb can be immediately converted into OMb with re-oxygenation (path 1 in Figure I-2; AMSA, 2012); however, DMb is often readily oxidized into MMb by free radicals and reactive oxygen species, mainly hydrogen peroxide (path 2 in Figure I-2) . Metmyoglobin contains ferric iron oxidizing from ferrous iron and has its 6^{th} site of ferric iron occupied by water or nothing. The living muscle cells can reduce the MMb back into DMb via the metmyoglobin reducing system (path 3 in Figure I-2).

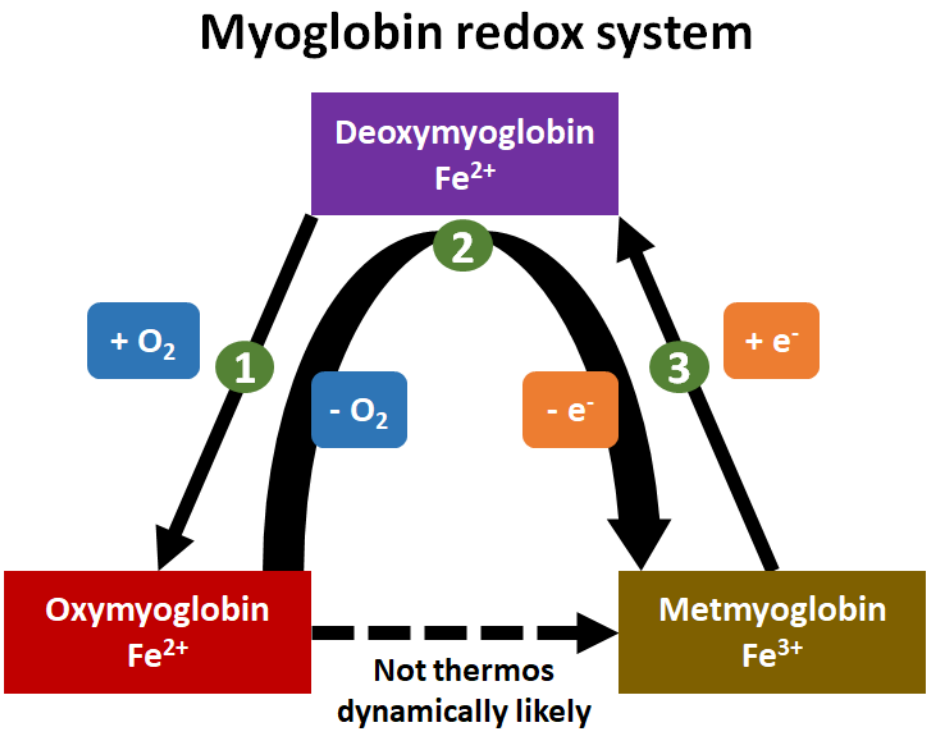

Figure I-2: Schematic of the interconversions of myoglobin redox forms in fresh meat
Path 1: DMb can be immediately converted into OMb with re-oxygenation under appropriate partial oxygen pressure. Path 2: Upon losing oxygen, DMb is often readily oxidized into MMb by free radicals and reactive oxygen species, mainly hydrogen peroxide. Path 3: MMb is reduced back into DMb by metmyoglobin reducing system or antioxidant (electron donor). This was adapted from AMSA, 2012.

In conclusion, the fresh meat color is governed by the amount of the three derivatives of myoglobin, including DMb, OMb, and MMb, and the dynamic change of myoglobin is affected by the other factors, such as metmyoglobin reducing system, lipid oxidation, and microbial spoilage (Suman *et al.*, 2014). Hence, controlling the dynamic change of myoglobin is one of the most attractive strategies to improve the color stability of meat.

1.2.3 Antioxidant system and myoglobin oxidation in muscle

Antioxidants can be categorized into different subgroups depending on the source, functionality, enzymatic or non-enzymatic, and natural or synthetic (Pisoschi & Pop, 2015; Yashin *et al.*, 2017). Antioxidants can be endogenously produced by the cells and exogenous acquired mainly through ingestion. The functionalities of antioxidants can be described as 1) scavenging free radicals or radical compounds, 2) chelating metal ions and 3) removing or repairing damaged/oxidized molecules. Enzymatic antioxidants are mostly endogenous and have multiple functions in alleviating oxidative stress.

Metmyoglobin cannot carry oxygen and can cause further oxidative stress by interacting with other proteins or lipids in the live cells (Faustman *et al.*, 2010). The formation of MMb is intervened mainly by the metmyoglobin reducing system in muscle (Faustman & Cassens, 1990). The metmyoglobin reducing system generates DMb by reducing MMb via a series of redox reactions and is classified into two antioxidant systems - enzymatic and non-enzymatic. The enzymatic antioxidant system utilizes NADH-cytochrome b_5 MMb reductase (enzyme), cytochrome b_5 (intermediate) and NADH (cofactor/electron donor). The non-enzymatic system employs an exogenous antioxidant (electron donor) to reduce MMb into DMb (Figure I-3; Bekhit & Faustman, 2005; Arihara *et al.*, 1995; Seideman *et al.*, 1984).

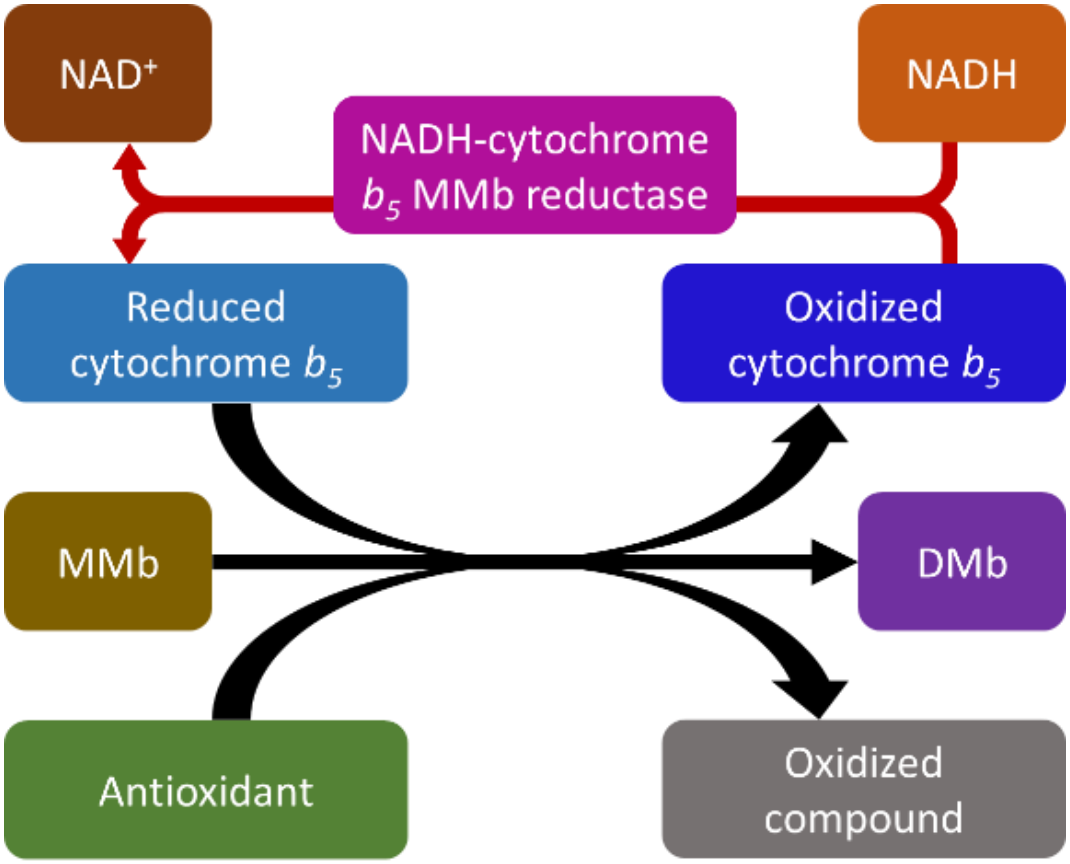

Figure I-3: Metmyoglobin reducing pathway
The pathway is classified into enzymatic system (upper part) and non-enzymatic system (lower part). Adapted from Faustman & Cassens, 1990 and original artwork by author.

1.3 Lipids

1.3.1 The functionality and structure of lipid in muscle

Lipids display a wide diversity in structure and play an important functional role in cells. Fatty acids comprise the fundamental structure of lipids and are classified as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA). Most natural fatty acids are composed of long aliphatic carbons chain with an even number between C_4 and C_{28}.

When stored in an adipocyte, three fatty acids are combined with a glycerol to form triglycerides, which are the main form of lipids (Bayly, 2014; Klein & Romijn, 2016). Another form of lipids are phospholipids which form the phospholipid-bilayer of cell membranes and cellular organelles (Edidin, 2003). Free fatty acids can be utilized as an energy source through mitochondria-beta oxidation (Schulz, 1991). The beta-oxidation is initiated by transporting fatty acids from the cytosol into the mitochondrial intermembrane space by long-chain acyl-coenzyme A (acyl-CoA) synthetase (ACS), which is located on the outer membrane of mitochondria (Schulz, 1991). During transportation, the fatty acid is combined with Coenzyme A (CoA) into fatty acyl-CoA by ACS with ATP consumption (Schulz, 1991). The CoA of fatty acyl-CoA is replaced by carnitine to form fatty acyl-carnitine by carnitine palmitoyl transferase I (Schulz, 1991). The fatty acyl-carnitine is transferred from the intermembrane space into the matrix of the mitochondria by carnitine-acylcarnitine translocase, and then the carnitine palmitoyl transferase II will convert fatty acyl-carnitine back into fatty acyl-CoA (Schulz,

1991). In the mitochondrial matrix, fatty acyl-CoA will go through a series of metabolic processes and eventually become beta-ketoacyl-CoA which can be further cleaved into shorter fatty acyl-CoA and acetyl-CoA catalyzed by β-ketothiolase with the addition of another CoA. The shorter fatty acyl-CoA can be used in the next beta-oxidation and the acetyl CoA can be utilized in the citric acid cycle to produce energy.

1.3.2 The mechanism of lipid peroxidation in muscle and meat

Lipid peroxidation is a chain reaction initiated by a free radical, such as reactive oxygen species (ROS). Reactive oxygen species commonly exist in cells as a hydroxyl radical, superoxide anion, or hydroperoxyl radical. Initiation, propagation and termination are three principal events that comprise lipid peroxidation (Min & Ahn, 2005; Barclay, 1993; Gardner, 1989).

The initiation of lipid peroxidation begins when a lipid fatty acid (LH) becomes a lipid radical ($L^{\bullet}$; hydrogen abstraction) by interacting with a ROS, mainly a hydroxyl radical ($HO^{\bullet}$; Reaction 1 in Figure I-4). Following initiation, the lipid radical will interact with oxygen and produce a lipid peroxyl radical ($LOO^{\bullet}$; Reaction 2 in Figure I-4). The lipid peroxyl radical will then interact with another intact fatty acid to from a lipid radical hydroperoxide (LOOH; Reaction 3 in Figure I-4). These last two reactions are known as propagation. Noteworthy, lipid hydroperoxide can be readily broken down through β-scission into a wide range of ketones, aldehydes, and other volatiles (Reaction 4 in Figure I-4; Gardner, 1989). The termination steps are very complicated and cannot be simplified or expressed as a reaction. It is defined that the end product

of the chain reaction are non-radical products. Mostly, termination is achieved by an enzymatic system, such as peroxidase, dismutase, etc., in cells (Bhabak & Mugesh, 2010).

$LH + ROS \rightarrow L^{\bullet} + \text{reduced compound}$ (Reaction 1)

$L^{\bullet} + O2 \rightarrow LOO^{\bullet}$ (Reaction 2)

$LOO^{\bullet} + LH \rightarrow L^{\bullet} + LOOH$ (Reaction 3)

$LOOH \rightarrow L^{\bullet} + LO$ (Reaction 4)

Figure I-4: Lipid peroxidation
The reaction 1 is the initiation. The reaction 2 to 4 is propagation. Adapted from Gardner, 1989.

Theoretically, polyunsaturated fatty acids (PUFA) are more susceptible to the lipid peroxidation than saturated fatty acids (SFA) due to lower carbon-hydrogen (C-H) bond dissociation energy. The peroxidation of PUFA is demonstrated by the rate of peroxidation of the lipid relative to the content of the bis-allylic C-H bond and the amount of available radicals *in vitro* (Gardner, 1989; Buettner, 1993). The weakest C-H bond dissociation energy is 75 to 80 kcal/mol at the bis-allylic methylene position compared to those at alkyl and allylic methylene positions (101 and 88 kcal/mol, respectively; Gardner, 1989; Koppenol, 1990). The hydroperoxyl radical with linoleic, linolenic and arachidonic acids have reaction rate constants of 1.2×10^3, 1.7×10^3 and 3.1×10^3 $M^{-1}s^{-1}$ and the numbers of bis-allylic methylene groups as 1, 2 and 3,

respectively (Choe & Min, 2006; Min & Ahn, 2005). Therefore, the amount of bis-allylic methylene in PUFA controls the rate of lipid oxidation in vitro. The cellular lipid peroxidation elevated as the availability of bis-allylic methylene increased (Wagner *et al.*, 1994). This evidence showed that the rate of lipid peroxidation in muscle/cells are positively correlated with the amount of the PUFA *in vivo*.

Thermodynamically, the lipid can directly react with singlet oxygen to form the lipid peroxyl radical in cells/muscle, but the hydrogen abstraction of PUFA requires less initial energy than this reaction (Buettner, 1993). Hence, it is important to remember that a reaction that is thermodynamically possible may not be kinetically feasible (Buettner, 1993).

In the presence of oxygen, unstable lipid radicals tend to react with oxygen to form a lipid peroxyl radical. Otherwise, the lipid radical can interact with other molecules to change the carbon double bond from the cis to the trans form and result in the formation of an abnormal conjugated diene. The lipid peroxyl radical with a higher standard reduction potential (+1.0 V) compared to PUFA can oxidize the adjacent PUFA into a lipid radical and become a lipid hydroperoxide. This lipid radical can be used in reaction 2 (Figure I-4) and continue the chain reaction.

Lipid hydroperoxide is an important non-radical intermediate of lipid peroxidation. Due to its polar properties, it can react with various molecules such as PUFA, oxygen, ferrous Fe compounds (especially myoglobin), and protein. Lipid hydroperoxides can react with either Fe (II) complex or Fe (III) complex to produce $LO^{\bullet}$

or LOO•, respectively (Reactions 5 & 6 in Figure I-5). The occurrence of reaction 5 is higher than the one of reaction 6 (Garnier-Suillerot *et al.*, 1984; Davies, 1989). This evidence supports the conclusion that the lipid oxidation could possibly facilitate myoglobin oxidation.

$$LOOH + Fe\ (II)\ complex \rightarrow Fe\ (III)\ complex + OH^- + LO\bullet \quad \text{(Reaction 5)}$$

$$LOOH + Fe\ (III)\ complex \rightarrow Fe\ (II)\ complex + H^+ + LOO\bullet \quad \text{(Reaction 6)}$$

Figure I-5: Lipid hydroperoxide and iron complex reactions
The lipid hydroperoxide can react with either Fe (II) complex or Fe (III) complex to produce LO• or LOO•. Adapted from Gardner, 1989.

1.3.3 Antioxidant system mitigates lipid peroxidation in muscle

Glutathione peroxidase (GPx), a selenocysteine-containing protein, is one of the major mammalian selenoenzymes that contain selenocysteine (E-SeH) at its active site (Flohe *et al.*, 1973; Bhabak & Mugesh, 2010; Niki *et al.*, 2005). The main biochemical function of glutathione peroxidase is to reduce hydroperoxides and lipid hydroperoxides to their corresponding water and alcohols. The catalytic mechanism of GPx to terminate lipid peroxidation is quite complex and involves the coenzyme Glutathione (GSH) and glutathione disulfide (GSSH). Using hydrogen peroxide (H_2O_2) as an example to illustrate the functionality of GPx, the termination steps start with the GPx-(E-SeH) reducing hydroperoxide into water (Reaction 7 in Figure I-6). The GPx-(E-

SeOH) is reduced back to GPx-(E-SeH) by a two-step reaction with two GSH molecules (Reaction 8 and 9 in Figure I-6), and the GSSG is formed. The GSSG will be reduced back to GSH by NADPH and an extra hydrogen ion (Reaction 10 in Figure I-6). The non-enzymatic antioxidants can be endogenous, such as GSH, and exogenous, such as ascorbic acid (vitamin C).

$$GPx\text{-}(E\text{-}SeH) + H_2O_2 \rightarrow GPx\text{-}(E\text{-}SeOH) + H_2O \quad \text{(Reaction 7)}$$

$$GPx\text{-}(E\text{-}SeOH) + GSH \rightarrow GPx\text{-}(E\text{-}SeSG) + H_2O \quad \text{(Reaction 8)}$$

$$GPx\text{-}(E\text{-}SeSG) + GSH \rightarrow GPx\text{-}(E\text{-}SeH) + GSSG \quad \text{(Reaction 9)}$$

$$GSSG + NAPDH + H^+ \rightarrow 2GSH + NAPD^+ \quad \text{(Reaction 10)}$$

Figure I-6: The catalytic mechanism of GPx converting hydroperoxide into water
The reaction 7 to 9 is the detailed steps of GPx converting hydroperoxide into water. The reaction 10 is the GSH generation by reducing GSSG using NAPDH. Adapted from Bhabak and Mugesh, 2010.

1.4 Shelf life of fresh meat

1.4.1 Major physiological changes of muscle associated with meat shelf life compared to live cells

Myoglobin oxidation and lipid peroxidation and the mechanism of antioxidants in muscle is quite different in meat. During postmortem conversion of muscle to meat (Kim *et al.*, 2018) muscle cells slowly deplete their oxygen and energy stores, mainly

adenosine triphosphate (ATP). Once the oxygen is depleted, the muscle cells cannot obtain the energy through the tricarboxylic acid cycle and electron transportation chain in mitochondria, but still attempt to produce ATP through a mechanism in which pyruvate converts to lactic acid. The accumulated lactic acid drops the pH of muscle cells to 5.6 to 5.8. Eventually, the postmortem process is completed, and the muscle cells are now recognized as meat (Ouali *et al.*, 2006; Matarneh *et al.*, 2017).

In molecular biology, the event of cell death can be categorized into apoptosis and necrosis (Bienert *et al.*, 2006; Edinger & Thompson, 2004). The apoptosis of cells is characterized with a shrinkage or reduction in cell size, a disintegrated nucleus, and increased permeability of the cell membrane. This process is recognized as programmed cell death and controlled by extrinsic and intrinsic signal pathways. The necrosis of cells is usually described as cell the membrane disappearing, resulting in the release of the cell constituents into the extracellular environment. This is not controlled by the cell and usually the exposed cell constituents will cause significant oxidative damages to the local region. Interestingly, the apoptosis is the pathway of the muscle cell death in meat (Kim *et al.*, 2018).

In living muscle cells, the ROS, mainly superoxide and hydrogen peroxide, can be trapped in confined areas, such as mitochondria and the cell membrane, and can be mitigated by the enzymatic antioxidant system (Bienert *et al.*, 2006; Murphy, 2009). The depleted energy and low pH environment of post-rigor meat turns off the majority of the enzymatic antioxidant system and the permeability of the cellular membrane

increases (Papuc *et al.*, 2017). This allows hydrogen peroxide to diffuse through the meat compared to selective transport in living muscle cells (Bienert *et al.*, 2006). Hence, the oxidation of meat, both myoglobin and lipid, is unstoppable.

Oxygen is an important source of ROS production in meat. Usually the diffusion depth of the oxygen is limited to the surface of meat; however, fabricated fresh meat, especially trimmings and ground meat, usually has increased surface area compared to the whole muscle. The increased surface area results in an elevated level of oxygen being diffused into meat. Thus, the oxidation of meat is a major issue negatively impacting meat quality.

1.4.2 Meat discoloration by myoglobin oxidation and off-odor by lipid peroxidation in meat

Meat discoloration is characterized as DMb oxidizing into MMb with the meat color being an undesirable brown color due to the combined effect of the presence of oxygen, the products of lipid peroxidation and the loss of functionality of the enzymatic antioxidant system (Faustman & Cassens, 1990; Mancini & Hunt, 2005; Seideman *et al.*, 1984). Although adequate oxygen levels are required to maintain the presence of the desirable OMB, oxygen also increases the production of reactive oxygen species (ROS), mainly superoxide and H_2O_2, in meat (Papuc *et al.*, 2017). The increase in ROS can oxidize DMb into MMb. Lipid oxidation is inevitable due to increasing levels of ROS. Unlike meat discoloration which can be identified by the brown color of meat, the lipid

oxidation cannot be visually detected. Consumers identify lipid oxidation by the smell of off-odors and flavors of the fresh meat (Shahidi & Pegg, 1994).

Previously mentioned in section 1.4.2, lipid hydroperoxide can be broken down through β-scission into a wide range of ketone, aldehyde, and other volatile compounds. Some of these products are responsible for the development of off-odors. Hexanal and pentanal were the dominant volatile aldehydes produced from fresh meat and cooked meat (Shahidi & Pegg, 1994). Different lipids and/or fatty acids produce different volatile compounds. The most abundant PUFA in beef is linoleic acid (18:2 n-6; 1-4% variable between breed types; (Smith *et al.*, 2006). Hexanal is one of the off-odor indicators and produced from the lipid peroxidation of omega 6 fatty acid, mainly linoleic acid in beef (Shahidi & Pegg, 1994; Smith *et al.*, 2006; Frankel, 1991; Calkins & Hodgen, 2007).

1.4.3 Myoglobin oxidation and lipid peroxidation affect each other

In meat, lipid peroxidation produces off-odors while myoglobin oxidation causes meat discoloration. These two reactions are highly interactive with each other and exacerbate both oxidation processes (Figure I-7**;** Faustman & Cassens, 1990; Faustman *et al.*, 2010; Lynch & Faustman, 2000).

The partial oxygen pressure (pO_2) will affect the interaction between lipid and myoglobin oxidation (Faustman *et al.*, 2010). Lipid oxidation occurs readily in high-oxygen atmospheres whereas MMb formation is favored in low-oxygen atmospheres. Thus, the interaction between lipid oxidation and myoglobin oxidation is not as great in

packaging where meat is exposed to either a high pO_2 or low pO_2 packaging atmosphere. Aerobically packaged or stored meat is more susceptible to the interaction between lipid oxidation and myoglobin oxidation compared with vacuum-package and modified-air package.

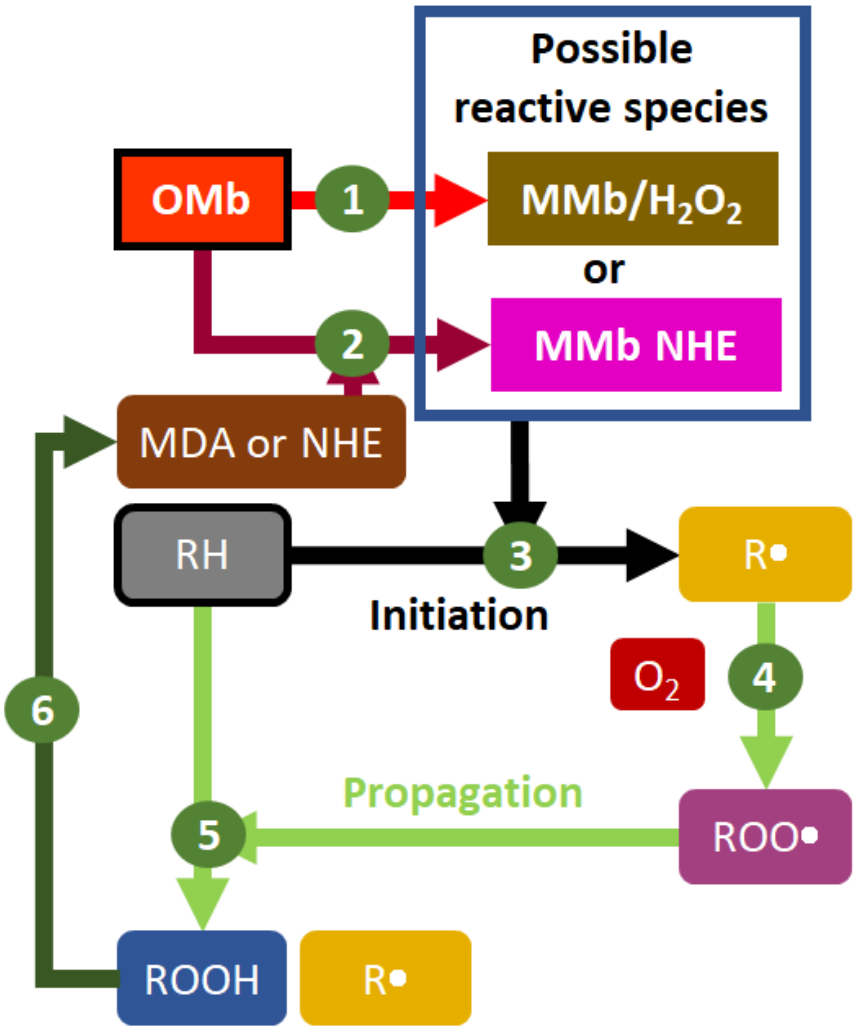

Figure I-7: Summary of potential interacting oxidation reactions between OMb and unsaturated fatty acids in lipid bilayers.
RH is an unsaturated fatty acid on cell membrane. R• is fatty acid radical. HO• and O_2• is reactive oxygen species. ROO• is fatty acid peroxyl radical. ROOH is fatty acid hydroperoxide. MDA (malondialdehyde) and HNE (4-hydroxy-2-nonenal) is the end product of lipid peroxidation. Myoglobin oxidation includes reactions 1 and 2. In reaction 1, the OMb is oxidized into MMb and then the MMb forms reactive species complex with hydrogen peroxide. In reaction 2, the MDA or NHE, products from lipid oxidation, can oxidize OMb into a MMb-NHE complex, a potential reactive species. Lipid oxidation includes reaction 3, 4, 5 and 6. In reaction 3, the generation of R• via the interaction between RH and reactive species, including MMb/H_2O_2 and MMb/NHE complex. In reaction 4, ROO• is generated by the interaction between R• and O_2. Then, the ROO• interacts with RH to form ROOH and R• in reaction 5. Finally, ROOH reacts with R• to form MDA or NHE in reaction 6. Adapted from Faustman & Cassens, 1990; Faustman et al., 2010; Lynch & Faustman, 2000 and original artwork by author.

1.4.4 Metmyoglobin reducing ability of meat

The mechanisms that reduce MMb to DMb has been referred to as metmyoglobin reducing activity (MRA) comprehensively (Bekhit & Faustman, 2005). Previously covered in section 1.1.3, the MRA in living muscle cells is mainly governed by the NADH-cytochrome b_5 MMb reductase, cytochrome b_5 and NADH. Although the meat itself cannot maintain this ability due to the depletion of NADH, it has been proposed that the generation of NADH can bring back the functionality of the NADH-dependent metmyoglobin reducing enzyme system. Therefore, the color stability of meat can be enhanced. Yeater (2016) reported an increased a* value of the beef muscle using NADH solution. Wet-aged (28 days) beef longissimus steaks which were vacuumed-packaged and stored in the absence of light at 5 ^{O}C had lower levels of NADH compared to those aged for 3 days (Mitacek *et al.*, 2019). There was no difference observed in NADH-dependent reductase activity for various aging times. This evidence further supports that the MRA of meat is dominantly governed by the regeneration of NADH. In other words, the regeneration of NADH can improve the redness of meat by the MRA of meat.

1.5 Microbial spoilage of meat

Fresh meat products, stored aerobically under refrigeration, are susceptible to growth of aerobic bacteria due to a nutrient-rich environment with high water activity. The uncontrolled microbial growth (>10^8 CFU/cm^2), the production of microbial

biofilms, and the metabolic activities of bacteria are the main causes of meat spoilage (Chaillou *et al.*, 2014; Gill & Newton, 1978). These microorganisms can be found on the surface of meat due to contamination during pre-harvest, harvest, and further processing.

The attachment of bacteria to meat surfaces involves two successive steps (Firstenberg-Eden, 1981; Wang, 2019). First, physical forces can transfer bacteria to the meat surface. Second, the bacteria can produce a biofilm which provides physical and chemical resistance to sanitization. The composition of the biofilm (also called extracellular polymeric substances) include polysaccharides, proteins, lipids and DNA. During the logarithmic growth phase, these aerobic bacteria can cause discoloration of meat due to oxygen consumption, and can produce off-flavors, off-odors, and slime (Seideman *et al.*, 1984; lacButler *et al.*, 1953). Therefore, it is important to control bacterial presence and/or growth to improve the color stability, flavor and aroma of fresh meat.

1.6 Enhanced color stability, reducing lipid peroxidation using lactate and sodium erythorbate and reduced off-odor using sodium bicarbonate

Strategies used to improve fresh meat color stability include pre-harvest strategies such as feed composition and vitamin E supplementation, and post-harvest strategies such as modified atmosphere packaging, vacuum skin packaging, and chemical treatment (Suman *et al.*, 2014). Among these strategies, investigating how

food-safe ingredients interact to maintain meat color and reduce lipid oxidation and off odors may provide scientific evidence to better explain the mechanism of meat color stability. Hence, it has the potential to accurately identify control measures to improve color stability.

Sodium lactate (NaL) is highly correlated with the regeneration of NADH, utilized in enzymatic antioxidant systems as well as the non-enzymatic MMb reducing systems (Suman & Joseph, 2013; Belskie *et al.*, 2015; Watts *et al.*, 1966). In the fresh meat industry, lactic acid or its salts have been primarily used as an antimicrobial agent. Furthermore, lactate improves color stability by increasing NADH generation and reducing the rate of metmyoglobin formation (Suman *et al.*, 2014; Mancini & Ramanathan, 2008; Kim *et al.*, 2006). Potassium lactate improves the color stability via metmyoglobin reducing ability (MRA), and 10% pumped beef strip loins with 2.5 % (0.2 M) potassium lactate significantly increased LDH activity, NADH concentration, MMb-reducing activity, and subsequent color stability during display (Kim *et al.*, 2006). Refrigerated ground beef incorporating 2.5 % (0.2 M) potassium lactate exhibited increased surface redness, minimized discoloration, and darkened color up to 4 days of storage (Suman *et al.*, 2010). While others proposed that the lactate enhances MRA through NADH generation (Figure I-8), it has been demonstrated that 100 and 200 mM sodium lactate directly incubated with oxymyoglobin for 48 h can reduce the amount of metmyoglobin formation (Mancini & Ramanathan, 2008). However, it was reported that 4.46 M sodium lactate applied directly to beef trimmings did not have any effect

on improving color stability. This might be due to the difference of methodologies compared to other research (Yeater, 2016). Interestingly, sodium lactate (20 g/kg) treated ground beef in vacuum package stored for 21 days had lower TBA value compared to the control (0.31 vs 0.46; (Sallam & Samejima, 2004). However, the mechanism for these results was unanswered. In conclusion, lactate can improve color stability and reduce lipid peroxidation.

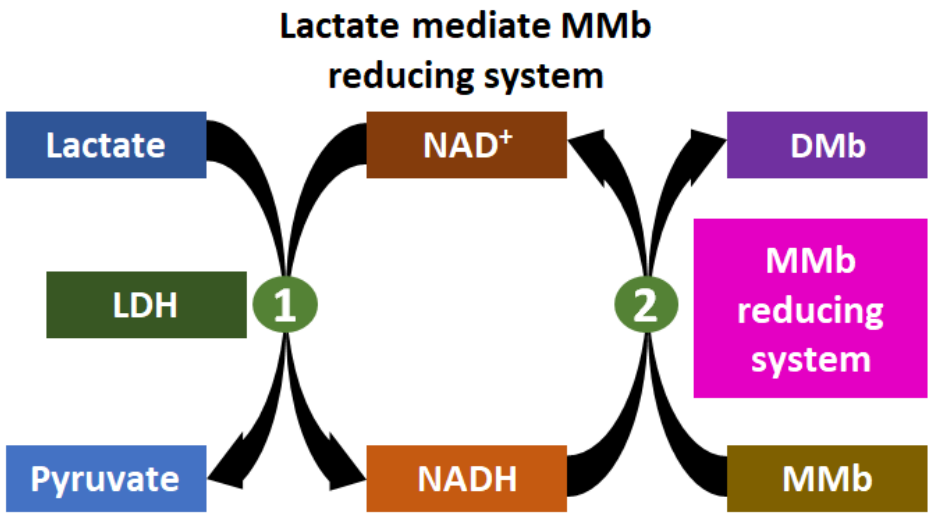

Figure I-8: Scheme of lactat-lactic dehydrogenase (LDH) system for generating NADH for metmyoglobin- reducing activity (MRA)
Reaction 1 presents that Lactate reduces NAD^+ to NADH via lactate dehydrogenase (LDH) and is oxidized into pyruvate. Reaction 2 presents that NADH reduces MMb to DMB via MMb reducing system. Adapted from Kim *et al.*, 2006.

Sodium erythorbate (NaE) is a food additive widely used in processed meat industry to facilitate the curing procedure. Cooked ground beef treated with 0.04 % and

0.06 % of erythorbic acid had higher a* values compared to the non-treated one upon 58 hours storage at 4°C (Phillips *et al.*, 2001). Also, the ground beef treated with 2.3 mM (0.05 %) of erythorbic acid or sodium erythorbate had higher a* values compared to the non-treated one upon 48 hours storage at 4°C (Sepe *et al.*, 2005). The beef trimmings treated with 0.1 M sodium erythorbate and stored for 48 hours had the highest OMb percentage compared to other treatments (Yeater, 2016). These three studies showed that erythorbic acid and its salt form can improve the color stability of ground beef through 6 days storage at 4°C. Also, 2.3 mM (0.05 %) erythorbic acid- or sodium erythorbate-treated ground beef had lower TBA values compared to the control at 48 hours storage at 4°C (Sepe *et al.*, 2005). However, there is no literature showing the direct evidence of improved MRA by erythorbic acid or its salt form.

Although it is better to stop or limit the progress of lipid oxidation and myoglobin oxidation regarding the color stability of fresh beef, the presence of off-odors in fresh meat stored for certain time is inevitable. Hence, the most appropriate intervention against this phenomenon is applying a treatment which can reduce/remove the off odor. Beef trimmings treated with sodium bicarbonate (NaB, 1.4 M) reduced aldehyde formation, which is a product of lipid oxidation compared with those aerobically stored for 9 days (Yeater, 2016). However, the mechanism of adsorption of off odor by NaB is still not clear.

1.7 Federal regulation of lactate, sodium erythorbate, and sodium bicarbonate in meat

Non-meat ingredients can be added into meat and food as food additives or used as a processing aid. In 21 CFR 184 and 182, potassium and sodium lactate and sodium erythorbate are identified generally recognized as safe (GRAS) substances (FDA, 2016). Under the 9 CFR 424 and FSIS directive 7120.1 Rev. 45, lactate can be used as an antimicrobial agent at 4.8 % (w/w) of total formulation, and the NaE can be used as a curing accelerator at a maximum 547 ppm (USDA, 2012). Sodium bicarbonate is regulated under 21 CFR 184.1736 and 9 CFR 424 with no limitation (USDA, 2012; FDA, 2016).

1.8 The strategies of future research on improving the shelf life of beef trimming

Although there are research studies that indicate that lactate and sodium erythorbate have a positive effect on the metmyoglobin reducing system in meat, the interaction between lipid oxidation, myoglobin oxidation and microbial spoilage has not been elucidated. Hence, there is a need to investigate the effect of potassium or sodium lactate, sodium erythorbate, and sodium bicarbonate on the metmyoglobin reducing system, lipid oxidation and microbial spoilage in fresh beef to better understand the interrelationships of these factors (Figure I-9).

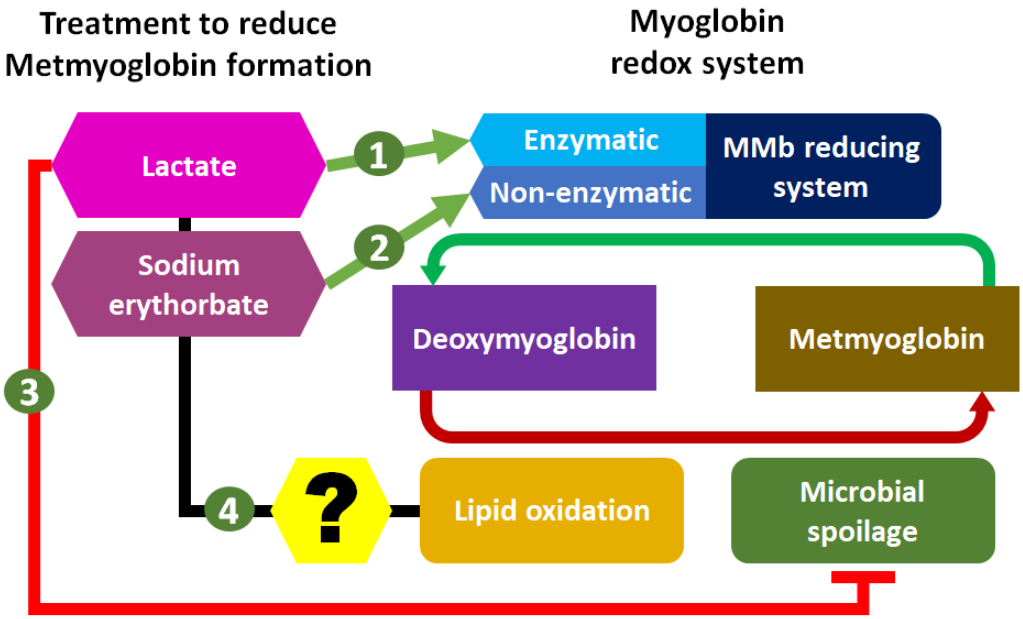

Figure I-9: The illustration of myoglobin redox system and the possible treatment to improve meat color stability.
Reaction 1 indicates that lactate can affect the MMb reducing system via enzymatic pathway, and reaction 2 indicates that erythorbate can improve MMb reducing system via non-enzymatic pathway. Reaction 3 suggests that lactate is capable of inhibit the microbial spoilage. Reaction 4 is an unanswered relationship between lipid oxidation and treatment. Original artwork by author.

The ability to develop a processing aid from a combination of lactate, NaE and NaB that can be applied to beef trimmings may prevent further deterioration of color, lipid, odor, or microbial growth, thereby enhancing further processed (i.e. ground beef patties) product shelf life at retail. The effect of treatments in different literature reviews on improving color stability, and reducing lipid oxidation were summarized (Faustman *et al.*, 2010); however, there was no attempt to develop a processing aid to target increased color stability, reduced lipid oxidation, controlled microbial spoilage, and mitigated off odors, mainly hexanal. We hypothesized that the ingredient

concentration level combinations containing NaL, NaE and NaB can be optimized to maintain color stability, reduce lipid oxidation, mitigate off odor and control microbial spoilage of aerobically stored beef trimmings. The objective of the proposed research is to determine the effect of sodium/potassium lactate, sodium erythorbate, and sodium bicarbonate based on literature reviews (Suman *et al.*, 2014; Mancini & Ramanathan, 2008; Kim *et al.*, 2006; Phillips *et al.*, 2001; Sepe *et al.*, 2005; Yeater, 2016) to improve the quality of aerobically stored beef trimmings used to manufacture ground beef products.

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CHAPTER II

THE EFFECT OF SODIUM LACTATE, POTASSIUM LACTATE, SODIUM ERYTHORBATE, AND SODIUM BICARBONATE ON FRESH BEEF TRIMMINGS FOR GROUND BEEF PRODUCTION

2.1 Overview

Studies were conducted to investigate the effect of sodium lactate (NaL), potassium lactate (KL), sodium erythorbate (NaE), and sodium bicarbonate (NaB) on extending the shelf life of fresh beef trimmings for ground beef production. The combinations of treatment solutions containing NaL or KL (0.1 to 1.5 M), NaE (1 mM to 0.6 M), and NaB (0.1 to 1.5 M) with water as a control were generated following a full factorial design. Beef trimmings fabricated from 14-day postmortem carcasses were aerobically stored for 6 days at 5^{o}C. After storage, beef trimmings were ground, treated with treatment solutions, reground and formed into beef patties wrapped with oxygen permeable film. The patties were stored under simulated retail conditions: 5°C, cool white fluorescent light (200 - 300 lux) and analyzed at day 0, 3, 6 and 9 of storage to assess the effectiveness of each treatment in preventing further quality deterioration. Objective color (L*, a*, and b*), 2-thiobarbituric acid (TBA) determinations, GC-MS for off-odor assessment and aerobic plate counts (APC) were conducted. The beef patties treated with NaB (1.5 M), NaE (0.1 - 0.8 M) and NaL (1 M) had the greatest effect on enhancing color stability, reducing lipid peroxidation and controlling microbial spoilage up to 6 days of retail storage in both studies. Results of these studies suggest that a

solution of NaL, NaE, and NaB can be beneficial in improving color stability, reducing lipid peroxidation, and controlling off-odors in aerobically-stored beef trimmings destined for ground beef production.

Key words: Beef trimmings, color, lipid oxidation, off-odor

2.2 Introduction

The discoloration of beef raw materials or retail products leads to economic loss, estimated to be more than $1 billion annually in the United States (Smith *et al.*, 2000). Color and its stability affect the marketability of beef because it is the first quality attribute seen by the consumer during purchase (Faustman & Cassens, 1990; Troy & Kerry, 2010).

Meat discoloration caused by metmyoglobin (MMb) accumulation is characterized as the meat color changing from a desirable cherry-red to an undesirable brown color (AMSA, 2012; Faustman & Cassens, 1990). The accumulation of MMb in meat is mainly caused by the oxidation of deoxymyoglobin (DMb) which is formed by interacting with reactive oxygen species, the products from lipid peroxidation, and/or the metabolites from microbial spoilage.

In meat, myoglobin oxidation and lipid peroxidation are highly interactive with each other to exacerbate the process of oxidation and contribute to the development of discoloration and off odors (Faustman & Cassens, 1990; Faustman *et al.*, 2010). Aerobically-stored meat is more susceptible to the interaction between lipid oxidation

and myoglobin oxidation compared to meat stored in vacuum- or modified-atmosphere packaging due to differences in partial oxygen pressure (Faustman *et al.*, 2010). Also, fresh meat products stored aerobically under refrigeration are vulnerable to aerobic psychrotrophic bacteria. These aerobic bacteria can cause discoloration due to their oxygen consumption and also produce off flavor, off odor, and slime as a result of metabolic processes (Chaillou *et al.*, 2014; Gill & Newton, 1978).

To improve color stability, reduce lipid peroxidation and inhibit microbial spoilage of aerobically stored fresh beef trimmings, the best approach may be the development of a processing aid solution containing compounds that are proven to maintain color, reduce lipid oxidation, and microbial spoilage. Sodium lactate (NaL), potassium lactate (KL) and sodium erythorbate (NaE) have been shown to improve color stability in beef (Suman *et al.*, 2014; Mancini & Ramanathan, 2008; Kim *et al.*, 2006; Phillips *et al.*, 2001; Sepe *et al.*, 2005). Sodium erythorbate has been reported to reduce lipid peroxidation (Sepe *et al.*, 2005). Sodium lactate is widely recognized as an effective antimicrobial reagent (de Wit & Rombouts, 1990; Sallam & Samejima, 2004). The presence of off-odors due to lipid peroxidation in fresh meat stored for certain time is inevitable. Sodium bicarbonate (NaB, 1.4 M) was reported to effectively reduce off-odor in aerobically-stored beef trimmings (Yeater, 2016). Based on these studies, we investigated the effect of single ingredient solutions containing either NaL, KL, NaE or NaB on improving color stability, reducing lipid peroxidation and controlling off-odors in aerobically stored beef trimmings destined for ground beef production.

2.3 Materials and Methods

2.3.1 Sample collection, preparation and treatments

Beef carcasses were obtained from the Rosenthal Meat Science and Technology Center at Texas A&M University. Beef trimmings (~50kg) were fabricated from beef forequarters (n = 5) 14 days postmortem, combined and aerobically stored (5°C) for an additional 6 days to simulate the collection, storage, transportation and receipt of a combo of beef trimmings. After 6 days of aerobic storage, the beef trimmings (~20% fat) were coarse ground (12 mm) and the single ingredient treatment/control (water) solution was applied to the coarse ground trimmings (~454 g) at 2% (w/w). The trimmings were reground (3 mm) and 120g of treated sample was placed into a Petri dish and overwrapped with oxygen permeable film (oxygen transmission rate: 21,700 cc/m^2/24h at 25°C) to form patties. Four samples per treatments with two replicates (n = 208) and two samples per treatments with three replicates (n = 312) were prepared for phase 1 and 2, respectively. The patties were stored under simulated retail conditions: 5°C, cool white fluorescent light (200 - 300 lux) and analyzed at day 0 and 5 (phase 1), and 0, 3, 6 and 9 (phase 2) of storage to assess the effectiveness of each treatment in preventing further quality deterioration.

2.3.2 Solution preparation

Sodium bicarbonate ($NaHCO_3$; Anhydrous, ACS reagent grade >99.5%) was purchased from Sigma Aldrich Co. (Milwaukee, WI). Potassium and sodium lactate ($C_3H_5KO_3$ and $C_3H_5NaO_3$) were procured from Corbion Purac (Purac Biochem,

Gorinchem, Netherlands). Sodium erythorbate ($C_6H_7NaO_6$; Zhenzhou Tuoyang Bioengineering CO., Henan Province, China) was purchased from a local food ingredient supplier. The treatments were prepared fresh according to Table II-1 by mixing each ingredient with the appropriate amount of double-distilled, deionized water. Sodium bicarbonate and NaE treatments required heating up to 70^{o}C to completely dissolve. For phase 1, the concentration levels of KL, NaL, NaE and NaB for both research phases were based on previous research (Yeater, 2016). The USDA and FDA regulations were reviewed to determine the concentrations of each ingredient that were within compliance for use in beef products as food additives or as a processing aid. In 21 CFR, KL, NaL, NaE and NaB are assumed to be generally recognized as safe (GRAS) substances (FDA, 2016). Under 9 CFR 424 and FSIS directive 7120.1 Rev. 45 (USDA, 2012), lactate can be used as an antimicrobial agent at a maximum 4.8 % (w/w) of the total formulation, and NaE can be used as curing accelerator at a maximum 547 ppm. Erythorbic acid can be used to delay discoloration in ground beef and ground beef patties at a maximum of 0.04 % (w/w). Sodium bicarbonate is regulated under 21 CFR 184.1736 and 9 CFR 424 with no limitations. The concentration levels of all single ingredient treatment solutions are summarized in Table II-1. For phase 2 the concentration levels of KL, NaL, NaE and NaB were based on phase 1. The concentration levels of all ingredient treatment solutions were increased to exceed regulatory guidelines to determine their impact on maintaining color, reducing lipid oxidation and microbial spoilage of aerobically stored beef trimmings (Table II-1). Only

NaB concentration levels remained the same due to solubility issues (precipitating out). Both phases of research assessed the impact on quality attributes (color, lipid oxidation, microbial growth) of beef patties manufactured from aerobically stored beef trimmings treated with single ingredient solutions within (phase 1) and outside (phase 2) federal guidelines during refrigerated retail storage (0 and 5 days, phase 1; 0, 3, 6 and 9 days, phase 2).

Table II-1: Concentrations/Combinations of treatments

	Phase 1			
Treatments	Concentration levels			
Potassium/sodium lactate (KL/NaL)	0.1 M	0.2 M	0.4 M	
Sodium erythorbate (NaE)	1 mM	2 mM	100 mM	
Sodium Bicarbonate (NaB)	0.1 M	1 M	1.5 M	
	Phase 2			
Treatments	Concentration levels			
Sodium Lactate (NaL)	0.1 M	0.5 M	1 M	1.5 M
Sodium erythorbate (NaE)	0.1 M	0.2 M	0.4 M	0.8 M
Sodium Bicarbonate (NaB)	0.1 M	0.5 M	1 M	1.5 M

2.3.3 pH determination

Ten grams of each treatment and control ground beef sample (n = 104 and 156 for phase 1 and 2, respectively) were 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).

2.3.4 Proximate composition

Untreated ground beef samples were collected and submerged in liquid nitrogen and powdered using a Waring blender (Model 33BL79, Waring Commercial, New Hartford, CT). Powdered samples (n = 3/replicate) were used to determine proximate composition according to AOAC (2005) procedures for moisture (AOAC 985.14 oven drying method), protein (AOAC 992.15 using a nitrogen analyzer, F528, Leco Corp., St. Joseph, MI), and fat (AOAC 985.15 subtracting 100% from moisture and protein; AOAC, 2019).

2.3.5 Objective color determination

Treated 3 mm ground beef patties overwrapped with oxygen permeable film (n = 208 and 312 for phase 1 and 2, respectively) 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 degree standard observers; Hunter Associates Laboratory, Inc., Reston, VA). The HunterLab Miniscan was calibrated with white and black tiles wrapped with oxygen permeable film before measurement. Illuminant D 65 was used for L*, a* and b* color values and illuminant A for spectral reflectance (474, 525, 572, and 700 nm). The percentage of deoxymyoglobin and metmyoglobin was calculated from the spectrum data and oxymyoglobin percentage was determined indirectly by subtracting their combined percentages (DMb% + MMb%) from 100 % (AMSA, 2012; Krzywicki, 1979).

2.3.6 Lipid oxidation determination

The thiobarbituric acid (TBA) assay method was used to determine lipid oxidation of the treated ground beef patties at each designated storage day (Tarladgis *et al.*, 1960). The mechanism of the TBA assay is based on the reaction between TBA and malonaldehyde (MDA) which results in a colored pigment. The concentration of MDA can be calculated by measuring the absorbance at 532 nm. Ten grams of each sample (n = 104 and 312 for phase 1 and 2, respectively) was homogenized with 50 mL of deionized water, 5mL of 0.5 % propyl gallate (PG) and 0.5 % ethylenediaminetetraacetic acid (EDTA) solution. The homogenous meat solution was transferred to a Kjeldahl distillation flask with 31.5 mL deionized water. The 2.5 mL of 4 N HCl and 5 to 6 boilling chips were added to the flask, and deionized water was added into the flask to make the total volume 100 mL. The mixture was heated and the first 50 mL of distillate was collected. The 5 mL of collected distillate was reacted with 5 mL of 0.02 M TBA agents in a test tube in a 100 ^{o}C water bath for 35 min. After incubation, the test tube was cooled in ice for 10 min and the optical density (O.D.) value of the sample was read at an absorbance of 532 nm. The TBA value was calculated from multiplying the O.D. value of sample by a constant of 7.8.

2.3.7 Off odor determination using gas chromatograph/mass spectrometry

Previously frozen samples (20 g; n = 28 and 156 for phase 1 and 2, respectively) were placed into glass jars (473 mL) with a 1.6 mm-thick Teflon piece under the metal lid at room temperature for thawing. After thawing and equilibrating at room

temperature, a solid-phase micro-extraction (SPME) portable field sampler (Supelco 504831, 75 µm carboxen/ polydimethylsiloxane [PDMS], Sigma-Aldrich, St. Louis, MO) was inserted through the lid in order to collect the headspace above each meat sample in the glass jar held at room temperature for 2 hr. After collection, the SPME was remove from the jar and injected into the injection port of a gas chromatograph GC (Agilent Technologies 7920 series GC, Santa Clara, CA), where the sample was desorbed at 280°C for 3 min. The sample was then loaded onto the multi-dimensional gas chromatograph through a first column (30 m × 0.53 mm ID/BPX5 [5% phenyl polysilphenylene-siloxane] × 0.5µm, SGE Analytical Sciences, Austin, TX) and then a second column (30 m × 0.53 mm ID [BP20—polyethylene glycol] × 0.50 µm, SGE Analytical Sciences). The GC temperature started at 40°C and increased at a rate of 7°C/min until reaching 260°C. The GC column then went to a mass spectrometer (MS; Agilent Technologies 5975 series MS, Santa Clara, CA) for quantification and identification using the Wiley Chemical Library. A 3-point external standard curve (1, 3 dichlorobenzene) was run to estimate concentration of volatiles from area data. Chemicals exceeded a quality report from the MS of 80 were used for analysis. The GC-MS data were reported as total ion counts.

2.3.8 Microbiological determination

Twenty grams (n = 104 and 312 for phase 1 and 2, respectively) of samples 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 milliliter 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 three sets of Petrifilms® (3M® Microbiology, St. Paul, MN). One set of aerobic count (AC) petrifilms were incubated for 48 h at 35°C before enumeration to quantify aerobic mesophiles (AM). One set of AC films was incubated for 7 days at 7°C to quantify aerobic psychrotrophs (AP; Salfinger & Tortorello, 2015).

2.3.9 Statistical analysis

For both phases of the study, least squares means (LSMeans) for a* values, TBA values, total ion counts, and AP counts were reported. The data was analyzed by two-way and one-way ANOVA using SAS JMP Pro 14 (SAS Institute, Inc., Cary, NC). The GLM and Standard Least Squares with $\alpha = 0.05$ was used for one-way and two-way ANOVA, respectively. The fixed effect for both phases was treatment and day for two-way ANOVA and treatment for one-way ANOVA. The significant differences among treatments means were determined using Tukey's HSD ($P < 0.05$).

2.4 Results and Discussions

2.4.1 Proximate composition and pH

The moisture, protein, and fat contents for phase 1 were 64.91 ± 0.52 %, 13.87 ± 0.81 % and 21.22 ± 0.86 %, respectively; and for phase 2 were 64.79 % ± 1.09%, 15.06 ± 0.46 % and 20.15 ± 0.79%, respectively (Table II-2). The fat content in both studies achieved the targeted 20 % fat content, a normal fat percentage found in many ground beef products.

For phase 1, the pH of all treatments was all significantly higher than the pH of control on day 0 ($P < 0.05$) while the pH of 1.5 M NaB-treated sample was significantly higher than other treatments and control ($P > 0.05$; Table II-3). For phase 2, the pH of treatments was significantly different compared to the pH of control on day 0, 3, 6 and 9 (Table II-4). The control pH on day 0 for phase 1 and 2 was 5.75 and 6.02, respectively. The muscle pH of 5.87 was reported as the approximate cut-off between normal and dark-cutting beef carcasses (Page *et al.*, 2001). Hence, the beef trimmings for phase 2 may have contained dark, firm, and dry beef. The pH of beef muscles treated with 0.10 M NaE, 1.43 M NaB, and 4.46 M NaL ranged from 5.76 to 5.83 and were not different ($P > 0.05$) compared to the untreated beef muscle (Yeater, 2016). The different pH results between these studies and Yeater (2016) might be caused by the different raw materials used (beef trimmings vs whole muscles) or the difference of storage time.

Table II-2: Least squares means of the proximate composition of 12 mm untreated ground beef

	Phase 1		
	Moisture (%)	Protein (%)	Fat (%)
Untreated Ground beef	64.91	13.87	21.22
SEM[1]	0.52	0.81	0.86
	Phase 2		
	Moisture (%)	Protein (%)	Fat (%)
Untreated Ground beef	64.79	15.06	20.15
SEM[1]	1.09	0.46	0.79

[1] The SEM present the standard error of mean of the untreated ground beef.

Table II-3: Phase 1 least squares means for ground beef patties pH values at day 0 and 5 of refrigerated storage

	Treatments		pH value	
	Ingredients	Concentration (M)	Day 0	Day 5
1	NaL	0.4	5.82^{cde}	5.77^{c}
2	NaL	0.2	5.81^{cde}	5.81^{bc}
3	NaL	0.1	5.83^{bcd}	5.77^{bc}
4	KL	0.4	5.80^{de}	5.82^{bc}
5	KL	0.2	5.81^{cde}	5.79^{bc}
6	KL	0.1	5.84^{bcd}	5.84^{bc}
7	NaE	0.1	5.84^{bcd}	5.74^{c}
8	NaE	0.002	5.79^{de}	5.70^{c}
9	NaE	0.001	5.89^{bc}	5.77^{c}
10	NaB	1.5	6.44^{a}	6.16^{a}
11	NaB	1	5.91^{b}	5.72^{c}
12	NaB	0.1	5.84^{bcd}	5.68^{c}
13	Control	N/A	5.73^{e}	6.07^{ab}
	SEM[1]		0.02	0.06
	p-value		0.000	0.000

[1] SEM- Standard error of mean for treatment and control means.

[abcde] Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

Table II-4: Phase 2 least squares means for ground beef patties pH values at day 0, 3, 6 and 9 of refrigerated storage

	Treatments		pH value			
	Ingredients	Concentration (M)	Day 0	Day 3	Day 6	Day 9
1	NaL	1.5	5.88^{bc}	6.13^{abcde}	6.01^{bcd}	5.93^{c}
2	NaL	1	6.08^{abc}	6.06^{bcde}	6.02^{bcd}	5.85^{c}
3	NaL	0.5	6.05^{abc}	6.06^{bcde}	5.92^{bcd}	5.76^{c}
4	NaL	0.1	6.04^{abc}	5.95^{de}	6.02^{bcd}	5.77^{c}
5	NaE	0.8	5.85^{c}	6.14^{abcde}	5.91^{bcd}	5.98^{abc}
6	NaE	0.4	5.91^{bc}	6.04^{cde}	5.89^{cd}	5.90^{c}
7	NaE	0.2	5.93^{bc}	5.94^{de}	5.8^{d}	5.93^{c}
8	NaE	0.1	6.04^{abc}	5.92^{e}	5.87^{cd}	5.96^{c}
9	NaB	1.5	6.29^{a}	6.37^{a}	6.29^{a}	6.27^{ab}
10	NaB	1	6.19^{ab}	6.26^{abc}	6.09^{abc}	6.29^{a}
11	NaB	0.5	6.08^{abc}	6.34^{ab}	5.91^{bcd}	5.97^{bc}
12	NaB	0.1	5.97^{abc}	6.23^{abcd}	5.90^{bcd}	5.88^{c}
13	Control	N/A	6.02^{abc}	6.16^{abcde}	6.13^{ab}	6.01^{abc}
	SEM[1]		0.13	0.07	0.05	0.08
	p-value		0.001	0.000	0.000	0.000

[1] SEM- Standard error of mean for treatment and control means.
^{abcde} Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

2.4.2 Objective color

For both phases, no differences were observed for treated and control patties for a* values on storage day 0 (19.83 to 22.32 and 17.88 to 20.11, respectively; $P > 0.05$, Table II-5 and II-6). For phase 1, 1.5 M NaB was the only treatment having greater a* value compared to the control on day 5 (13.63 vs 13.25; $P < 0.05$). For phase 2, 1.5 M NaB treatment had the greatest a* value compared to the control on both storage day 3 and 6 (10.97 vs 10.59, 16.11 vs 13.54, respectively; $P < 0.05$). Although 0.1 to 0.8 M

NaE and 0.1 M NaL treatments had greater a* value compared to the control on storage day 3 for phase 2 ($P < 0.05$), they had lower a* value compared to the control on storage day 6 ($P < 0.05$). No differences were observed for treated and control patties for a* value on storage day 9 for phase 2 ($P > 0.05$). The ground beef treated with 2.3 mM (0.05 %) sodium erythorbate had higher a* value compared to control upon 48 hours storage at 4 ^{o}C (Sepe *et al.*, 2005). Moreover, most research recognizes the capability of lactate to enhance color stability of meat through the regeneration of NADH by lactate dehydrogenase (Suman *et al.*, 2014; Mancini & Ramanathan, 2008; Kim *et al.*, 2006). However, it was reported that 4.46 M sodium lactate did not have any effect on improving color stability of beef trimmings (Yeater, 2016). These conflicted reports and our results might be due to the different methodologies. Interestingly, there was no research showing that the NaB could enhance the color stability of fresh beef trimmings.

Table II-5: Phase 1 least squares means for ground beef patties a* values at day 0 and 5 of refrigerated storage

	Treatments		a* value	
	Ingredients	Concentration (M)	Day 0	Day 5
1	NaL	0.4	20.39	11.81^{abc}
2	NaL	0.2	21.58	12.95^{abc}
3	NaL	0.1	21.22	13.27^{ab}
4	KL	0.4	20.84	10.63^{c}
5	KL	0.2	22.32	12.34^{abc}
6	KL	0.1	21.88	13.16^{ab}
7	NaE	0.1	21.36	12.57^{abc}
8	NaE	0.002	21.83	12.23^{abc}
9	NaE	0.001	21.43	10.93^{bc}
10	NaB	1.5	19.83	13.63^{a}
11	NaB	1	22.08	13.24^{ab}
12	NaB	0.1	20.95	13.07^{ab}
13	Control	N/A	22.25	13.25^{ab}
	SEM[1]		0.52	0.51
	p-value		0.033	0.000

[1] SEM- Standard error of mean for treatment and control means.

^{abcde} Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

Table II-6: Phase 2 least squares means for ground beef patties a* values at day 0, 3, 6 and 9 of refrigerated storage

	Treatments		a* value			
	Ingredients	Concentration (M)	Day 0	Day 3	Day 6	Day 9
1	NaL	1.5	19.51	9.43[b]	11.53[b]	10.00
2	NaL	1	20.11	10.12[b]	13.54[ab]	9.89
3	NaL	0.5	19.98	10.17[b]	13.20[ab]	10.17
4	NaL	0.1	19.26	10.88[ab]	11.59[b]	10.22
5	NaE	0.8	19.03	10.87[ab]	11.79[b]	10.85
6	NaE	0.4	19.61	11.24[ab]	12.75[b]	10.55
7	NaE	0.2	19.34	12.90[a]	13.14[ab]	10.46
8	NaE	0.1	19.58	11.21[ab]	12.62[b]	10.41
9	NaB	1.5	17.88	10.97[ab]	16.1[a]	10.83
10	NaB	1	18.31	10.74[b]	12.73[b]	10.19
11	NaB	0.5	18.85	10.13[b]	12.57[b]	10.19
12	NaB	0.1	19.49	10.59[b]	12.19[b]	10.39
13	Control	N/A	18.73	10.59[b]	13.31[ab]	10.88
	SEM[1]		0.47	0.44	0.65	0.34
	p-value		0.061	0.001	0.001	0.576

[1] SEM- Standard error of mean for treatment and control means.
[abcde] Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

2.4.3 Lipid peroxidation

No differences were observed for treated and control patties for lipid peroxidation analyses on storage day 0 for phase 1 (Table II-7). For phase 2, 1M NaL and 0.8 M NaE had lower TBA values compared to the control for phase 2 (0.87 and 0.85 vs 0.96, respectively; Table II-8). Sodium erythorbate (0.8 M) and 1.5 M NaB had smaller TBA value compared to the control (0.79 and 0.97 vs 1.31, respectively) for phase I, while for phase 2 , all NaE treatments (0.1 to 0.8 M) had lower TBA values

compared to the control on day 3, 6 and 9 (0.77 to 0.91 vs 1.38, 0.92 to 1.13 vs 1.70 and 0.93 to 0.99 vs 1.68, respectively). Although some treatments, such as NaB, had lower TBA values compared to the control on some storage days, their performance was not consistent throughout storage days 3 to 9. The higher concentration levels of NaL, especially 1 M NaL, seemed to intensify lipid peroxidation through storage day 3 to 9. treated The ground beef treated 2.3 mM (0.05 %) sodium erythorbate exhibited lower TBA values compared to the control at 48 hours storage at 4 ^{o}C (Sepe *et al.*, 2005).

Table II-7: Phase 1 least squares means for ground beef patties TBA values at day 0 and 5 of refrigerated storage

	Treatments		TBA value	
	Ingredients	Concentration (M)	Day 0	Day 5
1	NaL	0.4	1.14	1.41^{ab}
2	NaL	0.2	1.09	1.23^{abc}
3	NaL	0.1	1.06	1.45^{ab}
4	KL	0.4	1.06	1.66^{a}
5	KL	0.2	1.11	1.22^{abc}
6	KL	0.1	1.01	1.18^{abc}
7	NaE	0.1	0.89	0.79^{c}
8	NaE	0.002	0.96	1.37^{abc}
9	NaE	0.001	1.06	1.28^{abc}
10	NaB	1.5	1.10	0.97^{bc}
11	NaB	1	0.95	1.20^{abc}
12	NaB	0.1	1.02	1.12^{abc}
13	Control	N/A	1.08	1.31^{abc}
	SEM[1]		0.05	0.12
	p-value		0.215	0.000

[1] SEM- Standard error of mean for treatment and control means.

^{abcde} Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

Table II-8: Phase 2 least squares means for ground beef patties TBA values at day 0, 3, 6 and 9 of refrigerated storage

	Treatments		TBA value			
	Ingredients	Concentration (M)	Day 0	Day 3	Day 6	Day 9
1	NaL	1.5	0.96^{ab}	1.56^{a}	1.66^{a}	1.44^{abcd}
2	NaL	1	0.87^{b}	1.49^{ab}	1.72^{a}	1.72^{a}
3	NaL	0.5	1.02^{ab}	1.58^{a}	1.56^{abc}	1.55^{abc}
4	NaL	0.1	1.13^{a}	1.39^{abc}	1.58^{ab}	1.68^{ab}
5	NaE	0.8	0.85^{b}	0.77^{d}	0.92^{e}	0.93^{d}
6	NaE	0.4	1.06^{ab}	0.85^{cd}	0.94^{e}	0.98^{d}
7	NaE	0.2	1.01^{ab}	0.91^{bcd}	1.04^{de}	0.93^{d}
8	NaE	0.1	1.02^{ab}	0.91^{bcd}	1.13^{cde}	0.99^{d}
9	NaB	1.5	0.95^{ab}	1.06^{abcd}	1.20^{bcde}	1.19^{bcd}
10	NaB	1	0.94^{ab}	1.09^{abcd}	1.20^{bcde}	1.13^{cd}
11	NaB	0.5	0.90^{ab}	1.20^{abcd}	1.42^{abcd}	1.34^{abcd}
12	NaB	0.1	1.08^{ab}	1.31^{abcd}	1.52^{abc}	1.40^{abcd}
13	Control	N/A	0.96^{ab}	1.38^{abcd}	1.70^{a}	1.68^{ab}
	SEM[1]		0.05	0.13	0.09	0.11
	p-value		0.005	0.000	0.000	0.000

[1] SEM- Standard error of mean for treatment and control means.
^{abcde} Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

2.4.4 Off odor analysis

For phase 1, the total ion counts of hexanal showed no difference for treated patties on day 5, or control patties on day 0 and day 5 ($P > 0.05$; Table II-9). The treated beef patties were not analyzed on storage day 0 for phase 1 due to the remote possibility of aldehyde formation due to the treatments. Although no differences existed for total ion counts of hexanal for treated and control patties on storage day 0 and 3 for phase 2 ($P > 0.05$), there was a trend for NaE and NaB treated beef patties to

exhibit lower total ion counts of hexanal compared to the control (Table II-10). Sodium erythorbate (0.1 to 0.8 M), 0.5 M NaB, and 0.1 M NaB had the lowest hexanal counts compared to the control on storage day 6 ($P < 0.05$). No differences existed for total ion counts of hexanal for treated and control patties on storage day 9 for phase 2 ($P > 0.05$). Hexanal is an indicator of off-odor and is produced from the lipid peroxidation of an omega 6 fatty acid, primarily linoleic acid in beef (Shahidi & Pegg, 1994; Smith *et al.*, 2006; Frankel, 1991; Calkins & Hodgen, 2007). The effect of NaE (phase 2) on reducing off odor was better than observed for phase 1 due to the increased range of NaE concentration. The TBA values and hexanal counts for phase 2 indicated that NaE inhibited hexanal production by suppressing lipid peroxidation. Although NaB showed some effects in reducing off odor development, we surmise that the amount of hexanal produced exceeded the absorption capacity of NaB.

Table II-9: Phase 1 least squares means for ground beef patties the total ion counts of hexanal at day 5 of refrigerated storage

	Ingredients	Concentration (M)	Total ion counts
1	NaL	0.4	109,017
2	NaL	0.2	0
3	NaL	0.1	0
4	KL	0.4	65,706
5	KL	0.2	0
6	KL	0.1	128,896
7	NaE	0.1	0
8	NaE	0.002	14,497
9	NaE	0.001	150,785
10	NaB	1.5	39,258
11	NaB	1	107,464
12	NaB	0.1	84,097
13	Control – day 0	N/A	25,839
14	Control – day 5	N/A	25,043
		SEM[1]	32,904
		p-value	0.054

[1] SEM- Standard error of mean for treatment and control means.

[abcde]Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

Table II-10: Phase 2 least squares means for ground beef patties the total ion counts of hexanal at day 0, 3, 6 and 9 of refrigerated storage

	Treatments		Hexanal			
	Ingredients	Concentration (M)	Day 0	Day 3	Day 6	Day 9
1	NaL	1.5	194,574	113,641	172,498^{ab}	48,415
2	NaL	1	116,453	50,442	66,396^{ab}	25,286
3	NaL	0.5	164,689	114,753	144,209^{ab}	109,254
4	NaL	0.1	215,056	176,137	63,884^{ab}	7,630
5	NaE	0.8	6,709	10,426	2,363^{b}	0
6	NaE	0.4	17,326	4,403	0^{b}	8,920
7	NaE	0.2	28,548	18,792	0^{b}	0
8	NaE	0.1	306,480	24,821	4,363^{b}	4,924
9	NaB	1.5	77,389	93,437	156,122^{ab}	141,569
10	NaB	1	22,804	21,454	53,819^{ab}	117,047
11	NaB	0.5	31,088	21,249	3,466^{b}	53,446
12	NaB	0.1	80,114	114,267	14,945^{b}	120,205
13	Control	N/A	131,010	117,643	295,457^{a}	0
	SEM[1]		83,948	39,572	50,242	48,121
	p-value		0.335	0.072	0.006	0.304

[1] SEM- Standard error of mean for treatment and control means.
^{abcde} Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

2.4.5 Microbial spoilage

No differences were observed for treated and control patties for aerobic psychrotrophs (AP) counts on storage day 0 in both phases (5.85 to 6.18 and 5.79 to 6.2 $\log_{10}$ CFU/g, respectively; Table II-11). For phase 1, 0.4 M NaL had lower AP counts compared to the control on day 5 (7.35 vs 7.68 $\log_{10}$ CFU/g; $P < 0.05$. Figure II-3). For phase 2, 1 M NaL had the lowest AP counts compared to the control and other treatments through storage day 3 through 9 ($P < 0.05$; Table II-12). Although some

treatments, such as NaE, had lower AP counts compared to control on storage days 6, their performance was not consistent, especially during storage day 3 through 9. It was reported that 5% sodium lactate was effective in increasing the lag phase and reducing the growth rate of *Streptococcus faecalis* and *Staphylococcus aureus* (de Wit & Rombouts, 1990).

Table II-11: Phase 1 least squares means for ground beef patties aerobic plate counts (APC) of AP at day 0 and 5 of refrigerated storage

	Treatments		APC (log_{10} CFU/g)	
	Ingredients	Concentration (M)	Day 0	Day 5
1	NaL	0.4	6.18	7.35[b]
2	NaL	0.2	6.05	7.55[ab]
3	NaL	0.1	5.95	7.63[ab]
4	KL	0.4	6.03	7.53[ab]
5	KL	0.2	5.85	7.75[a]
6	KL	0.1	5.90	7.70[ab]
7	NaE	0.1	6.00	7.73[a]
8	NaE	0.002	5.93	7.80[a]
9	NaE	0.001	5.93	7.73[a]
10	NaB	1.5	6.13	7.60[ab]
11	NaB	1	6.08	7.75[a]
12	NaB	0.1	6.10	7.55[ab]
13	Control	N/A	5.98	7.68[ab]
	SEM[1]		0.13	0.07
	p-value		0.856	0.001

[1] SEM- Standard error of mean for treatment and control means.

[abcde] Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

Table II-12: Phase 2 least squares means for ground beef patties aerobic plate counts (APC) of AP at day 0, 3, 6 and 9 of refrigerated storage

	Treatments		APC (log_{10} CFU/g)			
	Ingredients	Concentration (M)	Day 0	Day 3	Day 6	Day 9
1	NaL	1.5	5.83	7.41^{cd}	8.69^{ab}	8.53^{bcd}
2	NaL	1	5.79	7.30^{d}	8.41^{c}	8.27^{e}
3	NaL	0.5	5.97	7.56^{bcd}	8.57^{abc}	8.47^{bcde}
4	NaL	0.1	5.80	7.48^{bcd}	8.67^{abc}	8.66^{abc}
5	NaE	0.8	6.21	7.97^{ab}	8.62^{abc}	8.63^{abcd}
6	NaE	0.4	6.09	7.92^{abc}	8.51^{bc}	8.43^{cde}
7	NaE	0.2	6.10	7.75^{abcd}	8.52^{bc}	8.38^{de}
8	NaE	0.1	6.12	7.69^{abcd}	8.49^{bc}	8.56^{bcd}
9	NaB	1.5	5.92	8.14^{a}	8.83^{a}	8.82^{a}
10	NaB	1	5.98	8.12^{a}	8.84^{a}	8.71^{ab}
11	NaB	0.5	6.07	7.83^{abc}	8.69^{ab}	8.62^{abcd}
12	NaB	0.1	5.95	7.86^{abc}	8.64^{abc}	8.52^{bcde}
13	Control	N/A	6.00	7.74^{abcd}	8.67^{abc}	8.56^{bcd}
	SEM[1]		0.12	0.11	0.06	0.05
	p-value		0.308	0.000	0.000	0.000

[1] SEM- Standard error of mean for treatment and control means.

^{abcde} Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

2.4.6 Interaction of treatments and day

The results from two-way ANOVA reported that the interaction between treatments and day significantly affected on a*values, TBA values, and AP counts for phase 1 ($P < 0.05$; Figure II-1, 2, and 3). For a*values, TBA values, and AP counts, there were no difference among treatments on at day 1, but there was significant difference among treatments at day 5 ($P < 0.05$; Figure II-1, 2, and 3). The results from two-way ANOVA reported that there was no interaction between treatments and day on a*values, TBA values, total ion counts of hexanal, and AP counts for phase 2 ($P > 0.05$).

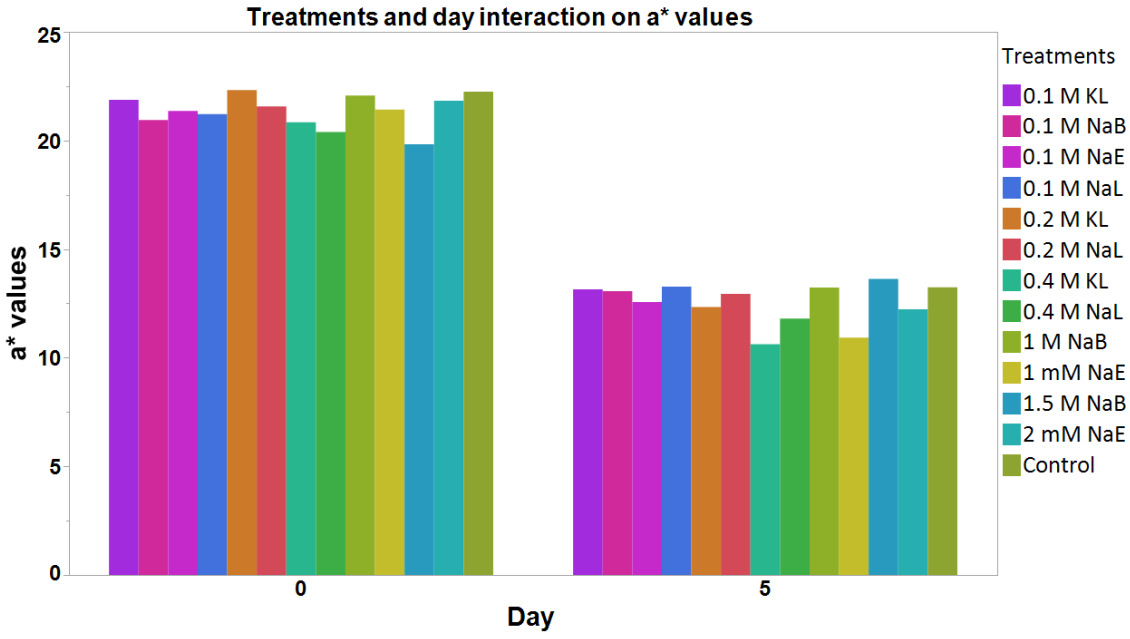

Figure II-1: Phase 1 bar graph for the interaction of treatments and day on a* values
The interaction of treatments and day was significantly affected a* values ($P < 0.05$, SEM: 0.56).

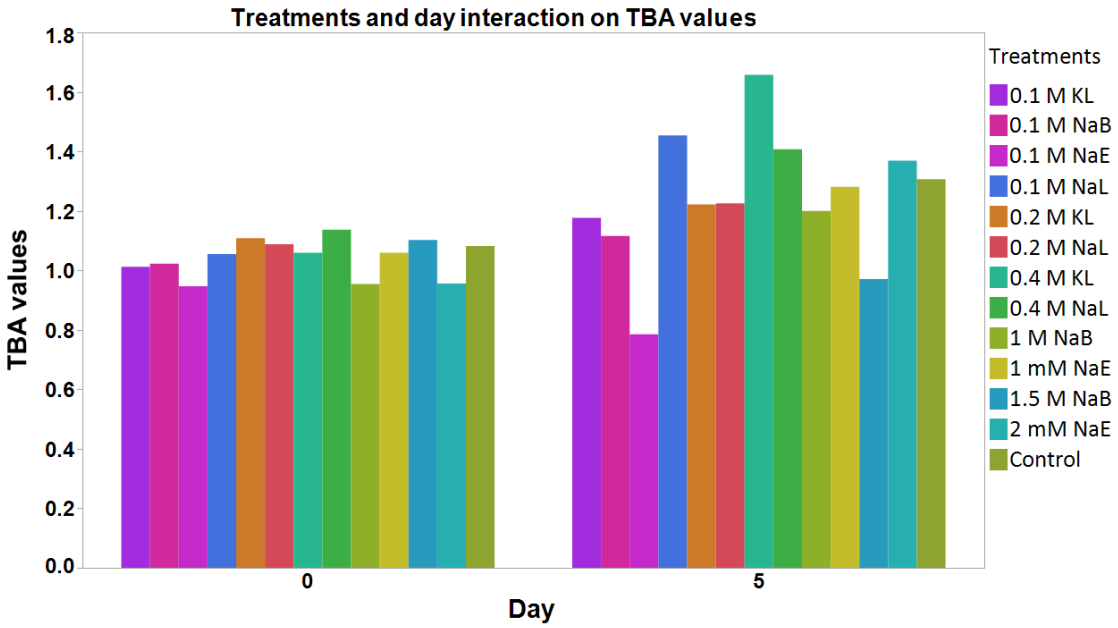

Figure II-2: Phase 1 bar graph for the interaction of treatments and day on TBA values
The interaction of treatments and day was significantly affected TBA values ($P < 0.05$, SEM: 0.09).

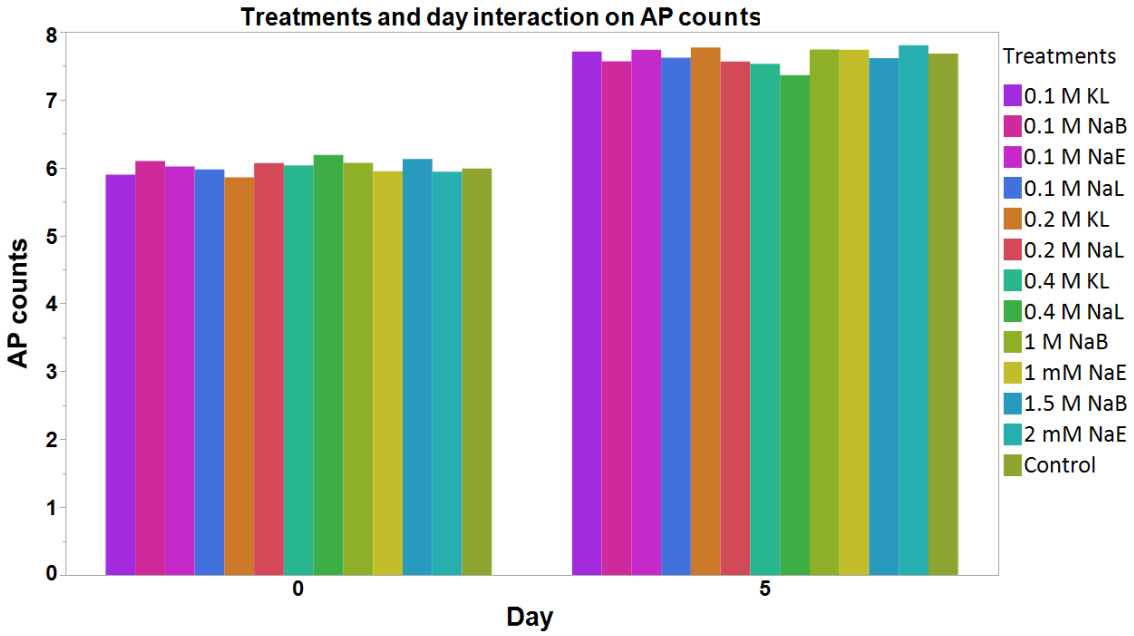

Figure II-3: Phase 1 bar graph for the interaction of treatments and day on AP counts
The interaction of treatments and day was significantly affected AP counts ($P < 0.05$, SEM: 0.10).

2.5 Conclusions

Beef trimmings treated with NaB (1.5 M), NaE (0.1 to 0.8 M) and NaL (1 M) had the greatest effect on enhancing the color stability, reducing the lipid peroxidation and controlling the microbial spoilage on ground beef patties compared to the control through day 6 of refrigerated retail storage for both phase 1 and 2. These treatments did not negatively impact the color, lipid peroxidation or microbial spoilage of ground beef on day 0. Although there was no treatment found to effectively mitigate off odor, results of this study indicate a trend in which patties treated with NaE and NaB had numerically reduced total ion counts of hexanal. A solution containing a combination of NaE and NaB applied to aerobically stored beef trimmings could potentially reduce the off odor of beef patties manufactured and subjected to retail storage conditions. Based on positive results, steps should be taken to optimize the positive impact of these individual ingredients to create a processing aid solution to enhance the color stability, reduce the lipid peroxidation, mitigate off odor and control the microbial spoilage off aerobically stored beef trimmings destined for fresh ground beef production.

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CHAPTER III

OPTIMIZATION OF CONCENTRATIONS OF SODIUM LACTATE, SODIUM ERYTHORBATE, AND SODIUM BICARBONATE TO ENHANCE THE SHELF LIFE OF BEEF TRIMMINGS FOR GROUND BEEF PATTIES

3.1 Overview

A 2^3 central composite response surface design (RSM) was used to generate 15 treatment combinations containing sodium lactate (NaL, 0.1 to 1.5 M), sodium erythorbate (NaE, 0.1 to 0.6 M), and sodium bicarbonate (NaB, 0.1 to 1.5 M) with water used as a control. Beef trimmings fabricated from 14 day-postmortem carcass were aerobically stored for 6 days at 5^{o}C. After storage, beef trimmings were ground, treated with various treatments, reground and formed into beef patties wrapped with oxygen-permeable film. The patties (2 per treatments) were stored under simulated retail conditions: 5°C, cool white fluorescent light (200 to 300 lux) and analyzed at day 0, 3, 6 and 9 of storage to assess the effectiveness of each treatment in preventing further quality deterioration. Objective color (L*, a*, and b*), 2-thiobarbituric acid (TBA) determinations, GC-MS for off-odor assessment and aerobic plate counts (APC) were conducted. Based on data, a* and TBA values were used to conduct multivariate RSM analyses for day 3 and 6. Multivariate RSM was conducted to overlap the contour plots of predicted a* and TBA values at day 3 and 6 to better approximate the optimal ingredient concentrations for a* values. The optimum concentration ranges of

solutions based on this analysis were NaL (0.43 to 0.57 M), NaE (0.35 M), NaB (1 M) with predicted a* values > 11 and TBA values < 0.52. Results of this study suggest that a combination of NaL, NaE, and NaB can be applied to improve color stability, reduce lipid oxidation, and control off-odor of ground beef patties.

Key words: Beef trimmings, color, lipid oxidation, off-odor, response surface methodology

3.2 Introduction

Most beef cuts in retail meat cases are about 18 to 33 days old after the animal harvesting (Smith *et al.*, 2000). The color of fresh beef products (i.e. steaks, ground beef) becomes an undesirable brown color when at least 60% of oxymyoglobin (bright cherry red color) is oxidized into the metmyoglobin. Consumers view the desirable cherry-red color of beef as an indicator of freshness and wholesomeness (Faustman & Cassens, 1990; Troy & Kerry, 2010). Unless it is discounted in price, discolored (brownish) beef products are not preferred by consumers who view them as being inferior in quality. The economic loss due to discoloration of beef raw materials/retail products was estimated to be more than $ 1 billion annually in the United States (Smith *et al.*, 2006).

The main factors contributing to meat discoloration are pH, oxygen, lipid peroxidation, and microbial spoilage (Mancini & Hunt, 2005; Suman & Joseph, 2013; Faustman & Cassens, 1990; Seideman *et al.*, 1984). For ground beef production, beef

trimmings are ground to achieve a specific fat content (i.e., 20% fat). Thus, ground beef is more susceptible to the interaction of myoglobin oxidation and lipid peroxidation due to the increased surface area (reduced particle size via grinding) and exposure to oxygen. Several studies indicate that oxidized lipid products and secondary aldehyde products could covalently modify myoglobin resulting in decreased myoglobin redox stability, and limiting the likelihood of metmyoglobin reduction via enzymatic processes (Faustman *et al.*, 1999; Mancini & Hunt, 2005; Lee *et al.*, 2003; Lynch & Faustman, 2000). These results provided an insight of the interaction of myoglobin oxidation and lipid peroxidation in meat.

Most meat science research had investigated the efficacy of using single active ingredients to either enhance color stability, reduce lipid oxidation, control microbial spoilage or all at once. Some studies showed increasing the shelf life of meat products. The treated ground beef with 2.3 mM (0.05 %) of sodium erythorbate exhibited higher a* values (redder color) and lower TBA values (less lipid oxidation) compared to a ground beef control at 48 hours storage at 4^{o}C (Sepe *et al.*, 2005). The treated beef patties with 500 ppm ascorbic acid had limited inhibition on myoglobin oxidation and effectively reduced lipid peroxidation (Sánchez-Escalante *et al.*, 2001). However, these studies did not employ a combination of these active ingredients to specifically address myoglobin oxidation, lipid peroxidation, off odor and microbial spoilage in meat.

Based on our preliminary research, sodium lactate (NaL), sodium erythorbate (NaE) and sodium bicarbonate (NaB) were selected to develop a processing aid that can

be applied to aerobically-stored beef trimmings to extend the shelf life of not only the trimmings, but also the ground products manufactured from them. Sodium bicarbonate and NaE were found to enhance color stability, NaE inhibited lipid peroxidation, NaE and NaB were effective in mitigating off odors and NaL controlling microbial spoilage of aerobically stored beef trimmings used in the manufacture of beef patties (preliminary research).

We hypothesize that the ingredient concentration level combinations containing NaL, NaE and NaB will be optimized to maintain color stability, reduce lipid oxidation, mitigate off odor and control microbial spoilage of aerobically stored beef trimmings. The objective of this study was to identify optimum concentrations of NaE, NaL and NaB and apply these ingredients as a processing aid solution to aerobically stored beef trimmings and evaluate the efficacy of these solutions on improving the shelf life attributes of beef patties manufactured from these treated trimmings.

3.3 Materials and Methods

3.3.1 Experimental design

Fifteen different combinations of the three variables were generated from 2^3 (three ingredients and two levels of concentration) central composite rotatable design for sodium lactate (NaL), sodium erythorbate (NaE) and sodium bicarbonate (NaB) with the center point replicated five times to assess lack-of-fit and ensure concentric variance (Cochran & Cox, 1957). Based on the previous research results (Chapter II), the

concentration range for the variables were NaL: 0.1 to 1.5 M, NaE: 0.1 to 0.6 M and NaB 0.1 to 1.5 M (Table III-1).

Table III-1: The combinations of treatments for 2^3 central composite response surface

Treatments[1]		NaL (M)	NaE (M)	NaB (M)
1	−−−	0.38	0.20	0.38
2	+−−	1.22	0.20	0.38
3	−+−	0.38	0.50	0.38
4	−−+	0.38	0.20	1.22
5	++−	1.22	0.50	0.38
6	+−+	1.22	0.20	1.22
7	−++	0.38	0.50	1.22
8	+++	1.22	0.50	1.22
9	000	0.80	0.35	0.80
10	00a	0.80	0.35	0.10
11	00A	0.80	0.35	1.50
12	0a0	0.80	0.10	0.80
13	0A0	0.80	0.60	0.80
14	a00	0.10	0.35	0.80
15	A00	1.50	0.35	0.80
16	Control	0.00	0.00	0.00

[1] The concentrations are extremely low, low, center, high and extremely high, as indicated by a, –, 0, + and A, respectively. The symbols --- to +++ are factorial points with a combination of low and high concentrations levels. The 000 represents the center point. The symbols 00a to A00 represent the star points. The control is treated with water and not used in response surface model.

3.3.2 Sample collection, preparation and treatments

Beef carcasses were obtained from the Rosenthal Meat Science and Technology Center at Texas A&M University. Beef trimmings (~50kg) were fabricated from beef forequarters (n = 5) 14 days postmortem, combined and aerobically stored (5°C) for an additional 6 days to simulate the collection, storage, transportation and receipt of a

combo of beef trimmings. After 6 days of aerobic storage, the beef trimmings (~20% fat) were coarse ground (12 mm) and the single ingredient treatment or control (water) solution was applied to the coarse ground trimmings (~454 g) at 2% (w/w). The trimmings were reground (3 mm) and 120g of treated sample was placed into a Petri dish and overwrapped with oxygen permeable film (oxygen transmission rate: 21,700 cc/m^2/24h at 25°C) to form patties. Each treatment had 2 samples, except the center point had 10 samples (n = 160). The patties were stored under simulated retail conditions: 5°C, cool white fluorescent light (200 to 300 lux) and analyzed at day 0, 3, 6 and 9 of storage to assess the effectiveness of each treatment in preventing further quality deterioration.

3.3.3 Solution preparation

Sodium bicarbonate ($NaHCO_3$; Anhydrous, ACS reagent grade >99.5%) was purchased from Sigma Aldrich Co. (Milwaukee, WI). Potassium and sodium lactate ($C_3H_5KO_3$ and $C_3H_5NaO_3$) were procured from Corbion Purac (Purac Biochem, Gorinchem, Netherlands). Sodium erythorbate (C6H7NaO6; Zhenzhou Tuoyang Bioengineering CO., Henan Province, China) was purchased from a local food ingredient supplier. The treatments were prepared fresh according to Table III-1 by mixing each ingredient with the appropriate amount of double-distilled, deionized water. Sodium bicarbonate and NaE treatments required heating up to 70 ^oC to completely dissolve.

3.3.4 pH determination

Ten grams of each treatment and control ground beef sample (n = 160) 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).

3.3.5 Proximate composition

Untreated ground beef samples were collected and submerged in liquid nitrogen and powdered using a Waring blender (Model 33BL79, Waring Commercial, New Hartford, CT). Powdered samples (n = 3) were used to determine proximate composition according to AOAC (2005) procedures for moisture (AOAC 985.14 oven drying method), protein (AOAC 992.15 using a nitrogen analyzer, F528, Leco Corp., St. Joseph, MI), and fat (AOAC 985.15 subtracting 100% from moisture and protein; AOAC, 2019).

3.3.6 Objective color determination

Treated 3 mm ground beef patties overwrapped with oxygen permeable film (n = 160) 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 degree standard observers; Hunter Associates Laboratory, Inc., Reston, VA). The HunterLab Miniscan was calibrated with white and black tiles wrapped with oxygen permeable film before measurement. Illuminant D 65 is used for L*, a*, and b* color values and illuminant A for spectral reflectance (474, 525, 572 and 700 nm). The

percentage of deoxymyoglobin and metmyoglobin was calculated from the spectrum data (DMb% = (2.375 * [1-(A474 – A700)/(A525 – A700)]) * 100 ; MMb% = (1.395 – [(A572 - A700)/(A525 – A730)]) * 100) and oxymyoglobin percentage was determined indirectly by subtracting their combined percentages (DMb% + MMb%) from 100 % (AMSA, 2012; Krzywicki, 1979).

3.3.7 Lipid oxidation determination

The thiobarbituric acid (TBA) assay method was used to determine lipid oxidation of the treated ground beef patties at each designated storage day (Tarladgis et al., 1960). The mechanism of the TBA assay is based on the reaction between TBA and malonaldehyde (MDA) which results in a colored pigment. The concentration of MDA can be calculated by measuring the absorbance at 532 nm. Ten gram of sample (n = 160) was homogenized with 50 mL of deionized water, 5mL of 0.5 % propyl gallate (PG) and 0.5 % ethylenediaminetetraacetic acid (EDTA) solution. The homogenous meat solution was transferred to a Kjeldahl distillation flask with 31.5 mL deionized water. The 2.5 mL of 4 N HCl and 5 to 6 boilling chips were added to the flask, and deionized water was added into the flask to make the total volume 100 mL. The mixture was heated and the first 50 mL of distillate was collected. The 5 mL of collected distillate was reacted with 5 mL of 0.02 M TBA agents in a test tube in a 100^{o}C water bath for 35 min. After incubation, the test tube was cooled in ice for 10 min and the optical density (O.D.) value of the sample was read at an absorbance of 532 nm. The TBA value was calculated from multiplying O.D. value of sample by a constant of 7.8.

3.3.8 Off odor determination using gas chromatograph/mass spectrometry

Ten grams of samples (n = 80) were placed into glass vials (20 mL) and stored at -80 ^{o}C until further analysis. The frozen glass vials containing samples were thawed at room temperature. After thawing and equilibrating at room temperature, a solid-phase micro-extraction (SPME) portable field sampler (Supelco 504831, 75 µm carboxen/ polydimethylsiloxane [PDMS], Sigma-Aldrich, St. Louis, MO) was inserted through the lid in order to collect the headspace above each meat sample in the glass vial held at room temperature for 2 hr. After collection, the SPME was remove from the vial and injected into the injection port of a gas chromatograph (GC; Agilent Technologies 7920 series GC, Santa Clara, CA), where the sample was desorbed at 280°C for 3 min. The sample was then loaded onto the multi-dimensional gas chromatograph through a first column (30 m × 0.53 mm ID/BPX5 [5% phenyl polysilphenylene-siloxane] × 0.5µm, SGE Analytical Sciences, Austin, TX) and then a second column (30 m × 0.53 mm ID [BP20—polyethylene glycol] × 0.50 µm, SGE Analytical Sciences). The GC temperature started at 40°C and increased at a rate of 7°C/min until reaching 260°C. The GC column then went to a mass spectrometer (MS; Agilent Technologies 5975 series MS, Santa Clara, CA) for quantification and identification using the Wiley Chemical Library. A 3-point external standard curve (1, 3 dichlorobenzene) was run to estimate concentration of volatiles from area data. Chemicals exceeded a quality report from the MS of 80 were used for analysis. The GC-MS data were reported as total ion counts.

3.3.9 Microbiological determination

Twenty grams of samples (n = 160) 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 milliliter 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 three sets of Petrifilms® (3M® Microbiology, St. Paul, MN). One set of aerobic count (AC) petrifilms was incubated for 48 hr at 35°C before enumeration to quantify aerobic mesophiles (AM). One set of AC films was incubated for 7 days at 7°C to quantify aerobic psychrotrophs (Salfinger & Tortorello, 2015).

3.3.10 Statistical analysis

For this study, least squares means for a* values, TBA values, total ion counts and AP counts were reported. The data was analyzed by one-way ANOVA using JMP Pro 14 (SAS Institute, Inc., Cary, NC). The GLM and Standard Least Squares with $\alpha = 0.05$ was used for one-way and two-way ANOVA, respectively. The fixed effect for both phases was treatment and day for two-way ANOVA and treatment for one-way ANOVA. The significant differences among treatments means were determined using Tukey's HSD ($P < 0.05$). For RSM and multivariate RSM analyses, the data was used to generate total quadratic polynomial linear regression models ($P < 0.05$) and contour plots to determine the optimum ingredient concentrations using JMP Pro 14 (SAS Institute, Inc., Cary, NC). The proportion of variance explained by the polynomial models obtained

was given by the multiple coefficient of determination, r^2 and the adequacy of the model was verified using a "lack of fit" test (Myers *et al.*, 2016).

3.4 Results and Discussions

3.4.1 Proximate composition

The moisture, protein, and fat contents were 60.34 ± 0.06 %, 18.95 ± 0.40 % and 20.71 ± 0.41 %, respectively (Table III-2). The targeted fat of 20 % fat was achieved, which is similar to the fat content of many types of ground beef products.

Table III-2: Least squares means of the proximate composition of 12 mm untreated ground beef

	Moisture (%)	Protein (%)	Fat (%)
Untreated Ground beef	60.34	18.95	20.71
SEM[1]	0.06	0.40	0.41

[1] The SEM present the standard error of mean for the treatment and control means.

3.4.2 Objective color

Most treated beef patties had lower a* values compared to the control on day 0 ($P < 0.05$), except treatment 3 and 11 (21.48 and 22.90, respectively; Figure III-1 and Table III-3). All treated patties had higher a* values compared to the control on day 3 ($P < 0.05$). Only treatment 13 had higher a* values compared to the control on day 6 ($P < 0.05$). Treatment 1, 2, 3, 7, 9, 11, 12 and 15 had higher a* values compared to the

control on day 9 (P < 0.05). However, the difference of a* values was minor (<5 difference) from day 0 to day 9 compared to the control.

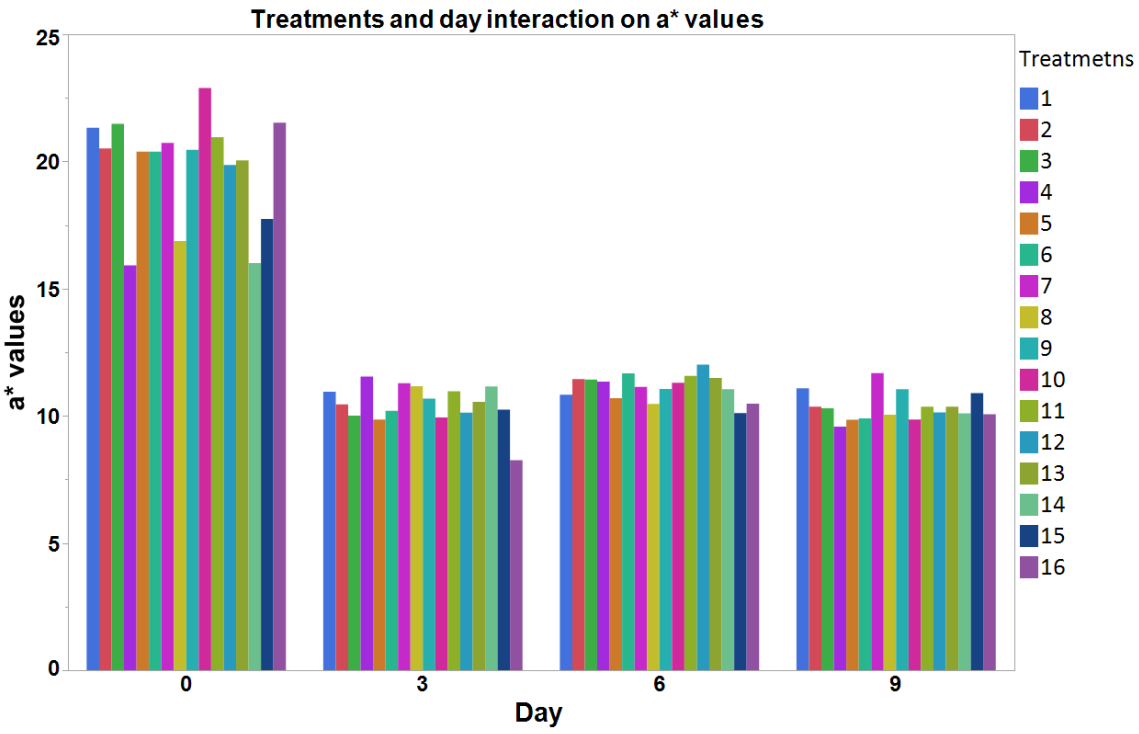

Figure III-1: Bar graph for the interaction of treatments and day on a* values
The interaction of treatments and day was significantly affected a* values (P < 0.05, SEM: 0.53).

Table III-3: Least squares means of the a* values of beef patties treated with solutions containing varying concentrations of NaL, NaE and NaB at 0, 3, 6 and 9 days of refrigerated storage

	Concentration (M)			Days of storage			
Treatments	NaL	NaE	NaB	Day 0	Day 3	Day 6	Day 9
1	0.38	0.20	0.38	21.33^{bc}	10.95^{abcd}	10.83^{ab}	11.09^{abc}
2	1.22	0.20	0.38	20.52^{def}	10.46^{cdef}	11.45^{ab}	10.37^{bcde}
3	0.38	0.50	0.38	21.48^{b}	10.01^{f}	11.43^{ab}	10.31^{bcde}
4	0.38	0.20	1.22	15.92^{j}	11.55^{a}	11.35^{ab}	9.58^{e}
5	1.22	0.50	0.38	20.39^{defg}	9.86^{f}	10.70^{ab}	9.85^{e}
6	1.22	0.20	1.22	20.39^{defg}	10.21^{def}	11.68^{ab}	9.90^{de}
7	0.38	0.50	1.22	20.73^{cde}	11.29^{ab}	11.19^{ab}	11.69^{a}
8	1.22	0.50	1.22	16.88^{i}	11.17^{abc}	10.48^{ab}	10.05^{de}
9	0.80	0.35	0.80	20.46^{ef}	10.68^{bcde}	11.06^{ab}	11.05^{ab}
10	0.80	0.35	0.10	22.90^{a}	9.94^{f}	11.30^{ab}	9.86^{e}
11	0.80	0.35	1.50	20.96^{bcd}	10.97^{abcd}	11.57^{ab}	10.37^{bcde}
12	0.80	0.10	0.80	20.05^{fg}	10.55^{bcdef}	11.49^{ab}	10.37^{bcde}
13	0.80	0.60	0.80	19.87^{g}	10.13^{ef}	12.02^{a}	10.14^{cde}
14	0.10	0.35	0.80	16.01^{j}	11.16^{abc}	11.05^{ab}	10.10^{cde}
15	1.50	0.35	0.80	17.74^{h}	10.25^{def}	10.11^{b}	10.90^{abcd}
16	0.00	0.00	0.00	21.53^{b}	8.26^{g}	10.49^{ab}	10.07^{cde}
	SEM[1]			0.03	0.04	0.09	0.05
	SEM[2]			0.07	0.09	0.21	0.12
	p-value			0.000	0.000	0.050	0.002

[1,2] The SEM[1] and SEM[2] present the standard error of mean for center point (treatment 9) and all other treatments means.

[abcde] Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

It was reported that 4.46 M sodium lactate did not have any effect on improving color stability of beef trimmings (Yeater, 2016). Pumped beef strip loins (10%) containing 2.5 % (0.2 M) potassium lactate significantly increased a* values and lactate dehydrogenase activity during vacuum package display compared to the control (Kim *et*

al., 2006). The differences in results were due to different forms of lactate used and method of application. It has been reported that erythorbic acid and its salt form can improve the beef color stability of ground beef, while the mechanism is still unanswered. The ground beef treated with 0.04 % and 0.06 % of erythorbic acid had higher a* value compared to the non-treated one upon 58 hours storage at 4 ^{o}C (Phillips *et al.*, 2001). Also, ground beef treated with 2.3 mM (0.05 %) of either erythorbic acid or sodium erythorbate had higher a* values compared to the non-treated samples during 48 hours storage at 4 ^{o}C (Sepe *et al.*, 2005). The beef muscles treated with 0.1 M sodium erythorbate and stored for 48 hours exhibited the highest oxymyoglobin (OMb) percentage compared to other treatments (Yeater, 2016). Interestingly, treatment solutions, including varying concentrations of both NaL and NaE, did not dramatically improve the color stability. Wet-aged beef longissimus steak (28 days) vacuumed package and stored in dark at 5 ^{o}C had lower levels of NADH and similar activity of NADH-dependent reductase activity compared to those aged for 3 days (Mitacek *et al.*, 2019). This evidence further supports that the MRA of meat is primarily governed by the regeneration of NADH. It is widely recognized that NaL could regenerate NADH by lactate dehydrogenase and restore the functionality of MRA in muscle (Suman *et al.*, 2014; Mancini & Ramanathan, 2008; Kim *et al.*, 2006). Theoretically, our treatments should be capable of regenerating NADH to maintain the color stability in the muscle (i.e., trimmings) unless the lactate dehydrogenase lost its functionality during aerobically storage (lower or no NADH regeneration). Or, NADH

regeneration by lactate dehydrogenase is functional but the NADH-dependent reducing system, such as NADH-cytochrome b_5 MMb reductase and cytochrome b_5, was damaged (MRA reduced or eliminated).

3.4.3 Metmyoglobin formation

The MMb percentage of treatments 4, 9 and 11 were lower compared to the control on day 0 ($P < 0.05$; Figure III-2 and Table III-4). All treatments were effective in reducing MMb formation compared to the control on day 3 and 9 ($P < 0.05$), except for treatment 8 on day 9. No difference in percent MMb was observed for treated and control patties on day 6 ($P > 0.05$). Lactate is known to improve the color stability of meat by increasing NADH generation through lactate dehydrogenase to reduce metmyoglobin formation (Suman *et al.*, 2014; Mancini & Ramanathan, 2008; Kim *et al.*, 2006). Also, 500 ppm ascorbic acid (the acid form of NaE) to ground beef packaged in modified air package (70 % O_2, 20% CO_2 and 10% N_2) had lower MMb formation compared to the control up to 20 days of storage (Sánchez-Escalante *et al.*, 2001). Our results support the positive effects of lactate and erythorbate in improving meat color stability.

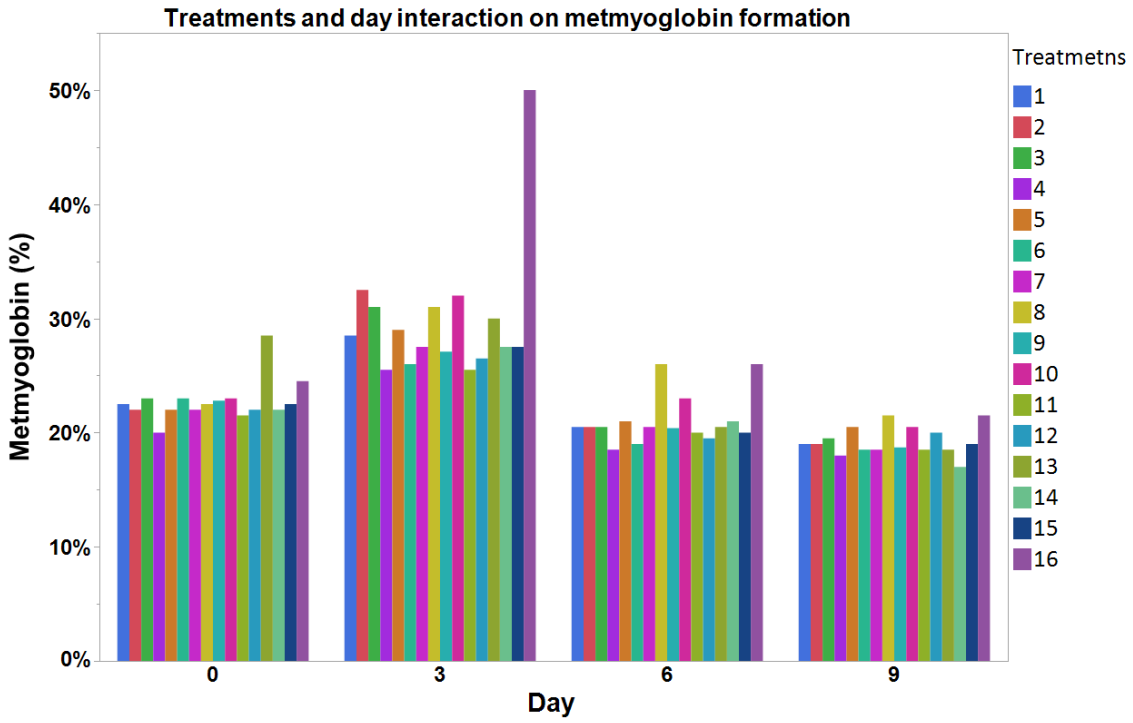

Figure III-2: Bar graph for the interaction of treatments and day on metmyoglobin formation
The interaction of treatments and day was significantly affected MMb formation ($P < 0.05$, SEM: 0.01).

Table III-4: Least squares means of the metmyoglobin percentage of beef patties treated with solutions containing varying concentrations of NaL, NaE and NaB at 0, 3, 6 and 9 days of refrigerated storage

	Concentration (M)			Days of storage			
Treatments	NaL	NaE	NaB	Day 0	Day 3	Day 6	Day 9
1	0.38	0.20	0.38	22.50 %^{ab}	28.50 %^{b}	20.50 %	19.00 %^{bcd}
2	1.22	0.20	0.38	22.00 %^{ab}	32.50 %^{b}	20.50 %	19.00 %^{bcd}
3	0.38	0.50	0.38	23.00 %^{ab}	31.00 %^{b}	20.50 %	19.50 %^{abc}
4	0.38	0.20	1.22	20.00 %^{b}	25.50 %^{b}	18.50 %	18.00 %^{cd}
5	1.22	0.50	0.38	22.00 %^{ab}	29.00 %^{b}	21.00 %	20.50 %^{ab}
6	1.22	0.20	1.22	23.00 %^{ab}	26.00 %^{b}	19.00 %	18.50 %^{bcd}
7	0.38	0.50	1.22	22.00 %^{ab}	27.50 %^{b}	20.50 %	18.50 %^{bcd}
8	1.22	0.50	1.22	22.50 %^{ab}	31.00 %^{b}	26.00 %	21.50 %^{a}
9	0.80	0.35	0.80	22.80 %^{b}	27.10 %^{b}	20.40 %	18.70 %^{bcd}
10	0.80	0.35	0.10	23.00 %^{ab}	32.00 %^{b}	23.00 %	20.50 %^{ab}
11	0.80	0.35	1.50	21.50 %^{b}	25.50 %^{b}	20.00 %	18.50 %^{bcd}
12	0.80	0.10	0.80	28.50 %^{a}	30.00 %^{b}	20.50 %	18.50 %^{bcd}
13	0.80	0.60	0.80	22.00 %^{ab}	26.50 %^{b}	19.50 %	20.00 %^{abc}
14	0.10	0.35	0.80	22.00 %^{ab}	27.50 %^{b}	21.00 %	17.00 %^{d}
15	1.50	0.35	0.80	22.50 %^{ab}	27.50 %^{b}	20.00 %	19.00 %^{bcd}
16	0.00	0.00	0.00	24.50 %^{ab}	50.00 %^{a}	26.00 %	21.50 %^{a}
	SEM[1]			0.03	0.01	0.01	0.00
	SEM[2]			0.07	0.02	0.01	0.00
	p-value			0.054	0.013	0.197	0.005

[1,2] The SEM[1] and SEM[2] present the standard error of mean for center point (treatment 9) and all other treatments means.

[abcde] Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

3.4.4 Lipid peroxidation and off odor

No difference was observed for treated and control patties for TBA on day 0 ($P > 0.05$; Table III-5). The TBA values for all treatments reduced lipid oxidation compared to the control on day 3, 6 and 9 ($P < 0.05$). Sodium-lactate-treated (20 g/kg) ground beef vacuum packaged and stored for 21 days had lower TBA values compared to the control (Sallam & Samejima, 2004). The treated ground beef with 2.3 mM (0.05 %) of sodium erythorbate had lower TBA values compared to the control at 48 hours storage at 4 ^{o}C (Sepe *et al.*, 2005). Our results supported the beneficial effects of lactate and erythorbate on reducing lipid peroxidation.

Table III-5: Least squares means of the total ion counts of hexanal of beef patties treated with solutions containing varying concentrations of NaL, NaE and NaB at 0, 3, 6 and 9 days of refrigerated storage

	Concentration (M)			Days of storage			
Treatments	NaL	NaE	NaB	Day 0	Day 3	Day 6	Day 9
1	0.38	0.20	0.38	0.0	0.0[f]	0.0[b]	0.0[b]
2	1.22	0.20	0.38	0.0	0.0[f]	0.0[b]	0.0[b]
3	0.38	0.50	0.38	0.0	15369.0[e]	0.0[b]	0.0[b]
4	0.38	0.20	1.22	0.0	0.0[f]	0.0[b]	0.0[b]
5	1.22	0.50	0.38	0.0	18448.0[d]	0.0[b]	0.0[b]
6	1.22	0.20	1.22	0.0	0.0[f]	41480.0[b]	0.0[b]
7	0.38	0.50	1.22	0.0	0.0[f]	0.0[b]	0.0[b]
8	1.22	0.50	1.22	0.0	0.0[f]	0.0[b]	0.0[b]
9	0.80	0.35	0.80	0.0	0.0[f]	6322.0[b]	0.0[b]
10	0.80	0.35	0.10	0.0	0.0[f]	0.0[b]	0.0[b]
11	0.80	0.35	1.50	0.0	0.0[f]	0.0[b]	0.0[b]
12	0.80	0.10	0.80	0.0	63842.0[b]	0.0[b]	0.0[b]
13	0.80	0.60	0.80	0.0	20956.0[c]	0.0[b]	0.0[b]
14	0.10	0.35	0.80	0.0	0.0[f]	0.0[b]	0.0[b]
15	1.50	0.35	0.80	0.0	0.0[f]	0.0[b]	0.0[b]
16	0.00	0.00	0.00	0.0	64253.0[a]	470859.0[a]	600736.0[a]
	SEM[1]			0.0	0.0	6322.0	0.0
	SEM[2]			0.0	0.0	14136.4	0.0
	p-value			N/A	N/A	0.001	N/A

[1,2] The SEM[1] and SEM[2] present the standard error of mean for center point (treatment 9) and all other treatments means.

[abcde] Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

No difference was observed for treated and control patties for total ion counts of hexanal on day 0 (Figure III-3 and Table III-6). All treatments had significantly lower total ion counts of hexanal compared to the control through day 3, 6 and 9 ($P < 0.05$). Hexanal is an indicator of off-odor and is produced from the lipid peroxidation of

omega 6 fatty acid, primarily linoleic acid in beef (Shahidi & Pegg, 1994; Smith *et al.*, 2006; Frankel, 1991; Calkins & Hodgen, 2007). The total ion counts of hexanal data validated the results of TBA values. All treatments were effective in reducing lipid peroxidation and eliminating off odors.

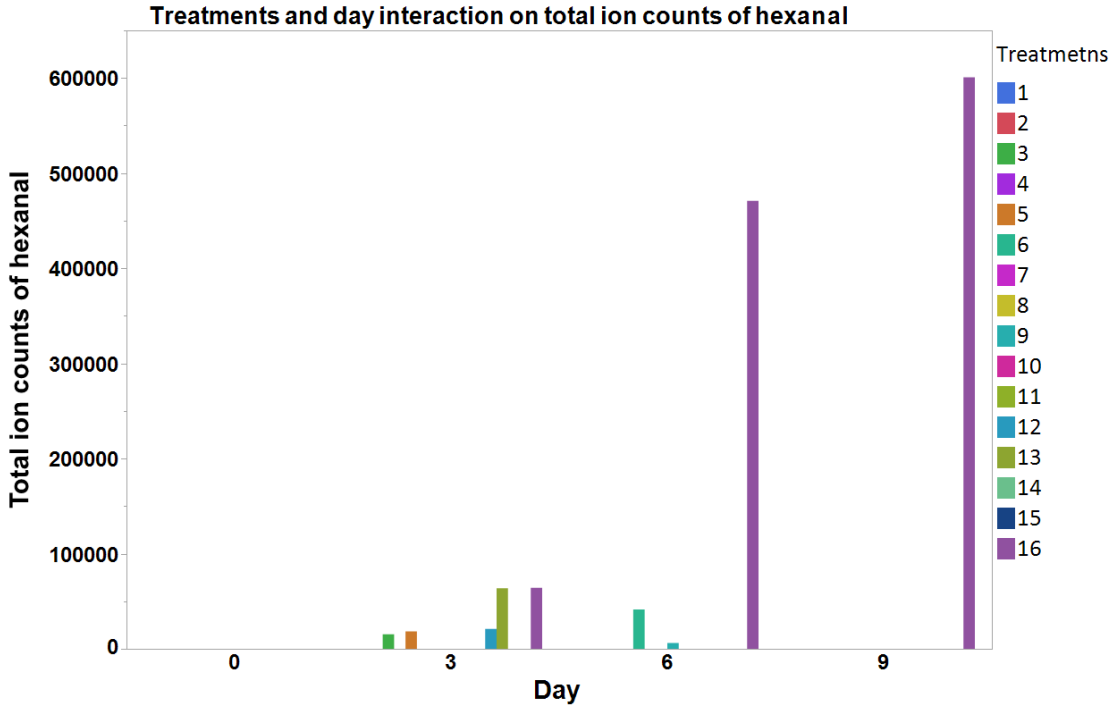

Figure III-3: Bar graph for the interaction of treatments and day on total ion counts of hexanal
The interaction of treatments and day was significantly affected total counts of hexanal ($P < 0.05$, SEM: 7068.21).

Table III-6: Least squares means of the TBA values of beef patties treated with solutions containing varying concentrations of NaL, NaE and NaB at 0, 3, 6 and 9 days of refrigerated storage

	Concentration (M)			Days of storage			
Treatments	NaL	NaE	NaB	Day 0	Day 3	Day 6	Day 9
1	0.38	0.20	0.38	0.61	0.47^{b}	0.57^{b}	0.54^{bcde}
2	1.22	0.20	0.38	0.54	0.56^{b}	0.51^{b}	0.45^{e}
3	0.38	0.50	0.38	0.55	0.52^{b}	0.58^{b}	0.48^{de}
4	0.38	0.20	1.22	0.54	0.53^{b}	0.51^{b}	0.50^{cde}
5	1.22	0.50	0.38	0.53	0.57^{b}	0.54^{b}	0.46^{e}
6	1.22	0.20	1.22	0.48	0.53^{b}	0.58^{b}	0.57^{bcd}
7	0.38	0.50	1.22	0.56	0.54^{b}	0.54^{b}	0.55^{bcde}
8	1.22	0.50	1.22	0.57	0.58^{b}	0.51^{b}	0.50^{cde}
9	0.80	0.35	0.80	0.57	0.50^{b}	0.51^{b}	0.53^{cde}
10	0.80	0.35	0.10	0.57	0.53^{b}	0.54^{b}	0.57^{bcd}
11	0.80	0.35	1.50	0.51	0.57^{b}	0.53^{b}	0.60^{bc}
12	0.80	0.10	0.80	0.55	0.47^{b}	0.52^{b}	0.62^{b}
13	0.80	0.60	0.80	0.53	0.54^{b}	0.54^{b}	0.49^{de}
14	0.10	0.35	0.80	0.43	0.58^{b}	0.57^{b}	0.49^{de}
15	1.50	0.35	0.80	0.63	0.56^{b}	0.56^{b}	0.57^{bcd}
16	0.00	0.00	0.00	0.62	0.71^{a}	0.74^{a}	0.74^{a}
	SEM[1]			0.01	0.01	0.01	0.01
	SEM[2]			0.02	0.01	0.01	0.01
	p-value			0.077	0.005	0.007	0.002

[1,2] The SEM[1] and SEM[2] present the standard error of mean for center point (treatment 9) and all other treatments means.

[abcde] Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

3.4.5 Microbial spoilage

No differences were observed for treated and control patties for APC from day 0 to day 6, but differences were observed on day 9 ($P < 0.05$; Figure III-4 and Table III-7). Sodium lactate (5%) was effective in increasing the lag phase and reducing the growth

rate phase of *Streptococcus fuecalis and Staphylococcus aureus* (de Wit & Rombouts, 1990). Sodium erythorbate (3%) was effective to lower aerobic plate counts

electrostatic coated ground turkey patties with sodium erythorbate reduced the total number of microorganisms after 9 days of refrigerated storage by an average of 2 logs (Barringer *et al.*, 2005). These reported researches were conflicting with our finding, the treatments had no effect on microbial spoilage of treated ground beef patties stored for 6 days, and this may due to the difference in methodology.

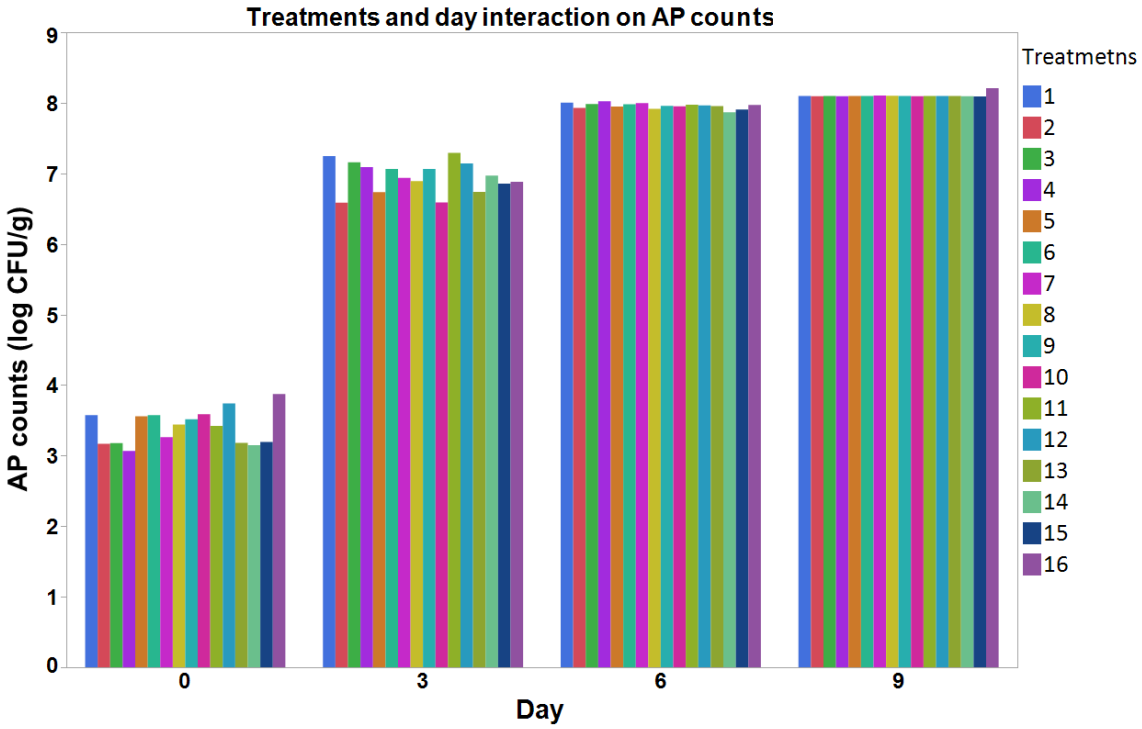

Figure III-4: Bar graph for the interaction of treatments and day on total ion counts of hexanal
The interaction of treatments and day was significantly affected total counts of hexanal ($P < 0.05$, SEM: 0.09).

Table III-7: Least squares means of aerobic psychrotrophs counts of beef patties treated with solutions containing varying concentrations of NaL, NaE and NaB at 0, 3, 6 and 9 days of refrigerated storage

	Concentration (M)			Days of storage			
Treatments	NaL	NaE	NaB	Day 0	Day 3	Day 6	Day 9
1	0.38	0.20	0.38	3.57	7.24	8.00	8.10^{b}
2	1.22	0.20	0.38	3.17	6.58	7.93	8.10^{b}
3	0.38	0.50	0.38	3.18	7.16	7.98	8.10^{b}
4	0.38	0.20	1.22	3.07	7.09	8.02	8.10^{b}
5	1.22	0.50	0.38	3.56	6.74	7.95	8.10^{b}
6	1.22	0.20	1.22	3.58	7.06	7.98	8.10^{b}
7	0.38	0.50	1.22	3.26	6.94	8.00	8.11^{b}
8	1.22	0.50	1.22	3.44	6.89	7.92	8.10^{b}
9	0.80	0.35	0.80	3.52	7.07	7.96	8.10^{b}
10	0.80	0.35	0.10	3.59	6.59	7.95	8.09^{b}
11	0.80	0.35	1.50	3.42	7.29	7.97	8.10^{b}
12	0.80	0.10	0.80	3.18	6.74	7.95	8.10^{b}
13	0.80	0.60	0.80	3.74	7.14	7.97	8.10^{b}
14	0.10	0.35	0.80	3.15	6.97	7.87	8.10^{b}
15	1.50	0.35	0.80	3.19	6.86	7.90	8.09^{b}
16	0.00	0.00	0.00	3.87	6.88	7.97	8.21^{a}
	SEM[1]			0.07	0.11	0.01	0.00
	SEM[2]			0.16	0.25	0.02	0.00
	p-value			0.227	0.252	0.099	0.001

[1,2] The SEM[1] and SEM[2] present the standard error of mean for center point (treatment 9) and all other treatments means.

[abcde] Means within a column of each parameter lacking a common superscript differ ($P < 0.05$).

3.4.6 Interaction of treatments and day

The results of two-way ANOVA reported that the interaction of treatments and day was significantly affected a* value, metmyoglobin (MMb) formation, total ion counts of hexanal and aerobic psychrotrophs (AP) counts ($P < 0.05$; Figure III-1, 2, 3, and 4). There was significant difference among treatments on a* values at day 0, 3, 6, and 9 ($P < 0.05$; Figure III-1). There was significant difference among treatments on MMb formation at day 0, 3 and 9 ($P < 0.05$; Figure III-2). There was significant difference among treatments on total ion counts of hexanal at day 3, 6, and 9 ($P < 0.05$; Figure III-3). There was significant difference among treatments on AP counts at day 9 ($P < 0.05$; Figure III-4).

3.4.7 Response surface methodology (RSM) and multivariate RSM analyses

Based on positive results observed for maintaining color stability (a* values) and reducing lipid oxidation (lower TBA values), these two variables were used to conduct RSM analyses to optimize ingredient concentrations based on results obtained on day 3 and 6 of ground beef patties storage. Day 9 was excluded due to a significant lack of fit. The RSM model of MMb formation was not significant ($P > 0.05$), hence it is not reported. The predicted value of hexanal was 0 for all treatments on day 3 and 6 and was not included in the model. The prediction of a* values on day 3 and 6 did not identify optimum ingredient concentrations for any treatment solution within the set concentration ranges ($r^2 = 0.94$ and 0.78, respectively; $P <0.05$; Figure III-5A and III-5B). The prediction of TBA values did determine the optimum ingredient concentrations for

NaL, NaE, and NaB on day 3 of beef patties storage: NaL (0.51 M), NaE (-5.21 M) and NaB (0.79 M; r^2 = 0.79, P <0.05; Figure III-5C). However, the predicted concentration level of NaE was outside the established data range. The prediction of TBA values found the optimum ingredient concentrations on day 6: NaL (0.74 M), NaE (0.35 M) and NaB (1.00 M; r^2 = 0.77, P <0.05; Figure III-5D). Since the optimum ingredient concentration for predicted a* (day 3 and 6) and TBA values (day 3) could not be determined, multivariate RSM was conducted to overlap the contour plots of a* and TBA values at day 3 and 6 to better approximate the optimal ingredient concentrations for these two variables (Figure III-5E). The proximal optimum concentration ranges of each ingredient based on this analysis were NaL (0.43 to 0.57 M), NaE (0.35 M) and NaB (1 M) with predicted a* values > 11 and TBA values < 0.52.

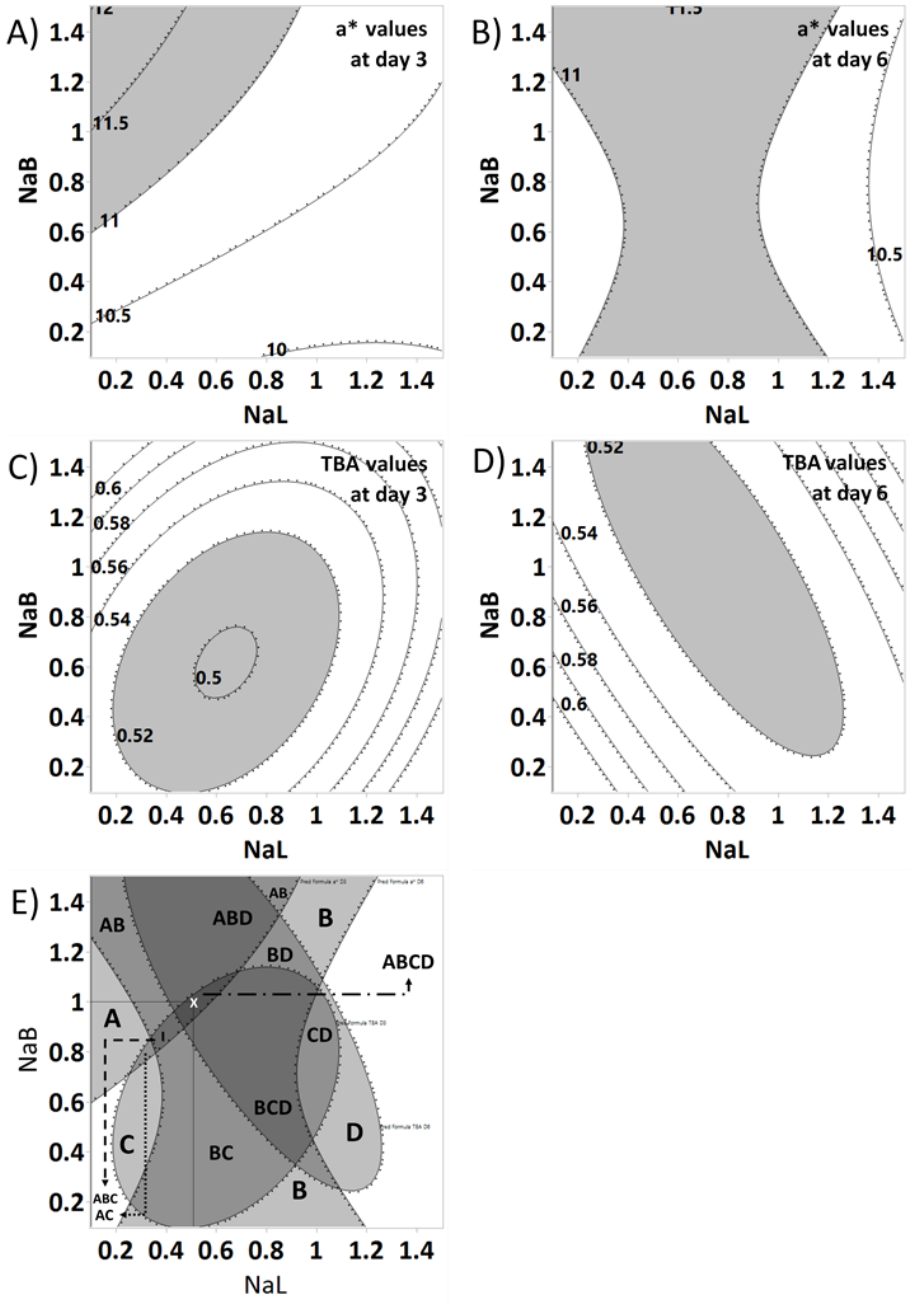

Figure III-5: Contour plots for predicted a* value and TBA value of the treated patties at day 3 and day 6

The NaE concentration was fixed at 0.35M. The grey area presented the desired target for predicted a* > 11 and TBA value <0.52. A) Predicted a* value at day 3 (r^2=0.94, P < 0.05) B) Predicted a* value at day 6 (r^2=0.78, P < 0.05) C) Predicted TBA value at day 3 (r^2=0.79, P < 0.05) D) Predicted TBA value at day 6 (r^2=0.77, P < 0.05) E) Contour plot of Multivariate RSM at day 3 and day6. The various combination of letters (AB, ABD, tec.) indicates the overlapped areas. The solid crossed reference lines indicate the optimal concentrations (X) determined by multivariate RSM: Na (0.43 to 0.57 M), NaE (0.35 M), NaB (1 M) with predicted a* values > 11 and TBA values < 0.52.

3.5 Conclusions

The results indicated that majority of treatments containing NaL, NaE, and NaB had positive effect on the treated beef patties on enhancing color, inhibiting MMb formation, reducing lipid peroxidation, and mitigating off odors for beef patties stored on day 3, 6 and 9. All treatments had a significant beneficial effect on mitigating off odors (reduced total ion counts of hexanal). Based on the ANOVA analysis, a* and TBA values were selected for RSM analysis for storage day 3 and 6. However, optimal ingredient concentrations were determined only for predicted TBA values for storage day 6. Therefore, multivariate RSM was employed to assist in determining proximal optimum ingredient concentrations for predicted values of both a* and TBA values. The proximal optimum concentration ranges of ingredients based on this analysis were NaL (0.43 to 0.57 M), NaE (0.35 M), NaB (1 M) with predicted a* values > 11 and TBA values < 0.52. Results of the current research suggests that a combination of NaL, NaE, and NaB (0.43 to 0.57, 0.35 and 1 M, respectively) could be applied to aerobically stored beef trimmings to improve color stability, reduce lipid oxidation, and mitigate off-odor in beef patties manufactured from these trimmings through 6 days of refrigerated retail storage. The development of a processing aid solution containing NaL, NaE and NaB at the concentrations determined in this study could be useful to enhance the shelf life attributes of beef trimmings that may possess less than desirable (poor color, slight off-odors/flavor development, microbial growth approaching) quality attributes for use in fresh beef products.

3.6 References

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CHAPTER IV

CONCLUSIONS AND FUTURE DIRECTION

Sodium bicarbonate (1.5 M), NaE (0.1 to 0.8 M) and NaL (1 M) had the greatest effect on enhancing the color stability, reducing the lipid peroxidation and controlling the microbial spoilage on ground beef compared to the control upon storage day 6 for the Chapter II study. Based on the results of phase 1 and 2, a 2^3 central composite design was used to optimize the concentrations levels of treatment combinations. For the Chapter III study, the treatment combinations of containing NaL, NaE and NaB had a positive impact on increasing a* values, lowering TBA values and reducing total ion counts of hexanal of treated beef patties compared to the control on day 3, 6 and 9 ($P < 0.05$) of storage. We employed multivariate RSM to potentially identify optimum ingredient concentrations predicted a* and TBA values on day 3 and 6. The predicted total ion counts of hexanal was 0 for all treatment combinations. The proximal optimum concentration ranges of solutions based on the multivariate response surface methogology analysis were NaL (0.43 to 0.57 M), NaE (0.35 M), NaB (1 M) with predicted a* values > 11 and TBA values < 0.52. Results of the research suggest that a combination of NaL, NaE, and NaB (0.43 to 0.57, 0.35 and 1 M, respectively) could be applied aerobically 6-days-stored beef trimmings (5 ^{o}C) at 2% addition to manufacture ground beef patties to improve color stability, reduce lipid oxidation, and mitigate off-odors after 6 days of refrigerated retail storage conditions.

Future studies should focus on the further investigation of NaL, NaE, and NaB (0.43 to 0.57, 0.35 and 1 M, respectively) to develop a processing aid that can enhancing the shelf-life of aerobically-stored beef trimmings. Additionally, further investigation of the percentage (w/w) of treatment solution application, the effect of time and application method would provide additional information to effectively utilize the processing aid solution developed from this research. Furthermore, we should explore the efficacy of the solution on different species, such as pork, chicken, turkey, etc. Moreover, there is a need to investigate the mechanisms/reactions that create the functionality of the solution and the interaction of myoglobin and lipid oxidation of treated samples. This will provide insight as to why the solution containing NaL, NaE and NaB had minor to no impact on color and microbial spoilage, respectively. From this investigation there would be the opportunity to improve the efficacy of the developed processing aid or create a better one to enhance the shelf life of aerobically stored trimmings.

APPENDIX A

SOLUTION PREPARATION

FOR CHAPTER II RESEARCH

1. Weigh the chemicals, including NaE and NaB.
2. Transfer distilled and deionized water into Erlenmeyer flask and place a stir bar inside the flask.
3. Transfer chemicals (NaE or NaB) into into Erlenmeyer flask contain distilled and deionized water.
4. Place the flask on the heating plate.
5. Turn on the heating (power 3) and stirring of the heating plate.
6. NaB and NaE were required heating up to 70 ^{o}C for completely dissolving.
7. Solutions were allowed to cool to room temperature before treatments application.
8. Dilute either NaL or KL into distilled and deionized water.

NOTE: the solutions were prepared freshly

FOR CHAPTER III RESEARCH

1. Weigh NaE and NaB.
2. Transfer distilled and deionized water into Erlenmeyer flask and place a stir bar inside the flask.
3. Transfer NaB into Erlenmeyer flask contain distilled and deionized water.
4. Place the flask on the heating plate.

5. Turn on the heating (power 3) and stirring of the heating plate.
6. NaB was required heating up to 70 ^{o}C for completely dissolving.
7. After NaB was dissolved, add NaE into the flask.
8. After NaE was dissolved, remove the flask from heating plate.
9. Add NaL into the flask and transfer it into the test tubes.
10. Solutions were allowed to cool to room temperature before treatments application.

APPENDIX B

BEEF PATTIE MANUFACTURE

1. The beef trimmings were collected from ANSC 307 and stored in container covered with plastic sheet for 6 days in aging cooler which set at 5 ^{o}C in Rosenthal Meat Science and Technology Center (RMSTC).
2. After 6 days storage, the beef trimmings were ground through 12 mm plate.
3. 907 g coarse ground beef were mixed with 18 mL treatments using a stand mixer (KitchenAid, Benton Harbor, MI).
4. The treatments were applied by manual pipette pump with 10 mL pipette tip and the application of treatment was done with an approximate speed as 0.45 mL/sec. The treatment application time was approximately first 20 sec of the total 60 sec of mixing.
5. After mixing, the mixed ground beef was ground through 3 mm plate. The table top grinder was rinsed with distilled water between each grinding of treated ground beef.
6. 120 g treated ground beef was filled into 100 X15 mm petri dish (CAT NO. 25384-302, VWR) and wrapped with oxygen permeable film (OTR: 21,700 cc/m^2/24h at 25°C; WP-MWL 18, western plastic, Temecula, CA).
7. The treated ground beef patties were then transferred to and stored in the food cooler in Kleberg Center room 329. The cooler was set at 5 ^{o}C for the duration of the study. Temperature was monitored each day both by the thermometer on the

outside of 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 200 - 300 lux and was measured at 4 locations in the cooler on each day of the study. Samples were stored on white plastic platters.

APPENDIX C

POWDERING SAMPLES FOR ANALYSIS

1. Fresh untreated coarse ground beef was prepared and collected after 6 days storage.
2. Fresh untreated coarse ground beef was placed into a wire straining basket and submerged into a container of liquid nitrogen until liquid nitrogen stopped bubbling
3. Frozen sample pieces were transferred to a stainless steel waring blender and blended until a homogenous powder was formed.
4. Powdered samples were transferred to a whirl pack back and stored frozen (-20 ^{o}C) until analysis.

APPENDIX D

AOAC 992.15 LECO F-528 RAPID NITROGEN/PROTEIN ANALYSIS

PERFORM 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 $\leq$ 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 ~.4000 grams of sample in tin foil cups, record weights.
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 E

AOAC 950.46 MOISTURE ANALYSIS

Equipment:

Gloves

Whatman Filter paper: #2 Qualitative Circles, 125 mm

Stapler with staples

#2 pencil

Desiccator with desiccant

Analytical balance/scale

Convection oven

**Gloves should be worn at ALL times

Procedure

1. Construct thimbles from Whatman #2 filter paper folded into a sleeve open at one end and stapled at the other end
2. Label thimbles with #2 pencil
3. Dry thimbles for a minimum of 12 hours at 100^{o}C using an air dry oven. Oven should not be overfilled. Only 1 pan per shelf and not stacked on desiccant. Metal pans should not tough any of the walls of the oven, as air must be able to circulate.
4. Ensure desiccator is properly equipped with functional desiccant, sealant, and is not overfilled with thimbles/samples

5. Desiccator should be opened by sliding lid to remove thimble/sample and then immediately sealed.
6. Transfer dried thimbles to desiccator
7. Cool thimbles in desiccator for 30 minutes
8. Record dried thimble weight and 1 staple to the nearest 0.0001g. This is “initial thimble weight”. See #5 for opening/closing desiccator and place thimble immediately on the scale. Record 1st weight.
9. Put 2-3 grams of powdered homogenous sample into thimble and record the weight plus 1 staple to the nearest 0.0001 grams. This is “initial thimble/sample weight”. Each sample should be performed in triplicate.
10. Fold over open end of the thimble and seal with a staple.
11. Place thimble on clean metal pan. Samples should be laid flat and not overlapping.
12. Dry in 100^{o}C dry oven for 16-18 hours. Oven should not be overfilled. Only 1 pan per shelf and not stacked on desiccant. Metal pans should not tough any of the walls of the oven, as air must be able to circulate.
13. Coll in desiccator for at least 1 hour. #4 should still be true.
14. Record dried thimble weight and 1 staple to the nearest 0.0001 gram. This is “dried thimble/sample weight”. See #5 for opening/closing desiccator and place thimble immediately on the scale

APPENDIX F

MEAT PH MEASUREMENT PROCEDURE

1. Place 10 g of the 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. Measure the pH of this slurry with a pH meter that has been calibrated with three standard buffer solutions. Three buffers used for calibration was at pH 4.0, 7.0 and 10.0.
4. Press read to begin pH measurement. “Stable” will appear when reading is finished. Record the pH of the slurry after the electrode has stabilized.
5. 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.
6. Always store the pH probe in CLEAN distilled water.

APPENDIX G

HUNTER LAB MINI SCAN XE PLUS OPERATING ROCEDURES

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 ensure 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

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

   Daylight Color was used with a 10 ^{o}C observer.

2. 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.
3. To take a reading, press the lightning bolt key and record the L*a*b* values.
4. The Mini Scan is now ready to read the next sample. Repeat the process.

PROCEDURE TO RECORD SPECTRAL REFLECTANCE DATA

1. Use left and right arrow keys to select the appropriate setup. (For my research, Spectral Data was used with illuminant D and a 10 ^{o}C observer)

2. 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.
3. To take a reading, press the lightning bolt key and record the reflectance values from 400-700 nm.
4. The Mini Scan is now ready to read the next sample. Repeat the process. Before taking readings on the second meat sample.
5. When all readings are complete, unplug it from the electrical source.
6. Be sure that the Mini Scan is clean, and that the aperture is clean before putting the machine away

APPENDIX H

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY PROTOCOL

1. For chapter II, 20 g of samples were wrapped in aluminum foil and stored at -80℃ until analysis. For chapter III, the 10 g samples are directly stored in sample vial (20 mL) at -80℃ until analysis.
2. The samples were thawed in the room temperature for 2 hr and the sample were placed into 486 mL jar and cover with Teflon lid. For chapter III, the sample vails containing samples were thawed in the room temperature for 2 hr.
3. After thawing, insert the SPME into the jar or sample vials.
4. Collect volatiles for 2 hours on the SPME.
5. 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.
6. On the computer program for GC/MS, load method (Beef) and wait for temperature to reach 40℃.
7. Click on the sample ID arrow, enter sample name, and click “ok and run method”.
8. When prompted, click the start button on the GC/MS.
9. Check that Status is “RUN” and that light is on inside GC/MS.
10. Insert SPME into injection port and lower filament (desorption occurs in first 3 minutes).

11. During run, click * on aroma trax program when an aromatic event begins
12. Click on the 0-100 scale when aromatic event ends.
13. Integrate the data by first opening the appropriate chromatogram in the GC/MS data analysis program.
14. Click Chromatogram, then “Select Integrator”, Select “RTE”.
15. Click Chromatogram, then “percent report”.
16. Click Chromatogram, then “Integrate”.
17. Click Spectrum, then “library search report”, choose “screen”.
18. 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.

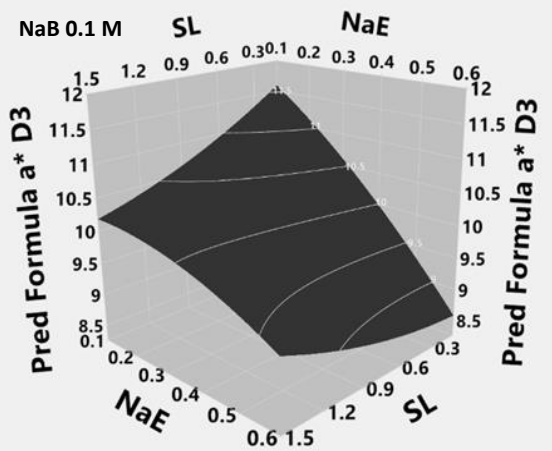
STATISTICAL NOTE

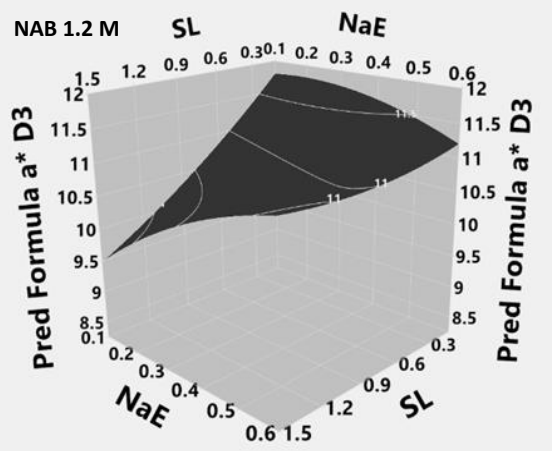
Samples that did not result in any quality peaks were entered as 0 for all volatiles. Only a truly missing data point would be entered as “.”.This approach

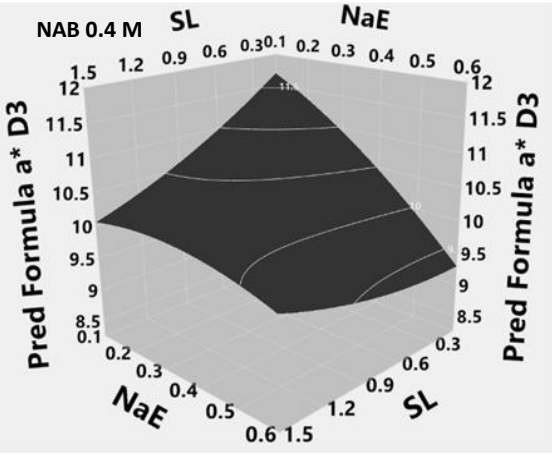
prevented any statistical differences in all studies volatile data but was the appropriate analysis.

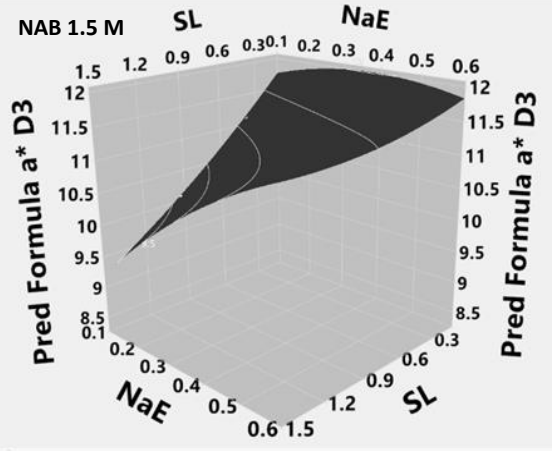
APPENDIX I

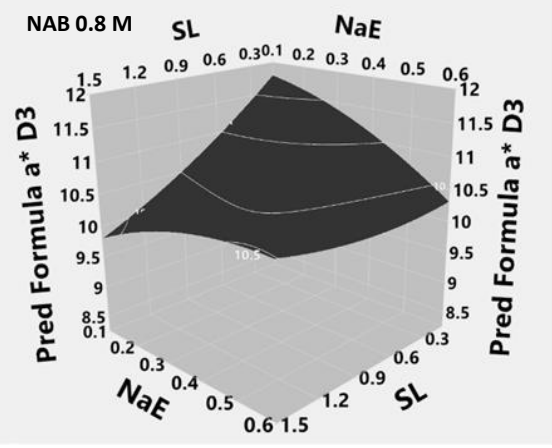
DATA FROM CHAPTER III



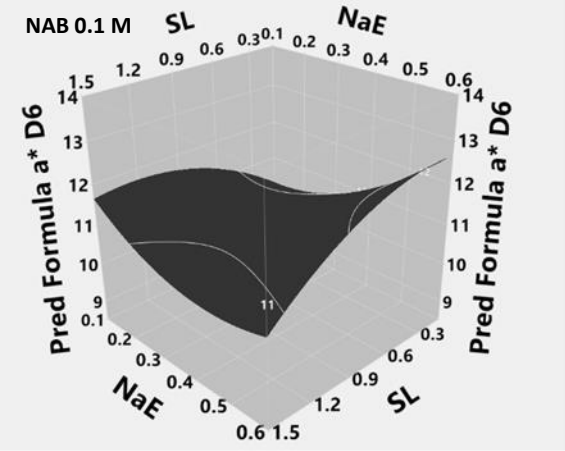


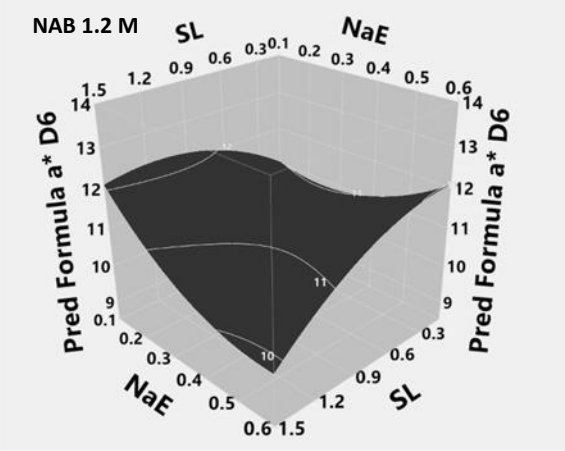


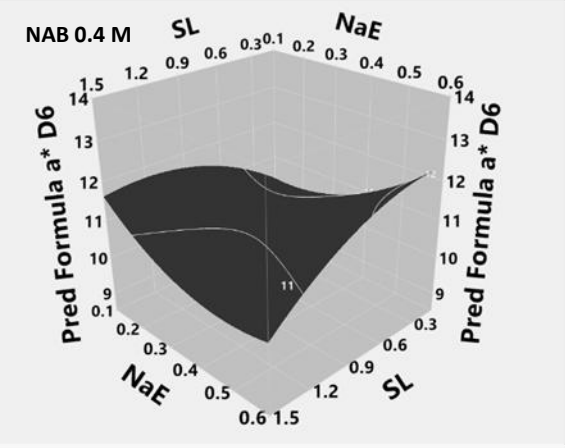


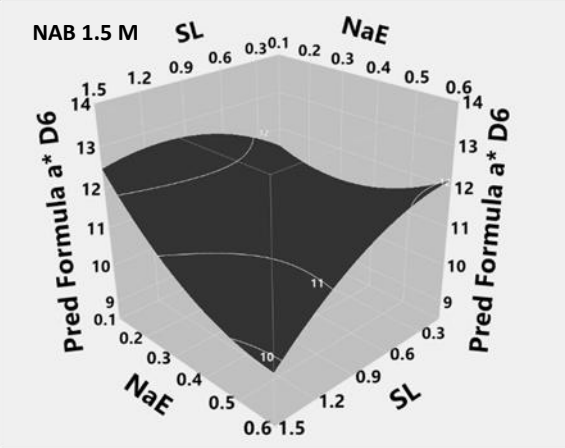


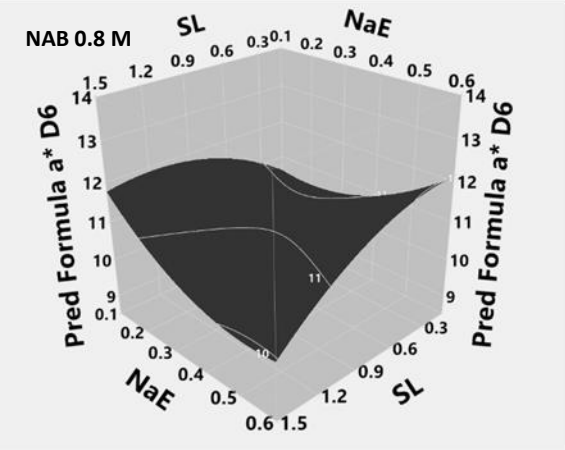
The response surface plot of predicted a* value on day 3. As the NaB level increased from 0.1 to 1.5 M, the NaL and NaE response surface plot shifted. The predicted formula of a* value at post-treatment day 3 was $12.17-4.34xNaE+0.06xNAB-1.75xNAL-3.43xNaE^2-0.21xNAB^2+0.30xNAL^2+0.42xNaExNAB+3.12xNaExNAL-0.58xNABxNAL$ and lack of fit was not significant (R^2=0.94, P<0.01).



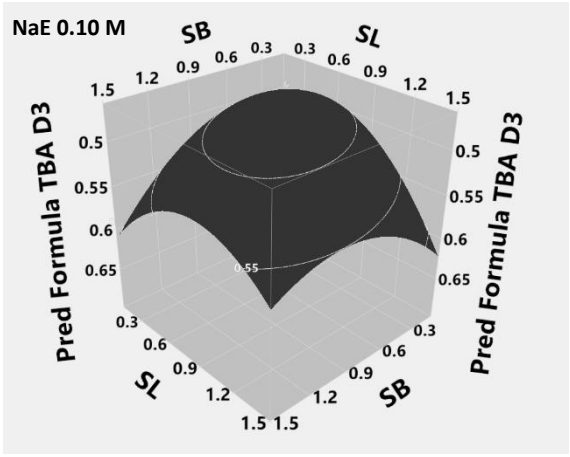


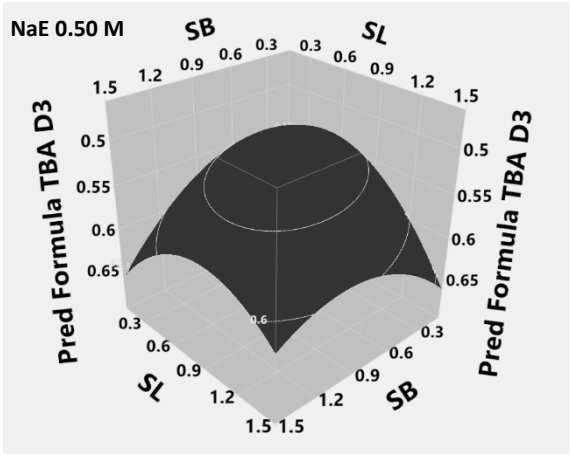


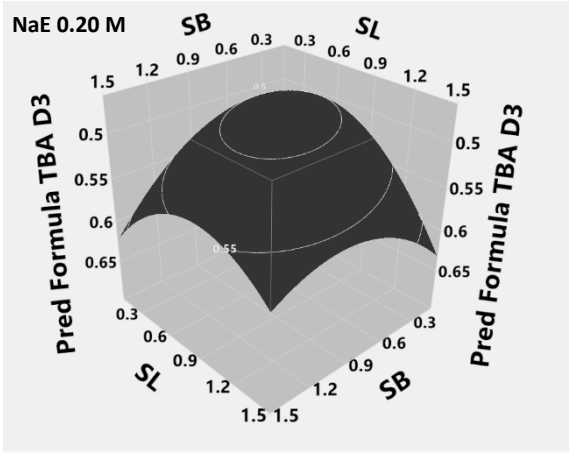


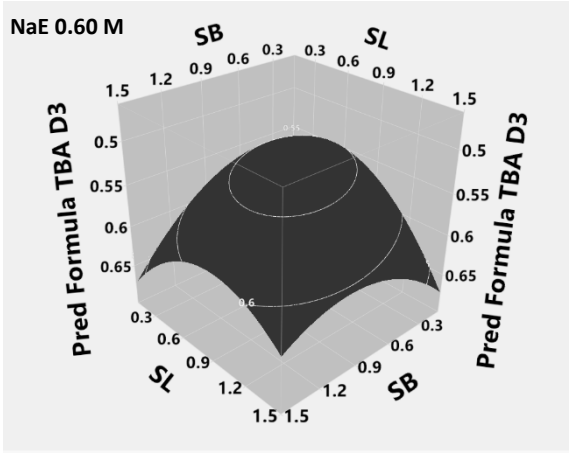


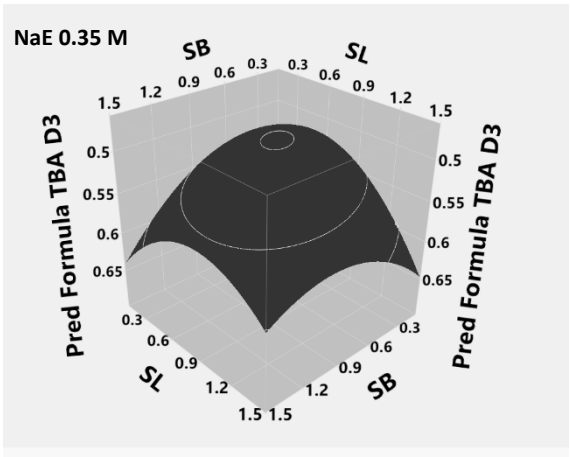
The response surface plot of predicted a* value at day 6. As the NaB level increased from 0.1 to 1.5 M, the NaL and NaE response surface plot shifted.
The predicted formula of a* value at post-treatment day 6 was $10.09-1.46xNaE+0.16xNAB+3.29xNAL+9.81xNaE^2+0.60xNAB^2-1.15xNAL^2-2.40xNaExNAB-4.75xNaExNAL-0.19xNABxNAL$ and the lack of fit was not significant. (R^2=0.78, P<0.01).



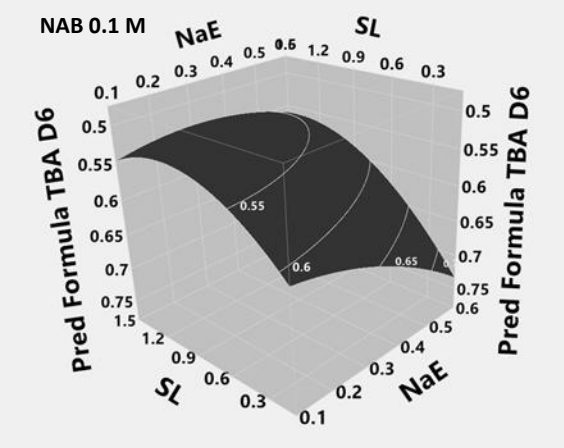


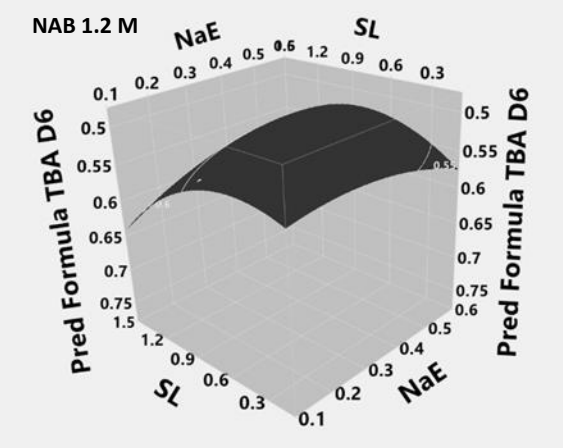


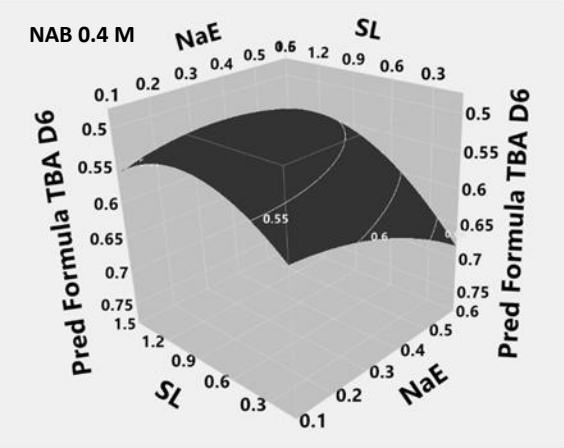


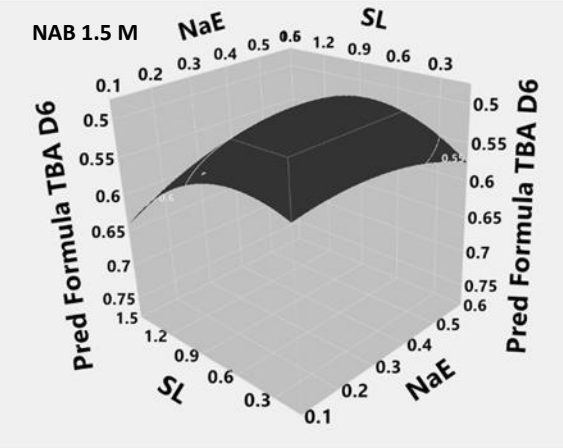


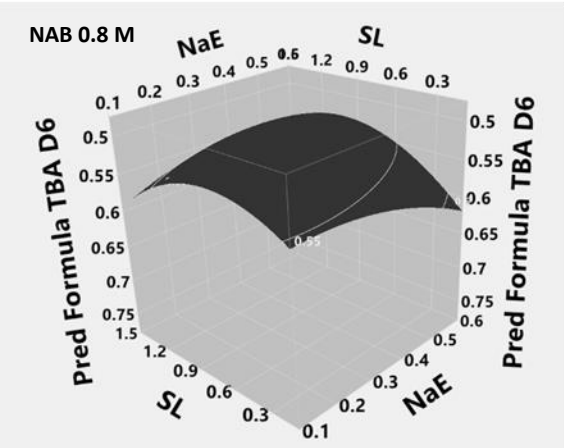
The response surface plot of predicted TBA value on day 3. As the NaE level increased from 0.1 to 1.5 M, the NAL and NaE response surface plot shifted.
The predicted formula of TBA value on day 3 was $0.51+0.11xNaE-0.07xNAB-0.11xNAL+0.01xNaE^2+0.09xNAB^2+0.12xNAL^2+0.01xNaExNAB-0.01xNaExNAL-0.08xNABxNAL$ and the lack of fit was not significant ($R^2=0.78$, $P<0.05$).











The response surface plot of predicted TBA value on day 6. As the NaB level increased from 0.1 to 1.5 M, the NaL and NaE response surface plot shifted.
The predicted formula of TBA value on day 6 was $0.64+0.05xNaE-0.12xNAB-0.21xNAL+0.32xNaE^2+0.05xNAB^2+0.10xNAL^2-0.16xNaExNAB-0.15xNaExNAL+0.11xNABxNAL$ and the lack of fit was not significant ($R^2=0.77$, $P<0.05$).