Evaluating fresh beef quality attributes and the effect of postmortem time using bioelectrical impedance and meta-analysis

by

Francisco Najar Villarreal

B.S., University of Nuevo León, 2014
M.S., Kansas State University, 2018

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2021

Abstract

This study assessed the application of new technologies to optimize freshness quality of beef. Phase one investigated quality attributes of beef *longissimus lumborum* (LL) during retail display using surface and internal bioelectrical impedance analysis (BIA). Beef loins (N = 18) obtained from three commercial processors with three postmortem aging times (PMT; 27, 34, and 37 d), were cut into steaks and displayed aerobically at 0-4°C for 15 d. Microbiological populations, surface BIA (S-BIA), internal BIA (I-BIA), pH, instrumental color, proximate composition, and lipid oxidation were assessed. Steaks with a longer PMT had lower (P < 0.05) BIA values and shorter shelf-life during retail display than steaks with lower PMT. Steaks aged 27 d had higher (P < 0.05) BIA values on d 9 and 12 of display than steaks aged 34 and 37 d. Overall, BIA values increased on steaks during retail display possibly due to changes in structural proteins and poor water holding capacity (WHC) and were correlated (r = -0.64 -0.56, 0.70, and 0.69) with redness, yellowness, aerobic plate counts, and TBARS, respectively. The needles used for the I-BIA method are invasive and may translocate bacteria into the muscle; therefore, the use of S-BIA method is recommended. In phase two, the S-BIA method was used to assess postmortem chemical changes in normal and high-pH beef LL steaks during 9 d of simulated retail display at 0-4°C. Beef strip loins (N = 20; PMT = 14 d) obtained from a commercial processor were sorted into two treatments, normal-pH (5.61–5.64) and high-pH (6.2–7.0) and cut into steaks. Surface BIA, oxygen consumption (OC), metmyoglobin reducing activity (MRA), protein degradation, and WHC were assessed. Surface BIA was 20% higher (P < 0.05) for high-pH meat than steaks from normal-pH meat. Low correlations were found between S-BIA and OC, MRA, protein degradation, and WHC. Surface BIA is a method that could be used to separate normal- and high-pH strip loins with potential for rapid, in-plant use to

identify dark-cutting beef; however, BIA is not as strongly correlated with changes in structural proteins after 14 d of aging and during retail display. For phase three, the color life threshold for LL and *psoas major* (PM) steaks during retail display and the effect of PMT on the display color life of LL and PM steaks using meta-analysis was determined. Lower bound estimates for a* as a borderline of LL and PM steaks were 20.24 and 20.99, respectively. The first 21 d PMT for LL steaks had the longest color life with 7 d of color life. Additionally, 22-28 and 29-65 d of PMT had 5 and 4 d, respectively, of color life for LL steaks. The borderline acceptability estimated for PM steaks with 0-7 d and 8-21 of PMT was 3 and 2 d of color life, respectively. Estimations from this meta-analysis demonstrate that using LL and PM subprimals having a PMT of 21 d or less and 7 d or less, respectively, would optimize retail display color life of aerobically packaged steaks.

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Acknowledgements

First and foremost, I would like to thank God for giving me and my family the opportunity to spend five wonderful years in Manhattan, KS, and being able to complete my graduate work. I am deeply and forever grateful to my major advisor, Dr. Elizabeth Boyle, who allowed me to study in a fantastic university. Throughout my time at KSU, I learned a lot from her professionally and internally as an individual. Her leadership and mentorship were key in my growth and I will use them in my entire career. The completion of my work would not have been possible without the generous guidance and support of committee members Drs. Christopher Vahl, Jayendra Amamcharla, and Justin Kastner. I am genuinely indebted to you for being agreeable to let me work on my project and providing your expertise and advice.

My gratitude to Dr. Justin Kastner for his spiritual guidance and mentorship. Thank you, Dr. Christopher Vahl, for your endless support and expertise in Statistics. I want to thank Dr. Jayendra Amamcharla for his valuable input and time when I needed it. I also want to express my gratitude to Drs. Terry Houser and Dr. John Gonzalez, though, they are no longer at KSU, they were key in my professional growth in graduate school and will be forever grateful for their kindness and support. I would like to express my appreciation to Dr. Kang for helping with the statistical analysis. I am deeply grateful to Dr. Melvin Hunt, who has been an amazing guide and teaching me about meat color. I am thankful to the meat science faculty members, including Dr. Travis O'Quinn, Dr. Michael Chao, Dr. Morgan Zumbaugh, and Jessie Vipham. I would like to thank my fellow meat science and food science graduate students, John Wolf, and Sally Stroda for your all your help and support. I would also like to thank the American Meat Science Association, especially Collette Kaster and Deidrea Mabry for their trust and support with my

meat science podcast. Thank you, Dr. Keith Cox, for allowing me to learn more about bioelectrical impedance and its application on agricultural foods.

A special thank you to my wife Daniela, thank you for being as authentic and wonderful as you are and being by my side even at the roughest times of graduate school. All these accomplishments would not have been possible without your love and patience. I definitely would not be the person I am today if I didn't have you by my side. I would also like to take this opportunity to acknowledge my family for all the love and support you have given me throughout my professional career. Thank you, Francisco, Adriana, and Diana. Finally, I wish that more young students from Mexico had the opportunity to study abroad with the support of National Council of Science and Technology. Thank you for supporting my scientific career and I will return to the Mexico's industry all my knowledge and expertise I gained at KSU.

Chapter 1 - Introduction

To date, the importance of quickly assessing the freshness of food remains among one of the biggest priorities in the food industry. Meat scientists have demonstrated several methods to accurately assess freshness and quality of agricultural foods, including the electric nose, near-infrared spectroscopy, hyperspectral imaging technology, torrymeter, and bioelectrical impedance analysis (BIA; Fletcher et al., 2018; Sujiwo et al., 2019) Most of the methods used for measuring quality in the food industry are generally destructive and costly. The industry's need for non-destructive and inexpensive methods, such as BIA, has led them to develop new technologies that can provide information based on the electrical characteristics of food products. This literature review explores some of the applications of BIA and its mechanisms to determine the freshness of food products.

Regardless of its nature, nearly all food products can be subjected to an electrical current and be electrically assessed (Nelson, 1973). The passive electrical characteristics of an object, which means carrying no electric source, can be measured using BIA (Pliquett, 2010). Applying a constant, low, and alternating current to a biological tissue produces an opposition to the dissemination of the frequency-dependent current and this is known as impedance. Moreover, an BIA device measures the reduction of voltage when an electrical current pass through an object (Mateos et al., 2013).

Myofibrils constitute 82 to 87% of the volume of the muscle cell and approximately 85% of water in the living muscle cell resides within the myofibrils (Lonergan and Lonergan, 2005). Water is highly conductive, and therefore it plays a role in BIA values. The cellular relative change in biological tissues, specially, in muscle tissue, can be estimated using impedance spectroscopy with multiple frequencies. This cellular activity occurs in 98% of cells in live

tissue, increasing postmortem (PMT) due to cellular inflammation, and then decreasing as a result of membrane alterations (Pliquett et al., 1995).

History

Physicians in the medical sciences have widely used BIA. In the beginning of the 1900s, Morse (1925) first used BIA in the medical sciences to study blood samples. Later, Callow (1936) was a pioneer as he was one of the first meat researchers who evaluated the electrical characteristics of meat. Bioelectrical impedance can be measured using single or multiple frequencies. Overall, BIA has been shown to accurately predict fat-free mass and body mass, and researchers concluded this technology is precise and portable (Kushner and Schoeller, 1986). Robert et al. (1992) demonstrated that BIA and other basic standard medical procedures may help clinicians by providing important information to efficiently monitor the nutrition of critically ill patients.

Meat quality

Food products, including meat, are composed of cells and are surrounded by an insulating membrane as any other biological structure. Cell membranes are known to form capacitive elements, resulting in higher resistance. Technologies like BIA are very sensitive to the change of permeability of cell membranes and can be used as a freshness indicator of meat (Pliquett, 2010). Swatland (1985) used BIA to evaluate the relationship between the quality of pork carcasses and its electrical properties. It is worthwhile to mention that the ultimate state of fresh meat affects BIA values; for instance, a pork *longissimus dorsi* muscle exhibiting drip loss (low-pH), after 3 d at 4 °C of storage may not appear fresh and can result in higher BIA values compared to normal-pH meat (Pliquett, 2010). The disruption and damage of cell membranes in meat occurs when meat undergoes freezing and thawing, affecting BIA values, making fresh and

frozen meat detectable using a BIA tool (Pliquett et al., 2003). This has been of particular interest to researchers and the meat industry in the last years. To preserve meat, fresh meat may undergo a freezing cycle; however, this step can produce severe quality defects and different BIA values after thawing. Recently, Battacone et al. (2019) demonstrated that beef *longissimus dorsi* muscle, after freezing at -18 °C, with two copper electrodes inserted into the muscle, had lower BIA values compared to its fresh counterparts.

Meat scientists have widely used BIA to study membrane integrity, aging, detection of frozen-thawed meat, pH, fat content, and tenderness (Damez and Clerjon, 2008). Scientists have also reported that BIA has potential to assess carcass composition, aging, and post-mortem changes in muscle cell membranes (Velazco et al., 1999; Byrne et al., 2000). Other researchers evaluated the correlation between BIA and fat and water content, salable yield, fat trim, and marbling scores and reported BIA to be highly correlated (Zollinger et al., 2010). Nonetheless, these BIA methods are invasive as the probes are inserted into the meat, leading to contamination (Damez and Clerjon, 2008). Additionally, an accurate fat content determination was demonstrated in different grinds of pork and beef using BIA; however, Marchello et al. (1999) reported that the smaller the grind size (0.32-cm plate), the more accurate BIA is to predict fat content.

Figure 1.1 visually illustrates how electrical impedance is applied to meat products. Two electrodes are placed onto the meat surface to induce a current flow, which measures the voltage. In 1995, Pliquett et al. determined that the rate of this change is related to the texture of meat, which can be expressed as purge and the loss of brightness in meat. A value known as Py has been previously established and used as a reliable parameter to rapidly characterize the quality and texture of fresh meat (Pliquett et al., 1995). However, to our knowledge, this alternative BIA

method is not currently commercially used by meat processors. To date, the application of BIA in the industry is minimal due to the lack of fundamental knowledge on the electrical properties of meat. Moving forward researchers may consider the use of BIA that is capable of externally scanning food products to avoid food safety concerns with internal BIA methods.

Shelf-life

Meat production and consumption have been steadily growing around the world in the past years (USDA, 2021), leading to an increased public concern for better practices to guarantee its quality. Meat is a highly perishable commodity and one of the main drivers for meat spoilage is bacteria growth (Ercolini et al., 2011). At the retail level, meat color is one of the major criteria for consumers when selecting meat purchases (Kropf, 1993). Myoglobin—a water-soluble protein responsible for the red color of fresh meat—is naturally-occurring in meat. There is a misconception about the term shelf-life among retail shoppers who often misunderstand meat discoloration considering it as an indicator of the end of its shelf-life due to the color changes present on the surface. Color and microbial shelf-life of meat are not necessarily the same, as changes in fresh meat color during retail display is a natural process and does not mean that a product is spoiled (USDA-FSIS, 2013).

Metmyoglobin, the ferric redox form of myoglobin, is formed as a result of the low oxygen partial pressure in meat along with a low pH decline, leading to the undesired brown color that is seen in muscle foods (Aberle, 2003). Along with meat discoloration, there are other quality attributes that may develop during storage, including slime formation, off-flavors and off-odors development (Jackson et al., 1997). Objective measurements such as total volatile base nitrogen (TVB-N), pH, and aerobic plate count (APC) are utilized to determine quality parameters in meat (Guan and Liu, 2011). Although performing a sensory analysis can be used to

evaluate meat freshness, this method is subjective and needs trained panelists (Guan and Liu, 2011). In a shelf-life study, Sujiwo et al. (2019) evaluated the freshness of beef aerobically-packaged *longissimus lumborum* steaks using BIA to measure the dielectrical properties of fresh meat. They reported a decrease of BIA values in steaks during the 18 d of retail display and found BIA values to be highly correlated (r = 0.85) with APC, a*, and b*. Nguyen and Nguyen (2015) found a high correlation between BIA and APC (r = 0.75) for aerobically packaged pork *psoas major* muscle. They also reported that BIA values decreased during a 15-d storage at 4°C and showed surface electrode BIA measurement had higher correlation coefficients than internal measurements.

pH

There are valuable quality attributes of meat that can be evaluated by determining its pH. In the meat industry, objective methods for measuring pH are needed as the traditional pH meter uses a glass pH electrodes that are typically very fragile and remain to be problematic due to the calibration technique. Homeostasis, a process that living organisms utilize to maintain biological functions, is interrupted after animal exsanguination. This leads to an interruption in the blood flow and oxygen depletion in the animal after harvest. In addition to homeostasis termination, the muscle will initiate the transition from aerobic to anaerobic metabolism. Glycogen is naturally stored in muscle fibers and it is depleted due to the anaerobic formation of lactic acid during slaughter, resulting in a pH decline (7.2 to 5.6). In pork. an increased and/or rapid formation of lactic acid has an impact on quality and leads to pale, soft, and exudative (PSE). On the other hand, it is known that the lack of lactic acid production after slaughter may yield dark, firm, and dry meat (DFD), which is most traditionally found in beef (Swatland, 1984). In 2016, beef

exhibiting DFD characteristics had an incidence of 1.9% in the meat industry (Boykin et al., 2017).

The electrical properties of the cell membranes in meat and the breakdown of various electrolyte compartments decrease during PMT metabolism (Swatland, 1980). This membrane disruption may be caused by the pH drop during rigor mortis; however, another important factor is the ATP depletion which also occurs in PMT metabolism (Swatland, 1980). The ATP is used by the majority of membrane bound ionic pumps found in muscle. Other studies that have looked at this pH effect with BIA. For example, BIA was correlated with pH in meat exhibiting PSE conditions (Chizzolini et al., 1993, Swatland, 1997). Figure 1.2 depicts the behavior of the drip channels from low and high drip loss pork *longissimus* muscles at three PMT's, including 0, 6, and 9 h (Annette Shäfer and Peter Purslow, unpublished results). They demonstrated that PSE pork had lower BIA values in pork samples due to the excess of extracellular fluid.

The structure and ion conductivity of meat are two important factors that affect BIA (Damez et al., 2008). Anisotropy is a phenomenon that exists in muscle as well in meat, which means that electrical properties of meat may change based on the direction of the electrical fields when examining the sample (Damez et al., 2008). Callow (1936) reported that meat is a biological tissue with a strong electrical anisotropy. Damez et al. (2008) described anisotropy as the muscle fibers existing in an intact muscle containing an electrolyte and encircled by an insulated phospholipid membrane. Therefore, when membranes begin to degrade in meat, its electrical anisotropy disappears (Damez et al., 2008). Impedance can be measured at a wide array of frequencies depending upon the type and purpose of measurement. Likewise, the conductivity of tissue may be assessed from low values and at low frequencies based on the volume of its extracellular fluid at a range (10–100 MHz) which is associated with the

conductivity of intra and extracellular ions (Castro-Giráldez et al., 2009). Recently, Afonso et al. (2020) used BIA at a frequency of 50 kHz on beef *longissimus thoracis* and *lumborum muscle*. They found strong correlations (r = 0.71) between BIA and intramuscular fat, total collagen, sarcomere length, and tenderness.

Postmortem effect

When rigor mortis sets in, BIA in meat declines very constantly along with the mechanical resistance of muscle fibers (Lepetit et al., 2002). Byrne et al. (2000) evaluated the relationship between BIA and other quality traits in beef vacuum-packaged *longissimus dorsi* muscle and the PMT impact on internal BIA values. They reported a decrease in BIA values when PMT was increased in beef *longissimus dorsi* muscle. They also concluded that as meat ages BIA values increase due to decreased extracellular fluid, which allows the flow of electric current. Conversely, increased extracellular fluid leads to lower BIA values due to membrane damage, resulting in poor water holding capacity (WHC).

Other proteins

In the seafood industry, BIA has been successfully used to determine fish freshness. More specifically, BIA is used to detect whether fish has been previously frozen and whether remained frozen or was subjected to a thawing step more than once. Vidacek et al. (2008) studied the electrical differences in freezing methods and cycles and reported a classification success rate of 78% using BIA, to detect sea bass (*Dicentrarchus labrax*) that had been through various freezing histories (Vidacek et al., 2008). Most recently, the shelf-life of poultry has been also studied using BIA (Sujiwo et al., 2018). In this study, they found that BIA values in aerobically packaged chicken *pectoralis major* muscle was highly correlated (r = 0.85) with T-VBN, pH, and APC counts during a 12-d cold storage (4°C) in a dark room.

Meat color

The primary sarcoplasmic protein in meat responsible for the red color meat is myoglobin. However, there are other proteins like hemoglobin and cytochromes that are also present in meat that can also play a role, to a lesser extent, in meat color. Myoglobin has various forms, including deoxymyoglobin, oxymyoglobin, metmyoglobin, and carboxymyoglobin that, based on its redox form and the ligand attached to myoglobin, may change the color of meat. Depending on the redox form of myoglobin, the intensity of redness (saturation) of the meat may be also affected (Ramanatham et al., 2020).

At the retail level, consumers are accustomed to the bright cherry-red color of meat and perceive this quality trait as critically important, and may result in meat being marked down or even discarded when the meat industry fails to meet this criterion. There are many factors that may affect the optical properties of meat, including muscle pH postmortem, types of packaging, and the rate of pH decline (Ramanatham et al., 2020). After harvest, the rate at which pH drops during the transformation of muscle to meat and from aerobic to anaerobic metabolism is key in achieving the natural color of normal pH meat. Muscle produces lactic acid, resulting in the acidification of meat from 7.2 to 5.6 and the extent of this pH decline has a tremendous effect on the ultimate quality characteristics of meat. This alteration in pH drop may cause some color changes, specially in dark cutting beef where occurs when beef undergoes antemortem stress, depleting muscle glycogen, which prevents muscle acidification yielding high-pH (approximately, $pH \geq 6$) meat.

High-pH meat exhibits larger spaces (inter and intra) in muscle fibers due to swelling, which results in increasing the ability of cells to hold more water (less free water) that ultimately improves light absorption and decreases reflectance, leading to darker colors (Ledward et al.,

1992; Hughes et al., 2014). Currently, there is a high prevalence of beef presenting dark, firm, and dry (DFD) conditions or high-pH meat in the meat industry (Mahmood et al., 2017; Zhang et al., 2018). In addition, the ultimate postmortem pH of meat can negatively affect the oxygen consumption (OC) and the MRA of myoglobin by changing the structure of muscle and enzymes in meat, affecting the bright color of fresh meat which is preferred by consumers (English et al., 2016).

The redox state of iron and the attached ligand may affect meat color. The chemical more readily available for mitochondria and oxygen-consuming enzymes is oxygen either from the atmosphere or within the packaging system used. Mitochondria and oxygen-consuming enzymes are constantly competing for oxygen (Tang et al., 2005), and this is affected by the postmortem time or pH. The time since harvest or postmortem time plays an important role in mitochondrial activity, affecting the reduction of deoxymyoglobin. In other words, as time PMT increases, mitochondria become less active, leading to faster discoloration (Mitacek et al., 2019).

It is well established that color influences consumers' purchasing decisions; however, ensuring tenderness to provide a good eating experience is necessary to promote the return of consumers. Postmortem aging is a common practice used by the meat industry to optimize tenderness and the industry uses a 14 d PMT as a baseline to meet tenderness expectations; however, greater than 14 d PMT may result in poor color stability during display (Ramanathan et al., 2020). Extended PMT can increase OC, affecting the consumer-preferred red color of beef as well as reducing the mitochondrial activity in muscle. For instance, steaks with > 42 d PMT were found to have less blooming compared to steaks aged 21 d (English et al., 2016). These authors concluded that the increased purge loss containing myoglobin present during the wet aging process may lead to this lack of blooming.

Food waste

For food to reach consumers' tables, 50% of U.S. agricultural lands (USDA-ERS, 2002), and 80% of freshwater (USDA-ERS, 2006) are used for food production. Food waste is a serious concern that accounts for $165 billion in losses each year (Buzby and Hyman, 2012). In other words, a 1% reduction in food loss would result in reducing the value of food loss by a substantial $1.66 billion. Since the world population is expected to increase to more than 9 billion people by 2050, global agricultural production will have to increase to sustainably meet the nutritional needs of this growing population (FAO, 2011). Reducing food loss by 15% could feed approximately 25 million Americans every year (Hall et al., 2009). Coleman et al. (2011) reported that 1 out of 6 Americans are food-insecure.

There is a continuous need to educate consumers and retail staff on how to identify food spoilage. In the U.S., meat discounted or discarded due to discoloration accounts for 15% of meat loss, leading to revenue losses up to $1 billion for the industry (Smith et al., 2000). As little of 20% discoloration is sufficient for consumers to reject meat (Djenane et al., 2001). A study by Buzby et al. (2014) estimated that 1.2 million metric tons of meat is not being utilized at the retail level leading to food loss and wasted food products across the U.S. supply chain.

Proposed Technology

The Seafood-CQR (Figure 1.3) is a non-invasive electronic device, developed by Seafood Analytics (Clinton Township, Michigan), currently used to measure the freshness of seafood products, including both whole fish and fillets. The device sends a low frequency electrical current through the fish and collects data based on its relative conductivity. Once conductivity is measured, the reader assigns a Certified Quality Number (CQN) to the seafood product, which is an indicator of freshness. In general, a higher CQN correlates to a fresher, and therefore higher-

quality, fish (Seafood Analytics, 2018). Currently, to our knowledge, there is no published data correlating the use of this technology with predicted shelf life of red meat products. This dissertation has three objectives: (1) this phase investigated the correlation between quality attributes of beef *longissimus lumborum* (LL) and BIA during retail display using surface and internal BIA; (2) to assess postmortem chemical changes in normal and high-pH beef LL steaks during 9 d of simulated retail display at 0-4°C; and (3) the color life threshold for LL and *psoas major* (PM) steaks during retail display and the effect of postmortem aging time on the display color life of LL and PM steaks using meta-analysis was determined.

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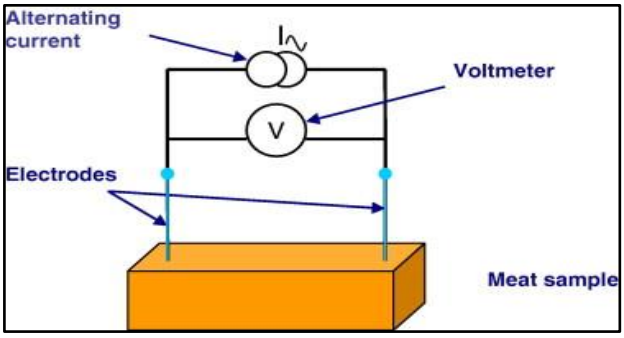
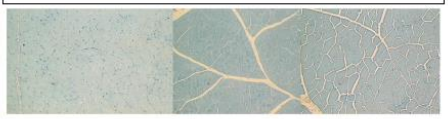


Figure 1.1 Impedance measurement on meat sample with the bipolar method. Adapted from Damez et al., 2008.

(a) Low drip loss muscle

Time (hr)	0	6	9
Impedance (Py)	70	70	70



(b) High drip loss muscle

Time (hr)	0	6	9
Impedance (Py)	70	20	10

Figure 1.2 Low drip and high drip pork longissimus muscle transverse section images using a light microscope.

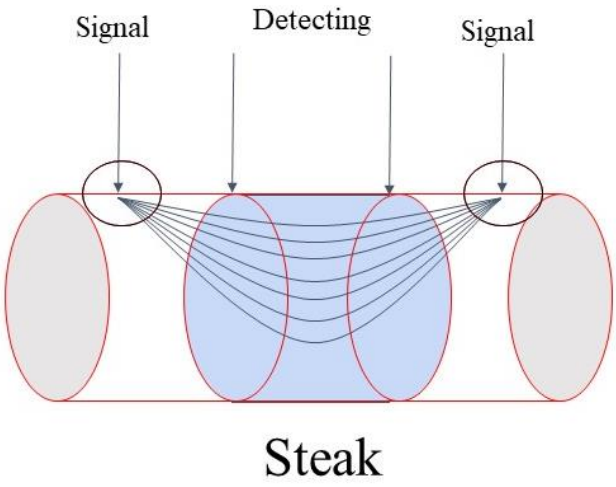


Figure 1.3 Image of bioelectrical impedance mechanism using 800 µA, 50 kHz, AC, 3.75 — 10.6 volts.

Chapter 2 - Correlation of bioelectrical impedance with freshness quality attributes of beef longissimus lumborum steaks

ABSTRACT

The quality attributes of beef *longissimus lumborum* during 15 days during retail display using surface and internal bioelectrical impedance analysis (BIA) were assessed. Beef loins (N = 18) were obtained from three commercial processors with three postmortem aging times (PMT) (27, 34, and 37 days). Loins were fabricated into 12 2.54-cm thick steaks, subdivided into six consecutively cut pairs, and randomly assigned to one of six display days (DD): 0, 3, 6, 9, 12, and 15. Steaks were assessed for surface BIA (S-BIA) and internal BIA (I-BIA). Three locations were analyzed within each steak, including top, middle, and bottom. Microbiological analysis, BIA, pH, instrumental color, proximate composition, and lipid oxidation were measured. There was a location × PMT × DD interaction ($P < 0.05$) for *longissimus lumborum* steaks for surface BIA (S-BIA). Among all three locations, steaks aged 27 d had higher ($P < 0.05$) S-BIA values on d 9 and 12 than steaks aged 34 and 37 d. There were no location × PMT × DD or two-way interactions ($P > 0.05$) for (I-BIA). Display day affected ($P < 0.05$) all instrumental color data regardless of PMT aging times. Among all PMT, steaks aged 27 d were 13 and 7% higher for a* and b* compared to 34 and 37 d PMT, respectively. There was a PMT day × DD interaction ($P < 0.05$) for aerobic plate counts (APC). From d 0 and 9 of display, APC counts of steaks aged 27 d PMT were 1 to 2.0 log CFU/cm^2 lower than steaks aged 34 and 37 d. Quality attributes, including a*, b*, APC, and TBARS were correlated (r = 0.70, -0.64, -0.56 , and 0.69),

respectively, with S-BIA. Overall, BIA values increased on aerobically packaged *longissimus lumborum* steaks and were correlated with various freshness quality parameters.[1]

INTRODUCTION

Food waste is an eminent worldwide issue. It has been estimated that approximately one-third of all edible food, or 1.3 billion metric tonnes, from the beginning of agricultural production fails to reach consumers tables (Gustavsson et al., 2011). A study by Buzby et al. (2014) estimated that 1.2 million metric tons of meat is not being utilized only at the retail level, which is due to food loss and wasted food products across the U.S. supply chain. In the U.S., meat is discounted or discarded due to discoloration which accounts for 15% of meat loss and leading to industry revenue losses up to $1 billion (Smith et al., 2000). As little as 20% surface discoloration is sufficient for consumers to reject meat (Djenane et al., 2001).

Measuring spoilage in meat is of utmost importance to determine freshness in the meat industry. Two of the major freshness quality traits that affect meat spoilage and can be objectively assessed include bacteria growth and lipid oxidation. Depending upon the environment in the meat packaging, microorganisms at levels of 10^7 -10^8 log CFU/cm^2 promote the formation of slime and off-odors, leading to spoilage (Ingram and Simonsen, 1980).

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Furthermore, lipid oxidation of meat is normally evaluated using the thiobarbituric acid reactive substances (TBARS) assay (Witte et al., 1970). Campo et al. (2006) determined a TBARS value of 2.0 mg MDA/kg caused trained panelists to reject product due to rancid odor. However, these laboratory-based methods are usually destructive, time consuming, and training-dependent.

The aforementioned drawbacks have set the path for quick and non-destructive methods to assess meat freshness and quality, including the electric nose, near-infrared spectroscopy, hyperspectral imaging technology, torrymeter, and bioelectrical impedance analysis (BIA) using a certified quality reader (CQR). The CQR is a technology that measures impedance as means to evaluate the freshness of meat by calculating algorithms that assess cellular condition. Bioelectrical impedance, a non-destructive analysis of meat, was first documented in the medical sciences at the beginning of 1900's (Morse, 1925). Callow (1936) was one of the first meat scientists who studied the electrical characteristics of meat. Some researchers have reported impedance as an effective technology to predict salable yields in beef carcasses (Marchello et al., 1994; Zollinger et al., 2010). In addition, Byrne et al. (2000) studied how electrical impedance affected vacuum-packaged *longissimus dorsi* muscle during post-mortem aging as well as its relationship to other quality traits. Additionally, in ground beef and pork, BIA was found to be an accurate predictor of fat content, especially at smaller the grind sizes (Marchello et al., 1999). To the best of our knowledge, little research has been conducted to study the effect of impedance on aerobically-packaged *longissimus lumborum* steaks during simulated retail display. Therefore, the effectiveness of BIA to determine the quality attributes of beef and its relationship with meat quality parameters during a 15 days of simulated retail display was analyzed.

MATERIALS AND METHODS

Sample collection

Beef strip loins (Top Choice; *N* = 18; IMPS #180; AMS-USDA, 2014), were obtained from three commercial processors, which had different postmortem age days, including 27, 34, or 37 d. Loins were cut into 12 steaks of 2.54-cm in thickness and were taken from the anterior to the posterior portion. They were prepared at the Kansas State University (KSU) Meats Laboratory. Steaks were subdivided into six consecutively cut pairs and were randomly assigned to one of six display days: 0, 3, 6, 9, 12, and 15. Within each pair, one steak was allocated to microbiological analysis and pH measurement. The paired steak was used for BIA, instrumental color assessment, proximate composition, and TBARS. Each steak was placed with the anterior-sliced end facing up on 17S Styrofoam trays (Dyne-a-pak Inc., Laval, QC, Canada) containing Dry-Loc moisture absorbent pads (ac-50, Cryovac, Duncan, SC) and overwrapped with polyvinyl chloride film (23,250 cm^3/m^2/24 h at 23 °C and 0% RH; Borden Packaging and Industrial Products, North Andover, MA).

Display case

Following packaging, steaks were moved to the KSU Color Laboratory and displayed in coffin-style retail cases (model DMF 8; Tyler Refrigeration Corp., Niles, MI) under fluorescent lights (32 W Del-Warm White 3000° K; Philips Lighting Co., Somerset, NJ) that emitted a constant 24-h case average intensity of 2,230 ± 34 lx. Case temperature was monitored using single channel temperature and humidity sensors (model OM-HL-SP-TH, Omega™, Norwalk, CT) and averaged 0.26 ± 0.95°C on steak package surfaces. Cases were defrosted twice daily (morning and evening) at 11°C for 30 min. Steaks were rotated twice a day in the cases from left

to right and front to back to account for minor variations in temperature and light intensity within the cases.

Impedance analysis

The freshness of longissimus lumborum steaks was assessed using BIA with a CQR (Seafood Analytics CQR, Model Quantum IV, RJL Systems, Clinton Twp., MI) device. Figure 2.1 depicts the location of the BIA measurements. This technology consists of a 4-electrode device of stainless-steel compression style electrodes. The outer pair electrodes send a low frequency electrical current (800μA, 50kHz), whose voltage capacity ranges between 3.75 to 10.60 V. After conductivity is measured, impedance is calculated using the resistance (R) and reactance (X) values that are displayed on the device digital screen. The formula used was impedance in series ($Z = \sqrt{(R^2+X^2)}$). The electrodes were cleaned after every measurement. Three locations within the steak, including top, middle, and bottom, from dorsal to ventral, were evaluated. Three readings were retrieved from each location of the electrodes and an average was calculated for each steak. The BIA readings were obtained on the surface of the steak by moderately compressing the electrodes up to the middle end (halfway). A template was created to be consistent along the S-BIA readings. Sample temperature was measured and ranged 0 to 10 °C. Similarly, the I-BIA of the samples was analyzed using needles inserted at 5 mm. A template was created to be consistent along the S-BIA readings.

Color measurements

Meat color readings for CIE L*, a*, and b* and reflectance from 400 to 700 nm were instrumentally assessed every sampling day using a HunterLab MiniScan™ EZ (Illuminant A, 2.54-cm diameter aperture, 10° observer; Model 4500; Reston, VA) according to the methods of Phelps et al. (2014). Readings were taken at 3 steak locations on each day of display and values

were used to calculate an average value for each steak. In addition, steak surface percentages of metmyoglobin and oxymyoglobin were calculated using reflectance values at 473, 525, 572, and 700 nm and using the equations of Krzywicki (1979), as published in the American Meat Science Association Meat Color Measurement Guidelines (AMSA, 2012). Additionally, a* and b* values were used to calculate chroma and hue angle values (AMSA, 2012).

Microbiological analysis and pH

At each sampling time, steak packages designated for microbial sampling, were aseptically opened in the KSU Microbiology Laboratory and two 21.6 cm^2 cores were removed from the steak surface using a sterile scalpel at a depth of 1.5±0.5 mm. Each core was outlined using a sterilized stainless-steel meat coring device. Excised steak samples were placed into sterile plastic bags (Whirl-Pak® bags, Nasco®, Fort Atkinson, WI, USA) containing 50 ml of sterile 0.1% peptone water (Bacto®; Flankin Lakes, NJ, USA). Excised cores were homogenized for 60 s using a stomacher (AES Chemunex™, Model AESAP1064. Bruz, France). Serial dilutions of this homogenate were prepared using 9 ml of 0.1% peptone water, and plated in duplicate on Petrifilm™ (3M, St. Paul, MN, USA) to enumerate aerobic plate counts (APC). Samples were incubated and enumerated according to manufacturer´s instructions. Bacteria populations were calculated, transformed logarithmically, and reported as log CFU/cm2. Following microbiological sampling, pH was measured using a calibrated pH probe (Model FC232, Hanna Instruments Inc.™, Woonsocket, RI) with a pH meter (Model HI 99163, Hanna Instruments Inc.™). To determine pH, the probe was inserted in duplicate on the side adjacent to the cores taken for microbial sampling.

Proximate composition

From the paired steak, an additional 50 g was excised, frozen using liquid nitrogen, and homogenized with a blender (Model 33BL79, Waring Products, New Hartford, CT). The homogeneous powder was placed in 11.4 × 22.9–cm plastic labeled Whirl-Pak® bags (Fisher Scientific, Fair Lawn, NJ) and stored at -80°C until used for proximate analysis determination and TBARS. The homogeneous powdered samples were transported to the KSU Analytical Laboratory to be analyzed for moisture and crude fat content by the SMART system 5 (CEM Corp., NC) following the AOAC Method (PVM-1 MEAT; AOAC, 2003). Additionally, the protein content was analyzed using the LECO FP-2000 Protein/Nitrogen Analyzer (Model 602-600, LECO Corp., St. Joseph, MI). The combustion method was used and the nitrogen percent was multiplied by 6.25 to determine the protein content of samples (Leco Corp., St. Joseph, MI).

Thiobarbituric Acid Reactive Substances

Lipid oxidation during simulated retail display was evaluated using the TBARS assay using procedures as described by Witte et al. (1970). Briefly, 5 g from the homogenous powder was weighed, blended with 45 ml ice cold trichloroacetic acid (TCA; 11%) solution, and homogenized using a Waring blender for 30 sec. One milliliter of filtrate was mixed with 1 ml of 2-thiobarbituric (TBA; 20 mM) solution. In parallel, a standard curve was created to calculate lipid oxidation using malondialdehyde bis (Millipore Corporation, Billerica, MA). Reaction was carried in the dark to exclude light. Samples and standards were heated in a 100°C water bath for 10 min. and then cooled in room temperature water for 5 min. Following cooling, 0.2 ml of standards and supernatant from each sample were transferred to 96 wells plate (in duplicates). Absorbance was read at 532 nm (Eon Microplate Spectrophotometer; BioTek Instruments, Inc., Winooski, VT). Values were expressed as milligrams of malonaldehyde/kilogram of muscle.

Statistical analyses

Statistical analysis was executed using the MIXED procedure in SAS/STATÒ software, Version 9.4 (SAS Institute Inc., Cary, NC, USA). The experimental design was a split-plot. For the whole plot, treatment was PMT, and the experimental unit was loin. For the sub-plot, treatment was DD and the experimental unit was the steak corresponding to loin and DD combination. Impedance measurement was collected at three locations of a steak at the surface and internally, leading to the multivariate response.

Data underwent natural-log transformation. Surface impedance and internal impedance were analyzed separately and was then analyzed under the linear mixed model. Fixed effects of the model include PMT, DD, location and all 2-way and 3-way interactions. Random effect of the model is loin×PMT. Each of the freshness quality attributes was analyzed, separately, under the linear mixed model with fixed effects being PMT, DD, and PMT×DD. The random effect of the model was loin×PMT. Least squares mean and its standard errors, back-transformed to the original scale when applicable, were reported for fixed effects. The adjustment for multiplicity was carried out using Tukey's method at the 0.05 significance level. The CORR procedure of SAS was used to calculate the correlation of S-BIA and I-BIA (on the natural-log scale). Impedance averaged across the three locations and DD was used. The codes of SAS for this phase are found in Appendix A.

RESULTS

Bioelectrical impedance

There was a location × PMT × DD interaction ($P < 0.05$) for *longissimus lumborum* steaks when assessed for S-BIA. Because all locations acted independent of one another, PMT × DD interactions were analyzed by steak location. Figure 2.2 shows the interaction for top(T)-S-

BIA. From d 0 to 3 of display, T-S-BIA values were similar ($P > 0.05$) for all PMT. On d 6, steaks aged 27 d were similar to steaks aged 34 d, but 26% higher than those aged 37 d. Moreover, steaks aged 34 and 37 d were similar ($P > 0.05$). On d 9, steaks aged 27 d were 19 and 24% higher ($P < 0.05$) than steaks with 34 and 37 d of aging, respectively. Top-S-BIA values of steaks aged 27 were higher ($P < 0.05$) than steaks aged 34 and 37 d on d 12. However, on d 15, steaks aged 27 and 34 d were similar ($P > 0.05$), but 18% higher than steaks aged 37 d. The interaction for middle(M)-S-BIA is presented in Figure 2.3. From d 0 to 3 of display, M-S-BIA values were similar ($P > 0.05$) for all PMT. On d 6, steaks aged 27 d were similar ($P > 0.05$) to steaks aged 34 d, but 17% higher ($P < 0.05$) than those aged 37 d. The M-S-BIA values of steaks aged 34 and 37 d were similar ($P > 0.05$) and 24 and 12% lower ($P < 0.05$) than those pertaining to steaks aged for 27 d on d 9 and 12, respectively. On d 15, steaks aged 34 d were similar ($P > 0.05$) to steaks aged 27 d, but 14% higher than steaks aged 37 d. In addition, steaks aged 27 and 37 d were similar ($P > 0.05$). Interaction for bottom(B)-S-BIA can be found in figure 2.4. From d 0 to 6 of display, all PMT were similar ($P > 0.05$). The B-S-BIA values of steaks aged 27 d were 24 and 15% greater ($P < 0.05$) than 34 and 37 d on d 9 and 12, respectively. Furthermore, B-S-BIA values of steaks aged 27 and 34 d were similar ($P > 0.05$) and 21% higher ($P < 0.05$) than steaks aged 37 on d 15 of retail display. Additionally, a location, PMT, and DD effect were found ($P < 0.05$). There was no three-way or two-way interaction ($P > 0.05$; Table 2.1) for *longissimus lumborum* steaks for I-BIA, but a main effect due to location, PMT, and DD was found ($P < 0.05$). The middle portion had lower ($P < 0.05$) I-BIA values than the top and bottom portion. Due to a technical problem with the I-BIA probe, data were removed on day 0 for the middle portion.

Instrumental color assessment

There were no PMT × DD interactions ($P > 0.05$) for a* and b* values; however, there was an interaction ($P < 0.05$; Figure 2.5) for L* values. On d 0, steaks aged 34 and 37 d were similar ($P > 0.05$), however, these two PMT were 6% darker ($P < 0.05$) compared to steaks aged 27 d. At d 3 of display, L* scores from all PMT were not different ($P > 0.05$). On d 6, steaks aged 27 and 34 d were similar ($P > 0.05$) but 6% lighter ($P < 0.05$) than those steaks pertaining to loins aged 37 d. From d 9 through d 15 of display, L* scores did not differ ($P > 0.05$) among all PMT. Postmortem aging time had no effect ($P > 0.05$) on L*. In contrast, steaks aged 27 d were more red (greater a* values; $P < 0.05$) and yellow (greater b* values; $P < 0.05$) by 10% and 5% ($P < 0.05$), respectively, compared to steaks aged 34 and 37 d PMT. No interactions were observed for surface metmyoglobin, deoxymyoglobin, or oxymyoglobin ($P > 0.05$). Display day affected ($P < 0.05$; Table 2.3) metmyoglobin, deoxymyoglobin, and oxymyoglobin accumulation. By day 0 and through 6 d of display, the surface metmyoglobin increased ($P < 0.05$), however, after d 9 the surface metmyoglobin remained constant ($P > 0.05$). Additionally, no PMT effect ($P > 0.05$) was found on all surface measurements. No PMT × DD interactions ($P > 0.05$) were found for hue angle and chroma values. Hue angle values increased ($P < 0.05$) over the 15-d retail display, indicating a less red over time. A decreased ($P < 0.05$) in chroma values was observed, following a similar pattern as redness values. There were no differences ($P > 0.05$) in hue angle values among all PMT. Chroma was 7% greater ($P < 0.05$) in steaks aged 27 d PMT than steaks aged 34 and 37 d PMT, indicating a more intense red in steaks with less PMT.

Aerobic plate counts

There was a PMT day × DD interaction ($P < 0.05$; Figure 2.6) for APC populations. Initially, steaks aged 27 d had the lowest ($P < 0.05$) APC populations with 2.3 log CFU/cm^2 in

comparison to steaks aged 34 and 37 d, which had a similar ($P > 0.05$) APC growth with 4.3 and 4.5 log CFU/cm^2, respectively. Aerobic plate counts from steaks aged 27 d were remained below ($P < 0.05$) compared to those from the two other steak age groups until 12 and 15 display day where they were no longer different and were > 6 log CFU/cm^2. Furthermore, steaks aged 37 d had a higher ($P < 0.05$) prevalence of APC populations than steaks aged 34 d only on d 3 and 6. Additionally, a PMT day and DD effect ($P < 0.05$) was found for APC populations.

TBARS

No PMT × DD interaction or PMT effect ($P > 0.05$) were found for TBARS; however, there was a DD effect ($P < 0.05$; Table 2.4). From d 0 to d 3, TBARS values were similar ($P > 0.05$) with 0.14 and 0.19 mg MDA/kg; however, lipid oxidation increased to 0.38 mg MDA/kg on d 6. By d 9 and 12, TBARS values remained constant ($P > 0.05$). At d 15 of display, lipid oxidation was the highest ($P < 0.05$) among all DD with 0.79 mg MDA/kg.

Proximate composition and pH

There was no PMT day × DD interaction ($P > 0.05$) or PMT day effect ($P > 0.05$) for protein, fat, and moisture content. A DD effect ($P < 0.05$; Table 2.4) was found for protein and moisture content. Although significant, the variation during retail display was around 1%. Steaks protein content was similar ($P > 0.05$) at all display days, except from d 6 to d 9 at which protein content decreased ($P < 0.05$) by 2%. Although it was observed that DD affected protein and moisture content ($P < 0.05$). A PMT × DD interaction was observed ($P < 0.05$; Figure 2.7) for the pH. On d 0, 6, 9, 12, and 15 of display, pH was similar ($P > 0.05$) among all PMT. At d 3, however, steaks aged 34 d had a higher ($P < 0.05$) pH than steaks aged 37 d, but was similar ($P > 0.05$) to steaks pertaining to loins aged 27 d. Overall the pH variation was limited and ranged from 5.48 to 5.65.

Correlations

Moderate negative correlations ($P < 0.01$; $r = -0.56$; Table 2.5) occurred between S-BIA values and instrumental color measurements, including a* and b*. No correlations ($P > 0.05$) were found for L* values for steaks aged 34 d. Although no correlation ($P > 0.05$) was found between S-BIA and moisture content for steaks aged 27 and 37 d, steaks aged 34 d were negatively correlated ($P < 0.01$; r = - 0.67). No correlation ($P > 0.05$) was found for steaks aged 27 and 37 d between S-BIA values and fat content. Aerobic plate counts and TBARS ($P < 0.01$; $r = 0.70$) were correlated with S-BIA values for all PMT. I-BIA was correlated ($P <0.05$; Table 2.6) with all the parameters tested except for the color lightness (L*) and protein content for PMT at 27 and 37 days.

DISCUSSION

Historically, numerous groups have studied BIA to predict or indicate various quality attributes in beef and other species, including pork, poultry, and fish (Marchello and Slanger, 1994; Marcello et al., 1999; Cox et al., 2011). In addition, few studies have evaluated meat freshness as means to predict the shelf-life of meat using electrical properties (Sujiwo et al., 2019). Sujiwo et al. (2019) in an attempt to measure freshness of *longissimus lumborum* steaks, used a torrymeter tool to measure the dielectrical properties of fresh meat, and reported a decrease of torrymeter values on aerobically-packaged *longissimus lumborum* steaks during a 18-d retail display. The torrymeter had an integrated system that utilizes a band pass filter at a wide range of frequencies. As a result, this provides a greater chance to detect the correct frequency at each sampling time. On the other hand, the CQR equipment used in this study utilizes a single frequency (50 Hz), thus limiting its potential to assess the freshness of beef.

The PMT effect of electrical impedance on *longissimus lumborum* steaks was also evaluated. When the PM increased, S-BIA values decreased during retail display in the current study. Steaks from loins with a longer PMT had lower S-BIA values during retail display compared to those steaks from loins with shorter aging time. These results are consistent with those from Byrne et al. (2000). They measured the internal impedance in muscle, and their results indicated that as PMT increased I-BIA values decreased in vacuum-packaged *longissimus dorsi* muscle. The commercial application for measuring internal impedance may pose a food safety concern because there is a risk of translocating bacteria into the meat, compromising the internal condition of the product. In a previous study, it was demonstrated that impedance and conductivity are good indicators of membrane integrity in pork (Kleibel et al., 1983). These results follow the pattern of other studies indicating a change in BIA values of meat during PMT. Recently, Chao et al., (2020) reported an increase in pork membrane degradation after PMT. This may help explain why higher BIA values are associated with increased PMT. Additionally, dorsal (top) and ventral (bottom) portion of the steak had a greater BIA values (data not shown) compared to the middle portion. Results previously reported by Goihl et al. (1992) have demonstrated the presence of a fat and moisture gradient difference between dorsal and ventral locations in fresh *longissimus lumborum* steaks which is likely to influence the impedance measurements. Time in display case affected S-BIA values. Regardless of PMT, from d 0 through d 9, a linear increase of the impedance measurement was observed. After d 9, S-BIA values had more variation and were inconsistent. As meat ages there is an increase in proteolysis (Huff-Lonergan and Lonergan, 2005), resulting in poor water holding capacity and possibly affecting BIA values.

Color is the most important quality attribute for consumers as it influences their purchasing decisions (Mancini and Hunt, 2005). It has been well established that increased PMT decreased color stability (Tang et al., 2005; Lindahl, 2011). Research has reported that aerobically-packaged *longissimus dorsi* steaks color stability was negatively affected when PMT increased (Lindahl, 2011). In the current study, an initial increase in L* for steaks aged 27 d was observed compared to steaks aged 34 and 37 d. These results are in contrast with those from Dietz (2014), as he found that gluteus medius steaks with increased postmortem aging time had higher initial L* compared to steaks with shorter PMT. Steaks aged 27 were more red and yellow, and had greater chroma values than steaks pertaining to loins with 34 and 37 d of aging. Our data on surface redness, yellowness and chroma follows other reported literature (Colle et al., 2015; English et al., 2016; Abraham et al., 2017), which indicated displaying steaks from loins with increased postmortem aging may lead to a faster red and yellow color loss. This study showed no differences among all PMT for hue angle values. However, Ramanathan et al. (2019) found that steaks from beef strip loins with less PMT had lower hue angle values. As expected, a decrease in surface oxymyoglobin and a simultaneous increase in metmyoglobin percentage was observed as display time increased. Phelps et al. (2016) reported similar results in *longissimus lumborum* steaks during a 7-d retail display.

Consumers often misunderstand meat discoloration and conclude that the product has reached the end of its usable shelf life. However, color shelf life and bacterial shelf life of meat are not always following one another, as changes in fresh meat color during retail display is a natural process and does not mean that a product is spoiled (USDA-FSIS, 2013). Spoilage in meat occurs when organoleptic properties are lost and bacterial degradation of amino acids triggers slime formation and off-flavor development on the meat surface (Gill, 1997). The

spoilage process is undergone when bacteria growth exceeds 107 log CFU/cm2 (Gill, 1982; Ingram and Simonsen, 1980). Overall, APC populations were relatively lower in steaks from loins aged 27 d, which ranged 2.39 to 6.35 log CFU/cm2 throughout the retail display, than their counterparts aged 34 and 37 d of aging. Following the aforementioned recommendations, steaks aged 27 and 34 d had a shelf-life of 15 d, respectively, and steaks aged 37 d had 12 d of shelf-life under the conditions used in this study.

At the end of the 15 d of display, steaks achieved a TBARS value of 0.79 mg MDA/kg. Similar results were found in *longissimus thoracis* steaks, in which TBARS reached 0.86 mg MDA/kg at the end of 18-d retail display (Sujiwo et al., 2019). A consumer panel determined that at 2.0 mg MDA/kg was sufficient to detect oxidation in beef (Greene and Cumuze, 1981). After d 9 of display, TBARS values were equal or greater than 0.6 mg MDA/kg, indicating these steaks had higher lipid oxidation. But nonetheless below the oxidation detection threshold.

It has been determined that impedance values may change due to proteolysis when the membrane of muscle tissue is affected during post-rigor (Reichert, 1996). The highest correlation was showed between BIA and a*, TBARS, and APC, which were negatively correlated for all PMT, reflecting an increase in BIA and a decrease in these quality attributes.

Sujiwo et al. (2019) conducted a retail display study using *longissimus thoracis* steaks and found similar results, in which APC, a*, and b* were highly correlated with torrymeter values. Torrymeter measures the phase angle with a wide range of frequencies. Sujiwo et al. (2019) used steaks with 24 h PMT, providing the opportunity for protein degradation to occur; therefore, a more consistent decrease was observed. Overall, these results indicate that BIA may be considered as a tool to assess correlations with freshness quality attributes, including redness, APC, and TBARS in beef during retail display. Additionally. the measurement of pH has been

historically used to evaluate the freshness of carcass and meat quality (Korkeala et al., 1986). Although weak correlations were found between pH and BIA in this study, other literature have previously reported a moderate to highly correlation (r = 0.91) between these two measurements (Sujiwo et al., 2019). In this study, pH variation is so little that it is not surprising that no correlation was found.

CONCLUSION

Overall, I-BIA and S-BIA values increased on aerobically packaged *longissimus lumborum* steaks and were correlated with various quality parameters, including redness, yellowness, APC, and TBARS. However, the needles used in the I-BIA method are invasive and may translocate bacteria into the muscle, therefore, the use of S-BIA method is recommended. Regardless of BIA method, lower BIA values serve as a tool to identify steaks from the *longissimus lumborum* that have undergone at least 34 d of PMT. Further research should be conducted to study the effect of S-BIA on protein degradation of structural proteins.

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Table 2.1 Internal impedance means[1] of beef *longissimus lumborum* steaks aged 27, 34, and 37 days and displayed for 15 days under fluorescent lighting at 0.26 ± 0.95°C

		Display day											
Location	PMT[2]	0		3		6		9		12		15	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Top	27 d	88.0	4.0	105.3	4.8	130.3	5.9	128.5	5.8	120.0	5.4	122.0	5.5
	34 d	81.0	3.7	91.5	4.1	112.3	5.1	108.4	4.9	113.5	5.1	124.4	5.6
	37 d	74.3	3.4	85.3	3.9	102.6	4.8	103.2	4.7	101.7	4.6	106.3	4.8
Middle	27 d	-	-	91.0	4.5	103.6	5.2	111.4	5.5	107.3	5.3	104.8	5.2
	34 d	-	-	79.9	4.0	95.4	4.8	91.1	4.5	95.0	4.7	114.9	5.7
	37 d	-	-	74.6	3.7	88.6	4.5	88.0	4.5	93.2	4.7	90.2	4.6
Bottom	27 d	87.1	4.5	101.8	5.3	114.6	5.9	124.9	6.5	126.3	6.5	128.7	6.7
	34 d	82.1	4.3	89.3	4.6	109.1	5.7	103.1	5.3	109.4	5.7	122.3	6.3
	37 d	74.1	3.8	82.1	4.3	97.0	5.0	97.0	5.0	104.7	5.4	99.6	5.2

[1]Back-transformed LSMEANS
[2]Postmortem aging time
[3]Location × PMT × DD interaction (P >0.05)
[4]Location × PMT interaction (P >0.05)
[5]Location × DD interaction (P >0.05)
[6]Location main effect (P <0.05)
[7]Postmortem aging time main effect (P <0.05)
[8]Display day main effect (P <0.05)

Table 2.2 Least square means for the display day effect for redness and blueness of beef *longissimus lumborum* steaks aged three different time periods and displayed for 15 d

	Display day							
	0	3	6	9	12	15	SEM	*P*-value
a*	33.05[a]	29.08[b]	21.33[c]	13.24[d]	11.50[de]	10.91[f]	1.01	< 0.01
b*	25.80[a]	23.54[b]	20.09[c]	18.04[d]	17.33[d]	16.98[d]	0.64	< 0.01

	Postmortem age				
	27 d	34 d	37 d	SEM	*P*-value
a*	21.45[a]	19.23[b]	18.88[b]	0.77	< 0.01
b*	21.10[a]	20.12[b]	19.66[b]	0.46	0.02

[ab]Means with different superscripts differ ($P < 0.05$).

Table 2.3 Least square means for the display day effect for redness and blueness of beef *longissimus lumborum* steaks aged three different time periods and displayed for 15 d

	Display day							
	0	3	6	9	12	15	SEM	*P*-value
Metmyoglobin	18.64[d]	25.44[c]	39.60[b]	60.02[a]	60.17[a]	58.84[a]	2.35	< 0.01
Deoxymyoglobin	3.00[ab]	5.85[a]	6.35[a]	5.29[a]	3.62[ab]	0.76[b]	2.17	0.11
Oxymyoglobin	78.35[a]	68.71[b]	54.15[c]	34.68[e]	36.20[de]	40.38[d]	3.37	< 0.01

	Postmortem age				
	27 d	34 d	37 d	SEM	*P*-value
Metmyoglobin	41.89	45.31	44.15	2.68	0.45
Deoxymyoglobin	4.51	3.04	4.89	2.86	0.79
Oxymyoglobin	53.59	51.64	51.00	1.80	0.32

[ab]Means with different superscripts differ ($P < 0.05$).

Table 2.4 Least square means for the display day effect for redness and blueness of beef *longissimus lumborum* steaks aged three different time periods and displayed for 15 d

	Display day							
	0	3	6	9	12	15	SEM	*P*-value
Protein	22.96[a]	22.74[ab]	22.73[ab]	22.35[b]	22.69[ab]	23.02[a]	0.20	0.03
Fat	5.02	4.41	5.34	5.48	5.35	5.60	0.49	0.19
Moisture	70.57[b]	71.52[a]	70.60[b]	70.40[b]	70.43[b]	69.69[b]	0.45	< 0.01
TBARS	0.14[d]	0.19[d]	0.38[c]	0.65[b]	0.60[b]	0.79[a]	0.049	< 0.01

	Postmortem age				
	27 d	34 d	37 d	SEM	*P*-value
Protein	22.87	22.59	22.77	0.25	0.55
Fat	5.44	5.60	4.56	0.57	0.17
Moisture	70.10	70.23	71.27	0.56	0.11
TBARS	0.42	0.50	0.46	0.06	0.40

[ab]Means with different superscripts differ ($P < 0.05$).

Table 2.5 Correlation coefficients between beef *longissimus lumborum* steak electrical measurements collected using S-BIA and redness, aerobic plate counts (APC), TBARS, and moisture content

PMT	L*	a*	b*	Protein	Moisture	Fat	APC	pH	TBARS
27 d	-0.56**	-0.66***	-0.56***	-0.12	-0.22	0.03	0.70***	-0.23	0.82***
34 d	-0.15	-0.64***	-0.69***	-0.06	-0.67***	0.45***	0.72***	-0.30*	0.69***
37 d	-0.43**	-0.70***	-0.65***	-0.13	0.20	0.27*	0.72***	0.18	0.72***

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

Table 2.6 Correlation coefficients between beef *longissimus lumborum* steak electrical measurements collected using I-BIA and redness, aerobic plate counts (APC), TBARS, and moisture content

PMT	L*	a*	b*	Protein	Moisture	Fat	APC	pH	TBARS
27 d	-0.10	-0.56***	-0.44**	-0.02	-0.68***	0.57***	0.58***	-0.29*	0.63***
34 d	0.06	-0.64***	-0.69***	-0.29*	-0.73***	0.58***	0.85***	-0.24*	0.67***
37 d	-0.25	-0.71***	-0.69***	0.16	-0.63***	0.40**	0.87***	0.45**	0.62***

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

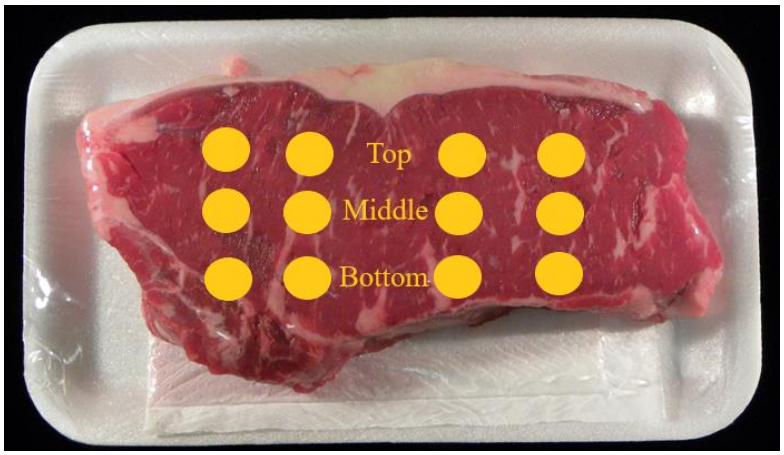

Figure 2.1 Illustration of anatomical locations of bioelectrical impedance measurements in *longissimus lumborum* steaks

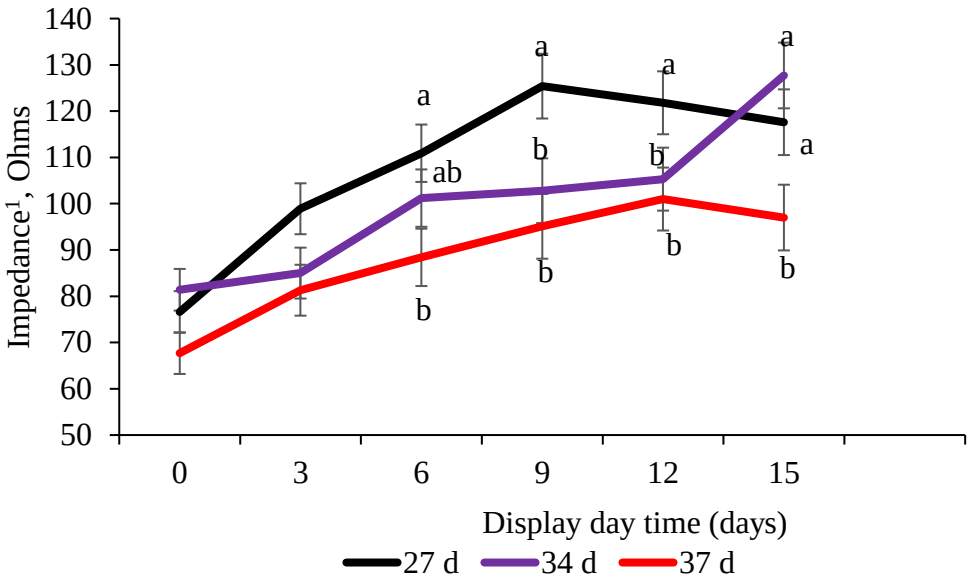

Figure 2.2 Interaction for top-bioelectrical impedance values of beef *longissimus lumborum* steaks aged 27, 34, and 37 days and displayed for 15 days under fluorescent lighting at 0.26 ± 0.95°C.

[ab]Means with different superscripts differ ($P < 0.05$).

[1]Back-transformed LSMEANS

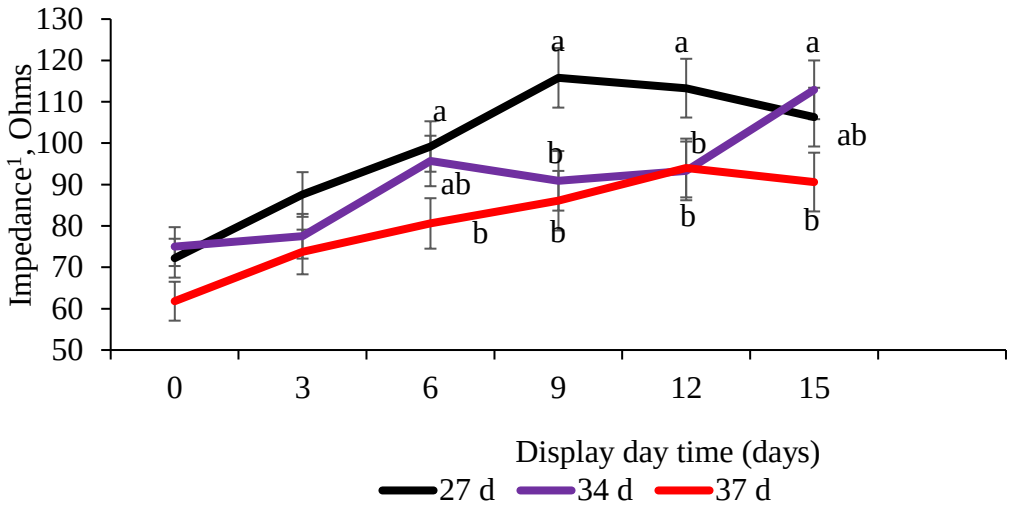

Figure 2.3 Interaction for middle-bioelectrical impedance values of beef *longissimus lumborum* steaks aged 27, 34, and 37 days and displayed for 15 days under fluorescent lighting at 0.26 ± 0.95°C.

[ab]Means with different superscripts differ ($P < 0.05$).

[1]Back-transformed LSMEANS

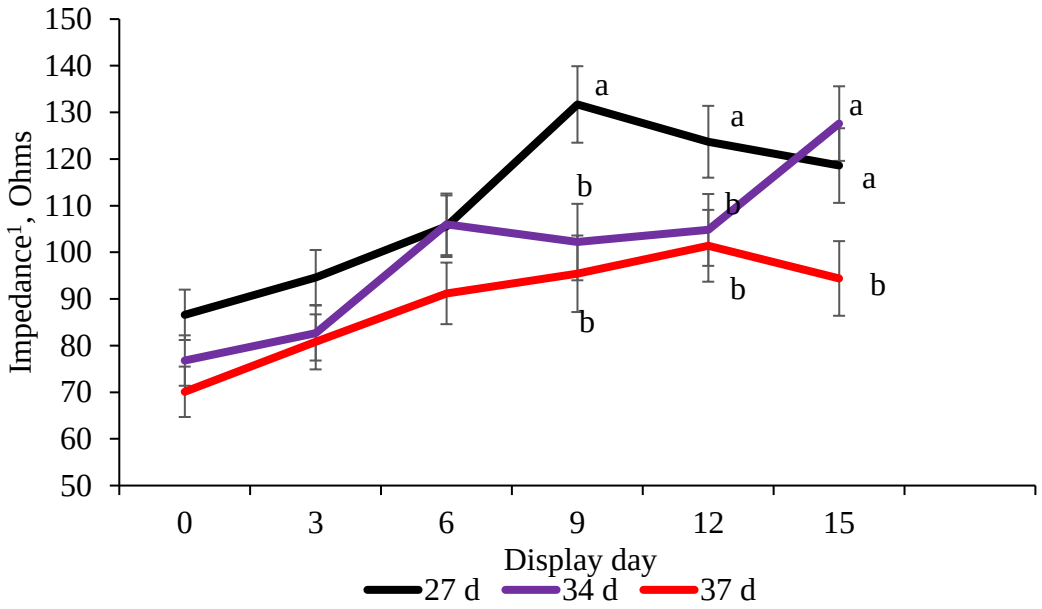

Figure 2.4 Interaction for bottom-bioelectrical impedance values of beef *longissimus lumborum* steaks aged 27, 34, and 37 days and displayed for 15 days under fluorescent lighting at 0.26 ± 0.95°C.

[ab]Means with different superscripts differ ($P < 0.05$).

[1]Back-transformed LSMEANS

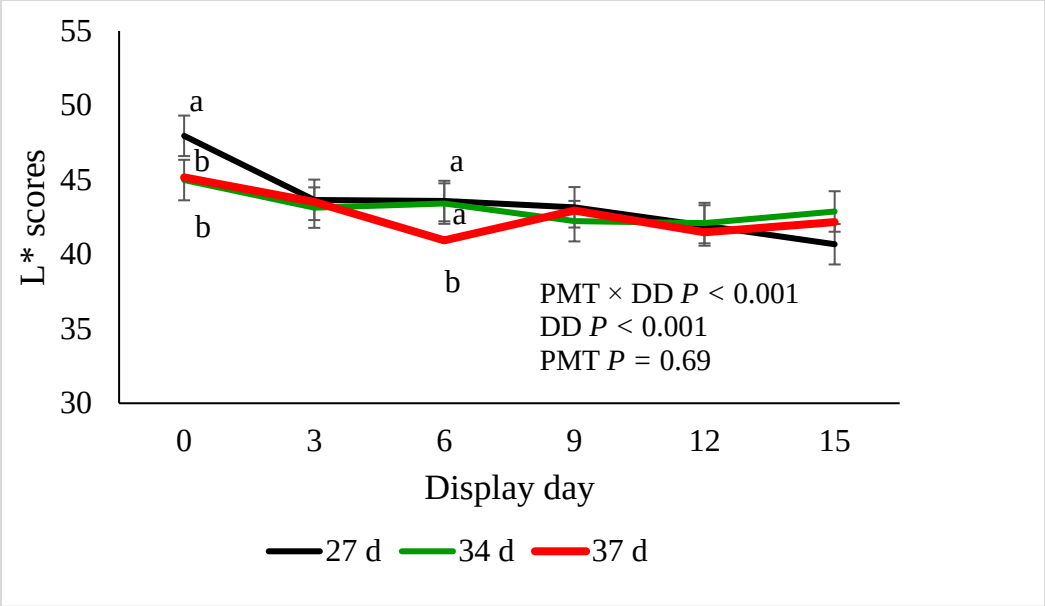

Figure 2.5 Interaction for lightness scores of beef *longissimus lumborum* steaks aged 27, 34, and 37 days and displayed for 15 days under fluorescent lighting at 0.26 ± 0.95°C.
[ab]Means with different superscripts differ ($P < 0.05$).

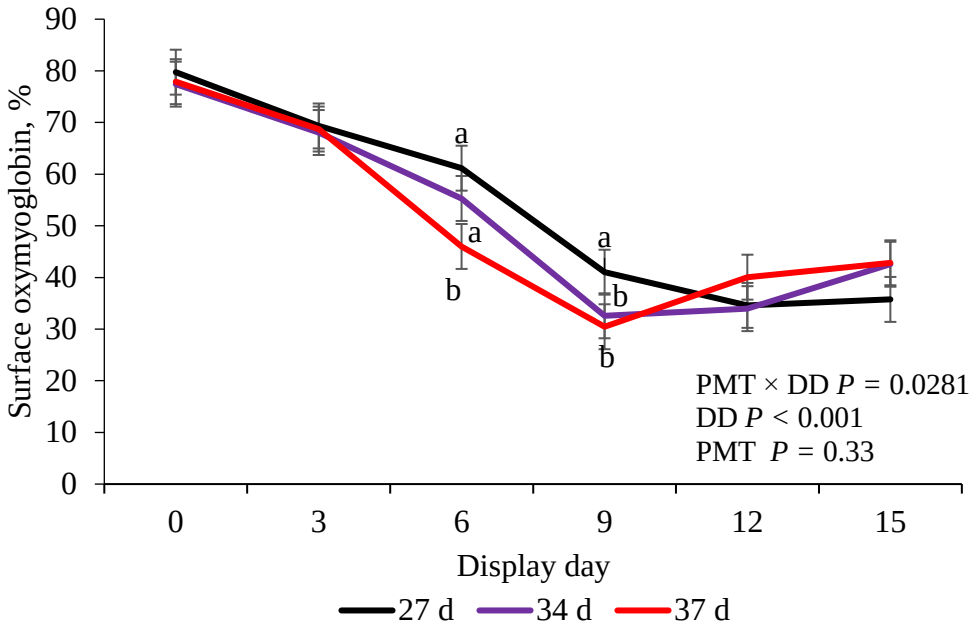

Figure 2.6 Interaction for percentage of surface oxymyoglobin of beef *longissimus lumborum* steaks aged 27, 34, and 37 days and displayed for 15 days under fluorescent lighting at 0.26 ± 0.95°C.

[ab]Means with different superscripts differ ($P < 0.05$).

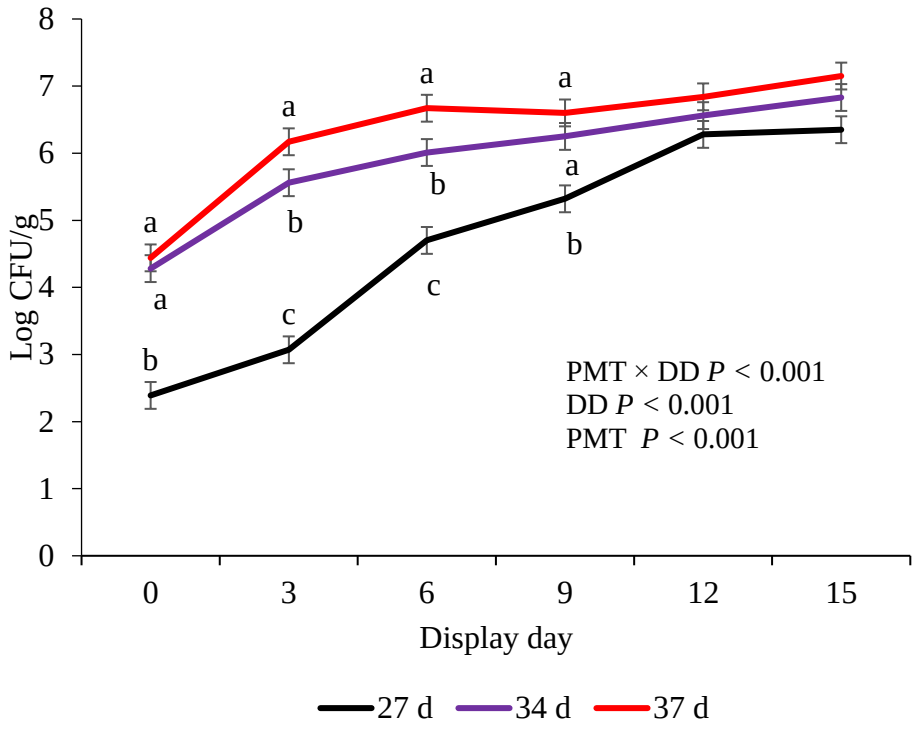

Figure 2.7 Interaction for APC populations of beef *longissimus lumborum* steaks aged 27, 34, and 37 days and displayed for 15 days under fluorescent lighting at 0.26 ± 0.95°C.
[abc]Means with different superscripts differ ($P < 0.05$).

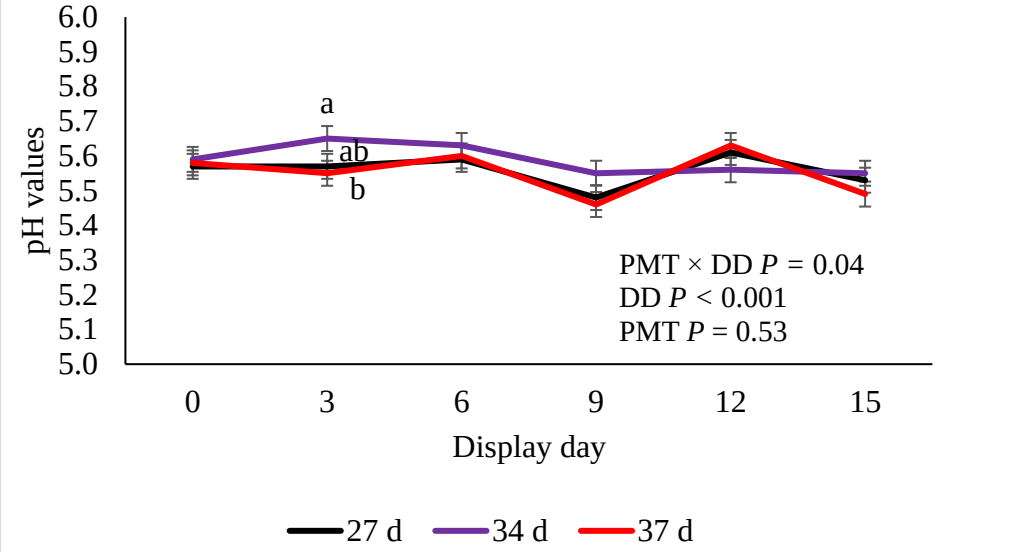

Figure 2.8 Interaction for pH values of beef *longissimus lumborum* steaks aged 27, 34, and 37 days and displayed for 15 days under fluorescent lighting at 0.26 ± 0.95°C.
[ab]Means with different superscripts differ ($P < 0.05$).

Chapter 3 - Beef longissimus lumborum steak pH affects bioelectrical impedance assessment

ABSTRACT

Postmortem chemical changes in normal- and high-pH beef strip loins (N = 20; postmortem age = 14 days) were assessed using surface bioelectrical impedance analysis (BIA) during simulated retail display. Two treatments, including normal (5.61 to 5.64; n = 11) and high-pH (6.2 to7.0; n = 9), were used. Loins were fabricated into five 2.54-cm thick steaks and randomly assigned to one of five display days (DD): 1, 3, 5, 7, and 9. Instrumental color, oxygen consumption (OC), metmyoglobin reducing activity (MRA), water holding capacity, pH, protein degradation, and BIA were measured. There was a meat-pH × DD interaction for OC and MRA (P <0.05). During retail display, high-pH steaks had a higher (P <0.05) OC and MRA than normal-pH steaks. Additionally, a meat-pH × DD interaction occurred for WHC and pH (P <0.05). The pH of high-pH steaks was higher (P <0.05) than normal-pH (approximately 1.5 units) during retail display. High-pH steaks had 10.5% greater (P <0.05) WHC than normal-pH beef on d 1 through d 5 and declined (P >0.05) after d 7. Normal-pH meat had a 43% higher (P <0.05) amount of degraded desmin than high-pH meat. Low correlations were found between BIA and other quality and chemical attributes of high and normal- pH steaks. Steaks with high-pH had three times lower (P <0.05) BIA compared to normal-pH meat. Day affected (P <0.05) BIA values. On d 1 and 3, BIA was not different (P >0.05) but were 16% lower (P <0.05) than on d 5. However, steaks surface BIA values were similar after d 5 (P >0.05). Surface BIA has potential for rapid, in-plant use to identify dark-cutting beef; however, BIA is not as strongly correlated with postmortem chemical changes that aerobically-packaged steaks undergo during retail display.

INTRODUCTION

In the U.S., the meat industry slaughters and processes 30-million head of cattle annually (USDA-NASS, 2018). Meat exhibiting dark cutting characteristics is an existing industry issue and occurred at an incidence rate of 3.2 and 1.9% of fed steers and heifers and heifers harvested in 2011 and 2016, respectively (Moore et al., 2012; Boykin et al., 2017). Dark cutting beef, also known as dark, firm, and dry (DFD) beef is not well accepted by beef packers mainly because consumers consider it unappealing and discriminate against this meat (Holdstock et al., 2014; Suman et al., 2014). Dark cutting beef pH is typically greater than 5.8 (McKeith et al., 2016). Furthermore, dark cutting beef are more prone to spoilage and are less tender than normal-pH meat (Tarrant, 1989; Wulf et al., 2002). As a result, producing dark beef represents an economic loss. It is known that pre-harvest stress contributes to early glycogen depletion, affecting the rate at which pH drops postmortem, which leads to a greater ultimate pH (Hendrick et al., 1959).

The meat industry continues to seek non-destructive technologies other than pH measurement to identify high-pH meat or dark cutting beef. There are biochemical differences between high and low pH meat. A recent study found high pH *longissimus lumborum* steaks had greater mitochondrial content than normal pH meat (Ramanathan et al., 2020). In addition, English et al. (2016) reported *longissimus lumborum* steaks with a pH of 6.4 presented increased oxygen consumption compared to those steaks with normal pH. The inherent biochemical characteristics of dark cutting beef will enhance the formation of deoxymyoglobin; thus, providing greater color stability (Mancini and Hunt, 2005).

Protein denaturation and water holding capacity (WHC) are also affected by ultimate pH of meat, as well as the rate at which pH drops, and these factors are important in determining the quality of fresh meat (Hughes et al., 2014). Meat with greater pH possesses proteins that have

higher WHC (Calkins and Hodgen, 2007). The relationship between WHC and bioelectrical impedance (BIA) has been previously reviewed by Hughes et al. (2014) and reported pork *longissimus* muscle exhibiting low drip loss had greater impedance values than samples with high drip loss.

Currently, new technologies are explored by the meat industry to assess pH of meat because traditional pH meters require more time for calibration. Over the years, researchers have evaluated this pH effect using BIA as well as the relationship of BIA with muscle pH and WHC in pork (Oliver et al., 1991; Chizzolini et al., 1993). Therefore, the objective of this study was to use surface BIA to assess postmortem chemical changes in normal- and high-pH beef *longissimus lumborum* steaks during simulated retail display.

MATERIALS AND METHODS

Product preparation

Beef strip loins (N = 20; Institutional Meat Purchase Specifications #180) were obtained from a commercial processor 14 d postmortem and shipped to the Kansas State University (KSU) Meats Laboratory. Loins were sorted into two treatments, normal-pH (5.61 to 5.64; n = 11) and high-pH (6.2 to7.0; n = 9), fabricated into five 2.54 cm thick steaks, taken from the anterior to the posterior portion of the loin, and randomly assigned to one of five display days (DD): 1, 3, 5, 7, and 9. Following fabrication, steaks were packaged on Styrofoam trays with a moisture absorbent pad with the anterior-sliced end facing up, and overwrapped with polyvinyl chloride film (23,250 cm^3/m^2/24 h at 23 °C and 0% RH; Borden Packaging and Industrial Products, North Andover, MA). Surface BIA, instrumental color, oxygen consumption, metmyoglobin reducing ability, WHC, pH, and protein degradation were assessed on each display day.

Display case and color measurement

Steaks were displayed in coffin-style retail cases (model DMF 8; Tyler Refrigeration Corp., Niles, MI) under fluorescent lighting (32 W Del-Warm White 3000° K; Philips Lighting Co., Somerset, NJ) that emitted a constant 24-h case average intensity of 2,230 ± 34 lx. Case temperatures were recorded on steak package surfaces using single channel temperature and humidity sensors (model OM-HL-SP-TH, Omega™, Norwalk, CT), and case temperatures ranged from 0 to 4°C. Cases were defrosted twice daily (morning and evening) at 11°C for 30 min and all steaks were rotated twice daily from left to right and front to back to account for minor variations in temperature and light intensity within cases. According to the methods of Phelps et al. (2014), steaks were instrumentally assessed every sampling day using a HunterLab MiniScan™ EZ (Illuminant A, 2.54-cm diameter aperture, 10° observer; Model 4500; Reston, VA) for CIE L*a* b* and reflectance from 400 to 700 nm. Readings were taken at 3 locations daily and values were averaged.

Oxygen consumption

Oxygen consumption was measured on each DD as described by Madhavi and Carpenter (1993) with some modifications from English et al. (2016). A 3 × 3 × 1.5 cm section was removed from each steak with little to no visible fat or connective tissue. Samples were allowed to bloom for 30 min at 4°C and immediately scanned using a HunterLab MiniScan™ EZ twice for surface color. Following the AMSA color guidelines (2012) the oxymyoglobin surface percent was determined using K/S ratios and equations.

Metmyoglobin reducing activity

The resistance to myoglobin oxidation was measured following the procedure described by Sammel et al. (2002) with modifications from English et al. (2016) on each DD. A 3 × 3 × 1.5 cm section with no visible fat or connective tissue was removed from the interior portion of the steak halves. Excised samples were submerged in a 0.3% solution of sodium nitrite (Sigma, St. Louis, MO) for 20 min to help the formation of metmyoglobin. Following submersion, sample surface was blotted to visually remove excess sodium nitrite solution, wrapped with PVC film, and scanned using metmyoglobin values (AMSA, 2012). Metmyoglobin reducing activity was calculated as percent initial MetMb formed on the sample surface.

Water holding capacity and pH

To determine the WHC of samples, a method described by Jauregui et al. (1981) was followed. Briefly, 5 g of muscle was weighed and transferred into a 50 mL conical tube. Twenty-five grams of 4-mm diameter glass beads was placed on bottom of the sample and then centrifuged at 900 ×g for 10 min. Following centrifugation, samples were reweighed, and the percent EM was calculated as [(initial weight-centrifuged weight)/initial weight] × 100. Sample pH was assessed using a calibrated pH probe (Model FC232, Hanna Instruments Inc.™, Woonsocket, RI) with a pH meter (Model HI 99163, Hanna Instruments Inc.™). The probe was inserted in duplicate on the side adjacent to the cores taken for OC and MRA determination.

Protein degradation

Samples were prepared and analyzed for desmin and TnT degradation at the University of Georgia following the procedures of Phelps et al. (2017) with minor modifications. Two-hundred milligrams of sample was homogenized in 5 mL of extraction buffer. The resulting

homogenate was then centrifuged at 1,500 ×g for 15 min at 20°C and the resulting supernatant was collected for analysis. Protein concentrations were determined using a Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL). Forty-five and 30 μg of protein were separated on 10% Novex Tris-Glycine Mini-Protein Gels (Invitrogen, Carlsbad, CA) for desmin and TnT, respectively. Proteins were separated by exposure to a constant amperage of 40 mA. Proteins were transferred onto nitrocellulose membranes using an iBlot 2 transfer unit (Thermo Fisher Scientific, Walham, MA). Blocking nonspecific antigen binding sites was performed by incubating membranes with 5% nonfat dry milk in 10 mM Tris, pH 8.0; 150 mM NaCl; and 0.1% Tween-20 (TBS-T) for 1 h at room temperature. Desmin and TnT blots were incubated for 20 h at 4°C with rabbit anti-desmin (1:15,000; Sigma-Aldrich Corporation, St. Louis, MO) and mouse anti-troponin-T (1:30,000; Sigma-Aldrich Corporation) antibodies diluted in blocking solution. After washing 4 times for 5 min with TBS-T, membranes were incubated with anti-rabbit and -mouse horseradish peroxidase–linked secondary antibody (Cell Signaling Technology, Inc., Danvers, MA) diluted 1:2,000 in blocking solution. Bands were visualized using a Pierce ECL Western Blotting Substrate kit (Thermo Fisher) and imaged using a iBright Western Blot Imaging System (Thermo Fisher Scientific). To measure band intensities a VisionWorksLS Image Acquisition and Analysis Software (Analytik Jena US LLC, Upland, CA) was used. Immunoreactive bands were located at 55 and 38 kDa for intact and degraded desmin. Immunoreactive bands were located at 40 for the intact form of Tnt and at 36, 34, and 30 kDa for the degraded form of Tnt. On each blot, the resulting bands were normalized to a pooled sample.

Impedance analysis

Steaks were assessed as described by Najar-Villarreal et al. (2021) using a CQR (Seafood Analytics CQR, Model Quantum IV, RJL Systems, Clinton Twp., MI) device. Briefly, BIA

values were calculated using the resistance (R) and reactance (X) values that are displayed on the device digital screen. The formula used was impedance in series ($Z= Xc + R^2/Xc$). Readings were collected at the top, middle, and bottom, and from dorsal to ventral of the steak. Three readings were retrieved from each location of the electrodes and an average was calculated for each steak. Bioelectrical impedance readings were obtained on the surface of the steak by moderately compressing the electrodes up to the middle end (halfway) using a template to consistently scan each steak. After every BIA measurement, moisture from previous steak was removed from the electrodes. Sample temperature was measured and ranged from 0 to 10 °C.

Statistical analysis

Data were analyzed using SAS version 9.4 (SAS Inst. Inc., Cary, NC) as a split-plot design. For the whole plot, treatment was meat-pH, and the experimental unit was loin. For the sub-plot, treatment was DD and the experimental unit was the steak. Fixed effects of the model included meat-pH, DD, and 2-way interactions. The Kenward–Roger approximation was utilized for all analyses. If a treatment effect was found to be significant ($P < 0.05$), the PDIFF option was used for mean separation. The CORR procedure of SAS was used to calculate the Pearson correlation coefficients. The codes of SAS for this phase can be found in Appendix B.

RESULTS AND DISCUSSION

Color measurements

There was no meat-pH × DD interaction ($P > 0.05$) for L* values; however, meat-pH × DD interactions ($P < 0.05$) for a* and b* values were found. Normal-pH steaks were 32% lighter ($P < 0.05$; Figure 3.1) than its counterpart steaks with higher pH. Regardless of pH, a DD effect ($P < 0.05$; Figure 3.2) was found for lightness. On d 1, steaks were 5.5% lighter ($P < 0.05$) than d 3 and 5 of display, which were similar ($P > 0.05$). By d 7 and 9, lightness decreased 4.1 and

6.5%, respectively. The interaction for redness is shown in Figure 3.3. Normal-pH steaks were 30, 19, 13, and 16% more red ($P < 0.05$) than high-pH beef on d 1, 3, 5, and 7, respectively. By d 9, redness values were similar ($P > 0.05$). Normal-pH steaks were 28% more yellow ($P < 0.05$; Figure 3.4) than high-pH over the display time. Yellowness values in normal-pH steaks decreased during retail display while high-pH steak yellowness increased from d 1 to d 3. After d 3, yellowness in high pH steaks went down.

Previous research observed a lightness increase on high-pH steaks compared to normal-pH steaks (Mitacek et al., 2018; Wu et al., 2020). It is well known that proteins in high-pH meat can hold more water and this increasing WHC can lead to the swelling of muscle fibers, resulting in less space at the muscle fibril level. As a result, the light scattering ability of meat is decreased as well as an increase in the light absorption of meat is observed, thus muscle has a darker appearance (Hughes et al., 2017). Following past literature, these results showed high-pH steaks had lower a* values and which is associated with high deoxymyoglobin content (Wu et al., 2020).

Oxygen consumption

There was a meat-pH × DD interaction for OC ($P < 0.05$; Figure 3.5). During retail display, high-pH steaks had a higher ($P < 0.05$) OC than normal-pH steaks. English et al. (2016) studied the impact of aging on the biochemical properties of DFD *longissimus lumborum* steaks and found OC was higher in high-pH steaks than those with normal pH. Ashmore et al. (1972) demonstrated that normal-pH (< 5.8) of meat affects the OC in mitochondria, which promotes blooming on the surface of meat in aerobic environments. On the other hand, high-pH meat improves OC, leading to a decreased oxygenation on meat surface that results in surface darkening (Price and Schweigert, 1987; Ledward et al., 1992).

Metmyoglobin reducing activity

There was a meat-pH × DD interaction for MRA ($P < 0.05$; Figure 3.6). During retail display, high-pH steaks had a higher ($P < 0.05$) MRA than normal-pH steaks. High-pH steaks MRA remained constant during the 9-retail display. The metmyoglobin reducing activity in normal-pH steaks was constant from d 1 to 7; however, by 9 MRA declined. It is known that high-pH meat has increased MRA values. These results are in agreement with those reported by English (2016) where DFD *longissimus lumborum* steaks reported greater MRA values than normal-pH steaks.

Water holding capacity and pH

There were meat-pH × DD interactions for WHC and pH ($P < 0.05$). As expected, pH of high-pH steaks was higher ($P <0.05$; Figure 3.7) than normal-pH (approximately 1.5 units) during retail display. High-pH steak pH from d 1 to 5 remained constant but increased by d 7. The pH of high-pH steak declined at the end of the retail display. The pH of normal-pH steaks remained the constant from d 1 to 9. High-pH steaks had 10.5% greater ($P < 0.05$; Figure 3.8) WHC than normal-pH beef on d 1 through 5 of display. However, WHC was similar ($P > 0.05$) between the two treatments after d 7 of display. It is known that meat with a higher pH has the ability to hold more water (Offer and Trinick, 1983; Calkins and Hodgen, 2007). Authors have previously reviewed the relationship among WHC, pH and BIA (Hughes et al., 2014). They presented a study where low and high drip loss pork *longissimus* muscles were used and they demonstrated that PSE pork (less WHC) had lower BIA values due to the excess of extracellular fluid.

Protein degradation

There was no meat-pH × DD interaction ($P > 0.05$) or DD effect for intact or degraded desmin, however, a meat-pH effect ($P < 0.05$) was found. The western blot for desmin is displayed in Figure 3.9. Normal-pH meat had 43% higher ($P < 0.05$; Figure 3.10) amount of degraded desmin than high-pH meat. No meat-pH × DD interactions were found for TnT 40, 36, 34, and 30 KDa ($P > 0.05$). No meat-pH or DD effects ($P > 0.05$) for degraded TnT (40, 36 and 34 KDa) were found. However, meat-pH affected ($P < 0.05$; Figure 3.11) degraded troponin-t (30 KDa). Normal-pH steaks had three times more ($P < 0.05$) degraded troponin-t (30 KDa) compared to those steaks with high pH. Additionally, no meat-pH × DD interaction ($P > 0.05$) or DD was observed for degraded portion. Irrespective of DD, the degraded portion was nearly two times greater ($P < 0.05$; Figure 3.12) in normal-pH steaks than high-pH steaks.

Tenderness is achieved through postmortem aging time (PMT) and is considered one of the most important factors influencing consumer palatability (Savell et al., 1987; Rhee et al., 2004). It has been established by Gruber et al. (2006) a universal aging time of 12-d PMT. The PMT process modifies membranes as well as intracellular and extracellular electrolytes, which can affect BIA with increasing frequencies. Desmin and TnT are cytoskeletal proteins that serve as indicators of meat tenderization. Intact desmin or degraded remained the same throughout the 9-d display time in the present study. These results did not follow other literature reported by Phelps et al. (2016) who found degraded desmin and TnT increased and intact desmin and TnT decreased during PMT on beef *semitendinosus* steaks. In the current study, beef *longissimus lumborum* with 14 d PMT, where most of proteolysis occurs, were used. As a result, few changes in these proteins were observed. Similarly, researchers have previously observed this increased

in degraded desmin in normal-pH beef *longissimus lumborum* with 13-d PMT aging compared to high-pH steaks (Grayson et al., 2016).

Impedance analysis

There was no meat-pH × DD interaction ($P > 0.05$) for BIA values; however, a meat-pH effect was found ($P < 0.05$; Figure 3.13). Steaks with high-pH were three times lower ($P < 0.05$) for surface BIA compared to those from loins with normal-pH. There was a day effect ($P < 0.05$; Figure 14), where BIA values were not different on d 1 and 3 of display ($P > 0.05$), but were 16% lower ($P < 0.05$) than d 5 of display. Steaks surface BIA values were similar after d 5 ($P > 0.05$). The meat industry deems DFD meat as a low value product due to lack of consumer acceptability (Holdstock et al., 2014). Thus, the separation of low-quality meat may be beneficial, preventing economic losses through the market development of low value meat (Castro-Giráldez et al., 2010). Historically, BIA has been evaluated to identify meats exhibiting different pH. Chizzolini et al. (1993) studied pork *semimembranosus* at 45 min and 24 h of PMT exhibiting DFD and PSE conditions using conductivity measurements and found low correlations between electrical measurements and pH. Additionally, their results indicated this method was unreliable for the early detection of DFD meat. Meat scientists have studied the effect of breed type on impedance measurements and found dairy-type cattle carcasses had greater BIA than native and brahman-type cattle (Page et al., 2001). These authors also reported a higher incidence of discounting DFD meat in dairy cattle, which could also explain the differences between high and normal pH in BIA values in this study.

Page et al. (2001) found a weak positive correlation between electrical impedance and muscle pH. It has been established BIA as a tool to assess the resistance of an electrical current in muscle, possibly associated with free water in muscle (Kauffman, 1997). Additionally,

researchers reported a positive correlation (r = 0.50) between drip loss and BIA at 1000 Hz on pork carcasses (Forrest et al., 2000). They concluded that this low correlation to the limited temperature correction system of the BIA device. In addition, Hughes et al. (2014) reported pork *longissimus* muscle with higher drip loss had lower BIA values.

Researchers evaluated the freshness of beef aerobically-packaged *longissimus lumborum* steaks with normal pH through the assessment of its electrical properties during a simulated retail display using a torrymeter (Sujiwo et al., 2019). Authors used beef loins with 1 d PMT and reported a linear decrease of torrymeter values on beef steaks during a 18-d retail display. The torrymeter uses a a band pass filter at a wide range of frequencies while the CQR equipment currently carries a default single frequency (50 Hz).

Correlations

In high-pH meat, BIA was negatively correlated (r = -0.35; $P < 0.05$) with pH and MRA. A positive correlation occurred between surface BIA and WHC (r = 0.28; $P < 0.05$). In normal-pH meat, BIA values were negatively correlated with degraded troponin-t (30 KDa) and intact troponin-t (40 KDa; r = -0.35; P <0.05). Surface BIA correlated with desmin and TnT degraded portion (r = 0.39; P <0.05) for normal-pH beef. In the beginning of the 1900's, Callow (1936) demonstrated that pH was related to electrical measurements. Researchers have previously studied BIA as means to assess pH; however, an early PMT measurement might not be reliable due to the softness and relaxation of muscle (Chizzolini et al., 1993). In accordance with past literature, the current study found a correlation between BIA and pH. Forrest et al. (2000) reported a positive correlation (r = 0.50) between drip loss using BIA on pork carcasses. However, this device assessed BIA internally which can be problematic in terms of contamination.

CONCLUSIONS

These results indicated that high-pH meat had higher BIA values, indicating that the CQR tool could identify normal-and high-pH strip loins. The CQR device has notorious potential for rapid, in-plant use to identify dark-cutting beef. However, BIA is not as strongly correlated with changes that aerobically-packaged steaks undergo during retail display. The low correlations between protein degradation and BIA observed in this study possibly occurred because most of proteolysis had already taken place in the first two weeks of PMT. Thus, low changes in protein degradation were observed in this study.

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Table 3.1. Correlation coefficients between electrical measurements[1] of high-pH and normal-pH beef *longissimus lumborum* steaks and water holding capacity, pH, oxygen consumption, metmyoglobin reducing ability, desmin, troponin-t, and degraded portion

Treatment	Water holding capacity	pH	Oxygen consumption	Metmyoglobin reducing ability	Desmin[2] KDa		Troponin-T[3] KDa				Degraded portion[4]
					55	38	30	34	36	40	
High-pH	0.11	-0.35*	-0.24	-0.40**	-0.07	-0.10	0.11	-0.01	-0.01	0.04	0.11
Normal-pH	0.28*	-0.04	0.03	-0.07	-0.13	0.23	-0.35**	0.12	-0.27*	0.39*	0.95

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
[1]Z = impedance ($Z = X_c + R^2/X_c$).
[2]Intact desmin (55 KDa); degraded desmin (38 KDa).
[3]Intact troponin-t (40 KDa); degraded troponin-t (30, 34, and 36 KDa).
[4]Degraded portion = degraded desmin + degraded troponin-t.

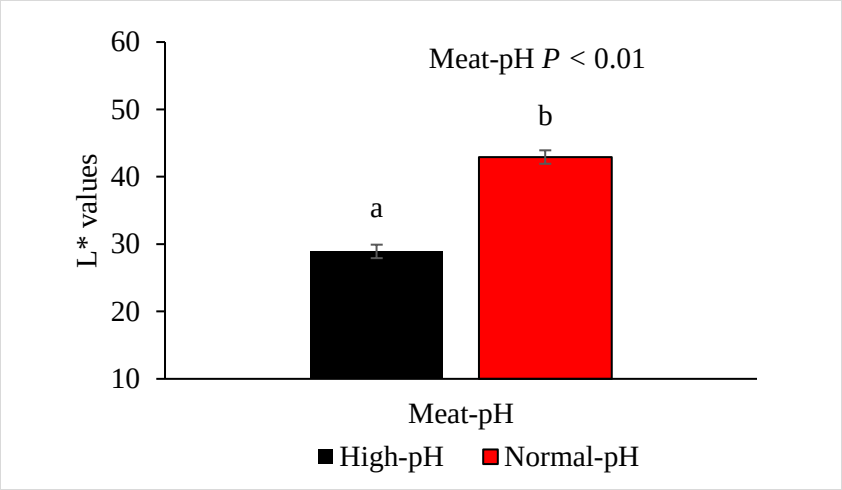


Figure 3.1 Lightness values of normal and high-pH beef *longissimus lumborum* steaks ($N = 100$) displayed under fluorescent lights at 0-4.4°C for up to 9 days.
[ab]Means with different superscripts differ ($P < 0.05$).

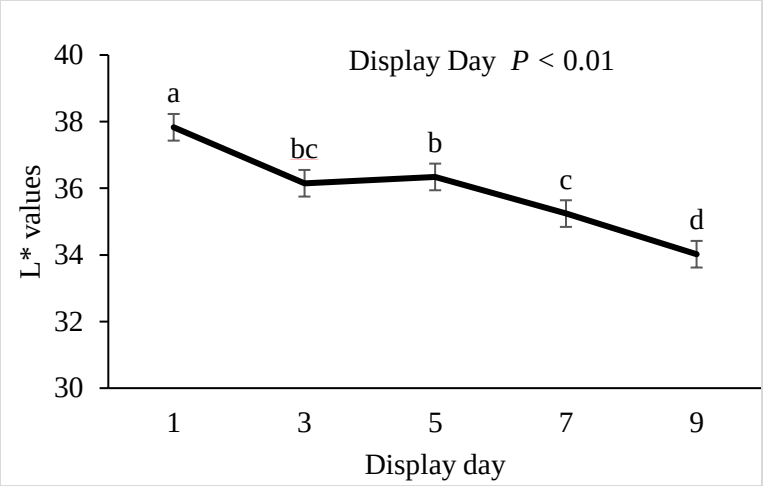


Figure 3.2 Surface bioelectrical impedance values of beef *longissimus lumborum* steaks (*N* = 100) displayed under fluorescent lights at 0-4.4°C for up to 9 days.
[ab]Means with different superscripts differ (*P* < 0.05).

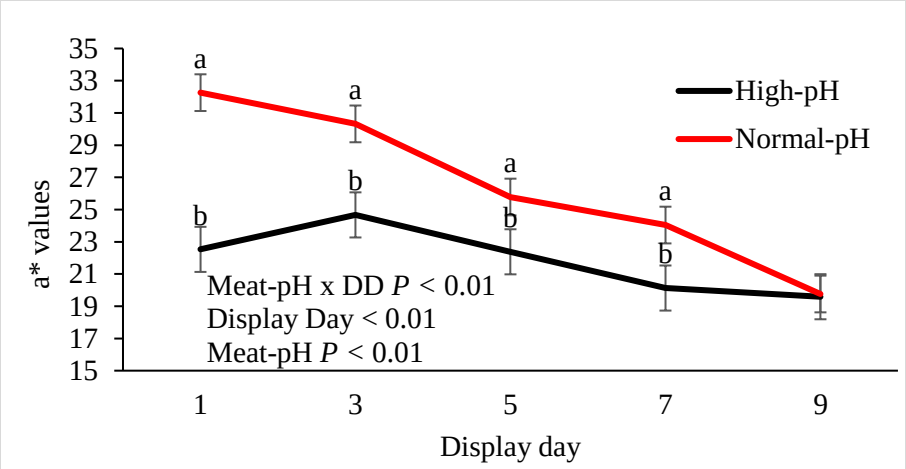


Figure 3.3 Interaction for redness values of beef *longissimus lumborum* steaks (N = 100) displayed under fluorescent lights at 0-4.4°C for up to 9 days.

[ab]Means with different superscripts differ (P < 0.05).

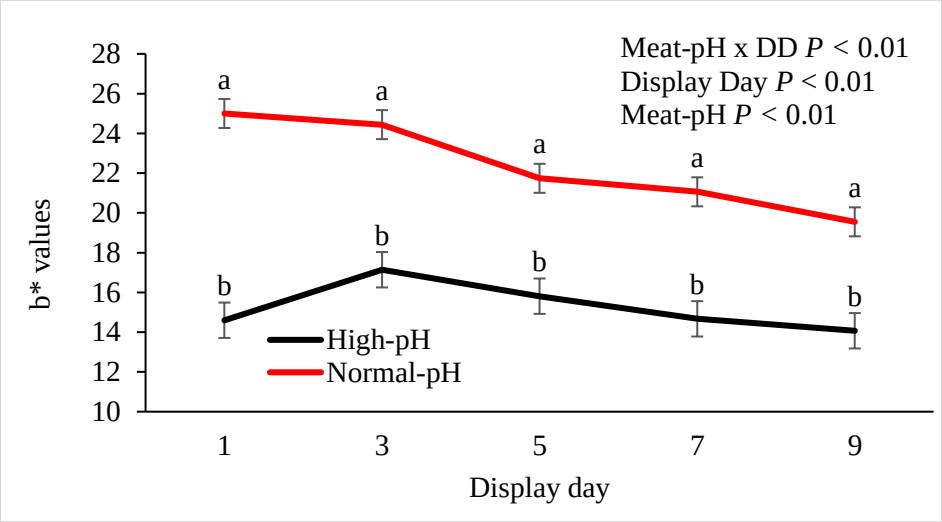


Figure 3.4 Interaction for yellowness values of beef *longissimus lumborum* steaks (N = 100) displayed under fluorescent lights at 0-4.4°C for up to 9 days.
[ab]Means with different superscripts differ (P < 0.05).

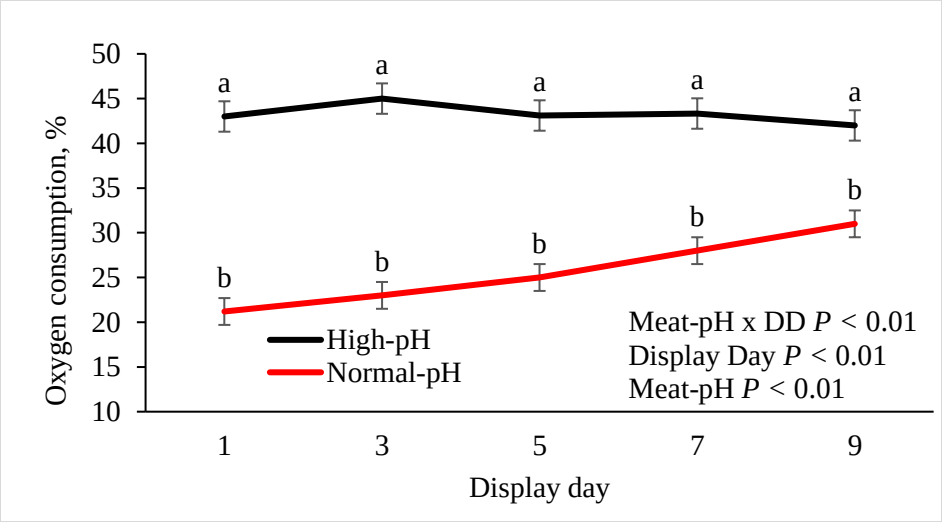


Figure 3.5 Interaction for oxygen consumption values of beef *longissimus lumborum* steaks (N = 100) displayed under fluorescent lights at 0-4.4°C for up to 9 days.
[ab]Means with different superscripts differ (P < 0.05).

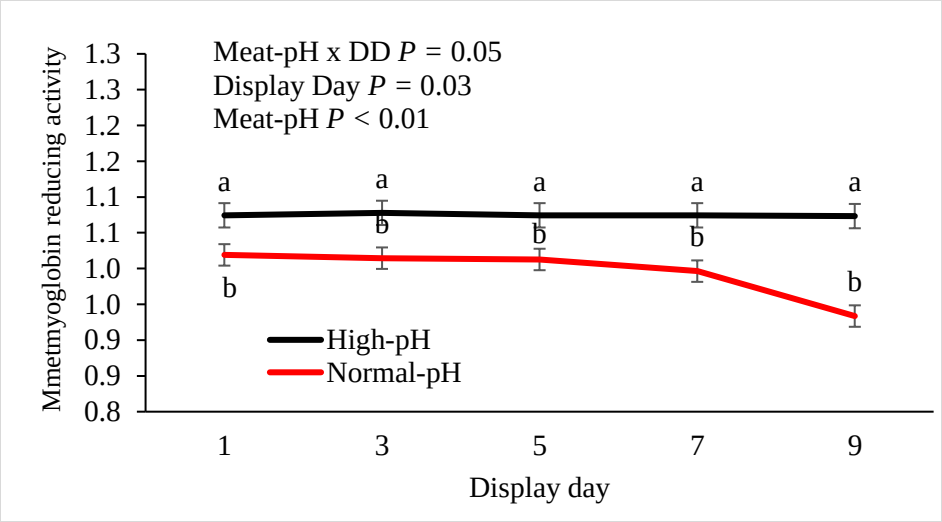


Figure 3.6 Interaction for metmyoglobin reducing activity of beef *longissimus lumborum* steaks (N = 100) displayed under fluorescent lights at 0-4.4°C for up to 9 days.
[ab]Means with different superscripts differ (P < 0.05).

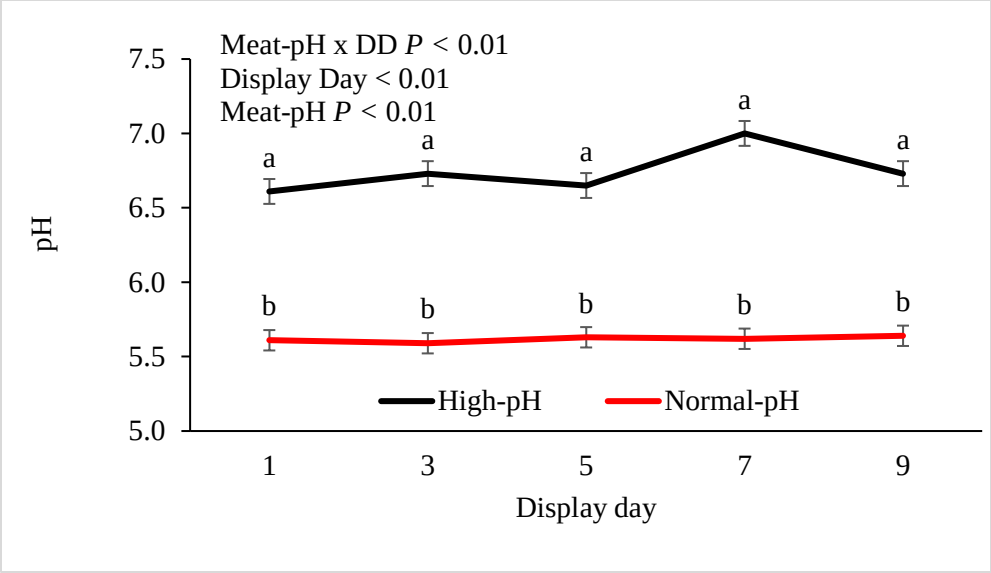


Figure 3.7 Interaction for pH values of beef *longissimus lumborum* steaks (N = 100) displayed under fluorescent lights at 0-4.4°C for up to 9 days.

[ab]Means with different superscripts differ (P < 0.05)

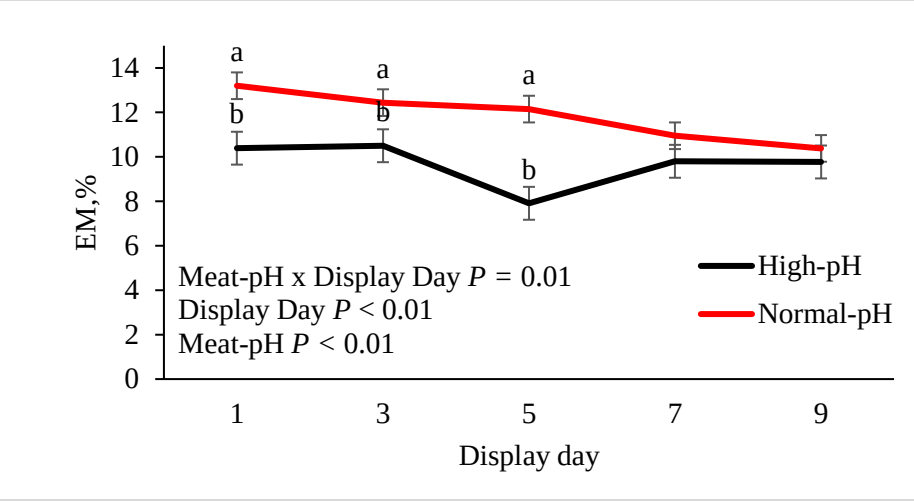
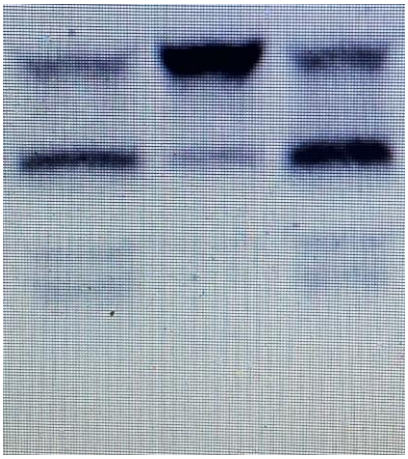


Figure 3.8 Interaction for water holding capacity of beef *longissimus lumborum* steaks (N = 100) displayed under fluorescent lights at 0-4.4°C for up to 9 days.
[ab]Means with different superscripts differ (P < 0.05).



Normal-pH High-pH Normal-pH

Figure 3.9 Desmin western-blot for normal and high-pH beef *longissimus lumborum* steaks displayed under fluorescent lights at 0-4.4°C for up to 9 days.

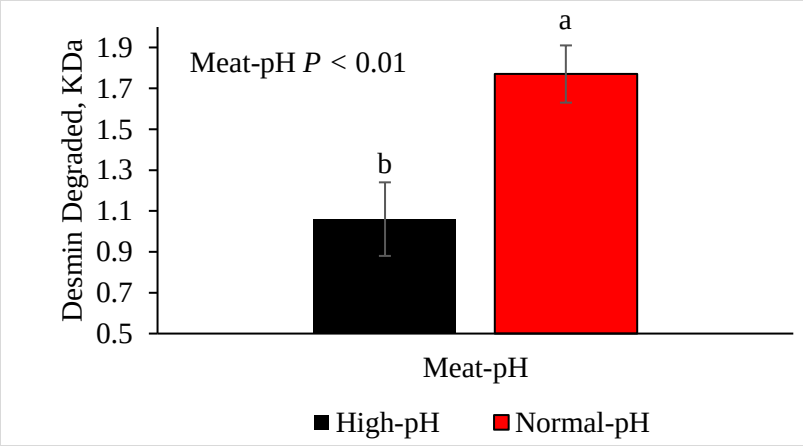


Figure 3.10 Degraded desmin amount of beef *longissimus lumborum* steaks displayed under fluorescent lights at 0-4.4°C for up to 9 days.

[ab]Means with different superscripts differ (P < 0.05).

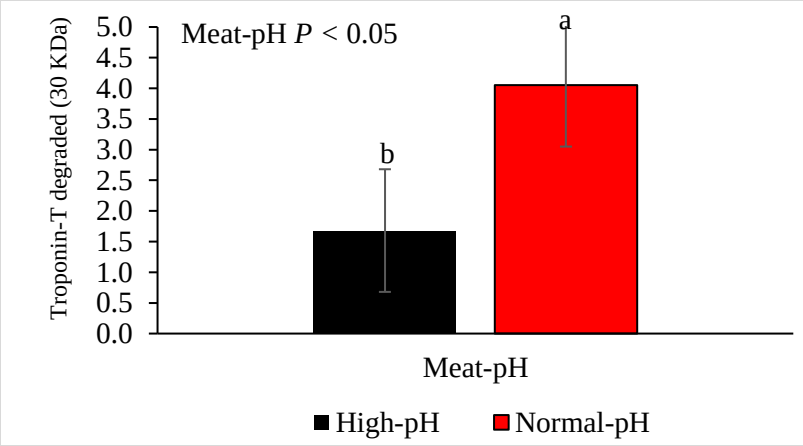


Figure 3.11 Degraded troponin-T (30 KDa) amount of beef *longissimus lumborum* steaks displayed under fluorescent lights at 0-4.4°C for up to 9 days.

[ab]Means with different superscripts differ ($P < 0.05$)

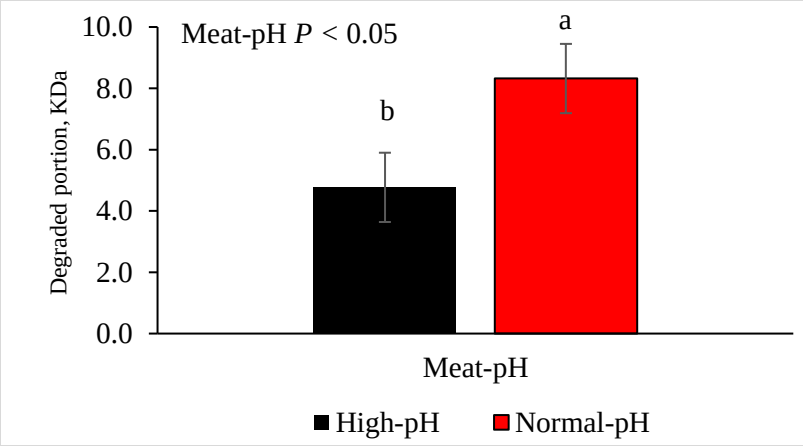


Figure 3.12 Degraded portion of beef *longissimus lumborum* steaks displayed under fluorescent lights at 0-4.4°C for up to 9 days.

[ab]Means with different superscripts differ ($P < 0.05$).

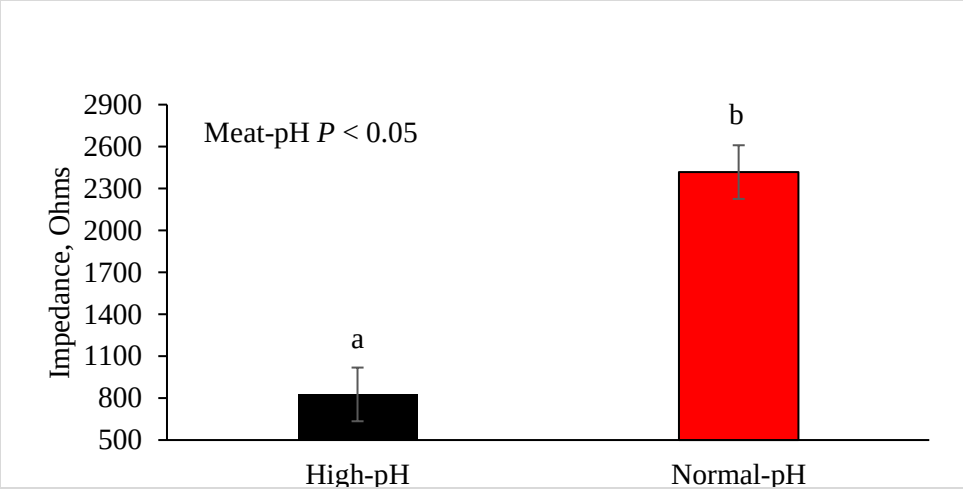


Figure 3.13 Surface bioelectrical impedance values of normal and high-pH beef *longissimus lumborum* steaks ($N = 100$) displayed under fluorescent lights at 0-4.4°C for up to 9 days.

[ab]Means with different superscripts differ ($P < 0.05$).

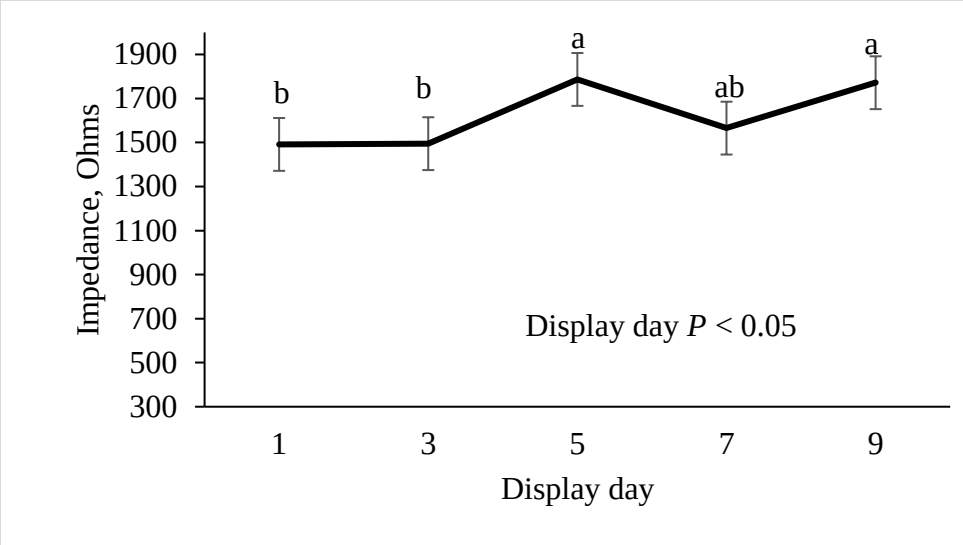


Figure 3.14 Surface bioelectrical impedance values of beef *longissimus lumborum* steaks ($N = 100$) displayed under fluorescent lights at 0-4.4°C for up to 9 days.
[ab]Means with different superscripts differ ($P < 0.05$).

Chapter 4 - Determining the longissimus lumborum and psoas major beef steak color life threshold and effect of post-mortem aging time using meta-analysis

ABSTRACT

The color life threshold for beef *longissimus lumborum* (LL) and *psoas major* (PM) steaks during retail display and the effect of postmortem aging time (PMT) on the display color life of LL and PM steaks using meta-analysis was determined. In phase one, data were retrieved from 13 and 3 referred journal articles, for LL and PM, respectively, that included a* and subjective visual scores. The total display day observations for LL and PM were 148 and 27, respectively. Lower bound estimates using a 95% confidence interval for a* as a borderline for the display color life of LL and PM steaks were 20.24 and 20.99, respectively. For phase two, data were retrieved from 26 and 10 referred journal articles, for LL and PM, respectively, that included a* and PMT. The total display day observations for LL and PM in phase two were 255 and 71, respectively. For LL steaks, the actual PMT was grouped into five categories: 0-7 d; 8-14 d; 15-21 d; 22-28 d; and 29-65 d. Additionally, the PMT of PM steaks was grouped into two categories: 0-7 d and 8-21 d. The first 21 d PMT for LL steaks had the longest color life with 7 d of color life. Additionally, 22-28 and 29-65 d of PMT had 5 and 4 d, respectively, of color life for LL steaks. The borderline acceptability estimated for PM steaks with 0-7 d and 8-21 of PMT was 3 and 2 d of color life, respectively. Estimations from this meta-analysis demonstrate that using LL and PM subprimals having a PMT of 21 d or less and 7 d or less, respectively, would optimize retail display color life of aerobically packaged steaks.

Introduction

Meat color is unstable, leading to discoloration, and is considered one of the major criteria for consumers, serving as an indicator of freshness and wholesomeness when selecting their meat purchases (Kropf, 1993). There are several factors affecting the appearance of fresh meat color, including processing, packaging, distribution, and display temperature (Mancini and Hunt, 2005). These variables also affect the rate at which the process of meat discoloration occurs, resulting in revenue loss at the retail level. Discoloration of meat has been heavily researched through objective and subjective methods via instrumental and trained panelists, respectively, during shelf-life studies as well as their relationship to determine color life thresholds (Hunt et al., 2004; Colle et al., 2015; Steele et al., 2016). Visual color scores determined by trained panelists have been associated with a strong correlation with consumers purchasing intent when beef is not red (Carpenter et al., 2001). As a result, Mancini and Hunt (2005) stated visual score is the gold standard to know consumer liking responses.

Traditionally, two categories, color stable and color labile muscle, have been established based on the biochemical characteristics that affect the color stability of beef muscles (McKenna et al., 2005). The *longissimus lumborum* (LL) muscle or strip loin lies under the color stable category and exhibits excellent color stability properties during retail display (Seyfert et al., 2006; Joseph et al., 2012). On the other hand, the *psoas major* (PM) or tenderloin, a color labile muscle, has less color life when displayed (Seyfert et al., 2006). Historically, the comparison between these muscles have served as a good model due to the difference in their muscle biochemistry. In addition, the LL and PM are commonly accessible at the retail store due to their popularity among meat shoppers.

Postmortem aging time (PMT) plays a key role in meat discoloration during retail display. Colle et al. (2015) concluded that extended PMT had a strong impact on the color life of various muscles. They also indicated that extended PMT in USDA Select strip loins longer than 14 d is detrimental for its color life. The 2010/2011 National Beef Tenderness Survey (NBTS) reported that PMT for vacuum-packaged subprimals under refrigerated conditions ranged from 1 to 358 d and 9 to 67 d at the retail level and foodservice, respectively (Guelker et al., 2013). According to the 2015 NBTS, PMT of strip loins at retail were shown to vary from 6 to 101 d with a post-fabrication storage average of 27.2 d (Martinez et al., 2017).

Meta-analysis is used to combine data from several studies to develop a single conclusion that has greater statistical power. To the best of our knowledge, there have been no meta-analysis that has evaluated color life of fresh meat in the literature. Therefore, the objective of this study was to determine the color life threshold for LL and PM steaks during retail display using published visual and instrumental color data and the effect of PMT on the display color life of LL and PM steaks.

Materials and Methods

Meta-analysis

Phase One

An electronic literature search was conducted to retrieve studies that have evaluated the effects of display day on LL and PM muscle using spectrophotometers with illuminant A. A literature search was conducted via the Kansas State University Libraries utilizing the CABI search engine using articles from 2000 to 2020. The search was restricted to studies presented in English in peer-reviewed journals. Visual scores from each experiment for LL were standardized for an 8 point-line scale where 1 = very bright red, 2 = bright red, 3 = dull red, 4 = slightly dark

red, 5 = moderately dark red, 6 = dark red to tannish red, 7 = dark reddish tan, 8 = tan to brown. Additionally, the PM visual color values were used based on a 5-point scale that included: 1 = very bright cherry red, 2 = bright cherry red, 3 = slightly dark red to tannish red, 4 = moderately grayish tan to brown, 5 = tan to brown, where 3.5 was considered borderline acceptable by the trained panelist. Studies used in this meta-analysis are shown in Table 4.1. There were 5 and 3 identified visual color score thresholds for LL and PM, respectively, in the literature and an average was calculated to be used in the model for LL and PM. Response variables for visual scores including meat color, meat discoloration, and muscle darkening were considered. Considering these criteria, the final database resulted in 13 and 3 papers for LL and PM, respectively, using illuminant A. The total display day observations for LL and PM were 148 and 27, respectively.

Phase Two

Similarly, a research procedure was conducted to study the PMT effect on color life of LL and PM using illuminant A to assess meat color. Considering these criteria, the final database resulted in 26 and 10 papers for LL and PM, respectively. For LL steaks, the actual PMT were grouped into five categories: 0-7 d; 8-14 d; 15-21 d; 22-28 d; 29-65 d. Each category consisted of 5 to 16 experiments totaling 48 experiments. For PM steaks, the actual postmortem aging times were grouped into two categories: 0-7 d; 8-21 d. Each category consisted of 11 and 5 experiments. The total display day observations for LL and PM were 255 and 71, respectively.

Selection Criteria for Inclusion and Exclusion

In order to be included in the final database for LL and PM, experiments had to meet the following criteria: 1) colorimeter type; 2) aperture size; 3) number of scans; 4) display days; 5) steak thickness; 6) pH of meat; 7) storage temperature; 8) objective color measures; 9) subjective

color measures; 10) oxygen permeable packaging (PVC) or modified atmosphere packaging with 80% O_2 and 20% CO_2; and 11) lighting type. Studies evaluating the effect of enhancement solutions on LL meat color were considered for this study. In addition to these parameters, the variable PMT was included and studies evaluating the effect of enhancements or other packaging different than oxygen permeable packaging for LL and PM steaks were not considered for phase two. To estimate a* redness values, studies assessing meat color with illuminant A were excluded if visual color data was not reported in hedonic scales. Furthermore, experiments had to provide display day means and SEM to be included in the meta-analysis.

Statistical analysis

Statistical analysis was performed using SAS 9.4 (SAS Institute, Inc., Cary, NC) PROC MIXED. The inverse variance weighting in meta-analysis was carried out via the WEIGHT statement followed by a variable equal to the inverse of the variance of the reported mean response. The phase one analysis implemented a hierarchical linear model (Singer, 1998; Sullivan et al. 1999) with the reported a* mean being the response variable and illuminant A mean being the linear regressor (i.e. fixed effect). Experiment was defined as the combination of paper, actual postmortem aging time, and study repeat. There were 29 experiments for LL steaks and 6 experiments for PM steaks. The model contained three random components: two represented the variation of intercept and slope at the experiment level; the other represented the random error at the display-day-by-experiment level. The variance-covariance of the intercept and slope was taken as unstructured. The phase two analysis implemented a hierarchical linear model with display day and postmortem aging time being the regressors. Fixed effects of the model included postmortem aging group (a categorical variable), display day (linear effect of a numeric variable), display day squared (quadratic effect of a numeric variable), interactions of

postmortem aging group with display day (linear effect heterogeneous with respect to postmortem aging group) as well as interaction of postmortem aging group and display day squared (i.e. quadratic effects heterogeneous with respect to postmortem aging group). The model contained four random components: three represented the variation of intercept, linear coefficient and quadratic coefficient at the experiment level; the other represented the random error at the display-day-by-experiment level. The variance-covariance of regression coefficients were taken as unstructured. Only a* redness was included in the model because all studies reported this measure. Using a confidence interval of 95%, a* threshold was calculated using 5.9 and 3.5 using illuminant A for LL and PM muscles, respectively.

Results and Discussion

Phase One

The estimates for a* redness using 5.9 and 3.5 as a borderline acceptability for color life of LL and PM steaks with a 95% confidence interval can be found in Table 4.2. For LL steaks, the visual color score threshold estimated in phase one for LL was 22.15 for the estimate, and 24.07 and 20.24 for the higher and lower bounds, respectively, for a* redness using a 95% confidence interval. In addition, the visual color score threshold for PM steaks was 22.37 for the estimate, and 23.75 and 20.99 for the higher and lower bounds, respectively, for a* redness using a 95% confidence. It has been previously reported that for a response known to decrease over time, the lower one-sided 95 percent confidence limit should be used (U.S. Department of Health and Human Services (HHS)-FDA-Center for Veterinary Medicine, 2014). Thus, the a* color values lower bounds, 20.24 and 20.99, were selected as borderline acceptability for LL and PM, respectively. Figure 4.1 depicts the adequacy of the model for phase one for LL and PM. The plots of residuals vs. predicted values suggests that the estimations calculated were precise for

LL and PM steaks. In addition, the studentized residuals plots suggest that normality assumption was met and no evidence for outliers and heteroscedasticity was observed for LL and PM.

Historically, LL, also known as the strip loin, is a heavily researched muscle, and it is widely used in meat science literature. Overall, this muscle provides a good lean tissue area to be assessed by researchers. As a result, a great number of referred journal articles for LL were found in the literature compared to the number found for the PM muscle. These two muscles are popular among consumers and are normally found in display cases at the retail level.

Simulated retail display time ranged from d 0 to d 15 among all experiments. Temperature averages for simulated display studies were -3 to 7 ºC. Mancini et al. (2002) conducted a national retail survey and reported an average display case temperature of 4.4 °C at retail. The pH of LL and PM steaks ranged from 5.50 to 5.82 in the papers used in this study. To objectively assess the color of fresh meat, colorimeters or spectrophotometers such as the Minolta-branded instrument (Chiyoda-ku, Tokyo, Japan) or the Hunter Associates Laboratory instrument (Reston, VA, USA) have been used in 644 and 339 articles, respectively (Tapp et al., 2011). Some of the specifications that can be used to assess color on meat products are illuminant A, C, and D65, which measure tristimulus values, included L*, a*, and b*. Upon the completion of the search of meat color papers, nearly 50% (data not shown) were journal articles reporting data using illuminant D65 and/or C, but these data are not comparable with illuminant A. The light source or type of illuminant plays an important role in the color being measured on meat and meat products, and the American Meat Science Association (AMSA, 2012) color guidelines recommend the use of illuminant A due to the higher proportion of long, red wavelengths, which have been determined to have higher correlations with visual color scores. Illuminant's C and D65 are known to resemble daylight, which ultimately affects the resulting

color measured by the apparatus (Tapp et al., 2011). Holman et al. (2017) used consumers to predict color acceptability with D65 and found that a* redness was best to predict color acceptability. Therefore, a* values were the instrumental color attribute that was selected in this study. It is noteworthy to mention that a* was reported in the literature in higher proportion compared to L* and b*.

It has been previously reported that there were some inconsistencies in publications when reporting apparatus specifications, and some authors failed to thoroughly describe the essential specifications when assessing meat color as recommended by the AMSA Meat Color Measurement Guidelines (2012). For instance, Tapp et al. (2011) conducted a survey of 1068 published (1998 to 2007) manuscripts and found that 3% of studies failed to include instrument type, 52.4% failed to report number of scans on each sample, and 73.6% failed to include aperture size. The number scans reported in the experiments used in this analysis ranged from 2-4 scans per sample. In addition, a standardized method to visually assess beef color was not observed across the experiments reviewed for this study; researchers used hedonic and 100% scales interchangeably in their results. Since this meta-analysis followed AMSA (2012) color guidelines, papers using other types of visual color scales were not comparable to one another and were excluded.

Estimations for the meta-analysis were calculated using visual color scores and a* instrumental color data from papers using illuminant A. In past literature, metmyoglobin formation or discoloration on the surface of 20% has been widely used in the literature as an acceptable color threshold to determine borderline acceptability using instrumental color results (Hood and Riordan, 1973). It is noteworthy to mention that this research was published more than 50 years ago. Therefore, the estimations calculated in this study represent the most current

data published within the last 20 years (2000-2020). Additionally, it reflects current beef production practices and may indicate a good color life of LL and PM steaks. The borderline thresholds estimated using the present data set may only be used for LL and PM, while other meta-analyses should be performed for other muscles depending upon the literature that is available.

Phase Two

Several studies have shown that antemortem factors can affect meat color, including age, sex, genetics, and nutrition (Faustman and Cassens 1990; Suman and Joseph, 2013). Meat scientists conducting meat color research using high forage/grass feeding systems have found that the meat produced metabolizes energy differently (more oxidative) and can result in darker lean meat (Muir et al., 1998; Vestergaard et al., 2000). It is well established that the ultimate pH of meat plays a role in meat color. Generally, high-pH meat is biochemically different and has shown increased oxygen consumption than normal-pH meat (English et al., 2016). Thus, those experiments using high-pH treatments were excluded from the meta-analysis. Overall, most of the meat used in the current studies was procured and sourced from a commodity cattle production system in the U.S., which are primarily cattle finished on a concentrate diet.

To date, meat packers utilize PMT as means to guarantee tenderness and 14 d of PMT is a meat industry standard to ensure a good consumer eating experience; however, undergoing PMT that exceeds 14 d may lead to poor color stability (Ramanathan et al., 2020). English et al. (2016) compared LL aged 21, 42, 62 and reported that extended aging had a detrimental effect on color stability during retail display. They demonstrated that LL steaks with >42 d of PMT bloomed less than LL steaks with 21 d PMT and deducted this lack of blooming was due to the increased purge loss containing myoglobin during PMT (English et al., 2016). In addition, these

authors reported that extended aging increased oxygen consumption, which may influence the consumer-preferred red color of beef. Postmortem strategies to optimize the color life of fresh meat is key throughout the supply chain. Other exogenous factors influencing beef color are storage, display conditions, packaging, and the addition of antioxidants, among others (Faustman and Cassens 1990; Mancini and Hunt, 2005; Suman and Joseph, 2013). For phase two, papers that included beef packaged in PVC film and modified atmosphere packaging (MAP) 80% O_2/20% CO_2 were used for the meta-analysis, but other types of MAP such as CO were excluded.

Plots of residuals vs. predicted values and studentized residuals plots (Figure 4.2) for phase two for LL indicate the model was adequate. However, an outlier in the studentized residuals plots and line of distribution for the PM in phase two was observed. The estimates of color life of LL steaks during retail display are shown in Figure 4.3. The a* value used for LL was 20.24. The first 21 d of PMT were found to have the longest color life with 7 d of color life for LL steaks. Additionally, the color life of LL steaks with PMT 29-65 d and 22-28 d was 5 and 4 d of color life, respectively. For PM, the estimates of color life of LL are shown in Figure 4.4. The estimated time before borderline acceptability for 0-7 d PMT was 3 d for PM steaks. The color life of PM steaks with 8-21 d of PMT was only 2 d. Colle et al. (2015) reported the same decline in redness color-life of LL steaks for extended postmortem aging time. They reported a* values of LL steaks decreased during simulated retail display when strip loins were aged 14 d or longer. In addition, English et al. (2016) compared LL aged 21, 42, 62 d and reported that extended aging had a detrimental effect on color stability during retail display.

Conclusion

These estimations may be applicable when procuring commodity meat with a normal pH from a grain-fed feeding system. Using meat produced from cattle raised under different feeding systems, primarily grass-fed that is typically found in other countries, may not provide an accurate estimation due to their inherent color differences. Overall, knowing the postmortem age of LL and PM subprimals could serve as a tool for retailers to identify the potential display color life of LL and PM steaks displayed under aerobic packaging conditions. Estimations from this meta-analysis demonstrate that using LL and PM subprimals having a PMT age of 21 d or less and 7 d or less, respectively, would optimize retail display color life of aerobically packaged steaks.

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Table 4.1. Summary of papers using illuminant A used in the regression analysis to predict redness of *longissimus lumborum* (LL) *and psoas major* (PM) steaks[1].

Authors	Source[2]	Colorimeter	Aperture size	Scans[3]	Display days	Steak thickness	pH[4]	T ºC[5]	Lighting Type[6]	Muscle[1]
Steele et al. (2016)	J	HunterLab MiniScan™ EZ	31.8 mm	3	0, 1, 2	2.54 cm	5.62	1.2 ºC	F, LED	LL
Colle et al. (2015)	J	Hunter MiniScan EZ	25 mm	2	0, 1, 2, 3, 4	2.54 cm	5.62	3.0 ºC	N	LL
Kim et al. (2006)	J	HunterLab MiniScan® XE Plus	2.54 cm	3	2, 9, 14	2.54 cm	5.85	1.0 ºC	F	LL
Rogers et al. (2010)	J	HunterLab MiniScan® XE Plus	2.54 cm	3	0, 2, 4	2.54 cm	NR	0.9±2.3 ºC	F	LL
Grobbel (2008)	J	HunterLab MiniScan® XE Plus	2.54 cm	3	0, 7	2.54 cm	5.50	2.0 ºC	F	LL
Seyfert et al. (2006)	J	HunterLab MiniScan® XE Plus	2.54 cm	3	0, 1, 2, 3, 4, 5, 6, 7	2.54 cm	5.62	1.7±3.2 ºC	F	LL, PM
English et al. (2015)	D	HunterLab MiniScan® XE Plus	2.50 cm	2	0, 1, 2, 3, 4, 5, 6	2.54 cm	5.60	2.0±1 ºC	F	LL
Mitacek et al. (2018)	J	HunterLab MiniScan® XE Plus	2.50 cm	3	0, 1, 2, 3, 4, 5, 6	2.50 cm	5.50	2.0±1 ºC	F	LL
Seyfert et al. (2007)	J	HunterLab MiniScan® XE Plus	2.54 cm	3	0, 4, 7	2.54 cm	5.50, 5.60	0.2±3.1 ºC	F	LL, PM
Hutchison (2007)	D	HunterLab MiniScan® XE Plus	2.54 cm	3	0, 1, 2, 3, 4, 5, 6	2.54 cm	5.75	2.0±5.0 ºC	F	LL
Gonzalez et al. (2009)	J	HunterLab MiniScan® XE Plus	2.54 cm	2	0, 1, 2, 3, 4, 5	1.27 cm	NR	2.0±3.0 ºC	F	LL
Daniel et al. (2009)	J	HunterLab MiniScan® XE Plus	2.54 cm	3	0, 1, 2, 3, 4, 5, 6	2.54 cm	5.6	2.0 ºC	F	LL
Limsupavanich (2005)	D	HunterLab MiniScan® XE Spectrophotometer	3.18 cm	3	0, 1, 3, 5	NR	5.50, 5.80	0.0±3.0 ºC	F	LL, PM
Abraham et al. (2017)	J	HunterLab MiniScan® XE Spectrophotometer	2.50 cm	2	0, 1, 3, 5, 7	2.50 cm	5.61, 5.72	2.0±1.0 ºC	F	LL, PM
Nair et al. (2018)	J	HunterLab LabScan XE Colorimeter	2.54 cm	3	0, 3, 6	1.92 cm	NR	2.0 ºC	D	LL, PM
Joseph et al. (2012)	J	HunterLab LabScan XE Colorimeter	2.54 cm	4	0, 5, 9	2.54 cm	5.53, 5.66	2.0 ºC	F	LL, PM
Phelps et al. (2014)	J	HunterLab MiniScan™ EZ	2.54 cm	3	0, 2, 4, 5, 6, 7	2.54 cm	5.61	3.0±2.0 ºC	F	LL
Phelps et al. (2016)	J	HunterLab MiniScan™ EZ	2.54 cm	3	0, 1, 2, 3, 4, 5, 6, 7	2.54 cm	5.65	0.3 ±0.9 ºC	F	LL
Purohit et al. (2015)	J	HunterLab MiniScan™ EZ	2.54 cm	3	1, 5, 9	2.54 cm	5.82, 5.85	2.0±1.0 ºC	F	LL, PM
Ramanathan et al. (2011)	J	HunterLab MiniScan® XE Plus	2.54 cm	3	0, 5, 13	1.91 cm	5.60	1.0 ºC	D	LL
Ramanathan et al. (2018)	J	HunterLab MiniScan® XE Plus	2.50 cm	2	0, 1, 2, 3	2.50 cm	5.60	2.0±1.0 ºC	F	LL
Mancini et al. (2018)	J	HunterLab Miniscan XE Plus	2.54 cm	2-3	0, 1, 2, 3, 4, 5, 6,	2.54 cm	NR	4.0 ºC	F	LL, PM
King et al. (2011a)	J	HunterLab MiniScan® XE Plus	25 mm	2	0, 1, 3, 6, 9	2.54 cm	5.59	1.0 ºC	F	LL

Kim et al. (2016)	J	HunterLab MiniScan™ EZ	25 mm	3	1, 4, 7	2.50 cm	NR	2.5 ºC	F	LL
McKenna et al. (2005)	J	HunterLab MiniScan® XE Plus	31.8 mm	3	0, 1, 2, 3, 4, 5, 6,	2.54 cm	5.77, 5.73	2.2±2 ºC	F	LL, PM
Canto et al. (2016)	J	CM-600D Konica Minolta Sensing	8 mm	3	0, 3, 6, 9	2.54 cm	5.52	4.0 ºC	NR	LL, PM
Wu et al. (2020)	J	Model SP62 X-Rite, Inc	8 mm	4	0, 3, 5, 7	2.50 cm	5.53	2.0±1.0 ºC	LED	LL
Najar et al. (2020)	J	HunterLab MiniScan™ EZ	2.54 cm	3	0, 3, 6, 9, 12, 15	2.54 cm	5.56	0.0±4.0 ºC	F	LL
Sakomoto (2017)	D	HunterLab MiniScan® XE Plus	2.50 cm	3	0, 2, 3, 4, 6	2.00 cm	5.34-5.58	3.0±1.0 ºC	F	LL
Phelps et al. (2020)	J	HunterLab MiniScan™ EZ	2.54 cm	3	0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10	2.54 cm	NR	2.0±1.0 ºC	F	LL
Canto et al. (2015)	J	HunterLab MiniScan® XE Plus	2.54 cm	2	0, 11	2.54 cm	5.70	1.0 ºC	F	LL
King et al. (2011b)	J	HunterLab MiniScan® XE Plus	25 mm	2	0, 1, 2, 3, 4, 5, 6,	2.54 cm	5.58	1.0 ºC	F	LT
Kim et al. (2009)	J	HunterLab MiniScan® XE Plus	31.8 mm	3	0, 1, 2, 3, 4, 5, 6, 7	2.54 cm	5.71, 5.73	1.0 ºC	F	LL, PM

[1]Muscles used in this study: *longissimus lumborum* (LL); *psoas major* (PM).
[2]Source of data: peer-review journal (J); dissertation (D).
[3]Number of scans per steak within the study.
[4]The pH average of muscles: *longissimus lumborum* (LL); *longissimus thoracis* (LT); *psoas major* (PM).
[5]Temperature of simulated retail display.
[6]Type of lighting used during retail display: fluorescence (F); LED; natural (N); dark (D)

Table 4.2. Estimations[1] for a* redness using illuminant A for 5.9 and 3.5 discoloration for *longissimus lumborum* (LL) and *psoas major* (PM) steaks, respectively.

Measurement	Estimate	Lower Bound	Upper Bound
LL	22.15	20.24	24.07
PM	22.37	20.99	23.75

[1]These values were estimated using a 95% confidence interval.

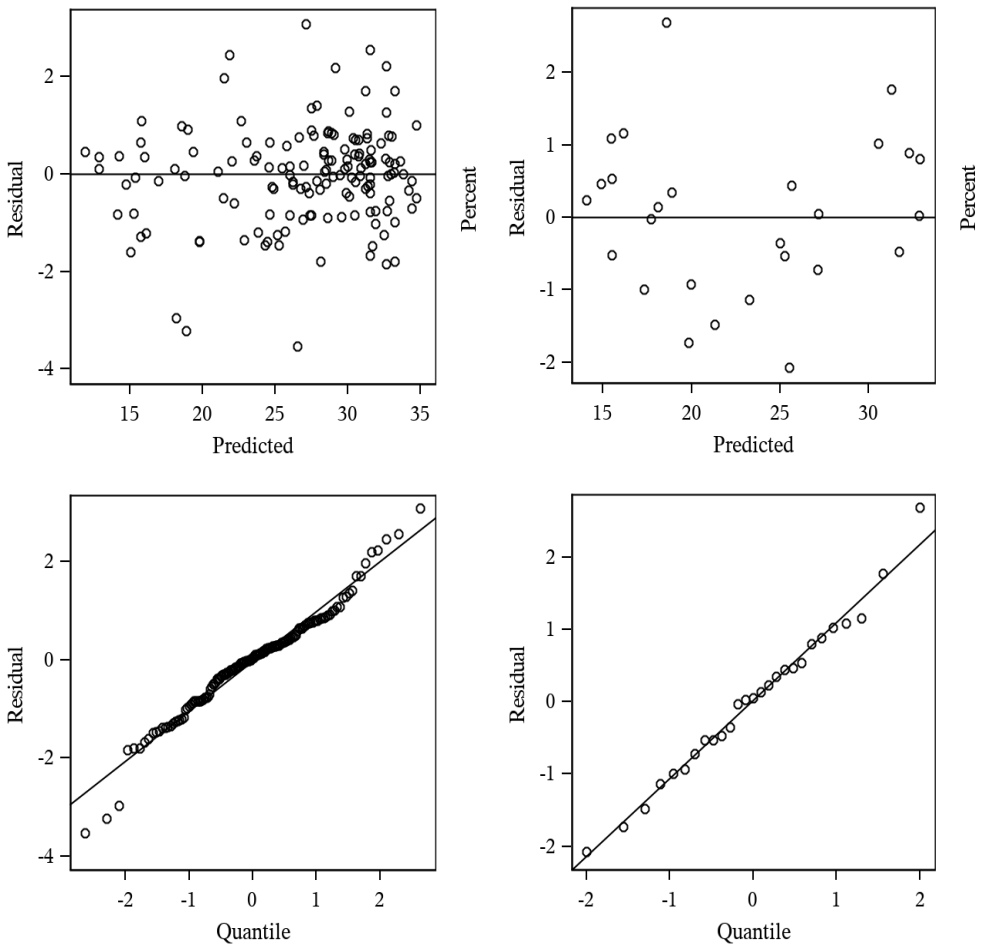

Figure 4.1 Plots of actual vs. predicted values relative to the line of distribution and studentized residual of a* redness for *longissimus lumborum* (a) and *psoas major* (b) in phase one.

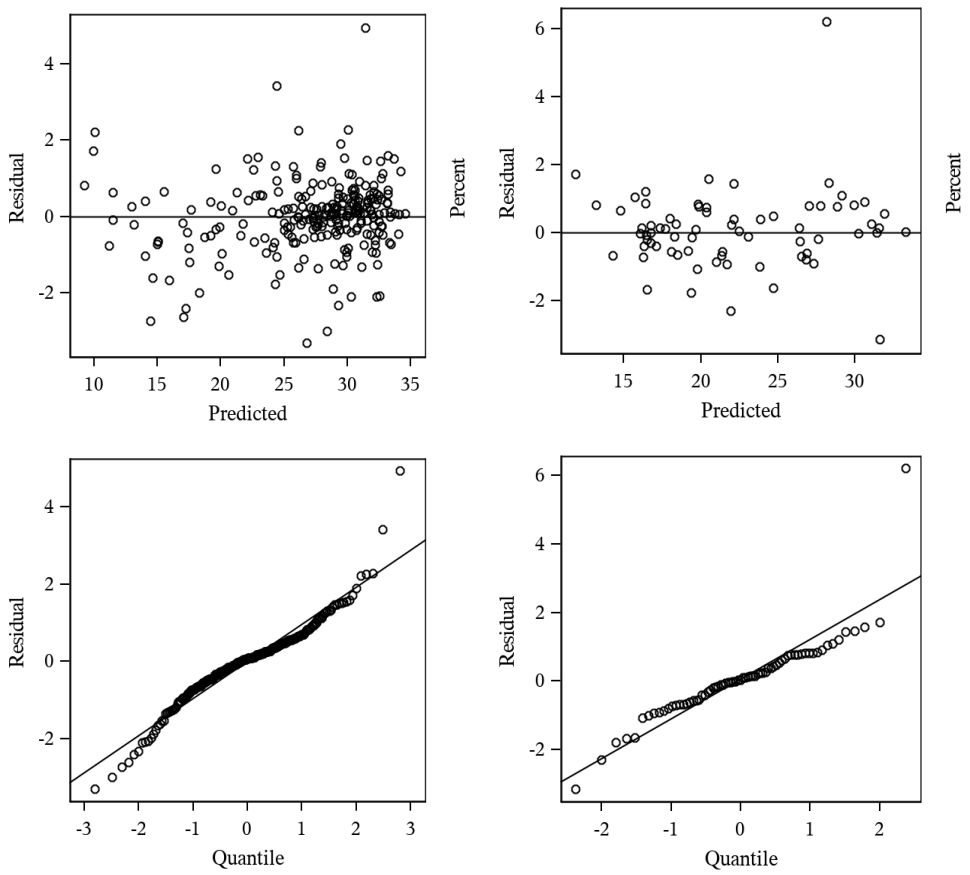

Figure 4.2 Plots of actual vs. predicted values relative to the line of distribution and studentized residual of a* redness for *longissimus lumborum* (a) and *psoas major* (b) in phase two.

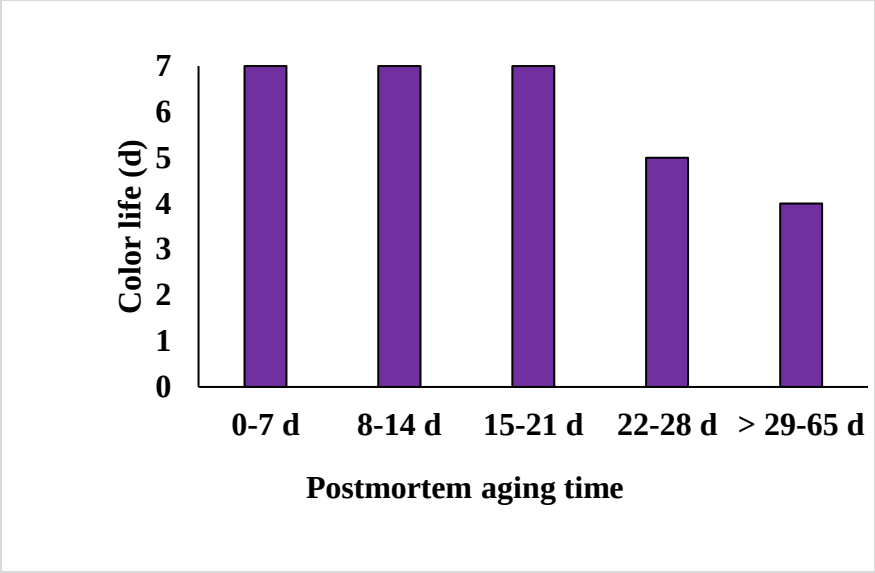

Figure 4.3 Estimates of color life of *longissimus lumborum* steaks during retail display using a* redness scores.

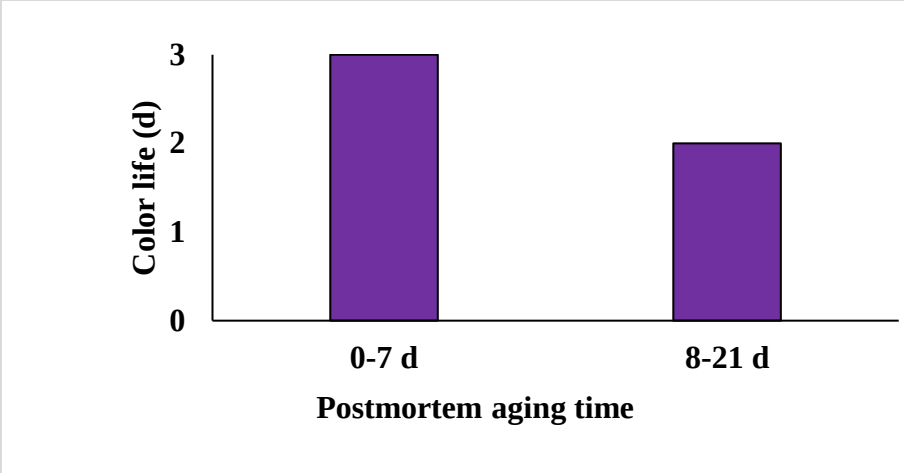

Figure 4.4 Estimates of color life of *psoas major* steaks during retail display using a* redness scores.

Appendix A - SAS Codes Chapter 2

The SAS codes below were produced by Dr. Qing Kang from the Department of Statistics at Kansas State University.

Surface BIA

```
%let path1=C:\Users\qkang\OneDrive - Kansas State University\Francisco
Najar_Villarreal\Impedence;
libname dat1 "&path1\data";
proc format;
value $ep '01'='APC'  '02'='PH'  '03'='L*'  '04'='a*'  '05'='b*'
'06'='Protein'
           '07'='Moisture' '08'='Fat' '09'='TBARS'
           '10'='Top'  '11'='Middle'  '12'='Bottom' '13'='Met' '14'='Deoxy'
'15'='Oxy';
value dd 1='Day 0' 2='Day 3' 3='Day 6' 4='Day 9' 5='Day 12' 6='Day 15';
run;
PROC IMPORT OUT= dat1.raw_data_surface
            DATAFILE= "&path1\data\Surface by location spreadsheet 12-06-
2019_stat.
csv"
            DBMS=CSV REPLACE;
     GETNAMES=YES;
     DATAROW=2;
RUN;

data  dat1.raw_data_surface;set dat1.raw_data_surface;
drop var28 var29 var30;
run;
proc sort data=dat1.raw_data_surface;by treatment loin steaks day;
proc transpose data=dat1.raw_data_surface(drop=number1 number2 TS1 TS2 MS1
MS2 BS1 BS2) out=dat1.data_long_analysis1;by treatment loin steaks day;
run;
data dat1.data_long_analysis1;format Endpoint $ep. storage_day dd.;set
dat1.data_long_analysis1;
label Storage_Day='Display Day' Day_of_Aging='Day of Aging' ;
if treatment=1 then Day_of_Aging='27 Days';
else if treatment=2 then Day_of_Aging='37 Days';
else if treatment=3 then Day_of_Aging='34 Days';
if _name_='APC' then Endpoint='01';
else if _name_='pH' then Endpoint='02';
else if _name_='L_' then Endpoint='03';
else if _name_='a_' then Endpoint='04';
else if _name_='b_' then Endpoint='05';
else if _name_='Protein' then Endpoint='06';
else if _name_='Moisture' then Endpoint='07';
else if _name_='Fat' then Endpoint='08';
else if _name_='TBARS' then Endpoint='09';
else if _name_='TS3' then Endpoint='10';
else if _name_='MS3' then Endpoint='11';
else if _name_='BS3' then Endpoint='12';
else if _name_='met' then Endpoint='13';
else if _name_='deoxy' then Endpoint='14';
```

```
else if _name_='oxy' then Endpoint='15';
Storage_Day=day;
rename col1=resp;
run;

data analysis;format location $ep.;
set dat1.data_long_analysis1;
Location=endpoint;
if resp=0 then resp=.;
logresp=log(resp);
if storage_day^=1 then i=1; else i=0;
where endpoint in ('10','11','12');
run;
options orientation=portrait nodate;
ods rtf file = "&path1\SASoutput\Analysis1_SASoutput.doc" ;
title 'Summary Statistics';
proc tabulate data=dat1.data_long_analysis1;
class endpoint Day_of_Aging loin Storage_Day;
var resp;
table endpoint*Storage_Day,Day_of_Aging*resp=' '*(n mean min max);
where endpoint in ('10','11','12');
run;
ods graphics on;
/*proc mixed data=analysis order=internal plots(only)=(STUDENTPANEL);
title "Data w/o transformation";
class Day_of_Aging loin Storage_Day Location ;
model resp=Day_of_Aging Storage_Day Location
           Day_of_Aging*Storage_Day Day_of_Aging*Location
Storage_Day*Location
           Day_of_Aging*Storage_Day*Location
            /ddfm=kr htype=3;
repeated Location Storage_Day/subject=loin*Day_of_Aging type=UN@cs ;
ods select studentpanel;
run;
*/

proc mixed data=analysis order=internal plots(only)=(STUDENTPANEL) covtest;
title "Log transformed Data";
class Day_of_Aging loin Storage_Day Location ;
model logresp=Day_of_Aging Storage_Day Location
           Day_of_Aging*Storage_Day Day_of_Aging*Location
Storage_Day*Location
           Day_of_Aging*Storage_Day*Location
            /ddfm=kr htype=3;
repeated Location Storage_Day/subject=loin*Day_of_Aging type=UN@cs ;
lsmeans Day_of_Aging*Storage_Day*Location;
ods exclude lsmeans;
ods output lsmeans=lsm covparms=covp tests3=t3;
run;

proc mixed data=analysis order=internal ;
class Day_of_Aging loin Storage_Day Location ;
model logresp=Day_of_Aging Storage_Day Location
           Day_of_Aging*Storage_Day Day_of_Aging*Location
Storage_Day*Location
           Day_of_Aging*Storage_Day*Location
            /ddfm=kr htype=3;
```

```
repeated Location Storage_Day/subject=loin*Day_of_Aging type=UN@cs ;
lsmeans Day_of_Aging*Storage_Day*Location;
slice Day_of_Aging*Storage_Day*Location/sliceby=location*storage_day pdiff
adjust=tukey adjdfe=row;
ods exclude all;
ods output slicediffs=sdiff;
run;
ods select all;
proc mixed data=analysis order=internal;
title "Partition Display Day into Day 0 vs Days 3-15";
class Day_of_Aging loin Storage_Day Location i;
model logresp=Day_of_Aging Storage_Day Location
           Day_of_Aging*Storage_Day Day_of_Aging*Location
Storage_Day*Location
           i*Day_of_Aging*Location Day_of_Aging*Storage_Day*Location
            /ddfm=kr htype=3;
repeated Location Storage_Day/subject=loin*Day_of_Aging type=UN@cs ;
ods select tests3;ods output tests3=t3_;
run;
title "Test the 3-way interactoin for Display Days 3-15";
proc print data=t3_ noobs;
where effect='Day_of*storag*locati';
run;
proc mixed data=analysis order=internal;
title "Partition Display Day into Day 0 vs Days 3-15";
class Day_of_Aging loin Storage_Day Location i;
model logresp=Day_of_Aging Storage_Day Location
           Day_of_Aging*Storage_Day Day_of_Aging*Location
Storage_Day*Location
           i*location
           Day_of_Aging*Storage_Day*Location
            /ddfm=kr htype=3;
repeated Location Storage_Day/subject=loin*Day_of_Aging type=UN@cs ;
ods select tests3;ods output tests3=t3__;
run;
title "Test the 2-way interaction btw Display Day and Location for Display
Days 3-15";
proc print data=t3__ noobs;
where effect='storage_day*location';
run;
ods rtf close;

options orientation=landscape nodate;
ods rtf file = "&path1\SASoutput\Analysis1_report_tables_figures.rtf"
style=monochromeprinter;
proc sort data=t3;by effect;
data pvalue;format lable $40.;
merge t3 t3_(where=(effect='Day_of*storag*locati') rename=(probf=p_))
         t3__(where=(effect='storage_day*location') rename=(probf=p_));
            by effect;
if effect='Day_of_Aging' then do;lable='Day of Aging';order=1;end;
if effect='storage_day' then do;lable='Display Day';order=2;end;
if effect='location' then do;lable='Location';order=3;end;
if effect='Day_of_Ag*storage_da' then do;lable='Day of AgingXDisplay
Day';order=4;end;
if effect='Day_of_Agin*location' then do;lable='Day of
AgingXLocation';order=5;end;
```

```
if effect='storage_day*location' then do;lable='Display
DayXLocation';order=6;end;
if effect='Day_of*storag*locati' then do;lable='Day of AgingXDisplay
DayXLocation';order=7;end;
run;
proc sort data=pvalue;by order;
title 'P-value for Fixed Effects';
proc report data=pvalue nowd split='^';
column lable probf p_;
define lable/center 'Fixed Effect' order=data group;
define probf/center 'Display Day:^0,3,6,9,12,15' f=pvalue6.3
style={cellwidth=1.2in};
define p_/center 'Display Day:^3,6,9,12,15' f=pvalue6.3
style={cellwidth=1.2in};
run;
data sdiff;format location $ep. storage_day dd. ratio $18.;set sdiff;
if index(slice,'Top') then location='10';
if index(slice,'Middle') then location='11';
if index(slice,'Bottom') then location='12';
if index(slice,'0') then storage_day=1;
if index(slice,'3') then storage_day=2;
if index(slice,'6') then storage_day=3;
if index(slice,'9') then storage_day=4;
if index(slice,'12') then storage_day=5;
if index(slice,'15') then storage_day=6;
ratio=put(exp(estimate),5.2)||' ('||put(adjp,pvalue6.3)||')';
run;
proc sort data=sdiff;by location storage_day day_of_aging _day_of_aging;
proc transpose data=sdiff out=sdiff_ prefix=ratio_;
by location storage_day day_of_aging;
var ratio;
id _day_of_aging;
run;
proc sort data=lsm;by location storage_day day_of_aging;
data lsm;merge lsm sdiff_;by location storage_day day_of_aging;
lsm=exp(estimate);se=lsm*stderr;
run;
title 'Fixed effect estimates';
proc report nowd spanrows data=lsm;
column location storage_day day_of_aging lsm se ('Ratio (Adj. P-value)to'
ratio_34_days ratio_37_days);
define location/center 'Location' group order=data;
define storage_day/center 'Display Day' group order=data;
define day_of_aging/center 'Day of Aging' order=data;
define lsm/center 'Median' f=8.1;
define se/center 'S.E.' f=8.1;
define ratio_34_days/center '34 Days of Aging';
define ratio_37_days/center '37 Days of Aging';
break after location/page;
run;
data plot;format Location $ep. lsm 8.0;set lsm;
upper=lsm+lsm*stderr;lower=lsm-lsm*stderr;
run;
title 'Figs. 2,3,4';
ods graphics/noborder height=7 in width=5 in outputfmt=png;
proc sgpanel data=plot nocycleattrs;
styleattrs datacontrastcolors=(black purple red);
```

```
panelby location/rows=3 novarname layout=panel headerbackcolor=white;
scatter x=storage_day y=lsm/group=day_of_aging yerrorupper=upper
                            yerrorlower=lower groupdisplay=cluster
clusterwidth=0.2
                            markerattrs=(symbol=plus) noerrorcaps;
series x=storage_day y=lsm/group=day_of_aging groupdisplay=cluster
clusterwidth=0.2 name='a' lineattrs=(pattern=1);
rowaxis label='Impedence(ohms) Median +/- S.E.' values=(50 to 150 by 20)
minor minorcount=1;
colaxis label='Display Day';
keylegend 'a'/noborder title='Day of Aging';
run;
data covp;set covp;
label cov='Coefficient of Variation' se='S.E.' location='Location';
cov=sqrt(exp(estimate)-1);
se=exp(estimate)/sqrt(exp(estimate)-1)/2*stderr;
if index(covparm,'UN(1,1') then do;
          location='Top   ';output;end;
if index(covparm,'UN(2,2') then do;
          location='Middle';output;end;
if index(covparm,'UN(3,3') then do;
          location='Bottom';output;end;
run;
title 'Random effect estimates';
proc report data=covp nowd;
column location cov se;
format cov percent8.1 se percent8.1;
run;
ods rtf close;
```

Internal BIA

```
%let path1=C:\Users\qkang\OneDrive - Kansas State University\Francisco
Najar_Villarreal\impedance\Internal;
libname dat1 "&path1\data";
proc format;
value $ep '01'='APC'  '02'='PH'  '03'='L*'  '04'='a*'  '05'='b*'
'06'='Protein'
          '07'='Moisture' '08'='Fat' '09'='TBARS'
          '10'='Top'  '11'='Middle'  '12'='Bottom' '13'='Met' '14'='Deoxy'
'15'='Oxy';
value dd 1='Day 0' 2='Day 3' 3='Day 6' 4='Day 9' 5='Day 12' 6='Day 15';
run;
PROC IMPORT OUT= dat1.raw_data_internal
            DATAFILE= "&path1\data\Spreadsheet for Needle Internal
Impedance_stat.csv"
            DBMS=CSV REPLACE;
     GETNAMES=YES;
     DATAROW=2;
RUN;

data  dat1.raw_data_internal;set dat1.raw_data_internal;
drop var28 var29 var30 var31;
run;
proc sort data=dat1.raw_data_internal;by treatment loin steaks day;
proc transpose data=dat1.raw_data_internal(drop=number1 number2 TI1 TI2 MI1
MI2 BI1 BI2)
 out=dat1.data_long_analysis1;by treatment loin steaks day;
run;
data dat1.data_long_analysis1;format Endpoint $ep. storage_day dd.;set
dat1.data_long_analysis1;
label Storage_Day='Display Day' Day_of_Aging='Day of Aging' ;
if treatment=1 then Day_of_Aging='27 Days';
else if treatment=2 then Day_of_Aging='37 Days';
else if treatment=3 then Day_of_Aging='34 Days';
if _name_='APC' then Endpoint='01';
else if _name_='pH' then Endpoint='02';
else if _name_='L_' then Endpoint='03';
else if _name_='a_' then Endpoint='04';
else if _name_='b_' then Endpoint='05';
else if _name_='Protein' then Endpoint='06';
else if _name_='Moisture' then Endpoint='07';
else if _name_='Fat' then Endpoint='08';
else if _name_='TBARS' then Endpoint='09';
else if _name_='TI3' then Endpoint='10';
else if _name_='MI3' then Endpoint='11';
else if _name_='BI3' then Endpoint='12';
else if _name_='met' then Endpoint='13';
else if _name_='deoxy' then Endpoint='14';
else if _name_='oxy' then Endpoint='15';
Storage_Day=day;
rename col1=resp;
run;

data analysis;format location $ep.;
set dat1.data_long_analysis1;
```

```
Location=endpoint;
*if resp=0 then resp=.;
logresp=log(resp);
if Storage_day^=1 then i=1; else i=0;
where endpoint in ('10','11','12');
run;
options orientation=portrait nodate;
ods rtf file = "&path1\SASoutput\Analysis1_SASoutput_Internal_Impedance.doc"
;
title 'Summary Statistics';
proc tabulate data=dat1.data_long_analysis1;
class endpoint Day_of_Aging loin Storage_Day;
var resp;
table endpoint*Storage_Day,Day_of_Aging*resp=' '*(n mean min max);
where endpoint in ('10','11','12');
run;
ods graphics on;
proc mixed data=analysis order=internal plots(only)=(STUDENTPANEL) covtest;
title "Log transformed Data";
class Day_of_Aging loin Storage_Day Location ;
model logresp=Day_of_Aging Storage_Day Location
           Day_of_Aging*Storage_Day Day_of_Aging*Location
Storage_Day*Location
           Day_of_Aging*Storage_Day*Location
            /ddfm=kr htype=3;
repeated Location Storage_Day/subject=loin*Day_of_Aging type=UN@cs ;
lsmeans Day_of_Aging*Storage_Day*Location;
ods exclude lsmeans;
ods output lsmeans=lsm covparms=covp tests3=t3;
run;

proc mixed data=analysis order=internal ;
class Day_of_Aging loin Storage_Day Location ;
model logresp=Day_of_Aging Storage_Day Location
           Day_of_Aging*Storage_Day Day_of_Aging*Location
Storage_Day*Location
           Day_of_Aging*Storage_Day*Location
            /ddfm=kr htype=3;
repeated Location Storage_Day/subject=loin*Day_of_Aging type=UN@cs ;
lsmeans Day_of_Aging*Storage_Day*Location;
slice Day_of_Aging*Storage_Day*Location/sliceby=location*storage_day pdiff
adjust=tukey adjdfe=row;
ods exclude all;
ods output slicediffs=sdiff;
run;
ods select all;
ods rtf close;

options orientation=landscape nodate;
ods rtf file =
"&path1\SASoutput\Analysis1_report_tables_figures_Internal_Impedance.rtf"
style=monochromeprinter;
proc sort data=t3;by effect;
data pvalue;format lable $40.;
merge t3;
if effect='Day_of_Aging' then do;lable='Day of Aging';order=1;end;
if effect='storage_day' then do;lable='Display Day';order=2;end;
```

```
if effect='location' then do;lable='Location';order=3;end;
if effect='Day_of_Ag*storage_da' then do;lable='Day of AgingXDisplay
Day';order=4;end;
if effect='Day_of_Agin*location' then do;lable='Day of
AgingXLocation';order=5;end;
if effect='storage_day*location' then do;lable='Display
DayXLocation';order=6;end;
if effect='Day_of*storag*locati' then do;lable='Day of AgingXDisplay
DayXLocation';order=7;end;
run;
proc sort data=pvalue;by order;
title 'P-value for Fixed Effects';
proc report data=pvalue nowd split='^';
column lable probf ;
define lable/center 'Fixed Effect' order=data group;
define probf/center 'Display Day:^0,3,6,9,12,15' f=pvalue6.3
style={cellwidth=1.2in};
run;
data sdiff;format location $ep. storage_day dd. ratio $18.;set sdiff;
if index(slice,'Top') then location='10';
if index(slice,'Middle') then location='11';
if index(slice,'Bottom') then location='12';
if index(slice,'0') then storage_day=1;
if index(slice,'3') then storage_day=2;
if index(slice,'6') then storage_day=3;
if index(slice,'9') then storage_day=4;
if index(slice,'12') then storage_day=5;
if index(slice,'15') then storage_day=6;
ratio=put(exp(estimate),5.2)||' ('||put(adjp,pvalue6.3)||')';
run;
proc sort data=sdiff;by location storage_day day_of_aging _day_of_aging;
proc transpose data=sdiff out=sdiff_ prefix=ratio_;
by location storage_day day_of_aging;
var ratio;
id _day_of_aging;
run;
proc sort data=lsm;by location storage_day day_of_aging;
data lsm;merge lsm sdiff_;by location storage_day day_of_aging;
lsm=exp(estimate);se=lsm*stderr;
run;
title 'Fixed effect estimates';
proc report nowd spanrows data=lsm;
column location storage_day day_of_aging lsm se ('Ratio (Adj. P-value)to'
ratio_34_days ratio_37_days);
define location/center 'Location' group order=data;
define storage_day/center 'Display Day' group order=data;
define day_of_aging/center 'Day of Aging' order=data;
define lsm/center 'Median' f=8.1;
define se/center 'S.E.' f=8.1;
define ratio_34_days/center '34 Days of Aging';
define ratio_37_days/center '37 Days of Aging';
break after location/page;
run;
data plot;format Location $ep. lsm 8.0;set lsm;
upper=lsm+lsm*stderr;lower=lsm-lsm*stderr;
run;
title 'Figs. 2,3,4 ';
```

```
ods graphics/noborder height=7 in width=5 in outputfmt=png;
proc sgpanel data=plot nocycleattrs;
styleattrs datacontrastcolors=(black purple red);
panelby location/rows=3 novarname layout=panel headerbackcolor=white;
scatter x=storage_day y=lsm/group=day_of_aging yerrorupper=upper
                            yerrorlower=lower groupdisplay=cluster
clusterwidth=0.2
                            markerattrs=(symbol=plus) noerrorcaps;
series x=storage_day y=lsm/group=day_of_aging groupdisplay=cluster
clusterwidth=0.2 name='a' lineattrs=(pattern=1);
rowaxis label='Impedance(ohms) Median +/- S.E.' values=(50 to 150 by 20)
minor minorcount=1;
colaxis label='Display Day';
keylegend 'a'/noborder title='Day of Aging';
run;
data covp;set covp;
label cov='Coefficient of Variation' se='S.E.' location='Location';
cov=sqrt(exp(estimate)-1);
se=exp(estimate)/sqrt(exp(estimate)-1)/2*stderr;
if index(covparm,'UN(1,1') then do;
          location='Top   ';output;end;
if index(covparm,'UN(2,2') then do;
          location='Middle';output;end;
if index(covparm,'UN(3,3') then do;
          location='Bottom';output;end;
run;
title 'Random effect estimates';
proc report data=covp nowd;
column location cov se;
format cov percent8.1 se percent8.1;
run;
ods rtf close;
```

Quality attributes

```
%let path1=C:\Users\qkang\OneDrive - Kansas State University\Francisco
Najar_Villarreal\Impedance\Surface;
libname dat1 "&path1\data";
proc format;
value $ep '01'='APC'  '02'='PH'  '03'='L*'  '04'='a*'  '05'='b*'
'05_1'='Chroma'  '05_2'='Hue Angle'
          '06'='Protein'
           '07'='Moisture' '08'='Fat' '09'='TBARS'
           '13'='Metmyoglobin' '14'='Deoxymyoglobin'
           '15'='Oxymyoglobin' '16'='Impedance';
value dd 1='Day 0' 2='Day 3' 3='Day 6' 4='Day 9' 5='Day 12' 6='Day 15';
run;
PROC IMPORT OUT= dat1.raw_data_surface_v2
            DATAFILE= "&path1\data\Surface by location spreadsheet 12-06-
2019_stat hue and chroma.csv"
            DBMS=CSV REPLACE;
     GETNAMES=YES;
     DATAROW=2;
RUN;

proc sort data=dat1.raw_data_surface_v2;by treatment loin steaks day;run;

data raw_data;set dat1.raw_data_surface_v2;
Impedance=(TS3+MS3+BS3)/3;
run;
proc sort data=raw_data;by treatment loin steaks day;
proc transpose data=raw_data(drop=number1 number2 TS1-TS3 MS1-MS3 BS1-BS3)
out=dat1.data_long_Analysis2_v2;
by treatment loin steaks day;
run;
data dat1.data_long_Analysis2_v2;format Endpoint $ep. storage_day dd.;set
dat1.data_long_Analysis2_v2;
label Storage_Day='Display Day' Day_of_Aging='Day of Aging' ;
if treatment=1 then Day_of_Aging='27 Days';
else if treatment=2 then Day_of_Aging='37 Days';
else if treatment=3 then Day_of_Aging='34 Days';
if _name_='APC' then Endpoint='01';
else if _name_='pH' then Endpoint='02';
else if _name_='L_' then Endpoint='03';
else if _name_='a_' then Endpoint='04';
else if _name_='b_' then Endpoint='05';
else if _name_='Chroma' then Endpoint='05_1';
else if _name_='Hue_angle' then Endpoint='05_2';
else if _name_='Protein' then Endpoint='06';
else if _name_='Moisture' then Endpoint='07';
else if _name_='Fat' then Endpoint='08';
else if _name_='TBARS' then Endpoint='09';
else if _name_='Impedance' then Endpoint='16';
else if _name_='met' then Endpoint='13';
else if _name_='deoxy' then Endpoint='14';
else if _name_='oxy' then Endpoint='15';
Storage_Day=day;
rename col1=resp;
run;
```

```
options orientation=portrait nodate;
ods rtf file = "&path1\SASoutput\Analysis2_SASoutput_v2.doc";
title 'Summary Statistics';
proc tabulate data=dat1.data_long_Analysis2_v2;
class endpoint Day_of_Aging loin Storage_Day;
var resp;
table endpoint*Storage_Day,Day_of_Aging*resp=' '*(n mean min max);
where endpoint in ('05_1','05_2');
run;
proc sort data=dat1.data_long_Analysis2_v2;by endpoint day_of_aging
storage_day;run;
ods graphics off;
title ' ';
%macro by_endpoint_analysis();
proc datasets library=work;
   delete fitstatistics lsmeans_main lsmeans_int estimates tests3
difference_main difference_int;
run;
%do i=1 %to 2;
data set1;set dat1.data_long_Analysis2_v2;
if &i=1 then name='05_1';
else if &i=2 then name='05_2';
if endpoint^=name then delete;
run;
proc mixed data=set1 ;by endpoint;
class Day_of_Aging loin Storage_day;
model resp=Day_of_Aging Storage_day Day_of_Aging*Storage_Day/ddfm=kr;
repeated/subject=loin*Day_of_Aging type=UN;
ods exclude all;
ods output fitstatistics=fs1;
run;
proc mixed data=set1 order=data;by endpoint;
class Day_of_Aging loin Storage_day;
model resp=Day_of_Aging Storage_day Day_of_Aging*Storage_Day/ddfm=kr;
repeated/subject=loin*Day_of_Aging type=AR(1);
ods exclude all;
ods output fitstatistics=fs2;
run;
proc mixed data=set1 order=data;by endpoint;
class Day_of_Aging loin Storage_day;
model resp=Day_of_Aging Storage_day Day_of_Aging*Storage_Day/ddfm=kr;
repeated/subject=loin*Day_of_Aging type=cs;
ods exclude all;
ods output fitstatistics=fs3;
run;
data fs;merge fs1(rename=(value=value1 endpoint=endpoint1) where=(descr="AIC
(Smaller is Better)"))
              fs2(rename=(value=value2) where=(descr="AIC (Smaller is
Better)"))
              fs3(rename=(value=value3) where=(descr="AIC (Smaller is
Better)"));
if min(value1,value2,value3)=value1 then type='UN   ';
else if min(value1,value2,value3)=value2 then type='AR(1)';
else if min(value1,value2,value3)=value3 then type='CS   ';
run;

data _null_;set fs;
```

```
call symputx('type', type);
run;
%put type;
ods select all;
proc mixed data=set1 order=data;by endpoint;
class Day_of_Aging loin Storage_day;
model resp=Day_of_Aging Storage_day Day_of_Aging*Storage_Day/ddfm=kr;
lsmeans Day_of_Aging*Storage_Day;
slice Day_of_Aging*Storage_Day/sliceby=Storage_Day pdiff adjust=tukey
adjdfe=row;
repeated/subject=loin*Day_of_Aging type=&type;
ods exclude slicediffs slicetests;
ods output tests3=t3 lsmeans=lsm_int slicediffs=diff_int;
run;
proc mixed data=set1 order=data;by endpoint;
class Day_of_Aging loin Storage_day;
model resp=Day_of_Aging Storage_day Day_of_Aging*Storage_Day/ddfm=kr;
lsmeans Day_of_Aging Storage_Day/pdiff adjust=tukey adjdfe=row;
repeated/subject=loin*Day_of_Aging type=&type;
ods select lsmeans;
ods output lsmeans=lsm_main diffs=diff_main;
run;
proc append data=t3 base=tests3;
run;
proc append data=lsm_int base=lsmeans_int;
run;
proc append data=lsm_main base=lsmeans_main;
run;
proc append data=diff_main base=difference_main;
run;
proc append data=diff_int base=difference_int;
run;
proc append data=fs base=fitstatistics;
run;
%end;
%mend;
%by_endpoint_analysis();
proc sort data=tests3;by endpoint effect;
proc transpose data=tests3 out=table1 prefix=p_;by endpoint;
var probf;
id effect;
run;
options orientation=portrait nodate;
ods rtf file = "&path1\SASoutput\Analysis2_report_tables_and_figures_v2.rtf"
style=monochromeprinter;
ods select all;
title 'P-value for Test of Fixed Effect';
proc report data=table1 nowd spanrows split="&";
column endpoint p_day_of_aging p_storage_day p_day_of_ag_Storage_Da;
define endpoint/"Endpoint" center;
define p_day_of_aging/"Day of Aging" f=pvalue.
style(column)=[cellwidth=1.5in] center;
define p_storage_day/"Storage Day" f=pvalue. style(column)=[cellwidth=1.5in]
center;
define p_day_of_ag_Storage_Da/"Day of Aging X Storage Day" f=pvalue.
style(column)=[cellwidth=2in] center;
where endpoint^='16';
```

```
run;

data difference_main;set difference_main;
diff=put(estimate,6.3)||' ('||put(adjp,pvalue6.3)||')';
run;
proc sort data=difference_main;by effect endpoint day_of_aging _day_of_aging;
proc transpose data=difference_main out=set1 prefix=diff_;
by effect endpoint day_of_aging ;
var diff;
id _day_of_aging;
where effect='Day_of_Aging';
run;
proc sort data=difference_main;by effect endpoint storage_day _storage_day;
proc transpose data=difference_main out=set2 prefix=diff_;
by effect endpoint storage_day ;
var diff;
id _storage_day;
where effect='storage_day';
run;
proc sort data=lsmeans_main;by effect endpoint day_of_aging;
data set1;merge lsmeans_main(where=(effect='Day_of_Aging')) set1;by endpoint
day_of_aging;
rename stderr=se;
run;
data set2;merge lsmeans_main(where=(effect='storage_day')) set2;by endpoint
storage_day;
rename stderr=se;
run;
title 'Fixed effect estimates: Day of Aging Main Effect';
proc report nowd spanrows data=set1;
column endpoint day_of_aging estimate se ('Diff. (Adj. P-value) to'
diff_34_days diff_37_days);
define endpoint/center 'Endpoint' group order=data;
define day_of_aging/center 'Day of Aging';
define estimate/center 'LS Mean' f=8.3;
define se/center 'S.E.' f=8.3;
define diff_34_days/center '34 Days of Aging';
define diff_37_days/center '37 Days of Aging';
run;
title 'Fixed effect estimates: Display Day Main Effect';
proc report nowd spanrows data=set2;
column endpoint storage_day estimate se ('Diff. (Adj. P-value) to' diff_day_3
diff_day_6 diff_day_9 diff_day_12 diff_day_15);
define endpoint/center 'Endpoint' group order=data;
define storage_day/center 'Display Day';
define estimate/center 'LS Mean' f=8.3;
define se/center 'S.E.' f=8.3;
define diff_day_3/center 'Display Day 3';
define diff_day_6/center 'Display Day 6';
define diff_day_9/center 'Display Day 9';
define diff_day_12/center 'Display Day 12';
define diff_day_15/center 'Display Day 15';
run;
ods rtf close;
```

Correlations Surface BIA

```
%let path1=C:\Users\qkang\OneDrive - Kansas State University\Francisco
Najar_Villarreal\Impedance\Surface;
libname dat1 "&path1\data";
proc format;
value $ep '01'='APC'  '02'='PH'  '03'='L*'  '04'='a*'  '05'='b*'
          '05_1'='Chroma'  '05_2'='Hue Angle'  '06'='Protein'
           '07'='Moisture' '08'='Fat' '09'='TBARS'
           '13'='Metmyoglobin' '14'='Deoxymyoglobin'
           '15'='Oxymyoglobin' '16'='Impedance';
value dd 1='Day 0' 2='Day 3' 3='Day 6' 4='Day 9' 5='Day 12' 6='Day 15';
run;
ods graphics off;
ods exclude all;
title ' ';
options orientation=portrait nodate;
ods rtf file="&path1\SASoutput\Analysis3_SASoutput_v2.rtf"
style=monochromeprinter;

%macro covp1();
ods exclude all;
proc datasets library=work;
   delete covparms1 covparms2;
run;
ods select all;
%do j=1 %to 2;
data analysis;set dat1.data_long_Analysis2_v2;
if endpoint='16' then do;resp=log(resp); output;end;
if &j=1 and endpoint='05_1' then output;
else if &j=2 and endpoint='05_2' then output;
run;
proc sort data=analysis;by day_of_aging;
proc mixed data=analysis order=internal covtest;by day_of_aging;
class Day_of_Aging Storage_Day Endpoint loin ;
model resp=Endpoint/ddfm=kr s noint;
random endpoint/subject=loin type=unr;
repeated endpoint/subject=loin*storage_day type=unr ;
ods select COVParms solutionf;
ods output covparms=covp1;
run;

proc mixed data=analysis order=internal covtest;
class Day_of_Aging Storage_Day Endpoint loin ;
model resp=Endpoint Day_of_Aging  Day_of_Aging*endpoint/ddfm=kr s noint;
random endpoint/subject=loin*day_of_aging type=unr;
repeated endpoint/subject=loin*day_of_aging*storage_day type=unr ;
ods select COVParms solutionf;
ods output covparms=covp2;
run;
data covp1;format endpoint $ep.;set covp1;
if &j=1 then endpoint='05_1';
else if &j=2 then endpoint='05_2';
run;
data covp2;format endpoint $ep.;set covp2;
if &j=1 then endpoint='05_1';
```

```
else if &j=2 then endpoint='05_2';
run;
proc append data=covp1 base=covparms1 force;
run;
proc append data=covp2 base=covparms2 force;
run;
%end;
%mend;
%covp1();


ods rtf close;
data table5;
merge covparms1(where=(Covparm='Corr(2,1)' and Subject='storage_day*Loin' and
Day_of_Aging="27 Days")
                rename=(estimate=rho27 stderr=se27))
      covparms1(where=(Covparm='Corr(2,1)' and Subject='storage_day*Loin' and
Day_of_Aging="34 Days")
                rename=(estimate=rho34 stderr=se34))
      covparms1(where=(Covparm='Corr(2,1)' and Subject='storage_day*Loin' and
Day_of_Aging="37 Days")
                rename=(estimate=rho37 stderr=se37))
      covparms2(where=(Covparm='Corr(2,1)' and
Subject='Day_of_*storage*Loin')
                rename=(estimate=rho stderr=se));
run;
data table5;set table5;
r27=put(rho27,percentn6.0)||' ('||put(se27,percent5.0)||')';
r34=put(rho34,percentn6.0)||' ('||put(se34,percent5.0)||')';
r37=put(rho37,percentn6.0)||' ('||put(se37,percent5.0)||')';
r=put(rho,percentn6.0)||'('||put(se,percent5.0)||')';
run;
title ' ';
ods rtf file="&path1\SASoutput\Analysis3_report_table_v2.rtf"
style=monochromeprinter;
proc report data=table5 nowd spanrows split='$' ;
column endpoint ('Correlation (S.E.) with Surface Impedance(Average of Top,
Middle, Bottom)$
                 w.r.t Display Day Effect' r27 r34 r37 r);
define endpoint/'Endpoint' center;
define r27/'27 Days of Aging' style(column)=[cellwidth=1.4in] center;
define r34/'34 Days of Aging' style(column)=[cellwidth=1.4in] center;
define r37/'37 Days of Aging' style(column)=[cellwidth=1.4in] center;
define r/'Across Days of Aging' style(column)=[cellwidth=1.8in] center;
run;
ods rtf close;
```

Correlations Internal BIA

```
%let path1=C:\Users\qkang\OneDrive - Kansas State University\Francisco
Najar_Villarreal\impedance\Internal;;
libname dat1 "&path1\data";
proc format;
value $ep '01'='APC'  '02'='PH'  '03'='L*'  '04'='a*'  '05'='b*'
'06'='Protein'
          '07'='Moisture' '08'='Fat' '09'='TBARS'
          '13'='Metmyoglobin' '14'='Deoxymyoglobin'
          '15'='Oxymyoglobin' '16'='Impedance';
value dd 1='Day 0' 2='Day 3' 3='Day 6' 4='Day 9' 5='Day 12' 6='Day 15';
run;
ods graphics off;
ods exclude all;
title ' ';
options orientation=portrait nodate;
ods rtf file="&path1\SASoutput\Analysis3_SASoutput_Internal_Impedance.rtf"
style=monochromeprinter;

%macro covp1();
ods exclude all;
proc datasets library=work;
   delete covparms1 covparms2;
run;
ods select all;
%do j=1 %to 12;
data analysis;set dat1.data_long_Analysis2;
if endpoint='16' then do;resp=log(resp); output;end;
if &j<10 and endpoint=compress('0'||&j) then output;
else if &j>=10 and endpoint=compress(' '||(&j+3)) then output;
run;
proc sort data=analysis;by day_of_aging;
proc mixed data=analysis order=internal covtest;by day_of_aging;
class Day_of_Aging Storage_Day Endpoint loin ;
model resp=Endpoint/ddfm=kr s noint;
random endpoint/subject=loin type=unr;
repeated endpoint/subject=loin*storage_day type=unr ;
ods select COVParms solutionf;
ods output covparms=covp1;
run;

proc mixed data=analysis order=internal covtest;
class Day_of_Aging Storage_Day Endpoint loin ;
model resp=Endpoint Day_of_Aging  Day_of_Aging*endpoint/ddfm=kr s noint;
random endpoint/subject=loin*day_of_aging type=unr;
repeated endpoint/subject=loin*day_of_aging*storage_day type=unr ;
ods select COVParms solutionf;
ods output covparms=covp2;
run;
data covp1;format endpoint $ep.;set covp1;
if &j<10 then endpoint=compress('0'||&j);
else if &j>=10 then endpoint=compress(''||&j+3);
run;
data covp2;format endpoint $ep.;set covp2;
if &j<10 then endpoint=compress('0'||&j);
```

```
else if &j>=10 then endpoint=compress(' '||&j+3);
run;
proc append data=covp1 base=covparms1 force;
run;
proc append data=covp2 base=covparms2 force;
run;
%end;
%mend;
%covp1();
ods rtf close;
data table5;
merge covparms1(where=(Covparm='Corr(2,1)' and Subject='storage_day*Loin' and
Day_of_Aging="27 Days")
                rename=(estimate=rho27 stderr=se27))
      covparms1(where=(Covparm='Corr(2,1)' and Subject='storage_day*Loin' and
Day_of_Aging="34 Days")
                rename=(estimate=rho34 stderr=se34))
      covparms1(where=(Covparm='Corr(2,1)' and Subject='storage_day*Loin' and
Day_of_Aging="37 Days")
                rename=(estimate=rho37 stderr=se37))
      covparms2(where=(Covparm='Corr(2,1)' and
Subject='Day_of_*storage*Loin')
                rename=(estimate=rho stderr=se));
run;
data table5;set table5;
r27=put(rho27,percentn6.0)||' ('||put(se27,percent5.0)||')';
r34=put(rho34,percentn6.0)||' ('||put(se34,percent5.0)||')';
r37=put(rho37,percentn6.0)||' ('||put(se37,percent5.0)||')';
r=put(rho,percentn6.0)||'('||put(se,percent5.0)||')';
run;
title ' ';
ods rtf file="&path1\SASoutput\Analysis3_report_table_Internal_Impedance.rtf"
style=monochromeprinter;
proc report data=table5 nowd spanrows split='$' ;
column endpoint ('Correlation (S.E.) with Internal Impedance(Average of Top,
Middle, Bottom)$
                 w.r.t Display Day Effect' r27 r34 r37 r);
define endpoint/'Endpoint' center;
define r27/'27 Days of Aging' style(column)=[cellwidth=1.4in] center;
define r34/'34 Days of Aging' style(column)=[cellwidth=1.4in] center;
define r37/'37 Days of Aging' style(column)=[cellwidth=1.4in] center;
define r/'Across Days of Aging' style(column)=[cellwidth=1.8in] center;
run;
ods rtf close;
```

Appendix B - SAS Codes Chapter 3

Surface BIA

pH and Water Holding Capacity

```
proc glimmix plots=residualpanel;
class trt day loin;
model EM= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;

proc glimmix plots=residualpanel;
class trt day loin;
model pH= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;
```

Impedance

```
proc glimmix plots=residualpanel;
class trt day loin;
model x6= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;
```

Instrumental Color

```
proc glimmix plots=residualpanel;
class trt day loin;
model l= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;

proc glimmix plots=residualpanel;
class trt day loin;
model a= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;

proc glimmix plots=residualpanel;
class trt day loin;
model b= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;
```

Oxygen Consumption and Metmyoglobin Reducing Activity

```
proc glimmix plots=residualpanel;
class trt day loin;
model oc= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;

proc glimmix plots=residualpanel;
class trt day loin;
model mra= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;
```

Protein Degradation

```
proc glimmix plots=residualpanel;
class trt day loin;
model desminintact= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;

proc glimmix plots=residualpanel;
class trt day loin;
model desmindegraded= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;

proc glimmix plots=residualpanel;
class trt day loin;
model tnt40= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;

proc glimmix plots=residualpanel;
class trt day loin;
model tnt36= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;

proc glimmix plots=residualpanel;
class trt day loin;
model tnt34= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;
```

```
proc glimmix plots=residualpanel;
class trt day loin;
model tnt30= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;

proc glimmix plots=residualpanel;
class trt day loin;
model degradedportion= trt|day / ddfm=kr;
random loin(trt);
lsmeans trt|day / pdiff lines;
run;
```