PREDICTING WATER HOLDING CAPACITY AND FORMS OF WATER LOSS IN BEEF SOURCES

by

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LIST OF ABBREVIATIONS

Description	Abbreviation
Cooked Area	CA
Cooked Surface Area	CSA
Cooked Weight	CW
Dark, Firm, Dry	DFD
Electric(al) Conductivity	EC
Meat Cooking Shrinkage	MCS
Microsiemens	µS
Raw Area	RA
Raw Surface Area	RSA
Raw Weight	RW
Siemens	S
Surface Area	SA
Warner-Bratzler Shear Force	WBSF
Water Holding Capacity	WHC

I. INTRODUCTION

Background

Consumer satisfaction with beef products is a major concern for the beef industry and has become a significant subject in meat research. When a consumer is deciding on a protein source for consumption, one of the main factors involved in deliberating between meat products is appearance (Bass et al., 2014), juiciness and tenderness (Muchenje et al., 2009). Products that have a watery appearance are often rejected by consumers and become a source of industry resulting in a decrease in revenue. Products that have a dark and dry appearance are subject to the same fate and again cause revenue loss to the beef industry. For the beef industry, beef that has a dark appearance, often termed “dark cutters” or “dark, firm and dry” (DFD) meat, is the most significant concern of the two extremes and is a source of reduction in the industry’s potential for profit. The meat products from these dark cutting animals are often considered undesirable for human consumption in the form of retail cuts, such as a rib-eye, which is where beef producers make larger profits (Bass et al., 2014). Instead, these products are designated for animal feed or mixed meat products as a way to recover some of the profit lost. Consumers are often correct to reject these products because the cooked version of these products is unsuitable, and is either too tough to satisfy the consumer or is lacking in palatability. These cuts are also prone to greater shrinkage during cooking, resulting in a smaller finished portion of product. Ensuring that the high quality standards that are demanded by consumers are met is one of the main priorities in the meat industry, regardless of the product.

Consumers demand products that are tender, juicy and most of all, visually appealing. To alleviate this problem, researchers have been tasked with understanding the underlying mechanisms of muscle structure and water properties of beef to decrease the occurrence of unacceptable beef products that consumers reject and to promote a higher degree of quality and consistency.

Purpose of This Study

The purpose of this study is to describe and research factors that affect water-holding capacity (WHC) and the forms of water in beef. Specifically, this study will investigate the different forms of water and which forms are lost during the cooking process. This research will examine beef from two different sources and determine any significant differences in WHC (water form loss and ionic strength).

Limitations of This Study

All nutritional values reported are from third party sources and are cited in the review of literature. The capacity of the lab facility used for this study had an impact on how the number of samples that could be processed and availability of instruments typically used to explore quality traits related to WHC.

Implications of This Study

This study provides a better understand of the effects of WHC and water forms on beef quality attributes. Water binding in beef muscle can be associated with factors such as physiological characteristics, pH, ions and to a lesser extent, genetics. Having knowledge of these factors can help cattle producers manipulate factors to promote a higher quality beef product, decrease economic losses and satisfy consumer demands. The methods used in this study could also potentially help the beef industry maintain the

high-quality standard set by consumers and increase the degree of consistency in beef products.

Research Questions

1. Is there a difference in water form loss in beef of different sources?
2. Is there a difference in ionic strength in beef from different sources?
3. Is there a difference in WHC in beef from different sources?

II. LITERATURE REVIEW

WHC is the ability of post mortem muscle to retain water (J. K. Apple, 2007; Hughes, Oiseth, Purslow, & Warner, 2014; Muchenje et al., 2009; Gerald Offer & Trinick, 1983; Pearce, Rosenvold, Andersen, & Hopkins, 2011; Schäfer, Rosenvold, Purslow, Andersen, & Henckel, 2002; Toldra, 2011; Traore et al., 2012)(J. K. Apple, 2007; Hughes et al., 2014; Gerald Offer & Trinick, 1983; Pearce et al., 2011; Schäfer et al., 2002; Toldra, 2011; Traore et al., 2012). WHC is an important meat quality attribute because it can be used to determine juiciness in steaks (Huff Lonergan, Zhang, & Lonergan, 2010; Muchenje et al., 2009; Reardon, Mullen, Sweeney, & Hamill, 2010). Products with abnormal WHC are subject to watery or dry appearances and are considered to be a profit loss factor in the meat industry (Devine, Wells, Lowe, & Waller, 2014; G. Offer et al., 1989; Reardon et al., 2010). Therefore, normalizing WHC in meats is an important step in eliminating undesirable meat products from the market. In order to do so, muscle structure must be evaluated because many structural components change as a muscle enters rigor mortis and starts the aging process that can alter quality traits (Bond & Warner, 2007). Composition and water characteristics need to be evaluated to understand the underlying problems that can lead to an abnormal WHC in a post mortem muscle.

Muscle Structure

Though most believe that beef muscle is primarily made up of protein, the largest structural component of beef is water. Water accounts for 75% of lean muscle tissue composition, with 85% of it located in the myofibrillar protein network (Bertram, Purslow, & Andersen, 2002; Huff-Lonergan & Lonergan, 2005; Muchenje et al., 2009;

Pearce et al., 2011). Protein is the second largest component of beef muscle and accounts for approximately 18 - 20% of lean muscle (Bertram et al., 2002; Huff Lonergan et al., 2010). The largest concentration of protein found in beef are myosin molecules which account for 43 - 45% of all protein found in muscle followed by actin (22%) and titin (8%) (Huff Lonergan et al., 2010; Puolanne & Halonen, 2010). Minerals, vitamins and carbohydrates represent the smallest components of muscle tissue composition (Figure 1). Each of these structural categories are inter-related when considering WHC and characteristics from each component are important factors that influence a muscle's capacity to retain its own water (Hughes et al., 2014). Protein muscle structure is complex and has various components to make up the entirety of the muscle, but all protein components are comprised of amino acids, the building blocks of protein. Amino acids are organic compounds that are either synthesized by the body or acquired through the diet. These amino acids come together to form polypeptide chains that are formed by DNA translation for various functions. Polypeptide chains formed for structural muscle come together to form thick and thin filaments (myosin and actin, respectively) that make up a sarcomere. The sarcomere also contains titin (or connectin), a massive protein that accounts for approximately 8% of the protein in the sarcomere, along with other proteins and enzymes (Puolanne & Halonen, 2010). These sarcomeres line up end to end to form a long fiber, or a myofibril. In muscle, there are many myofibrils and are arranged in a bundle to create a myofibrillar protein network. This protein network, or fascicle, is grouped together with other fascicles comprised of myofibrils and is surrounded by the perimysium to form a muscle belly that is connected to a bone by a tendon. Knowledge of muscle structure components is a critical step in understanding the underlying

mechanisms of how muscle retains and loses water during and after it undergoes the conversion of muscle to meat.

Myofibrillar Protein Network

Myofibrils make up a network in the muscle and is the location of approximately 80 - 85% of the water found in animal muscle (Hughes et al., 2014; Pearce et al., 2011; Tornberg, 2005). Due to the large concentration of water, the myofibrillar protein network is a critical aspect of WHC study. WHC is dependent on the myofibrillar protein network during the conversion of muscle to meat when significant changes occur to the structure of muscle and it can be expected that differences in muscle structure will be evident in differences in WHC (Bertram et al., 2002). After exsanguination, one of the first physiological functions halted is blood circulation. Without blood circulating through the body, oxygen cannot reach cells and the concentration of oxygen in the muscle tissue drops rapidly until there is no more available oxygen left for mitochondria to use. When this happens the mitochondria still synthesizes ATP by switching to anaerobic respiration, resulting in lactate production (Hudson, 2012). In living organisms, lactate produced by anaerobic respiration is transferred and discarded from the organisms system, but since blood circulation cannot occur *post mortem*, lactate accumulates in the cell and alters the pH of *post mortem* muscle to approximately 5.6 (Toldra, 2011). This significant drop in pH further decreases ATP production, and has serious consequences for the myofibrillar network resulting in structural changes that affect the muscle's ability to retain water. As pH falls, proteins become acidic and their net charge decreases and approaches zero, which results in hydrophobicity (Ouali et al., 2006) and facilitates packing of the myofibril structure giving rise to less space for water

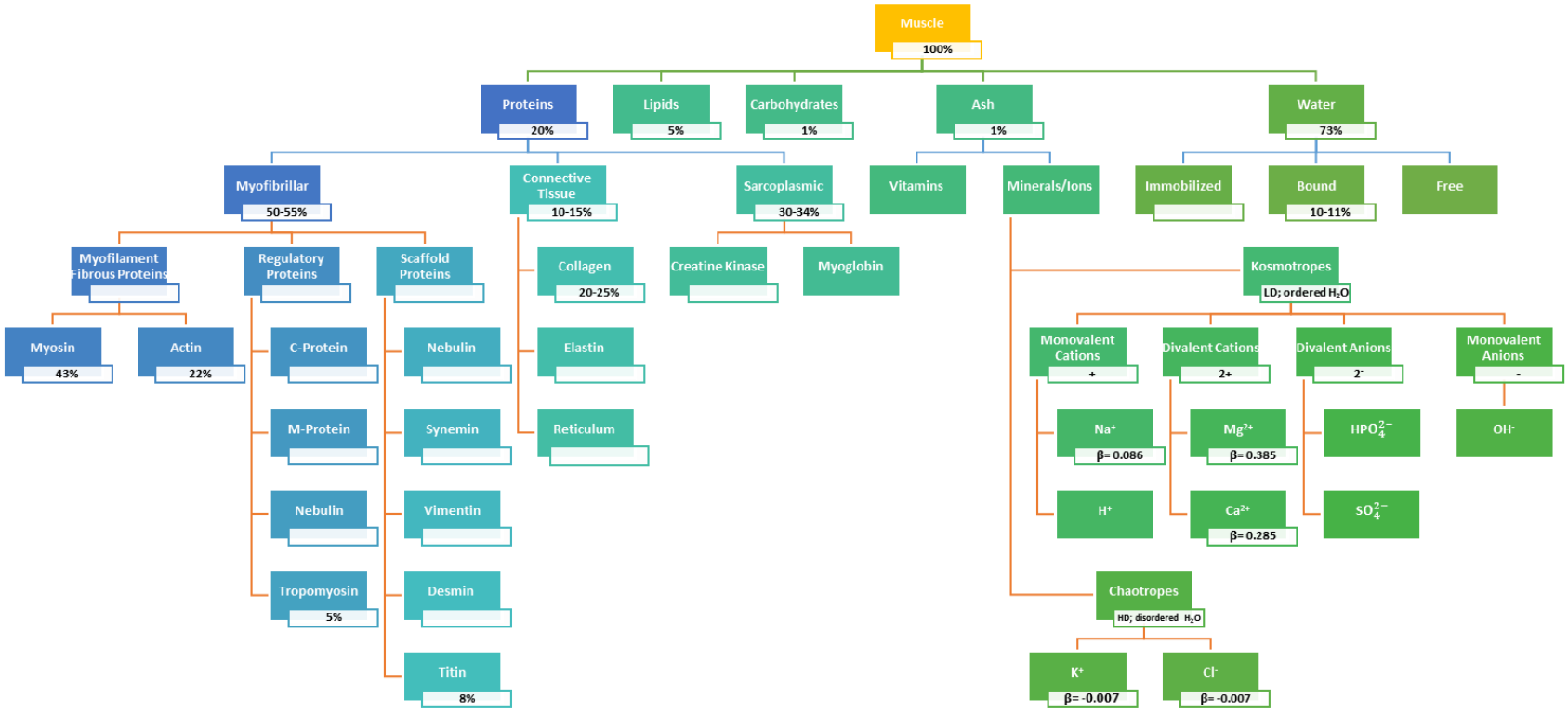


Figure 1. Muscle composition flow chart

to occupy (Huff-Lonergan & Lonergan, 2005). In addition to the accumulation of lactate and a drop in pH, depletion of oxygen (anoxia) results in a disturbance of intracellular osmosis that occurs due to of an increase of hydrogen ions from lactic acid formation and a reduction in electrostatic repulsion between proteins in the myofibrillar network (Pearce et al., 2011).With this drop in repulsion between fibers a "shrinking" of intracellular space occurs, which is the location of a majority of water that is held in the muscle (Ouali et al., 2006). Since intracellular space decreases, extracellular spaces increases, causing drip channels to be created thus allowing water to be released from the muscle. Another critical factor that contributes to the loss of water in the process of conversion of muscle to meat is the depletion of ATP in the muscle. At this point in the rigor mortis process, protein denaturation begins and actomyosin is formed when myosin becomes irreversibly bound to actin filaments, causing the muscle to become rigid (Pearce et al., 2011). The rigidity of the muscle further increases myofibrillar shrinkage resulting in even less intracellular space, forcing water to be expelled from between the myofilaments and out of the muscle. These factors are involved in the repulsion of water from the muscle, or drip loss, which can give meat a watery appearance and negatively affects the consumer's visual appraisal of meat products.

Water

Water accounts for 75% of lean muscle weight and can be considered a structural component of muscle (Huff-Lonergan & Lonergan, 2005; Toldra, 2011). Water is a dipole molecule where there are both partially negative and partially positive areas on the molecule. Since water has these dipolar characteristics, it is attracted to many molecules including proteins, ions and other water molecules. The level of juiciness in meat is

strongly related to how water behaves in muscle (Guignot, Vignon, & Monin, 1993; Muchenje et al., 2009; Ouali et al., 2006; Pearce et al., 2011). As mentioned previously, most water in muscle tissue occupies the space between thick and thin filaments in the myofibrils with a fraction of water held in place by electrostatic attraction to proteins (Bond & Warner, 2007). Water located in the muscle tissue is often referred to as "myowater".

Molecular Water Forms

When describing water by its molecular forms, it can be divided into three categories: ordered water (low density water), disordered liquid water (high density water) and ice (Puolanne & Halonen, 2010). Ordered water, also called low density water, is characterized by small or multivalent ions (see Fig. 1), has a higher freezing point and is less reactive than disordered water (Puolanne & Halonen, 2010). Contrary to ordered water, disordered water, or high density water, is characterized by large monovalent ions (see Fig. 1), is very reactive due to weak hydrogen bonds and tends to have a lower freezing point than ordered water (Puolanne & Halonen, 2010). The molecular form of water influences WHC and water loss because the molecular form of water determines how water reacts with other molecules in the vicinity and therefore can dictate how water will interact with proteins. The ions present are also a significant contributing factor on the molecular form of water and thus ions have a role in determining WHC in muscle.

Forms of Water in Beef Muscle

Water in muscle can be categorized into three different groups: free, bound (sometimes called bulk), or immobilized (also called entrapped). Free water is water in

the tissue that can flow from the tissue easily and is located in sarcomeres. Free water can be redistributed by physical forces or can be repelled from the muscle (Pearce et al., 2011). Bound water is defined as water that is bound to proteins and represents less than ten percent of the total water found in muscle (Pearce et al., 2011). This water is tightly bound to proteins and therefore is not allowed to move freely. Instead, bound water stays tightly bound to proteins even when forces are applied and is not greatly affected by the conversion of muscle to meat (Pearce et al., 2011). It is hypothesized that water is pushed and pulled by polar and non-polar regions on protein that causes it to assume a structured formation similar to ice (Puolanne & Halonen, 2010). Bound water has been determined to account for the smallest portion of water found in muscle (10 - 11% or 0.5 g of water for every gram of protein) (Bertram et al., 2002). The last category of water, immobilized water, is greatly affected by rigor mortis processes. It is held in place by steric forces and represents approximately 85% of water in muscle (Huff-Lonergan & Lonergan, 2005). How water acts and what form it is found in (molecular form or its relationship to protein) has a significant influence on quality characteristics of meat.

Physiological Influences on Myowater

There are several physiological factors that can influence the post mortem ability of muscle proteins to bind to water. One factor that needs to be considered is temperature. The rate at which temperature falls in muscle after slaughter can negatively impact water binding (Cheng & Sun, 2008). This factor cannot be manipulated as much as other factors due to the need and importance of chilling beef carcasses after slaughter for food safety purposes. Another physiological factor that affects water binding that can be manipulated are changes at the molecular level after slaughter. Processes such as the shrinkage of

myofilaments and the irreversible formation of actomyosin cross bridges greatly affects water binding characteristics of muscle (Cheng & Sun, 2008; Puolanne & Halonen, 2010). The formation of these cross bridges along with a shortening of sarcomeres can decrease the size of muscles cells and can expel intracellular water to the extracellular space and then potentially outside of the muscle (Huff-Lonergan & Lonergan, 2005). As the muscle cells shrink in diameter, gaps are formed between muscle cells (drip channels) which promotes the mobilization of water from inside the muscle cell to quickly exit the muscle entirely (Huff-Lonergan & Lonergan, 2005; Hughes et al., 2014; G. Offer et al., 1989; Gerald Offer & Trinick, 1983; Schäfer et al., 2002). The extent of shrinkage is correlated to pH, which will be discussed in a later section.

Genetic Influences on Water Binding

There are not many genetic driven characteristics found to influence WHC in beef muscle beyond differences seen between breeds. One gene that has been identified, a mutant myostatin gene that causes muscular hypertrophy (double muscling), has been found to significantly decrease water binding ability in beef muscle (Cheng & Sun, 2008; Muchenje et al., 2009). More research on genetics and how it may affect water binding has been reported in pork muscle where the Halothane gene has been determined to be correlated with WHC (Jason K. Apple et al., 2001; Calvo, Toldrá, Aristoy, López-Bote, & Rey, 2016; Cheng & Sun, 2008; Huff-Lonergan & Lonergan, 2005; Pearce et al., 2011). Further research is required to assess if genetics play a larger role in water binding characteristics in beef muscle.

Effects of pH

The pH of muscles cells greatly effects the ability of protein to bind water. The average pH of a muscle cells in a living animal is close to neutral at 7.1 but falls to a value closer to 5.0 post mortem. In postmortem muscle, oxygen is no longer delivered to cells in the body due to a lack of blood circulation, causing the oxygen concentration to drop in postmortem muscle. This drop decreases the redox potential of the cell and inhibits the mitochondrial system after the first several hours post mortem (Scheffler, Matarneh, England, & Gerrard, 2015; Toldra, 2011). Many researchers in this field have dismissed the notion that mitochondria play a role in post mortem changes in the muscle, but new evidence is emerging that suggests that mitochondria concentration in muscle and extent of functionality may have a role in pH decline in post mortem muscle (Scheffler et al., 2015). In anaerobic conditions, mitochondria hydrolyzes ATP to ADP and P_i, essentially acting in reverse (Hudson, 2012). It has been suggested that since mitochondria are independent bacteria that have evolved into to cellular organelles that it should be expected that the mitochondria would try to maintain homeostasis even at the expense of the host (Hudson, 2012). This process ultimately results in the degradation of myoglobin which gives beef a dark red appearance resulting in it being classified as DFD and decreases the potential for profit (Bass et al., 2014; Kreikemeier, Unruh, & Eck, 1998). Along with having a negative effect on WHC, DFD meat lacks tenderness, which also effects potential profit for beef producers (Gruber et al., 2010). In consuming the ATP available to the cell, the mitochondria may put pressure on the cell to create more ATP (Hudson, 2012). With this system inhibited, a cascade of events follows that ends in a significant decrease in water binding allowing for an increased quantity of water to be

released in the form of drip loss. When the mitochondrial system fails, cell respiration stops and the cells must switch to anaerobic pathways to produce ATP. To generate energy in the form of ATP from glucose and glycogen, cells metabolizes glucose and glycogen to pyruvate and four ATP molecules. However, for this to occur NAD^+ must be reduced to NADH, which would then enter the mitochondria to be oxidized back into NAD^+ to give the glycolysis system a constant supply of NAD^+. This last step of glycolysis is impossible in an anaerobic state, so the cell change the process so that pyruvate is reduced to lactate by lactate dehydrogenase which allows NADH to be oxidized to NAD^+ and glycolysis is allowed to continue (Salway, 2004). The shortcoming of this modified process is that lactate accumulates waiting to be oxidized to pyruvate when oxygen becomes available again. In postmortem muscle, oxygen does not become available again, which is a problem in oxygen deprived, post mortem muscle where lactate is allowed to accumulate and pH is altered.

Effects of Lactate Accumulation on pH

As lactate accumulates in the muscle, the pH of muscle drops dramatically from approximately 7.0 to 5.6-5.9 (Hudson, 2012; Scheffler et al., 2015; Toldra, 2011). This processes has been summarized by the term "glycolytic potential" which refers to the concentration of glycogen and glucose and their influence on the rate of anaerobic glycolysis (Hudson, 2012). Here, the higher the concentration of carbohydrates available for metabolism at the time of death, the more lactate can be expected as a result of anaerobic glycolysis (Hudson, 2012). As a result, it has been suggested that beef cattle producers should decrease the amount of digestible carbohydrates to help improve water binding (Cheng & Sun, 2008). The rate and extent of pH drop in muscle post mortem is

an important factor for predicting meat quality attributes because a low pH can speed up protein denaturation and is followed by the formation of actomyosin after ATP has been depleted (Toldra, 2011). The formation of actomyosin is an irreversible act and can also be called a "cross-bridge". The formation of actomyosin has a negative effect on water in the muscle because the water that was initially bound the myosin and actin protein molecules are disrupted and are released; it is estimated that approximately 2,500 water molecules are un-bound from protein in response to this event (Puolanne & Halonen, 2010). pH also plays a role in determining the degree of swelling in muscle tissue. Swelling is at its greatest at a pH of 3 and decreases as the pH increases to about 5 where swelling is at a minimum (Puolanne & Halonen, 2010). Both of these factors, protein denaturation and the formation of actomyosin cross bridges, decrease the ability of protein to bind water.

Effects of Isoelectric Point

As discussed in the previous section, pH has an important role in influencing protein's ability to bind water. pH represents the concentration of hydrogen ions, or the net value of reactions that produce and deplete hydrogen ions (Scheffler et al., 2015). Hydrogen ions also carry a positive charge (+1) which can be reflected by the reactivity of a certain molecule at a certain pH. As pH falls in post-mortem beef, the hydrogen ion concentration decreases and so does the electrical charge of the muscle. When the pH of beef falls to approximately 5.0 – 5.3, it is said to have reached its isoelectric point, or the pH at which a particular molecule carries no electrical charge (Huff-Lonergan & Lonergan, 2005). At this pH, the total negative and positive charges of a protein are essentially equal and are not able to attract water molecules (J. K. Apple, 2007; Cheng &

Sun, 2008; Toldra, 2011). As stated previously, water is either bound, free or immobilized, and since at the isoelectric point protein is unable to effectively bind water, most water in the muscle is either free or held in place by steric forces.

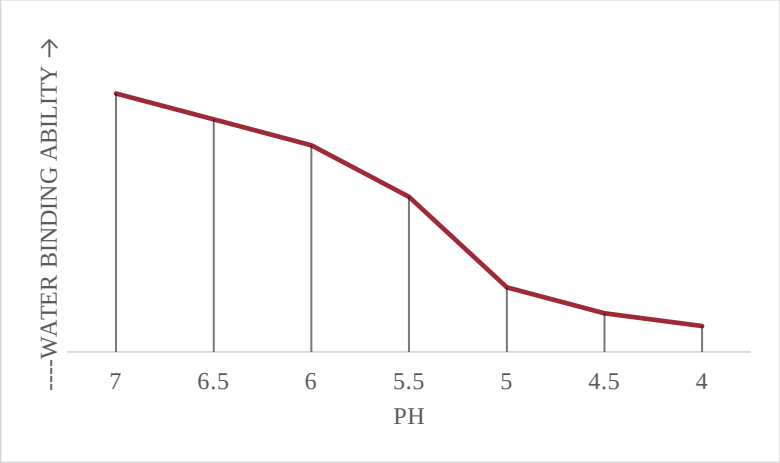


Figure 2. Water binding ability at different pH values. Adapted from *Principles of Meat Science* (p. 111) by E. Aberle, J. Forrest, & D. Gerrard, 2001, Dubuque, IA: Kendall/Hunt Publishing Co. Copyright 2001.

Another critical factor in water binding that is affected by the isoelectric point is the amount of space in the myofibril (J. K. Apple, 2007; Huff-Lonergan & Lonergan, 2005). When pH is at a normal level, proteins create space between myofibrils by repelling each other, but when muscle reaches the isoelectric point, this repulsion force is diminished and the myofibril becomes more tightly packed together, reducing the space for immobilized water to be held (Huff-Lonergan & Lonergan, 2005; Toldra, 2011). Pre and post-slaughter handling can greatly affect the ultimate pH of meat and therefore can greatly effect water binding characteristics.

Cooking Losses and Shrinking in Beef

During the heating and cooking of beef, many structural changes occur to the muscle. Proteins in the muscle undergo structural changes that change quality characteristics that are dependent on muscle structure such as water holding and tenderness (Tornberg, 2005). Cooking processes force muscle tissue to exhibit protein denaturation, shrinking of muscle fibers , shrinking connective tissue, aggregation of

various proteins and a decrease in the structural integrity of cell membranes (Barbera & Tassone, 2006; Põldvere, Tänavots, Saar, Sild, & Lepasalu, 2016; Tornberg, 2005). As sarcoplasmic protein is heated and denaturation and aggregation occur the protein forms a gel. When proteins are denatured and extensively aggregated, a turbid gel is formed which is indicative of poor WHC (Tornberg, 2005). When protein aggregation occurs at a lesser extent, a clear gel is formed as a result and is accompanied by a better WHC (Tornberg, 2005). These changes induced by the heating process have serious implications for the WHC of beef. As heating occurs, muscle structure changes as muscle and connective tissues shrink, reducing the space that held approximately 80% of water found in muscle prior to cooking (Barbera & Tassone, 2006; P. P. Purslow, Oiseth, Hughes, & Warner, 2016; Tornberg, 2005). This leads to water being expelled from the muscle and therefore leads to a decrease of the WHC of beef and the texture of meat (Barbera & Tassone, 2006; Põldvere et al., 2016; Peter P. Purslow, 2005). Since this phenomenon occurs during the heating of beef to 37- 75 °C (Barbera & Tassone, 2006) and water loss tends to be more extreme at higher temperatures but it is also dependent on the ionic strength and pH of the muscle tissue (P. P. Purslow et al., 2016; Tornberg, 2005). Cooking loss and meat cooking shrinkage are important illustration methods of the water quality characteristics in beef.

Ions

In addition to containing the highest concentration of water in beef muscle, the myofibrillar network also contains a large amount of mineral ions. The type and quantity of these ions greatly influence the water binding characteristics of beef muscle proteins. The electrolyte composition of beef muscle is comprised of potassium, magnesium,

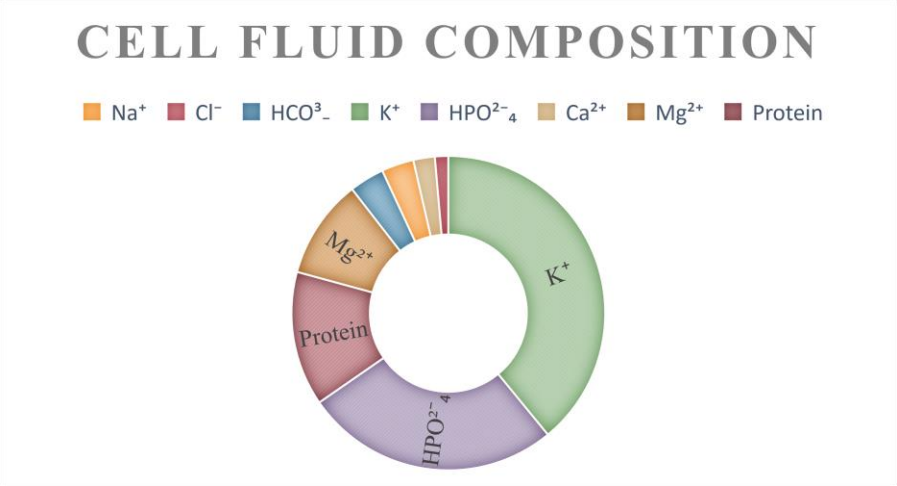


Figure 3. Cell fluid composition. Cell fluid is composed of Potassium, Hydrogen Phosphate, Magnesium, Phosphate, Sodium, Calcium and Chlorine.

sodium, chlorine, calcium and other electrolytes which is illustrated in Figure 3. Each of these ions have different effects on protein-water binding characteristics (Puolanne & Halonen, 2010). The concentration of these ions change as muscle enters rigor mortis and can play a role in decreasing electrostatic repulsion of proteins and the shrinking or swelling of the myofibrillar matrix (Bond & Warner, 2007). Manipulating these ions through feeding practices and post-mortem injections can impact water and protein binding in beef muscle.

Table 1

Mineral Composition of Raw Ground Beef [1]

Mineral	**Value/100g of beef**	**Units**
Calcium	18	mg
Iron	1.94	mg
Magnesium	17	mg
Phosphorus	158	mg
Potassium	269	mg
Sodium	66	mg
Zinc	4.18	mg
Copper	0.061	mg
Manganese	0.01	mg
Selenium	0.0151	mg

[1](U.S. Department of Agriculture, ARS, 2017)

Ions found in beef muscle can be classified into two groups: kosmotropes and chaotropes (Puolanne & Halonen, 2010). Kosmotropes are comprised of smaller ions such as sodium, magnesium and calcium where chaotropes are larger monovalent ions such as potassium (see Fig. 1) (Puolanne & Halonen, 2010). These ions interact with water in various ways and are partly responsible for the water characteristics in beef. Of all the mineral ions found in beef, the largest concentration of ions present in beef muscle are potassium ions. In living muscle, potassium has a significant role in physiological functions such as maintaining water balance, osmotic pressure, acid-base balance and regulating neuromuscular activity. The high concentration of potassium is critical for the animal's survival, but it has negative affects post mortem in terms of water binding. Potassium is classified as a chaotrope and promotes high density, disordered water (Puolanne & Halonen, 2010). As mentioned previously, disordered water is very reactive and tends to form weak hydrogen bonds which is not beneficial for the promotion of water binding in muscle. The second most abundant mineral present in beef muscle tissue is phosphorus. When phosphorus is present in post mortem muscle, it has an important influence on the pH of beef muscle. Phosphates are able to increase pH away from the isoelectric point, which as mentioned previously, will improve water binding abilities of proteins (Hoffman, Vermaak, & Muller, 2012). Phosphates also promote filament swelling and increased ionic strength, both of which promote water binding in muscle (Hoffman et al., 2012). Phosphorus is also able to form complexes with magnesium and calcium which helps to increase the solubilization of myosin and actin (Gerald Offer & Trinick, 1983). This act promotes an increase in the amount of water that can be bound to protein. Phosphorus in the diet is important because it helps regulate the amount of

calcium available post mortem which has a significant role in the tenderization process. It is important to note that phosphorus and calcium are readily available in young cattle, but as they get older phosphorus and calcium levels decrease (Montgomery et al., 2004). It is important to supplement the diet to keep both calcium and phosphorus at optimal levels.

Sodium is the third most abundant mineral present in beef muscle. In addition to promoting ordered water, sodium also increases the osmotic pressure causing the filaments to swell which exposes more protein side chains that can bind to water. (Cheng & Sun, 2008). After slaughter, the quantity of sodium being pumped out of the cell decreases significantly and ultimately stops, in turn increasing the amount of calcium and magnesium in the cell to three times the quantities that normally exist in living tissue (Bond & Warner, 2007). Chlorine, which usually becomes available to the animal with sodium in the form of salt, also has an important role in water binding due to its effect on protein structure. Chlorine promotes the swelling of protein structures by binding to filaments and creating more electrostatic repulsion forces (Cheng & Sun, 2008; Puolanne & Halonen, 2010). Though chlorine allows for more water to be held in the myofibrillar network, Cl^- can have a negative effect on water-binding when the pH of the muscle is below the isoelectric point.

Table 2

Hydration of Cell Fluid Electrolytes[1]

Mineral	No. of water molecules/ mineral
Calcium	30
Magnesium	18
Phosphorus	12
Sodium	18
Potassium	18
Manganese	18

[1](Dorvee & Veis, 2013)

Electrical Properties of Beef

The electric properties of meat are complex because of meat's anisotropic nature which results in varying electrical characteristics dependent on environmental variables (Lepetit, Salé, Favier, & Dalle, 2002). These properties can be characterized as either the meat's electrical conductivity (EC) or electrical impedance (Byrne, Troy, & Buckley, 2000; Lepetit et al., 2002). Electrical conductivity in meats represents the tissue's capability to conduct electricity (Byrne et al., 2000) and is generally expressed in siemens (S) or microsiemens (µS) which are used by the majority of meat researchers cited in this thesis. It should be noted that siemens is a relatively new term and was previously known as mho (℧). Meat is able to create an electrical charge due to the electrolytes found in beef muscle tissue along with water. EC can be used to evaluate water content and characteristics of muscle tissue (Põldvere et al., 2016) and the integrity of cell membranes (Byrne et al., 2000). Impedance on the contrary, describes "the total opposition to the flow of an alternating current at a given frequency" (Byrne et al., 2000) and is expressed in ohms (Ω). There is a relationship between ohms and siemens demonstrated in the equation below:

$$S = ℧ = \Omega^{-1} = \frac{A}{V}$$

Figure 4. Relationship between siemens (S),mho (℧),ohm (Ω), ampere (A) and voltage (V)

The electrical properties of meat are directly dependent on the water characteristics of the tissue. Ions in water give meat its charge, so the higher concentration of ions in water, the higher the EC. As discussed in an earlier section, there are three forms of water in meat: free, bound and immobilized. Free water is water that

has been pushed out of the muscle during rigor mortis and storage. This fluid will carry a substantial quantity of ions and augments the electoral conductivity of meat (Lee et al., 2000). Bound water is tightly bound to proteins, not ions. So, bound water does not add to the electrical properties of meat in the same manner as free water. Immobilized water also carries ions that gives myowater an electrical charge, and is the most affected by heating processes and changes form to free water and flows easily during this process. It would be logical to assume that raw meat with relatively higher EC values would be characterized by an abnormally high amount of free water and raw meat with relatively low EC would be indicative of drier meats (Lee et al., 2000). Since water is lost during cooking and heating processes, it follows that EC and impedance should be different in raw and cooked beef.

III. METHODS

Study Design

This research study was designed to predict relative estimates of WHC and forms of water from two different sources of beef. The EC and electrical impedance of raw beef loin steaks from two sources was contrasted to the EC of the control beef in its cooked form after cooking in a commercial smoker to predict relative WHC and water form loss. Tenderness was also used to evaluate quality characteristic of loin steaks. Ground beef from both sources was acquired and formed into burgers to investigate cooking loss, water loss and muscle shrinkage.

Sample Preparation and Cooking Treatments

All beef samples were acquired from and selected by two different sources: a local rancher (Wagyu breed) and a commercial grocery store (unknown breed). From each source, 12 loin steaks representing 6 animals (2 roasts per animal) and 20 pounds of ground beef were acquired. Raw meat analysis of the beef samples was preformed immediately upon arrival at the meat laboratory. Control and Wagyu loin steaks were cooked without humidity or smoke in a multi-purpose smokehouse (UltraSource Grand Prize™ 3) at 93° C (200° F). Control and Wagyu burgers were grilled on a George Forman Grill (Model: GR2080R) and the drippings/runoff was collected. All samples undergoing a cooking treatment were heated to an internal temperature of 71° C (160° F). Samples used for predicting water-form-ratios were collected from individually wrapped one-pound packages of ground beef that were obtained from the two sources of beef used.

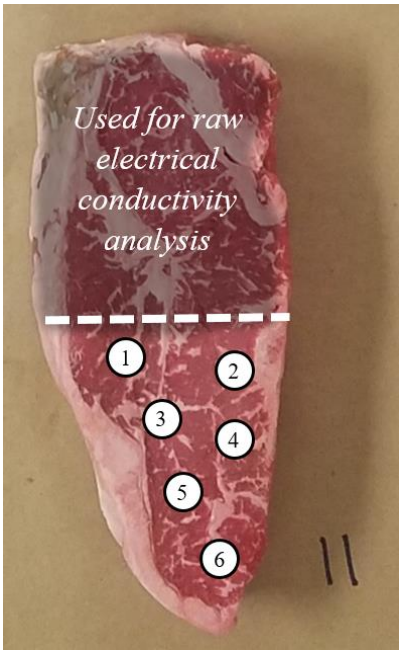


Figure 5. Diagram of how loin steaks were divided for testing purposes. The circles with numbers inside represent the location were core samples were taken and how they were numbered.

Electrical Conductivity of Raw Meat Samples

The ionic strength (electric conductivity) of raw and cooked loin steak beef samples was evaluated from both sources of beef. Four 50 g samples of raw beef were taken from each steak (N= 4 samples x 12 steaks= 48 per source) and evaluated for EC. The remaining portion of the cuts from which the six 50 g samples were set aside for further analysis (see Cooked meat predictions). All raw samples were emulsified with 40 ml of distilled water to create a dilution factor (DF) of 2 (see Figure 6). This colloidal solution was then transferred to a silicon vessel with two copper nails embedded into the side of the container. The silicon vessel was concealed in a plastic bowl with a lid and two holes cut out in the side of the container. The holes allowed for access to the two copper nails embedded in the silicon vessel to be reached by two test leads of a digital multimeter (16040T True RMS Multimeter, Southwire Tools & Equipment). After the emulsified meat slurry was transferred into the silicon vessel, the test leads of the multimeter were aligned to touch the two copper nails simultaneously (see Figure 7) and a conductivity reading was recorded for a duration of 60 seconds with a sampling rate of two per second. Between samples, all containers and/or vessels that were exposed to the samples were cleaned and sterilized with bleach prior to being used for the next test. Ambient temperature was recorded.

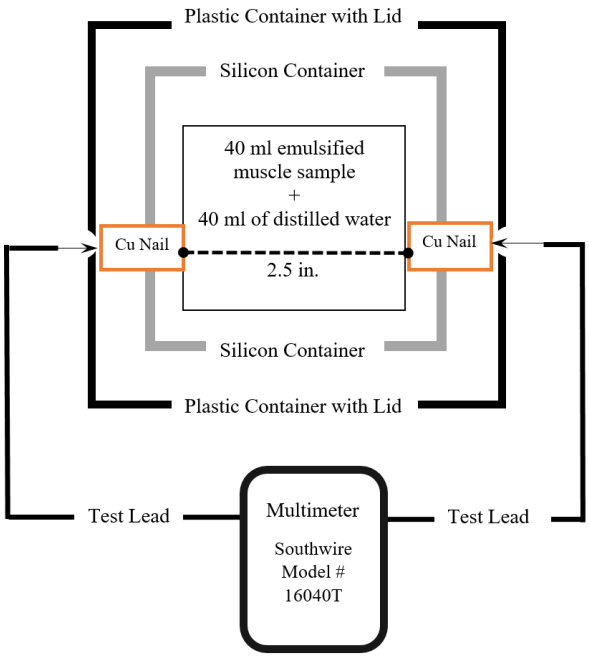


Figure 7. Diagram of electrical conductivity experimental design

$$DF = \frac{V_f}{V_i} = \frac{80\ ml}{40\ ml} = 2$$

Figure 6. Dilution factor determination (equation)

Cooked Meat Predictions

To further evaluate the quality characteristics of beef from both sources, the remaining portions of the loin steaks sampled for raw analysis were cooked in oven bags in a commercial grade smoker at 93° C (200° F) without humidity or smoke until the steaks reached an internal temperature of 71° C (160° F). Cooking time of the samples was recorded. After reaching the desired internal temperature, samples were removed from oven bags and allowed to rest overnight. Weight of beef samples was recorded prior to cooking, raw weight (RW), and after cooking, cooked weight (CW) to determine a cooking loss (CL) percentage using the following equation:

$$\text{CL } (\%) = \frac{\text{RW- CW}}{\text{RW}} \times 100$$

Figure 8. Cooking loss percentage determination (equation)

The amount of liquid that was contained in the oven bag was also recorded. After the cooked samples were allowed to cool and rest overnight, six cores were obtained from each steak (Figure 6). Tenderness of the cores was appraised by determining the Warner-Bratzler shear force value (WBSF). After determining the shear force value, cores 1-3 were emulsified with 20 ml of distilled water. The water and meat mixture was transferred to the apparatus described in the previous section to determine EC and a reading was recorded (Ω). The same procedures were followed for cores 4-6 and a conductivity reading was recorded. Ambient temperature was also recorded.

Burger Quality Characteristic Determinations

The drippings/run off of quarter-pound grilled ground beef patties was evaluated for both sources of beef. Each burger patty was cooked to an internal temperature of 71° C (160° F) on a George Foreman grill. Run off was collected by grilling the ground beef patties and saving the drippings/runoff from each pound. Patties were formed from individually wrapped one-pound packages of ground beef into four approximately equal parts to create four - 112 g patties. Patties formed from the same one-pound package of

Figure 9. Experimental design setup example for burger quality characteristic determinations

ground beef were grilled together on the George Foreman grill and the drippings/runoff from all four burgers was collected. Several measurements were taken prior and after cooking. These measurements included: weight (g), diameter and height before and after cooking. Patties were weighted before and after grilling to determine the weight difference due to cooking (see Fig. 4). The surface area (in^2) of the patties was determined before, raw surface area (RSA), and after grilling, cooked surface area (CSA) using the following equation:

$$SA_{cylinder} = 2\pi r^2 + 2\pi rh$$

Figure 10. Surface area of a cylinder (equation)

Meat cooking shrinkage (MCS) was determined using the following equation:

$$MCS = \frac{CSA - RSA}{CSA} \times 100$$

Figure 11. Meat cooking shrinkage determination (equation)

The fat and water in the ground beef drippings/run off was separated by gravitational separation. The total fat content lost in the runoff was weighed (g) and recorded. An apparent water loss was determined by subtracting the weight of the fat collected from the total weight loss of the burger patties.

To address variation in the heating element of the grill, patties from each pound of ground beef were numbered 1-4. This number corresponded to a spot on the grill (spot 1= top left, spot 2= top right, spot 3= bottom left, spot 4= bottom right) which remained constant throughout the duration of the experiment.

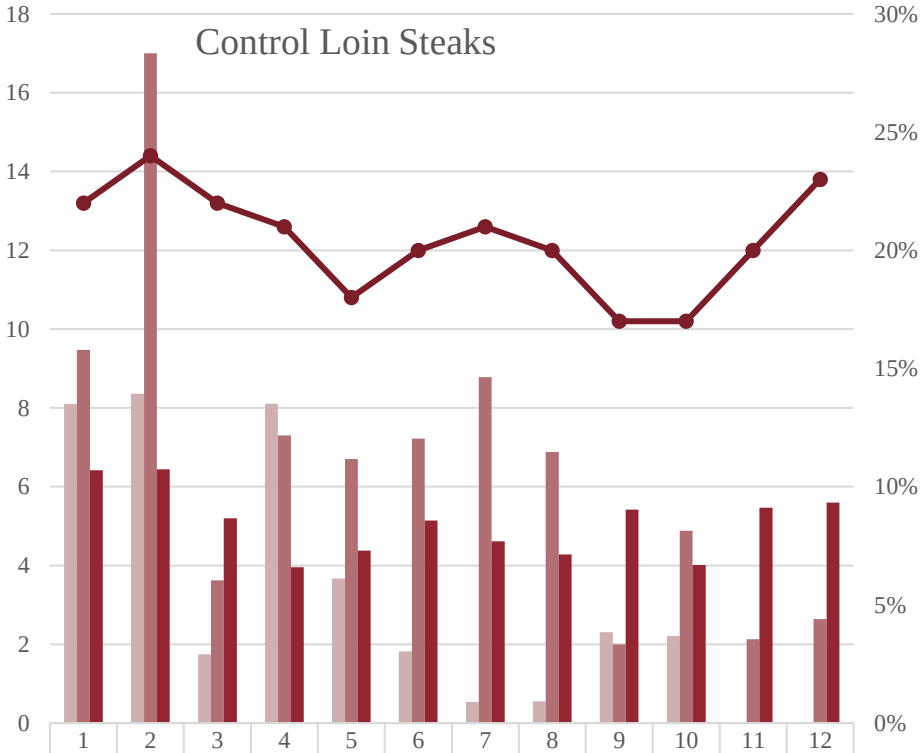
Statistical Analysis

IBM SPSS 22 and Microsoft Excel 2016 were used to analyze data collected in this study. Appropriate statistical procedures including Pearson correlations, regression and paired t-tests were used to examine relationships between quality traits and for the prediction of water forms.

IV. RESULTS

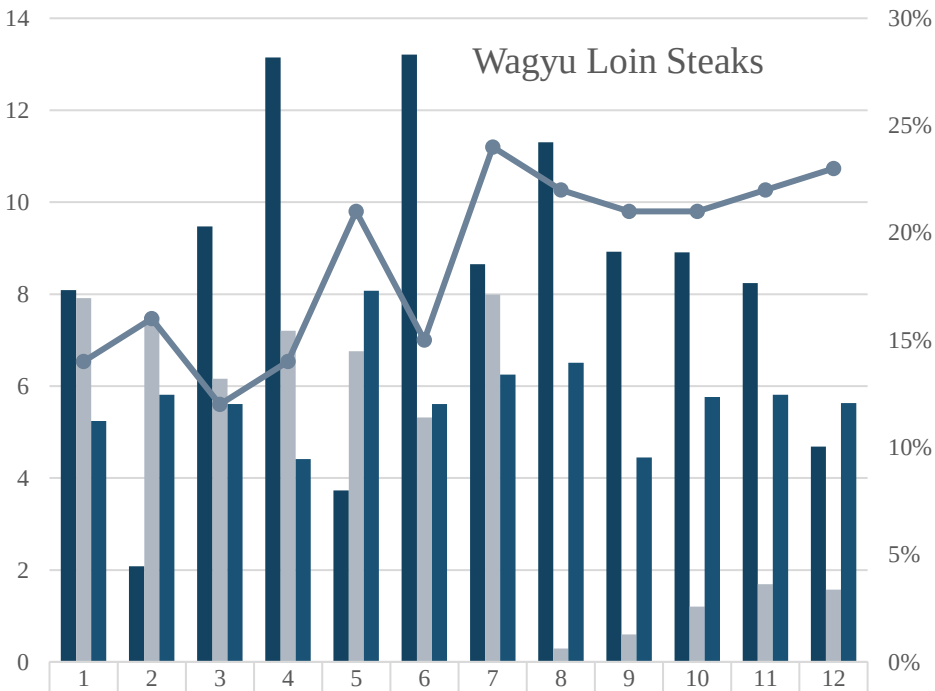
Control and Wagyu Loin Steaks

A summary of the results obtained from control and Wagyu loin steaks is presented in Table 3. These results are also presented visually in Figures 12 and 13. Paired t-tests were performed to evaluate differences in quality characteristics between Wagyu and control loin steaks with a 95% confidence interval. Each t-test compared values from 12 steaks from the 2 sources making the degrees of freedom 11. When looking at EC values for raw steaks, Wagyu loin steaks exhibited significantly higher EC (M = 8.45, SE = 1.0) than control loin steaks (M = 3.12, SE = 0.93), t(11) = 3.598, $P <$ 0.01, r =0.74 for raw samples. The EC values for cooked samples showed that control



	1	2	3	4	5	6	7	8	9	10	11	12
EC- Raw (µS)	8.1	8.36	1.75	8.11	3.67	1.82	0.54	0.55	2.31	2.21	0.01	0.01
EC-Cooked (µS)	9.47	17	3.62	7.3	6.7	7.22	8.78	6.88	2	4.88	2.13	2.64
WBSF (lbf)	6.42	6.44	5.2	3.96	4.38	5.14	4.61	4.28	5.42	4.01	5.47	5.6
Cooking Loss (%)	22%	24%	22%	21%	18%	20%	21%	20%	17%	17%	20%	23%

Figure 12. Control loin steaks



	1	2	3	4	5	6	7	8	9	10	11	12
EC- Raw (µS)	8.09	2.08	9.47	13.15	3.73	13.21	8.65	11.3	8.92	8.91	8.24	4.68
EC-Cooked (µS)	7.91	7.51	6.16	7.2	6.76	5.32	7.99	0.29	0.6	1.2	1.69	1.57
WBSF (lbf)	5.24	5.81	5.61	4.41	8.07	5.61	6.25	6.51	4.45	5.76	5.81	5.63
Cooking Loss	14%	16%	12%	14%	21%	15%	24%	22%	21%	21%	22%	23%

Figure 13. Wagyu loin steaks

steaks had numerically higher, but not significantly different values (M = 6.55, SE = 1.20) when compared to EC values for Wagyu cooked samples (M = 4.52, SE = 0.91). There was a difference between raw and cooked EC values for both control and Wagyu steaks. For control steaks, raw samples had lower EC values (M = 3.12, SE = 0.93) than cooked samples (M = 6.55, SE = 1.20), t(11) = -3.86, $P < 0.01$, r = 0.76.

Wagyu steaks however showed an opposite trend where the EC of raw samples (M = 8.45, SE = 1.00) were higher than the EC of cooked samples (M = 4.52, SE = 0.91), t(11) = 2.71, $P < 0.05$, r = 0.63. In control steaks, steak 2 had the highest EC values for both raw (8.36 µS) and cooked (17 µS) samples. This steak also had the highest WBSF value (6.44 lbf) and the highest percentage of cooking loss (24%). Two steaks, steak 9

and 10 (or animal 5) had the lowest cooking loss (17%) and were characterized by lower raw and cooked EC values (steak 9 = 2.31 µS and 2 µS respectively; steak 10 = 2.21 µS and 4.88 µS, respectively). These two steaks had WBSF values (steak 9 = 5.42 lbf, steak 10 = 4.01 lbf) in the median range. On average, control steaks had higher but not significantly different average cooking loss, lower WBSF but not significantly different values and a higher but not significantly different quantity of water collected from oven bags.

In contrast to the control loin steaks, the Wagyu steak with the highest EC in raw samples had a cooked EC value in the median range. WBSF values that fell in the median range and the fourth smallest cooking loss percentage. The steak with the highest cooking loss had the highest EC value for cooked samples, a WBSF value in the median range and an EC value for raw samples in the median range. The steak with the lowest WBSF value had the second highest EC value for raw samples and a cooking loss percentage that was the second lowest.

Loin Steak Correlations

Pearson correlation coefficients are illustrated in Table 4 for both control and Wagyu loin steaks. In control loin steaks there was a significant correlation between raw EC values and cooked EC values ($P < 0.01$). Cooked EC values were correlated with cooking loss and the quantity of water collected from oven bags after steaks were cooked ($P < 0.05$). Cooking loss was also correlated with WBSF values ($P < 0.05$). In Wagyu loin steaks, there was a correlation between cooked EC and cooking loss ($P < 0.05$). There were no significant correlations between raw EC and other quality characteristics.

The various relationships between control and Wagyu loin steak quality characteristics are presented visually in figures 14-20.

Control and Wagyu Burgers

Water form predictions in control and Wagyu burgers quality characteristic values are summarized in Table 5. Both control and Wagyu burgers had the same average cook time to reach an internal temperature of 160 ºF relative to the spot they were cooked at, but, control burgers had lower cooked weights and higher muscle shrinkage on average. Burgers cooked in spot 4 took more time on the grill to reach the desired internal temperature of 160 ºF compared to cooking times the other three spots. Control burgers cooked in spot 4 had lower cooked weights (M = 70.70, SE = 0.74) compared to Wagyu burgers cooked in spot 4 (M = 74.1, SE = 0.42), t(19) = -3.847, $P < 0.01$, r = 0.66. Muscle shrinkage was more pronounced in control burgers cooked in spot 1 (M = 43.75, SE = 3.46) than Wagyu burgers cooked in spot 1 (M = 32.60, SE = 2.78), t(19)= 2.498, $P < 0.05$, r = 0.50. Shrinkage in spot 2 was greater for control burgers (M = 40.60, SE = 3.62) when compared to Wagyu burgers (M = 30.45, SE = 1.99), t(19) = 2.203, $P < 0.05$, r = 0.45. A similar situation also occurred in spot 3 where Wagyu burgers (M = 34.20, SE = 1.61) shrunk less than control burgers (M = 46.60, SE = 2.77), t(19) = -5.437, $P < 0.01$, r = 0.78. In spot 4, control burgers shrunk more (M = 49.15, SE = 3.124) than Wagyu burgers (M = 33.25, SE = 2.973), t(19) = 4.498, $P < 0.01$, r = 0.72. Though Wagyu burgers had higher cook weights, lower cooking loss and lower muscle shrinkage, they had on average higher estimated water loss values. Water loss was higher for Wagyu burgers cooked in spot 1 (M = 37.95, SE = 2.25) than control burgers (M = 22.70, SE = 2.25), t(19) = 5.369, $P < 0.01$, r = 0.78. control burgers cooked in spot 2 also had less

water loss (M = 23.05, SE = 2.80) than Wagyu burgers (M = 38.80, SE = 0.85), t(19) = -5.226, $P < 0.01$, r = 0.77. In spot 3, control burgers also lost less water (M = 28.20, SE = 2.30) than Wagyu burgers (M = 28.20, SE = 2.30) than Wagyu burgers (M = 41.60, SE = 1.15), t(19) = -5.124, $P < 0.01$, r = 0.76. Burgers cooked in spot 4 followed the same pattern as the burgers cooked in spots 1-3 where control burgers lost less water (M = 28.90, SE = 2.35) when compared to Wagyu burgers (M = 39.40, SE = 0.734), t(19) = -3.966, $P < 0.01$, r = 0.67.

Burger Correlations

Pearson correlation coefficients for control and Wagyu burgers are presented in Table 6. Shrinkage was significantly correlated to weight loss and cooking loss in both control and Wagyu burgers, but Pearson correlation coefficients for control burgers were more negative. The estimated water loss in Wagyu burgers was more highly correlated to weight loss (0.920) and cooking loss (0.921) when compared to control burger correlation coefficients (0.653 and 0.652, respectively). Figures 21-26 give a visual representation of these results.

Table 3

Summary of Values Obtained from Control and Wagyu Loin Steaks

	Control					Wagyu				
Steak #	Raw EC (µS)	EC of Cores (µS)	WBSF (lbf)	Cooking Loss (%)	H_2O Collected (g)	Raw EC (µS)	EC of Cores (µS)	WBSF (lbf)	CL (%)	H_2O Collected (g)
1	8.10±0.37	9.47±2.76	6.42±0.75	22	46	8.09±0.8	7.91±0.97	5.24±1.38	14	40
2	8.36±0.31	17.00±1.50	6.44±1.59	24	60	2.08±0.11	7.51±0.20	5.81±0.70	16	30
3	1.75±0.54	3.62±2.16	5.20±2.09	22	40	9.47±0.52	6.16±0.36	5.61±1.07	12	22
4	8.11±0.46	7.30±0.19	3.96±1.32	21	60	13.15±0.64	7.20±0.56	4.41±0.69	14	18
5	3.67±1.77	6.70±0.16	4.38±1.35	18	50	3.73±3.25	6.76±0.14	8.07±1.09	21	26
6	1.82±0.08	7.22±0.11	5.14±2.00	20	18	13.21±0.39	5.32±0.34	5.61±1.25	15	16
7	0.54±0.92	8.78±0.25	4.61±1.66	21	48	8.65±0.64	7.99±0.61	6.25±2.28	24	50
8	0.55±0.94	6.88±2.14	4.28±1.02	20	70	11.30±0.62	0.29±0.08	6.51±0.98	22	50
9	2.31±1.79	2.00±1.50	5.42±2.38	17	14	8.92±0.49	0.60±0.09	4.45±0.81	21	76
10	2.21±0.25	4.88±1.31	4.01±0.63	17	26	8.91±0.55	1.20±0.01	5.76±0.77	21	48
11	0.01±0.00	2.13±2.05	5.47±1.00	20	26	8.24±2.80	1.69±0.08	5.81±1.75	22	36
12	0.01±0.00	2.64±2.65	5.60±2.35	23	16	4.68±1.49	1.57±0.04	5.63±1.39	23	22
Total Means:	3.12±3.21	6.55±4.33	5.08±1.70	20.42±2.23	39.13±19.13	8.14±3.51	4.52±3.04	5.76±1.48	18.75±4.2	36.17±17.6

Table 4

Pearson Correlation Coefficients Between Meat Quality Characteristics

	Raw EC	WBSF	Cooking Loss	Water Collected	Cooked EC
Control Loin Steaks					
Raw EC					
WBSF	0.288				
Cooking Loss	0.318	0.571[b]			
Water Collected	0.469	-0.107	0.459		
Cooked EC	0.680[a]	0.309	0.502[b]	0.598[b]	
Wagyu Loin Steaks					
Raw EC					
WBSF	-0.442				
Cooking Loss	-0.247	0.390			
Water Collected	-0.125	0.247	0.568[b]		
Cooked EC	-0.155	0.105	-0.547[b]	-0.288	

[a] Correlation is significant $P < 0.01$
[b] Correlation is significant $P < 0.05$

Table 5

Pearson Correlation Coefficients for Weight Loss, Cooking Loss, Shrinkage and Estimated Water Loss[b]

	Weight Loss	Cooking Loss	Shrinkage	Water Loss[b]
Control Burgers				
Weight Loss				
Cooking Loss	0.998			
Shrinkage	-0.510	-0.502		
Water Loss[b]	0.653	0.652	-0.538	
Wagyu Burgers				
Weight Loss				
Cooking Loss	0.997			
Shrinkage	-0.308	-0.332		
Water Loss[b]	0.920	0.921	-0.262	

[a] Correlation is significant $P < 0.01$
[b] "Water Loss" is estimated an estimated value

Table 6

Quality Characteristic Means for Control and Wagyu Burgers

	Control Burgers				Wagyu Burgers			
	Spot 1	Spot 2	Spot 3	Spot 4	Spot 1	Spot 2	Spot 3	Spot 4
Quality Characteristic								
Cook Time (mins)[1]	5.36±0.76^{a}	5.95±1.09^{b}	6.68±1.18^{bc}	7.18±0.84^{cd}	5.36±0.76^{a}	5.95±1.09^{b}	6.68±1.18^{bc}	7.18±0.84^{cd}
Cook Weight	75.80±4.15^{ab}	75.30±6.43^{ab}	71.40±6.02^{bc}	70.70±3.33$^{cd¤}$	75.20±2.19^{ab}	74.65±3.05^{ab}	72.60±4.21^{c}	74.10±1.90
Cook Loss (%wt)	32.40±3.69^{ab}	32.80±5.79^{ab}	36.30±5.34^{bc}	37.00±2.83$^{cd¤}$	32.85±2.08^{ab}	33.35±2.82^{ab}	35.30±3.85^{c}	33.90±1.89
Shrinkage (%SA)	43.75±15.47$^{\#}$	40.70±16.19†	46.60±12.38‡	49.15±13.97$^{¤}$	32.60±12.45	30.45±8.88	34.20±7.21	33.25±13.30
Water Loss (%wt)[2]	22.70±10.06$^{ab\#}$	23.05±12.51^{ab†}	28.20±10.29^{bc‡}	28.90±10.49$^{cd¤}$	37.95±3.73^{ab}	38.30±3.81^{ab}	41.60±5.12^{c}	39.40±3.28

%wt: percentage of weight; %SA: percent change in surface area

[1] cook times are statistically equal relative to the cooking spot between beef sources; [2] This is an estimated water loss percentage; Different letters within a row indicate significant differences within sources; #: significant difference between sources for burgers cooked in spot 1; †: significant difference between sources for burgers cooked in spot 2; ‡: significant difference between sources for burgers cooked in spot 3; ¤: significant difference between sources for burgers cooked in spot 4. Significance is $P < 0.05$.

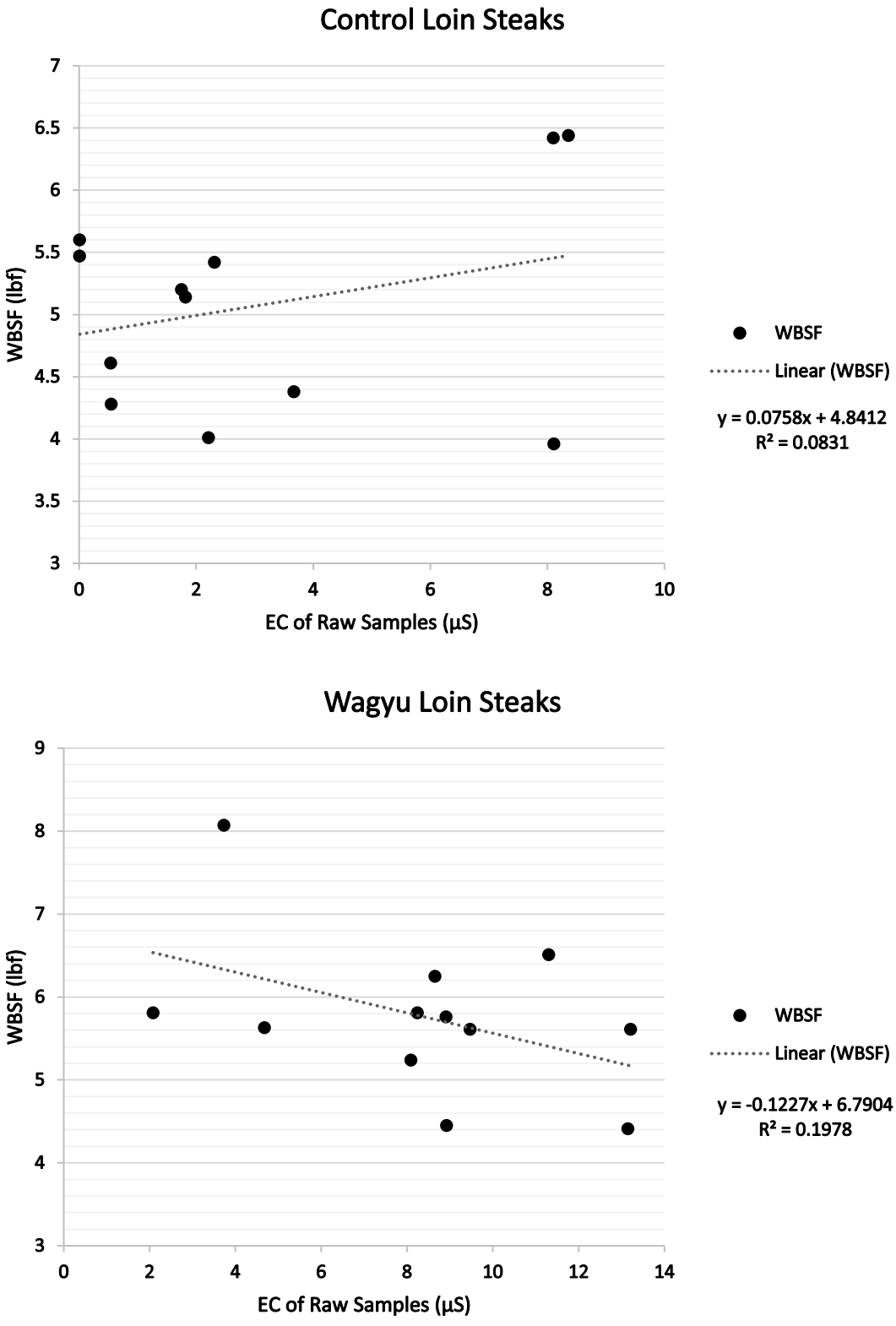


Figure 14. Relationship between EC and WBSF in Control and Wagyu loin steaks

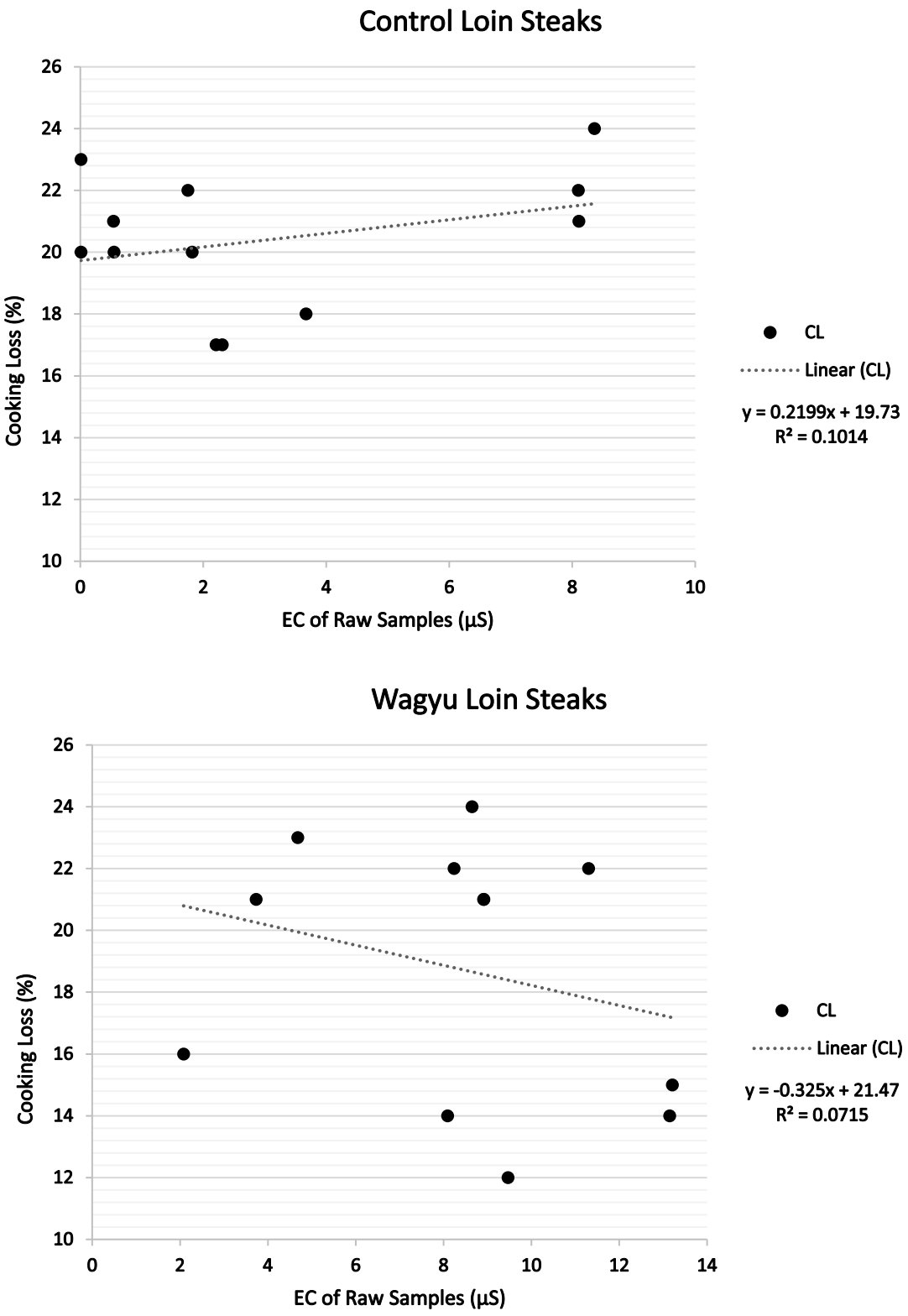


Figure 15. Relationship between EC and Cooking Loss in Control and Wagyu loin steaks

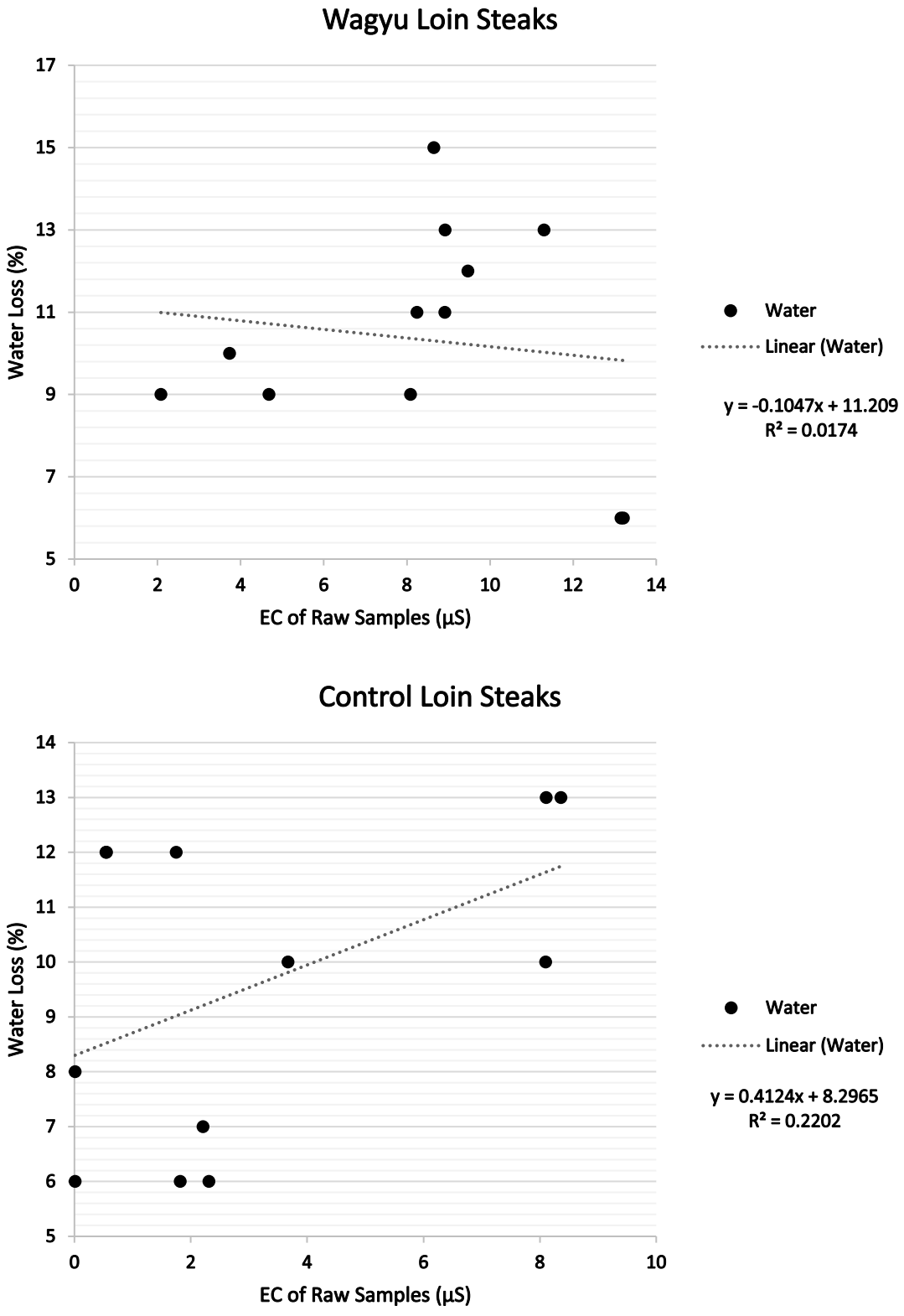


Figure 16. Relationship between EC and Water Loss (%) in Control and Wagyu loin steaks

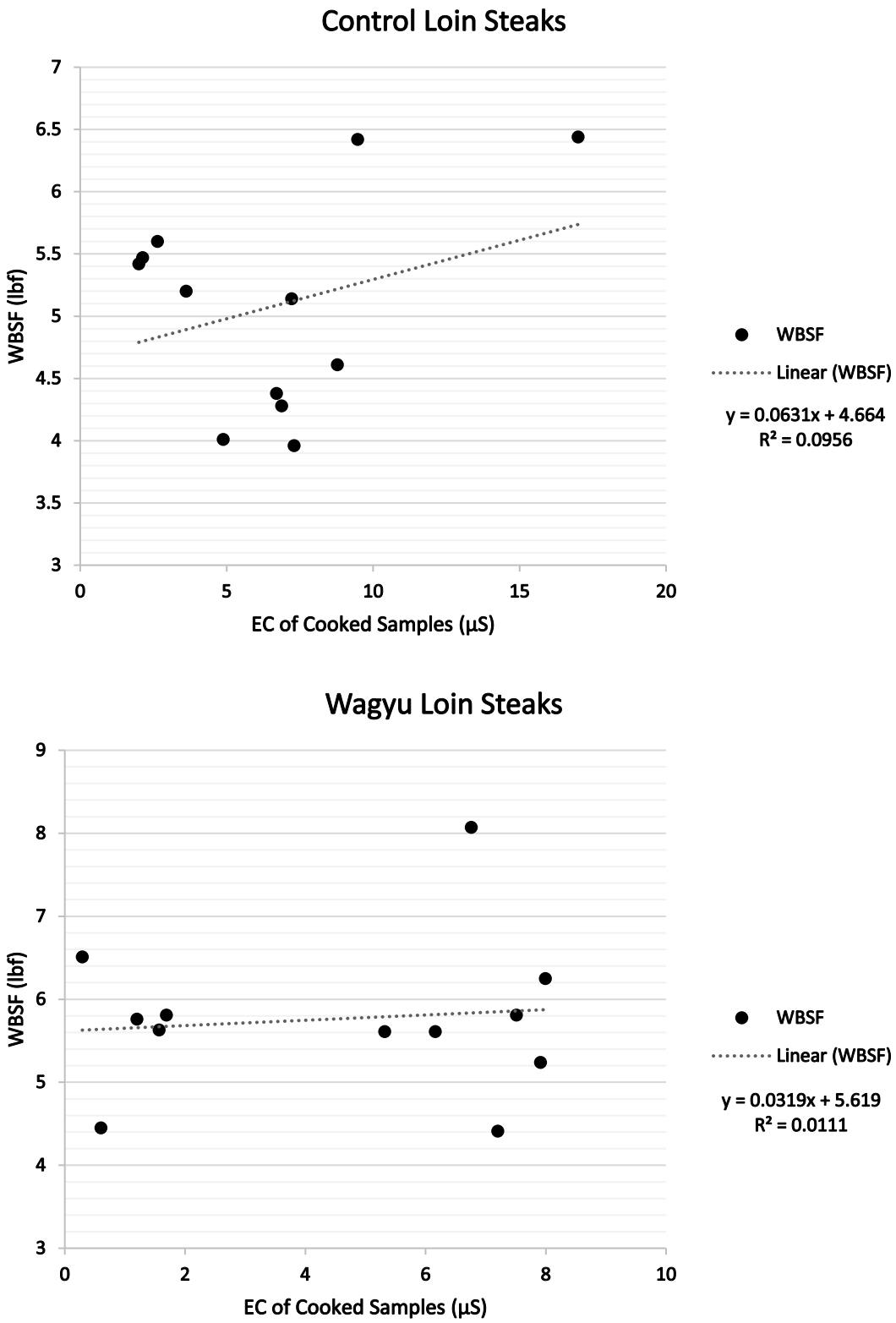


Figure 17. Relationship between EC and WBSF in Control and Wagyu loin steaks

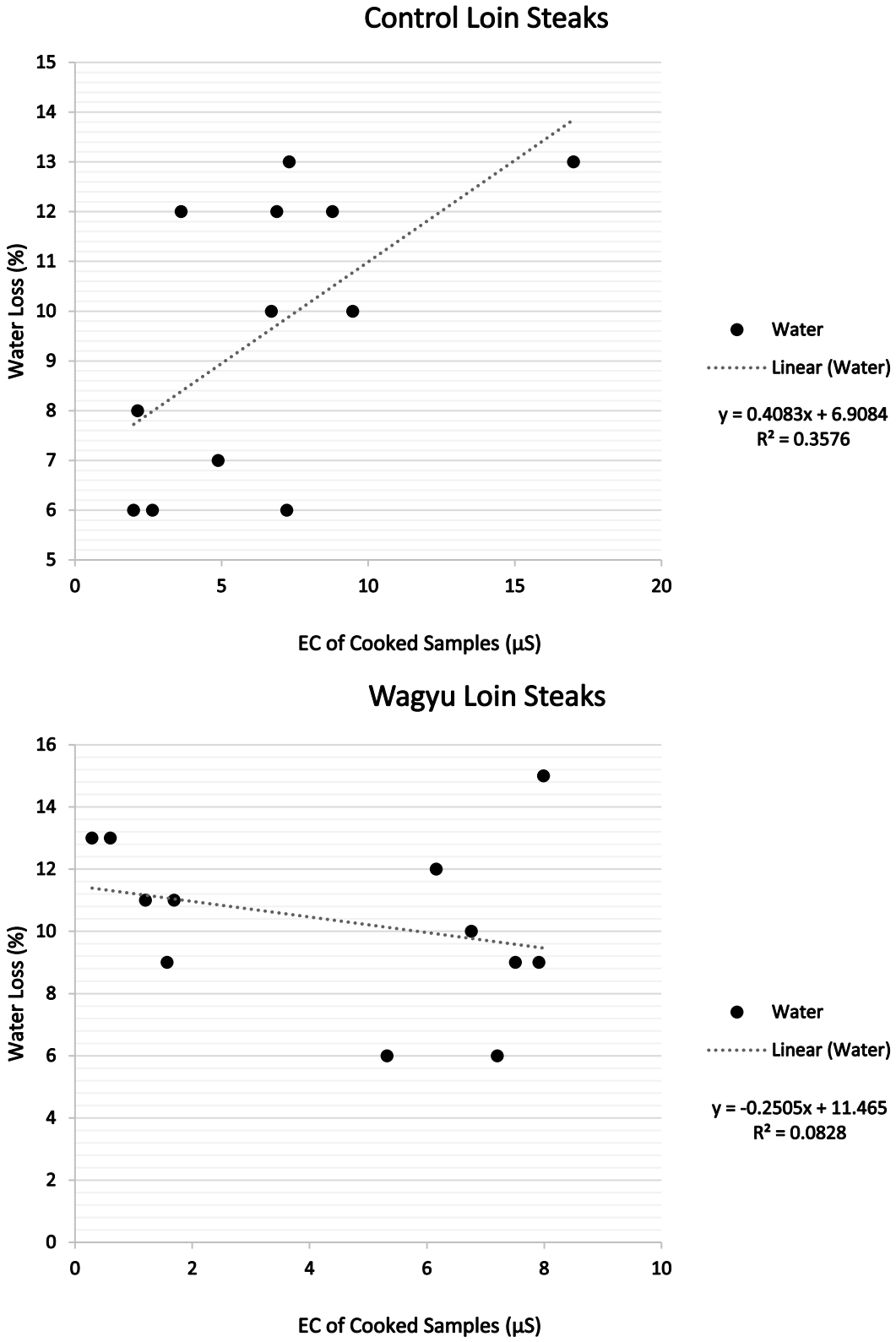


Figure 18. Relationship between EC and Cooking Loss (%) in Control and Wagyu Loin steaks

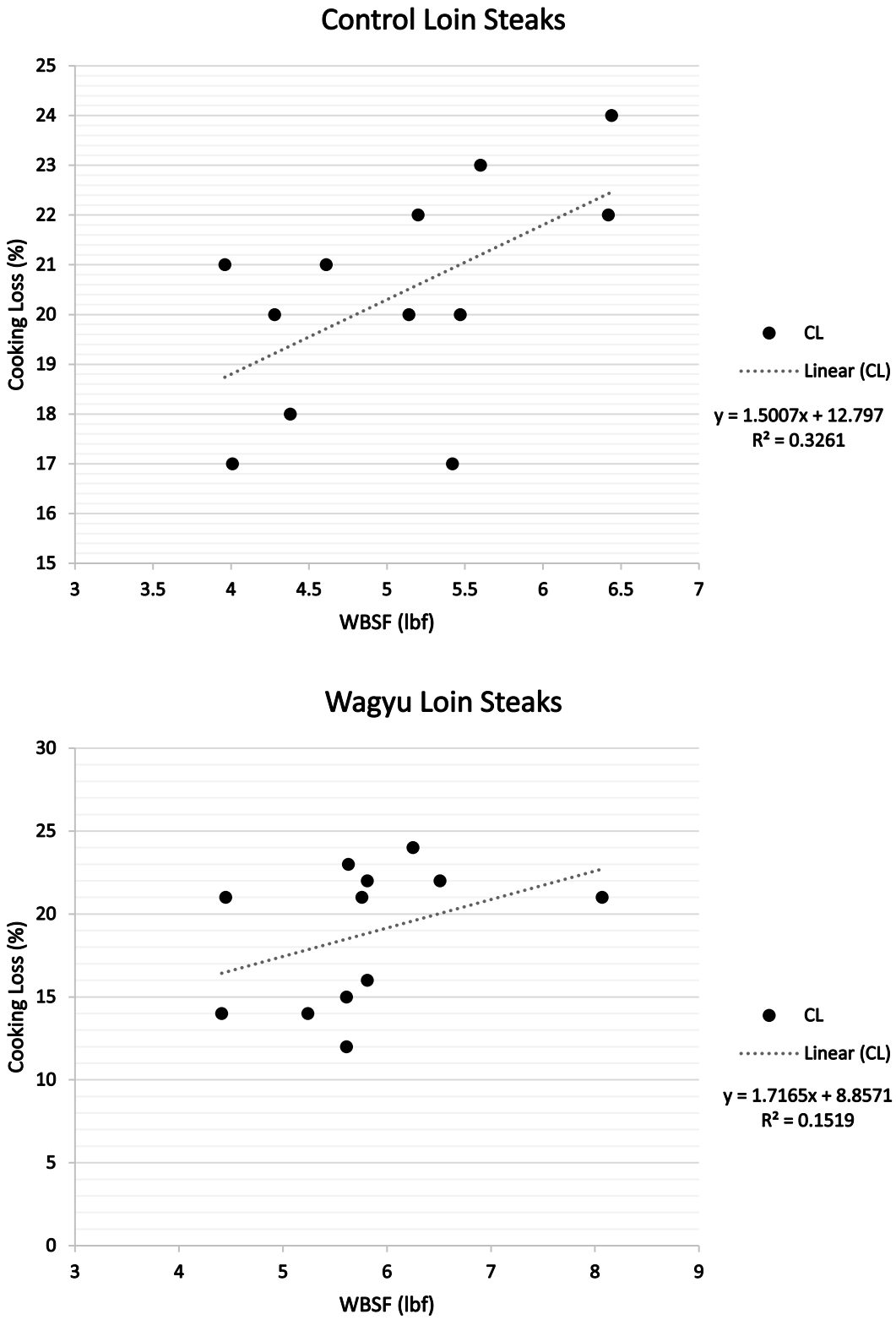


Figure 19. Relationship between WBSF and Cooking Loss (%) in Control and Wagyu loin steaks

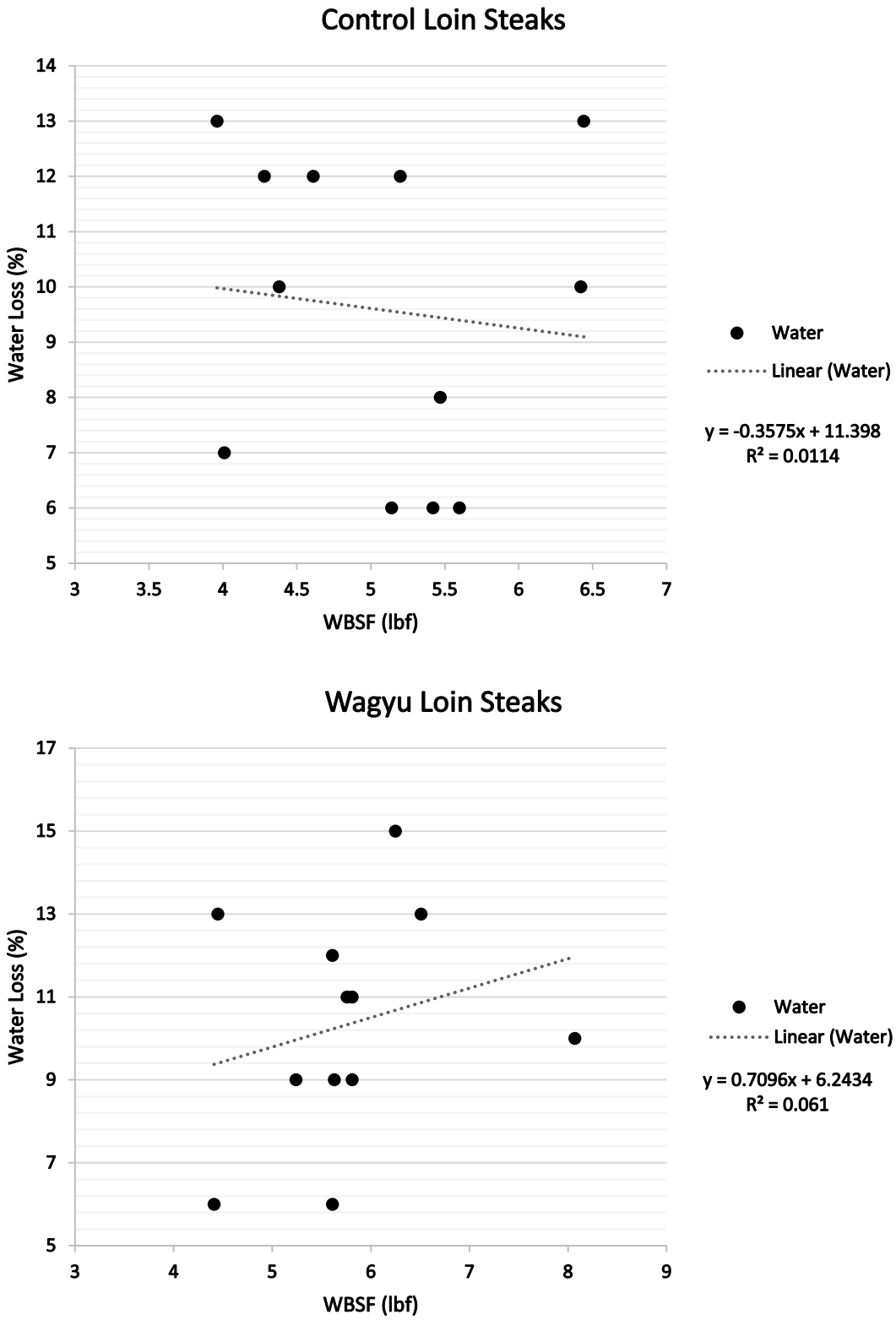


Figure 20. Relationship between WBSF and Water Loss (%) in Control and Wagyu loin steaks

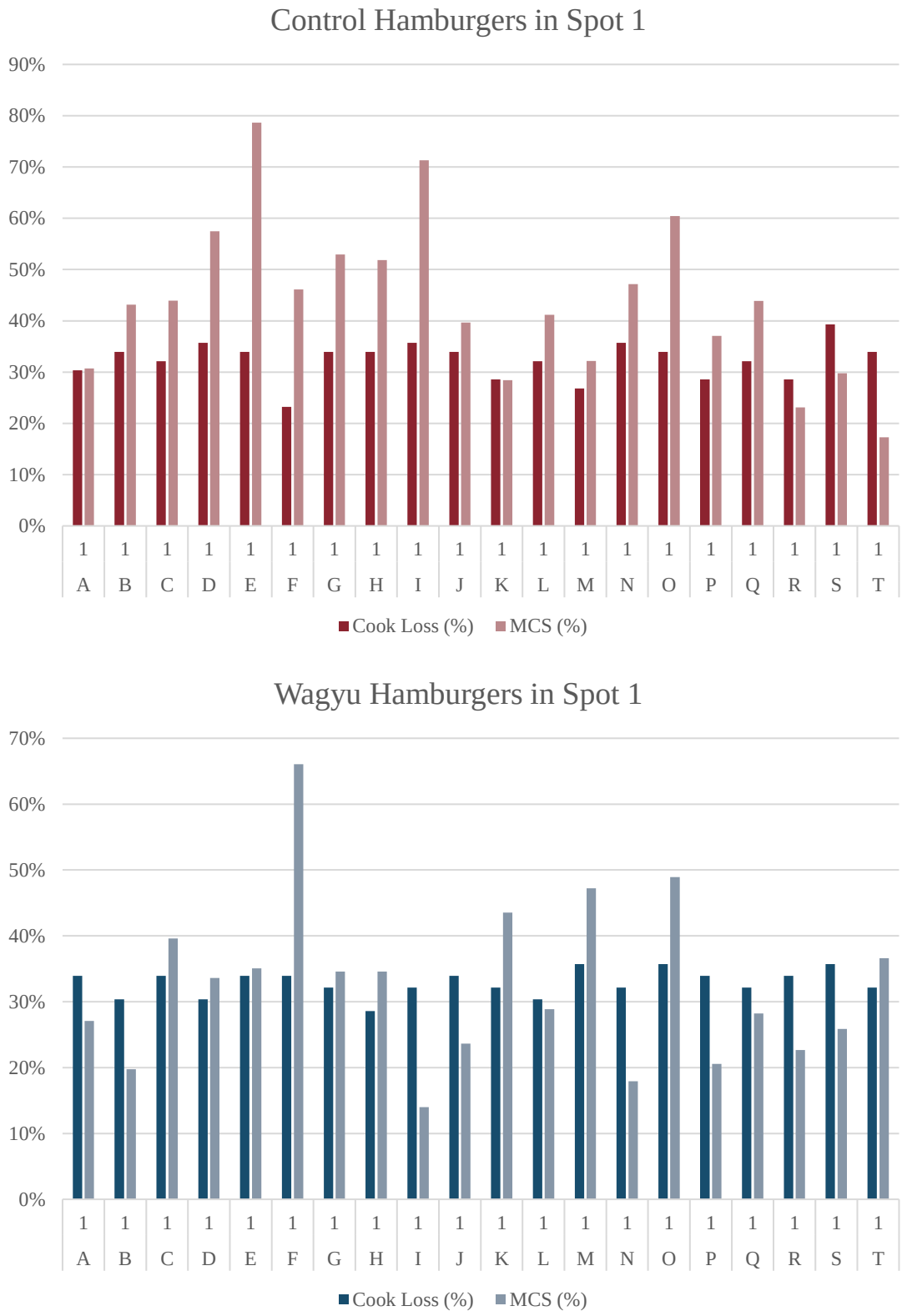


Figure 21. Cooking Loss (%) and muscle shrink (%) in Control and Wagyu burgers cooked in spot 1

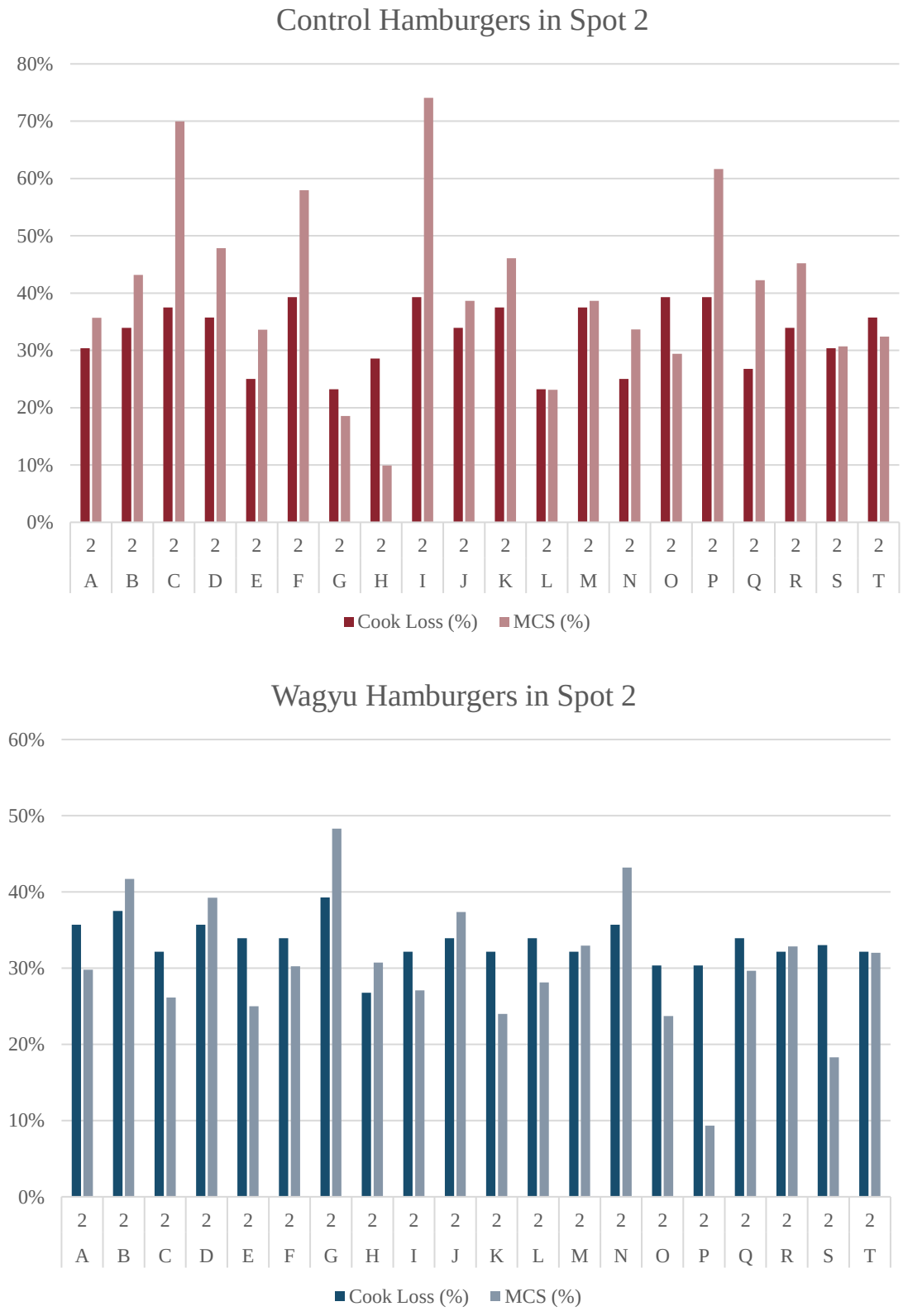


Figure 22. Cooking loss (%) and muscle shrink (%) of Control and Wagyu burgers cooked in spot 2

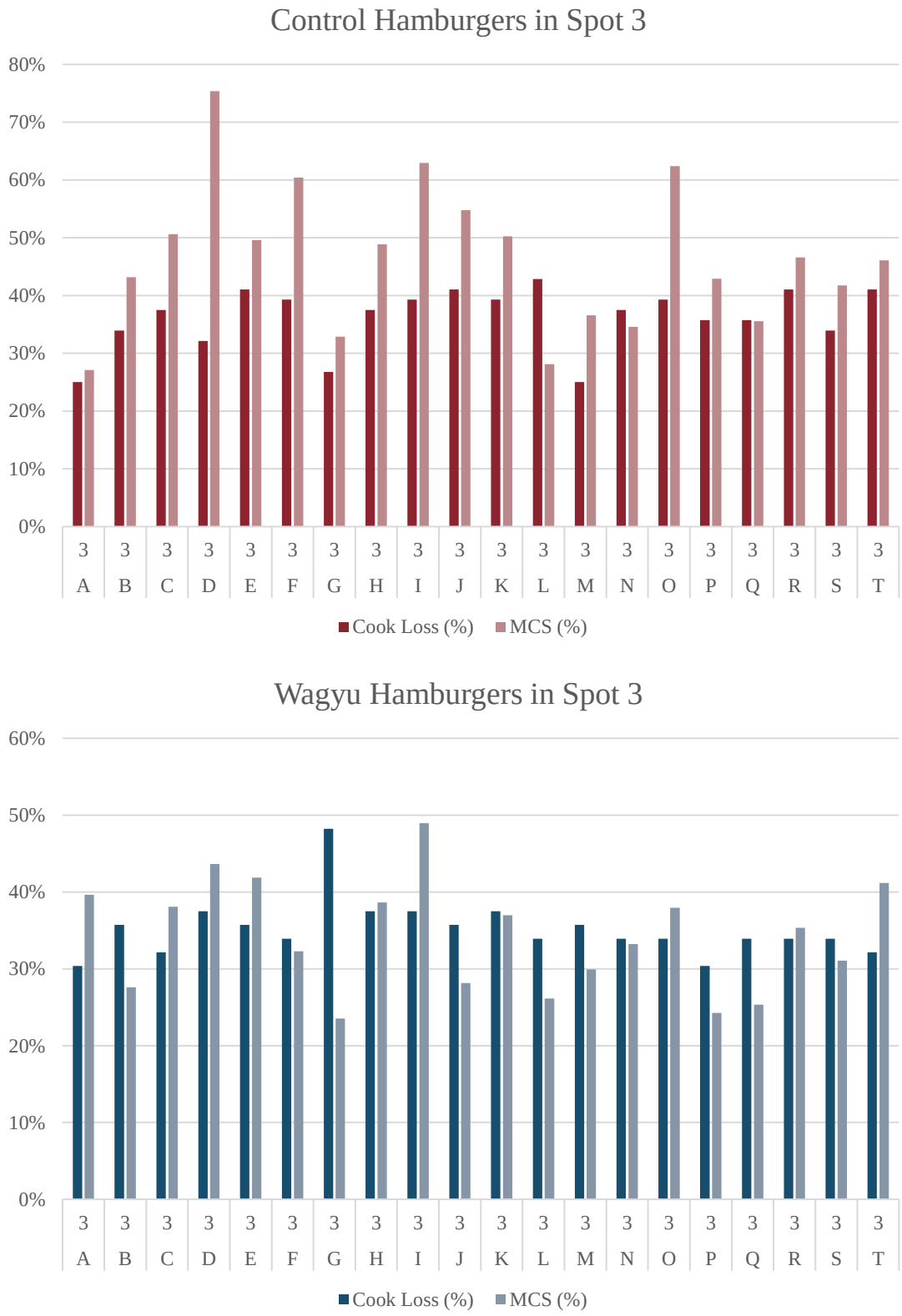


Figure 23. Cooking loss (%) and muscle shrink (%) in Control and Wagyu burgers cooked in spot 3

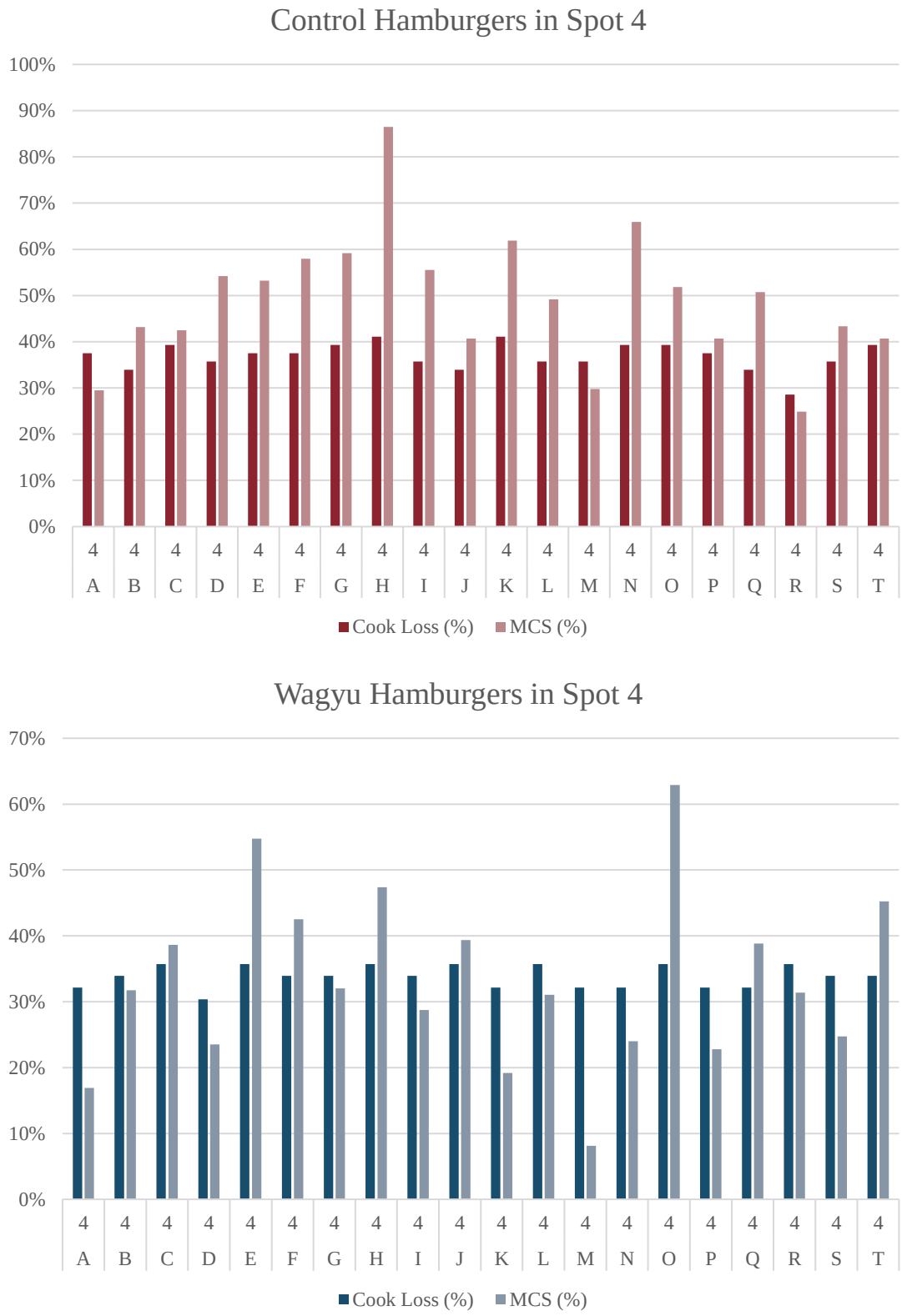


Figure 24. Cooking loss (%) and muscle shrink (%) in Control and Wagyu burgers cooked in spot 4

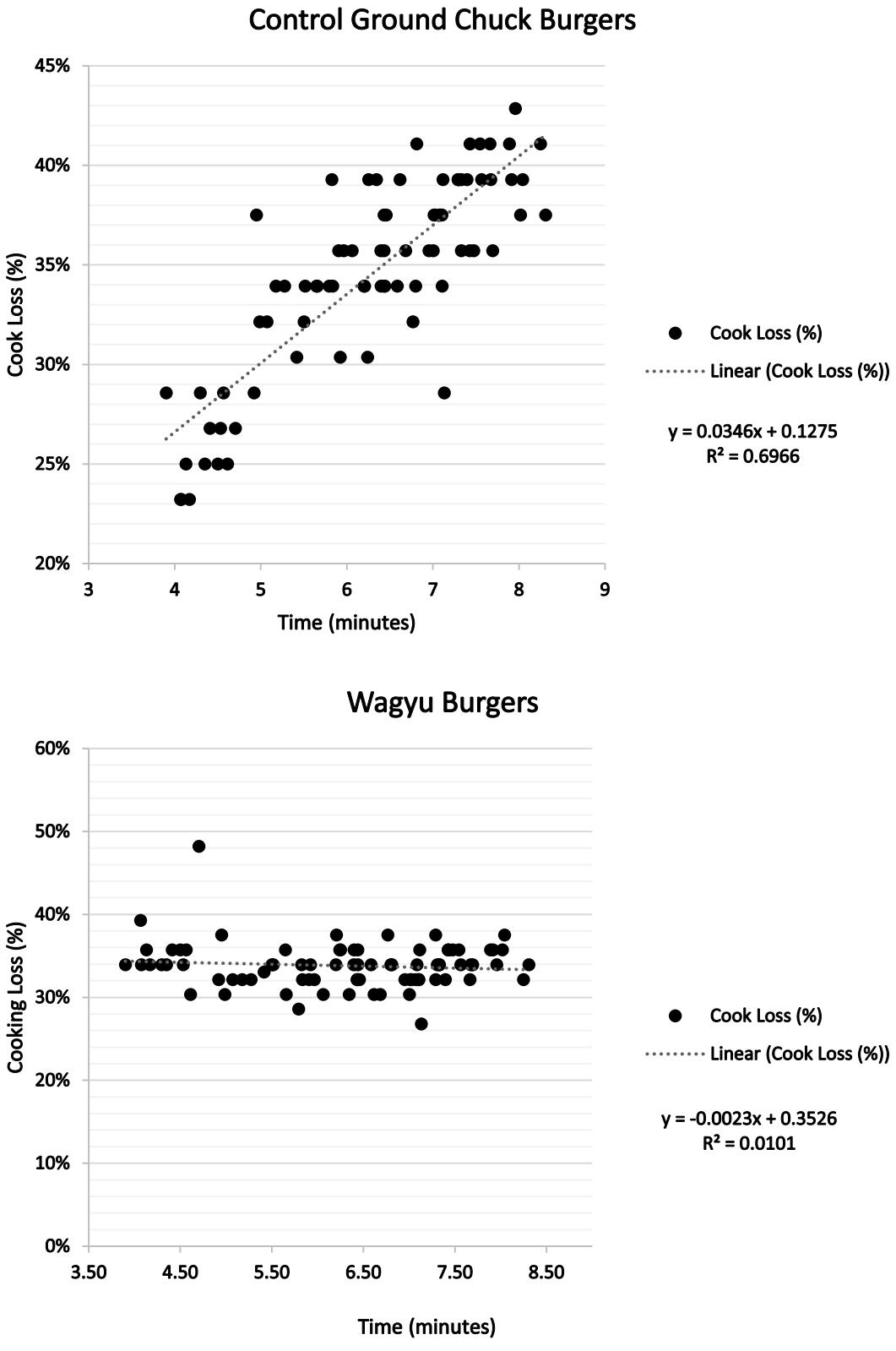


Figure 25. Relationship between cooking time and cooking loss for Control and Wagyu burgers

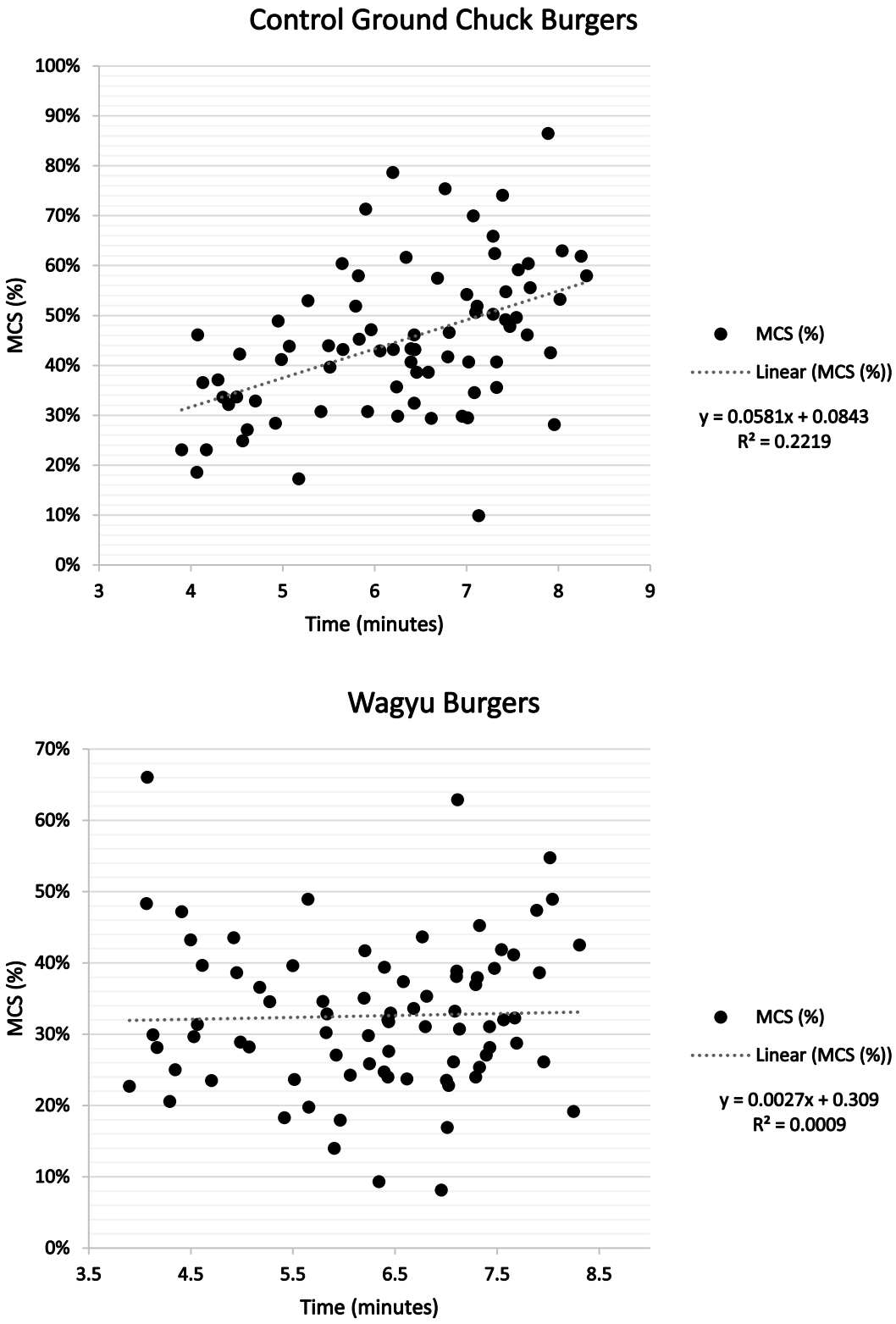


Figure 26. Relationship between time and muscle shrink in Control and Wagyu burgers

V. DISCUSSION

The proposed conclusions presented in this paper are derived from loin steak and burger results from both sources in coordination with literature reviewed for this study. Data gathered from loin steaks and burgers from both beef sources were used to address the following topics: water form loss, ionic strength and WHC.

Water Form Loss

As mentioned previously, water in meat exists in three forms: bound, immobilized and free. Bound water represents approximately 5% of water found in muscle with the other 95% is an un-known ratio of free and immobilized water (Bertram et al., 2002). This study was designed to predict relative estimates of water form ratios rather than absolute values. Since bound water is not significantly impacted by rigor-mortis, ageing or cooking processes, this form of water is not discussed in this section. Instead, water form loss will be discussed as a free water to immobilized water ratio. To do this, water quality characteristics must be examined. In this study, the EC of raw and cooked meat, cooking loss, water loss and shrinkage where the parameters chosen to investigate water form losses in Wagyu and control beef. Wagyu loin steaks had a higher EC value in raw steaks (8.14 µS± 3.51) when compared to control steaks (3.12µS±3.21). A higher EC value in Wagyu raw loin steaks indicates that the Wagyu steaks contained more free water and/or ions. During the rigor mortis process, the concentration of extracellular divalent cations (such as calcium and magnesium) increase to approximately three times the quantity normally found in living tissue as the pH drops (Puolanne & Halonen, 2010). Ions such as calcium and magnesium that are increasing in quantity during rigor mortis fall into the kosmotropic category (Fig.1). These cations support low-density, ordered

water which tends to be more stable, less mobile and has a hydrating effect on proteins. If the concentration of kosmotropes is relatively high, an interaction forms between water and ions and water results in adopting charges from the ions (Puolanne & Halonen, 2010).

The differences seen between raw and cooked beef EC also suggest that Wagyu loin steaks had more free water prior to cooking. Control steaks had a lower EC before cooking when compared with Wagyu steaks, but after cooking control steaks had numerically higher EC values when compared to Wagyu steaks. This seems to indicate that prior to cooking, control steaks had more immobilized water than free water and during cooking the immobilized water changed forms and became free water. In Wagyu meat, the opposite was seen where EC was higher prior to cooking and decreased in value after cooking. This combined with the fact that Wagyu steaks had a numerically lower EC of cooked samples compared to control steaks indicates that Wagyu steaks had more free water than immobilized and the shifting of immobilized to free water was less severe. From this, it seems likely that Wagyu steaks had a higher concentration of ions relative to control loin steaks.

Cooking loss percentages and cooked weights are useful for determining water holding capacity, but their values also represent fat and small fragments of meat that are lost during cooking. In this study, runoff that resulted from cooking burgers on a table-top grill was collected so that fat could be separated from water present in the drippings. The weight of fat was measured and the amount of water lost during cooking was estimated from that value. The amount of run-off/drippings was similar in both sources, but control run-off/drippings contained a higher amount of fat compared to the fat

collected from Wagyu burger run-off/drippings. Though Wagyu burgers lost more water, cooking losses and shrinkage percentages were still lower than what was seen in control burgers. It seems likely that Wagyu burgers had a higher amount of free water loss and a lower loss of the other less mobile forms of water.

Ionic Strength

Ionic strength is a measurement of ion concentration and is directly related to the electrical conductivity of meat, the ions have an electrical charge of their own or their charge can be reflected by the overall electrical conductivity of the water due to the interaction between kosmotropic ions (i.e. calcium, magnesium) and water. To examine ionic strength, this study analyzed EC of raw and cooked loin steaks. The differences seen between the two sources of beef were used to ascertain relative ionic strengths. The control and Wagyu steaks had significantly different raw EC values ($P < 0.01$) where Wagyu EC values were higher. Having a significant difference between control and Wagyu loin steak raw EC values indicates that prior to cooking, Wagyu loin steaks had a higher ionic strength. After steaks were cooked the difference in electrical conductivity between the two types of beef diminished and become less significant. A significant difference in EC values for raw control and Wagyu loin steaks indicates a significant difference in ionic strength in the two different sources of beef.

Water Holding Capacity

A prediction of relative WHC was made by examining EC of raw and cooked samples, estimated water loss, cooking loss and muscle shrink from both sources of beef. Though Wagyu burgers lost more water during the cooking process than control burgers and had a slightly larger difference in raw and cooked EC values, Wagyu had lower

cooking loss percentages in loin steaks and burgers. Muscle shrinkage disrupts water distribution in muscle tissue by reducing the space that holds approximately 80% of all water in the muscle. As the space contracts during the cooking process, the immobilized water that normally resides there will be released and will have changed from immobilized water to free water. When comparing the muscle shrink percentages from both sources of beef, Wagyu burgers exhibited lower muscle shrinkage percentages than the control burgers. With a relatively lower muscle shrink percentage, it can be postulated that the water in Wagyu beef did not change forms as extensively as water changed forms in control beef. From this, water can be characterized as being less mobile and more stable. This description also represents low-density, ordered water that has a high concentration of divalent cations (i.e. kosmotropes such as calcium and magnesium). This conclusion is also supported by differences seen in EC of raw samples from both sources of beef discussed in the Water Form Loss section of this chapter. The characterization of water as less mobile and more stable supports the claim that the Wagyu steaks had a higher concentration of kosmotropes and also suggests that the Wagyu meat had a better WHC than the control steaks when this characterization is considered with other quality traits such as cooking loss and water loss.

LITERATURE CITED

Aberle, E. D., Forrest, J. C., & Gerrard, D. E. (2001). *Principles of Meat Science* (4th ed.). Kendall Hunt Pub Co.

Apple, J. K. (2007). Effects of nutritional modifications on the water-holding capacity of fresh pork: A review. *Journal of Animal Breeding and Genetics*, *124*(SUPPL. 1), 43–58. https://doi.org/10.1111/j.1439-0388.2007.00686.x

Apple, J. K., Davis, J. R., Rakes, L. K., Maxwell, C. V., Stivarius, M. R., & Pohlman, F. W. (2001). Effects of dietary magnesium and duration of refrigerated storage on the quality of vacuum-packaged, boneless pork loins. *Meat Science*, *57*(1), 43–53. https://doi.org/10.1016/S0309-1740(00)00075-9

Barbera, S., & Tassone, S. (2006). Meat cooking shrinkage: Measurement of a new meat quality parameter. *Meat Science*, *73*(3), 467–474. https://doi.org/10.1016/j.meatsci.2006.01.011

Bass, P. D., Scanga, J. A., Chapman, P. L., Smith, G. C., Tatum, J. D., & Belk, K. E. (2014). Recovering value from beef carcasses classified as dark cutters by United States Department of Agriculture graders 1, (2005), 1658–1668. https://doi.org/10.2527/jas.2007-0688

Bertram, H. C., Purslow, P. P., & Andersen, H. J. (2002). Relationship between meat structure, water mobility, and distribution: A low-field nuclear magnetic resonance study. *Journal of Agricultural and Food Chemistry*, *50*(4), 824–829. https://doi.org/10.1021/jf010738f

Bond, J. J., & Warner, R. D. (2007). Ion distribution and protein proteolysis affect water holding capacity of Longissimus thoracis et lumborum in meat of lamb subjected to antemortem exercise. *Meat Science*, *75*(3), 406–414. https://doi.org/10.1016/j.meatsci.2006.08.005

Byrne, C. E., Troy, D. J., & Buckley, D. J. (2000). Postmortem changes in muscle electrical properties of bovine M. longissimus dorsi and their relationship to meat quality attributes and pH fall. *Meat Science*, *54*(1), 23–34. https://doi.org/10.1016/S0309-1740(99)00055-8

Calvo, L., Toldrá, F., Aristoy, M. C., López-Bote, C. J., & Rey, A. I. (2016). Effect of dietary organic selenium on muscle proteolytic activity and water-holding capacity in pork. *Meat Science*, *121*, 1–11. https://doi.org/10.1016/j.meatsci.2016.05.006

Cheng, Q., & Sun, D.-W. (2008). Factors affecting the water holding capacity of red meat products: a review of recent research advances. *Critical Reviews in Food Science and Nutrition*, *48*(2), 137–59. https://doi.org/10.1080/10408390601177647

Devine, C., Wells, R., Lowe, T., & Waller, J. (2014). Pre-rigor temperature and the relationship between lamb tenderisation, free water production, bound water and dry matter. *Meat Science*, *96*(1), 321–326. https://doi.org/10.1016/j.meatsci.2013.07.024

Dorvee, J. R., & Veis, A. (2013). Water in the formation of biogenic minerals: Peeling away the hydration layers. *Journal of Structural Biology*, *183*(2), 278–303. https://doi.org/10.1016/j.jsb.2013.06.007

Gruber, S. L., Tatum, J. D., Engle, T. E., Chapman, P. L., Belk, K. E., & Smith, G. C. (2010). Relationships of behavioral and physiological symptoms of preslaughter stress to beef longissimus muscle tenderness. *Journal of Animal Science*, *88*(3), 1148–1159. https://doi.org/10.2527/jas.2009-2183

Guignot, F., Vignon, X., & Monin, G. (1993). Post mortem evolution of myofilament spacing and extracellular space in veal muscle. *Meat Science*, *33*(3), 333–347. https://doi.org/10.1016/0309-1740(93)90005-3

Hoffman, L. C., Vermaak, A., & Muller, N. (2012). Physical and chemical properties of selected beef muscles infused with a phosphate and lactate blend. *South African Journal of Animal Sciences*, *42*(4), 317–340. https://doi.org/10.4314/sajas.v42i4.1

Hudson, N. J. (2012). Mitochondrial treason: A driver of pH decline rate in post-mortem muscle? *Animal Production Science*, *52*(12), 1107–1110. https://doi.org/10.1071/AN12171

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science*, *71*(1), 194–204. https://doi.org/10.1016/j.meatsci.2005.04.022

Huff Lonergan, E., Zhang, W., & Lonergan, S. M. (2010). Biochemistry of postmortem muscle - Lessons on mechanisms of meat tenderization. *Meat Science*, *86*(1), 184–195. https://doi.org/10.1016/j.meatsci.2010.05.004

Hughes, J. M., Oiseth, S. K., Purslow, P. P., & Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science*. https://doi.org/10.1016/j.meatsci.2014.05.022

Kreikemeier, K. K., Unruh, J. A., & Eck, T. P. (1998). Factors Affecting the Occurrence of Dark-Cutting Beef and Selected Carcass Traits in Finished Beef Cattle. *Journal of Animal Science*, *76*(2), 388–395.

Lee, S., Norman, J. M., Gunasekaran, S., van Laack, R. L. J. M., Kim, B. C., & Kauffman, R. G. (2000). Use of electrical conductivity to predict water-holding capacity in post-rigor pork. *Meat Science*, *55*(4), 385–389. https://doi.org/10.1016/S0309-1740(99)00166-7

Lepetit, J., Salé, P., Favier, R., & Dalle, R. (2002). Electrical impedance and tenderisation in bovine meat. *Meat Science*, *60*(1), 51–62. https://doi.org/10.1016/S0309-1740(01)00104-8

Montgomery, J. L., Blanton, J. R., Horst, R. L., Galyean, M. L., Morrow, K. J., Miller, M. F., & Wester, D. B. (2004). The online version of this article , along with updated information and services , is located on the World Wide Web at : Effects of biological type of beef steers on vitamin D , calcium , and phosphorus status 1, 2043–2049.

Muchenje, V., Dzama, K., Chimonyo, M., Strydom, P. E., Hugo, A., & Raats, J. G. (2009). Some biochemical aspects pertaining to beef eating quality and consumer health: A review. *Food Chemistry*, *112*(2), 279–289. https://doi.org/10.1016/j.foodchem.2008.05.103

Offer, G., Knight, P., Jeacocke, R., Almond, R., Cousins, T., Elsey, J., … Purslow, P. (1989). The structural basis of the water-holding, appearance and toughness of meat and meat products. *Food Microstructure*, *8*,(1), 151–170.

Offer, G., & Trinick, J. (1983). On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. *Meat Science*, *8*(4), 245–281. https://doi.org/10.1016/0309-1740(83)90013-X

Ouali, A., Herrera-Mendez, C. H., Coulis, G., Becila, S., Boudjellal, A., Aubry, L., & Sentandreu, M. A. (2006). Revisiting the conversion of muscle into meat and the underlying mechanisms. *Meat Science*, *74*(1), 44–58. https://doi.org/10.1016/j.meatsci.2006.05.010

Pearce, K. L., Rosenvold, K., Andersen, H. J., & Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes - A review. *Meat Science*. https://doi.org/10.1016/j.meatsci.2011.04.007

Põldvere, A., Tänavots, A., Saar, R., Sild, S., & Lepasalu, L. (2016). Effect of heat treatment at constant 120 °C temperature on the rheological and technological properties of pork. *Agronomy Research*, *14*(SpecialIssue 2), 1434–1441.

Puolanne, E., & Halonen, M. (2010). Theoretical aspects of water-holding in meat. *Meat Science*, *86*(1), 151–165. https://doi.org/10.1016/j.meatsci.2010.04.038

Purslow, P. P. (2005). Intramuscular connective tissue and its role in meat quality. *Meat Science*, *70*(3 SPEC. ISS.), 435–447. https://doi.org/10.1016/j.meatsci.2004.06.028

Purslow, P. P., Oiseth, S., Hughes, J., & Warner, R. D. (2016). The structural basis of cooking loss in beef: Variations with temperature and ageing. *Food Research International*, *89*, 739–748. https://doi.org/10.1016/j.foodres.2016.09.010

Reardon, W., Mullen, A. M., Sweeney, T., & Hamill, R. M. (2010). Association of polymorphisms in candidate genes with colour , water-holding capacity , and composition traits in bovine M . longissimus and M . semimembranosus. *MESC*, *86*(2), 270–275. https://doi.org/10.1016/j.meatsci.2010.04.013

Salway, J. G. (2004). *Metabolism at a Glance*. (V. Noyes, G. Jeffers, J. Rowley, & K. Charman, Eds.) (Third). Oxford: Blackwell Publishing.

Schäfer, A., Rosenvold, K., Purslow, P. P., Andersen, H. J., & Henckel, P. (2002). Physiological and structural events post mortem of importance for drip loss in pork. *Meat Science*, *61*(4), 355–366. https://doi.org/10.1016/S0309-1740(01)00205-4

Scheffler, T. L., Matarneh, S. K., England, E. M., & Gerrard, D. E. (2015). Mitochondria influence postmortem metabolism and pH in an in vitro model. *Meat Science*, *110*, 118–125. https://doi.org/10.1016/j.meatsci.2015.07.007

Toldra, F. (2011). Muscle foods: water, structure and functionality. *Main*, *9*(3), 173–177. https://doi.org/10.1177/108201303035048

Tornberg, E. (2005). Effects of heat on meat proteins - Implications on structure and quality of meat products. *Meat Science*, *70*(3 SPEC. ISS.), 493–508. https://doi.org/10.1016/j.meatsci.2004.11.021

Traore, S., Aubry, L., Gatellier, P., Przybylski, W., Jaworska, D., Kajak-Siemaszko, K., & Santé-Lhoutellier, V. (2012). Higher drip loss is associated with protein oxidation. *Meat Science*. https://doi.org/10.1016/j.meatsci.2011.11.033

U.S. Department of Agriculture, A. R. S. (2017). USDA National Nutrient Database for Standard Refernce, Release 28. Retrieved January 25, 2017, from http://www.ars.usda.gov/nutrientdata