

EVALUATION OF ALKALINE ELECTROLYZED REDUCED WATER TO REPLACE TRADITIONAL PHOSPHATE ENHANCEMENT SOLUTIONS

by

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(Under the Direction of Alex Stelzleni)

ABSTRACT

Two studies were conducted to evaluate the use of alkaline electrolyzed reduced water (AEW) as an enhancement solution for the replacement of traditional salt and phosphate solutions. The first study examined the effects of AEW on the water-holding capacity and sensory traits of pork loin chops when enhanced to 110% of green weight. The use of AEW did not improve the water-holding capacity or sensory traits of pork chops when compared to traditional enhancement solutions. Study 2 evaluated the use of AEW and its effects on color and lipid oxidation during retail display. Loins enhanced with AEW only did not possess similar color characteristics compared to non-enhanced loins or loins enhanced with an industry standard solution. Additionally, chops enhanced with AEW alone had greater lipid oxidation than all other treatments. Enhancement of pork chops with AEW is not an effective replacement for salt/phosphate solutions.

KEYWORDS: alkaline enhancement, pork, water holding capacity, shelf life

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B. S., California State University, Fresno, 2013

A Thesis Submitted to the Graduate Faculty of The University Of Georgia in Partial
Fulfillment of the Requirements of the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2015

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DEDICATION

The dedication of this work is to my father and mother, whose continued work and dedication to my success is unrivaled. Their support of me in all avenues of my life does not go unnoticed. Without the drive and passion for perfection of my father, as well as the mental strength and never quit attitude of my mother, I would never have been able to accomplish this feat. I owe you the world and will never be able to thank you for what you do for me.

ACKNOWLEDGEMENTS

I would like to thank the Boss-man himself, Dr. Alex Stelzleni; working with you on my Masters of Science degree has been an absolute pleasure. Under your direction I have learned more than just meat science, and for that I cannot thank you enough. I have truly enjoyed our conversations, and discussions about current topics as well as our current and future research. I would like to thank you for pushing me and challenging me every step of the way.

To my committee, Dr. Dean Pringle, and Dr. Yen-Con Hung, thank you very much for your outstanding help, as you have been more than available for my help when called upon. I look forward to working with you both in the future. I would also like to acknowledge Ms. Gina McKinney for intangible help in the lab. Your aid, and guidance in lab techniques and procedures is greatly appreciated.

My fellow cube farmers, Elyse Ford, Rebecca Kersey, Samantha Belanger, Matt Studstill, Jenna Lacey, Rachael Detweiler, Abby Jones, Caitlyn Haines and Cheyenne Thomas, your constant support and encouragement through the hardest of times is greatly appreciated. To Ryan Crowe, you have always been a cheerful attitude that I look forward to seeing each day. Dr. Kari Turner, you have been a wonderful friend to me through this process, always encouraging me and enabling me to keep strong and work hard. I am forever grateful for your friendship and look forward to working with you in the future. Lastly, Ms. Robin, and Ms. Terry for your constant support and encouragement. Thank you all!

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CHAPTER 1

INTRODUCTION

Moisture enhancement is used in today's pork industry to improve quality and maintain palatability perceptions (Holmer, Killefer, & McKeith, 2008). Consumers develop expectations about the quality of a meat product long before they eat it based on the quality cues available to them at retail (Bredahl, Grunert, & Fertin, 1998). Intrinsic quality cues include color, marbling, and to a lesser extent, the amount of moisture in the product (Bredahl et al., 1998). However, upon eating, consumers relate pork quality to tenderness, intramuscular fat, and final cooking temperature (Moeller, 2010). Davis and coworkers (1975) found that the greatest consumer satisfaction came from cooked chops with a high reflectance at 685 nm, low expressible juice, high pH, low moisture, and a high fat content. Reflectance of pork at 685 nm was found to be the best predictor of pork color as it related to consumer acceptance (Ockerman & Cahill, 1969). Moeller confirmed that tenderness, juiciness, and color, along with flavor are major aspects of pork quality (2010).

One of the most important goals of the pork industry is to produce a product that is profitable for the packer, processor, and retailer (Brewer, Jensen, Prestat, & Zhu, 2002). Pork enhancement is a method of adding weight to a product, making it more profitable, while simultaneously improving quality. It was stated that pork enhancement is not a method to improve low quality pork, but rather a method, used primarily in the

US, to improve overall quality of fresh pork (Hayes, Desmond, Troy, Buckley, & Mehra, 2006). Traditional enhancement solutions may include different types of phosphates, salts, lactates, and other ingredients to increase the moisture, sensory attributes, and consumer acceptability of whole muscle meat products (Brewer et al., 2002; Prestat, Jensen, Robbins, et al., 2002; Sutton, Brewer, & McKeith, 1997). Brewer and coworkers (2002) suggested that even though consumers buy enhanced pork products, they are increasingly concerned with ingredients such as phosphate and salt. Therefore clean label alternative ingredients that function similarly to traditional enhancement solutions are of value to both the pork industry and consumers. The objectives of this research were to evaluate alkaline electrolyzed reduced water as a clean label alternative to industry standard enhancement solutions for moisture retention, eating quality, and shelf life properties.

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CHAPTER 2

THE REVIEW OF LITERATURE

2.1 History Of Enhancement

Consumer acceptance of meat products in today's market is of the utmost importance to a successful meat business. With consumer demands for products that are both high quality and wholesome, meat processors are utilizing technology to ensure consumer satisfaction. Since the 1970's the poultry industry has been using enhancement with water, salt, and phosphates to improve eating quality of whole muscle, breast fillets (Brotsky, 1976; Robbins, Jensen, Ryan, Homco-Ryan, McKeith & Brewer, 2002). Since then the pork industry has incorporated enhancement technology to improve flavor and overall eating experience (Brewer, Jensen, Prestat, & Zhu, 2002; Prestat, Jensen, McKeith, & Brewer, 2002). Most enhancement solutions contain water, salt, phosphate, and/or flavor enhancers and shelf life extenders (Prestat et al., 2002; Robbins et al., 2002). Phosphates are well known to enhance the water-holding capacity, improve color, and improve cook yields of meat products (Prestat et al., 2002). Research on novel enhancement solutions is a growing field of study for the meat industry as they continually aim to satisfy consumer demands.

2.2 Traditional Enhancement Solutions

As noted previously, traditional enhancement solutions contain ingredients such as salt and phosphates, and may include flavor enhancers and shelf life extenders. It is

noteworthy that salt in conjunction with phosphates creates a synergistic effect on water-holding capacity, where alkaline phosphates are used primarily for the decrease in purge, and overall water loss, and salt is used to shift the pH water holding capacity curve increasing water holding capacity (Hamm, 1960; Romans, Costello, Carlson, Greaser, & Jones, 1994).

Phosphates used alone can increase yields by approximately 10%, and when used with other ingredients such as sodium and potassium salts, greater yields can be realized (Romans et al., 1994). Many different types of phosphates are available and many processors use blends of these phosphate groups for their different attributes such as, protein solubility, and buffering capacity (Long, Gál, & Buňka, 2014). Monophosphates are used as buffers to help change the meat's pH and aid in fresh meat color retention, while diphosphates are effective for the sequestration of Ca^{2+} and Mg^{2+} and thus aid in the separation of actomyosin after rigor mortis allowing for greater protein swelling (Long et al., 2014). Long chain phosphates are less effective on protein functionality but add to the solubility of the phosphate blends used in fresh meat (Long et al., 2014). Furthermore, the antioxidant properties of phosphates aid in the stabilization of color as well as the prevention of lipid oxidation, at levels at or below the legal phosphate inclusion limit of 0.5% (Romans et al., 1994).

Shelf life extenders and flavor enhancers such as potassium lactate aid in the prevention of oxidation, improve flavor, and have bactericidal properties. Inclusion of lactates in enhancement solutions can improve color and color stability during retail display when compared to enhancement with salt and phosphate solutions. Lactates work to restore NADH pools via lactate dehydrogenase enzymes postmortem (Lawrence,

Dikeman, Hunt, Kastner, & Johnson, 2004). Researchers have also investigated lactate salts as antimicrobials. Lactate salts work as an antimicrobial due to their lipophilic properties and their ability to cross cell membranes undissociated (de Wit & Rombouts, 1990). Once in the cell they dissociate due to the internal pH, creating a lower pH in the cell and a greater concentration of anions, thus disrupting cell function (de Wit & Rombouts, 1990). Lawrence et al. (2004) showed that lactate-enhanced beef strip loin steaks had improved beef flavor when compared to salt/phosphate enhancement solutions. Lactates, when added to salt/phosphate enhancement solutions, in pork have shown to improve pork flavor, increased tenderness, and increased juiciness compared to salt/phosphate enhancement solutions alone, suggesting that lactates enhance shelf life, from a color and microbial standpoint, as well as sensory properties (Jensen, Robbins, et al., 2003).

2.3 Water Holding Capacity:

Meat is comprised of 45 to 80% water (Romans et al., 1994). There are many things that can alter the percent of water in meat including the following: live animal composition, enhancement, drying, and cooking. However unless 100% desiccation is achieved, water is still present during mastication (Romans et al., 1985). The ability of meat to hold water is referred to as its water-holding capacity. Many of the physical properties commonly associated with meat quality, such as color, texture, firmness, juiciness, and tenderness are directly related to the amount and state of water within the meat system (Aberle, Forrest, Gerrard, & Mills, 2001). The loss of water due to a decrease in water holding capacity is not only a quality issue but one of economic importance. Increasing the water holding capacity and decreasing purge during cold

storage gives the industry more salable weight while improving the quality and eating experience for the end user. While purge, or water loss, is costly (Hamm, 1960), there are many ways to influence the water holding capabilities of a meat product. Water chemistry, ionic strength of enhancement solutions, meat pH, and the swelling of myofibrils are the traits that are manipulated when enhancing pork loins. However, ultimately changes in protein charges and myofibrillar structure are responsible for changes in water holding capacity (Hamm, 1960).

Based on water chemistry, there are three forms of water that are found in meat systems. These forms, bound, immobilized, and free water, are described by the nature in which they are bound in the meat system. The first form is the base layer of water, sometimes called bound water, or hydration water (Aberle et al., 2001). Hydration water is described by Hamm (1960) as the water molecules that are tightly bound to proteins at a concentration of no more than 0.5 g H₂O/g of protein and comprises about 5% of the total water in the meat system (Aberle et al., 2001; Offer & Trinick, 1983; Ranken, 1976). Hamm (1960) stated that changes in the water-holding capacity of meat are mainly due to actin and myosin or their complex, actomyosin, while others (Offer & Trinick, 1983) believe that globular proteins can also influence water retention in meat. Water molecules that are tightly bound are associated with the polar amino acid residues and when the structure of the sarcomere is changed bound water is hardly influenced (Aberle et al., 2001; Hamm, 1975). Hamm (1960) further clarifies that the base layer of water molecules are bound tightly to hydrophilic residues and when not in a suspension or solution the hydrophilic residues create a monomolecular layer of water hydrating each protein (Hamm, 1960). There are two types of hydrophilic groups that are responsible for

the strong binding of water to proteins: the polar groups of protein side chains, and the carboxyl and amino groups of peptide bonds. Furthermore, the properties of bound water molecules are slightly different than that of other chemical forms of water. Hydration water has a lower freezing point, lower vapor pressure, and lower dissolving power than that of normal water (Hamm, 1960).

Due to the polar nature of water molecules, other water molecules are attracted to and interact with the monolayer water molecules. These water molecules continue their attraction to each other, while diminishing binding strength as more layers are added to the hydration layer (Aberle et al., 2001). This layer constitutes the second type of water found in meat systems, referred to as immobilized water. Immobilized water is highly ordered in the meat system and is not affected by low or light pressure (Hamm, 1975). There is no distinct line that separates the immobilized water from other classifications of water remaining (Aberle et al., 2001; Hamm, 1975). Ranken (1976) referred to immobilized water as firmly bound water and estimated this layer accounts for approximately 40%-60% of the total water in meat. While the immobilized water is bound to the hydration water it is also organized in the protein filaments and the network of cellular protein membranes, as well as, by the electrostatic forces between polypeptide chains and cross linkages (Hamm, 1960). Fluctuations in the amount of immobilized water in meat systems are ultimately due to changes in protein charges and the protein structure of the myofilament (Hamm, 1960). Even though these concepts do not explain the immobilization of water in muscle (Hamm, 1960), a study proved that the immobilization of water is in fact due to the muscle structure and the solubility of myofibrillar proteins (Offer & Trinick, 1983). As the salt concentration of the muscle cell

is increased from 0.1M NaCl to 1.0M NaCl nearly the entire A-band is extracted and solubilized while the I-band is partially solubilized. Sarcomere length is unchanged while the sarcomere diameter is increased 2.8 times. Assuming the sarcomere to be cylindrical, that equated to an eightfold increase in volume allowing water to infiltrate the cell and remain bound to the solubilized A-band proteins (Offer & Trinick, 1983). The remaining portion of water in meat is referred to as free or loosely bound water (Ranken, 1976). The free water of meat systems is held in the protein structure by weak surface forces (Aberle et al., 2001). Free water is released through purge from the opened ends of fibers that expose the extra cellular space (Ranken, 1976) and light forces.

The pH of a meat system can be used as a mechanism for increasing water holding capacity. Raising or lowering the pH of the meat system away from the isoelectric point (pH at which a protein has no net charge) of the muscle proteins can have a positive influence on water holding capacity (Hamm, 1975). When moving away from the isoelectric point on either the basic or the alkaline side, small changes in pH give rise to relatively large changes in water holding capacity (Hamm, 1960). Although it is commonly thought that the pH of a meat system directly affects the water holding capacity, it is in fact the pH changing the structure of the myofibrillar proteins via charge repulsion, causing a swelling of the myofibrillar network that allows more water to be held (Hamm, 1960; Offer & Trinick, 1983). When the pH of the meat system is at the isoelectric point, the net charge on the myofilament proteins is approximately zero. If the pH was to change to a more alkaline environment, the amino acid side chains would become less dissociated making the overall charge more positive (Campbell & Farrell, 2012). The positive charges between the myofilament proteins would cause repulsion and

thus a swelling. A similar affect is seen when the pH of the system is made more acidic, the amino acid side chains lose their protons and the protein becomes more negatively charged (Campbell & Farrell, 2012). Like the alkaline portion, the acidic system creates negatively charged myofilaments that repel each other and swell the fiber (Campbell & Farrell, 2012; Offer & Trinick, 1983). The pH-hydration curve as described by Hamm (1959) is however subject to change (Hamm, 1960). There are several phase shifts that change or distort the curve causing more or less water holding capabilities in the same meat system (Hamm, 1960).

There are six different shifts that occur in the pH-hydration curve, two of which increase a portion of the curve, three that decrease a portion of the curve, and one that moves the curve in relation to pH. Shift one occurs when the water holding capacity is increased on the alkaline side of the pH-hydration curve with no alteration in the isoelectric point (Hamm, 1960). This cannot be due to an increase of negative protein charges because it is possible for carboxyl groups of amino acids to lose a hydrogen down as low as pH of 1.71 (Campbell & Farrell, 2012), which would affect the water holding capacity on the acidic end of the spectrum. Hamm (1960) credits this base-wise water holding capacity increase to the cleavage of metallic cross linkages such as Zinc, Magnesium, and Calcium; which would happen by way of ion exchangers eliminating cations. Shift three is an increase in water holding capacity over the entire pH-hydration curve via the destruction of the protein structure (Hamm, 1960), not the cleaving of electrostatic bonds and hydrogen. The quantity of electrostatic and hydrogen bonds is much higher at the isoelectric point than at the acidic and alkaline ends of the pH-hydration curve. If shift three was due to electrostatic and hydrogen bonds the change in

water holding capacity would be much greater at the isoelectric point than at the rest of the curve (Hamm, 1960). The disintegration of the protein structure by enzymatic activity causes peptide bond dissociation allowing the myofibrillar structure to swell.

Shift two is described as a decrease in water holding capacity in the basic spectrum of the isoelectric point (pHi) due to intramolecular bonds (Hamm, 1960). These bonds, important for protein structure and function, only happen in the alkaline portion of the pH-hydration curve. Declining pH immediately postmortem is caused by anaerobic metabolism and as ATP becomes depleted myosin permanently couples with actin to form actomyosin (Romans et al., 1994). These crosslinks prevent the muscle from swelling and cause a decline in water holding capacity for pH values above the pHi. Shift four is a decrease in water holding capacity over the entire range of the pH-hydration curve (Hamm, 1960). The shrinking of the myofiber and thus the loss of hydration water is caused by the formation of new, more stable protein crosslinks, as happens when proteins are denatured or cooked (Hamm, 1960). More stable bonds such as ester, disulfide, and peptide bonds cannot be broken by changing the pH of the system alone (Hamm, 1960). Shift five is the decrease in water holding capacity at the isoelectric point and an increase in the water holding capacity in the basic portion (Hamm, 1960). The decrease in water holding capacity in shift five is due to the formation of weak crosslinks that are present near the isoelectric point but can be broken by alkaline environments.

Shift six has two different shifts associated with similar phenomenon. In the first part of shift six the isoelectric point is moved to lower pH values (Hamm, 1960). This shift is due to the binding of anions by proteins. The binding of anions occurs in the basic, as well as the acidic range of the pH-hydration curve. In the basic end the bonding

of anions to the protein structure causes repulsion between the anions and the deprotonated carboxyl groups allowing for swelling of the myofiber (Hamm, 1960). In the acidic spectrum the binding of anions blocks the positively charged proteins that cause repulsion. The screening of these positively charged proteins tightens the macrostructure of the protein and decreases the water holding capacity (Hamm, 1960). The second cause of a shift six is the shift of the pH-hydration curve to a higher pH by the binding of cations, in the same respect as the opposing shift six (Hamm, 1960). Although these shifts are the explanation to most of water holding capacity changes it is unclear whether the addition of acids or bases cause irreversible changes. However denaturation of myofibrillar proteins are seen at pH values below 4.0 and above 10.0 (Hamm, 1960).

Calcium ions also play a major factor in the overall water holding capacity of a meat system (Hamm, 1960). Calcium ions are responsible for the contraction of the sarcomere in vivo, and similarly play a role in the inextensibility of muscle post rigor (Lawrie, 1966). The presence of calcium ions bonded to the protein structure creates actomyosin formation in the protein microstructure and tightens the intramolecular space, shrinking the fiber, and decreasing the water holding capacity (Hamm, 1960). Calcium ions also cause a shrinking of the myofiber due to the formation of salt bridges in the protein structure (Hamm, 1960).

Raising the water holding capacity is achieved by increasing the intermolecular charges swelling the myofibril and immobilizing more water. This has been demonstrated when pork loins were injected with four different enhancement solutions containing phosphates, salt, and either sodium lactate/acetate, potassium lactate/acetate, or no

lactate/acetate. Chops with the highest pH held the most water (Jensen, Homco-Ryan, et al., 2003) confirming the water holding capacity and pH curve proposed by Hamm (1975). Although Hamm (1975) suggested the relationship of pH and water holding abilities, Ranken (1976) reported that they found no correlation between pH and cook loss when a small sample size was examined. Ranken (1976) however also stated there is likely a correlation between meat ultimate pH and water holding capacity; however, it is likely controlled by the buffering capacity of the meat.

Phosphates, a common enhancement ingredient, are thought to be responsible for a shift in the pH of the meat system in enhanced products, however, even phosphates with a pH in the upper range of 9 to 10 have been shown to have a negligible effect the final pH meat (Ranken, 1976). The buffering capacity of meat is great enough that even using a phosphate in the upper region of the pH scales the final pH of the meat system will only be raised 0.1 to 0.2 pH units (Ranken, 1976). This further proves the theories of Offer and Trinick (1983), the mechanism for meat water holding capacity heavily relies on the solubilization of structural proteins and swelling of the sarcomere structure.

Changing the salt concentration of the meat system can drastically affect the structural components of the sarcomere, shifting of all water types to a more tightly bound state and causing swelling. Elevating the salt concentration of a meat system between 0.8M to 1.0M, or 4.6-5.8%, optimizes the water holding capacity of meat. At a salt concentration of 1.0M NaCl almost all C-protein, troponin, and tropomyosin, as well as some α -actinin, actin, and myosin would be extracted allowing for greater myofibrillar expansion causing a greater amount of water being stored interstitially (Offer & Trinick, 1983). Most meat systems in commerce use 2% or less salt due to consumer detection of

off flavors; however, salts in combination with phosphates have a synergistic effect on water holding capacity (Bendall, 1954; Hamm, 1960, 1975; Offer & Trinick, 1983; Ranken, 1976). Adding 3*mM* potassium phosphate to the meat system allowed for lower salt concentrations (0.3*M* NaCl) needed to attain the necessary protein extraction to maximize water holding (Offer & Trinick, 1983). Looking for an alternative to salt/phosphate enhancement solutions, Cerruto-Noya, VanOverbeke, and DeWitt (2009) found that enhancement with 0.1% ammonium hydroxide at a pH ~ 10 did not raise the pH of the meat system when compared to a phosphate enhancement solution (Cerruto-Noya et al., 2009). The authors further explained that the ammonium hydroxide was not effective because the pKa of phosphates is in the range of 6.1 – 7.1, thus the phosphates provided a stronger buffering agent than the meat itself. With the absence of the phosphate buffering capacity the meat was able to buffer the pH back down much lower than that of the phosphate solutions.

2.4 Pork Quality

2.4.1 Tenderness:

Tenderness, along with juiciness, and flavor are the primary determinates of pork quality to the consumer (Hayes et al., 2006). Pork tenderness increases with time post-mortem due to proteolysis by enzymes such as the Ca²⁺ dependent proteases (Koohmaraie, 1991). It has also been shown that intrinsic factors can affect pork tenderness including, swine breed, sex, water content, pH, marbling, water holding capacity, and sarcomere length (Chen et al., 2012; Jelenikova, Miyahara, & Pipek, 2008; G.-D. Kim et al., 2013). The effect of pH and water content on water holding capacity are highly correlated. In the instance of normal pork pH decline postmortem, ultimate pH is

approximately 5.5 (Krol, Roon, & Houben, 1988). While rapid glycolysis may produce a pork carcass with a pH similar to that of normal pork, the length of time to final pH is much shorter causing a condition known as pale, soft, and exudative (PSE; Krol et al., 1988). Pale soft and exudative pork experiences a loss in water holding capacity and begins to exudate as early as 5 hr postmortem (Krol et al., 1988). The loss in water and water holding capacity has been shown to be positively correlated to tenderness. An increase in water holding capacity increases muscle swelling which increases tenderness (Hamm, 1960). Additionally, extrinsic factors such as rate of chilling are also directly related to the tenderness and water holding capacity of pork (Shackelford, Wheeler, & King, 2012).

Intramuscular fat influences overall palatability (Kerry, Kerry, & Ledward, 2002; Wood et al., 2004) and may increase the perception of tenderness through several mechanisms (Savell & Cross, 1988). Even though marbling has been positively correlated to tenderness (Lo, McLaren, McKeith, Fernando, & Novakofski, 1992; Wood et al., 2004) the industry has seen a decrease in marbling due to increased selection pressure on pork cutability. However consumer panels have shown marbling only directly accounts for approximately 10% of the perceived tenderness in pork products (Rincker, Killefer, Ellis, Brewer, & McKeith, 2008). Although postmortem production practices can improve pork tenderness and juiciness, additional measures can be applied to help ensure a satisfactory eating experience, such as enhancement.

Moisture enhancement of pork loins at various levels of uptake, with water, salts, and phosphates, have been shown to be an effective method to reduce Warner-Bratzler shear force (Baublits, Mehaffey, Saha, Meullenet, & Sawyer, 2006). Warner Bratzler

shear force (WBSF) values can increase due to loss of water by way of purge and cooking loss (Kim et al., 2013) suggesting that if water is added it may compensate for the moisture lost and improve tenderness ratings. Additionally, needle penetration of muscle fibers increases tenderness via severance of muscle fibers (Glover, Forrest, Johnson, Bramblett, & Judge, 1977). Bertram et al. (2002) stated in a study on meat structure and water mobility and distribution that the ultra-structure of the meat proteins affects water holding capacity. Bertram et al. (2002) further expanded that sarcomere length is responsible for the retention of water, and water will be forced out of the intracellular space of the myofibrillar complex, as the sarcomere is contracted or shortened during rigor and chilling.

Final cooking temperature also affects the tenderness of pork products; where products cooked to 82°C were less tender than those cooked to 71°C (Baublits, Mehaffey, et al., 2006). At 65°C myofibrillar proteins begin to become less tender as they harden. However collagen starts to hydrolyze (in the presence of moisture) weakening the connective tissue structure and improving tenderness (Romans et al., 1994). The relationship between the amount of collagen and the shear strength of meat is positively correlated (Krol et al., 1988). Increased collagen content can be caused by the aging of the live animal and the inter- or intramolecular bonds of collagen crosslinking (Krol et al., 1988). While aging leads to increased collagen stability, final cooking temperature and cooking method can improve tenderness of high collagen content meats (P. Bouton, Harris, & Ratcliff, 1981).

Ultimate pH is also a major contributor to the tenderness of non-enhanced meat products. Postmortem pH in the range of 5.8 to 6.3 leads to an increase in shear strength

while low pH (< 5.3) and high pH (> 6.3) has been shown to increase tenderness (Watanabe, Daly, & Devine, 1996). These affects are predominantly attributed to the activation of enzymes that aid in the proteolytic degradation of myofibrillar proteins (Watanabe et al., 1996). Tenderness as measured by WBSF in enhanced pork was negatively correlated to the pH of chops post-enhancement (Sheard & Tali, 2004). Sheard and Tali (2004) also showed that pH and percent yield were positively correlated which shows that a higher pH would lead to increased water in the system, aiding in the muscle swelling and increasing tenderness as previously discussed. These findings further support the use of enhancement solutions as an effective aid in tenderization.

2.4.2 Sensory:

Pork sensory characteristics, including tenderness, juiciness, and flavor, can be affected by cooking temperature. As final temperature increases juiciness has been shown to decrease while moisture lost to cooking increased (Moeller et al., 2010). As previously stated, Baublits et al. (2006) found that pork tenderness increased as greater amounts of water were retained within the meat system after cooking. Utilizing moisture enhancement technology is an effective method to ensure meat retains moisture when heated to elevated internal temperatures (Baublits, Mehaffey, et al., 2006).

In addition to moisture retention, enhancement of pork has also been shown to have an effect on pork flavor compounds. More intense pork flavors were found when chops were enhanced to 6% of their green weight when compared to untreated chops, but chops enhanced to 12% were comparable to non-treated chops (Baublits, Mehaffey, et al., 2006). Holding final concentrations of salt and phosphate the same between the 6% and 12% enhancements, it was suggested that a dilution of flavor compounds by water was

likely the reason for pork flavor scores from the 12% enhancement were similar to untreated chops (Baublits, Mehaffey, et al., 2006). While enhancement has an effect on pork flavor, intramuscular fat content also contributes (Brewer, Zhu, & McKeith, 2001). Brewer, Zhu, and McKeith (2001) reported that 41.55% of consumers chose a lean chop over a medium or highly marbled chop while 40.14% chose medium marbled chops and only 18.31% chose a chop that was highly marbled. It was also found that although purchase intent is negatively correlated with marbling, when a blind sensory panel was performed consumers showed preference for the chops with higher marbling scores, rating them as more juicy, tender, and flavorful (Bray, 1966; Brewer et al., 2001). Furthermore, when non-enhanced chops were rated based on color score, trained sensory analysis showed the darker colored chops were rated as more tender, juicier, and less dry than chops of lighter colors (Norman, Lorenzen, Heymann, & Berg, 2003). This shows that although consumers, based on sight, desire a leaner, less marbled chop they prefer the sensory attributes of the fatter, higher marbled, and darker colored chops. This is addressed by the mechanisms of marbling perception on tenderness as stated by Savell and Cross (1988) with specific regard to the lubrication and bulk density theories. Lubrication theory gives the perception of a juicier product as the fat renders out causing a coating of the mouth and increased saliva production, while the bulk density theory gives the perception of increased tenderness as the density of fat is less than that of denatured proteins (Savell & Cross, 1988). Jensen et al. (2003) reported that enhancement solution retention improved trained sensory panelists' perceptions of pork flavor, juiciness, and tenderness. Holmer et al. (2008) also reported that chops with the greatest water holding capacity had the highest sensory ratings. However the chops with

the greatest water holding capacity also had the saltiest flavor. This is in contradiction to early research which found that factors that increase water holding capacity, such as pH, decrease the salty taste. Meat samples with the same amount of salt show a lower salty taste at higher pH values and a more salty taste at lower pH values (Hamm, 1960). The literature reveals that early reports were contradictory in regards to the relationship of subjective and objective measurements of juiciness and expressible juice. Hamm (1960) stated that this is the possible reason that many researchers did not find a correlation between juiciness and expressible juice, as they were looking at the composition rather than the amount of expressible juice. The correlation between juiciness and expressible juice can also be misinterpreted if cook loss is not the same or not accounted for across all samples (Hamm, 1960). Furthermore the methodology used in measuring expressible juice can cause differences in the correlations between expressible juice and perceived juiciness (Hamm, 1960).

2.5 Shelf Life

2.5.1 Color:

Color is a major indicator of shelf life stability. Brewer et al. (2002) reported that meat color is the primary consumer determinant of freshness and quality at the point of purchase, while Bredahl et al. (1998) showed that color is an important attribute for consumers when purchasing meat. Furthermore, consumers perceive discolored meat negatively (Troy & Kerry, 2010). Although many factors can influence meat color, myoglobin, especially the myoglobin state is the primary influence of meat color (AMSA, 2012). Myoglobin is an allosteric protein that can change its shape in the presence of particular ligands. Myoglobin is made up of eight α -helices, and a heme

porphyrin ring about a centrally located iron atom (AMSA, 2012). There are three primary states of myoglobin when referencing fresh meat color. The first is deoxymyoglobin, which is a purple or purplish-pink color that is present in vacuum-packaged meat products. Deoxymyoglobin is the absence of a ligand at the iron binding site in the heme ring or myoglobin (AMSA, 2012). Oxymyoglobin produces a bright reddish-pink color and the presence of diatomic oxygen in the iron binding site of the myoglobin heme ring. Third, metmyoglobin is the result of prolonged exposure to oxygen or low oxygen partial pressure and subsequently the oxidation of the iron in the heme ring from ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron. During the process of oxidation, diatomic oxygen is replaced with water and the color is reduced to a brown. While the most desirable color among consumers is oxymyoglobin, as days of shelf life increase so does the oxidation of oxymyoglobin to metmyoglobin (AMSA, 2012). Objective color can be measured via reflectance (nm) at specific wavelengths. Objective meat color is usually recorded utilizing *Commission Internationale de l'Eclairage* (CIE) color values of L^* , a^* , and b^* ; where L^* is lightness to darkness and is measured on a scale of 100 to 0, respectively; a^* is red to green, measured on a linear scale of 60 to -60; and b^* is measured on a linear scale from 60 to -60, denoting yellow to blue.

Various factors can affect meat color, including but not limited to, packaging, cutting or processing temperatures, atmospheric gases, and the microbial composition and their byproducts. Packaging can be used to extend shelf life by removing all the gases from the package, such as in vacuum-packaged product, or by replacement of atmospheric gases with blended gases. Vacuum packaging removes gases from a package and creates an anaerobic environment devoid of oxygen causing myoglobin to return to

the deoxymyoglobin state and produces the longest shelf life (AMSA, 2012). Packaging such as a polyvinyl chloride (PVC) over wrap are used in short shelf life applications. Due to the high oxygen permeability of PVC, the meat is allowed to bloom, or oxygenate which converts myoglobin to the oxymyoglobin state. Other packages, such as modified atmosphere packages, are used to change the gas composition in the package to extend the stability of oxymyoglobin through the addition of carbon monoxide, carbon dioxide, nitrogen, or increased elemental oxygen. Carbon monoxide as well as other gasses causes the heme ring to enter a more permanent reduced state (ferrous iron) and thus mimics the bright reddish pink color of oxymyoglobin.

The normal progression of color change in fresh meat is the conversion of deoxymyoglobin into metmyoglobin, first by the oxygenation of deoxy- to oxymyoglobin; then by the oxidation of ferrous iron in the porphyrin ring to the ferric state. In the first step oxygenation occurs as the deoxymyoglobin state of the porphyrin ring is exposed to oxygen. Although the valence of the iron is not changed the final coordination site of the iron is occupied by diatomic oxygen which also attaches to the histidine residue and alters the structure and stability of the myoglobin (Mancini & Hunt, 2005). From this ferrous state of oxymyoglobin, the oxidation process begins. Although it is often thought that discoloration happens on the surface of meat it is the subsurface oxidation that occurs first. Metmyoglobin forms beneath the superficial layer of oxymyoglobin due to low partial pressure of oxygen and above the interior deoxymyoglobin layer. As time progresses and the oxidation of oxymyoglobin to the ferric state continues the band of metmyoglobin thickens and becomes more visible as it rises to the surface (Mancini & Hunt, 2005). In vacuum-packaged meat, or a low pressure

environment, the reduction of met- to deoxymyoglobin relies heavily on inherent reducing enzymes, and NADH pools (Mancini & Hunt, 2005). With prolonged retail display, the reduction of ferric iron to ferrous iron cannot take place and the color fades to brown, due primarily to the low activity of reducing enzymes and depletion of NADH pools postmortem.

Literature has shown that postmortem enhancement with lactates can activate lactate dehydrogenase converting lactate to pyruvate and NADH, thereby reducing metmyoglobin to a ferrous state (Mancini, Kim, Hunt, & Lawrence, 2004). Additionally, there is a relationship between pH, water holding capacity, and meat color. It is said that there is not a satisfactory explanation for this relationship however it is likely that several factors are interacting, such as myoglobin state, fiber condition and structure, as well as the denaturation process (King & Whyte, 2006). Elevated meat pH extends meat color stability, which can be achieved by addition of phosphate salts, however raising the pH of the meat system also causes the meat to appear darker and drier (Cerruto-Noya et al., 2009). Pork enhanced with phosphates has been shown to have lower L^* , and a^* - values indicating a darker, more red chop through 8 days of retail display, indicating that phosphates increase the red color of meat while binding more water and allowing greater light absorption (Jensen, Robbins, et al., 2003). Jensen (2003) also reported that lactate and diacetate salts have a positive impact on enhanced pork color causing higher L^* , a^* , and b^* values across all days of retail display.

2.5.2 Lipid Oxidation:

Lipid oxidation, when referring to meat shelf life, is the deterioration of fat through free radicals. Lipid oxidation tends to increase as days of shelf life, in the

presence of oxygen, increase (Greene, 1969; Greene, Hsin, & Zipser, 1971). There is a relationship between the oxidation of lipids and the deterioration of meat color, or color oxidation (Cheng, Wang, & Ockerman, 2007; Faustman, Sun, Mancini, & Suman, 2010). As lipids continue to oxidize they create compounds that are responsible for off-odors, and flavors such as primary products like alkyl, alkoxy, and peroxy radicals that are ready to remove neighboring protons (Faustman et al., 2010). Secondary oxidation occurs as days on retail display increase producing secondary products such as hexanal, propanal, and malondialdehyde (Sakai, Yamauchi, Kuwazuru, & Gotoh, 1998; Siu & Draper, 1978). Malondialdehyde (MDA) is frequently used when measuring the amount of lipid oxidation present in a meat sample (Siu & Draper, 1978). The threshold level of detection of off-flavors in trained sensory panels of pork meat is 0.5 – 0.6 mg MDA/ kg meat (Greene & Cumuze, 1982; Lanari, Schaefer, & Scheller, 1995; Lauzurica et al., 2005; Tarladgis, Watts, Younathan, & Dugan, 1960). Published data shows much higher detection levels, around 2.0 mg/kg in beef, which was attributed partially to the experience, and sensitivity of the panelists, but mainly to the inherent differences between pork and beef (Campo et al., 2006).

In a study where beef *longissimus lumborum* steaks were enhanced with ammonium hydroxide and compared to phosphate-enhanced steaks packaged in high oxygen modified atmosphere packaging (MAP) it was found that the alkaline enhancement caused greater oxidation than phosphate enhancement (Cerruto-Noya et al., 2009). In addition to causing more oxidation, the alkaline enhancement caused threshold level MDA values according to the values previously stated for pork samples (Cerruto-Noya et al., 2009). The phosphate based enhancement solution with lower MDA values

was attributed to phosphates ability to act as an antioxidant, limiting free radical production (Pokorny, Yanishlieva, & Gordon, 2001).

2.6. Electrolyzed Water

Electrolyzed water is relatively new concept in the meat industry in which a solution of sodium or potassium chloride and other salt compounds are passed through an electrically charged membrane to obtain water consisting of two phases, acidic electrically oxidized water, and alkaline electrolyzed reduced water. In the patent description, the technology focus of inorganic chemistry was described as a pH controlled process that produces a product of pH 5 and a concentration of active chlorine that is stable at temperatures of 25° C for ≤ 38 hours (Hung, Chung, & Hung, 2003). Although two water phases can be produced, much of the recent focus has been on the use of acidic electrically oxidized water as a disinfectant and antimicrobial applications (Ding, Rahman, Purev, & Oh, 2010; Huang, Hung, Hsu, Huang, & Hwang, 2008). If plants were to incorporate this technology as a food safety mechanism, it may leave them with a by-product of alkaline electrolyzed reduced water. To comply with efficiency and sustainability standards, plants would benefit from being able to capture the alkaline electrolyzed reduced water and use it in production systems.

During electrolyzed reduced water generation a low concentration salt solution (0.1% NaCl) is passed through two chambers separated by a membrane. One chamber being charged with an anode and the other with a cathode. The water is then split into two portions across the membrane and exits the chamber via two different conduits (Hung et al., 2003). The process creates an acidic solution with a pH of approximately 2.7, an oxidation reduction potential greater than 1100 mV, and a free chlorine level ranging

from 10 to 80 PPM (Venkitanarayanan, Ezeike, Hung, & Doyle, 1999). The effectiveness of acidic electrically oxidized water on *Escherichia coli*, *Salmonella enteritidis*, and *Listeria monocytogenes* has been evaluated and found to be extremely effective against all three pathogens (Kim, Hung, & Brackett, 2000; Venkitanarayanan et al., 1999)

The basic, or alkaline form of electrolyzed reduced water is obtained from the cathode side of the electrolyzed water generator. Alkaline electrolyzed reduced water has a pH of approximately 11 with an oxidation/reduction potential approaching -80 mV, similar to that of phosphates (Kim et al., 2000). Although information on the efficacy of alkaline electrolyzed reduced water as an antimicrobial is limited, some reports show that its use as a antimicrobial is effective against the adhesion of fecal material in chicken carcasses (Kim et al., 2000). As a byproduct from the production of acidic electrically oxidized water, alkaline electrolyzed reduced water has attractive properties that may make it beneficial as a novel enhancement solution: it is relatively inexpensive, it is environmentally friendly, it has no added chemicals (with the exception of NaCl), and employees will not have to handle regulated substances. Additionally, factors that make alkaline electrolyzed reduced water attractive for potential use as an enhancement solution includes an elevated pH, and high ionic strength.

2.7. Consumer Trends Toward Clean Labels

Today's consumers are increasingly more conscious of the origin of their food, and are reading labels, more often, on the products they purchase. Consumer desire to purchase products without certain functional ingredients has facilitated the need for research to investigate and develop new ingredients with functional properties, satisfy the consumer emotionally, as well as remain profitable for the processor (Caswell &

Mojduszka, 1996). At the same time new ingredients and methods must be able to produce a product of similar quality with regards to tenderness, juiciness, and flavor; as well as from a color and shelf life view point. Current market place trends include moving toward clean labels such as uncured, and replacement of chemical names with common names as allowed by law. Consumers are also willing to pay a premium for label claims that indicate a more wholesome product (Umberger, Thilmany McFadden, & Smith, 2009). Ingredients such as alkaline electrolyzed reduced water, which can be labeled as water, may be able to fill a niche in enhancement products if found to be viable. In an American Meat Institute presentation by Anne-Marie Roerink entitled *The Power of Meat 2013* it was mentioned that 31% of consumers check nutrition facts panels when purchasing processed meats, and over 50% of consumers' purchasing decisions were somewhat or majorly influenced by label claims. A noteworthy conclusion can be drawn from the data; in 2011, there was no driver of purchase intent behind natural or organic purchases, however in 2013, 46% selected "Free of Substances I Want to Avoid" as a driver of purchase intent. As previously mentioned Brewer and coworkers (2002) suggested that consumers would buy enhanced pork products, but were concerned with ingredients such as phosphate and salt. In the 2010 National Meat Case Study, the number of products containing a "natural" label claim rose 10% from 2004 to 2010, during the same period the number of enhanced pork products declined 6%. These consumer trends are the driving force behind novel products such as alkaline electrolyzed reduced water enhanced pork loins. In order to capitalize on this growing market segment, and meet the demands of today's and tomorrow's consumers it is important that

the industry continue to find ingredients that can be applied with a clean label such as alkaline electrolyzed water, while maintaining functionality and quality standards

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CHAPTER 3

EVALUATION OF ALKALINE ELECTROLYZED WATER TO REPLACE TRADITIONAL PHOSPHATE ENHANCEMENT SOLUTIONS: EFFECTS ON WATER HOLDING CAPACITY, TENDERNESS, AND SENSORY¹

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Abstract

Sixty-four whole pork loins were randomly assigned to four treatments to evaluate the use of alkaline electrolyzed reduced water as a replacement for traditional enhancement solutions. Treatments included: alkaline electrolyzed reduced water (EOH; pH \approx 11.5), EOH plus 2.5% potassium-lactate (EOK), industry standard (IS; 0.35% Sodium Tri-polyphosphate, 0.14% sodium chloride, 2.5% potassium-lactate), and no enhancement (CON). After enhancement (target 110%) and a rest period, chops were cut (2.54-cm) to test treatment effects on water holding capacity, Warner-Bratzler shear force (WBSF), and sensory attributes. Despite its alkaline nature EOH chops exuded more water ($P<0.05$) than EOK, IS, or CON chops. Control chops were similar ($P>0.05$) to EOK, however CON and EOK both lost more moisture ($P<0.05$) than IS. The use of alkaline electrolyzed reduced water did not improve WBSF or sensory characteristics compared to IS treated chops. As a stand-alone enhancement solution alkaline electrolyzed reduced water was not a suitable replacement compared to industry standard solutions.

Keywords: Water holding capacity, enhancement, pork, water

3.1. Introduction

The commercial pork industry has taken advantage of enhancement as a process to provide consumers with more tender, juicy, and flavorful pork products (Brewer et al., 2002; Hayes et al., 2006). Typical enhancement solutions may contain ingredients including water, salt, and phosphates. Additionally, enhancement solutions may contain additives such as lactates, and acetates that have been shown to improve shelf life stability, color, and flavor (Brewer, McKeith, Martin, Dallmier, & Meyer, 1991; de Wit & Rombouts, 1990; Sutton, Brewer, & McKeith, 1997). Fresh meat enhancement improves palatability largely due to: 1) disruption of the myofibrillar network via severance of the contractile, structural, and connective tissue proteins by needle penetration (Tyszkiewicz, Jakubiec-Puka, Wieczorek, & Klossowska, 1997), and 2) causing an increase in the swelling of the myofibrillar structure by increased water holding capacity due to the effect the alkaline salts and phosphates have on the protein charges (Baublits, Pohlman, Brown Jr, & Johnson, 2006; Offer & Trinick, 1983).

In the United States approximately 57% of fresh pork is enhanced (*Description Designation for Raw Meat and Poultry Products Containing Added Solutions*, 2014) of these fresh enhanced products 79.5% are enhanced by needle injection (Muth, Ball, & Coglaiti, 2012). Although enhancement solutions have been widely used and have been successful in helping to ensure a satisfactory eating experience, the consuming public has become circumspect of chemicals used in their foods, even if they have a functional role (Brewer, 1998). As consumers' demand and willingness to pay for perceived clean-label items increases (Zurawicki, 2015) the meat industry must respond to maintain viability. However, novel clean-label, and all natural ingredients must be able to retain the

functionality of the original ingredients. Alkaline electrolyzed water (AEW) is one such ingredient that may hold promise as a replacement of salts and phosphates in enhancement solutions and can be labeled simply as water.

Electrolyzed water is produced by passing a dilute salt solution through a membrane with an electrical current flowing across it producing both acidic electrically oxidized water ($\text{pH} \approx 2.5$) and AEW with an approximate pH of 10.8 (Huang et al., 2008). Acidic and alkaline electrolyzed water have shown to possess bactericidal properties when used in equipment (K. S. Venkitanarayanan, G. O. Ezeike, Y.-C. Hung, & M. P. Doyle, 1999) and meat (Ding et al., 2010) applications. From plant use and cost savings mechanism, electrolyzed water is easy and economical to produce and use (C. Kim et al., 2000). If a plant were to invest in electrolyzed water as an antimicrobial, they could potentially use both phases by incorporating the AEW in enhancement solutions in lieu of traditional salt phosphate solutions due to its high alkalinity and ionic strength (Huang et al., 2008). Therefore, the objectives of this study were to determine if pork loins enhanced with one of two AEW solutions were comparable to traditionally enhanced pork for water holding capacity and palatability characteristics.

3.2. Materials and methods

3.2.1. Pork Loin Procurement and Enhancement

Sixty-four Institutional Meat Purchase Specifications 413 whole boneless pork loins (*longissimus thoracis et lumborum*) were procured 2 d postmortem from a multi-national pork supplier (Smithfield Farmland, Duncan, NC) across two replicates (32 loins per replicate) and transported ($0 \pm 2^\circ\text{C}$) 575 km to The University of Georgia Meat Science Technology Center (MSTC, Athens, Ga). The pork loins for each replicate were

randomly selected from the mornings fabrication line, vacuum packaged and boxed accordingly to plant standard operating procedures. Upon arrival at the MSTC, the pork loins were placed in cold ($2 \pm 2^{\circ}\text{C}$), dark storage until 4 d postmortem. At 4 d postmortem the loins were randomly assigned to one of four treatments (8 loins / treatment • replicate⁻¹ for a total of 16 loins per treatment) to test the efficacy of novel enhancement solutions on pork loin water holding capacity and palatability characteristics. The four treatments included: 1) alkaline electrolyzed reduced water (EOH; pH \approx 11.76), 2) EOH with 2.5% potassium lactate (EOK; pH \approx 10.92; Hawkins, Minneapolis, MN), 3) water with 0.35% Sodium Tri-polyphosphate (ICL Performance Products, Bolingbrook, IL), 0.14% sodium chloride (Morton Salt Inc., Chicago, IL), 2.5% potassium lactate (IS; pH \approx 6.78), and 4) no enhancement (CON).

After treatment randomization whole loins where enhanced (except CON) to a target of 110% of green weight (Injectamatic PI21, Koch Equipment LLC, Kansas City, MO, USA). The enhanced loins were allowed to rest and purge for 15 min. Weights were recorded for green weight, immediately after enhancement, and after 15 min rest to determine immediate and final percent enhancement solution uptake (Table 1).

After the post enhancement rest period, the whole loins were cut into 2.54 cm chops. Starting from the anterior end, the loin was squared, and the squared end was used to measure pH using a pH probe (model WD-35649-50, Oakton Instruments, Vernon Hills, IL) placed directly into the muscle. Two chops were removed for Warner-Bratzler shear force (WBSF), the following 7 chops were used for moisture retention/water holding capacity determination, an area of 7 chops (\approx 18 cm) was removed, and then two additional chops were cut for trained sensory analysis. Chops for WBSF and sensory

analysis were immediately vacuum packaged (B-620 series; 30–50 cm³ O₂/m²/24 h/101,325 Pa/23 °C; Cryovac Sealed Air Corporation, Duncan, SC, USA) and frozen (-20°C) until further analysis.

3.2.2. Water Analysis

Water analysis was calculated following two methods: 1) on an individual basis where moisture loss was calculated in succession based on the chop starting weight of each assay; and 2) on a 100% basis where moisture loss was calculated as part of a whole where moisture loss from each assay contributed to the total moisture loss. Water loss on a percent basis was performed using a 100% starting weight; each subsequent assay's starting weight was based upon chop weight after total water loss to that point, as indicated by $\omega_1 = 100$; $\omega_2 = 100 - [\text{Free Drip on Percent Basis (FDPB)}]$; $\omega_3 = \omega_2 - [\text{expressible juice loss due to vacuum packaging on percent basis (EJVPB)}]$; $\omega_4 = \omega_3 - [\text{expressible juice loss due to gravimetric force on percent basis (EJGPB)}]$.

3.2.2.1. Free Drip:

Free drip was measured using the methods as outlined by Honikel and Hamm (1994). Immediately after cutting, chops were weighed, then hung from a hook and placed in a whirl-pak bag (Fort Atkinson, WI) so as to not touch the meat to the bag. Chops were hung in a 2°C cooler for 24 h and reweighed. This is reported as free drip using the following equations:

$$\text{Free Drip} = (\text{enhanced weight} - \text{drip weight}) / \text{enhanced weight}$$

$$\text{FDPB} = \text{Free Drip}$$

Chops were then vacuum packaged, held for 4 d in dark, cold storage at $2 \pm 2^{\circ}\text{C}$ to simulate distribution, and then removed from storage on their respective days of shelf life (0, 5, 10, 15, 20, 25, 30).

3.2.2.2 Expressible Juice (EJ):

Vacuum packaged chops were pulled on their respective days, unpackaged and weighed. Expressible juice due to vacuum (EJV) was calculated following:

$$\text{EJV} = (\text{post free drip weight} - \text{post package weight}) / \text{post drip weight}$$

$$\text{EJVPB} = \omega_2 * X_1, \text{ where } X_1 = \text{EJV} / 100$$

Following calculations of EJV expressible juice due to gravimetric force (EJG) were measured by the methods as outlined by Prevolnik, Čandek-Potokar, and Škorjanc (2010) as modified by Honikel and Hamm (1994). After post-packaging weights were taken from EJV duplicate 5 g samples were removed from each chop. The two 5 g samples were individually wrapped in one #2 Whitmann filter paper, placed in a 50 ml conical screw cap centrifuge tube and centrifuged at $8,400 \times g$ for 30 min at 4°C . Samples were promptly removed from the centrifuge tubes with forceps, filter paper was removed, the sample was blotted and reweighed. Expressible juice due to gravimetric force was calculated following:

$$\text{EJG} = (\text{post EJV packaging weight} - \text{centrifugal weight}) / \text{post EJV packaging weight}$$

$$\text{EJGPB} = \omega_3 * X_2, \text{ where } X_2 = \text{EJG} / 100$$

3.2.2.3. Bound water:

After samples were centrifuged and weighed they were cut into three equal pieces, placed onto a dried aluminum pan, and put into a drying oven at 105°C for 24 h as

outlined by Bouton (1972). After the drying period samples were removed from the oven and a final weight was recorded. Bound water weight was calculated by:

Dry Loss = (EJG weight – dried weight) / EJG weight

Dry Loss on percent basis = $\omega_4 * X_3$, where X_3 = Dry Loss / 100

3.2.3. Cookery, Warner-Bratzler Shear Force, and Sensory Attributes

Twenty-four hours prior to cooking, chops were thawed in a $2 \pm 2^\circ\text{C}$ cooler. Cooking procedures were performed in accordance with the AMSA (1995) using Clamshell grills (George Formen, Saltotn Inc., Miramar, FL). Chops were cooked to an internal temperature of 70°C . Internal temperature was monitored using copper-constantan thermal couplers (Omega Engineering, Stamford, CT, USA) placed in the approximate geometric center of the chop. All weights were recorded for thaw loss and cook loss and the chops were cooled in a $2 \pm 2^\circ\text{C}$ cooler for 24 h after cooking. Once cooled four to six 1.27 cm cores were taken from each chop parallel to the long axis of the muscle fiber, being careful not to include extra connective tissue or fat seams in the core. Cores were then sheared once perpendicular to the long axis of the muscle fiber using an Instron Universal Testing Machine (Instron Dual Column Model 3365, Instron Corp., Norwood, MA, USA) with a Warner-Bratzler shear head, 51 kgf load cell with a crosshead speed of 25 cm/min. The peak shear force (kgf) for each core was recorded (Bluehill software, Instron Corp.) and analyzed to obtain an average value for each chop.

An 8-member trained panel (AMSA, 1995) was used to evaluate the effects of each enhancement solution on organoleptic properties of the pork chops. Pork loin chops were thawed and cooked as outlined for WBSF. After cooking samples were cut for panelists using a sample sizer as described by AMSA (1995) by cutting off all fat and

epimysial connective tissue. Samples (1.27 cm x 1.27 cm x chop thickness) cubes were cut and placed in warmed yogurt makers (Euro Cuisine, Inc., Los Angeles, CA, USA) until sampled (\approx 5 mins). Two cubes per sample were served unsalted, and unspiced with a glass of water and saltless soda crackers to cleanse panelist palate between samples. Fourteen samples were given to each panelist every day (seven samples per session, and two sessions each day) with 3 hr between the start of each session. Panelists sampled and recorded traits in a dark room with positive air flow, and illuminated with blue and yellow lighting to mask degree of doneness and color. Samples were given to panelist through a breadbasket with individual walls separating each panelist. Chops were evaluated on tenderness, pork flavor intensity, juiciness (1 – extremely tough, extremely bland, extremely dry, and 8 – extremely tender, extremely intense, extremely juicy) and off-flavors (1 – none detected, and 6 – extreme off-flavor). If off flavors were detected panelists were asked to describe the flavor in one word with the aid of a pre-arranged lexicon.

3.2.4 Statistical Analysis:

Data were analyzed using Proc Mixed version of SAS (version 9.3), as a completely randomized split-plot design where loin was the whole-plot and chop within loin as the sub-plot. Loin within replication by treatment was included as the random variable. Loin was considered the experimental unit and chop was considered the observational unit. Moisture analysis included day in the model and the treatment by day interaction was tested. If an interaction was detected data were reanalyzed by day. For sensory analysis chop end point temperature and panelist effects were tested, however were not significant and subsequently removed from the model. The multiple value

recordings for sensory analysis (panelist) and WBSF (core) from each chop were not averaged before statistical analysis to account for within chop variation. Sample size was determined utilizing operating characteristic curves as outlined by Ferris, Grubbs, and Weaver (1946) and (Montgomery, 2001). Means were separated using the PDIFF option of LSMEANS at $\alpha = 0.05$.

3.3. Results and Discussion

3.3.1 Whole Loin Water Loss

After loin treatment randomization there was no difference in green loin weights ($P > 0.05$; Table 3.1). Immediately post-enhancement EOH and EOK loins weighed more ($P < 0.05$) than CON loins, while IS loins were similar ($P > 0.05$) to all treatments. This was attributed to the numerically greater initial weight of EOH and EOK loins compared to IS loins coupled with the increased ($P < 0.01$) initial enhancement solution pickup of EOH loins compared to EOK and IS. After a 15 min rest period EOH loins maintained a greater percentage of enhancement pickup ($P < 0.01$) compared to EOK and IS; however, post-rest weights were similar ($P > 0.05$) among all treatments. The pH values of green loins were similar ($P > 0.29$) across all treatments, as well as the pH of loins post enhancement ($P > 0.30$). However EOH-treated loins had numerically decreased pH (0.04) after enhancement while loins treated with EOK had numerically higher pH (0.09) compared to initial pH. The numeric decrease in pH of EOH loins was not expected as the AEW only solution had a pH ≈ 11.76 .

Similar results had been reported where loins injected with an alkaline food grade ammonium hydroxide solution had a lower pH, and alkaline enhanced loins lost more water after enhancement (Cerruto-Noya et al., 2009). Alkaline hydrolysis of proteins into

short chain peptides and amino acid residues in the microstructure of the meat can cause a buffering effect due to an increasing number of functional groups releasing their protons which in turn can cause a regulation of pH (Campbell & Farrell, 2012).

Furthermore, the pH water holding capacity scale as described by Hamm (1960) shows a decline in water holding capacity around pH 10.5 which further supports the statement of decreased protein functionality. The pH value of IS (6.78) solution which was below the peak pH of 10.5 on the pH water holding capacity scale may relate to more of the functional groups on individual amino acids remaining protonated due to their pKa (Campbell & Farrell, 2012). With the pH of EOH and EOK solutions being elevated beyond the pKa of all amino acids the surplus of hydroxide ions allows for potential attacks on intramolecular bonds between residues, hydrating them and cleaving proteins into smaller segments (Campbell & Farrell, 2012), thereby increasing the buffering capacity of these proteins.

3.3.2. Chop Water Loss:

When free drip was analyzed chops treated with EOH lost the most water , followed by EOK-treated chops (Table 3.2). Industry standard enhanced chops and CON chops lost similar ($P \geq 0.96$) amounts but lost less ($P < 0.01$) than both EOK and EOH. For subsequent moisture loss on an individual assay basis there was a treatment by day interaction ($P < 0.01$) for EJY and EJG (Figure 3.1a and 3.1b respectively). For EJY, regardless of day, IS had the least percent of moisture loss ($P < 0.05$). On d 0 and 5, CON, EOH, and EOK were similar ($P > 0.06$) for moisture loss. However, after 10 d of storage, EOH exhibited a greater percent moisture loss than CON or EOK, which remained similar to each other ($P > 0.05$) for the remainder of shelf life display. For EJG,

all treatments lost similar ($P > 0.05$) amounts of water on d 0, however after 5 d and after 10 d IS and EOH chops lost the least water ($P < 0.01$). After 15 d and 20 d CON chops lost less water than EOH chops, which were similar ($P > 0.05$) to IS and EOK. All chops were similar ($P > 0.05$) after 25 d, while EOH chops had less moisture loss ($P < 0.01$) than all other treatments after 30 d.

There was not a treatment by day interaction for dry loss or total loss and main effects for treatment and day are shown in Table 3.2 and 3.3, respectively. The effect of treatment on dry loss showed that CON and EOH lost more water ($P < 0.01$) than IS, while EOK was similar ($P > 0.05$) to both EOH and IS. Total loss showed that EOH had a greater moisture loss ($P < 0.01$) than all other treatments. Industry standard chops lost the least ($P < 0.01$) amount of moisture, while CON and EOK were similar ($P > 0.05$). The effect of day of retail display on dry loss was not significant, while the effect of day on total loss of chops on a per assay basis was not analyzed due to the non-additive nature of the model.

When moisture loss was analyzed on a 100% basis there were significant interactions ($P < 0.01$) for EJV, EJG, and dry loss (Figure 3.2a, 3.2b, 3.2c, respectively). For expressible juice due to vacuum packaging, IS lost the least ($P < 0.01$) moisture across all days of shelf life display. After 0 and 5 d, EOH, EOK, and CON were similar ($P > 0.05$); however throughout the remainder of display EOH lost more ($P < 0.01$) moisture than CON and EOK, with the exception of 10 d and 20 d where EOH and CON were similar ($P > 0.05$). For EJG, regardless of day, EOH chops lost less ($P < 0.05$) moisture than IS-treated chops. Additionally after 15 d of display EOH chops exhibited less moisture loss ($P < 0.05$) than all other treatments with the exception of d 15 and 20

where EOH exuded similar ($P > 0.05$) amounts of moisture as EOK and CON. For dry loss on a 100% basis, IS-treated chops lost more moisture ($P < 0.05$) than all other treatments regardless of day. Chops treated with EOH exuded the least moisture ($P < 0.05$) across all days of display with the exception of 5 d and 10 d, on which it was similar ($P > 0.05$) to EOK, which was similar ($P > 0.05$) to CON across all days of display. There was not an interaction between treatment and day for total moisture loss, therefore the main effects are shown in Table 3.2 and 3.3, respectively. The main effect of treatment showed that both AEW-treated chops lost a greater amount of moisture ($P < 0.05$) than CON and IS-treated chops, which were similar ($P > 0.05$). As expected, the effect of day on total water loss showed that as days on display increased moisture loss also increased.

It is important to look at water loss on a 100% basis so conclusions may be made about the retention or loss of the various forms of water found in the meat system. Free drip water loss is the most loosely bound water in the chop, typically referred to as free water and is the first type of water to be lost. Free water will either be lost before packaging, or inside the package to a soaker pad or in the case of vacuum packaged meat, to the vacuum bag. The current study as noted during the free drip portion found that EOH increased the amount of free water prevalent in the chop. Cerruto-Noya et al. (2009) reported similar findings when an ammonium hydroxide enhancement solution was compared to a salt/phosphate enhancement solution in pork loin chops. Industry standard solutions, although enhanced to 110% of their green weight lost similar amounts of moisture to the CON chops during free drip. This means that the additional water present in the IS chops was shifted to a more immobilized state increasing its hydrostatic

interaction to a more stable state (Hamm, 1960) due to the increased ionic strength. Chops treated with EOK showed slight improvements compared to EOH in shifting water to a more hydrostatically attractive state. We also note that the retention of water throughout the vacuum force assay by IS chops explains their water loss in the gravimetric stages being slightly higher than that of EOH. Phosphates play a key role in the retention of water, and to a lesser extent pH of the solution as demonstrated by Everts et al. (2010), who reported that the addition of ammonium hydroxide to salt/phosphate solutions did not enhance their water hold capacity over that of salt/phosphate solutions alone. Previous research has shown that increases in the pH of a meat system can increase WHC (Robbins et al., 2002; Sindelar, Prochaska, Britt, Smith, & Osburn, 2003; Wynveen et al., 2001), however it is clear that individual enhancement solution pH is not the sole factor in determining the WHC of pork products (Bertram, Meyer, Wu, Zhou, & Andersen, 2008). These effects of pH on water holding capacity, parallels the results found in the current study where EOH solution which had the greatest pH but lost the most water over time. Bertram (2008) went on to say that an elevated pH could give rise to protein denaturation.

3.3.3. Cookery, Warner-Bratzler Shear Force, and Sensory Attributes:

Control, EOH, and EOK chops lost similar ($P > 0.05$) amounts of moisture during thawing. Thaw loss for IS chops was less than CON, EOH, and EOK by 82.15, 86.67, and 83.51%, respectively (Table 3.4). Moisture loss due to cooking showed that EOH chops lost the greatest percent of moisture ($P < 0.05$) followed by EOK and CON, which were similar ($P > 0.05$) and then IS chops, which were similar to CON chops. Total moisture loss during the thawing and cooking process showed that EOH chops had the

greatest loss ($P < 0.05$) and IS chops retained the most moisture with CON and EOK chops being intermediate and similar ($P > 0.05$) to each other.

Shear force (Table 3.4) was lower in IS and EOK-enhanced chops ($P < 0.05$) than CON or EOH chops. The values reported in the current study are similar to those previously reported (Jensen, Robbins, et al., 2003) for non-enhanced chops compared to those enhanced with a traditional salt/phosphate solution. Trained sensory tenderness ratings paralleled that of WBSF with IS chops being the most tender ($P < 0.05$) and EOH and CON being similar to each other ($P > 0.05$; Table 3.4). Industry standard enhanced chops also had the most intense pork flavor ($P < 0.05$) followed by EOK and CON chops ($P > 0.05$) and finally EOH chops, which had the least intense pork flavor, but were similar ($P > 0.05$) to CON chops. As expected from the moisture loss and cooking data IS chops were rated as being the juiciest ($P < 0.05$) while chops from all other treatments were similar ($P > 0.05$) to each other. Considering the moisture loss and cook loss data, it was not expected that EOH chops would be similar to CON and EOK chops since the previous assays showed they expressed the most moisture even under light forces. There were differences detected for off-flavor with IS chops having stronger ($P < 0.05$) off-flavor detected than CON and EOH chops; however, chops from all treatments were rated between no off-flavor and threshold levels of detection.

Although Chi-square analysis for off-flavor descriptors was not significant ($P \geq 0.10$; data not shown), 67.9% of the IS-treated chops, that were considered to have off flavor, were described as salty. Sensory tenderness and instrumental tenderness showed that IS-treated chops were more tender than CON and EOH-treated chops. It has been documented that phosphate based enhancement solutions enhance the tenderness and

juiciness of pork loin chops (Jensen, Robbins, et al., 2003; Jones-Hamlow et al., 2015; D. Sutton et al., 1997). Alkaline electrolyzed water-treated chops were not effective in the improvement of tenderness or juiciness, which was also found when steaks were enhanced with sodium hydroxide (Cerruto-Noya et al., 2009). Results of this study were contradictory to those of Tobin, O'Sullivan, Hamill, and Kerry (2013) who reported a negative correlation to salt content and meat flavor in sausages which was attributed to the detection of salt masking meat flavor; where the current study shows that the addition of salt in small quantities improved the pork flavor intensity. Furthermore, in the present study the addition of potassium lactate was shown to increase the detection of pork flavor over CON and EOH-enhanced chops. The addition of lactates and their beneficial effects on meat flavor have been documented, as well as, some of their connections to off flavors (Brewer et al., 1991; Jensen, Robbins, et al., 2003).

3.4. Conclusions

The use of AEW as a replacement for traditional salt/phosphate based solutions did not improve water holding capacity or moisture retention. Furthermore, AEW did not improve pork loin tenderness or sensory characteristics compared to non-enhanced controls. The addition of potassium lactate to AEW improved moisture retention and sensory characteristics compared to AEW alone, however it still performed below traditional enhancement solutions. The use of AEW without functional additives is not recommended.

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Table 3.1: Least Squares Means for Loin Enhancement Characteristics

Traits ²	Treatments ¹				SEM	<i>P</i> - value
	CON	EOH	EOK	IS		
Grn Wt.	3.40	3.23	3.27	3.17	0.08	0.20
Enh. Wt.	3.40 ^b	3.78 ^a	3.74 ^a	3.57 ^{ab}	0.10	0.04
Rest Wt.	3.40	3.59	3.58	3.47	0.09	0.43
PU Initial, %	-	16.74 ^a	13.96 ^b	12.59 ^b	0.71	<0.01
PU Final, %	-	10.90 ^a	9.26 ^b	9.47 ^b	0.42	<0.01
pH Grn	5.80	5.75	5.66	5.85	0.07	0.29
pH Enh	5.80	5.71	5.75	5.86	0.05	0.30

^{abc}Means within a row that do not have common superscripts are different ($P < 0.05$).

¹CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution.

²Grn Wt.: Weight of pre-enhanced loins; ENH. Wt.: Weight of loins immediately after enhancement; Rest Wt.: Weight of enhanced loins after 15 min rest period; PU, Initial: Percent of moisture pickup when compared to Enh. Wt.; PU, Final: Percent of moisture pickup when compared to Rest Wt.; pH Grn: pH of the loin pre-enhancement; pH Enh; pH of the loins post-enhancement.

Table 3.2: Least Squares Means for the Main Effects of Enhancement Solution on Percent Moisture Loss When Calculated Based on Individual Assay (Top) and as a Cumulative Percent of Total Moisture Loss (100% Basis; Bottom).

	Treatments ¹					
Trait ²	CON	EOH	EOK	IS	SEM	<i>P</i> - value
Ind. Assay						
Free Drip	1.53 ^c	8.06 ^a	4.65 ^b	1.56 ^c	0.34	<0.01
EJV	7.24 ^b	9.69 ^a	6.85 ^b	3.60 ^c	0.37	<0.01
EJG	19.18 ^b	21.69 ^a	19.54 ^b	19.09 ^b	0.25	<0.01
Dry Loss	29.53 ^a	29.33 ^{ab}	28.56 ^{bc}	28.44 ^c	0.27	0.01
Total Loss	57.50 ^b	68.80 ^a	59.61 ^b	52.69 ^c	0.80	<0.01
100% Basis						
Free Drip	1.53 ^c	8.06 ^a	4.65 ^b	1.56 ^c	0.34	<0.01
EJV	7.13 ^b	8.89 ^a	6.64 ^b	3.54 ^c	0.30	<0.01
EJG	18.51 ^a	16.11 ^b	18.04 ^a	18.48 ^a	0.32	<0.01
Dry Loss	51.33 ^b	47.31 ^c	50.49 ^b	54.68 ^a	0.67	<0.01
Total Loss	78.50 ^b	80.38 ^a	79.84 ^a	78.27 ^b	0.20	<0.01

^{abc}Means within a row that do not have common superscripts are different (*P* < 0.05).

¹CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution.

²Free Drip: Moisture loss after hanging for 24h; EJV: Expressible juice, vacuum packaging, or moisture lost under vacuum pressure; EJG: Expressible juice, gravimetric, or moisture lost due to gravimetric force; EJT: Expressible juice total, or the combination of EJV and EJG; Dry Loss: moisture lost after EJG samples are place

in drying oven for 24*h*; Total Loss: Total moisture lost, or the sum of free drip, EJT, and dry loss.

Table 3.3: Least Squares Means for the Main Effect of Days on Retail Display on Percent Moisture Loss When Calculated Based on Individual Assay (Top) and as a Cumulative Percent of Total Moisture Loss (100% Basis; Bottom).

	Days of Shelf Life Display								
Trait ¹	0	5	10	15	20	25	30	SEM	<i>P</i> - value
Ind. Assay									
Free Drip	-	-	-	-	-	-	-	-	-
EJV	4.04 ^d	5.63 ^c	6.53 ^b	7.16 ^b	7.91 ^a	8.13 ^a	8.52 ^a	0.30	<0.01
EJG	21.54 ^a	20.28 ^b	19.73 ^{bc}	18.84 ^d	19.40 ^{cd}	19.03 ^d	20.30 ^b	0.23	<0.01
Dry Loss	29.09	28.84	29.01	28.75	29.40	28.83	28.82	0.26	0.47
Total Loss	-	-	-	-	-	-	-	-	-
100% Basis									
Free Drip	-	-	-	-	-	-	-	-	-
EJV	3.86 ^e	5.36 ^d	6.24 ^c	7.01 ^b	7.54 ^{ab}	7.75 ^a	8.10 ^a	0.28	<0.01
EJG	18.32 ^a	18.07 ^a	17.90 ^{ab}	17.91 ^{ab}	17.79 ^{abc}	17.28 ^{bc}	17.21 ^c	0.24	<0.01
Dry Loss	52.35 ^a	51.61 ^{ab}	51.10 ^{bc}	50.76 ^{cd}	49.99 ^e	50.55 ^{cde}	50.30 ^{de}	0.34	<0.01
Total Loss	78.53 ^c	79.10 ^b	79.13 ^b	79.56 ^{ab}	79.23 ^{ab}	79.53 ^{ab}	79.65 ^a	0.19	<0.01

^{abc}Means within a row that do not have common superscripts are different ($P < 0.05$).

¹Free Drip: Moisture loss after hanging for 24*h*; EJV: Expressible juice, vacuum packaging, or moisture lost under vacuum pressure; EJG: Expressible juice, gravimetric, or moisture lost due to gravimetric force; EJT: Expressible juice total, or the combination of EJV and EJG; Dry Loss: moisture lost after EJG samples are place in drying oven for 24*h*; Total Loss: Total moisture lost, or the sum of free drip, EJT, and dry loss.

²Due to free drip measurements starting simultaneous and only running 24*h* no day effect was analyzed.

Table 3.4: Least Squares Means for Cooking, Shear Force, and Sensory Characteristics of Enhanced Pork Loins Chops

	Treatments ¹					
Trait	CON	EOH	EOK	IS	SEM	<i>P</i> - value
Cooking						
Thaw Loss, %	7.06 ^a	9.45 ^a	7.64 ^a	1.26 ^b	1.56	<0.01
Cook Loss, %	10.86 ^{bc}	14.33 ^a	11.39 ^b	9.62 ^c	0.61	<0.01
Total Loss, %	17.20 ^b	22.43 ^a	18.15 ^b	10.76 ^c	1.48	<0.01
WBSF	2.09 ^a	2.18 ^a	1.59 ^b	1.46 ^b	0.12	<0.01
Sensory ²						
Tenderness	4.95 ^c	4.61 ^c	5.71 ^b	6.75 ^a	0.23	<0.01
Pork Flavor	4.88 ^{bc}	4.59 ^c	5.11 ^b	5.73 ^a	0.14	<0.01
Juiciness	4.29 ^b	4.01 ^b	4.44 ^b	5.45 ^a	0.18	<0.01
Off Flavor	1.12 ^b	1.09 ^b	1.16 ^{ab}	1.33 ^a	0.06	0.03

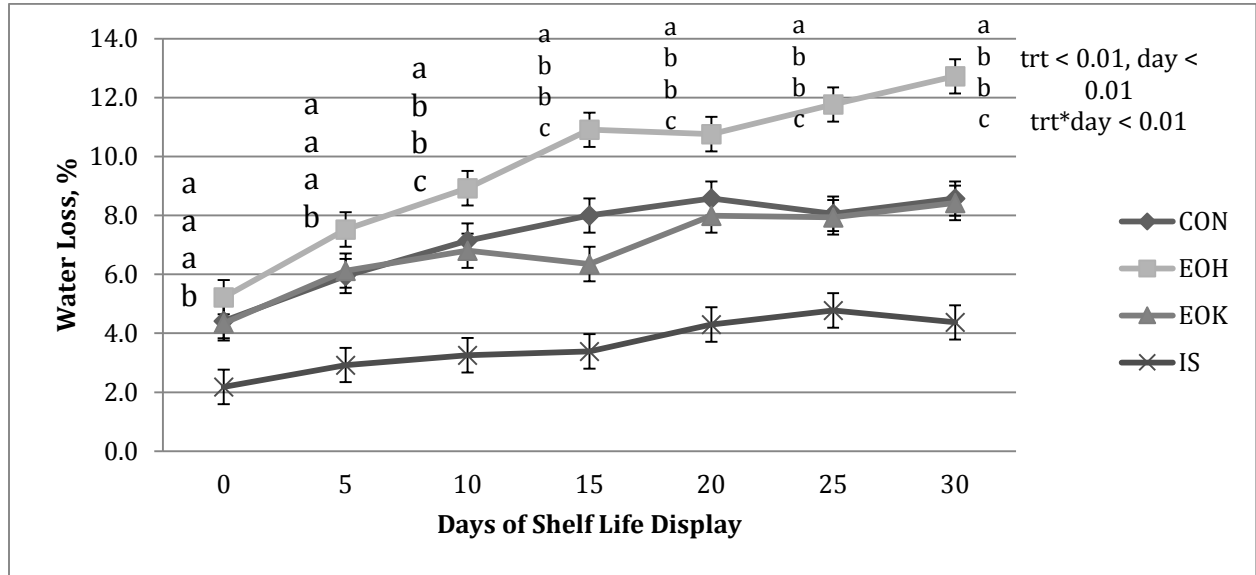
^{abc}Means within a row that do not have common superscripts are different ($P < 0.05$).

¹CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution.

²Sensory attributes were ranked on 1 – 8 scale for tenderness, pork flavor intensity, and juiciness (1 – extremely tough, extremely bland, extremely dry, and 8 – extremely tender, extremely intense, extremely juicy) while off flavor was ranked on a 1 – 6 scale (1 – none detected, and 6 – extreme off-flavor).

Figure 3.1:

A.



B.

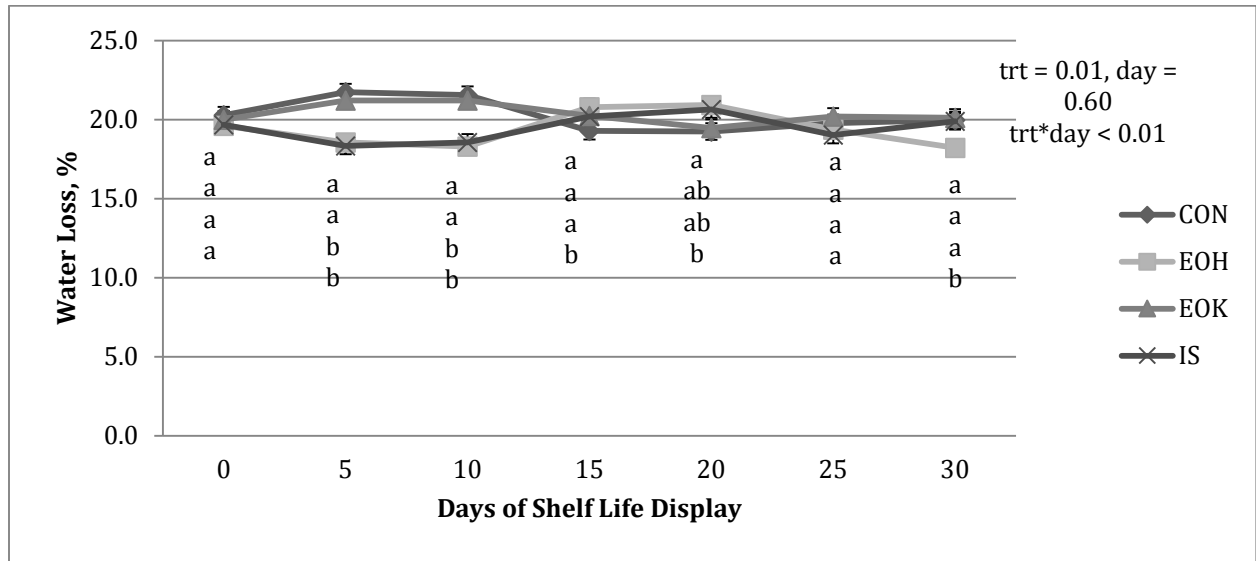
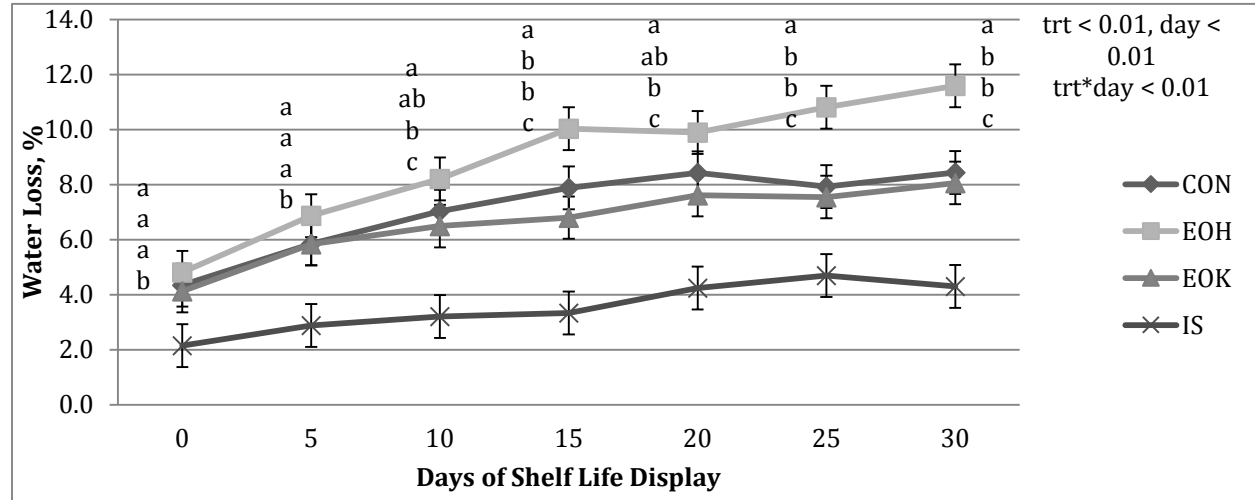


Figure 3.1a: Effects of treatments on expressible juice due to vacuum force of pork loin chops for 30 days of shelf life display on an individual assay basis. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution. SEM = 0.60 ^{abc}Means within each day that do not have common superscripts are different ($P < 0.05$).

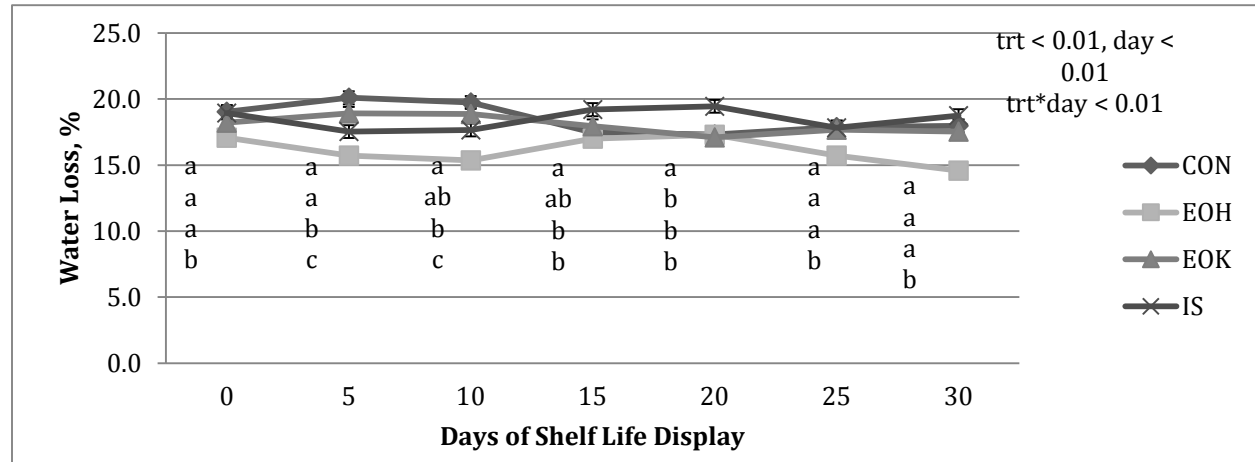
Figure 3.1b: Effects of treatments on expressible juice due to gravimetric force of pork loin chops for 30 days of shelf life display on an individual assay basis. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution. SEM = 0.47 ^{abc}Means within each day that do not have common superscripts are different ($P < 0.05$).

Figure 3.2:

A.



B.



C.

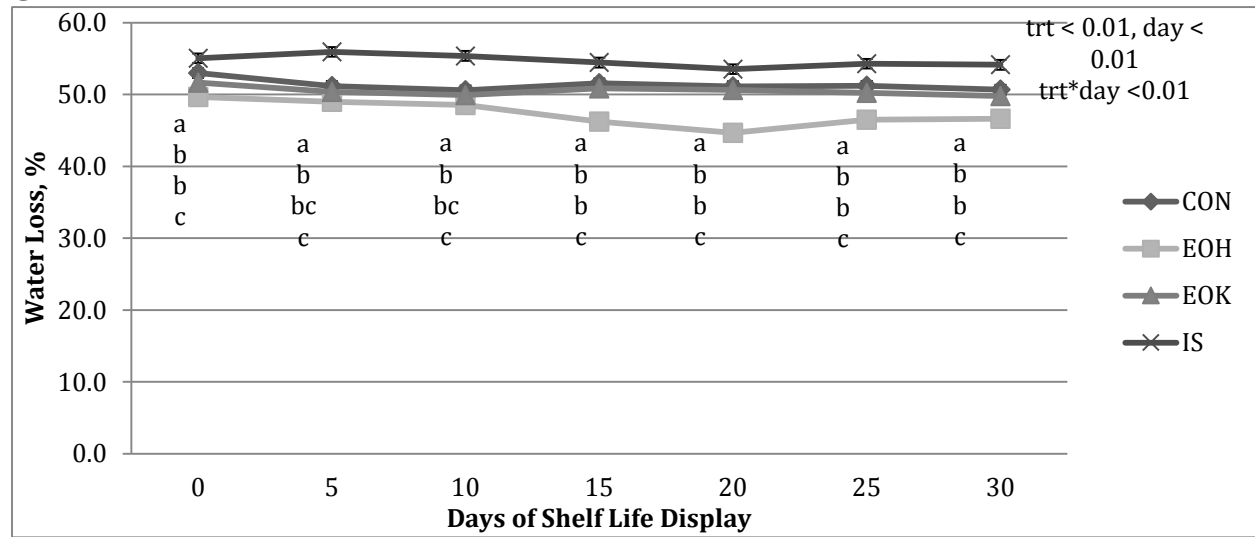


Figure 3.2a: Effects of treatments on expressible juice due to vacuum force of pork loin chops for 30 days of shelf life display on a 100% basis. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution. SEM = 0.55^{abc} Means within each day that do not have common superscripts are different ($P < 0.05$).

Figure 3.2b: Effects of treatments on expressible juice due to gravimetric force of pork loin chops for 30 days of shelf life display on a 100% basis. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution. SEM = 0.49^{abc} Means within each day that do not have common superscripts are different ($P < 0.05$).

Figure 3.2c: Effects of treatments on dry loss of pork loin chops for 30 days of shelf life display on a 100% basis. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution. SEM = 0.68^{abc} Means within each day that do not have common superscripts are different ($P < 0.05$).

CHAPTER 4

EVALUTATION OF ALKALINE ELECTROLYZED WATER TO REPLACE TRADITIONAL PHOSPHATE ENHANCEMENT SOLUTION: EFFECTS ON SHELF LIFE COLOR AND LIPID OXIDATION²

² Rigdon, M., and A.M. Stelzleni. To be submitted to *Meat Science*

Abstract

Sixty-four whole pork loins were randomly assigned to four treatments to evaluate the use of alkaline electrolyzed reduced water for replacement of traditional enhancement solutions. Treatments included: alkaline electrolyzed reduced water (EOH; pH \approx 11.5), EOH plus 2.5% potassium lactate (EOK), industry standard (IS; 0.35% Sodium Tri-polyphosphate, 0.14% sodium chloride, 2.5% potassium lactate), and no enhancement (CON). After enhancement (110%), chops were cut (2.54-cm) to test treatment effects on shelf life color and lipid oxidation. Despite the alkaline nature of the enhancement solution, EOH chops were less red ($P<0.05$) and produced more metmyoglobin ($P<0.05$) than IS chops across all days of simulated retail display. Subjective color panelists rated the average color of EOH chops as brighter purplish-pink ($P<0.05$) and having less darkening ($P<0.05$) from muscle discoloration than both IS and CON. Alkaline electrolyzed water enhanced chops exhibited greater ($P<0.05$) lipid oxidation than all other treatments. Enhancement with EOH as a stand-alone enhancement solution is not a suitable replacement for industry standard solutions.

Keywords: Alkaline electrolyzed water, enhancement, pork, color, oxidation

4.1. Introduction

Enhancement solutions containing salt and phosphates have been used by the pork industry for many years to improve the overall quality of pork products (Miller, 1998). Functional non-meat ingredients in today's commercial pork products are used for their ability to improve and stabilize color, and increase shelf life stability (Miller, 1998). Phosphate salts are among these functional non-meat ingredients and are used for adjusting meat pH, improving protein solubility, and increasing water holding capacity (Long et al., 2014). Meat pH has a direct influence on meat color, with higher meat system pH values resulting in darker color. The darker color of high pH meat products is a result of the meat's ability to bind more water which causes the muscle to swell absorbing more light (Lawrie, 1991; John R. Romans et al., 1994). Although pH has an influence on the color of a meat product, myoglobin accounts for approximately 90% of the pigmentation (Damodaran, Parkin, & Fennema, 2008). Myoglobin, made of 153 amino acids, is one single polypeptide chain, which attaches to a porphyrin ring containing a central iron atom, and is responsible for the overall hue in meat (Damodaran et al., 2008).

The main factor in consumer acceptance of meat products is meat color (Damodaran et al., 2008), however Brewer et al. (2002) also showed that meat color is the primary determinant of freshness and quality, at the point of sale. On the contrary, discoloration is determined as a defect in meat products according to consumers (Troy & Kerry, 2010). As previously mentioned, phosphates aid in the retention and stability of meat color, however consumers have become weary of chemicals in their food, regardless of their safety and role in satisfactory eating experience (Brewer, 1998). While

consumers demands and willingness to pay for clean-label meat products increases (Zurawicki, 2015) new clean-label non-meat ingredients must be tested that retain the functionality, safety, and performance of traditional ingredients such as phosphate salts. Alkaline electrolyzed water (AEW) may hold promise as a replacement for traditional non-meat ingredients for ensuring satisfactory eating experience and its ability to be labeled as water. Therefore, the objective of this study was to determine the effects of AEW on enhanced pork loin color and lipid oxidation.

4.2. Materials and methods

4.2.1. Pork Loin Procurement and Enhancement

Sixty-four Institutional Meat Purchase Specifications 413 whole boneless pork loins (*longissimus thoracis et lumborum*) were procured 2 d postmortem from a multi-national pork supplier (Smithfield Farmland, Duncan, NC) across two replicates (32 loins per replicate) and transported ($0 \pm 2^{\circ}\text{C}$) 575 km to The University of Georgia Meat Science Technology Center (MSTC, Athens, Ga). The pork loins for each replicate were randomly selected from the mornings fabrication line, vacuum packaged and boxed accordingly to plant standard operating procedures. Upon arrival at the MSTC the pork loins were placed in cold ($2 \pm 2^{\circ}\text{C}$) dark storage until 4 d postmortem. At 4 d postmortem the loins were randomly assigned to one of four treatments (8 loins / treatment • replicate⁻¹ for a total of 16 loins per treatment) to test the efficacy of novel enhancement solutions on pork loin shelf life color and lipid oxidation. The four treatments included: 1) alkaline electrolyzed reduced water (EOH; pH \approx 11.5), 2) alkaline electrolyzed reduced water with 2.5% potassium lactate (EOK; Hawkins, Minneapolis, MN; pH \approx 10.92), 3) water with 0.35% Sodium Tri-polyphosphate (ICL Performance Products,

Bolingbrook, IL), 0.14% sodium chloride (Morton Salt Inc., Chicago, IL), 2.5% potassium lactate (IS; pH \approx 6.78), and 4) no enhancement (CON).

After treatment randomization whole loins were enhanced (except CON) to a target of 110% of green weight (Injectamatic PI21, Koch Equipment LLC, Kansas City, MO, USA). The enhanced loins were allowed to rest and purge for 15 min before cutting.

After post enhancement rest period, the whole loins were cut into 2.54 cm chops. Starting from the anterior end 9 chops (\approx 23 cm) were removed for other analyses. The following 7 chops (2.54 cm) were cut, vacuum packaged (B-620 series; 30–50 cm³ O₂/m²/24 h/101,325 Pa/23 °C; Cryovac Sealed Air Corporation, Duncan, SC, USA) and randomly assigned to 0, 5, 10, 15, 20, 25, and 30 d of shelf life. After day of display assignment chops were boxed and placed in cold dark storage (2°C \pm 2°C) for 4 d to simulate transportation time.

4.2.2. Objective Color:

After transportation simulation, vacuum-packaged chops were placed in a coffin style retail display case (3 \pm 2°C, with two defrost cycles every 24-h; MIX-E, Hussmann, Bridgeton, MO) with 24-h continuous fluorescent lighting (Octron/ECO; 30000K; F032/830/ECO; Sylvania Company, Versailles, KY) at a range of 1400 - 1900 lux for 30 d. Objective color was performed using a Hunter-Lab MiniScan EZ (Hunter Associates Laboratory, Reston, Virginia) with illuminate A and 10° viewing angle on d 0, 5, 10, 15, 20, 25, and 30, standardized using a white tile, and black tile standard before each use. *Commission Internationale de l'Eclairage* (CIE) L* (Lightness), a* (Redness), and b* (Yellowness) were measured and recorded on d 30 chops in triplicate and averaged on each sampling day to assess change in color over time. Values for hue angle (H* =

arctangent [b^* / a^*]), and chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) were calculated to evaluate the redness and vividness of chops. Reflectance readings were taken at 630 nm, 610 nm, 580 nm, 572 nm, 525 nm, and 474 nm for the calculation of redness, deoxymyoglobin (DMb), oxymyoglobin (OMb), and metmyoglobin (MMb). Redness, either due to OMb or DMb was calculated using the ratio $R_{630}:R_{580}$. Deoxymyoglobin, OMb, and MMb values were calculated using the reflectance at isobestic wavelength ratios of $R_{474}:R_{525}$, $R_{610}:R_{525}$, and $R_{572}:R_{525}$, respectively (AMSA, 2012).

4.2.2. Subjective Color:

A five-member panel was used to evaluate color characteristics of pork loin chops. Panelists were selected based on Farnsworth-Munsell 100 hue color test to determine panelist ability to detect hue, or differences in color. Panelists with scores of less than 40 were selected. On d 0, 5, 10, 15, 20, 25, and 30 of simulated retail display panelists evaluated chops used for instrumental color based on average color, muscle darkening, percent discoloration, and purge characterization on d 30 chops. Average color and percent darkening were measured on an 8 point scale (where 8 = extremely dark purplish-pink, 96 – 100% discoloration; 7 = dark purplish-pink, 80 – 95% discoloration; 6 = moderate purplish-pink, 60 – 79% discoloration; 5 = slight dark purplish-pink, 40 – 59% discoloration; 4 = slight purplish-pink, 20 – 39% discoloration; 3 = moderately bright purplish-pink, 5 – 19% discoloration; 2 = bright purplish-pink, 0 – 4% discoloration; 1 = extremely bright purplish-pink, 0% discoloration) modified from Hunt et al. (1991) and Stivarius, Pohlman, McElyea, and Waldroup (2002). Muscle darkening of enhanced chops were evaluated on a 7 point scale (where 7 = very dark; 5 = moderately dark; 3 = slightly dark; 1 = no darkening) as described in AMSA (2012).

Purge discoloration was evaluated on a 6 point scale (6 = dark brown; 5 = light brown; 4 = dark purplish-red; 3 = red; 2 = pink; 1 = clear) modified from AMSA (2012).

4.2.3 Lipid Oxidation:

Lipid oxidation was performed using the method of thiobarbituric acid reactive species (TBARS) as outlined by Ahn et al. (1998) with modifications. After simulated retail display time chops were removed from the retail case and placed into the freezer (-20°C) until further analysis could be performed. Chops were removed from the freezer 24 h prior to analysis and placed into a cooler at 4°C for 18 – 24 h. The chops were removed from vacuum packages and the subcutaneous fat and epimysial connective tissue was removed. Chops were minced and mixed by hand to form a representative sample. Subsequently, 5 g of sample were placed into a 50 ml conical screw cap centrifuge tubes. Fifteen milliliters of distilled water was added to the tubes and homogenized (IKA, Wilmington, NC) on high for 30 s. The tubes were centrifuged (CR 312, Jouan INC., Winchester, VA) at 1850 x g for 10 min at room temperature. Two milliliters of supernatant was removed and added to duplicate 13x100 ml culture tubes with 100 µl BHT (7.2%) and 4 ml of TBA-TCA solution and each tube was vortexed. Tubes were heated in a hot water bath (90°C) for 15 min, removed and cooled in a room temperature water bath for 10 min. Samples were centrifuged at 3000 x g (CR 312, Jouan INC., Winchester, VA) for 15 min at room temp and the absorbance of the supernatant was measured using a spectrophotometer (V-630 UV-Visible/NIR, Jasco Analytical Instruments, Easton, MD) at 531 nm and fitted to a standard curve. Lipid oxidation was reported as mg malondialdehyde/kg.

4.2.4 Statistical Analysis:

Data were analyzed using Proc Mixed of SAS (version 9.3), as a completely randomized split-plot design where loin was the whole-plot and chop within loin was the sub-plot. Loin within replication by treatment was included as the random variable. Loin was considered the experimental unit and chop was considered the observational unit. Main effects and all treatment by day interactions were tested when applicable. Objective and subjective color analysis included day in the model and the treatment by day interaction was tested. Panelist was also included as a covariate for subjective color; however, panelist was not a significant factor. If an interaction was detected data were reanalyzed by day. Sample size was determined utilizing operating characteristic curves as outlined by Ferris et al. (1946) and (Montgomery, 2001). Means were separated using the PDIFF option of LSMEANS at $\alpha = 0.05$.

4.3. Results Discussion

4.3.1 Objective Color

For L^* there was no treatment by day interaction ($P = 0.25$). Values for L^* (Table 4.1) showed EOH-treated chops were the lightest color ($P < 0.05$), IS chops were the darkest ($P < 0.05$), and CON and EOK were intermediate and similar ($P > 0.05$). As days of display increased all chops became lighter in color ($P < 0.05$) with the exception of d 20, which was lighter ($P < 0.05$) than d 30 but similar ($P > 0.05$) to d 25 (Table 4.2).

On d 0 through d 10 all chops were similar ($P > 0.05$) in redness (a^* ; Figure 4.1a). However, from d 15 to d 30 CON chops exhibited greater ($P < 0.05$) a^* values than EOH chops. Industry standard enhanced chops and EOK chops remained similar ($P > 0.05$) to EOH chops through 20 d. After 25 d of display IS chops had greater ($P < 0.05$) a^* values

than EOH, however EOK and EOH chops remained similar ($P > 0.05$). Values for b^* (Figure 4.1b) were greater ($P < 0.05$) for EOH chops compared to IS chops through 10 d. After 15 d b^* was similar ($P > 0.05$) for all treatments; however after 20 d IS chops were less yellow ($P < 0.05$) than CON and EOK. After 30 d of display EOK chops were greater ($P < 0.05$) than EOH and CON chops, with IS chops being similar ($P > 0.05$) to all treatments. Overall chroma values (Figure 4.1c) were similar ($P > 0.05$) for vacuum packaged chops throughout display with the exception of d 20 and d 30. On d 20 CON chops were more vivid than IS and EOH chops which were similar ($P > 0.05$) to each other. After 30 d IS chops were similar ($P > 0.05$) to EOK and CON chops but more saturated ($P < 0.05$) than EOH chops.

There was not a treatment by day interaction for hue value or OMb ($P > 0.05$), therefore, the effects for treatment and day are presented in Tables 4.1 and 4.2, respectively. Chops from EOH and EOK were similar ($P > 0.05$) and less red ($P < 0.05$) than CON and IS ($P > 0.05$). As days of simulated retail display increased chops became less red ($P < 0.05$), as expected. Control, EOK, and IS chops were similar ($P > 0.05$) in OMb content, while EOH chops had less ($P < 0.05$) OMb than both CON and IS chops, but was similar ($P > 0.05$) to EOK chops. Oxymyoglobin content decreased ($P < 0.05$) as days on simulated retail display increased, with the exception of d 15 which had a greater reflectance ratio ($P < 0.05$) than d 5 and d 10. Furthermore, d 30 ratios were similar ($P > 0.05$) to those on d 5, d 10 and d 15.

For redness, deoxymyoglobin, and metmyoglobin ratios there were treatment by day interactions ($P < 0.01$). When chops were analyzed for redness ratios (Figure 4.2a), regardless of day, EOH chops were less red ($P < 0.01$) than both IS-treated and CON

chops, which were similar ($P > 0.05$) across all days of retail display except for d 30 where IS chops were more red ($P < 0.05$) than CON chops. Additionally, across all days of simulated retail display EOK and CON chops were similar ($P > 0.05$). From d 0 to d 20 of retail display EOH and EOK chops were similar ($P > 0.05$), however after 25 and 30 d, EOH chops were less red ($P < 0.05$) than EOK chops. Throughout the course of shelf life EOH-treated chops had lower ($P < 0.01$) DMb ratios than CON chops, with the exception of d 25 and 30 where they were similar ($P > 0.05$; Figure 4.2b).

Deoxymyoglobin ratios showed that regardless of day IS and CON chops had similar ($P > 0.05$) DMb content. Furthermore, IS chops had a greater ($P < 0.05$) DMb reflectance ratio than EOH with the exception of d 0 and 25. Additionally, EOK and CON chops were similar in DMb content through 15 d of display. After 20 d EOK chop DMb reflectance ratio decreased and was lower than ($P < 0.01$) than CON chops for the remainder of display. Regardless of day, the treatments using AEW were similar ($P > 0.05$) for MMb content. Across all days of simulated retail display EOH and EOK chops had greater ($P < 0.01$) MMb reflectance ratio (Figure 4.2c) than IS chops; indicating a higher MMb content. Furthermore, IS chops were similar ($P > 0.05$) to CON chops across all days of simulated retail display, with the exception of d 30 where IS chops had less ($P < 0.01$) MMb than those from CON. Additionally EOK chops were similar ($P > 0.05$) to CON chops across all days, except for d 30 where EOK chops had greater ($P < 0.05$) MMb than CON chops.

Similar results for L^* and a^* were found when beef strip steaks were enhanced with ammonium hydroxide and compared to steaks enhanced with a salt/phosphate solution, where steaks treated with the alkaline enhancement solution were lighter, and

less red (Cerruto-Noya et al., 2009). These results were attributed to the increased water holding capacity, and pH of phosphate-injected steaks. In the current study, hue and a^* values were similar across treatment and day of display, as days on display increase, redness measured by either hue or a^* decreased. Additionally, the decrease of redness ratios, the increase of MMb and decrease of OMb over the course of the study is congruent with the findings of decreasing redness noted for a^* . Although AEW has a low oxidation reduction potential (Huang et al., 2008) and low oxidation reduction potential has been associated with color stability (Ahn & Nam, 2004) the low oxidation reduction potential of the EOH treatment (-800mV) did not improve color stability and redness compared to IS. Lactate treatments are reported to have no effect on MMb formation after 14 d of incubation *in vitro* (Nair, Suman, Li, Ramanathan, & Mancini, 2014). However, it is reported that the effects of lactates on meat color are due to their effects on enzyme and metabolic activity post-mortem and their antioxidant properties (Nair et al., 2014). This explains the greater color stability of EOK chops when compared to EOH chops, as well as aiding in the color stability of IS chops. Y. H. Kim et al. (2006) also reported that the inclusion of lactates in beef enhancement have improved color due to replenishment of NADH and the subsequent reduction of MMb to OMb or DMb. Phosphates have also been reported to have beneficial effects on fresh meat color and stability through their antioxidant properties (Long et al., 2014; John R. Romans et al., 1994). Further, removing oxygen, as seen in a vacuum packaged pork chop, will aid in the retention of color and decrease MMb production (Damodaran et al., 2008).

4.3.2. Subjective Color:

There was a treatment by day interaction ($P < 0.01$) for subjective average color (Figure 4.3a). Regardless of day panelist rated IS chops as being darker purplish-pink ($P < 0.05$) than all other treatments. Additionally, EOH chops were rated as being brighter purplish-pink ($P < 0.05$) than all other treatments, with the exception of d 5 where EOH and EOK chops were similar ($P > 0.05$). For all days of retail display CON and EOK chops were similar ($P > 0.05$) being rated between moderately bright and bright purplish-pink. Pork chop darkening due to enhancement, as rated by panelists, did not exhibit a treatment by day interaction ($P = 0.84$). Similar to overall color scores, IS chops were darker ($P < 0.05$) than all other chops, while EOH chops were lighter ($P < 0.05$) than both CON and EOK chops, which were similar ($P > 0.05$; Table 4.3). Chops became darker as day on display increased ($P < 0.01$; Table 4.4). Neither treatment ($P = 0.11$) nor day ($P = 0.32$) affected percent darkening of chops (Table 4.3 and 4.4, respectively).

Subjective purge color scores exhibited a treatment by day interaction ($P < 0.01$) as shown in Figure 4.3b. After d 0 and d 5 all treatments showed similar ($P > 0.05$) purge color scores. After d 10 IS chop purge maintained a lighter color ($P < 0.05$) than all other treatments. Control, EOH and EOK had similar ($P > 0.05$) purge color on d 10 and 15, however after 20 d EOH and EOK chop purge became more brown ($P < 0.05$).

Average color as rated by color panelist showed that EOH chops turned less dark purplish-pink which followed the same trends of L^* values of lightness. Additionally panelist ratings for muscle darkening show similar results to L^* and chroma, where darkness of chops increased over time. Cerruto-Noya et al. (2009) reported similar findings to those of the current study where color panelists rated phosphate enhanced

chops as being able to maintain color stability when compared to those enhanced with ammonium hydroxide. Increase of browning in purge color is attributed to the excessive purge in the package, and subsequently the sarcoplasmic protein, myoglobin in the purge, oxidizing (Aberle et al., 2001). Although changes in objective color, and standard errors were small, similar trends were noted in subjective color panelist ratings, indicating color change differences between treatments were evident.

4.3.3. Lipid Oxidation:

There was not a treatment by day interaction ($P = 0.59$) for lipid oxidation. The EOH chops had greater oxidation ($P < 0.05$; Table 4.3) than all other treatments. Day of display did not affect lipid oxidation ($P = 0.67$; Table 4.4). Lipid oxidation of EOH chops over that of phosphate-enhanced chops was found to be similar to that of beef loin steaks enhanced with ammonium hydroxide (Cerruto-Noya et al., 2009). Additionally the use of phosphates can act as antioxidants for protection against lipid oxidation (Pokorny et al., 2001). Furthermore, the association of protein (myoglobin) oxidation and lipid oxidation (Cheng et al., 2007; Faustman et al., 2010) would suggest that the EOH-treated chops, with greater MMb formation, would have higher lipid oxidation. However, even after 30 d of display all chops were below the lipid oxidation threshold for fresh pork noted by Lanari et al. (1995).

4.4. Conclusions:

The use of AEW as a replacement for traditional salt/phosphate based solutions did not aid in color improvement or stability. The addition of potassium lactate to AEW improved color attributes as well as lipid oxidation values. The use of AEW is not recommended as a color stabilizer and is detrimental to lipid oxidation.

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Table 4.1: Least Squares Means for Loin Chop Objective Color Characteristics

Traits	Treatments ¹				SEM	<i>P</i> - value
	CON	EOH	EOK	IS		
L*	56.18 ^b	59.09 ^a	56.36 ^b	53.20 ^c	0.49	<0.01
Hue	37.40 ^b	39.24 ^a	39.00 ^a	36.97 ^b	0.53	<0.01
Oxymyoglobin	1.49 ^a	1.45 ^b	1.47 ^{ab}	1.50 ^a	0.01	<0.05

^{abc}Means within a row that do not have common superscripts are different ($P < 0.05$).

¹CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution

Table 4.2: Least Squares Means for the Main Effect of Days on Retail Display on Objective Color Characteristics.

Trait	Days of Shelf Life Display							SEM	<i>P</i> - value
	0	5	10	15	20	25	30		
L*	54.35 ^e	55.61 ^d	56.00 ^c	56.18 ^c	57.28 ^a	57.13 ^{ab}	56.90 ^b	0.27	<0.01
Hue	37.72 ^c	37.34 ^c	37.45 ^c	37.73 ^c	38.51 ^b	38.85 ^b	39.46 ^a	0.31	<0.01
Oxymyoglobin	1.51 ^a	1.48 ^c	1.47 ^c	1.49 ^b	1.45 ^d	1.45 ^d	1.48 ^{bc}	0.01	<0.01

^{abc}Means within a row that do not have common superscripts are different ($P < 0.05$).

Table 4.3: Least Squares Means for Loin Chop Subjective Color and Lipid Oxidation.

	Treatments ¹					
Trait	CON	EOH	EOK	IS	SEM	<i>P</i> - value
Subjective Color						
Darkening ²	2.55 ^b	2.14 ^c	2.50 ^b	2.98 ^a	0.34	<0.01
Percent Darkening ³	1.90	1.97	1.99	2.36	0.24	0.11
TBARS	0.07 ^b	0.12 ^a	0.07 ^b	0.06 ^b	0.02	0.03

^{abc}Means within a row that do not have common superscripts are different ($P < 0.05$).

¹CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution.

²Muscle Discoloration was measured by panelists on a 7-point scale (7 = very dark; 5 = moderately dark; 3 = slightly dark; 1 = no darkening).

³Percent darkening was measured by panelists on an 8 point scale (8 = 96 – 100% discoloration; 7 = 80 – 95% discoloration; 6 = 60 – 79% discoloration; 5 = 40 – 59% discoloration; 4 = 20 – 39% discoloration; 3 = 5 – 19% discoloration; 2 = 0 – 4% discoloration; 1 = 0% discoloration).

Table 4.4: Least Squares Means for the Main Effect of Days on Retail Display on Subjective Color Characteristics and Lipid Oxidation.

	Days of Shelf Life Display								
Trait	0	5	10	15	20	25	30	SEM	<i>P</i> - value
Subjective Color									
Darkening ¹	2.13 ^{de}	2.10 ^e	2.28 ^d	2.70 ^c	2.76 ^{bc}	2.89 ^{ab}	2.93 ^a	0.25	<0.01
Percent Darkening ²	2.11	2.08	2.09	2.07	2.11	2.01	1.91	0.22	0.32
TBARS	0.06	0.10	0.10	0.06	0.08	0.08	0.10	0.02	0.67

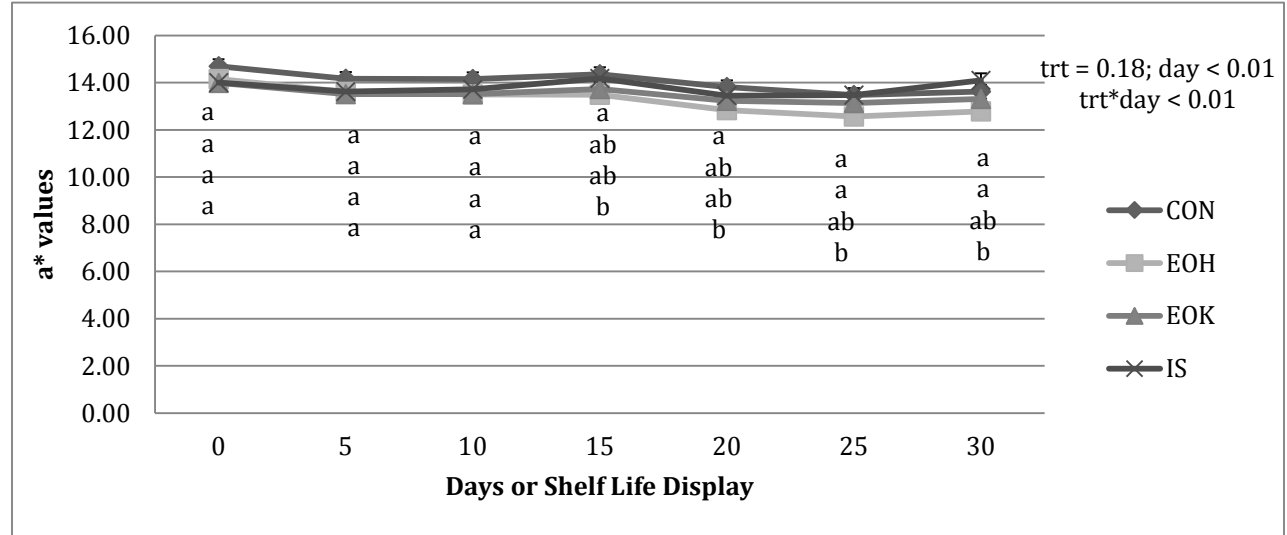
^{abc}Means within a row that do not have common superscripts are different ($P < 0.05$).

¹Muscle Discoloration was measured by panelists on a 7-point scale (7 = very dark; 5 = moderately dark; 3 = slightly dark; 1 = no darkening).

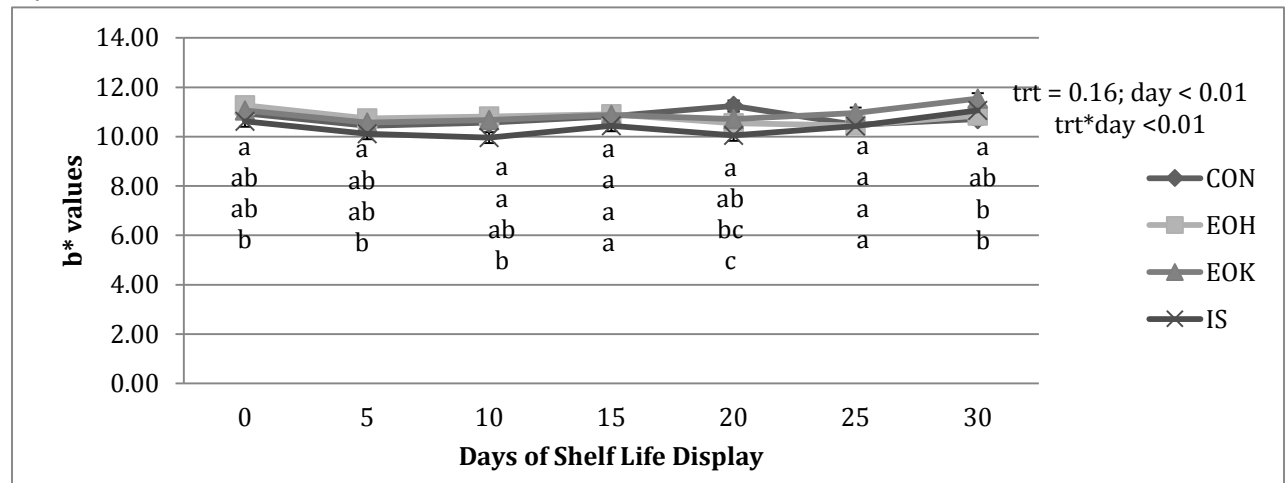
²Percent darkening was measured by panelists on an 8 point scale (8 = 96 – 100% discoloration; 7 = 80 – 95% discoloration; 6 = 60 – 79% discoloration; 5 = 40 – 59% discoloration; 4 = 20 – 39% discoloration; 3 = 5 – 19% discoloration; 2 = 0 – 4% discoloration; 1 = 0% discoloration).

Figure 4.1.

A.



B.



C.

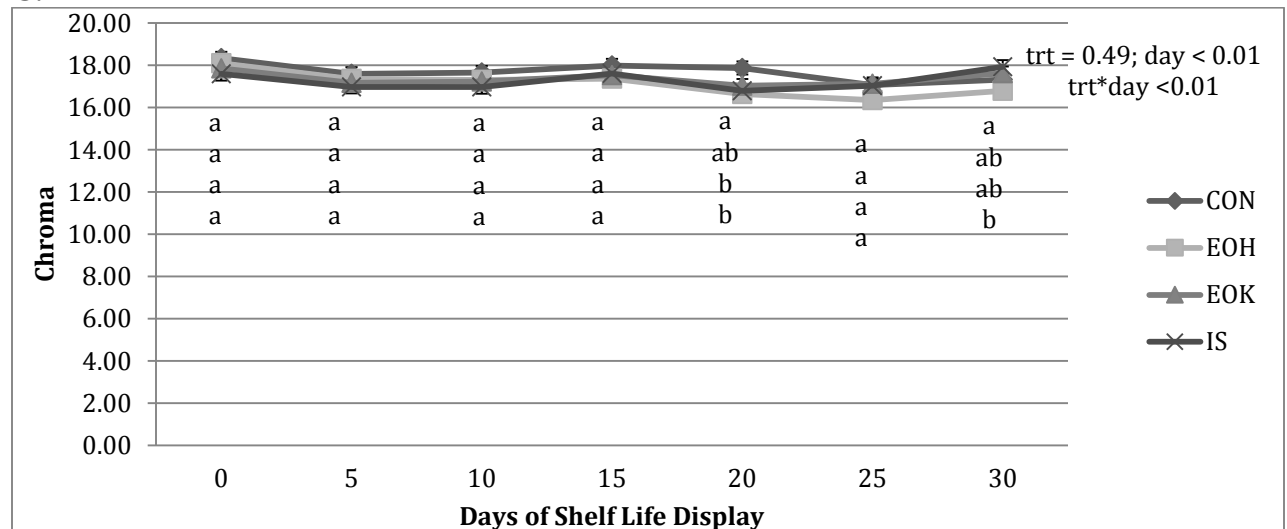


Figure 4.1a: Effects of treatments on a* values of pork loin chops for 30 days of shelf life display. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution. SEM = 0.29

^{abc}Means within each day that do not have common superscripts are different ($P < 0.05$).

Figure 4.1b: Effects of treatments on b* values of pork loin chops for 30 days of shelf life display. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution. SEM = 0.22

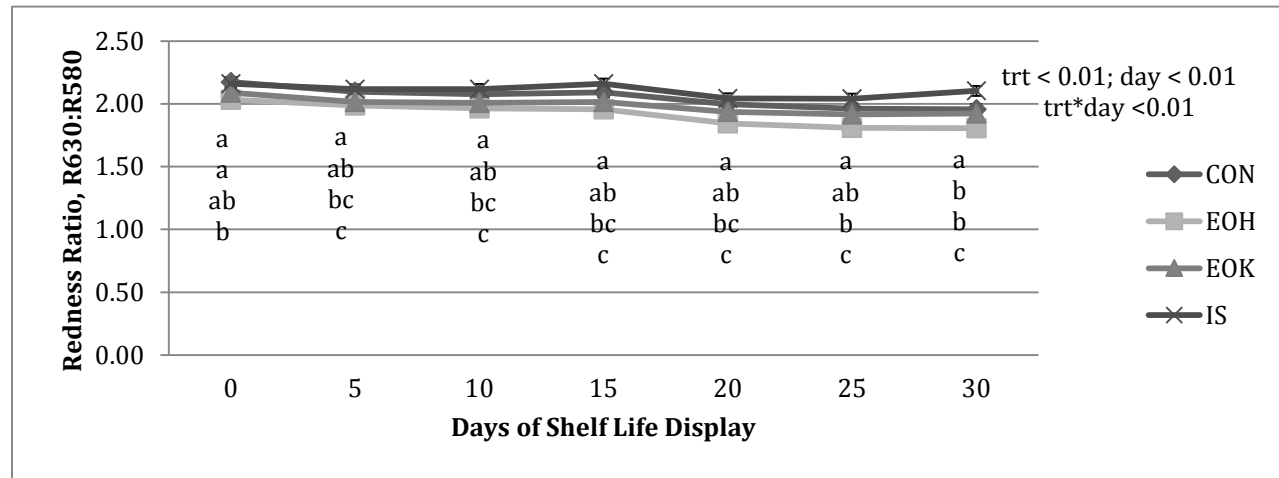
^{abc}Means within each day that do not have common superscripts are different ($P < 0.05$).

Figure 4.1c: Effects of treatments on chroma of pork loin chops for 30 days of shelf life display. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution.

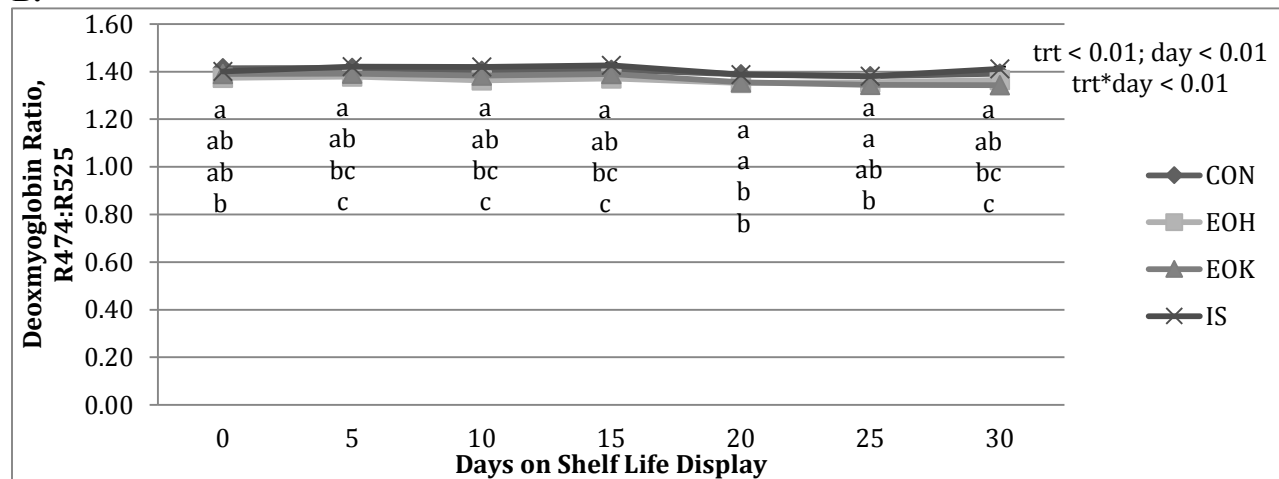
^{abc}Means within each day that do not have common superscripts are different ($P < 0.05$). SEM = 0.31

Figure 4.2.

A.



B.



C.

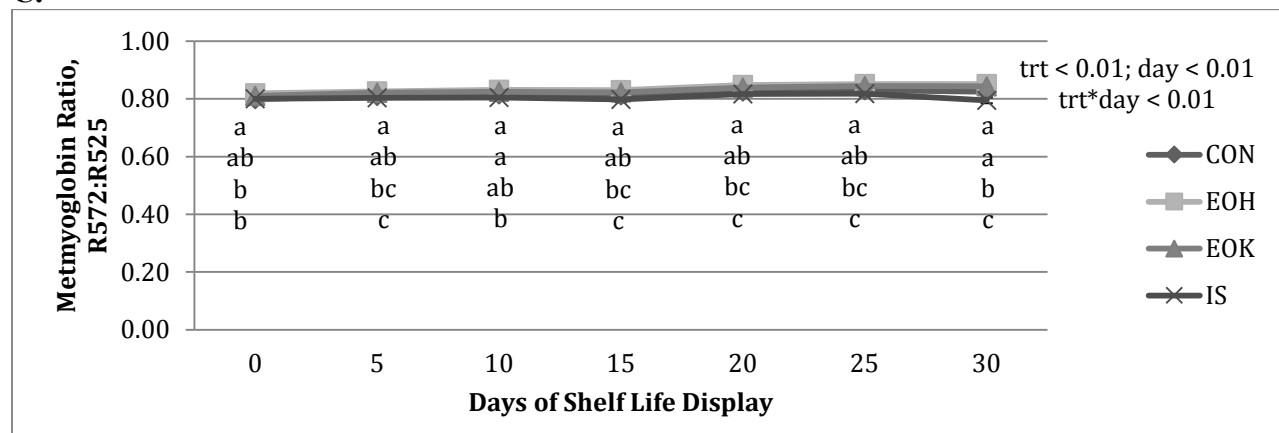


Figure 4.2a: Effects of treatments on Redness ratios ($R_{630}:R_{580}$) of pork loin chops for 30 days of shelf life display. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution. SEM = 0.04

^{abc}Means within each day that do not have common superscripts are different ($P < 0.05$).

Figure 4.2b: Effects of treatments on deoxymyoglobin ratios ($R_{474}:R_{525}$) of pork loin chops for 30 days of shelf life display. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution. SEM =

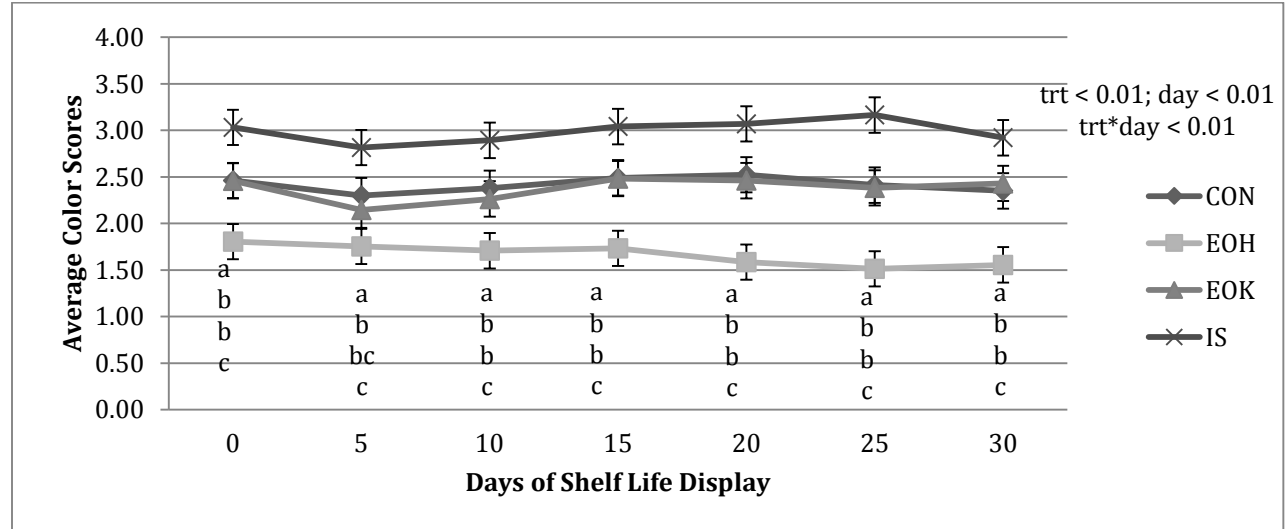
0.01 ^{abc}Means within each day that do not have common superscripts are different ($P < 0.05$).

Figure 4.2c: Effects of treatments on metmyoglobin ratios ($R_{572}:R_{525}$) of pork loin chops for 30 days of shelf life display. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution. SEM =

0.01 ^{abc}Means within each day that do not have common superscripts are different ($P < 0.05$).

Figure 4.3.

A.



B.

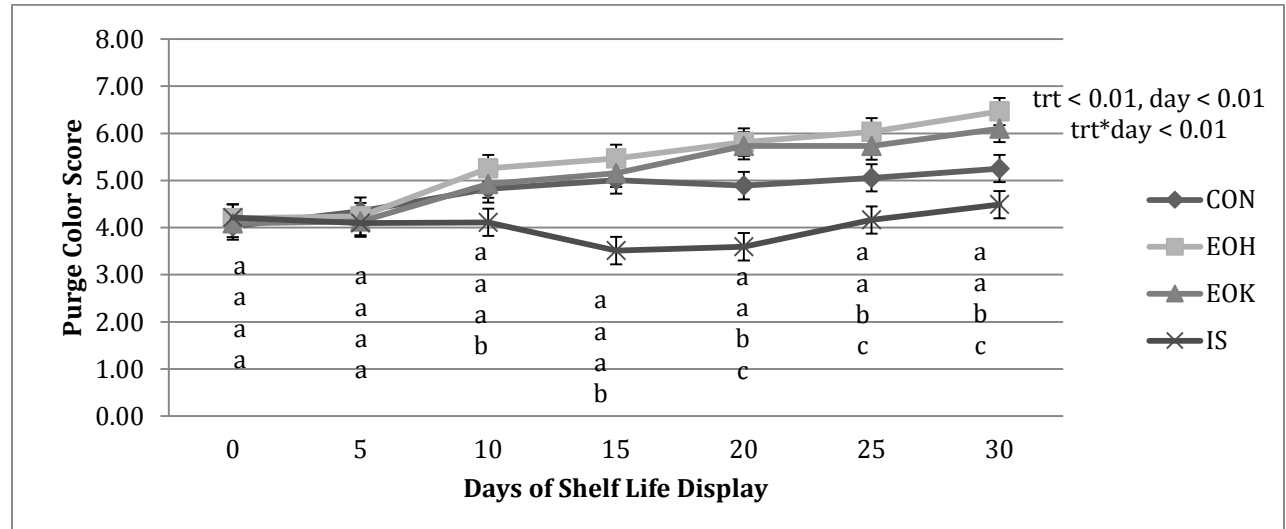


Figure 4.3a: Effects of treatments on objective average color scores of pork loin chops for 30 days of shelf life display. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution. Average color was ranked on an 8 point scale, where 8 = extremely dark purplish-pink; 7 = dark purplish-pink; 6 = moderate purplish-pink; 5 = slight dark purplish-pink; 4 = slight purplish-pink; 3 = moderately bright purplish-pink; 2 = bright purplish-pink; 1 = extremely bright purplish-pink. SEM = 0.19 ^{abc} Means within each day that do not have common superscripts are different ($P < 0.05$).

Figure 4.3b: Effects of treatments on objective purge color scores of pork loin chops for 30 days of shelf life display. CON: Control; EOH: Alkaline electrolyzed water; EOK: Alkaline electrolyzed water with potassium lactate; IS: Industry standard enhancement solution. Purge color was ranked on a 6 point scale, where 6 = dark brown; 5 = light brown; 4 = dark purplish-red; 3 = red; 2 = pink; 1 = clear. SEM = 0.28 ^{abc} Means within each day that do not have common superscripts are different ($P < 0.05$).

CHAPTER 5

CONCLUSIONS

This research shows that alkaline electrolyzed water (AEW) is not an effective replacement for salt/phosphate enhancement solutions commonly used in the industry. Alkaline electrolyzed water did not increase the water holding capacity of pork loin chops when compared to industry standard solutions. Also, treating chops with AEW did not improve tenderness or sensory characteristics. Potassium lactate increased the water holding capacity and sensory characteristics of AEW-treated chops, however this solution was still ineffective as a replacement for traditional enhancement solutions.

Alkaline electrolyzed reduced water was detrimental to pork loin color and lipid oxidation. Chops treated with AEW were less red and had a greater amount of metmyoglobin formation than industry standard solutions. Subjective sensory panelists confirmed objective color results and rated AEW-treated chops as darker. Furthermore, chops treated with AEW alone had increased lipid oxidation. Based on this research the use of AEW as a replacement enhancement solution for traditional solutions is not advised.